Inflammatory mechanisms associated with type 1 diabetes mellitus and oral diseases

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Abstract

Diabetes is a well-known risk factor for periodontal disease; however, the pathogenic links between periodontal disease and type 1diabetes (T1DM) are not completely understood. Therefore, this study evaluated, longitudinally over 6 months, the impact of periodontal disease and its treatment on clinical outcomes, glycated haemoglobin (HbA1c), high-sensitivity C-reactive protein (hsCRP), lipids and local and systemic levels of pro-inflammatory biomarkers [matrix metalloproteinase-9 (MMP-9), B-cell activating factor, resistin, epithelial neutrophil activating peptide-78/CXCL5 (ENA-78/CXCL5) and interleukin-8, (IL-8)] in patients with T1DM.

57 T1DM and 43 non-T1DM patients were recruited. Pre-treatment, T1DM patients had significantly lower diastolic BP, non-HDL and cholesterol compared to non-T1DM patients. T1DM periodontally healthy patients had significantly higher bleeding on probing (BOP) scores compared to non-T1DM periodontally healthy patients. Serum MMP-9, resistin and ENA-78/CXCL5 levels were significantly higher in T1DM patients compared to non-T1DM patients. Furthermore, T1DM periodontitis patients had significantly higher serum MMP-9 levels compared to non-T1DM periodontitis patients. Regardless of diabetes status, GCF MMP-9 levels were significant predictors of clinical periodontal condition. Moreover, T1DM periodontally healthy patients had significantly higher GCF MMP-9 and IL-8 levels compared to non-T1DM periodontally healthy patients.

In T1DM and non-T1DM patients, all clinical periodontal parameters significantly improved at 3 and 6 months following non-surgical periodontal management (NSM), and both groups demonstrated significant reductions in GCF MMP-9 levels at month 6 following NSM. Furthermore, following NSM, GCF IL-8 levels significantly reduced at 3 and 6 months in T1DM patients and at month 3 in non-T1DM patients. In T1DM patients, HbA1c showed 0.45% and 0.90% reductions at 3 and 6 months following NSM, respectively, although these reductions were not statistically significant.

In conclusion, NSM led to significant reductions in GCF MMP-9 and IL-8 levels, and these inflammatory mediators may play a role in the pathogenesis of periodontitis in patients with T1DM.

Dedication

This thesis is dedicated to,

My mother, Dr. Meena Desai

A strong, intelligent, kind and gentle soul, for her unconditional love and constant support, who taught me how to be strong, kind and compassionate, and who made me believe that knowledge is important, as the eyes cannot see what the mind doesn't know.

My father, Dr. Shyam Desai

Who is a gentleman par excellence and an inspiration to many, for earning an honest living for us, for his vision and belief in me and for teaching me that hard work always pays off and integrity is everything.

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List of abbreviations

A. actinomycetemcomitans	*Aggregatibacter actinomycetemcomitans*
AGE	advanced glycation end product
AP	aggressive periodontitis
AAP	American Academy of Periodontology
ADA	American Diabetes Association
ANOVA	analysis of variance
ADDQoL-19	Audit of Diabetes Dependent Quality of Life-19
BAFF	B-cell activating factor
BMI	body mass index
BOP	bleeding on probing
BP	blood pressure
β-cell	beta cell
BSA	bovine serum albumin
CAL	clinical attachment loss
CEJ	cementoenamel junction
COX-2	cyclooxygenase-2
CPITN	community periodontal index for treatment needs
CRF	case report form
CV	coefficient of variation
CVD	cardiovascular disease
DG	type 1 diabetes mellitus patients with gingivitis
DH	type 1 diabetes mellitus patients with healthy periodontal tissues
DMFS	decayed, missing and filled surfaces
DP	type 1 diabetes mellitus patients with periodontitis
EDTA	ethylenediaminetetraacetic acid
EFP	European Federation of Periodontology
ELISA	enzyme-linked immunosorbent assay
ENA-78	epithelial neutrophil activating peptide-78
ESRD	end-stage renal disease
FMD	full-mouth debridement
FMI	full-mouth instrumentation
GCF	gingival crevicular fluid

GDP	general dental practitioner
GIC	glass ionomer cement
H	health
HbA1c	glycated haemoglobin
HDL	high density lipoprotein
HG	non-type 1 diabetes mellitus patients with gingivitis
HH	non-type 1 diabetes mellitus patients with healthy periodontal tissues
HLA	human leukocyte antigen
HP	non-type 1 diabetes mellitus patients with periodontitis
HRQoL	health-related quality of life
HSA	human serum albumin
hsCRP	high-sensitivity C-reactive protein
IDF	International Diabetes Federation
IL-8	interleukin-8
IMD	index of multiple deprivation
LLOD	lower limit of detection
LPS	lipopolysaccharide
LOA	loss of attachment
MAMP	microbe-associated molecular pattern
mGI	modified gingival index
MMP	matrix metalloproteinase
MMP-9	matrix metalloproteinase-9
MPO	myeloperoxidase
n	number
non-HDL	non-high density lipoprotein
NS	not significant
NSM	non-surgical periodontal management
OHIP-14	Oral Heath Impact Profile-14
OHIP-49	Oral Heath Impact Profile-49
OHRQoL	oral health-related quality of life
OHI	oral hygiene instructions
P	periodontitis
PBS	phosphate buffered saline

PD	probing depth
PDI	Periodontal Disease Index
PGE_2	prostaglandin E_2
P. gingivalis	*Porphyromonas gingivalis*
PI	plaque index
PMN	polymorphonuclear leukocytes
QoL	quality of life
RAGE	advanced glycation end products receptor
RANTES	regulated on activation, normal T cell expressed and secreted
RCT	randomised controlled trial
RI	relative intensity
RIA	radioimmunoassay
ROS	reactive oxygen species
RSD	root surface debridement
SD	standard deviation
T1DM	type 1 diabetes mellitus
T1DM+AP	type 1 diabetes mellitus and aggressive periodontitis
T1DM+H	type 1 diabetes mellitus and healthy tissues
T1DM+P	type 1 diabetes mellitus and periodontitis
T1DM-LD	long duration type 1 diabetes mellitus
T1DM-ND	newly diagnosed type 1 diabetes mellitus
T1DM-PC	poorly-controlled type 1 diabetes mellitus
T1DM-SD	short duration type 1 diabetes mellitus
T1DM-WC	well-controlled type 1 diabetes mellitus
T2DM	type 2 diabetes mellitus
T. forsythia	*Tannerella forsythia*
TIMP	tissue inhibitors of metalloproteinase
TLR	toll-like receptor
TNF-α	tumour necrosis factor alpha
OHQoL-UK	UK oral health-related quality of life
W-BQ12	Well-being Questionnaire 12
W-BQ22	Well-being Questionnaire 22
WHO	World Health Organisation

1 Chapter 1. Introduction

1.1 Periodontal disease

1.1.1 Definition and classification

Periodontal disease is defined as, "any inherited or acquired disorder of the tissues surrounding and supporting the teeth (periodontium)" (Pihlstrom et al. 2005). These disorders may be developmental, traumatic, inflammatory, genetic, neoplastic or metabolic in origin (Armitage 2004; Jordan 2004). The term "periodontal disease" commonly refers to gingivitis and periodontitis caused by pathogenic bacteria within the dental plaque biofilm that forms adjacent to tooth surfaces on a daily basis (Pihlstrom et al. 2005). The disease progress is also influenced by host susceptibility, age and smoking (Pantlin 2008).

Gingivitis is defined as "inflammation of the gingiva in which the connective tissue attachment to the tooth remains at its original level". Gingivitis is the mildest form of periodontal disease affecting approximately 50-90% of adults worldwide (Albandar and Rams 2002). The inflammation is confined to the soft-tissue compartment of the gingival epithelium and connective tissue and is readily reversible by means of simple and effective oral hygiene practices (Pihlstrom et al. 2005). Gingivitis always precedes the development of periodontitis, and no evidence from around the world indicates the onset of periodontitis without gingival inflammation (Albandar and Rams 2002). Periodontitis occurs when this inflammation extends deep into the periodontal tissues causing loss of the supporting connective tissue and alveolar bone, leading to pocket formation or deepened crevices between the soft tissues and the tooth root. Severe periodontitis can result in tooth mobility, pain and discomfort, impaired mastication and eventual tooth loss (Pihlstrom et al. 2005).

Periodontal disease encompasses a wide range of disease presentations, and hence the recognition of these diseases requires an accurate diagnosis to be made (Highfield 2009). Various systems of classification of periodontal disease have arisen which assist clinicians in identifying the different presentations in relation to aetiology, pathogenesis and treatment options. Classification systems also allow clinicians and researchers from around the world to communicate effectively in a common language. The most commonly used classification systems are those of the American Academy of Periodontology (AAP). Table 1.1 presents

the classification of periodontal disease as modified from the International Workshop for Classification of Periodontal Disease and Conditions (Armitage 1999).

I. Gingival diseases
A. Plaque induced
1. Gingivitis associated with dental plaque only
2. Gingival diseases modified by systemic factors
3. Gingival diseases modified by medications

B. Non-plaque induced
1. Gingival diseases of specific bacterial origin
2. Gingival diseases of viral origin
3. Gingival diseases of fungal origin
4. Gingival lesions of genetic origin
5. Gingival manifestations of systemic conditions
6. Traumatic lesions
7. Foreign body reactions
8. Not otherwise specified

II. Chronic periodontitis
A. Localised
B. Generalised

III. Aggressive periodontitis
A. Localised
B. Generalised

IV. Periodontitis as a manifestation of systemic disease
A. Associated with haematological disorders
B. Associated with genetic disorders

V. Necrotizing periodontal diseases
A. Necrotizing ulcerative gingivitis
B. Necrotizing ulcerative periodontitis

VI. Abscesses of the periodontium
A. Gingival abscess
B. Periodontal abscess
C. Pericoronal abscess

VII. Periodontitis associated with endodontic lesions

VIII. Developmental or acquired deformities
A. Localised tooth-related factors that modify or predispose to plaque-induced gingival diseases or periodontitis
B. Mucogingival deformities and conditions around teeth
C. Mucogingival deformities and conditions on edentulous ridges
D. Occlusal trauma

Table 1.1: Overview of the classification of periodontal diseases and conditions.

“Localised” suggests periodontal disease involving ≤30 % of sites and “generalised” suggests periodontal disease involving ≥30 % sites (Armitage 1999).

The severity of periodontal disease can be characterised on the basis of the degree of clinical attachment loss (CAL) as: slight (CAL of 1 or 2 mm), moderate (CAL of 3 or 4 mm) and severe (CAL ≥5 mm) (Armitage 1999). Currently within the published literature there appears to be a conflict and lack of consistency with regard to the definition of what constitutes a periodontal case. Table 1.2 shows two different case definitions of periodontal disease which have been used for epidemiological surveys in past research studies (Tonetti and Claffey 2005; Page and Eke 2007).

Case definition	**5th European Workshop** (Tonetti and Claffey 2005)	**American Academy of Periodontology** (Page and Eke 2007)
Incipient or moderate periodontitis	Presence of proximal attachment loss of ≥3 mm in ≥2 non-adjacent teeth.	Presence of ≥2 interproximal sites with CAL of ≥4 mm (not on same tooth) or ≥2 interproximal sites with PD ≥5 mm (not on same tooth).
Substantial or severe periodontitis	Presence of proximal attachment loss of ≥5 mm in ≥30% of teeth present.	Presence of ≥2 interproximal sites with CAL of ≥6 mm (not on same tooth) and ≥1 interproximal site with PD ≥5 mm.

Table 1.2: Case definition of periodontal disease used in past research studies.

Both these studies have used CAL at interproximal sites of non-adjacent teeth as their main criteria for defining periodontitis; despite this, there is recognition that the diagnosis and detection of periodontal disease cannot be based on measurement of a single variable. CAL is a measure of the cumulative lifetime experience of periodontitis, and hence provides very little evidence of the current inflammatory condition of the periodontal tissues and therefore it is essential to consider additional measurements, such as bleeding on probing (BOP) and probing depth (PD) measurements. Additionally, both sets of criteria took into account the potential error in measuring CAL, to exclude cases without periodontitis. Hence, the threshold for interproximal CAL was set at ≥6 mm (Page and Eke 2007) or ≥5 mm (Tonetti and Claffey 2005).

The AAP's case definition defines 'severe periodontitis' as being present if there is a minimum of two teeth with 6 mm CAL and one tooth with PD of 5 mm (Page and Eke 2007). Based on this definition, it would seem possible to include a subject into the study who has only minimal levels of disease or attachment loss caused by overhanging restorations or at the

distal aspect of second molars where a third molar has been extracted. The benefits of having a high threshold for identifying periodontitis cases must be weighed against an ethical issue of missing periodontal cases, and if the criteria for case definitions are to provide a robust basis for research, it is essential to include cases which have suitable levels of disease to generate data, from which valid conclusions can be made. With regard to substantial extent and severity of periodontal disease, the 5th European Workshop in Periodontology provided a more robust inclusion criteria, requiring subjects to have interproximal CAL of ≥5 mm present in ≥30% of teeth to define the presence of periodontitis (Tonetti and Claffey 2005).

Further to these case definitions, the AAP updated the case definition for population-based surveillance of periodontitis by providing and including a case definition for mild periodontitis (Eke et al. 2012). The initial report (Page and Eke 2007) did not define mild periodontitis as it primarily focused on validating the utilization for self-reported questions for predicting the prevalence for moderate to severe periodontitis. In the updated case definition, criterion for assessing moderate to severe periodontitis remain unchanged as previously published (Page and Eke 2007) and are included in Table 1.3.

Updated case definition	**American Academy of Periodontology**
No periodontitis	No evidence of mild, moderate, or severe periodontitis.
Mild periodontitis	Presence of ≥2 interproximal sites with CAL ≥3 mm, and ≥2 interproximal sites with PD ≥4 mm (not on the same tooth) or one site with PD ≥5 mm.
Moderate periodontitis	Presence of ≥2 interproximal sites with CAL of ≥4 mm (not on same tooth) or ≥2 interproximal sites with PD ≥5 mm (not on same tooth).
Severe periodontitis	Presence of ≥2 interproximal sites with CAL of ≥6 mm (not on same tooth) and ≥1 interproximal site with PD ≥5 mm.

Table 1.3: Updated case definition for periodontal disease (Eke et al. 2012).

From a public health perspective, it is important to track mild periodontitis in populations as this type of disease is most responsive to preventive care and oral hygiene practices to control and prevent periodontitis and is crucial for predicting those populations at risk for developing moderate to severe periodontal disease in the future. It is also essential to include mild periodontitis in case definitions in research studies as excluding this would underestimate the

burden of periodontal disease especially in the younger population who are more likely to have a/the mild form of disease (Eke et al. 2012). Previously, Eke and colleagues determined the accuracy of periodontal prevalence assessment methods by comparing two methods of periodontal examination: partial-mouth periodontal examination and full-mouth ‘gold standard’ periodontal examination, and found that partial-mouth periodontal examination greatly underestimated the prevalence of periodontal disease by at least 50%, leading to high levels of misclassification of periodontal cases (Eke et al. 2010).

1.1.2 Epidemiology of periodontal disease

Over the past 20 years, epidemiological studies have attempted to provide information regarding the extent and severity of periodontal disease in various populations. In 2009, the UK Adult Dental Health Survey reported the prevalence of approximately 66% of adults, aged ≥55 years, having moderately advanced chronic periodontitis [with loss of attachment (LOA) ≥4 mm] and 25% of adults, having severe periodontitis (with LOA ≥6 mm) and only a small proportion (4%) had LOA ≥9 mm. Additionally, 17% of adults had healthy periodontal tissues and good periodontal health was more commonly seen in adults <45 years of age. A majority (37%) of adults had mild levels of disease, with PD restricted to a range between 4-6 mm (White et al. 2011). This survey revealed that although periodontal disease is prevalent in the UK population, severe periodontal disease occurs in only a relatively small portion of individuals (Steele and O'Sullivan 2011). Furthermore, visible plaque and calculus were present in 66% and 68% of adults, respectively (Chadwick et al. 2011; Steele and O'Sullivan 2011).

Similar findings were reported in prevalence studies of other populations, for example, the recent update on the prevalence of periodontitis in adults (aged ≥30 years) in the US (the National Health and Nutrition Examination Survey 2009 to 2012) reported that 46% of adults, representing 64.7 million people had periodontitis, with only 8.9% having severe periodontitis (Eke et al. 2015). A previous prevalence study in the US of 7,447 people, found that although over 90% of people aged ≥13 years experienced LOA, only 15% of them showed signs of severe disease (with LOA ≥5 mm) (Brown et al. 1996). Another study in the US of 9,698 people concluded that mild periodontitis is widespread in the population however, moderate to severe periodontitis affects only a small portion of people (3.1-9.5%) (Albandar et al. 1999). In contrast, a study of 853 Brazilians reported a much higher prevalence of advanced periodontal disease, with 52% of individuals showing severe

periodontal destruction (LOA ≥7 mm) (Susin et al. 2004). A prevalence study in Xinjiang a rural area in China, reported the prevalence of mild, moderate and severe periodontitis was 28.9%, 10.2% and 8% respectively (Awuti et al. 2012). An epidemiological survey in the US from 1988 to 2000, reported a reduction in the prevalence of advanced periodontal disease from 7.3% to 4.2% (Borrell et al. 2005). Despite these results being lower than previous prevalence estimates for advanced periodontal disease, there still remains a significant number of individuals who experience periodontal disease which could lead to tooth mobility and subsequent tooth loss.

The differences in periodontal disease prevalence rates reported over the past years may be due to methodological variations, such as, the practice of full-mouth versus partial-mouth examinations. Additionally, the past few years have seen clear improvements in periodontal health care, awareness and improved provision of dental care (Steele and O'Sullivan 2011). Smoking also plays a vital role in the development of periodontal disease (Kinane and Chestnutt 2000) and it has been estimated that up to 50% of cases of periodontitis are caused by smoking (Tomar and Asma 2000). The current smoking prevalence rate for smoking in England is 19% (Niblett 2015). The last 40 years have seen a decline in the percentage of smokers within Western populations (Pierce 1989; Molarius et al. 2001), which may have led to a decrease in prevalence rates of periodontal disease in different populations.

1.1.3 Pathogenesis of periodontal disease

Periodontitis was for many years, considered to be an almost ubiquitous condition in which dental plaque was known to be the sole aetiological factor. Landmark publications have changed our way of thinking about periodontitis, such as the initiating role of plaque bacteria in gingivitis (Loe et al. 1965), the histological evidence of inflammation in the periodontium (Page and Schroeder 1976), recognition of the differences in disease susceptibility among individuals (Loe et al. 1986) and the important role the host response plays in disease progression (Page et al. 1997).

Initiating role of the plaque biofilm

Periodontitis results from a complex interaction between the immune system and microorganisms (Sanz et al. 2011), which can be further modified by acquired and environmental risk factors (Zee 2009). Dental plaque is a microbial biofilm causing periodontal disease. Biofilms are defined as “matrix-enclosed bacterial populations adherent

to each other and/or to surfaces" (Socransky and Haffajee 2002). Initial biofilm formation involves the adsorption of salivary mucins and proteins resulting in formation of an acquired pellicle. Oral bacteria bind to this pellicle and to each other in a highly specific succession of species. In healthy periodontal sites, the biofilm consists mostly of Gram-positive bacterial species, while only about 15% Gram-negative species are found. In contrast, diseased periodontal sites demonstrate an increase in Gram-negative species to approximately 50% (Tanner et al. 1996). Accompanying this shift in microbial composition from health to disease is an increase in the total amount of bacteria from approximately 10^2-10^3 during periodontal health, 10^4-10^6 during gingivitis and rising as high as 10^5-10^8 during periodontitis (Tanner et al. 1996). Although there is evidence that the microbial biofilm plays a role in the aetiology of periodontal disease, it is less clear whether it initiates periodontal disease non-specifically or specifically. The non-specific plaque hypothesis states that, "periodontal disease is due to bacterial accumulation, irrespective of its composition". This implies that no one specific species of bacteria is more significant than the other in its ability to cause disease. In contrast, specific plaque hypothesis states that, "periodontal disease is the result of an infection with a single specific pathogen". This theory may help explain why although many patients have substantial plaque deposits, only a minority suffer from severe disease. However, to date, no one specific pathogen has been linked to chronic gingivitis or periodontitis (Hasan and Palmer 2014). The multiple pathogen hypothesis states that, "periodontal disease is the result of infection with a relatively small number of interacting bacterial species". However, the major difficulty lies in establishing the possible combination of species that are important (Hasan and Palmer 2014).

The concept that not all microbial biofilms cause periodontal destruction was highlighted in the consensus report of the World Workshop on Clinical Periodontics in 1996, which concluded that *Porphyromonas gingivalis* (*P.gingivalis*), *Aggregatibacter actinomycetemcomitans* (*A.actinomycetemcomitans*) (previously known as *Actinobacillus actinomycetemcomitans*) and *Tannerella forsythia (T.forsythia)* (previously known as *Bacteroides forsythus*) (known as red complex microorganisms) must be considered as chief periodontal pathogens (Hur 1996) with the following recognition that *Fusobacterium nucleatum* is also a member of this group (Teles et al. 2006). Molecular techniques and cluster analysis of subgingival plaque have shown that certain bacterial species co-exist in "complexes" (Figure 1.1) and have demonstrated a strong association between *P.gingivalis* and greater PD and increased BOP in periodontal disease (Socransky et al. 1998). Another

study showed increased proportion of red and orange complex species (*Prevotella intermedia*, *Fusobacterium nucleatum* and *Eubacterium nodatum*) in patients with periodontitis compared to subgingival plaque from patients with healthy tissues (Ximenez-Fyvie et al. 2000). Additionally, the relationship between clinical periodontal parameters and red and orange complex species is also mirrored for supragingival plaque samples (Haffajee et al. 2008). However, these bacterial species have also been identified in plaque from patients with healthy tissues (Loomer 2004; Sanz and Quirynen 2005) highlighting the complex interplay between host response and bacterial challenge involved in pathogenesis of periodontal disease.

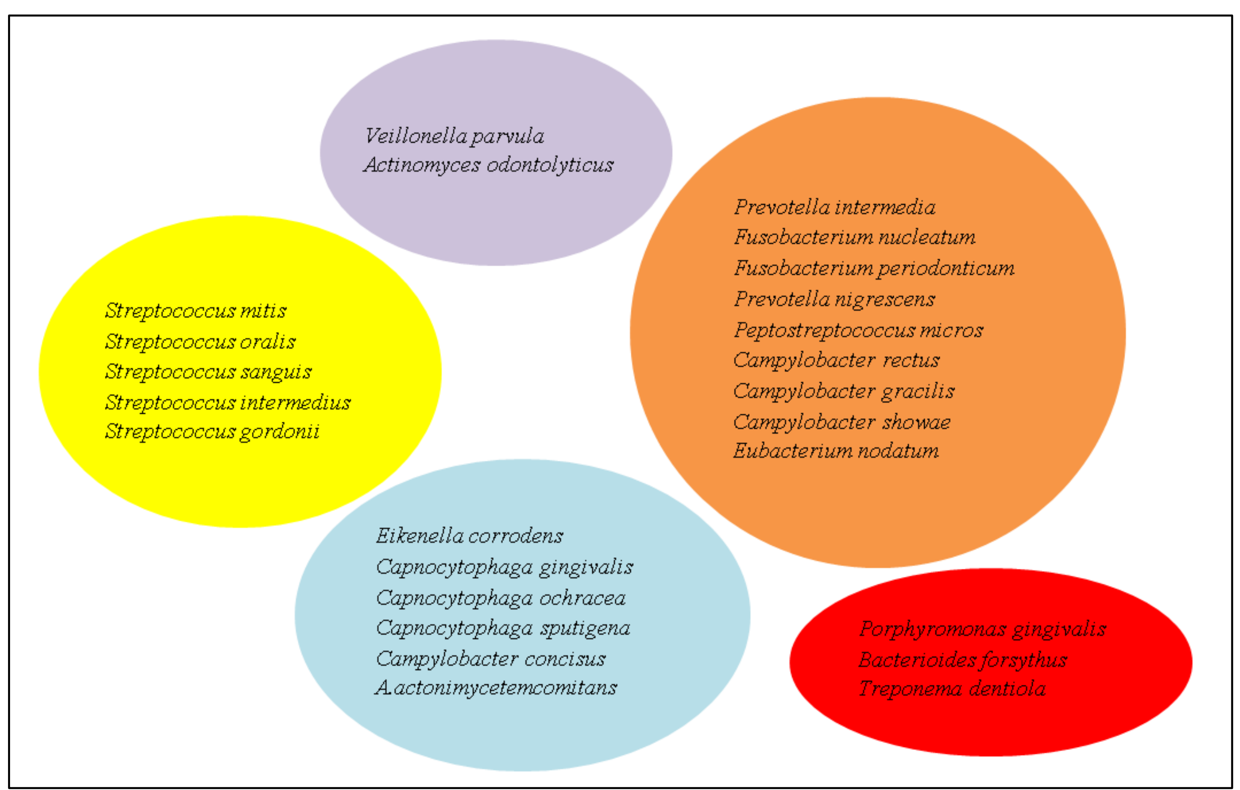


Figure 1.1: Overview of microbial complexes in subgingival plaque.

In subgingival plaque certain microbial species have been found to frequently occur together in “complexes”. This figure shows a diagrammatic representation of these “complexes” (Socransky et al. 1998).

The dental plaque is a source of a number of antigens including leukotoxin, lipoteichoic acid, peptidoglycan, lipopolysaccharides (LPS), fimbriae and extracellular enzymes (Travis et al. 1997; Fives-Taylor et al. 1999). The bacterial challenge stimulates an inflammatory response and causes direct damage to the periodontal tissues. For example, gingipains produced by *P.gingivalis* facilitate bacterial invasion into the tissues and contribute to periodontal tissue destruction (Genco et al. 1999a; Imamura 2003; Andrian et al. 2004), furthermore, LPS from Gram-negative bacteria like *P.gingivalis*, stimulate host responses via specific host receptors (Dixon et al. 2004). Indeed studies over the past 20 years, have suggested the initiating role of pathogenic bacteria in periodontal pathogenesis, confirming a limited number of bacterial species associated with severe periodontal disease (Tanner et al. 1996; Socransky et al. 1998). However, variations in disease experiences are not matched with microbial factors and individuals may harbour pathogens without displaying progressive periodontal disease (Cullinan et al. 2003). Hence, although periodontitis is related to the existence of certain pathogenic bacteria in the subgingival biofilm (Socransky et al. 1998; Haffajee et al. 2008), the presence of a pathogenic biofilm alone does not cause periodontal disease. A host-bacteria interaction and the complexity of the subsequent inflammatory response are essential for the development and progression of periodontal disease.

Host response

The accumulation of dental plaque causes inflammation to develop within the periodontal tissues. The blood vessels within the periodontal tissues dilate and become more permeable, allowing fluid and defence cells to accumulate at the infection site. In order to combat the pathogenic bacteria, first, a large number of neutrophils [polymorphonuclear leukocytes (PMNs)], followed by lymphocytes accumulate within the tissues, crossing the junctional epithelium and migrating into the periodontal pocket (Page and Schroeder 1976). The PMNs are a critical component of the innate immune system; they maintain periodontal health when subjected to constant bacterial challenge from the plaque biofilm. PMNs are protective by intent, by their ability to phagocytose and kill microorganisms. The vital role PMNs play in innate immunity is highlighted in congenital disease such as Chediak-Higashi syndrome and leukocyte adhesion deficiency syndrome, in which genetic defects alter the functional responses of the PMNs, leading to recurrent microbial infections and severe periodontal disease in these patients (Lekstrom-Himes and Gallin 2000). However, along with their protective function, the PMNs release potent lysosomal enzymes, cytokines and reactive oxygen species (ROS) which cause destruction of the periodontal tissues (Van Dyke and

Vaikuntam 1994; Johnstone et al. 2007). Contributing to the destructive process in periodontitis is neutrophil hyperactivity leading to the overproduction of antimicrobial and tissue-damaging ROS (Fredriksson et al. 2003). The host response is essentially protective by intent, but results in local tissue destruction, sometimes referred to as ‘collateral damage’ (Preshaw and Taylor 2011).

If dental plaque is left undisturbed there is a continued cycle of microbial challenge and host inflammatory responses. Hence, in addition to tissue damage caused by the pathogenic bacteria, the residing tissue cells and the infiltrating host defence cells contribute to connective tissue breakdown and alveolar bone loss (Bartold and Narayanan 2006). Handfield *et al.* demonstrated that host cells respond to bacteria by activating intra-cellular signalling pathways leading to cytokine secretion *in vitro* (Handfield et al. 2008). The activation of the inflammatory host response relies on the ability of the host cells to recognise the presence of pathogenic bacteria and their by-products. Within the periodontal tissues, a diverse collection of host receptors enables the host cells to recognise microbe-associated molecular patterns (MAMPs) and orchestrate an immune-inflammatory response which reflects the bacterial challenge. An example of this type of periodontal pathogenesis is the ability of host receptors such as LPS-binding protein, membrane-associated CD14 and Toll-like receptors (TLRs) to recognise bacterial LPS and fimbriae (Dixon and Darveau 2005).

In vitro research experiments have demonstrated that bacteria within the periodontal tissues stimulate the secretion of a wide range of pro-inflammatory cytokines such as interleukin-1α (IL-1α), IL-1β, IL-6, IL-8 and IL-12 (Sandros et al. 2000; Kusumoto et al. 2004). Furthermore, when TLRs interact with the periodontal pathogens there is a release of a similar range of inflammatory cytokines and antimicrobial proteins from the host cells (Jotwani et al. 2003; Dixon et al. 2004; Eskan et al. 2007; Eskan et al. 2008), highlighting the important role host receptors play in the immune-inflammatory response in periodontal disease. The activation of specific host receptors by bacterial MAMPs allows the periodontal tissues to direct an immune-inflammatory response appropriate to the pathogens present within the biofilm. However, this defence mechanism of the host causes the majority of periodontal tissue destruction leading to the clinical signs of periodontal disease.

The persistent nature of the plaque biofilm also results in the activation of the adaptive immune responses, leading to infiltration of T and B cells into the periodontal tissues (Page and Schroeder 1976). An appropriate adaptive immune response to the bacterial challenge

relies on the balanced production of different subsets of T cells by the host tissues. The production of Th1 cells leads to cell-mediated immune responses, with the activation of macrophages and the induction of B cells to produce opsonising antibodies, which facilitate bacterial killing. Conversely, the production of Th2 cells provides humoral immunity, with activation of B cells to produce neutralising antibodies. Th1 and Th2 cells have been found to release different but overlapping sets of cytokines, but despite extensive research, their contribution to periodontal destruction has yet to be clearly defined. Some studies support the hypothesis that Th1 cells are associated with healthy periodontal sites and Th2 cells are associated with periodontal disease progression (Gemmell and Seymour 1994; Bartova et al. 2000). However, elevated Th1 and reduced Th2 cells, in sites with periodontal disease have been found in a few studies (Salvi et al. 1998; Takeichi et al. 2000). Interestingly, some studies have demonstrated the involvement of both Th1 and Th2 cells in periodontal disease (Gemmell et al. 1999; Berglundh et al. 2002). Despite the lack of consensus about the role of different T cells, it remains clear that the balance of cytokines produced by innate and adaptive immune responses is a key contributing factor in whether the periodontal disease remains stable or progresses (Okada and Murakami 1998).

Biomarkers in periodontal disease

Tissue destruction in periodontal disease results primarily from an upregulated immune-inflammatory response stimulated by prolonged exposure to plaque bacteria. This inflammatory response is characterised by increased local production of pro-inflammatory cytokines [particularly IL-1β, IL-6 and tumour necrosis factor-α (TNF-α)] which result in breakdown of collagen fibres, osteoclastic activation and impaired wound healing, leading to the clinical signs of disease. The vital role cytokines play in the host-inflammatory immune response has been demonstrated by analysing samples from both human and animal studies (Gemmell et al. 1997; Landi et al. 1997; Okada and Murakami 1998). Cytokines are soluble proteins which bind to specific receptors on target cells, initiating intracellular signalling cascades which result in phenotypic changes in the target cells via altered gene regulation and are effective at low concentrations (Seymour and Taylor 2004; Preshaw and Taylor 2011). Within the periodontal tissues, cytokines are produced by the infiltrating host defence cells (lymphocytes, neutrophils and macrophages) and the resident periodontal tissue cells (fibroblasts and epithelial cells) (Takashiba et al. 2003). Most cytokines are self-regulatory, having the ability to induce their own expression and have pleotropic actions on a number of cell types (Taylor et al. 2004). Cytokines are fundamental to immune and inflammatory

responses, but also contribute to tissue breakdown; for example, IL-1β, IL-6 and TNF-α have biologic activity which underpin tissue damage in chronic periodontitis.

Periodontal research has largely focused on investigating IL-1β concentrations in gingival crevicular fluid (GCF) and gingival tissues. IL-1β is believed to play a major role in periodontal pathogenesis, and several studies have found elevated GCF IL-1β levels in patients with periodontitis (Preiss and Meyle 1994; Figueredo et al. 1999; Engebretson et al. 2002; Zhong et al. 2007) and improvements in periodontal health were accompanied by significant reductions in GCF IL-1β levels following periodontal treatment (Engebretson et al. 2002; Thunell et al. 2010). Additionally, IL-1β has proved to be a potent inducer for connective tissue degradation and bone resorption via the induction of matrix metalloproteinases (MMPs) (Birkedal-Hansen 1993). Elevated levels of IL-1β and TNF-α have been found in gingival biopsies obtained from diseased sites compared to healthy sites (Stashenko et al. 1991). Both IL-1β and TNF-α can cause upregulation of adhesion molecules on endothelial cells and leukocytes, stimulate chemokine production (which in turn recruit leukocytes to sites of inflammation) and induce the expression of inflammatory mediators such as prostaglandins and MMPs which have the ability to potentiate inflammatory responses (Preshaw and Taylor 2011).

MMPs are known to play a crucial role in the regulation of periodontal tissue turnover in health and disease (Uitto et al. 2003; Sorsa et al. 2004; Sorsa et al. 2006; Li et al. 2012; Salazar et al. 2013). MMPs are controlled and inhibited by tissue inhibitors of metalloproteinases (TIMPs). A balance between MMPs and TIMP activities can maintain tissue integrity, while an excessive production of MMPs or TIMPs can result in increased tissue degradation (Jacqueminet et al. 2006). MMPs can also process cytokines and a variety of other bioactive non-matrix substrates such as chemokines, immune mediators and growth factors, thereby mediating pro- and anti-inflammatory processes (Sorsa et al. 2006; Giannobile 2008; Hernandez et al. 2011; Butler and Overall 2013). The primary source of MMPs in the oral cavity is the PMNs, which enter the oral cavity through the gingival sulcus (Gangbar et al. 1990; Overall et al. 1991). MMP-9 is present within the granules of PMNs (Hartog et al. 2003), but is also expressed by a variety of other cells in the healthy and diseased periodontium (Schiott and Loe 1970; Sorsa et al. 2004). The main collagenase in periodontitis is MMP-8 followed by MMP-9 (Sorsa et al. 1995). MMP-8, MMP-9 and MMP-13 in GCF, are the most widely reported MMPs in sites with active periodontal disease

(Lee et al. 1995; Choi et al. 2004; Tuter et al. 2005; Beklen et al. 2006; Kumar et al. 2006; Soder et al. 2006). Additionally, studies have reported significantly elevated plasma MMP-3, MMP-8 and MMP-9 levels (Marcaccini et al. 2009a), GCF MMP-8, MMP-9, TIMP-2 and myeloperoxidase (MPO) levels (Marcaccini et al. 2010) and serum levels of MMP-1, MMP-3, MMP-9, IL-2, IL-8 and cyclooxygenase-2 (COX-2) (Li et al. 2012) in patients with chronic periodontitis compared to healthy controls.

Chemokines are synthesized by various cell types, including epithelial, endothelial and stromal cells, such as leukocytes, monocytes, fibroblasts, mast and bone cells. Based on functionality, chemokine molecules can be homeostatic and inflammatory (Moser et al. 2004). Homeostatic chemokines are expressed in lymphoid tissues and bone marrow and play a crucial role in immune surveillance, haematopoiesis and adaptive immune responses (Murphy et al. 2000; Moser et al. 2004; Esche et al. 2005). While the expression of homeostatic chemokines is constitutive, the inflammatory chemokines are induced by stimuli such as pathogens, cytokines, and growth factors, by cell-to-cell contact or by chemokines themselves. Chemokines found in both GCF and gingival tissue are thought to play an important role in the immunopathogenesis of periodontal disease (Silva et al. 2007). The first cytokine identified to have chemotactic activity was IL-8/CXCL8, which was found to be a chemoattractant of PMNs. IL-8 has been detected in periodontally healthy tissues and has been associated with PMNs-associated low subclinical inflammation (Payne et al. 1993; Mathur et al. 1996). After cessation of tooth brushing, a rapid increase in GCF IL-8 levels was found preceding clinical signs of periodontal disease (Garlet et al. 2005). The levels of IL-8 in GCF and gingival tissues were found to be drastically increased and correlated with disease severity in patients with periodontitis (Tsai et al. 1995). In contrast, one study reported lower GCF IL-8 levels in periodontitis patients compared to healthy controls (Chung et al. 1997). Plasma concentrations of a chemokine, neutrophil chemoattractant and activator known as epithelial neutrophil activator-78 (ENA-78)/CXCL5 were significantly elevated in smokers with periodontitis compared to non-smokers with periodontitis (Lappin et al. 2011). Additionally, the authors reported that periodontitis patients had significantly elevated plasma levels of pro-inflammatory cytokine IL-6 and chemokine ENA-78/CXCL5 compared to periodontally healthy subjects (Lappin et al. 2011).

1.1.4 Quantification of biomarkers

It is essential to have sensitive methods for the precise quantification of biomarkers while assessing levels in clinical samples, especially in GCF samples, for which sample volumes are very small. Various techniques are available to detect and quantify biomarkers, such as bioassay, radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), and multiplex assays for the simultaneous quantification of multiple biomarkers.

Bioassays have been used to study and monitor the effects of biomarkers on biological systems *in vitro*, for example, to assess the impact of adding specific cytokines on responses of cells in culture. For example, primary human gingival fibroblasts cultured with IL-1β or TNF-α of different concentrations, showed a concentration-dependent stimulation of production of IL-6 mRNA and IL-6 protein by IL-1β and TNF-α (Palmqvist et al. 2008). Further to this, the impact of IL-6 on the osteoblastic differentiation of primary human periodontal ligament cells in culture was assessed by quantifying alkaline phosphatase staining histochemically (Iwasaki et al. 2008). Samples usually contain many different biomarkers and the contamination by more active substances may influence the results. Hence, this technique is not ideal for biomarker quantification in clinical periodontal studies.

The principle of the RIA is based on the competition between the antigen (within a sample) and a radio-labelled homologous antigen for a limited number of specific antibody binding sites. Subsequently, the amount of radio-labelled homologous antigen is quantified by a liquid scintillation counter. The amount of radio-labelled homologous antigen present is inversely proportional to the mediator concentration in the clinical sample, which is calculated from a standard curve generated from known amounts of mediator. RIAs can be used to detect various mediators. For example, a study demonstrated the use of RIA to detect GCF prostaglandin-E_2 (PGE_2) levels in patients with gingivitis and periodontitis. Patients with periodontitis had significantly higher PGE_2 levels in GCF as compared to those with gingivitis (Offenbacher et al. 1981). RIAs are very sensitive testing methods, despite this, they are lengthy to perform and the availability of more rapid assays to assess biomarker levels precludes their use in clinical studies.

ELISAs are non-competitive immunoassays, which are based on the principle of capturing test antigens by an antibody which is coated onto the wells of the microtiter plate. A washing step removes any free antigen, and then a second antibody is added which binds to the antigen present on the plate. Following this, the plate is washed to remove any unbound

antibody and then, a ligand is added. The ligand is a molecule which binds to the antibody on the plate, and itself is covalently coupled to an enzyme such as peroxidase. Following this, a washing step is performed which removes the free ligand. The bound ligand is then visualised by the addition of a chromogen, which is a colourless substrate and when acted upon by the enzyme of the ligand, produces a visible coloured end product. The colour intensity and visibility in the reaction wells is determined by optical density scanning of the plate, and comparison with a standard curve determines the quantity of the test antigen present. ELISA testing has been used in studies to quantify biomarker levels in GCF, saliva and plasma samples in patients with periodontitis (Zhong et al. 2007), and also to quantify biomarker levels in GCF following periodontal management (Engebretson et al. 2002). ELISA can only be performed to test a single biomarker per sample. This is a major limitation given the increasing recognition that biomarkers function in networks in periodontal disease pathogenesis (Preshaw and Taylor 2011).

Most recently, methods to simultaneously quantify biomarkers have been developed, known as high-throughput multiplex immunoassays. Based on ELISA technology, two basic assay formats have been developed: planar array assays and micro-bead assays. In planar assays, various capture antibodies are spotted on a 2-dimensional array, acquiring defined positions on a pre-coated microtiter plate. A standard curve is then used to quantify biomarker levels. In the micro-bead assays, various antibodies are conjugated onto different populations of micro-beads, which can be differentiated by using fluorescence intensity in a flow cytometer. Similarly, this method also utilises a standard curve to quantify the unknown biomarker levels. The MULTI-ARRAY (Meso Scale Discovery) and the Luminex-based Bio-Plex (Bio-Rad Laboratories) platforms are most suitable for biomarker quantification (Fu et al. 2010). The micro-bead assay was used to quantify multiple biomarkers assessing changes in serum levels in patients with diabetes, following periodontal therapy (O'Connell et al. 2008). Although these multiplex immunoassays are advantageous, an important limitation is their high cost, limiting their use in large clinical studies (Thunell et al. 2010).

1.1.5 Factors influencing the susceptibility to periodontal disease

Cigarette smoking has long been recognised as a risk factor for periodontal disease and subsequent tooth loss (Tomar and Asma 2000; Genco and Borgnakke 2013). A risk factor analysis suggests that 40% of cases with chronic periodontitis may be due to tobacco smoking (Brothwell 2001). Smokers are approximately 3-4 times more susceptible to

periodontal disease compared to non-smokers (Tomar and Asma 2000; Calsina et al. 2002) and those having a longer smoking history are at a higher risk of developing periodontal disease (Linden and Mullally 1994; Hyman and Reid 2003). The importance of smoking as a risk factor for periodontal disease has been supported by a consistency in results across several studies, the strength and dose-response of the association, the temporal sequence of smoking and periodontal disease and biologic plausibility (Genco and Borgnakke 2013). Grossi *et al.* demonstrated that the amount of CAL was greater as number of pack years increased, and loss of alveolar crest height was positively correlated with the number of pack years of smoking (Grossi et al. 1995). Mechanisms by which smoking can adversely impact the periodontium have been reviewed by Heasman *et al.* and these effects can be divided into several categorises related to the effect of cigarette smoking on: microbiology, gingival blood flow, PMNs phagocytosis, cytokine production, CD3, CD4 and CD8+ T-cell subsets and periodontal healing (Heasman et al. 2006). Chronic smoking has a long-term effect on the periodontal tissues and impairs gingival circulation (Bergstrom and Bostrom 2001; Dietrich et al. 2004). Smoking leads to peripheral vasoconstriction possibly associated with low doses of nicotine. Vasoconstriction leads to reduced gingival bleeding and hence it may appear that smokers have less gingivitis compared to non-smokers. The compromised microvasculature response in smokers may lead to reduced oxygen tension within the periodontal pocket, hence favouring the overgrowth of anaerobic bacteria such as, *P. gingivalis*, *T. forsythia* and *Treponema denticola* increasing the risk for development and progression of periodontal disease (Zambon et al. 1996; Genco and Borgnakke 2013). Furthermore, smoking alters the composition of the plaque biofilm, by increasing the pathogenic bacteria which cause periodontal disease (Eggert et al. 2001; Haffajee and Socransky 2001; Shchipkova et al. 2010). Smoking has deleterious effects on the functioning of PMNs, which include impaired migration and chemotaxis (Seow et al. 1994), and increased PMN elastase, leading to degranulation in the neutrophils, making neutrophils more prone to bacterial challenge (Soder et al. 2002). Increased concentrations of GCF levels of TNF-α have been detected in smokers, suggesting a more destructive inflammatory process (Fredriksson et al. 2002; Genco and Borgnakke 2013). Smoking also leads to elevated numbers of CD3, CD4 and CD8+ T-cell subsets within the periodontal tissues, associated with greater periodontal breakdown (Loos et al. 2004). Research has confirmed the benefits of quitting smoking as a part of periodontal management, which proved to be beneficial with greater PD reductions in smokers who quit smoking compared to those who did not (Preshaw et al. 2005). A 10-year radiographic follow-up study, showed that progressive alveolar bone loss significantly

reduced in those who quit smoking during the study compared to continual smokers (Bolin et al. 1993). Furthermore, smoking cessation is found to alter the subgingival microbial recolonization. Fullmer *et al.* reported that following non-surgical periodontal management (NSM), the microbial profile in smokers remained similar to baseline, whereas the biofilm composition altered reflecting a less pathogenic subgingival microbiota in those who quit smoking, (Fullmer et al. 2009). There is need for long-term follow-up studies of those who quit smoking and non-smokers to determine more clearly the benefit of smoking cessation on the periodontium. However, existing studies strongly suggest that a part of periodontal management must involve an attempt at smoking cessation (Genco and Borgnakke 2013).

Besides smoking, systemic conditions such as cardiovascular disease (CVD), pregnancy and diabetes mellitus may increase the susceptibility to periodontal disease. The 2013 consensus report of the European Federation of Periodontology (EFP)/ AAP Workshop on Periodontitis and systemic diseases concluded that there was strong and consistent epidemiological evidence that periodontitis increases the risk of developing future CVD (Tonetti et al. 2013). Periodontal disease contributes to a low-grade systemic infection and inflammatory burden, leading to cardiovascular events and stroke in susceptible individuals. The impact of periodontitis on markers of inflammation in serum such as, high-sensitivity C-reactive protein (hsCRP), IL-6, plasminogen factors, white blood cell counts, and on serum lipids, brachial artery flow rate, intima media thickness suggests that periodontitis has a negative impact on such CVD surrogates (Kinane et al. 2008). Chronic periodontitis leads to the entry of pathogenic bacteria or their by-products into the blood stream. These bacteria have the ability to activate the host inflammatory response by a variety of mechanisms. The host's immune response support atheroma formation, maturation and exacerbation. Additionally, there is a correlation between the subgingival microbiota and pathogenic bacteria detected in vascular lesions. Periodontal treatment often elicits a transient increase in systemic inflammatory or pro-thrombotic mediators and a decrease in endothelial cell function in the first 24-48 hours (D'Aiuto et al. 2013). This occurs mostly due to bacteraemia and trauma following treatment. Hence, it would be beneficial to minimize potential bacteraemia by emphasizing oral hygiene and carrying out periodontal treatment in multiple sessions rather than performing a single intensive treatment session (Tonetti et al. 2013).

Pregnancy-related periodontal disease has been associated with adverse outcomes such as low birth-weight babies, pre-term birth, growth restriction, pre-eclampsia, miscarriage and/or still birth (Sanz et al. 2013). Maternal periodontitis has the ability to directly or indirectly

influence the health of the foetal-maternal unit. Research has identified two major pathways: one is the direct pathway, where the oral bacteria reach the foetal-placental unit and the indirect pathway, where inflammatory mediators circulate and impact the foetal-placental unit. The consensus report of the Joint EFP/ AAP Workshop on Periodontitis and systemic diseases reported that periodontal management has been shown to be safe and effective leading to an improved periodontal condition in pregnant women, and NSM, with or without adjunctives such as systemic antibiotics, does not reduce overall rates of pre-term birth and low birth weight (Sanz et al. 2013).

Diabetes mellitus is emerging as a worldwide epidemic whose complications have a significant impact on quality of life (QoL), longevity and healthcare costs. The bidirectional relationship between diabetes and periodontal disease has been long established, hyperglycaemia is associated with adverse periodontal outcomes and severe periodontitis adversely affects glycaemic control in patients with diabetes and glycaemia in non-diabetic individuals. There is a direct dose-dependent relationship between the severity of periodontitis and diabetes complications. Emerging evidence supports an increased risk for diabetes onset in patients with moderate-to-severe periodontitis (Chapple et al. 2013). The relationship between diabetes and periodontal disease is the main focus of this research study, and will be discussed in detail later.

1.1.6 Management of periodontal disease

The most important and initial step in periodontal management is communication with the patients, behavioral change and risk assessment and management. Most forms of periodontitis can be treated with NSM, described as ‘root surface’ debridement (RSD), which involves the disruption and removal of the plaque biofilm and calculus, without intentional removal of the root structure, in order to reduce the bacterial load, thereby reducing tissue inflammation (Turani et al. 2013). Mechanical debridement, is the “gold standard” for management of periodontal disease which aims to disrupt the subgingival biofilm, eliminate and reduce pathogenic bacteria, therefore allowing a shift in the microbial population to those commonly associated with health (Preshaw et al. 2004). The presence of supra- or sub-gingival calculus impedes effective oral hygiene, hence calculus removal remains a key aim for periodontal treatment as it improves access for cleaning by the patient (Turani et al. 2013). In most patients NSM in combination with good plaque control is sufficient to stabilize the disease process (Heitz-Mayfield et al. 2002). The concept of complete removal

of calculus deposits is viewed as unrealistic, and periodontal healing occurs despite the presence of residual calculus, as detected microscopically (Nyman et al. 1986; Cobb 2002).

While considering which method to use for NSM, a systematic review found no statistically significant differences in PD reduction for moderate pockets (5-7 mm) and deep pockets (≥7 mm), clinical attachment gain and BOP between 'traditional' treatment strategies (e.g. quadrant-wise basis at two week intervals) and single visit 'full-mouth' debridement (FMD) strategies (Farman and Joshi 2008). A few studies highlight that the FMD approach required less instrumentation time to achieve similar results as quadrant-wise therapy (Koshy et al. 2005; Wennstrom et al. 2005). Although single visit FMD requires less chair-side time, higher level of post-operative pain may be experienced using this approach (Apatzidou and Kinane 2004; Wennstrom et al. 2005). The use of subgingival antiseptics (e.g. chlorhexidine irrigation) does not significantly or predictably improve treatment outcomes of FMD. Therefore, all treatment modalities (i.e. quadrant-wise RSD, full-mouth RSD with chlorhexidine, full-mouth RSD without chlorhexidine) can be utilised in the management of periodontitis (Sanz et al. 2008). While considering instrumentation techniques, no differences in effectiveness have been reported between hand instruments or powered scalers (sonic or ultrasonic) (Kinane 2005), and outcome of treatment was comparable when treating patients with chronic periodontitis using either of the methods (Wennstrom et al. 2005; Aslund et al. 2008). However, powered instruments are quicker, and current treatment regimens focus on utilising ultrasonic instrumentation for biofilm disruption, using multiple and overlapping light strokes of the instrument (Kinane 2005).

In clinical studies, the use of tooth loss as a marker of disease is complicated, as it may require a long-term follow-up period. Therefore, surrogate markers of periodontal treatment commonly used are reduction in PD and BOP, and gain in clinical attachment (Hujoel 2004). The two most useful targets to set for treatment outcomes are BOP and the number of PD sites ≥5 mm (Turani et al. 2013). For example, a good target to aim for would be to achieve a BOP score of <20%, however this figure can seem a little esoteric to patients. After recording pre-treatment periodontal indices, it is important to highlight the sites measuring ≥5 mm and count them as a measure of extent of disease to help set a target. This enables something to aim for and a very crucial sense of achievement for both the clinician and the patient once this target has been achieved (Turani et al. 2013). Studies generally do not specify a set of predefined criteria for success of periodontal treatment. There appears to be an assumption that if a patient attends a number of treatment sessions their needs have been

met. Within clinical trials it is difficult to manage patients until the end-point, hence at the end of the study the patients may be categorised based on their treatment outcomes as responders and non-responders (Hujoel 2004; Armitage 2008). The presence or absence of BOP as a marker for inflammation of the periodontal tissues is a reliable marker to detect and monitor periodontal inflammation (Lang et al. 1996), but despite this BOP has proved to be a poor marker to determine risk of disease progression (Lang et al. 1986). In contrast, the absence of BOP suggests the absence of inflammation and therefore is an important indicator of periodontal health and stability (Lang et al. 1990; Joss et al. 1994).

The benefits of successful periodontal therapy have also been demonstrated in biological samples collected from patients, pre- and post-periodontal therapy. For example, levels of inflammatory markers such as MMP-3, MMP-8 and MMP-9 in plasma (Marcaccini et al. 2009a), MMP-8, MMP-9, TIMP-2 and MPO levels in GCF (Marcaccini et al. 2010), IL-8 levels in GCF (Goutoudi et al. 2012) and MMP-1, MMP-3, MMP-9, IL-2, IL-8 and COX-2 in serum (Li et al. 2012), significantly decreased following successful periodontal therapy in patients with periodontitis. These findings collectively suggest that efficient periodontal therapy plays a major role in reducing the overall inflammatory burden seen in periodontitis.

1.2 Diabetes mellitus

1.2.1 Definition and classification

"Diabetes is a group of metabolic diseases characterised by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both" (ADA 2014). The American Diabetes Association (ADA) classified diabetes mellitus into four general categories based on the aetiopathogenesis of the disease which are namely: type 1 diabetes mellitus (T1DM) (previously known as insulin-dependent diabetes mellitus), type 2 diabetes mellitus (T2DM) (previously known as non-insulin dependent diabetes mellitus), other specific types of diabetes and gestational diabetes mellitus (Table 1.4). Of these four categories, T1DM and T2DM are the most common forms which comprise the bulk of cases reported.

T1DM occurs due to the destruction of the β-cells of the pancreas, usually leading to absolute insulin deficiency, whereas T2DM occurs due to the progressive defect in insulin secretion on the background of insulin resistance. T2DM is the most prevalent form of diabetes, resulting from insulin resistance, with or without a secretory defect. T2DM usually occurs with increasing age, and is commonly associated with environmental and genetic risk factors.

T2DM is usually preceded by a long-duration of abnormal glycaemic control and is an integral part of the metabolic syndrome associated with dyslipidaemia, hypertension and hyperglycaemia. T2DM has a stronger genetic aetiology compared to T1DM, however environmental factors such as smoking, diet, exercise and obesity have an impact on the development of T2DM (Stumvoll et al. 2005). Gestational diabetes usually occurs in the second or third trimester of pregnancy and can put the mother and the baby at risk of developing T2DM in future. It is currently estimated that one in seven births is affected by gestational diabetes (International Diabetes Federation 2015). The other specific types of diabetes relates to diabetes occurring as a result genetic defects in β-cell function and insulin action, disease of the exocrine pancreas, endocrinopathies, drug-induced diabetes, infections, and immune mediated and genetic syndromes (ADA 2015).

I. Type 1 diabetes (β-cell destruction usually leading to absolute insulin deficiency)
- A. Immune mediated
- B. Idiopathic

II. Type 2 diabetes (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly secretory defect with insulin resistance)

III. Other specific types
- A. Genetic defects of β-cell function
  1. MODY 3 (Chromosome 12, HNF-1α)
  2. MODY 1 (Chromosome 20, HNF-4α)
  3. MODY 2 (Chromosome 7, glucokinase)
  4. Other very rare forms of MODY (e.g. MODY 4: Chromosome 13, insulin promoter factor-1; MODY 6: Chromosome 2, *NeuroD1*; MODY 7:Chromosome 9, carboxyl ester lipase)
  5. Transient neonatal diabetes (most commonly ZAC/HYAMI imprinting defect on 6q24)
  6. Permanent neonatal diabetes (most commonly KCNJ11 gene encoding Kir6.2 subunit of β-cell K_{ATP} channel)
  7. Mitochondrial DNA
  8. Others
- B. Genetic defects in insulin action
  1. Type A insulin resistance
  2. Leprechaunism
  3. Rabson-Mendenhall syndrome
  4. Lipoatrophic diabetes
  5. Others
- C. Diseases of the exocrine pancreas
  1. Pancreatitis
  2. Trauma/ pancreatectomy
  3. Neoplasia
  4. Cystic fibrosis
  5. Hemochromatosis
  6. Fibrocalculous pacreatopathy
  7. Others
- D. Endocrinopathies
  1. Acromegaly
  2. Cushing's syndrome
  3. Glucagonoma
  4. Pheochromocytoma
  5. Hyperthyroidism
  6. Somatostatinoma
  7. Aldosteronoma
  8. Others
- E. Drug or chemical induced
  1. Vacor
  2. Pentamidine
  3. Nicotinic acid
  4. Glucocorticoids
  5. Thyroid hormone
  6. Diazoxide
  7. β-Adrenergic agonists
  8. Thiazides
  9. Dilatin
  10. γ-Interferon
  11. Others
- F. Infections
  1. Congenital rubella
  2. Cytomegalovirus
  3. Others
- G. Uncommon forms of immune-mediated diabetes
  1. Stiff-man syndrome
  2. Anti-insulin receptor antibodies
  3. Others
- H. Other genetic syndromes sometimes associated with diabetes
  1. Down syndrome
  2. Klinefelter syndrome
  3. Turner syndrome
  4. Wolfram syndrome
  5. Friedreich ataxia
  6. Huntington chorea
  7. Laurence-Moon-Biedl syndrome
  8. Myotonic dystrophy
  9. Porphyria
  10. Prader-Willi syndrome
  11. Others

IV. Gestational diabetes mellitus

Table 1.4: Etiologic classification of diabetes (ADA 2014).

1.2.2 Epidemiology of diabetes

Diabetes mellitus is one of the largest global health emergencies of the 21st century. Every year more and more people are diagnosed with this condition resulting in life-threatening complications. The estimated global prevalence of diabetes for adults (aged 20-70 years) for 2015 was 415 million, and it is estimated it will affect 642 million by 2040 (International Diabetes Federation 2015). In 2015, the International Diabetes Federation (IDF) estimated seven countries to have greater than 10 million people with diabetes: Brazil, China, India, Indonesia, Mexico, Russian Federation and the US (Figure 1.2). Furthermore, in 2015 the prevalence of diabetes in the adult population was highest in Tokelau (30%), followed by Nauru, Mauritius, Cook Islands, Marshall Islands, Palau, Kuwait, Saudi Arabia, Qatar and New Caledonia. Diabetes affects individuals in both rural and urban settings, with 35% of cases in rural areas and 65% in urban (International Diabetes Federation 2015). In the UK it is estimated that one in 16 people has diagnosed or undiagnosed diabetes (Hex et al. 2012). Since 1996, the number of individuals diagnosed with diabetes in the UK has more than doubled from 1.4 million to almost 3.5 million (Diabetes UK 2015). Today, there are approximately 3.5 million people in the UK who have been diagnosed with diabetes, and it is estimated that this figure will increase to 5 million by the year 2025. It is also estimated that almost 549,000 people in the UK have undiagnosed diabetes (Diabetes UK 2015).

Whilst T1DM is less common, it is increasing by approximately 3% each year, particularly among children. Every year, approximately 86,000 children develop T1DM and when insulin is not provided, the life expectancy for a child with T1DM is very short. The number of children (aged 0-14 years) with T1DM worldwide is 542,000. The top ten countries for number of children with T1DM are the US, Brazil, China, UK, Russian Federation, Saudi Arabia, Germany, Nigeria and Mexico (International Diabetes Federation 2015).

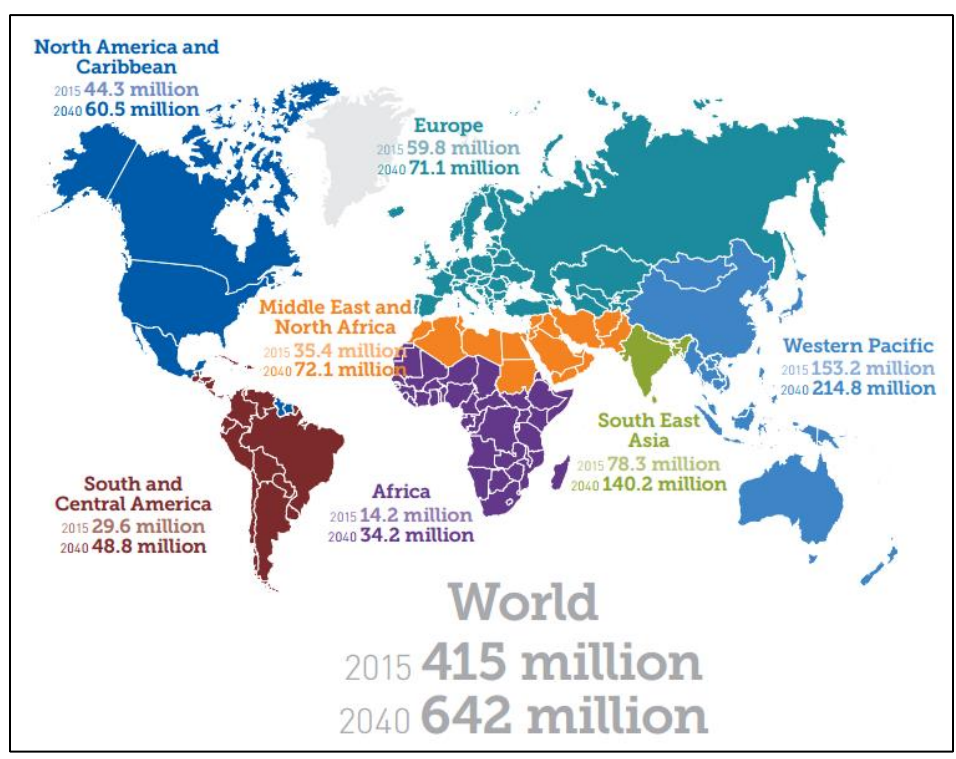


Figure 1.2: Estimated number of people with diabetes mellitus worldwide and per region in 2015 and 2040 (aged 20-79 years) (International Diabetes Federation 2015).

1.2.3 Type 1 diabetes mellitus

T1DM is a chronic autoimmune disease in which an individual's immune system selectively destroys the insulin producing β-cells of the pancreas, resulting in hyperglycaemia as a result of lack of insulin production (Skyler 2007). Why this occurs is not fully understood. T1DM accounts for only 5-10% of all cases of diabetes and slightly affects males more commonly than females (Ostman et al. 2008). T1DM usually occurs in children and young adults with peaks in presentation occurring between 5-7 years of age and at/near puberty (Harjutsalo et al. 2008). However this opinion has changed over the last decade, and age at symptomatic onset is no more a restricting factor for the diagnosis of T1DM (Leslie 2010). T1DM appears very suddenly and is currently incurable. The risk factors associated with T1DM include genetics, family history of diabetes and other environmental influences. The underlying mechanisms leading to increase in incidence rates of T1DM are still unclear, but could possibly be due to viral infections and/or changes in environmental risk factors. Genetic influence or more children being born from mothers with T1DM does not solely explain the rapid rate of increased incidence globally (Soltesz et al. 2007). Therefore, in addition to genetic factors, environmental factors also play a crucial role in the development of T1DM (Atkinson et al. 2014). Currently, interest is growing to establish the influence of environmental factors on the pathogenesis of T1DM. A plethora of environmental factors have been purported to influence the epidemiology of T1DM, such as infant and adolescent diets (Knip et al. 2010), viruses (Yeung et al. 2011; Stene and Rewers 2012), vitamin D and vitamin D pathway constituents (Svoren et al. 2009; Blanton et al. 2011; Cooper et al. 2011).

1.2.4 Diagnosis of diabetes

Historically, a diagnosis of diabetes was made with fasting blood glucose level higher than 7 mmol/L (126 mg/dL), or any blood glucose ≥11.1 mmol/L (200 mg/dL) with symptoms of hyperglycaemia or an abnormal 2-hour oral glucose tolerance test (ADA 2012). In 2009, the ADA modified their guidelines for diagnosis of diabetes and included glycated haemoglobin (HbA1c), a test to determine the average blood glucose concentrations over 3 months, of ≥6.5% as a method to diagnose diabetes (International Expert Committee 2009). HbA1c is a surrogate marker of plasma glucose and gives an indication of a patient's long-term glycaemic control and is utilised to set appropriate management goals allowing patients to achieve adequate glucose control (Home 2008). The widespread clinical use of HbA1c to monitor long-term glycaemic control has been established following publication of data from

the UK Prospective Diabetes study, demonstrating a relationship between HbA1c levels and diabetes-related complications caused by hyperglycaemia (Stratton et al. 2000). The biomechanical basis for glycosylation of haemoglobin has been established (Higgins and Bunn 1981). Glucose covalently interacts with the primary amine groups on lysine residues of haemoglobin to produce HbA1c. Glycosylation of haemoglobin is the best studied example of intracellular advanced glycation end products (AGEs) formation. HbA1c forms slowly and is irreversible during the 120-day life span of the red blood cells. The extent to which HbA1c accumulates depends upon the average glucose concentration in plasma during the preceding 2-3 months. Thus, HbA1c has proved to be a reliable index of diabetic control (Gabbay et al. 1977). HbA1c as a marker for glycaemic control has several advantages including, convenience (fasting not required), greater preanalytical stability and fewer day-to-day perturbations during periods of illness and stress. However, these advantages should be balanced by the limited availability of HbA1c testing in certain areas of the world, greater cost, and the incomplete correlation between HbA1c and average glucose in certain patients. Additionally, it is important to take into consideration the patient’s age, ethnicity and anaemias/hemoglobinopathies as interpretation of HbA1c levels may vary across individuals based on these categories (ADA 2015).

In 1998, the ADA classified diabetes control as good, moderate, and poor metabolic control. An individual is said to have a good metabolic control if HbA1c is <7%, moderate metabolic control if HbA1c lies between 7-8% and poor metabolic control if HbA1c is >8%. Analysis of data from a large prospective, multicentre study demonstrated a strong correlation between HbA1c and plasma glucose levels, which confirmed a predictable relationship between HbA1c and hyperglycaemia (Rohlfing et al. 2002). The understanding of this relationship allows patients and clinicians to target, on a daily basis, appropriate plasma glucose levels achieving the recommended HbA1c goal of 6.5% (NICE 2008).

Diagnosis of T1DM

At disease onset, T1DM is associated with the classic triad of symptoms of polyphagia, polydipsia and polyuria, in addition to overt hyperglycaemia, which remain the diagnostic hallmarks in children and adolescents, but to a lesser extent in adults with T1DM (Atkinson et al. 2014). Other symptoms associated with T1DM include lack of energy, extreme tiredness, sudden weight loss and blurred vision (International Diabetes Federation 2015). Individuals at increased risk for developing T1DM are often diagnosed by serological

evidence of the autoimmune pathologic process occurring within the pancreatic islets and specific genetic markers (ADA 2014). Despite efforts being made to standardise diagnosis of T1DM, the typology and causes remain unclear especially among adults, where the diagnosis of T1DM versus T2DM can be challenging. Approximately 5-15% of adults diagnosed with T2DM may actually have T1DM with islet autoantibodies present (Tuomi 2005). If this is the case then perhaps 50% of actual cases with T1DM are misdiagnosed as T2DM, suggesting that the number of T1DM cases is vastly underestimated.

It is critical to accurately diagnose T1DM in order to avoid complications and provide optimal care. Additionally, the accurate recognition of diabetic ketoacidosis at the advent of T1DM, is key to survival (Usher-Smith et al. 2011). A majority of T1DM cases present as an immune, if not autoimmune-mediated disorder, which means that the disease pathogenesis has an immunological contribution (e.g. autoantibodies or genetic associations). However, not all T1DM patients have these characteristics, and although not commonly used, this has led to the classification of type 1A diabetes (autoimmune), comprising 70-90% of cases having self-reactive immunological autoantibodies and type 1B diabetes (idiopathic), represents those patients whose pathogenesis remains unclear. Other factors which complicate diagnosis of T1DM include the increasing problem of obesity and increasing diverse genetic mixtures due to social changes and migration (Atkinson et al. 2014).

1.2.5 Pathogenesis of T1DM

Most reports on T1DM pathogenesis, suggests that T1DM develops following the autoimmune destruction of the insulin-secreting β-cells of the pancreas (Atkinson and Eisenbarth 2001; Bluestone et al. 2010; Todd 2010; Atkinson 2012). This results in absolute deficiency in the production of endogenous insulin (Daneman 2006). The existence of a chronic inflammatory infiltrate affecting the islets of the pancreas at symptomatic onset of T1DM is the basis of this observation (Atkinson et al. 2014). Our understanding of the pathogenesis of T1DM is derived from analysis of serum, pancreatic specimens, and peripheral blood lymphocytes obtained from T1DM patients (Bingley 2010; Roep and Peakman 2011). Research related to these constituents has proposed that a series of functional defects in the β-cells, immune system, bone marrow and thymus collectively contribute to the pathophysiology of T1DM (Atkinson et al. 2014).

Within the pancreas, specific destruction of β-cells occur when macrophages, dendritic cells, CD4+ and CD8+ T lymphocytes infiltrate the pancreatic islets (Atkinson and Eisenbarth 2001). As β-cell depletion reaches an advanced stage, there is an acute onset of symptoms with a likelihood of accumulation of ketone bodies in the blood due to lipolysis in the absence of insulin necessary for glycolysis, leading to fatal, acute ketoacidosis (Daneman 2006). Another principle is that in patients with longstanding T1DM, the pancreas is devoid of insulin-producing cells and the residual β-cells are incapable of regeneration. Both concepts of pathogenesis have been debated in the past (Butler et al. 2007; Gregg et al. 2012). It has been suggested that although a majority of patients with longstanding T1DM have very few β-cells, if any, there is evidence of β-cell regeneration in infants and young children, but not in adolescents or adults (Keenan et al. 2010; Gregg et al. 2012).

Studies analysing the pancreas of patients with recent onset T1DM suggest that approximately 70% of islets show complete absence of insulin (Keenan et al. 2010; Gregg et al. 2012), approximately 20% islets contain insulin, only 1% of insulin-deficient islets are inflamed, and many pancreas have insulin-containing non-inflamed islets that seem normal (Gepts 1965). Although symptoms of T1DM occur when 90-95% of the β-cells are destroyed, diagnosis of T1DM can occur when nearly two-thirds of the islets are devoid of β-cells (Foulis and Stewart 1984; Willcox et al. 2009). The most predominant cells within the insulitis lesion are the CD8+ T cells, followed by the macrophages (CD68+), CD4+ T cells, B lymphocytes (CD20+), and plasma cells (CD138+) (Willcox et al. 2009). A key distinguishing feature between T1DM and T2DM is the presence of autoantibodies against β-cell autoantigens. At disease onset, a majority (90%) of the newly diagnosed T1DM patients have one or more of the following antibodies present: those reactive to insulin, glutamic acid decarboxylase, insulinoma-associated autoantigen 2, and zinc transporter 8. These autoantibodies are present months or years before symptomatic onset, and usually appear at 6 months to 2 years of age in genetically susceptible individuals (Atkinson et al. 2014).

T1DM is a polygenic disorder, having nearly 40 loci known to influence disease susceptibility (Oresic et al. 2008). The human leukocyte antigen (HLA) region on chromosome 6 probably provides one-half of the genetic susceptibility leading to the risk of T1DM. Additionally, of the several HLA types, HLA class II demonstrates the strongest association with T1DM, where its haplotypes confer to the greatest disease susceptibility and provide disease resistance. Additionally, class I major histocompatibility complex molecules

also seem to influence the risk for T1DM. Most of the loci associated with T1DM risk are believed to involve immune responses, in support of the notion that genetic influences involve pathways which collectively contribute to immune responsiveness. This mechanism may explain the variations in rates of disease progression to T1DM in adults versus children, where minor variations have been noted in genetic susceptibility (Howson et al. 2011).

The natural history of T1DM model that was originally put forward in 1986, and updated in 2001, and modified subsequently, proposes that at birth individuals possess various degrees of susceptibility for T1DM. Although the proposed model has stood the test of time some modifications have been made based on the knowledge gained over the years (Figure 1.3). For example, environmental influences can occur *in utero*, and could possibly continue during the first few months or years of life, thus influencing onset and continuance of β-cell autoimmunity. Physiological events, such as immune system development and turnover of β-cells, may contribute to disease pathogenesis (Atkinson et al. 2011). Genetic susceptibility facilitates inherent immune dysregulation and results in early β-cell destruction, i.e. altered autoantibodies and amino acids associated with T1DM. In most patients, changes in glucose tolerance and insulin secretion occur months to decades after detection of multiple islet autoantibodies (Bonifacio and Ziegler 2010). It is unclear as to why not all individuals with anti-β cell autoimmunity progress to overt disease (<5% with T1DM associated autoantibody progress) (Eisenbarth 2007). Metabolic changes in T1DM are associated with reduced C-peptide response (Sosenko et al. 2010a), increased glucose fluctuations (Sosenko et al. 2010b), and an overall rise in plasma glucose (Ferrannini et al. 2010) prior to disease onset. Once a critical amount of β-cells are destroyed, symptomatic onset occurs and need for exogenous insulin-replacement arises. This symptomatic onset occurs after a silent phase which lasts for months to years, which could, in genetically susceptible individuals with multiple autoantibodies, be considered asymptomatic T1DM. Loss of β-cell mass possibly affects the performance of the residual β-cells and other islets cells. Following diagnosis, the ability to retain remaining β-cell function (assessed by C-peptide production) is heterogeneous, with respect to time taken to reach an undetectable stage, while a number of individuals who despite decades of T1DM have the ability to produce C-peptide (Keenan et al. 2010). Therefore, T1DM is considered to be a heterogeneous disease, influenced by age of onset, genetics, and intensity of diabetes management on the ability to sustain β-cell function (Atkinson et al. 2014).

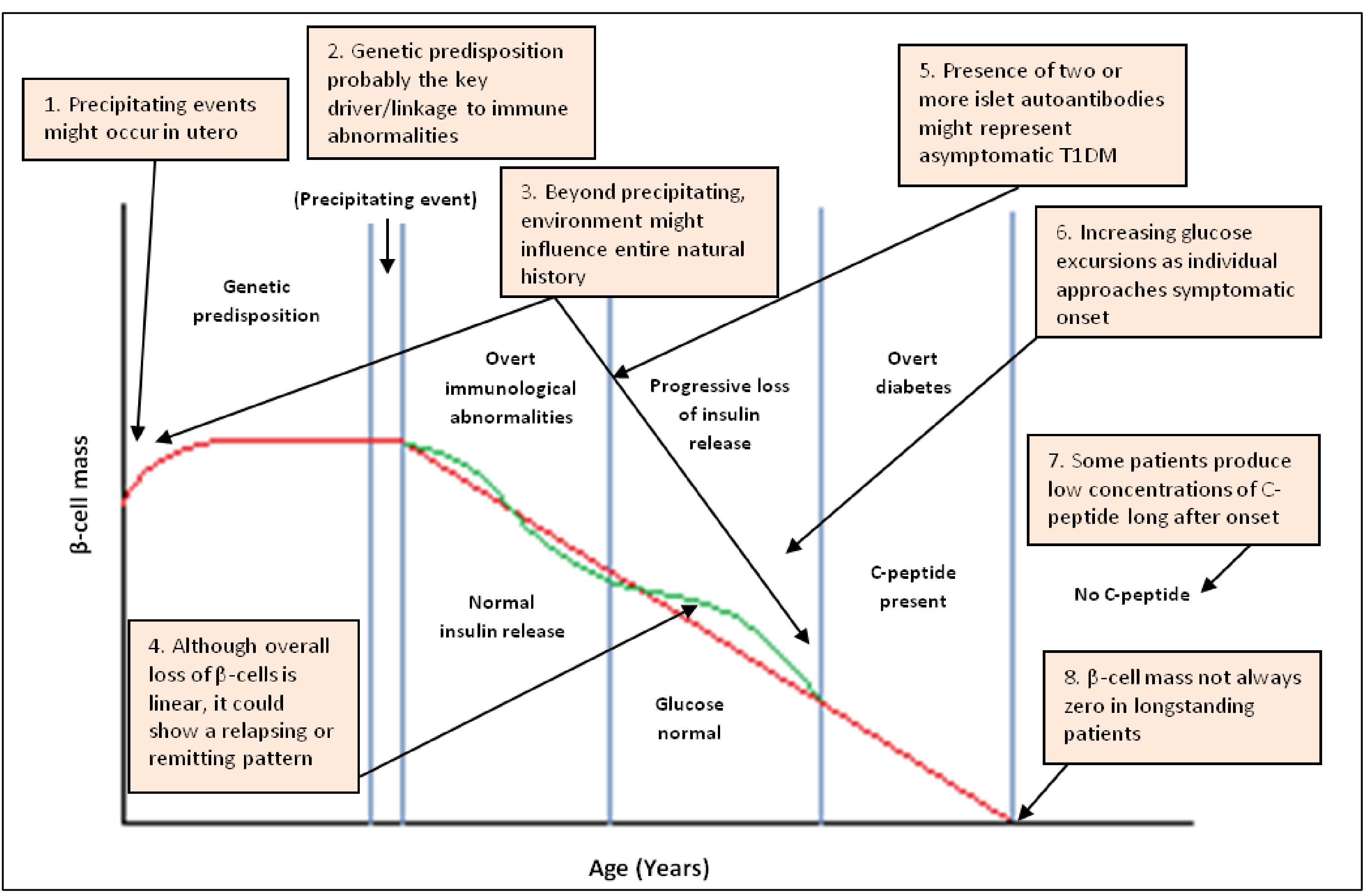


Figure 1.3: Model of the natural history of T1DM.

Originally proposed by (Eisenbarth 1986) and re-created by (Atkinson et al. 2014).

1.2.6 Complications associated with diabetes

Patients with diabetes are at a higher risk of acquiring infections and life threatening health problems than non-diabetic individuals. Consistently chronic hyperglycaemia is associated with long-term dysfunction, damage and failure of various organs, notably the heart, kidneys, eyes, nerves and blood vessels (ADA 2014). Diabetes-related complications are classified as microvascular or macrovascular. CVD is becoming a more common diabetes-related macrovascular complication as patients with T1DM live longer (Melendez-Ramirez et al. 2010). Diabetic patients have a higher incidence of peripheral arterial, atherosclerotic cardiovascular and cerebrovascular disease. Additionally, hypertension and lipoprotein metabolism abnormalities are often detected in diabetic patients (ADA 2014). T1DM patients have a ten-times greater risk for cardiovascular incidents than matched controls (Orchard et al. 2006). The Pittsburgh Epidemiology of Diabetes Complications study of T1DM reported that cardiovascular events in adults <40 years of age to be 1% per year and were three-times greater in patients older than 55 years (Maser et al. 1991). The Epidemiology of Diabetes Interventions and Complications study, which observed T1DM patients for long-term complications, reported that intensive diabetes management reduced the risk of cardiovascular incidents by 42% compared to conventional management (Nathan et al. 2005).

The microvascular diabetes-related complications include retinopathy with possible loss of vision, nephropathy with potential renal failure; autonomic neuropathy leading to cardiovascular, genitourinary and gastrointestinal symptoms and sexual dysfunction; peripheral neuropathy increasing risk for foot ulcers, lower-limb amputations and Charcot joints (ADA 2014). The risk of developing microvascular complications reduces with intensive insulin therapy. Diabetes can also pose a threat to oral health. There is an increased risk for periodontal inflammation in patients with poor glycaemic control. It is important to effectively manage periodontitis in patients with diabetes as optimal oral hygiene is the key to prevent tooth loss, promote a healthy diet and improve metabolic control (International Diabetes Federation 2015).

1.2.7 Management of T1DM

Patients with diabetes should receive appropriate care from a physician-coordinated team and diabetes self-management education must be an integral component of diabetes care. Glycaemic control is fundamental for the management of diabetes. Prospective randomised

controlled trials (RCTs) and epidemiological studies have demonstrated that good glycaemic control is associated with decreased rates of neuropathy, retinopathy, nephropathy and CVD complications (ADA 2002). Diabetes-related complications can be delayed or prevented by maintaining blood glucose, cholesterol and blood pressure levels close to normal as possible. Most complications can be identified in their early stages by regular screening programmes which allow management preventing them from becoming more serious (International Diabetes Federation 2015).

The management of T1DM warrants the need for immediate exogenous insulin replacement, a treatment modality whose therapeutic practice lasts a lifetime (Atkinson et al. 2014). T1DM therapy in modern countries often incorporates the usage of insulin analogues and mechanical technologies such as, continuous glucose monitors and insulin pumps for improved management (Hirsch 2009). The IDF recommends that of all the different types of insulin available, as a minimum, quick-acting human insulin and a long-acting NPH (Neutral Protamine Hagedorn) insulin must be available to all T1DM patients (International Diabetes Federation 2015). Following initial diagnosis and metabolic stabilization, few T1DM patients maintain the capacity to produce endogenous insulin. Although, the secretion of endogenous insulin is typically low, maintenance is important as it is correlated with less severe hypoglycaemia and less retinopathy at later stages of diabetes (Steffes et al. 2003). Thus, preserving endogenous insulin secretion following disease onset is progressively a therapeutic goal, and may involve mechanical technologies, intensive insulin therapy, or immune intervention to disrupt destruction of β-cells (Atkinson et al. 2014).

Several methods of insulin therapy exist for metabolic optimization. Using multiple daily injections, basal insulin is provided by long-acting insulin analogue and prior to meals, rapid-acting insulin is administered, based on carbohydrate grams consumed, i.e. basal-bolus therapy. Over the last decade, the utilization of continuous subcutaneous insulin infusions (insulin pumps) has increased considerably (Pickup 2012). A RCT in adults with T1DM reported lower HbA1c levels with sensor augmented pump therapy compared to insulin injection therapy, and a higher proportion of patients reaching HbA1c target levels (Bergenstal et al. 2010). A meta-analysis reported that insulin pumps lower HbA1c levels more than daily multiple insulin injections in T1DM adults, having comparable rates of hypoglycaemia (Yeh et al. 2012). However, whether insulin pumps are superior overall than multiple daily injections for the optimum management of T1DM is debatable, as outcomes

reported in research studies have substantially varied (Pickup et al. 2011). In addition to enhanced insulin preparations and delivery methods, advancements to optimize metabolic control and lessen hypoglycaemia include self-monitoring blood glucose reports, point-of-care HbA1c tests, and real-time glucose monitoring system (Atkinson et al. 2014). Real-time glucose monitoring system has proved to reduce time spent in hypoglycaemia and lower HbA1c levels effectively (Juvenile Diabetes Research Foundation Continuous Glucose Monitoring Study et al. 2008; Juvenile Diabetes Research Foundation Continuous Glucose Monitoring Study 2010), and is the most appropriate method for highly motivated patients who have continuous poor metabolic control and are to wear this monitoring device willingly (Ahmet et al. 2011). With real-time glucose monitoring and insulin pumps improving diabetes care, both technologies are now being used together, known as sensor-augmented pump therapy. A clinical trial comparing multiple daily injections with sensor-augmented pump therapy demonstrated a significant improvement in HbA1c levels with less hypoglycaemia in the group receiving sensor-augmented pump therapy (Bergenstal et al. 2010; Bergenstal et al. 2011).

For future diabetes care, efforts are being made to combine insulin pumps and real-time glucose monitors with computer algorithms that could facilitate interpretation of glycaemic control data for patients and optimise glycaemic therapy. This new system has been tested in T1DM adults and has so far reported favourable outcomes (Breton et al. 2012), with improvements and reduction in risk of nocturnal hypoglycaemia compared to conventional insulin-pumps (Buckingham et al. 2010; Garg et al. 2012). Furthermore, new insulin analogues, incretins and other hormones such as insulin degludec, incretin GLP-1, hormone pramlintide, and leptin hormone therapy are being tested for their ability to enhance management of T1DM (Atkinson et al. 2014).

1.3 Periodontal disease and diabetes

1.3.1 Epidemiological association between T1DM and periodontal disease

The prevalence and severity of periodontal diseases are higher in patients with diabetes compared to healthy individuals (Grossi et al. 1997; Poplawska-Kita et al. 2014). Patients with diabetes are at a three-fold increased risk of developing periodontitis compared to non-diabetic individuals (Mealey and Oates 2006). A number of reports on the relationship between T1DM and periodontal disease have included children and adolescents. T1DM as such does not cause gingivitis or periodontitis, but T1DM is found to alter the response of periodontal tissues to local factors (Newman et al. 2006). Previous studies have indicated that gingival inflammation is significantly increased in diabetic children compared to healthy children (Bernick et al. 1975; Gislen et al. 1980; Cianciola et al. 1982), and diabetic children were found to have more gingival inflammation in spite of having similar plaque index scores (Sandholm et al. 1989b; de Pommereau et al. 1992).

Table 1.5 presents a summary of research studies related to T1DM and periodontal disease. Cross-sectional studies found gingivitis to be more prevalent in T1DM patients compared to non-diabetic controls (Hugoson et al. 1989; Siudikiene et al. 2006). T1DM patients were found to have significantly higher amounts of plaque and gingival inflammation (Novaes et al. 1991; Aren et al. 2003; Orbak et al. 2008), LOA, bleeding to plaque ratio, PD measurements (Dakovic and Pavlovic 2008; Silvestre et al. 2009) and greater amounts of alveolar bone loss (Novaes et al. 1991) compared to non-diabetic controls. Longer duration of T1DM is associated with increased gingival inflammation and PD measurements (Aren et al. 2003; Xavier et al. 2009), greater CAL and missing teeth (Al-Shammari et al. 2006) and more severe upper and lower anterior alveolar bone loss (Hugoson et al. 1989). A few studies reported that factors such as smoking, diabetes-related complications (Al-Shammari et al. 2006) and poor metabolic control (Dakovic and Pavlovic 2008; Xavier et al. 2009; Hodge et al. 2012; Poplawska-Kita et al. 2014) were associated with periodontal disease severity (Silvestre et al. 2009). A study including both T1DM and T2DM patients reported an increase in periodontal destruction in all patients with diabetes, with higher amounts of plaque, gingival inflammation, bleeding sites and LOA in these patients compared to healthy controls (Lalla et al. 2006a). The evidence of periodontal disease was indeed present in controls, but the presence of diabetes clearly conferred a significant risk (Lalla et al. 2006a).

Some studies found no differences in clinical periodontal parameters between patients with T1DM and non-diabetic controls (Akyuz and Oktay 1990; Novaes et al. 1991; de Pommereau et al. 1992; Sbordone et al. 1998). One study investigated the prevalence of periodontal disease using the Community Periodontal Index for Treatment Needs (CPITN) and reported that 44% of the T1DM patients aged >35 years had a CPITN score of 3 or 4 (suggesting that at least one tooth had PD >3.5 mm) compared to 12.5% of the non-diabetic controls having similar scores (Pinducciu et al. 1996). Another study utilised the Periodontal Disease Index (PDI) and found no significant differences in clinical periodontal parameter scores between T1DM patients and controls (Luczaj-Cepowicz et al. 2006).

Longitudinal (5 years) observational (Firatli 1997) and experimental gingivitis (21 days) (Salvi et al. 2010) studies revealed no differences in periodontal parameters between T1DM patients and healthy controls prior to and post-study. In patients with T1DM, with the exception of CAL which significantly increased after 5 years, no other changes in periodontal parameters were noted at the 5-year follow-up (Firatli 1997). An uncontrolled 2-year longitudinal study utilizing a partial-mouth periodontal assessment method, found age, smoking and duration of T1DM to be critical risk factors for the increased prevalence of severe periodontal disease, and the presence of periodontal disease to be associated with other diabetes-related complications (Moore et al. 1999). A longitudinal study including both T1DM and T2DM patients, collectively reported a significant increase in CAL and % of PD sites ≥5 mm at the 5-year follow-up, particularly in uncontrolled diabetic patients (Demmer et al. 2012). The authors concluded that in addition to diabetes status, increasing age, lower education, current smoking habit, elevated hsCRP and baseline CAL, were predictors of tooth loss (Demmer et al. 2012).

1.3.2 Inflammatory mechanisms linking T1DM and periodontal disease

Inflammation is the key feature of pathogenesis linking diabetes and periodontal disease. Diabetes alters the host environment, and increases a patient's vulnerability to periodontal disease due to alterations in the inflammatory response to microbial challenge (Mealey and Rose 2008a; Salvi et al. 2008). Both T1DM and T2DM are associated with elevated levels of markers of inflammation (Dandona et al. 2004). The inflammatory state gives rise to diabetes-related microvascular and macrovascular complications, and chronic hyperglycaemia is known to activate pathways which increase inflammation, oxidative stress and apoptosis (Brownlee 2005). Past research has demonstrated that consequences of

hyperglycaemia over time, such as vascular damage and hyperlipidaemia, not only result in diabetes-related micro- and macro-vascular complications, but also increase a risk for development of severe periodontal disease (Noack et al. 2000b). Both gingivitis and periodontitis are considered as hyperglycaemia-associated diabetic complications (Taylor 2001; Lalla et al. 2007c).

A large body of evidence has established the prevalence, severity and extent of periodontal disease in patients with diabetes. However, understanding the mechanism behind the cross-susceptibility between the two conditions is currently lacking (Hodge et al. 2012; Chapple et al. 2013; Moodley et al. 2013). One hypothesis is that chronic hyperglycaemic precipitates the formation of AGEs by the irreversible, non-enzymatic glycation of lipids and proteins leading to loss of protein functionality (Sima and Glogauer 2013). The binding of AGE to its receptor (RAGE), found on the surface of immune and resident tissue cells, causes an increase in production of inflammatory mediators such as, IL-β, IL-6, and TNF-α (Lalla et al. 2001; Kim et al. 2005; Lin et al. 2009; Sima and Glogauer 2013). Once in the circulation, AGE can initiate a chronic, low-level inflammatory response via the AGE-RAGE axis (Katz et al. 2005; Hein et al. 2006; Katz et al. 2007; Nah et al. 2007). AGE production results in the formation of ROS and enhances oxidative stress, leading to endothelial cell changes which contribute to vascular injury, implicated in diabetes-related complications (Vlassara 2001). The accumulation of AGEs within the periodontal tissues is also known to upregulate periodontal inflammation in patients with diabetes. Within the periodontium, a number of cells express RAGE, including fibroblasts, oral keratinocytes, and immune cells (resident and invading), such as monocytes and macrophages (Brett et al. 1993; Bierhaus et al. 1996; Katz et al. 2005). The interaction of AGE and RAGE within the periodontium leads to an inflammatory response through the upregulation of molecules such as MMPs (Nah et al. 2007) and osteolytic activators (Hein et al. 2006) which potentially damage the periodontal ligament and alveolar bone. Additionally, AGE enhances the respiratory burst in PMNs, which significantly increases local tissue destruction in periodontitis (Wong et al. 2003). Moreover, AGEs have a detrimental effect on bone metabolism, leading to impaired bone formation and repair (Santana et al. 2003), and decreased extracellular matrix formation (Cortizo et al. 2003). The increased susceptibility to periodontitis in patients with diabetes may also be due to the apoptosis of the matrix-producing cells which significantly limits the repair of inflamed periodontal tissues.

Patients with diabetes are known to exhibit defects in PMN activity such as impaired chemotaxis, microbicidal functions and phagocytosis (Alba-Loureiro et al. 2007). The PMN require energy for proper functioning, hence PMN defects may be attributed to the metabolic changes which occur in diabetes (Alba-Loureiro et al. 2006). Depressed PMN chemotaxis and defective PMN apoptosis are more likely to be found in diabetic patients with severe periodontitis compared to those with diabetes and mild periodontitis, leading to increased PMN retention within the periodontal tissues, and increasing tissue destruction by the continuous release of MMPs and ROS (Manouchehr-Pour et al. 1981; Graves et al. 2006).

Figure 1.4 is a schematic representation of the proposed two-way relationship between diabetes and periodontitis (Preshaw et al. 2012). The heart of the two-way interaction between the two diseases is the exacerbated and dysregulated inflammatory responses (orange box). The hyperglycaemic state gives rise to distinct pro-inflammatory effects which impact on various body tissues, including the periodontal tissues. The adipokines (produced by adipose tissue) include TNF-α, IL-6 and leptin. The hyperglycaemic state causes deposition of AGEs in the periodontal tissues, and binding to the receptor RAGE resulting in release of cytokines and altered inflammatory responses. In the diabetic state, PMN function is altered, resulting in an increased respiratory burst and delayed apoptosis leading to increased periodontal destruction. The local production of cytokines in periodontal tissues may, in turn, affect glycaemic control through systemic exposure and impact on insulin signalling (red arrow). All these factors contribute towards the dysregulated inflammatory responses within the periodontal tissues in response to the bacterial challenge in the subgingival tissues, and which are further exacerbated by smoking (Preshaw et al. 2012).

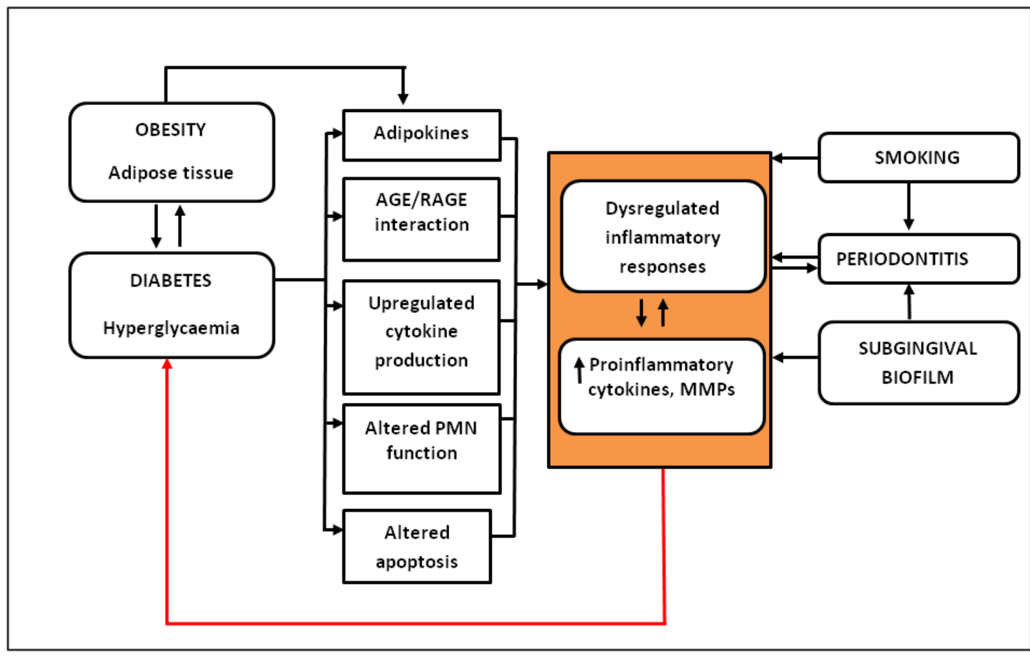


Figure 1.4: A schematic representation of the proposed two-way relationship between diabetes and periodontitis (Preshaw et al. 2012).

A few clinical studies have demonstrated that T1DM is associated with elevated levels of circulating inflammatory mediators (Antonelli et al. 2008; Van Sickle et al. 2009; Redondo et al. 2014). However, evidence relating to circulating levels of cytokines and chemokines in patients with T1DM and periodontal disease is notably lacking, and only a number of studies have investigated the link between these two conditions (Salvi et al. 1997b; Salvi et al. 1997a; Salvi et al. 2010; Passoja et al. 2011; Poplawska-Kita et al. 2014; Surlin et al. 2014; Lappin et al. 2015). These studies have focused on analysing varied biomarkers in collected samples of saliva, GCF, gingival tissue, serum and plasma.

Table 1.5 presents a summary of research studies related to T1DM and periodontal disease. A previous cross-sectional study showed that T1DM patients with periodontal disease (gingivitis or periodontitis) had significantly higher GCF IL-1β and PGE_2 levels compared to non-diabetic individuals with similar levels of periodontal disease (Salvi et al. 1997a). Additionally, in the same study, monocytes from T1DM patients, when challenged with LPS produced significantly higher concentrations of IL-1β, PGE_2 and TNF-α than monocytes from healthy controls (Salvi et al. 1997b; Salvi et al. 1997a). It has been previously demonstrated that T1DM patients were more likely to have decreased levels of the marker for bone formation, osteocalcin, signifying a decrease in their ability to form bone in the

presence or absence of periodontitis (Lappin et al. 2009). Serum levels of IL-6 (Passoja et al. 2011; Cutando et al. 2015) and TNF-α (Poplawska-Kita et al. 2014) have been found to play a significant role in periodontal tissue destruction, especially in those patients in whom T1DM is poorly-controlled (Poplawska-Kita et al. 2014). Elevated circulating levels of the chemokine IL-8 have been involved in poor clinical outcomes in T1DM patients. Elevated GCF (Salvi et al. 2010), salivary (Dakovic et al. 2013), and plasma (Lappin et al. 2015) IL-8 levels have been implicated in the cross-susceptibility between T1DM and periodontal disease. Recently, plasma levels of chemokine ENA-78/CXCL5 along with IL-8 have been found to be elevated in patients with T1DM (with and without periodontitis) compared to non-diabetic subjects with healthy periodontal tissues (Lappin et al. 2015).

MMPs can be considered as host-modulatory agents, as they are capable of altering and activating proteins and certain chemokines (Giannobile 2008). Increase in GCF IL-1β, and activation of plasma MMP-2 and MMP-9 have been found in patients with diabetes compared to healthy controls (Mealey and Rose 2008a). The expression of MMP-7, MMP-8, MMP-9 and MMP-13 was found in gingival biopsies from patients with T1DM and aggressive periodontitis, with the expression of MMP-8 and MMP-13 being more intense in T1DM patients with severe periodontitis compared to T1DM patients with moderate periodontitis and non-diabetic patients with moderate periodontitis (Surlin et al. 2014). An experimental periodontitis study in Wistar rats with and without T1DM demonstrated an increase in collagenolytic activity of MMP-9 in the gingival tissues and higher MMP-9 levels in rats with T1DM than controls (Silva et al. 2008). The significant increase in MMP-9 levels during the inflammatory process suggests that this enzyme was involved in the inflammatory mechanism in both normal and diabetic rats (Silva et al. 2008). Some studies have reported significant positive correlations between biomarker levels and patients' clinical periodontal parameters (Passoja et al. 2011; Poplawska-Kita et al. 2014; Lappin et al. 2015), and HbA1c levels (Lappin et al. 2015) and hsCRP levels (Passoja et al. 2011). While other authors reported no such association between biomarkers levels and periodontal or biochemistry data (Dakovic et al. 2013).

1.3.3 Association between periodontal status and glycaemic control

The most significant diabetes risk factor for severe periodontitis seems to be hyperglycaemia. For instance, the US National Health and Nutrition Examination Survey III found a significantly higher prevalence of severe periodontitis in adults with HbA1c >9% than non-

diabetic individuals after controlling for sex, age, ethnicity, education and smoking (Tsai et al. 2002). Periodontitis can alter the periodontal tissues into a pro-inflammatory environment via increases in inflammatory mediator levels, which additionally play a crucial role in impairing insulin signalling and worsening glucose intolerance (Lagervall and Jansson 2007; Merchant et al. 2011). Previous research has demonstrated that intensive glycaemic control can delay or prevent the onset and slow the progression of microvascular complications associated with T1DM (DCCT 1993). Diabetes is a well-known risk factor for tooth loss in patients with periodontal disease (Lagervall and Jansson 2007; Orbak et al. 2008), and evidence suggests that inadequate metabolic control is the key factor causing this complication and that the number of remaining teeth decreases with increasing HbA1c levels (Demmer et al. 2012).

A number of studies have reported on the association between periodontal status and level of glycaemic control in patients with T1DM. Some authors have reported a significant impact of glycaemic control on periodontal disease severity, with increases in HbA1c levels corresponding to increased periodontal destruction (Aren et al. 2003; Dakovic and Pavlovic 2008; Silvestre et al. 2009; Xavier et al. 2009; Hodge et al. 2012; Poplawska-Kita et al. 2014; Lappin et al. 2015). Hodge *et al.* reported a higher prevalence of severe periodontal disease (27.2%) in poorly-controlled T1DM patients (HbA1c >7.5%) compared to non-diabetic controls (20.5%) (Hodge et al. 2012). A recent study by Poplawska-Kita *et al.*, found the prevalence of periodontitis was 59.5% in poorly-controlled T1DM patients (HbA1c >6.5%) and 40% in those with well-controlled T1DM (HbA1c ≤6.5%) (Poplawska-Kita et al. 2014). Severe periodontitis was more prevalent in poorly-controlled T1DM patients (26%) than in the well-controlled diabetics (20%) and healthy controls (5%). Additionally, clinical periodontal findings in well-controlled diabetics were comparable to those of the healthy controls, indicating that good metabolic control is a key factor in protecting patients with diabetes from the development of periodontal disease. The authors concluded that strict metabolic control plus good oral hygiene practices might protect patients with diabetes from progressive periodontal disease and subsequent tooth loss (Poplawska-Kita et al. 2014). A longitudinal observational study including both T1DM and T2DM patients reported that uncontrolled diabetes (HbA1c >7.0%) was associated with greater CAL and increased severity of periodontal disease relative to diabetes-free individuals, whereas in those patients with controlled diabetes, no such disease progression was observed at the 5-year follow-up (Demmer et al. 2012).

Table 1. 5: Principal studies investigating links between T1DM and periodontal disease.

Author, Year	Subjects	Age (years)	Study design	Principal findings
(Cutando et al. 2015)	T1DM: 17 T2DM: 13 Controls: 30	Diabetics: 24-58 (43.1±12.4) Controls: 31-68 (47.0±10.3)	Longitudinal	Patients with diabetes and periodontal disease had significantly higher serum TNF-α (1.79±0.19 pg/ml), IL-6 (0.57±0.07 pg/ml) and CRP (0.39±0.11, mg/L) levels compared to healthy subjects (0.82±0.17 pg/ml, 0.38±0.05 pg/ml, 0.21±0.08 mg/L, respectively), (P<0.001).
(Lappin et al. 2015)	T1DM+periodontitis (T1DM+P): 34 T1DM+healthy tissues (T1DM+H): 28 Periodontitis (P): 23 Healthy tissues (H): 19	20-56 (36.4±9.9)	Cross-sectional	Significantly higher plasma IL-8 levels in the T1DM+H, T1DM+P and P group compared to the H group, (P<0.001). The T1DM+P group had significantly higher plasma IL-8 levels compared to the P group, (P<0.05). Significantly higher plasma ENA-78/CXCL5 in the T1DM+H, T1DM+P and P group compared to the H group, (P<0.01, P<0.001 and P<0.05, respectively). No significant difference between T1DM+P and P group for plasma ENA-78/CXCL5 levels. Plasma IL-8 and ENA-78/CXCL5 levels and BOP correlated significantly with PD, CAL and HbA1c levels.
(Poplawska-Kita et al. 2014)	T1DM: 107 [Well-controlled (T1DM-WC): 22 Poorly-controlled (T1DM-PC): 85] Controls: 40	T1DM-WC: 34.8±10.9 T1DM-PC: 37.9±3.7 Controls: 32.3±1.0	Cross-sectional	Severe periodontitis was more frequent in T1DM-PC compared to T1DM-WC patients. T1DM-PC patients had significantly higher HbA1c (9.8±2.4%) compared to T1DM-WC patients (6.0±0.6%) (P<0.01). T1DM-PC patients had significantly higher fasting glucose and CRP levels compared to T1DM-WC patients and healthy controls. T1DM patients with periodontitis had significantly higher serum TNF-α levels compared to those without periodontitis, (P<0.001). Serum TNF-α levels correlated significantly with number of sextants with PD 4-5 mm.
(Surlin et al. 2014)	T1DM+Aggressive periodontitis (T1DM+AP): 5 [moderate: 3 & severe: 2] Aggressive periodontitis alone (AP): 4	T1DM+AP: 19-29 AP: 18-28	Cross-sectional	The expression of MMP-7, -8, -9 and -13 in the gingival tissues was positive in all patients with T1DM+AP. The gingival expression of MMP-7 was positive in all T1DM+AP patients. The expression of MMP-8 and -13 was positive in all cases with T1DM+AP, but was more intense in those with severe periodontitis.

Study	Subjects	Age	Design	Findings
(Dakovic et al. 2013)	T1DM with periodontitis: 10 T1DM without periodontitis: 10 Controls: 20	7-18	Cross-sectional	Salivary IL-8 levels were significantly higher in T1DM patients (474.47±716.76 pg/ml) compared to healthy controls (101.99±68.32 pg/ml), (*P*<0.005). No significant difference in salivary IL-8 levels between T1DM patients with and without periodontitis.
(Demmer et al. 2012)	All subjects: 2,626 Incident-T2DM: 79 Controlled-T2DM: 80 Uncontrolled-T2DM: 72 Controlled-T1DM: 43 Uncontrolled-T1DM: 72 Controls: 2,280	20-81	Longitudinal observational	Relative to non-diabetic controls, uncontrolled T1DM and T2DM were statistically significantly associated with progression of CAL and 4% greater increase in % of 5 mm PD sites/mouth during 5-years of follow-up, whereas controlled T1DM and T2DM was not associated with CAL or PD progression.
(Hodge et al. 2012)	T1DM: 203 [Well-controlled (T1DM-WC): 34 Poorly-controlled (T1DM-PC): 169] Controls: 112	20-55	Cross-sectional	Prevalence of severe periodontitis was higher in all T1DM patients (24.1%) and T1DM-PC patients (27.2%) compared to controls (20.5%). CAL was significantly higher in all T1DM patients and T1DM-PC patients compared to controls, (*P*<0.001).
(Salvi et al. 2010)	T1DM: 9 Controls: 9	16-35 (25.6±5.8)	Longitudinal observational	Results of the experimental gingivitis study revealed significantly elevated GCF IL-1β and MMP-9 levels in T1DM patients compared to healthy controls, showing differences between the 2 groups at 7-21 days and 7-14 days, respectively following cessation of oral hygiene practices.
(Xavier et al. 2009)	T1DM: 168	7-19 (13.0±3.5)	Cross-sectional	Of the T1DM patients, 20.8% had gingivitis and 5.9% had periodontitis. Diabetes duration and poor glycaemic control were significantly associated with periodontal disease severity.
(Silvestre et al. 2009)	T1DM: 90 Controls: 90	T1DM: 32.5±8.02 Controls: 31.0±7.38	Cross-sectional	T1DM patients had significantly higher BOP, PD and LOA (50.5±26.4%, 3.12±0.8 mm and 1.29±1.1 mm) compared to healthy controls (18.4±18.5%, 2.18±0.5 mm and 1.29±0.4 mm), (*P*<0.01). In the presence of similar plaque levels, T1DM patients were more vulnerable to periodontal disease than controls. Diabetes duration, diabetes-related complications and poor glycaemic control were significantly associated with periodontal disease severity.

(Dakovic and Pavlovic 2008)	T1DM: 187 Controls: 178	6-18	Cross-sectional	T1DM patients had significantly higher plaque index (PI), modified gingival index (mGI), bleeding/plaque ratio and PD measurements (0.9±0.3, 0.7±0.3, 1.1±2.03 and 1.5±0.3 mm) compared to healthy controls (0.7±0.2, 0.5±0.3, 0.69±1.28 and 1.4±0.2 mm), (P<0.05). Severity of periodontal disease correlated with metabolic control and duration of diabetes.
(Orbak et al. 2008)	T1DM: 50 Controls: 50	5-14	Cross-sectional	T1DM patients had significantly higher gingival inflammation than healthy controls. The amount of plaque, inflammation and calculus increased with age in both groups.
(Lalla et al. 2006a)	T1DM & T2DM: 182 Controls: 160	6-18	Cross-sectional	Periodontal destruction was increased in diabetic children compared to controls and periodontal disease severity increased with increase in age. Diabetic children had significantly higher amount of plaque (1.2±0.4), gingival inflammation (1.2±0.3), % of bleeding sites (23.6±23.9%) and LOA (1.8±1.1 mm) compared to controls (1.1±0.03, 1.0±0.3, 10.2±13.6% and 0.8±0.9 mm, respectively), (P<0.001).
(Luczaj-Cepowicz et al. 2006)	T1DM: 50 Controls: 50	14	Cross-sectional	No significant differences for PDI between T1DM patients and controls. Only maximum scores of the PDI, mGI and papillary bleeding index were significantly higher in T1DM children compared to controls, (P<0.05).
(Siudikiene et al. 2006)	T1DM: 68 Controls: 68	10-15	Cross-sectional	Despite having similar oral hygiene habits, children with T1DM were more prone to calculus accumulation and gingivitis compared to non-diabetic children.
(Al-Shammari et al. 2006)	T1DM: 72	18-65	Cross-sectional	Greater CAL and missing teeth were associated with longer duration of diabetes. Smoking and diabetes-related complications were associated with severity of periodontal disease.
(Aren et al. 2003)	T1DM-Newly diagnosed (T1DM-ND): 16 T1DM-Long duration (T1DM-LD): 16 Controls: 16	T1DM-ND 12.8±5.8 T1DM-LD: 12.7±3.8 Controls: 12.4±1.9	Cross-sectional	Both T1DM groups had significantly higher PI scores (1.29±1.36 and 1.53±1.51) compared to controls (0.39±0.46), (P<0.05). Significantly higher mGI and PD were found in T1DM-LD patients (0.62±0.98 and 2.62±1.44 mm) compared to T1DM-ND patients (0.21±0.31 and 2.07±0.81 mm), (P<0.05). Glycaemic control affected PD, salivary pH, buffering capacity and peroxidase activity.
(Moore et al. 1999)	T1DM: 320	32.1±0.43	Longitudinal observational	Periodontitis was associated with diabetes-related complications [retinopathy (39%), nephropathy (18%), peripheral neuropathy (22%) and peripheral vascular disease (9%)]. Smoking, older age and longer duration of T1DM was associated with increased prevalence of severe periodontal disease.

(Firatli 1997)	T1DM: 44 Controls: 20	T1DM: 12.2±3.88 Controls: 12.3±4.26	Longitudinal observational	No significant difference in periodontal status between the 2 groups at baseline and at 5-year follow-up. Only significant change in T1DM group was increase in CAL at the-5 year follow-up. CAL, glucose, fructosamine and HbA1c levels were significantly higher in T1DM patients compared to controls. In T1DM patients, CAL was significantly correlated with duration of diabetes, while fructosamine levels significantly correlated with mGI scores.
(Pinducciu et al. 1996)	T1DM: 131 Controls: 20	5-65	Cross-sectional	16% of T1DM patients had a CPITN score of 3 or 4. For patients >35 years, 44% of T1DM patients had a CPITN score of 3 or 4 compared to 12.5% of healthy controls.
(Novaes et al. 1991)	T1DM: 30 Controls: 30	5-18	Cross-sectional	T1DM patients had significantly higher plaque (1.23) and gingival inflammation (0.58) compared to controls (0.81 and 0.15, respectively). Although PD did not differ between groups, upper and lower anterior alveolar bone loss was higher in T1DM patients compared to controls.
(Hugoson et al. 1989)	T1DM-short duration (T1DM-SD): 72 T1DM-long duration (T1DM-LD): 82 Controls: 77	20-70	Cross-sectional	Patients with T1DM-LD had increased alveolar bone loss and PD of 4 and 5 mm compared to healthy controls. Irrespective of diabetes duration, gingivitis was more prevalent in T1DM patients than healthy controls.

Table 1.5: Principal studies investigating links between T1DM and periodontal disease.

1.3.4 Periodontal treatment outcomes in T1DM patients

The high prevalence of diabetes together with the increased risk for periodontal disease and impaired wound healing requires an investigation of the healing response to periodontal therapy in patients with diabetes. Patients with T1DM have been found to have a good response to appropriate periodontal therapy and their response to therapy is similar to non-diabetic controls (Westfelt et al. 1996; Christgau et al. 1998). A number of interventional studies have been conducted to determine the effects of periodontal disease and its treatment on clinical periodontal parameters in patients with T1DM (Table 1.6). Some investigations have found a significant short- and long-term improvement in clinical periodontal parameters following appropriate periodontal management (Bay et al. 1974; Seppala and Ainamo 1994; Smith et al. 1996; Westfelt et al. 1996; Christgau et al. 1998; Llambes et al. 2005). Other investigations have reported significant improvements in clinical periodontal measurements when NSM was provided with and without adjunctive doxycycline (Martorelli de Lima et al. 2004; Llambes et al. 2005). These authors also reported that improvements in PD and CAL (Martorelli de Lima et al. 2004) and reductions in BOP and PD sites measuring ≥6 mm (Llambes et al. 2005) were more evident when doxycycline was used. Periodontal treatment with and without adjunctive antibiotic therapy has been found to not only improve the periodontal condition but also contribute to improved glycaemic control in uncontrolled diabetic patients (Grossi et al. 1997; Taylor 2001; Singh et al. 2008). Westfelt *et al.* demonstrated that periodontal health could be maintained in both T1DM and non-diabetic patients over a 5-year period with non-surgical and subsequent surgical periodontal treatment (Westfelt et al. 1996). The long-term response of periodontal therapy and stability of the periodontal condition in patients with T1DM will largely depend on the level of oral hygiene, the periodontal maintenance provided and level of glycaemic control (Llambes et al. 2005).

1.3.5 Impact of periodontal treatment on glycaemic control

The prevalence, severity and progression of periodontal disease were found to be higher in patients with diabetes; despite this, these patients have a good response to periodontal treatment (Seppala et al. 1993). The response to periodontal treatment in T1DM patients has been found to be similar to the response seen in non-diabetic controls. However, it is crucial that diabetes is well-controlled, as periodontal disease recurrences will be more frequent and more difficult to control in patients with poor metabolic control (Seppala et al. 1993; Tervonen and Karjalainen 1997). A few investigations have found no significant influence of

metabolic control on periodontal healing (Tervonen et al. 1991; Westfelt et al. 1996). There is a lack of evidence in the literature regarding the effects of periodontal therapy on glycaemic control in patients with T1DM. Only a few interventional studies have been conducted to determine the effects of periodontal disease and its treatment on glycaemic control in T1DM patients (Table 1.6).

Williams and Mahan reported that reductions in blood glucose levels and insulin requirements were seen following periodontal treatment in diabetic patients with "gross evidence of periodontal disease" during the 3-month follow-up (Williams and Mahan 1960). A number of investigations into the effect of periodontal treatment on metabolic control in patients with T1DM have reported no significant change or improvement (Miller et al. 1992; Seppala et al. 1993; Aldridge et al. 1995; Smith et al. 1996; Llambes et al. 2008). A few investigations including both T1DM and T2DM patients have reported a significant improvement in HbA1c levels following periodontal treatment (Williams and Mahan 1960; Wolf 1977), whereas others reported no such effect (Westfelt et al. 1996; Christgau et al. 1998). Some authors have reported that NSM could improve metabolic control especially when adjunctive doxycycline is given (Miller et al. 1992; Grossi et al. 1996; Grossi et al. 1997; Iwamoto et al. 2001). Doxycycline has both host-response modifying effects and antimicrobial properties, and a probable non-enzymatic inhibitory effect on glycation (Hungund and Panseriya 2012).

1.3.6 Impact of periodontal treatment on inflammation in T1DM

In patients with diabetes, clinical interventional trials have shown a significant reduction of acute phase protein levels, such as fibrinogen (Christgau et al. 1998), and CRP (Lalla et al. 2007a) following periodontal therapy. Research studies related to T2DM have shown a significant reduction of the pro-inflammatory cytokine, TNF-α following successful periodontal therapy, whereas although other pro-and anti-inflammatory cytokines such as IL-4, IL-6, IL-8 and IL-10 reduced post-treatment, this reduction was not statistically significant (Correa et al. 2010). In contrast, some studies reported no changes in biomarker levels in diabetic patients following periodontal therapy (Talbert et al. 2006; Lalla et al. 2007a; O'Connell et al. 2008; Geisinger et al. 2016). Factors such as periodontal status and the adjunctive use of systemic antibiotics may possibly explain these outcome differences (Correa et al. 2010).

Research studies related to the effect of periodontal treatment on levels of local and systemic biomarkers in T1DM patients are notably lacking. An uncontrolled study reported that after periodontal therapy, no statistically significant changes were seen for serum IL-6 levels, however, those patients with higher serum IL-6 levels presented with poorer periodontal healing as compared to those with low levels of IL-6 following treatment (Passoja et al. 2011). A recent study in T1DM and T2DM patients assessed the effect of topical application of melatonin (1% orabase cream) on serum TNF-α, IL-6 and CRP levels compared to healthy controls treated with a placebo orabase cream (Cutando et al. 2015). The authors reported a significant decrease in gingival index scores, PD measurements, serum IL-6 and CRP levels in diabetic patients 20 days following treatment suggesting that melatonin could modulate the inflammatory action of these inflammatory markers in periodontal disease, though the authors did not propose a mechanism of action in this regard.

Clearly there is lack of evidence demonstrating the effect of periodontal treatment on inflammatory markers in patients with T1DM and this area of research needs further investigation.

Table 1.6: Studies investigating the impact of periodontal treatment on periodontal health, glycaemic control and biomarker levels.

Author, Year	Sample size (n)	Age (years)	Duration (weeks)	Treatment provided	Changes in periodontal health	Changes in biomarkers	Changes in HbA1c
(Cutando et al. 2015)	T1DM: 17 T2DM: 13 Controls: 30	Diabetics: 24-58 (43.1±12.4) Controls: 31-68 (47.0±10.3)	3	Diabetics: Topical melatonin application Controls: Placebo orabase cream	Significant improvement in mGI and PD.	Significant decrease in IL-6 and CRP 20 days following treatment, (P<0.001). Serum TNFα, IL-6 and CRP significantly correlated with mGI and PD pre- and post-treatment.	Data not reported
(Passoja et al. 2011)	T1DM: 80 (at baseline) T1DM: 58 (treated & reviewed 8 weeks after NSM)	T1DM at baseline: 38.6±12.3 T1DM after NSM: 39.5±12.6	8	NSM	Improvements in PI, PD and BOP. Response of NSM was poorer in patients with higher post-treatment serum IL-6 levels than those with low IL-6 levels.	No significant change in serum IL-6 following NSM. Significant association between serum IL-6 levels and periodontal inflammation (BOP and PD ≥4 mm) pre- and post-NSM.	NSM had no significant influence on HbA1c.
(Llambes et al. 2008)	T1DM: 60 T1DM-group 1: 30 T1DM-group 2: 30	T1DM: 35.3±9.0	12	NSM +/- doxycycline	Data not reported	Data not reported	Mean baseline HbA1c was 7.64±1.81% in group 1 (NSM + doxycycline) and 7.51±1.36% in group 2 (NSM - doxycycline), and following treatment HbA1c was 7.71±1.74% and 7.45±1.29%, respectively. There was a non-significant HbA1c variation of 0.07% in group 1 and -0.06% in group 2.

(Llambes et al. 2005)	T1DM: 60 T1DM-group 1: 30 T1DM-group 2: 30	T1DM: 35.3±9.0	12	NSM +/- doxycycline	Significant improvement in PI, BOP, PD and CAL in both groups. The reduction in BOP and PD sites ≥6 mm was more evident when doxycycline was used.	Data not reported	Data not reported
(Martorelli de Lima et al. 2004)	T1DM: 22 T1DM-treatment A: 11 T1DM-treatment B: 11	T1DM: 35-55	48	NSM +/- doxycycline	Significant improvement in PD and CAL in both treatment groups. Statistically significant difference in PD and CAL, between groups only at month 12, favouring adjunctive doxycycline group.	Data not reported	Data not reported
(Christgau et al. 1998)	T1DM: 7 T2DM: 13 Controls: 20	Diabetics: 20-60 Controls: 30-67	16	NSM	Significant improvement in PI, Papillary Bleeding Index and BOP in all groups.	Data not reported	NSM had no significant influence on HbA1c.
(Westfelt et al. 1996)	T1DM: 14 T2DM: 6 Controls: 20	45-65	240	NSM +/- surgical therapy	Significant improvement in PI, PD, BOP and CAL following treatment.	Data not reported	No significant change in HbA1c between baseline-24 months and 24 months-60 months.
(Smith et al. 1996)	T1DM: 18	26-57	8	NSM	Significant improvement in mGI, PD, CAL.	Data not reported	Non-significant increase in HbA1c of 0.10%, from 8.18% before to 8.28% following NSM.

(Aldridge et al. 1995)	Study 1 T1DM: 16 & controls: 15 (with gingivitis & early periodontitis) Study 2 T1DM: 12 & controls: 10 (with advanced periodontitis)	Study 1 16-40 Study 2 20-60	8	NSM	Data not reported	Data not reported	NSM had no significant influence on HbA1c.
(Seppala et al. 1993; Seppala and Ainamo 1994)	At 1 year follow-up T1DM: 38 [Well-controlled (T1DM-WC): 12 & Poorly controlled (T1DM-PC): 26] At 2 year follow-up T1DM: 22 [T1DM-WC: 6 & T1DM-PC: 16]	T1DM: 35-56 T1DM-WC: 43.4±4.7 T1DM-PC: 47.8±6.0	24	NSM	Both well-controlled and poorly-controlled groups responded well and equally to periodontal treatment.	Data not reported	Non-significant reduction in HbA1c levels in both groups.
(Miller et al. 1992)	T1DM: 9	Data not published	24	NSM + doxycycline	Improvement in BOP.	Data not reported	Non-significant reduction in HbA1c from pre- (9.44±1.69 %) to post-treatment (9.01±2.01 %). Only those patients with consistent improvements in BOP scores showed a decrease in HbA1c post-treatment.
(Bay et al. 1974)	T1DM: 57 Controls: 49	20	1	NSM	Significant improvement in PI and mGI in both groups.	Data not reported	Data not reported

(Wolf 1977)	T1DM & T2DM: 91	16-60	48	NSM + surgical therapy	Data not reported	Data not reported	Following treatment, a limited comparison of 23 responders and 23 non-responders revealed that significant reduction in periodontal inflammation had a non-significant association with improved metabolic control. The authors concluded that this was “statistically indicative” of a beneficial effect.
(Williams and Mahan 1960)	T1DM: 8 Type not specified: 1	20-32	12	NSM + surgical therapy + antibiotic	Data not reported	Data not reported	Significant reduction in insulin requirement and noticeable reduction in blood glucose levels was seen in 7 out of 9 diabetic patients.

Table 1.6: Studies investigating the impact of periodontal treatment on periodontal health, glycaemic control and biomarker levels.

1.3.7 Other oral manifestations of diabetes

The oral health of patients with diabetes has been the subject of several studies in recent years, and while these patients are acknowledged to have a greater susceptibility to periodontal disease, even very early in life (Lalla et al. 2006a), the possibility of detecting an increased prevalence of caries in patients with T1DM is controversial. It has been proposed that hyperglycaemia is associated with reduced salivary secretion and increased glucose levels, notably in cases of severe insulin insufficiency (Harrison and Bowen 1987a; Karjalainen et al. 1996) and consequently a heightened cariogenic challenge can be expected in such individuals. Additonally, the presence of caries in diabetic patients was associated with increasing age, gingival recession and the presence of diabetes-related nephropathy (Moore et al. 2001a).

Dental caries is a multifactorial infectious disorder involving various factors needing to coincide at a given point and time. The development of caries is dependent on the presence of microorganisms, the host (tooth), nutrition, diet and the immune capacity of an individual. Twetman *et al.* reported a high proportion of caries-causing bacteria such as *Streptococcus mutans* in the aerobic flora of patients with diabetes (Twetman et al. 1989). Other authors reported a decrease in salivary *lactobacilli* counts in these patients due to dietary restrictions (Swanljung et al. 1992; Collin et al. 1998), while Iughetti *et al.* observed similar counts of *lactobacilli* and *Streptococcus mutans* in patients with and without diabetes (Iughetti et al. 1999).

To date, there is no clear evidence to support an association between dental caries and T1DM, and epidemiological studies relating to the effect of T1DM on the prevalence of caries in children and adults have yielded contradictory results. On one hand, some authors have reported lower caries experience (Matsson and Koch 1975; Leeper et al. 1985; Kirk and Kinirons 1991; Siudikiene et al. 2006; Orbak et al. 2008), while others have reported an increased presence of caries (Albrecht et al. 1988; Jones et al. 1992; Moore et al. 2001b; Lopez et al. 2003; Miralles et al. 2006) whereas some studies have shown similar caries experience in patients with T1DM compared to matched controls (Faulconbridge et al. 1981; Tenovuo et al. 1986; Harrison and Bowen 1987b; Twetman et al. 1989; Swanljung et al. 1992; Edblad et al. 2001; Siudikiene et al. 2008; Tagelsir et al. 2011). Miko *et al.'s* study found that adolescents with T1DM had significantly higher mean Deacayed Missing and

Filled teeth index scores, with fewer decayed teeth and more filled teeth compared to healthy controls (Miko et al. 2010).

Earlier studies have reported low caries incidence in diabetic populations, mainly explained by the lifelong sucrose-free diet, restricted carbohydrate intake and insulin treatment regimens, with results indicating that diet modification had a positive impact on caries prevention (Matsson and Koch 1975; Leeper et al. 1985; Sarnat et al. 1985; Kirk and Kinirons 1991; Tavares et al. 1991; Lamster et al. 2008). Previous research has also demonstrated that frequent main meals, a starch-rich diet and longer eating time in children with diabetes is associated with comparable caries prevalence in diabetics and healthy controls (Sarnat et al. 1985). However, modern management of diabetes, characterised by flexibility of insulin treatment and blood glucose monitoring allows for less rigid meal planning and reduces the significance of dietary factors as an indicator for possible variations in caries development (Twetman et al. 2002; Siudikiene et al. 2006). Children with T1DM were also found to have more daily main meals and fewer snacks than non-diabetic children, who had fewer main meals per day but consumed more snacks (Siudikiene et al. 2006).

The probable relationship between cariogenic experience and metabolic control of diabetes is an investigation of much interest. Due to disturbed glucose metabolism, these patients are considered to be at a higher risk for dental caries (Siudikiene et al. 2005b; Siudikiene et al. 2006). Poor metabolic control has been associated with reduced salivary secretion, elevated salivary glucose concentrations and pronounced yeast growth (Harrison and Bowen 1987a; Reuterving et al. 1987; Karjalainen et al. 1996; Karjalainen et al. 1997). Therefore, chronic hyperglycaemia is known to cause shifts in the ecology and composition of saliva resulting in a cariogenic environment in the oral cavity. Some authors have found that caries-active diabetic children have significantly higher HbA1c levels than caries-inactive diabetic children (Twetman et al. 1992; Karjalainen et al. 1997). Other studies reported that poorly-controlled T1DM patients had a higher incidence of caries compared to matched controls (Twetman et al. 1992; Canepari et al. 1994; Karjalainen et al. 1997; Miko et al. 2010). Twetman *et al.* reported that poorly-controlled T1DM patients developed three times more carious lesions during the study compared to those with well-controlled T1DM (Twetman et al. 2002). Siudikiene *et al.* showed that among the variables associated with caries risk, age and level of metabolic control were significantly associated with caries experience in T1DM children (Siudikiene et al. 2005b). Higher counts of *Streptococci mutans*, *lactobacilli* and yeasts were

found in poorly-controlled diabetics when compared to those with well-to-moderately controlled T1DM (Syrjala et al. 2003; Siudikiene et al. 2006). Additionally, poor metabolic control has led to changes in certain behavioural factors such as poor adherence to diabetes treatment regimens and oral health recommendations (Syrjala et al. 2003). The level of untreated dental decay was considerably higher in diabetic children, reflected by a significantly lower dental attendance in these patients than matched controls (Tagelsir et al. 2011). However, a few studies were unable to relate the prevalence of caries in T1DM patients to good or poor metabolic control (Leeper et al. 1985; Thorstensson et al. 1989; Edblad et al. 2001; Miralles et al. 2006).

Saliva is essential for the preservation and maintenance of oral health, therefore the comprehensive evaluation of salivary flow rate must be included in the management of oral diseases in patients with diabetes (Moore et al. 2001b). Lower salivary flow rates and self-reported xerostomia have been frequently seen in patient with diabetes (Sreebny et al. 1992; Ben-Aryeh et al. 1993; Moore et al. 2001b; Siudikiene et al. 2006), especially if poorly-controlled (Harrison and Bowen 1987a; Harrison and Bowen 1987b). Diminished salivary flow has been linked to high caries prevalence in diabetic patients (Twetman et al. 1992; Karjalainen et al. 1997; Moore et al. 2001a; Siudikiene et al. 2006). Hyperglycaemia-induced reduced salivary flow rate is mainly characteristic of periods of poor metabolic control, during which there is a possibility of glucose leakage into the oral cavity, thereby facilitating the growth of acidogenic bacteria and development of carious lesions (Karjalainen et al. 1996; Siudikiene et al. 2006). Good metabolic control can prevent salivary changes such as high glucose content and acidic pH, whereas a good diabetic diet rich in fibre and low in carbohydrates, can delay plaque accumulation and proliferation of acidogenic bacteria (Karjalainen et al. 1997; Orbak et al. 2008). A 2-year longitudinal study by Siudikiene *et al.* demonstrated that salivary flow rates (stimulated and unstimulated) remained significantly lower in T1DM children compared to matched healthy controls (Siudikiene et al. 2008). Whereas another study found no differences in salivary flow rate between patients with diabetes and healthy controls (Miralles et al. 2006). Multivariable regression analysis demonstrated that children with higher 2-year decayed, missing and filled surfaces (DMFS) index scores were more likely to have T1DM, be older in age, and have higher salivary glucose concentrations compared to those with lower 2-year DMFS scores (Siudikiene et al. 2008).

1.4 Quality of life

1.4.1 Definition and dimensions

Since the 1990s, the research field in matters relating to quality of life (QoL) has increased greatly. In 1994, the World Health Organization (WHO) defined QoL as "an individual's perception of their position in life in the context of the culture and value system in which they live and in relation to their goals, expectations, standards and concerns." Considerable agreement confirms that QoL is an elusive, complex and multidimensional concept (Felce and Perry 1995). Additionally, QoL can also be defined as the "value assigned to duration of life as modified by impairments, functional status, perceptions and social opportunities that are influenced by disease, injury, treatment or policy" (WHO 1994).

An individual is said to have a good QoL if his or her hopes and experiences are matched; whereas a poor QoL is one in which an individual's hopes are not matched with their experiences. QoL has a tendency to change with time, age and experience (Calman 1984). As QoL assessments represent the effect an illness has on an individual, as perceived by the individual, and yields additional information to epidemiological or clinical data, it is often utilized as an outcomes measurement (Wandell 2005). QoL measures have also been characterized as "the ultimate goal of all health interventions" (Rubin and Peyrot 1999).

QoL is a dynamic construct and has a tendency to vary between individuals; despite this its dimensions and the content of each instrument measuring QoL are somewhat similar and typically involve assessment of:

- Physical function - for example, mobility, self-care
- Emotional function - for example, depression, anxiety
- Social function - for example, intimacy, social support, social contact
- Role performance - for example, work, housework
- Pain
- Other symptoms - for example, fatigue, nausea, disease specific symptoms (Fitzpatrick et al. 1992).

Health-related QoL

In 1948, the WHO stated that disease is defined by the pathological process which affects the functioning and pathological integrity of the body. However, health is defined as, "a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity" (WHO 1948). Health-related QoL (HRQoL) is defined as, "the value assigned to duration of life as modified by the impairments, functional states, perceptions, and social opportunities that are influenced by disease, injury, treatment, or policy" (Patrick and Erickson 1988). Although the definition of HRQoL varies across studies, the consensus in the literature identifies three major dimensions of HRQoL: physical symptoms, functional capacity and perceptions of well-being (Chen and Hunter 1996). HRQoL primarily assesses the relationship between an individual's health status and their QoL (Allen 2003). Research related to HRQoL highlights two aspects: the "objective measure", which is the functional status of an individual, and the "subjective measure" which is an individual's opinion of their own health affecting their QoL (Testa and Simonson 1996; Muldoon et al. 1998).

Oral health-related QoL

An important aspect of HRQoL is "oral health-related QoL" (OHRQoL) (Naito et al. 2006), which is defined as "the impact of oral disorders on aspects of everyday life that are important to patients and persons, with those impacts, being of sufficient magnitude, whether in terms of severity, frequency or duration, to affect an individual's perception of their life overall" (Locker and Allen 2007). Over the last two decades, a number of patient-centred assessment tools have been developed and validated to evaluate patients' subjective oral health with reference to how it affects their inter-personal relationships, daily activities and psychological well-being (McGrath and Bedi 2002). The psychosocial experience of oral condition determines whether patients will seek treatment and follow advice, and could therefore influence treatment planning and treatment process (Ng and Leung 2006).

Previous research suggests that oral health can have a profound negative impact on an individual's QoL in terms of physical functioning and social well-being (Needleman et al. 2004; Naito et al. 2006; Locker and Quinonez 2011), and it is often difficult to dissociate general health from oral health with regards to impacts on QoL (Fontanive et al. 2013). Over the past few years, the increase in awareness that QoL is an important outcome for oral care has led to the development of a wide range of instruments to measure OHRQoL. The main requirements of QoL assessments are:

- Reliability
- Validity
- Sensitive to change
- Multidimensional construct
- Appropriate to question or use
- Practical utility (Fitzpatrick et al. 1992; Allen 2003).

Dimensions of QoL

In 1995, a conceptual model for HRQoL was proposed by Wilson and Cleary, which provides a causal pathway linking HRQoL and traditional clinical variables (Wilson and Cleary 1995). Conceptually, Wilson and Cleary's model links five health concepts on a continuum, these being: symptoms, physiological factors, general health perceptions and HRQoL. Starting at the clinical level (biological, objective) at one end of the continuum and moving outwards to the individual's interaction with the environment to perceive a level of QoL (psychological, subjective) at the other end, this model integrates both social science and biomedical paradigms.

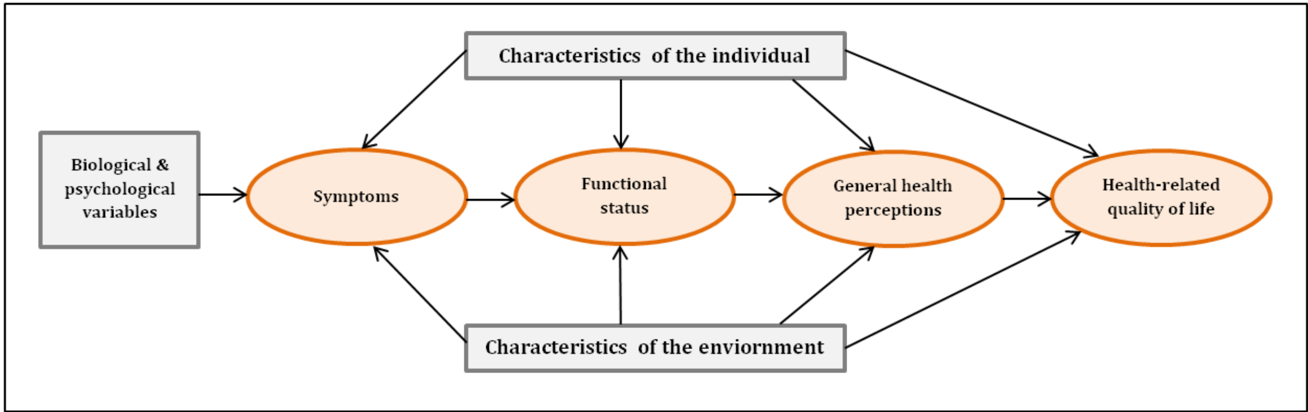


Figure 1.5: Model linking HRQoL to physical function, psychosocial function, social function and environmental characteristics (Wilson and Cleary 1995).

1.4.2 Impact of periodontal disease and treatment on QoL

Periodontal disease in its initial stages is generally asymptomatic, and patients are unaware of their condition and hence, may underestimate their need for treatment. However, as the disease progresses, it results in a number of signs and symptoms such as tooth mobility, eating difficulties, pain, discomfort and compromised aesthetics, which can be readily perceived by

patients and are highly relevant from their point of view. Those who suffer from this condition often report a considerable negative impact on their daily lives and are known to experience in particular, physical functioning, psychosocial and pain impacts on their QoL due to their oral health status (Cunha-Cruz et al. 2007; O'Dowd et al. 2010; Durham et al. 2013; Desai et al. 2014; Jansson et al. 2014; Simona et al. 2014).

Locker in 1988 stated that periodontal disease causing inflammation and destruction of the periodontium giving rise to multiple signs and symptoms may have a considerable impact on an individual's day-to-day QoL (Locker 1988). Past research related to periodontal disease has usually focused on clinical and pathological mechanisms of the disease process rather than the impact the disease has on a patient's QoL (O'Dowd et al. 2010). Decades ago, Cohen and Jago proposed the use of socio-dental indicators, incorporating functional, psychological and social consequences of oral diseases for patients, and not merely evaluation of the signs and symptoms of various diseases (Cohen and Jago 1976). In doing so, clinicians would better understand the impact of oral diseases such as periodontitis on QoL, and would assist in providing appropriate care by addressing patient needs and main concerns and assist in evaluating treatment outcomes from a patient's point of view (McGrath and Bedi 1999).

Several studies have evaluated the impact of periodontal disease on OHRQoL. Needleman *et al.* utilised the UK oral health-related QoL (OHQoL-UK) measure and found that periodontal disease had substantial physical, psychological and social impact on OHRQoL as patients complained of halitosis, discomfort and unaesthetic facial appearance (Needleman et al. 2004). These impairments caused social impairments, financial setbacks and the patient's appeared worried, with reduced happiness and self-confidence (Needleman et al. 2004). Ng and Leung utilised the Oral Heath Impact Profile (OHIP-14S, Chinese version) and comparing OHIP-14S summary scores of patients with healthy or low CAL (CAL $\leq$2 mm) and high or severe CAL (CAL >3 mm) revealed significant differences in the domains of functional limitation, physical pain, psychological discomfort, physical and psychological disabilities, demonstrating a significant correlation between OHRQoL and periodontal disease (Ng and Leung 2006). Patel *et al.* assessed smiling patterns and found that those with an increased number of mobile and missing teeth, and more gingival recession opened their mouth less widely while smiling or covered their mouth while laughing. Poor periodontal health prevented these patients from expressing positive emotions leading to impairments in

self-confidence, social interactions and QoL (Patel et al. 2008). O' Dowd *et al.* examined the impact periodontal disease had on the daily lives of patients by conducting semi-structured interviews to record daily life experiences (O'Dowd et al. 2010). The authors found that periodontal disease had a negative impact with regards to causing functional limitations, discomfort and physical, emotional and social disability (O'Dowd et al. 2010). Durham *et al.* identified, while using the OHIP-49 measure, that patients with chronic periodontitis reported significantly poorer OHRQoL compared to those without chronic periodontitis, with significant functional, psychological and social impacts on OHRQoL (Durham et al. 2013). Another study by Desai *et al.* reported that patients with chronic periodontitis had the greatest impacts on the functional limitation and psychological disability and discomfort domains of the OHIP-49 (Desai et al. 2014). Loss of periodontal structures can negatively affect masticatory performance and OHRQoL (Borges Tde et al. 2013). Jansoon *et al.* reported that patients having severe marginal bone loss experienced worse OHRQoL compared to those with minor or no marginal bone loss, as assessed by the OHIP-14. The authors emphasized the need for prevention and early periodontal treatment, as severe periodontitis leads to considerable negative impacts on OHRQoL (Jansson et al. 2014).

Patient-based outcome measures are subjective measures used to capture patients' perspective of disease and treatment and complement conventional clinical surrogate measures (Hujoel 2004; Tsakos et al. 2012). Traditionally, surrogate markers such as PD and LOA have been used to define and measure periodontal disease (Armitage 1996; Renvert and Persson 2002; Armitage et al. 2003; Savage et al. 2009), but these measures do no capture the total impact of treatment on a patient's health status (Saito et al. 2010). Patient's perception regarding the provided care and HRQoL is increasingly recognized as a crucial outcome of care (Locker 2004). Patient-based outcome measures are more meaningful in assessing patient's daily lives than objective changes in PD and LOA measurements (Locker 1988; Naito et al. 2006; Ng and Leung 2006), and are useful in detecting QoL changes before and after periodontal treatment (Needleman et al. 2004). Although there is sound evidence to support the clinical efficacy of surgical and NSM, data related to patient-based outcomes are limited and more information is required regarding the impact of periodontal therapy on QoL (Heitz-Mayfield et al. 2002; Hung and Douglass 2002; Tunkel et al. 2002; Van der Weijden and Timmerman 2002; Allen 2003; Locker and Allen 2007).

Needleman *et al.'s* cross-sectional study found a correlation between OHQoL-UK scores and the number of teeth with PD ≥5 mm, also patients who had undergone periodontal care and

were in the maintenance phase had significantly higher OHQoL-UK scores (indicating better QoL) compared to patients who received no periodontal therapy (Needleman et al. 2004). Surgical therapy alone appeared to have no effect on OHRQoL (Ozcelik et al. 2007), whereas surgical therapy supplemented with Emdogain led to an improved OHRQoL as indicated by the OHIP-14 responses (Bajwa et al. 2007). Aslund *et al.* investigated the impact of two modes of delivery of NSM (ultrasonic versus hand instruments) on OHRQoL and on patient experience of pain in patients with mild to moderate periodontitis (Aslund et al. 2008). The authors reported that NSM was generally well tolerated, clinical outcomes of therapy were similar in both modes of therapy and NSM had a small positive impact on pain and contributed to changing patients' perception from a negative effect of oral health on QoL to a positive one (Aslund et al. 2008). Jowett *et al.* reported that patients with periodontal disease had significantly greater impacts on QoL compared to dentally healthy patients and, following 24-hour RSD the impact was significantly reduced but still greater than the QoL experienced by the dentally healthy patients (Jowett et al. 2009). Saito *et al.* reported that pain, eating and chewing and psychological functioning were the most affected OHRQoL domains in patients with periodontitis, and a majority (97%) of patients perceived their oral health as poor. On the other hand NSM resulted in a higher proportion of patients rarely or never having pain and eating difficulties, suggesting that NSM had the potential to ameliorate perception of oral health (Saito et al. 2010). Jönsson *et al.'s* study to determine the minimally important differences 1-year following NSM in chronic periodontitis patients found that the OHRQoL significantly improved following therapy in these patients as assessed by the General Oral Health Assessment Index and the OHQoL-UK (Jonsson and Ohrn 2014). A systematic review of the impact of periodontal therapy on OHRQoL found that routine NSM can moderately improve OHRQoL in patients with periodontal disease (Shanbhag et al. 2012). Wong *et al's.* study reported that successful NSM resulted in improvements in physical pain, psychological discomfort and disability domains of the OHIP-14S and the authors emphasized that clinicians must capitalize upon the positive psychological OHRQoL impacts of NSM for subsequent patient motivation during maintenance therapy (Wong et al. 2012). The benefits of successful NSM were also seen in a previous study, in which patients perceived physical, psychological and social changes in OHRQoL following NSM, additionally, the positive effect of NSM was more pronounced in patients with PD of >7 mm (Brauchle et al. 2013).

1.4.3 QoL in patients with diabetes and tools available for assessment

Diabetes can have a profound impact on health and QoL in terms of physical, psychological and social well-being (Glasgow et al. 1997). Diabetes is related to an increased risk of developing macrovascular and microvascular complications, and national and international guidelines state that the overall goal for diabetes treatment is to prevent complications, while preserving a good QoL for the patient (Wandell 2005). Diabetes management, taking insulin therapy, can substantially influence QoL either positively, for instance, by reducing high blood sugar level symptoms, or negatively, for example, by increasing low blood sugar level symptoms. Patients often feel challenged by their illness and the substantial day-to-day management demands that come with it. The diabetes-related psychosocial toll is often a great one, and this toll can in turn affect self-care behavior, long-term glycaemic control, the risk of developing diabetes-related complications and QoL (Rubin and Peyrot 1999).

The prevalence of depression is known to be three times higher among patients with diabetes compared with the general population (Bradley 1994). Psychopathological conditions such as anxiety and depression can negatively affect QoL, glycaemic control and the adherence to treatment, which in turn increases health care costs and the risk for acquiring diabetes-related complications (Rubin and Peyrot 1992; Jacobson 1996; Jacobson et al. 1997; Lustman et al. 1998; Pouwer et al. 1999; Ciechanowski et al. 2000; Lustman et al. 2000; Talbot and Nouwen 2000; Anderson et al. 2001). Goldney *et al.'s* study involved personal interviews of patients with and without diabetes, and found the prevalence of depression in patients with diabetes was 24% compared with 17% of the non-diabetic controls (Goldney et al. 2004). Psychological conditions can be effectively treated, but their accurate recognition is often hampered in clinical practice (Penn et al. 1997; Kessler et al. 1999; Anderson et al. 2001). Intensified treatment regimens might be associated with changes in the psychological condition of the patient, and hence scales are needed to be sensitive to these changes and to the positive aspects of well-being (Bradley 1994).

The findings related to duration of diabetes and QoL are mixed. A few studies reported that an increase in duration of diabetes is associated with decreased QoL (Aalto et al. 1997; Glasgow et al. 1997; Klein et al. 1998), while others reported no significant relation between diabetes duration and QoL (Parkerson et al. 1993; Peyrot and Rubin 1997). Past research has been consistent in finding that the presence of two or more diabetes-related complications is associated with a poorer QoL (Peyrot and Rubin 1997). Some researchers have found an

increase in depression and negative life experiences during 2-years following diagnosis of diabetic retinopathy; the psychological disruptions existed irrespective of the severity of visual impairment and were existent even after lost vision was regained (Wulsin et al. 1987). Other researchers found that the presence of CVD, neuropathy or end-stage renal disease (ESRD) was associated with a poorer QoL (Ahroni et al. 1994; Peyrot and Rubin 1997). The presence of ESRD in T1DM patients was associated with marked increase in functional impairment (Rodin 1990); and reduced perceived health and greater health worries were associated with nephropathy in patients with T1DM (Parkerson et al. 1993). Trief *et al.* study of insulin-treated patients demonstrated that the number of complications was a strong predictor of disease impact and treatment satisfaction scores of the Diabetes Quality of life questionnaire (Trief et al. 1998). The results of research related to the association between treatment regimens and QoL in diabetic patients are varied, some indicate that an increase in treatment intensity in T2DM patients from exercise and diet alone, to oral medications, to insulin leads to a worsening of QoL (Rubin and Peyrot 1999). Jacobson *et al.* reported that diabetes-related worries were higher in patients taking oral medications compared to those who were controlling their diabetes with exercise and diet alone, also those who were on insulin therapy reported less satisfaction and higher burden of illness than those taking oral blood glucose lowering medication or no medication at all (Jacobson et al. 1994). Similarly, some studies found lower scores (indicating poorer QoL) on Bradley's Well-being and Treatment Satisfaction in T2DM patients taking insulin therapy compared to those who were not (Petterson et al. 1998). In contrast, a study of T1DM patients found no association between treatment with twice-daily versus multiple insulin injection regimens (Eiser et al. 1992). The past decade has brought a burgeoning of research related to the association between glycaemic control and QoL in patients with diabetes. A few studies have suggested that a relation does exist, especially when QoL is assessed by diabetes-specific measures rather than generic questionnaires (Rubin and Peyrot 1999). Mazze *et al.* found lower levels of anxiety and depression in T1DM patients with good glycaemic control compared to those with moderate or poor glycaemic control (Mazze et al. 1984).

Methodologically, it is essential to use multidimensional assessments, including both generic and diabetes-specific measures as a guide to evaluate treatment interventions and to assess QoL (Rubin and Peyrot 1999). Generic measures assess concepts which are relevant to everyone, i.e. they are not disease- or treatment-specific and are measures which are applicable to both health and illness groups (Speight et al. 2009). Though these measures

appear to have an advantage of permitting comparison across disease entities, they are less suitable for measurement within disease type (Bradley et al. 1999). Even a well-designed generic QoL measure will not address specific diabetes-related life aspects such as insulin injections, dietary restrictions, hypoglycemia and self-monitoring of blood glucose, which might be critical to a patient's HRQoL.

Over the years, several research studies have added disease-specific measures to generic ones, in order to increase the ability of the measures to identify trends most relevant to the HRQoL of patients with a specific disease. Some studies have advocated a 3-level approach, incorporating disease-specific measures, generic measures, and finally situation-specific (condition or intervention) measures. Diabetes-specific measures are more likely to be sensitive to change and responsive to subgroup differences. These measures usually assess functioning levels, the impact and satisfaction of treatment, worries about the future effects of diabetes, worries about diabetes-related social and vocational issues, sexual functioning and overall well-being. (Speight et al. 2009).

Previous research has found that patients with diabetes are more concerned about social and physical function, emotional and mental health, and burden of illness and treatments on daily life than with clinical parameters such as HbA1c, BP or lipid levels (Barr 1995; Krumholz 2008). Thus, patient-reported outcome measures are relevant and meaningful outcomes. Furthermore, evidence states that when HRQoL of patients with diabetes is appropriately measured and the results are incorporated into health care management, positive improvements in patient outcomes occur (Magwood et al. 2008; Tapp et al. 2010). Improvements in QoL, glycaemic control and a reduction in short-term diabetes-related complications have been observed when a combination of treatment and education approach has been used in patients with diabetes (Norris et al. 2002; Khanna et al. 2012).

The Well-being questionnaire

The Well-being Questionnaire (W-BQ) is one of the measures used to identify psychological problems in patients with diabetes. The questionnaire was originally designed in 1982 for use in a study organised by the WHO evaluating new treatment options for the management of diabetes (Bradley 1994). The original W-BQ consists of 22 items or questions divided among subscales evaluating depressed mood, anxiety, energy and positive well-being and an overall total well-being score can be calculated by combining the four subscales. Questions

concerning somatic states were not included as they may lead to criterion contamination in diabetic patients, in whom somatic symptoms such as loss of appetite or fatigue might result from the physical state due to diabetes, rather than from depression.

In 1994, the WHO/IDF advised the use of the W-BQ22 to monitor psychological well-being in diabetic patients and that this measure should be one of the routine clinical procedures employed in the management of diabetes (Bradley and Gamsu 1994). The W-BQ22 has been utilised in studies evaluating the effects of new treatment regimens such as comparing subcutaneous insulin infusion pumps to conventional insulin-injections (Bradley 1992), insulin treatment in patients previously treated with tablets (Bradley and Lewis 1990; Jennings et al. 1991) and the influence of educational interventions in patients with tablet-treated T2DM (Lewis 1994). The W-BQ22 was further developed in the early 1990s to create the short-form, the W-BQ12 which has a balanced selection of positive and negative items, and consists of 12 items and can be scored on three subscales measuring negative well-being, energy and positive well-being (Bradley 1994). The W-BQ12 has been widely used, particularly in clinical trials, and is available in more than 35 languages. To ensure that data obtained from the questionnaires are meaningful, it is important that they are validated, i.e. proved to be valid and reliable measures of the specific targeted concepts (Speight et al. 2009). Pouwer *et al.* carried out a study to evaluate the validity and reliability of the W-BQ12 in a group of both T1DM and T2DM patients (Pouwer et al. 1999). The authors concluded the W-BQ12 to be a reliable and valid measure of psychological well-being for patients with diabetes. Compared to the original 22-item questionnaire, the 12-item questionnaire is easier to administer and hence was considered to be a useful tool for both researchers and clinicians to assess the psychological health and well-being in patients with diabetes (Pouwer et al. 1999).

A few studies have utilised the W-BQ12 to assess QoL in patients with diabetes. Pouwer *et al.* utilised the computerised version of the W-BQ12 to assess the differences in treatment strategies between psychological monitoring versus standard care in diabetic patients over a year (Pouwer et al. 2001). Assessment of the W-BQ12 revealed that patients who received psychological monitoring reported significantly lower negative well-being, and significantly higher energy and general well-being compared to those who received standard care. The authors concluded that although the W-BQ12 is a great measure to assess psychological well-being in a few minutes, diabetes-related emotional disturbances, such as fear of

hypoglycaemia and worries about complications, cannot be detected easily with this instrument (Pouwer et al. 2001). A previous study of T2DM patients utilised the W-BQ12 along with other generic and diabetes-specific measures to assess the effects of intensive diabetes treatment versus routine care on patient reported outcomes after 5 years, and found no differences in W-BQ12 scores between the two interventions (Van den Donk et al. 2013). Although the W-BQ was designed for use in diabetic patients, the 12-item version is not actually diabetes-specific. However, the W-BQ12 has proved sensitive to the positive benefits of new treatment regimens for diabetes, mostly as a result of its ‘energy’ and ‘positive well-being’ subscales (Bradley 1994; Witthaus et al. 2001).

The Audit of Diabetes Dependent Quality of Life

In 1999, Bradley *et al.* developed the Audit of Diabetes Dependent Quality of Life (ADDQoL), an instrument aimed to measure QoL in both TIDM and T2DM patients (Bradley et al. 1999). The ADDQoL was designed to provide an individualized questionnaire to measure of the impact of diabetes on QoL (Bradley et al. 1999). It consists of diabetes-specific questions which capture any negative psychological impact that diabetes may have on the patient. The ADDQoL has important advantages over generic questionnaires as it allows patients to indicate which aspects of life apply to them; the perceived importance of each aspect of life on their QoL and whether the impact of diabetes on that aspect of life is positive or negative. The selection of the ADDQoL domains was based on past research experience and derived from discussions with healthcare professionals in the field. In addition, on a routine diabetes clinic, 12 in-depth face-to-face interviews were conducted where the interviewer elicited domains of importance for the interviewee’s QoL which were most affected by diabetes (Bradley and Speight 2002).

The ADDQoL-19 consists of two overview items designed for audit purposes: a generic domain “present QoL” and a diabetes-specific domain “impact of diabetes on QoL”. Further 19 domains or questions are concerned with the impact of diabetes on specific life aspects. The 19 life domains are as follows: leisure activities, working life, local or long-distance journeys, holidays, physical health, family life, friendships and social life, close personal relationships, sex life, physical appearance, self-confidence, motivation to achieve things, people’s reactions, feelings about the future, financial situation, living conditions, dependence on others, freedom to eat, and freedom to drink. The 19 questions ask respondents to rate how their life would be if they did not have diabetes. For all applicable domains, the

respondents have to rate the impact of diabetes on the life aspect and the importance of that life aspect on their QoL. For each domain, a weighted impact score is calculated and lower scores reflect a poorer QoL. Lastly, a total ADDQoL-19 score is the mean of all the weighted impact scores of all applicable domains (Bradley et al. 1999; Bradley and Speight 2002; Costa et al. 2006).

The ADDQoL has proven to be an acceptable, valid and reliable measure with good internal consistency (El Achhab et al. 2008). The ADDQoL has proven sensitive to the benefits of switching from a traditional insulin regime to flexible, intensive insulin therapy (Group 2002). Whereas generic measures used to quantify health status may be strongly affected by non-diabetic co-morbidity in patients with diabetes, ADDQoL scores remain unaffected by co-morbidity. They are affected by diabetes-related complications (Bradley et al. 1999; Woodcock et al. 2001) but not by unrelated conditions (Woodcock et al. 2001). A longitudinal study utilised the ADDQoL-19 along with other generic (W-BQ12) and diabetes-treatment satisfaction measures to assess the effects of intensive diabetes treatment versus routine care in patients with T2DM (Van den Donk et al. 2013). The authors reported no differences in health status, well-being and treatment satisfaction between the two treatment regimes. Another previous study utilised the ADDQoL-19 to evaluate the QoL in patients with T2DM (aged ≥65 years) (Turk et al. 2013). The authors reported that poorer QoL was significantly associated with heart attack episodes and to the perception of not having diabetes under control. The findings of their study did highlight the impact of T2DM on QoL with particular reference placed on the freedom to eat, family life and dependence on others (Turk et al. 2013).

The ADDQoL has received certain criticisms for its complex structure which focuses on a hypothetical situation that patients with diabetes may or may not be able to imagine, i.e. 'if I did not have diabetes, my (domain) would be… ("very much better" to "very much worse")'. In 2006, the Food and Drug Administration guidance draft on the utilization of patient-reported outcome measures in clinical trials stated that it does not recommended the use of 'items that ask patients to respond hypothetically or that give the patients the opportunity to respond on the basis of their desired condition rather than on their actual condition', due to the unreliability of self-reported data based on hypothetical situations (FDA 2006). Furthermore, despite the development of the ADDQoL Senior (Speight et al. 2003), it is possible that elderly patients or those with lower literacy levels may find the ADDQoL a

difficult task to complete due to its complex structure. Additionally, critics have questioned the requirement for weighting of items according to personal importance, suggesting that such weightings have little consequence on the overall scores. However, taking into account that an individual's preferences and priorities are cardinal to understanding their distinctive QoL, the ADDQoL has better face or content validity compared to many other measures used to assess QoL in patients with diabetes (Speight et al. 2009).

1.4.4 Impact of periodontal status and treatment on QoL in patients with diabetes

Knowing that patients with diabetes have a higher prevalence and severity of periodontal disease compared to non-diabetics, it is of importance to evaluate the impact of periodontal disease on QoL in patients with diabetes (Albandar 2002). Both periodontitis (Needleman et al. 2004; O'Dowd et al. 2010) and diabetes (Goldney et al. 2004; Wandell 2005) have been found to have negative impacts on QoL. Patients with diabetes are known to have limited awareness of the potential effect of diabetes on their oral health, and of the potential effects that periodontal disease has on their general health (Bissett et al. 2013). Hence, there is a need to ensure that diabetic patients are better informed of their risk for acquiring periodontal disease and the negative impact it can have on their oral and general health and overall well-being (Allen et al. 2008).

A majority of studies to date have focused on the impact periodontal disease has on the QoL in patients without diabetes. Only a few QoL studies have focused on the impact that diabetes and periodontal disease together have on an individual's QoL. One such study identified that T2DM patients have a significant impact on their OHRQoL in the domains of general health, social functioning, physical functioning and role functioning when compared to non-diabetic patients (Sandberg and Wikblad 2003). A previous study evaluated the impact of periodontal disease on QoL in patients with diabetes (n=159) utilizing the OHIP-14 (Drumond-Santana et al. 2007). The patients completed the OHIP-14 prior to clinical examination (PD, CAL and BOP) to evaluate how their oral health interfered with their QoL in the past year. The clinical examination revealed that 15.7% of patients had healthy tissues, 35.2% had gingivitis and 49.1%, 27.7% and 21.4% had mild, moderate and advanced periodontitis, respectively. A significant association was seen between QoL and severity of periodontal disease, and PD, CAL ≥4 mm and BOP were associated with poorer QoL. The authors concluded that diabetic patients with periodontitis had a greater negative impact on QoL in comparison to those with healthy tissues and gingivitis (Drumond-Santana et al.

2007). Allen *et al.* assessed the attitudes and awareness of the risk for periodontal disease and OHRQoL in patients with T1DM (n=27) and T2DM (n=74) (Allen et al. 2008). The authors found that only 33% of the patients were aware of their increased risk for periodontal disease (40% of T1DM). Only 43% had attended a dentist within the past year, while 34% had not attended a dentist for more than 5 years. 37% of the patients attended the dentist for treatment once a year, while 63% attended only when they had dental problems. Assessment of the OHIP-20 revealed that 66% of the patients had problems related to food lodgment between their teeth and under their dentures, and 43% reported an unsatisfactory diet due to problems with their teeth. Unfortunately, their study did not include a control group, hence no comparisons were made to OHRQoL in non-diabetic patients (Allen et al. 2008).

A recent study by Irani *et al.* investigated the impact of periodontal status and treatment on OHRQoL in patients with (n=61) and without T2DM (n=74) using the OHIP-49 (Irani et al. 2015). The authors reported no significant differences in the overall OHIP-49 summary score between patients with and without T2DM, suggesting that T2DM had no impact on the overall OHRQoL. However, within the non-diabetic group patients with chronic periodontitis and gingivitis had poorer OHRQoL compared to those with periodontal health with evidence of improvements in QoL being noted, with significant reductions in the psychological discomfort and psychological disability domains of OHIP-49, following periodontal treatment (Irani et al. 2015). The authors reported that the lack of significant differences in OHIP-49 scores among T2DM patients with periodontitis, gingivitis and healthy tissues could possibly indicate that patients with diabetes are less concerned about their oral health than they are about other health problems that they have to manage as part of their diabetes. Potentially, systemically healthy patients who have chronic periodontitis might be more concerned about the signs and symptoms of periodontal disease compared to patients with diabetes, who need to address other pressing health issues, which might lead to lower expectation of oral health or better coping with the impact of periodontitis (Irani et al. 2015). Drumond-Santana *et al.'s*, Allen *et al.'s* and Irani *et al.'s* studies utilized versions of the OHIP questionnaire, to assess OHRQoL in patients with diabetes and periodontal disease. The OHIP has been used for many years to assess the impact of oral conditions on OHRQoL particularly in context to prosthodontic patients, however its use in patients with periodontal disease and its ability to detect a meaningful change following periodontal treatment has yet to be established (Irani et al. 2015). The OHIP is useful in measuring OHRQoL, however the

OHIP contains no diabetes-specific questions hence might not to the full extent assess HRQoL in patients with diabetes.

Given the important interactions between periodontal disease and diabetes, it is good practice to ensure optimal oral health care for patients with diabetes. Investigations have shown an improvement in clinical periodontal status following NSM, based on clinical periodontal parameters. Findings related to patient-based outcomes of periodontal disease are limited, especially in relation to other comorbidities of systemic disease, such as T1DM. Considering that QoL measures are being increasingly used to give context to patient-centred outcomes of disease and treatment, it is important to explore the impact of periodontal status and treatment on QoL especially in patients with T1DM as this group has been largely under researched.

1.5 Aims

1. To study the presentation of periodontal disease and caries in a local population of young adults with T1DM.
2. To investigate associations between glycaemic control and periodontal and oral health.
3. To evaluate the effect of periodontal treatment on glycaemic control in T1DM patients with periodontitis.
4. To study the local and systemic production of biomarkers and the initial response to therapy by monitoring markers of inflammation before and after periodontal treatment.
5. To assess the QoL in patients with T1DM before and after periodontal treatment.

2 Chapter 2. Materials and methods

2.1 Ethical approval

For this research study, ethical approval was obtained from the Sunderland NHS Research Ethics Committee (ref 06/Q0904/16). The application to the ethics committee included a protocol for the study, which highlighted the possible ethical issues pertaining to the study. The main ethical issues were related to the collection of samples for analysis (GCF and blood). The samples were collected purely for research purposes and would otherwise not be collected. The purpose and reason for collecting samples was made clear to the prospective participants in the information sheet. The collection of GCF was non-invasive, painless and quick, however, the collection of blood samples had a possibility of being associated with some discomfort and there could have been a potential for unwanted events (e.g. bruising). To minimise the risks associated with venepuncture, trained and experienced clinicians were asked to obtain the blood samples.

The periodontal examination and treatment provided as part of the study, constituted routine clinical care. A possible benefit of this study was that the T1DM patients received oral and periodontal examinations and if they were found to have periodontal disease, treatment was offered to them, as part of the study. All participants in the study were also given information and instructions on how to better maintain their oral health. All data recorded and samples collected were stored securely and anonymously, using a coding system. The information that was generated as part of this research study did not have an impact on the patient's clinical care and treatment other than that relating to any required periodontal clinical management.

2.2 Patient recruitment and discharge

T1DM patients were recruited from databases held by Dr. Jolanta Weaver at the Queen Elizabeth Hospital in Gateshead and Professor Roy Taylor at the Newcastle Diabetes Centre. Some of these patients had periodontal disease, and others, were periodontally healthy. Patients on this database were evaluated regularly by Dr. Weaver, Professor Taylor and their teams. The identified patients were then sent a letter informing them about the research study and inviting them to participate on an 'opt-in' basis. If the patients then contacted the research team to indicate their interest in participating in the study, a short telephone screening was carried out to make sure they fitted within the inclusion and exclusion criteria. Following this, the patients were sent a detailed information sheet giving them information

about the arrangements for them to attend the dental hospital for a pre-treatment screening appointment.

Recruitment of the non-T1DM patients involved the identification of suitable patients who met the inclusion and exclusion criteria and were either those referred from general dental practices to the Department of Restorative dentistry within the Newcastle Dental Hospital or were patients seen on student treatment clinics in the School of Dental Sciences. Each non-T1DM patient was matched to a previously recruited T1DM patient. Patients were matched based on age (within 5 years), gender, smoking status and periodontal diagnosis. Suitable non-T1DM patients were approached during their appointment at the dental hospital, informed about the study and what their potential participation in it would involve, and were given the opportunity to decide whether they wanted to participate.

Recruited patients fulfilled the following inclusion criteria: 16-50 years old, male or female, with a minimum of 20 natural teeth. The exclusion criteria included: pregnancy, any condition requiring prophylactic antibiotics prior to dental treatment, immunosuppression, bleeding disorders, and prolonged bleeding due to medication, drug-induced gingival overgrowth, any medical condition that could compromise safe participation in the study, or any patient who had undergone NSM in the past 6 weeks.

At the screening appointment, patients who were diagnosed with periodontitis were offered the necessary treatment and were then monitored as part of the longitudinal component of the study. The patient's general medical practitioner and general dental practitioner (GDP) were informed via a letter, of the patient's involvement in the study. Following completion of the study, the patients were discharged back to their GDP with a written discharge letter containing details of the periodontal maintenance plan for the GDP to follow. For patients without periodontitis (those with healthy periodontal tissues and gingivitis), their participation in the study was limited to the screening appointment only. These patients were given oral hygiene advice and dental prophylaxis at the screening appointment. If the clinical examination revealed any other oral or dental problems, such as caries, appropriate management was arranged either with their GDP or in the dental hospital, for patients who were not registered with a GDP.

2.3 Consent

At the start of the pre-treatment screening appointment, written informed consent was obtained from all patients. This involved a clinician confirming that the patient had understood the written information leaflet they had received about the study. Following this, the clinician verbally confirmed the background and aims of the study, and explained the potential benefits and risks that their participation in the study involved. Every patient was given the opportunity to ask questions and a choice to opt out of participating in the study. Patients who wished to participate in the study were then asked to sign two copies of the consent form, one of which was retained in the patients' hospital notes and the other copy was given to the patient to keep.

2.4 Power calculation and estimation of sample size

It was difficult to provide a definitive power calculation at the planning stage of the study due to the paucity of research studies that have investigated this area of research previously. Using data from a study of patients with T2DM (Kiran et al. 2005) it was estimated that 17-20 patients would be required to provide an 85% power for detecting significant changes in HbA1c over 6 months, assuming $\alpha=0.05$, $\delta=0.7\%$ and $\sigma=0.9\%$. However, in order to identify these patients, and assuming a prevalence rate of periodontitis of 10% in this young cohort, then approximately 200 patients would be required for baseline assessment. Of these, it was anticipated that approximately half would require further treatment for gingivitis, and approximately 10% (up to 20) would need treatment for periodontitis. Those patients who received treatment would be those diagnosed with periodontitis. These patients would be monitored longitudinally for 6 months following periodontal treatment, with assessments carried out by the research team at months 3 and 6.

2.5 Periodontal disease case definition

At the pre-treatment screening appointment, all patients received a full-mouth periodontal examination, which included recording PI, mGI, PD measurements, recession, LOA and BOP at 6 sites per tooth. Clinically indicated radiographs were obtained, following which, clinical and radiographic data were used to confirm the periodontal diagnosis based on the diagnostic criteria (Table 2.1). Robust case definitions for periodontal status were used to avoid the misclassification of patients. A difficult diagnosis was resolved by discussion between the two clinicians.

Periodontal diagnostic criteria	
Healthy periodontal tissues	• BOP ≤15 %. • No PD sites >4 mm. • No LOA [disregard localised recession (e.g. due to tooth brush trauma)]. • No bone loss.
Gingivitis	• BOP >15 %. • No sites with PD >4 mm, except for up to 5 sites with 5 mm PD (e.g. at the distal surface of last standing molars). • No LOA [disregard localised recession (e.g. due to tooth brush trauma)].
Periodontitis	• ≥6 sites with PD of ≥5 mm at separate teeth. • LOA and/or bone loss present.

Table 2.1: Case definitions for healthy periodontal tissues, gingivitis and periodontitis.

2.6 Clinical protocol

2.6.1 Plaque index

The Silness and Loe (Silness and Loe 1964) PI was used to assess the amount of plaque present on the surfaces of teeth, as follows:

0 No plaque

1 A film of plaque adhering to the free gingival margin and the adjacent area of the tooth. The plaque may be seen *in situ* only after application of disclosing solution or by probing the tooth surface.

2 Moderate accumulation of soft deposits within the gingival pocket, or on the tooth and gingival margin which can be seen with the naked eye.

3 Abundance of soft matter within the gingival pocket and/or on the tooth and gingival margin.

In order to record the PI score, the tooth was dried with a gentle stream of air and no disclosing solution was used. Plaque scores of ‘2’ or ‘3’ were easily identified with visual examination. If no plaque was seen with direct unaided visual examination, the probe was

swept along the gingival margin to be able to differentiate between a score of '1' (if plaque was present at the probe tip) or a score of '0' (if plaque was absent at the probe tip). The plaque score was recorded on 6 sites per tooth and four target teeth were selected; from these selected teeth GCF samples were also taken (section 2.8). The PI scores were immediately recorded by an assistant.

2.6.2 Modified gingival index

The degree of gingival inflammation present was assessed using the mGI (Lobene et al. 1986) as follows:

0	No inflammation
1	Mild inflammation; slight change in colour, little change in texture of any portion, but not the entire gingival margin or papillary unit.
2	Mild inflammation; criteria as above but involving the entire gingival margin or papillary unit.
3	Moderate inflammation; glazing, redness, oedema and/or hypertrophy of the gingival margin or papillary unit.
4	Severe inflammation; marked redness, oedema and/or hypertrophy of the gingival margin or papillary unit with spontaneous bleeding, congestion or ulceration.

In order to record the mGI score, the gingival tissues were dried with a gentle stream of air. A visual examination was carried out to allocate a score at 6 sites per tooth. A score of '0' was assigned if no gingival inflammation was present and a score of '4' was assigned if the gingival tissues were severely inflamed and there was evidence of marked swelling or redness, spontaneous bleeding and/or ulceration. A score of '1' was assigned if only part of the gingival tissue was inflamed. A score of '2' and '3' was assigned if mild or moderate gingival inflammation, respectively, had affected the entire gingival margin or papillary unit.

2.6.3 Probing depth

PD measurement was carried out using a University of North Carolina (UNC) 15 manual periodontal probe (Dentsply, Addlestone, UK) and was recorded as the distance from the

gingival margin to the base of the gingival sulcus or pocket. The periodontal probe was inserted into the gingival sulcus and advanced apically, along the long axis of the tooth, until resistance of the tissue was felt at the base of the gingival sulcus or pocket. The PD measurements were recorded in millimetres (mm) by direct visualisation of the markings on the probe. PDs were recorded for all teeth present excluding the 3rd molars, and measurements were taken at 6 sites per tooth.

2.6.4 Bleeding on probing

The BOP scores were recorded immediately following PD measurements within one aspect of a quadrant (for example, the buccal aspect of the upper right quadrant). The probing sites were re-examined by visual examination to determine post-probing bleeding. Bleeding status was recorded as the presence or absence of bleeding from the pocket base following probing and was determined dichotomously at 6 sites per tooth.

2.6.5 Recession

Recession was measured using the UNC 15 probe and was taken as the distance from the cementoenamel junction (CEJ) to the gingival margin. The measurement was recorded whilst the probe was inserted into the gingival sulcus during PD measurement. When the CEJ was located above the gingival margin, recession was recorded by direct visualisation of the probe markings. When the CEJ was located below the gingival margin (e.g. in the case of false pocketing), the clinician estimated the position of the CEJ in relation to the gingival margin and a negative recording in mm was made. Recession was recorded for all teeth present excluding the 3rd molars, and measurements were taken at 6 sites per tooth.

2.6.6 Loss of attachment

The LOA measurement was the sum of the PD and recession, and therefore is, the distance from the CEJ to the base of the gingival sulcus or pocket. LOA was calculated for all teeth present excluding the 3rd molars.

2.6.7 Smoking status

Smoking habits self-reported of each patient were assessed at the pre-treatment screening visit and at month 6, according to whether the patients were current, non-, or ex-smokers. The smoking extent of the current and ex-smokers were further quantified according to the standardised measure of pack years, which equates the number of cigarettes (packs per day)

smoked by each patient by the number of years smoked. A pack year equates to smoking 1 pack of 20 cigarettes per day for 1 year.

2.6.8 Demographic data

At the pre-treatment screening appointment, demographic data were recorded which included age and gender.

2.6.9 Diabetes history

Diabetes history was recorded for all T1DM patients at the pre-treatment screening visit. Recorded data included years since diagnosis, age at diagnosis, family history of diabetes, method of diabetes control, patient-perceived level of glycaemic control in the past one year (good/moderate/poor), presence of macrovascular and microvascular diabetes complications and current medications.

2.6.10 Physical examination

The physical examination involved taking the patient's blood pressure (BP), height and weight. The BP was recorded using the patient's right upper arm, with them being seated and using an automated BP machine. Height and weight were recorded in order to calculate the body mass index (BMI) by dividing the weight [in kilograms (kg)] by the square of the height [in metres (m)].

2.6.11 Oral examination

The oral examination comprised examination of soft tissues to identify any lesions, or problems such as xerostomia or oral candidiasis. The number of teeth present and missing, and denture-use was also recorded. The dentition was then assessed using pre-defined dental examination criteria (Kelly et al. 2000), recording the number of teeth for each category as shown in Table 2.2.

Diagnostic criteria for hard tissue examination	
Sound and untreated teeth	• No evidence of caries into dentine or restorations. • Including caries restricted to enamel.
Restored teeth (1 to 3 surfaces)	• Amalgam, composite, glass ionomer cement (GIC), fissure sealants, onlays, inlays and ¾ crowns – up to and including 3 surfaces. • Including veneers, shims and adhesive retainers for resin retained bridges.
Extensively restored teeth (4 or more surfaces)	• Amalgam, composite, GIC, onlays or inlays - 4 or more surfaces. • Temporary or permanent crowns, including full-coverage crowns and conventional bridge abutments.
Carious teeth	• Visual examination - manifests as showing under an occlusal surface or marginal ridge. • Cavitated – but without pulpal involvement. • Temporary dressing placed for treatment of caries.
Broken down or teeth with pulpal involvement	• Teeth so broken down that it is inconceivable that there is no pulpal involvement.

Table 2.2: The dentition was examined and assessed against these pre-defined examination criteria (Kelly et al. 2000).

2.6.12 Oral health behaviour

At the pre-treatment screening appointment, patients were asked closed questions regarding their oral health behaviour which included questions regarding their frequency of tooth brushing, interproximal cleaning, as well as the timing and reason for their last dental visit to their GDP.

2.6.13 Non-surgical periodontal management and follow-up

Following the pre-treatment screening appointment, patients diagnosed with periodontitis received comprehensive targeted NSM. The treatment day was the baseline time point, and was within 2 months of the screening appointment. The baseline treatment constituted giving personalised oral hygiene instructions (OHI) and using a full-mouth instrumentation approach (Quirynen et al. 2000) to disrupt and remove biofilm and calculus. A combination of hand instrumentation, using Gracey curettes and flexichange scalers (Dentsply, Addlestone, UK), and ultrasonic instrumentation, using a Cavitron Select machine (Dentsply, Addlestone, UK), were used to perform full-mouth instrumentation. Simultaneously, periodontal pockets were irrigated with 0.2% chlorhexidine gluconate (Kent express, Kent, UK). Effective tooth brushing and interproximal cleaning techniques were demonstrated to the patients.

Following the baseline treatment appointment, the patients were seen at week 3 and week 6 for OHI and further prophylaxis to disrupt reforming plaque biofilm deposits. A periodontal examination was then carried out at month 3 and 6 after the initial instrumentation to assess periodontal healing after treatment. Additional periodontal treatment in order to eliminate inflammation was undertaken at months 3 and 6, as clinically indicated (Table 2.3). Pre-treatment screening was undertaken on all patients, however, only those patients diagnosed with periodontitis proceeded beyond the screening appointment. Patients diagnosed with gingivitis received OHI and full-mouth instrumentation and prophylaxis, as required at the screening appointment. For patients with gingivitis and healthy periodontal tissues, their participation in the study was limited to the screening appointment itself and they were not followed-up further after this appointment.

		Pre-treatment screening	Month 0 *	Week 3 *	Week 6 *	Month 3 *	Month 6 *
Informed consent		•					
Demographic data		•					
Medical history		•					•
Diabetes history #		•					
Smoking status		•					
Physical exam		•					•
Oral health history		•					
Oral examination		•		•	•	•	•
GCF samples		•				•	•
Periodontal examination	PI	•				•	•
	mGI	•				•	•
	PD	•				•	•
	Recession	•				•	•
	LOA	•				•	•
	BOP	•				•	•
Blood samples	HbA1c	•				•	•
	hsCRP	•				•	•
	Lipids	•				•	•
Initial periodontal therapy	OHI & FMI		•				
Prophylaxis				•	•	•	•
Additional periodontal therapy	As required					•	•

Table 2.3: An overview of the protocol followed highlighting the procedures undertaken at each time point in the study.

GCF; gingival crevicular fluid, PI; plaque index, mGI; modified gingival index, PD; probing depth, LOA; loss of attachment, BOP; bleeding on probing, HbA1c; glycated haemoglobin, hsCRP; high-sensitivity C-reactive protein, OHI; oral hygiene instructions, FMI; full-mouth instrumentation, #; not taken for non-T1DM patients, *; only in patients with periodontitis.

2.6.14 Data collection and storage

All patients were allocated an identification number and each of them had a case report form (CRF) in which all data were recorded at the time of examination by the clinician or an assistant. The results from the clinical biochemistry laboratory were also recorded in the patient's CRF. Subsequently, data were entered into a statistical software package.

2.7 Statistical analysis

Statistical analyses of the data were conducted using statistical software IBM SPSS Statistics, version 22. Firstly, all variables to be tested were assessed for normality using the Shapiro Wilk test, supplemented with histograms. For normal or parametric variables, means and standard deviations were calculated. Where normality was rejected, for non-parametric variables, medians and interquartile ranges were calculated. Chi-squared tests were used to analyse discrete variables. The significance of all tests was assessed at the 5% level.

Cross-sectional pre-treatment data were analysed, based on diabetes status using independent samples t-tests or Mann-Whitney tests for parametric or non-parametric variables, respectively. Following this, cross-sectional data were analysed, based on diabetes status and periodontal diagnosis, with one-way analysis of variance (ANOVA) or the Kruskal Wallis test for parametric and non-parametric variables, respectively. For post hoc analysis, independent samples t-tests or Mann-Whitney tests were used for parametric and non-parametric data, respectively. A *P*-value of <0.05 was considered significant. For multiple comparisons, the p-values were corrected using the Bonferroni-Holm test. The associations between clinical data and biomarker levels were assessed using Pearson's correlation coefficient (r), if both variables were normally distributed, or using Spearman's correlation coefficient (rho) if both variables were not normally distributed. Scatter diagrams were also constructed to illustrate associations.

Longitudinal parametric data were analysed with repeated measures ANOVA and paired samples t-tests for post hoc analyses. Longitudinal non-parametric data were analysed with the Friedman test, with the Wilcoxon Mann-Whitney test applied for post hoc analyses. A *P*-value of <0.05 was considered significant. For multiple comparisons, the p-values were corrected using the Bonferroni-Holm test. At each time point (pre-treatment, months 3 and 6), for patients diagnosed with periodontitis only, cross-sectional data were analysed using

independent samples t-tests or Mann-Whitney tests for parametric and non-parametric data, respectively.

2.8 Sampling, elution and storage of GCF

GCF was collected with Periopaper strips (Oraflow Inc, New York) and the volume was quantified using a calibrated Periotron 6000 (Preshaw et al. 1996). According to the manufacturer's instructions, prior to its use, the Periotron was allowed to 'warm up' and then zeroed with a blank (dry) Periopaper. The dial was adjusted until the digital display indicated a zero reading.

To minimise sample contamination by blood, GCF was collected prior to probing the periodontal pockets. At the pre-treatment screening appointment, 4 GCF samples were collected from each patient, from the mesio-buccal aspect of the four 1st molars. If the 1st molar was absent in any quadrant, the sample was collect from the 2nd molar, then the 2nd premolar, 1st premolar, and canine or incisor teeth (the sampled teeth were designated target teeth). The area was isolated using cotton rolls and a saliva ejector, and the teeth were dried with a gentle stream of air. If supragingival plaque was present prior to sampling, it was carefully and gently removed with a curette. A Periopaper was carefully placed into the gingival sulcus until mild resistance was felt and was held in position for 30 seconds.

The Periopaper was immediately transferred to the jaws of the Periotron to minimise evaporation errors. Care was taken to ensure that the Periopaper was in the exact standardised position between the jaws, with the black line on the paper positioned at the outer rim of the jaw plate. After "mode II" illuminated on the Periotron display, the GCF volume (in Periotron units) was recorded by the assistant. The Periopaper was then placed into a 0.5 ml sterile plastic microtube (Sarstedt, Leicester, UK) containing 150 µl autoclaved and filtered phosphate buffered saline (PBS). Each sample was stored in a separate microtube and each microtube was labelled with the patient's study number, tooth number and the date. Between samples, the jaws of the Periotron were cleaned with an alcohol swab and allowed to dry. At the chairside, the GCF samples were kept on ice and transferred within 20 minutes of sampling, to the laboratory and were frozen at -80°C (Cutler et al. 1999) to await subsequent elution and analysis. The same procedure was followed to collect GCF samples at months 3 and 6 from the same 4 sites in patients diagnosed with periodontitis, who were entered into the longitudinal phase of the study.

For the elution of the GCF from the Periopapers, the samples were thawed on ice for 15 minutes following which, 50 µl of 1% bovine serum albumin (BSA) in PBS (w/v) was added. The GCF samples were then centrifuged (Sigma 3K10 centrifuge) for 60 minutes at 300 rpm, at 4 °C, following which a second centrifuge step was carried out at 1200 rpm for 2 minutes, at 4 °C. Lastly, the Periopapers were removed with college tweezers (with the ends of the tweezers being rinsed with PBS between samples) and the eluted GCF samples were frozen again at -80°C, until further analyses.

Prior to analysis, the stored GCF samples were thawed on the benchtop and the 4 GCF samples collected from each patient, were pooled into a single microtube and these pooled GCF samples were further analysed by ELISA (section 2.15).

2.9 Collection of venous blood samples

A tourniquet was applied to the patient's arm 8 cm above the antecubital fossa or the hand, and the veins were allowed to engorge with blood. Venous access was achieved using a 21 gauge and 1.5 inch Vacutainer needle (NHS Supply Chain, Derbyshire, UK) and a needle holder for 16 mm diameter tubes (NHS Supply Chain, Derbyshire, UK). The venous blood sample was taken to fill the following three Vacutainer plastic tubes (BD, Oxford, UK): 3 ml ethylenediaminetetraacetic acid (EDTA) tube (lavender top), 5 ml serum separation tube (gold top) and 9 ml serum separation tube (gold top). Once the blood samples were collected, the tourniquet was loosened, the needle was removed and pressure was applied with a cotton wool to the sample site until haemostasis was achieved. All blood samples in the Vacutainer tubes were slowly inverted at least five times and then left to stand upright for 30 minutes prior to transferring them to the appropriate laboratory for analysis.

2.10 Clinical biochemistry analysis

The 3 ml EDTA (lavender top) and 5 ml serum separation (gold top) tubes were labelled with adhesive labels from the patient's hospital notes and were sent to the Clinical Biochemistry Department of the Newcastle upon Tyne Hospitals NHS Foundation Trust Royal Victoria Infirmary for analysing the level of HbA1c, hsCRP, triglycerides, cholesterol, high density lipoprotein (HDL) and non-high density lipoprotein (non-HDL) for each patient. The samples were analysed as per the standard operating procedures of the Clinical Biochemistry Laboratory.

2.11 Serum separation and storage

The 9 ml serum separation tube (gold top) was kept on ice at chair side and transferred, within 2 hours, to the laboratory. The sample was then centrifuged at 1500 x g for 15 minutes at 4 °C to separate the serum at the top of the tube via the formation of a polymer barrier. The serum was aliquoted into six 0.5 ml microtubes using a Pasteur pipette. The microtubes were labelled with the patient number and date, and were frozen at -80 °C for further analyses.

2.12 Calculating GCF volume

In order to calculate GCF volume, firstly, a quadratic equation was generated as part of the calibration of the Periotron 6000 (Preshaw et al. 1996) which was undertaken every 12 weeks during the study. Software package Excel was used to solve the quadratic equation to calculate GCF volume from the Periotron units.

2.13 Quantification of protein

The serum and GCF samples were collected from 2006 to 2008 and were stored in the -80 °C freezer until analysis. Given the long period of storage (albeit at -80 °C), a concern was that protein levels may have reduced due to sample degradation. In order to determine the amount of protein present, the samples were tested following the Bradford method for protein quantification. An assay which was originally described by Bradford (Bradford 1976) has become the preferred method for quantifying protein in samples in many laboratories. The Bradford assay is based on the binding of the dye Coomassie Blue G520 to protein present in sample. Following this the quantity of protein can be estimated by measuring the absorbance of the solution at 595 nm.

The assay procedure was carried out on all baseline serum samples using a sterile, flat bottom 96-well cell culture plate (Cellstar, Greiner Bio-One, Germany). The first step involved diluting 0.4 g of BSA powder (Sigma, Poole, UK) in 2 ml of distilled water. This dilution resulted in a protein solution of concentration 200 mg/ml (tube A). The 200 mg/ml solution was then further diluted by taking 200 µl from tube A and diluting it into 1800 µl of distilled water, which resulted in a solution of protein concentration of 20 mg/ml (tube B). Following this the standard curve (Figure 2.1) was prepared following the protocol in Table 2.4.

µg/ml	Standard / tube	mg/ml	Procedure
	A	200	0.4 g BSA in 2 ml distilled water
20000	B	20	200 µl of 200 mg/ml (tube A) + 1800 µl distilled water
4000	1		200 µl of 20 mg/ml + 800 µl distilled water
3500	2		175 µl of 20 mg/ml + 825 µl distilled water
3000	3		750 µl of tube 1 + 250 µl distilled water
2500	4		125 µl of 20 mg/ml (tube B) + 875 µl distilled water
2000	5	2	200 µl of 20 mg/ml + 1800 µl distilled water
1500	6		750 µl of tube 5 + 250 µl distilled water
1000	7		500 µl of tube 5 + 500 µl distilled water
750	8		300 µl of tube 7 + 100 µl distilled water
500	9		300 µl of tube 7 + 300 µl distilled water
250	10		300 µl of tube 9 + 300 µl distilled water
125	11		200 µl of tube 10 + 200 µl distilled water
25	12		100 µl of tube 10 + 900 µl distilled water
0	13		Only distilled water

Table 2.4: Standard curve protocol for the Bradford assay.

BSA; bovine serum albumin.

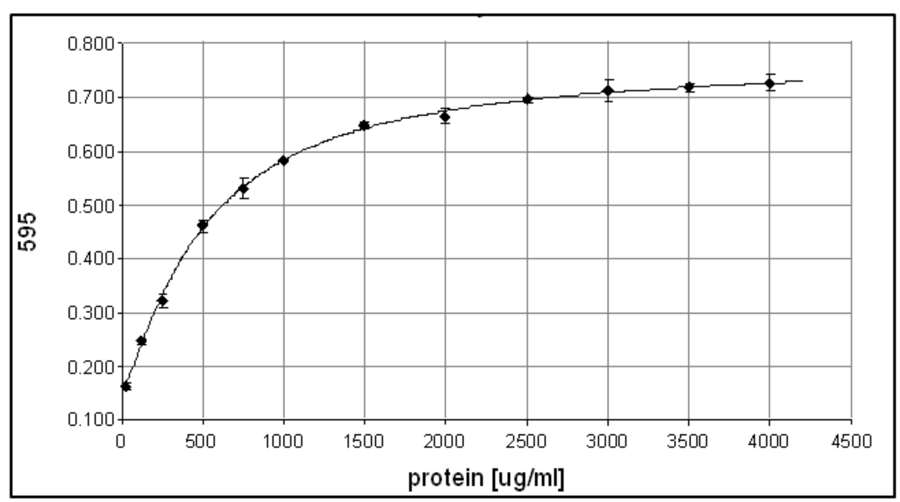


Figure 2.1 The Bradford assay standard curve.

X-axis represents the absorbance of protein at 595 nm and Y-axis represents the quantity of protein in µg/ml.

Following the preparation of standards, the serum samples were diluted in a ratio of 1:300, in distilled water. Following this, 10 µl of each standard and diluted serum sample was pipetted in triplicate in a 96-well multidish. Additionally, 150 µl of Bradford assay reagent (Pierce, Thermo Scientific) was added to each well of the 96-well multidish. The multidish was incubated for 5 minutes at room temperature, following which the absorbance was measured at 595 nm using a plate reader (Synergy HT, BioTek, USA) following the Bradford protocol. Prior to reading, it was made sure that there were no air bubbles to avoid reading errors. The Bradford protocol subtracts the water blank from all the standards and samples, it then uses the mean absorbance values for the standards to generate a standard curve (Figure 2.1).

2.14 Cytokine array analysis

The Proteome Profiler Human XL Cytokine Array Kit (R&D Systems) was used to determine the multiple cytokines present in the serum samples collected for the study. The cytokine array is a membrane-based sandwich immunoassay and has the ability to detect 102 human cytokines simultaneously. The cytokine array capture and control antibodies are spotted in duplicate on a nitrocellulose membrane, and bind to specific target antibodies present in the sample. The array is capable of determining cytokines present in cell lysates, cell culture supernatants, plasma, serum, human milk, saliva, and urine or tissue lysates. The samples are diluted and added to a multidish containing the nitrocellulose membranes and are incubated overnight at 2-8 °C. Following this, the captured proteins are detected with biotinylated detection antibodies and are then visualised using chemiluminescent detection reagents. The amount of analyte bound to the membrane is proportional to the signal produced on a radiograph.

The nitrocellulose membranes were placed in separate wells of a multidish (number of the membrane facing upwards) and blocked with Array Buffer 6. The multidish was incubated for 1 hour at room temperature on a 3D rocking platform shaker (STR9 Stuart Scientific, UK). While the membranes were blocking, the serum samples were diluted in Array Buffer 6. After 1 hour, the Array Buffer 6 was aspirated from the wells of the multidish and the diluted serum samples were added to the membranes and incubated overnight at 2-8 °C, on a rocking platform shaker. The next day, the membranes were placed in separate petri dishes (Cellstar, Greiner Bio-One, Germany) and washed with 20 ml wash buffer solution for 10 minutes, and this was repeated twice for a total of three washes. Following this, 1.5 ml of Detection Antibody cocktail diluted in Array Buffer 4/6 was added to the multidish

containing the membranes and incubated for 1 hour at room temperature, on a rocking platform shaker. The wash step was repeated. Following the washing step, 2 ml of Streptavidin-HRP was added on to each membrane and incubated for 30 minutes at room temperature, on a rocking platform shaker. The wash step was repeated. Following the washing step, the membranes were removed from the petri dish and placed on a transparent plastic sheet and 1 ml of Chemi Reagent Mix was pipetted evenly over each membrane. The bottom plastic sheet was covered with a similar top sheet for 1 minute. Excess Chemi Reagent Mix was gently removed and the membranes within the sheet were placed inside a radiographic cassette for exposure.

The radiograph (Kodak, USA) of the membranes was scanned and the intensity of the membrane spots were analysed. Each cytokine binds to their specific spot on the membrane. The intensity of each spot was quantified using computer software GeneTools, Syngene and graphs were plotted to compare intensities of the detected cytokines.

2.15 Enzyme-linked immunosorbent assay

To measure MMP-9, B-cell activating factor (BAFF), resistin and ENA-78/CXCL5 levels in serum samples and MMP-9 and IL-8 in GCF samples, Human Quantikine ELISA developmental system (R&D Systems) was used. The assay employs the quantitative sandwich enzyme immunoassay principle. A monoclonal antibody which is specific for each biomarker has been pre-coated onto their individual microplates. The standards and samples are pipetted into the wells and any MMP-9, BAFF, resistin, ENA-78/CXCL5 or IL-8 present is bound by the immobilized antibody. The unbound substances are washed away, following which an enzyme-linked polyclonal antibody specific for each biomarker is added to each well. Following a wash step to remove any unbound antibody-enzyme reagent, substrate solution is added to each well and colour develops in proportion to the amount of MMP-9, BAFF, resistin, ENA-78/CXCL5 or IL-8 bound in the initial step. The colour development is stopped and the intensity of the colour present is measured.

All assays were performed at room temperature. The procedure for the Human Quantikine ELISA, involved the addition of 100 µl of Assay Diluent (specific for each biomarker) to each well followed by addition of the standards and diluted samples. The plate was incubated at room temperature either on the benchtop or on a horizontal orbital microplate shaker (0.12” orbit) set at 500±50 rpm (specific for each biomarker). Following this, the contents of each well were aspirated and washed with wash buffer, repeating the process three times for a

total of three or four washes (as indicated for each biomarker). Following the washing step, 200 µl of Human Conjugate (specific for each biomarker) was added to each well and the plate was incubated either on the benchtop or on a horizontal orbital microplate shaker (0.12" orbit) set at 500±50 rpm (specific for each biomarker). Following this, the washing step was repeated and 200 µl of Substrate Solution was added to each well and the plate incubated for 30 minutes at room temperature, on the benchtop. Care was taken to ensure the plate was protected from light. Lastly, 50 µl of Stop solution was added to each well and the colour in the well changed from blue to yellow. The optical density of each well was determined within 30 minutes, using a microplate reader set to 450 nm and 550 nm. Table 2.5 and 2.6 present the protocol for performing the Human Quantikine ELISA experiment for each biomarker for serum and GCF samples, respectively.

	MMP-9	**BAFF**	**Resistin**	**ENA-78/CXCL5**
Assay diluent	Assay diluent RD1-34	Assay diluent RD1-111	Assay diluent RD1-19	Assay Diluent RD1W
Dilution	100-fold	2-fold	5-fold	2-fold
Conjugate	Human MMP-9 Conjugate	Human BAFF Conjugate	Human resistin Conjugate	Human ENA-78 Conjugate
Calibrator diluent	Calibrator diluent RD5-10	Calibrator diluent RD6Q	Calibrator diluent RD5K	Calibrator diluent RD6-1
Amount of standards & samples	100 µl per well	50 µl per well	100 µl per well	50 µl per well
Incubation of standards & samples	2 hours on a horizontal orbital microplate shaker (0.12” orbit) set at 500±50 rpm	3 hours on a horizontal orbital microplate shaker (0.12” orbit) set at 500±50 rpm	2 hours on benchtop	2 hours on benchtop
Incubation of conjugate	1 hour on shaker	1 hour on shaker	2 hours on benchtop	2 hours on benchtop

Table 2.5: Protocol for Human Quantikine ELISA for biomarker levels in serum.

MMP-9; matrix metalloproteinase-9, BAFF; B-cell activating factor, ENA-78/CXCL5; epithelial neutrophil activating peptide-78.

	MMP-9	IL-8
Assay diluent	Assay diluent RD1-34	Assay diluent RD1-85
Conjugate	Human MMP-9 Conjugate	Human IL-8 Conjugate
Calibrator diluent	Calibrator diluent RD5-10	Calibrator diluent RD5P (diluted 1:5)
Amount of standards & samples	100 µl per well	50 µl per well
Incubation of standards & samples	2 hours on a horizontal orbital microplate shaker (0.12” orbit) set at 500±50 rpm	2 hours on benchtop
Incubation of conjugate	1 hour on shaker	1 hour on benchtop
Dilution	100-fold	None

Table 2.6: Protocol for Human Quantikine ELISA for biomarker levels in GCF.

MMP-9; matrix metalloproteinase-9 and IL-8; interleukin-8.

2.16 Assessment of quality of life

At baseline prior to any periodontal treatment, QoL in patients with T1DM was assessed using two validated measures routinely used to assess QoL in patients with diabetes, the W-BQ12 and the ADDQoL-19 questionnaire. Only the T1DM patients enrolled in the study were asked to complete both questionnaires, using manual-self complete mode of administration. For T1DM patients with healthy periodontal tissues and gingivitis their participation in the study was restricted to the baseline appointment where they were given OHI and oral prophylaxis. Only T1DM patients with periodontitis underwent NSM and were seen for follow-up and necessary treatment at months 3 and 6, and were once again asked to complete the both questionnaires.

2.16.1 The Well-being Questionnaire 12

The W-BQ12 consists of 12 questions divided into 3 subscales, asking questions related to an individual's negative well-being, energy levels and positive well-being (Table 2.7, Appendix A). The W-BQ12 can be scored as 3 subscales, each of 4 items or questions: negative well-being (item 1 to 4), energy (item 5 to 8) and positive well-being (item 9 to 12). All the negative well-being questions are negatively worded, all the positive well-being questions are positively worded, and, the energy subscale consists of 2 positive and 2 negative questions. The responses to each question are on a Likert response scale and range from score '0' to score '3'. Score '0' indicates that the item applied to the individual 'not at all' and score '3' indicates that the item applied to the individual 'all the time', over the past few weeks (Table 2.8). Scores of each subscale were calculated separately (Table 2.9). A higher score in each subscale indicates more of the mood described, indicating a greater sense of positive well-being, energy level and negative well-being, and from these subscale scores an overall general well-being score can be generated (Table 2.9). A higher overall general well-being score indicates a greater QoL.

The associations between W-BQ12 scores and clinical data (age, duration of diabetes, diabetic complications, HbA1c and mean PD) were assessed using Pearson's correlation coefficient (r), if both variables were normally distributed, or using Spearman's correlation coefficient (rho) if both variables were not normally distributed.

Negative Well-being	Energy	Positive Well-being
1. "I have crying spells or feel like it" 2. "I feel downhearted & blue" 3. "I feel afraid for no reason at all" 4. "I get upset easily or feel panicky"	5. "I feel energetic, active or vigorous" 6. "I feel dull or sluggish" 7. "I feel tired, worn out, used up or exhausted" 8. "I have been waking up feeling fresh & rested"	9. "I have been happy satisfied, or pleased with my personal life" 10. "I have lived the kind of life I wanted to" 11. "I have felt eager to tackle my daily tasks or make new decisions" 12. "I have felt I could easily handle or cope with any serious problem or major change in my life"

Table 2.7: The 12 items of the W-BQ12.

All the time			Not at all
3	2	1	0

Table 2.8: Likert scale responses to the W-BQ12.

Negative Well-being	Energy	Positive Well-being	General Well-being
Add all 4 items	6 + item 5 – item 6 – item 7 + item 8	Add all 4 items	12 – Negative well-being + Energy + Positive well-being

Table 2.9: Equation used to calculate the W-BQ12 scores.

2.16.2 The Audit of Diabetes Dependent Quality of Life-19

The ADDQoL-19 consists of 19 questions or domains and 2 overview items (Appendix B). Overview item 1 is a generic assessment of QoL and states, "*In general, my present quality of life is…*", and its response ranges from score '3' indicating 'excellent' to score '-3' indicating 'extremely bad' (Table 2.10). Overview item 2 assesses the impact of diabetes on QoL and states, "*If I did not have diabetes, my quality of life would be…*" and its response ranges from score '-3' indicating 'very much better' to score '1' indicating 'worse' (Table 2.11). The 2 overview items are scored separately.

The 19 domains of the ADDQoL include questions pertaining to the impact of diabetes on specific life aspects (Table 2.12), and ask the respondents to rate how their life would be if they did not have diabetes. Each question has two parts, the impact of the life aspect (impact rating) and the importance of that life aspect (importance rating). The responses to the 'impact rating', range from score '-3' indicating 'very much greater' to score '+1' indicating 'less' (Table 2.13). The responses to the 'importance rating', range from score '+3' indicating 'very important' to score '0' indicating 'not at all important' (Table 2.14).

In order to calculate the ADDQoL-19 scores, firstly, the responses of the 'impact rating' and the 'importance rating' are multiplied to obtain a 'weighted impact score'. Following this, an overall ADDQoL-19 score is generated by the sum of the 'weighted impact score' ratings of applicable domains divided by the number of applicable domains. The overall ADDQoL-19 score ranges from '-9' to '+3', where '-9' indicates the 'maximum negative impact of diabetes' and '+3' indicates the 'maximum positive impact of diabetes'. Lower scores reflect on a poorer QoL, whereas a score of '0' indicates that the individual's QoL is not affected by diabetes at all. Interpreting the overall ADDQoL-19 score further involves dividing the patients into 2 groups by using quartiles, the group of patients having an overall ADDQoL-19 score below the lower quartile were considered to have a poorer QoL compared to those having a score above the lower quartile. Such a cut off strategy was utilized previously in literature (Chung et al. 2012; Turk et al. 2013).

The associations between ADDQoL-19 scores and clinical data (age, duration of diabetes, diabetic complications, HbA1c and mean PD) were assessed using Pearson's correlation coefficient (r), if both variables were normally distributed, or using Spearman's correlation coefficient (rho) if both variables were not normally distributed.

Overview item 1: *In general, my present quality of life is….*						
Excellent	Very good	Good	Neither good nor bad	Bad	Very bad	Extremely bad
3	2	1	0	-1	-2	-3

Table 2.10: ADDQoL-19 overview item 1 and its responses.

Overview item 2: *If I did not have diabetes, my quality of life would be….*				
Very much better	Much better	A little better	The same	Worse
-3	-2	-1	0	1

Table 2.11: ADDQoL-19 overview item 2 and its responses.

1.	Leisure activities
2.	Working life
3.	Journeys
4.	Holidays
5.	Physical health
6.	Family life
7.	Friendship & social life
8.	Personal relationships
9.	Sex life
10.	Physical appearance
11.	Self-confidence
12.	Motivation
13.	People's reaction
14.	Feelings about the future
15.	Financial situation
16.	Living conditions
17.	Dependence on others
18.	Freedom to eat
19.	Freedom to drink

Table 2.12: ADDQoL-19 domains.

Impact rating	Very much greater	Much greater	A little greater	The same	Less
	-3	-2	-1	0	1

Table 2.13: Impact rating responses to the ADDQoL-19 domains.

Importance rating	Very important	Important	Somewhat important	Not at all important
	3	2	1	0

Table 2.14: Importance rating responses to the ADDQoL-19 domains.

3 Chapter 3. Investigation of the general and oral health status of patients with T1DM prior to non-surgical periodontal management

3.1 Introduction

Periodontitis and diabetes are both highly prevalent diseases, and the association between these two conditions has been well recognised by dental professionals for many years. A number of epidemiological studies have unequivocally confirmed diabetes as a major risk factor for periodontal disease, and that individuals with diabetes are at an approximately 2 to 3 fold increased risk of developing periodontitis compared to those without diabetes, particularly if their glycaemic control is poor (Khader et al. 2006; Mealey and Ocampo 2007; Chavarry et al. 2009). Individuals with diabetes are found to have more severe and extensive destruction of periodontal tissue compared to those free from diabetes (Thorstensson and Hugoson 1993; Sandberg et al. 2000). Longitudinal studies have demonstrated a higher incidence of progressive periodontal disease in patients with diabetes (Seppala et al. 1993; Firatli 1997; Taylor et al. 1998). Evidence to support the negative impact of periodontal disease on diabetes has also been established. Studies have reported the increased risk of developing diabetes-related complications such as cardiovascular problems, retinopathy, neuropathy and nephropathy where patients have advanced periodontal disease (Karjalainen et al. 1994; Thorstensson et al. 1996; Moore et al. 1998; Moore et al. 1999).

The severity of periodontal disease in patients with T1DM has not been consistently reported. A number of studies have demonstrated no significant differences in the periodontal status of patients with T1DM compared to non-diabetic subjects (Firatli 1997; Lalla et al. 2006b; Kaur et al. 2009), whereas other studies have demonstrated that T1DM patients have higher levels of periodontal tissue loss compared to non-diabetic subjects (Bridges et al. 1996; Firatli et al. 1996; Alpagot et al. 2001; Silvestre et al. 2009). Also, most studies have focused primarily on children and adolescents, and only a number of studies have focused on the prevalence and severity of periodontal disease in adults with T1DM.

The aim of this chapter is to report on the presentation and severity of periodontal disease and dental caries, oral hygiene status, oral health behaviour, and the associations between glycaemic control and periodontal health in adult patients with and without T1DM.

3.2 Results

3.2.1 Demographics

A total of 100 patients were recruited into this study, 57 T1DM patients and 43 non-T1DM patients. Demographic findings for the T1DM and non-T1DM patients are summarised in Table 3.1. Of note, no statistically significant differences were found between the two groups for gender, ethnicity, IMD rank, smoking status and pack years of smoking, (P>0.05). This demonstrates that the two groups were appropriately matched for gender, ethnicity and IMD, demonstrating that both groups were from similar ethnic origin, resided in similar areas and had similar lifestyles. However, the age differed significantly between the T1DM [median (IQR), 28.0 (23.0-32.5) years, range 18 to 35 years] and non-T1DM [40.0 (35.0-47.0) years, range 26 to 50 years] patients, suggesting that T1DM patients were from a younger age group compared to the non-T1DM patients.

With reference to smoking habits, no statistically significant differences were found for smoking status and pack years between T1DM and non-T1DM patients (P>0.05), suggesting that the two groups were appropriately balanced for smoking status. Current smokers were 21.1% in the T1DM group and 14% in the non-T1DM group. Also, 22.8% of T1DM and 30.2% of non-T1DM patients had a previous history of smoking. However a majority of the T1DM (56.1%) and non-T1DM (55.8%) patients were non-smokers and had never smoked previously. The demographic findings did highlight a difference between the two groups for diastolic BP, the non-T1DM group having significantly higher diastolic BP [80.0 (73.0-88.0) mmHg] compared to the T1DM group [74.0 (68.3-80.0) mmHg], (P<0.05). No statistically significant differences between the T1DM and non-T1DM groups were found for systolic BP, (P>0.05). With reference to BMI, no statistically significant differences were identified between the T1DM (mean±SD 26.3±4.66 kg/m^2) and non-T1DM (25.3±4.52 kg/m^2) patients, (P>0.05). However, while considering BMI category, the T1DM group contained a higher proportion of obese (21.1%) and morbidly obese (5.30%) patients compared to the non-T1DM group, of which 4.70% were either, obese and morbidly obese. These differences in proportions were not statistically significant, (P>0.05) (Table 3.1 and Figure 3.1).

Tables 3.2 and 3.3 summarize demographic data following further categorisation of the T1DM and non-T1DM patients based on periodontal diagnosis. When the demographic data of six categories [T1DM patients with healthy periodontal tissues (DH); T1DM patients with gingivitis (DG); T1DM patients with periodontitis (DP); non-T1DM patients with healthy

periodontal tissues (HH); non-T1DM patients with gingivitis (HG) and non-T1DM patients with periodontitis (HP)] were analysed, there were no statistically significant differences between groups for gender. Within the T1DM group, age differed significantly, the DP patients were significantly older [32.0 (27.0-34.0) years] compared to the DH patients [25.0 (19.5-28.0) years], ($P<0.05$). All the non-T1DM groups (HH, HG and HP) were significantly higher in age than the T1DM groups (DH, DG and DP), ($P<0.001$). Based on periodontal diagnosis no statistically significant differences were found for ethnicity and IMD rank, ($P>0.05$).

When considering smoking habits, within the T1DM group, the DP group contained a higher proportion of ex-smokers (42.1%) compared to the DG group (6.90%), whereas the DG group had a higher proportion of current smokers (27.6%) compared to the DP group (21.1%). Similar findings were found within the non-T1DM group, where the HP group had a significantly higher proportion of ex-smokers (47.1%) compared to the HG group (23.5%). There was a 17.6% prevalence of current smokers in both HG and HP groups. The DH and HH groups had no current smokers. No statistically significant differences were found between the T1DM and non-T1DM groups for pack years ($P>0.05$).

The systolic BP was significantly higher in DH patients [139 (126.8-148) mmHg] compared to the HH patients [120 (114.5–133) mmHg], ($P<0.05$) (Table 3.3). Within the non-T1DM group, the HG patients had significantly higher systolic BP [135 (126-150) mmHg] compared to the HH patients [120 (114.5-133) mmHg], ($P<0.05$). The diastolic BP was significantly higher in the HG patients [80.0 (75.0–88.0) mmHg] compared to the DG patients [74.0 (68.0-80.5) mmHg], ($P<0.05$). No statistically significant differences were found between groups for BMI and BMI category, ($P>0.05$).

	T1DM (n=57)	Non-T1DM (n=43)	*P*
Gender [n (%)]			
Male	28 (49.1)	20 (46.5)	NS
Female	29 (50.9)	23 (53.5)	
Age (years)	28.0 (23.0-32.5)	40.0 (35.0-47.0)	< 0.001
Ethnicity [n (%)]			
Caucasian	56 (98.2)	43 (100)	NS
Asian	1 (1.80)	-	
IMD rank	12621 (7095-23926.8)	20804 (8511-24491)	NS
Smoking status [n (%)]			
Current	12 (21.1)	6 (14.0)	
Ex	13 (22.8)	13 (30.2)	NS
Never	32 (56.1)	24 (55.8)	
Pack years	7.76 ± 6.49	9.97 ± 7.90	NS
Systolic BP (mmHg)	132.5 (121.3-144.8)	131 (119-140)	NS
Diastolic BP (mmHg)	74.0 (68.3-80.0)	80.0 (73.0-88.0)	< 0.05
BMI (kg/m^2)	26.3 ± 4.66	25.3 ± 4.52	NS
BMI category [n (%)]			
Underweight	2 (3.50)	4 (9.30)	
Normal weight	20 (35.1)	17 (39.5)	NS
Overweight	20 (35.1)	18 (41.9)	
Obese	12 (21.1)	2 (4.70)	
Morbidly obese	3 (5.30)	2 (4.70)	

Table 3.1: Demographic data comparing T1DM and non-T1DM patients.

Mean ± SD presented for parametric data, median (IQR) presented for non-parametric data and n (%) presented for discrete variables. P-values determined using chi-squared test for discrete variables; Mann-Whitney U tests for continuous non-parametric variables and Independent t-test for continuous parametric variables. *P* indicates significant difference between T1DM and non-T1DM patients. BP; blood pressure, BMI; body mass index, IMD; index of multiple deprivation, NS; not significant.

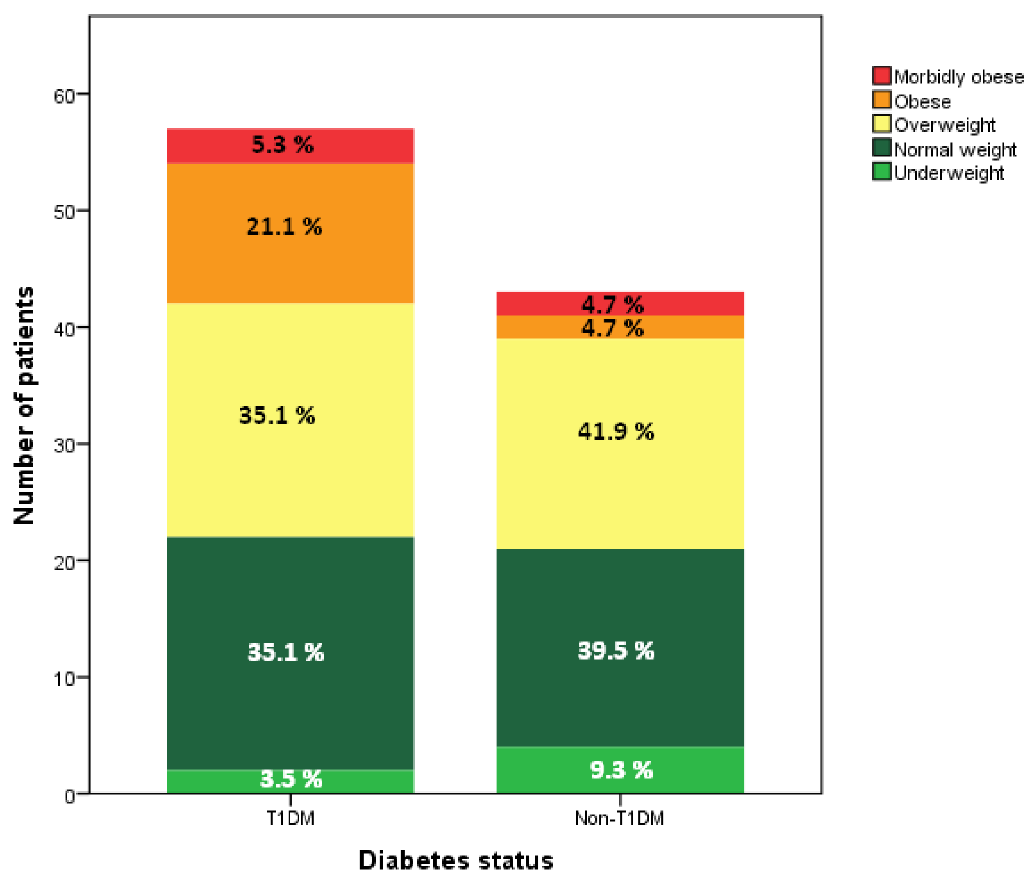
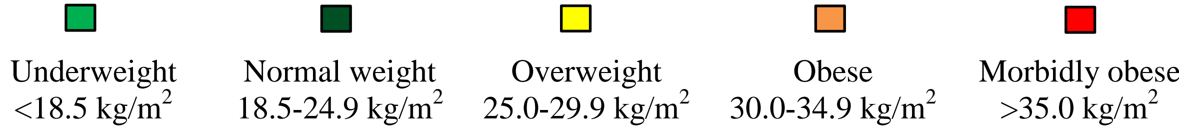


Figure 3.1: Categorization of T1DM and non-T1DM patients based on BMI.

T1DM patients (underweight n=2, normal weight n=20, overweight n=20, obese n=12, morbidly obese n=3) and non-T1DM patients (underweight n=4, normal weight n=17, overweight n=18, obese n=2, morbidly obese n=2).



		Health (DH n=9) (HH n=9)	Gingivitis (DG n=29) (HG n=17)	Periodontitis (DP n=19) (HP n=17)	*P**
Gender [n (%)]	**T1DM**				
	Male	5 (55.6)	15 (51.7)	8 (42.1)	NS
	Female	4 (44.4)	14 (48.3)	11 (57.9)	
	Non-T1DM				
	Male	1 (11.1)	11 (64.7)	8 (47.1)	NS
	Female	8 (88.9)	6 (35.3)	9 (52.9)	
	P	NS	NS	NS	
Age (years)	**T1DM**	25.0 (19.5-28.0)	28.0 (22.0-32.5)	32.0 (27.0-34.0) ***	< 0.01
	Non-T1DM	44.0 (39.0-46.5)	38.0 (34.5-45.5)	39.0 (34.5-47.0)	NS
	P	< 0.001	< 0.001	< 0.001	
Ethnicity [n (%)]	**T1DM**				
	Caucasian	9 (100)	29 (100)	18 (94.7)	NS
	Asian	-	-	1 (5.30)	
	Non-T1DM				
	Caucasian	9 (100)	17 (100)	17 (100)	NS
	Asian	-	-	-	
	P	NS	NS	NS	
IMD rank	**T1DM**	10362 (4602.5-26277)	14826.5 (9267.3-24299.8)	11188 (4987-22317)	NS
	Non-T1DM	17069 (11006.5-24979)	20804 (10438-25035)	21857 (7830.5-23202.5)	NS
	P	NS	NS	NS	
Smoking status [n (%)]	**T1DM**				
	Current	-	8 (27.6)	4 (21.1)	NS
	Ex	3 (33.3)	2 (6.90)	8 (42.1)	
	Never	6 (66.7)	19 (65.5)	7 (36.8)	
	Non-T1DM				
	Current	-	3 (17.6)	3 (17.6)	NS
	Ex	1 (11.1)	4 (23.5)	8 (47.1)	
	Never	8 (88.9)	10 (58.8)	6 (35.3)	
	P	NS	NS	NS	
Pack years	**T1DM**	3.58 ± 2.98	7.73 ± 6.42	8.83 ± 7.12	NS
	Non-T1DM	N/A	9.65 ± 9.74	9.44 ± 6.87	NS
	P	NS	NS	NS	

Table 3.2: Demographic data comparing T1DM and non-T1DM patients based on periodontal diagnosis.

Mean ± SD presented for parametric data, median (IQR) presented for non-parametric data and n (%) presented for discrete variables. P-values were determined using chi-squared test for discrete variables, Kruskal-Wallis test with Mann-Whitney U post hoc tests for continuous non-parametric variables and one-way ANOVA test with post-hoc independent t-test for continuous parametric variables. *P** indicates overall p-value comparing across periodontal categories within T1DM or non-T1DM groups. P-values under columns (*P*) relate to comparisons between T1DM and non-T1DM groups. ***P<0.001 indicates statistically significant differences compared to health within the T1DM group. DH; T1DM, periodontal health, DG; T1DM, gingivitis, DP; T1DM, periodontitis, HH; non-T1DM, periodontal health, HG; non-T1DM, gingivitis, HP; non-T1DM, periodontitis, IMD; index of multiple deprivation, NS; not significant.

		Health (DH n=9) (HH n=9)	Gingivitis (DG n=29) (HG n=17)	Periodontitis (DP n=19) (HP n=17)	*P**
Systolic BP (mmHg)	**T1DM**	139 (126.8-148)	133 (124-147.5)	125 (113-138)	NS
	Non-T1DM	120 (114.5-133)	135 (126-150) *	124 (118-143)	< 0.05
	P	< 0.05	NS	NS	
Diastolic BP (mmHg)	**T1DM**	79.0 (73.0- 88.5)	74.0 (68.0-80.5)	72.0 (66.0-80.0)	NS
	Non-T1DM	80.0 (70.0-82.0)	80.0 (75.0-88.0)	80.0 (69.0-89.5)	NS
	P	NS	< 0.05	NS	
BMI (kg/m^2)	**T1DM**	26.0 ± 4.75	26.0 ± 4.67	27.0 ± 4.80	NS
	Non-T1DM	24.9 ± 4.18	26.2 ± 4.42	24.7 ± 4.91	NS
	P	NS	NS	NS	
BMI category [n (%)]	**T1DM**				
	Underweight	0 (0.00)	1 (3.40)	1 (5.30)	
	Normal weight	4 (44.4)	11 (37.9)	5 (26.3)	
	Overweight	4 (44.4)	10 (34.5)	6 (31.6)	NS
	Obese	0 (0.00)	6 (20.7)	6 (31.6)	
	Morbidly obese	1 (11.1)	1 (3.40)	1 (5.30)	
	Non-T1DM				
	Underweight	0 (0.00)	2 (11.8)	2 (11.8)	
	Normal weight	6 (66.7)	4 (23.5)	7 (41.2)	
	Overweight	2 (22.2)	9 (52.9)	7 (41.2)	NS
	Obese	1 (11.1)	1 (5.90)	0 (0.00)	
	Morbidly obese	0 (0.00)	1 (5.90)	1 (5.90)	
	P	NS	NS	NS	

Table 3.3: Demographic data comparing T1DM and non-T1DM patients based on periodontal diagnosis.

Mean ± SD presented for parametric data, median (IQR) presented for non-parametric data and n (%) presented for discrete variables. P-values were determined using chi-squared test for discrete variables, Kruskal-Wallis test with Mann-Whitney U post hoc tests for continuous non-parametric variables and one-way ANOVA test with post-hoc independent t-test for continuous parametric variables. *P** indicates overall p-value comparing across periodontal categories within T1DM or non-T1DM groups. P-values under columns (*P*) relate to comparisons between T1DM and non-T1DM group. *$P<0.05$ indicates statistically significant differences compared to health within the non-T1DM group. DH; T1DM, periodontal health, DG; T1DM, gingivitis, DP; T1DM, periodontitis, HH; non-T1DM, periodontal health, HG; non-T1DM, gingivitis, HP; non-T1DM, periodontitis, BP; blood pressure, BMI; body mass index, NS; not significant.

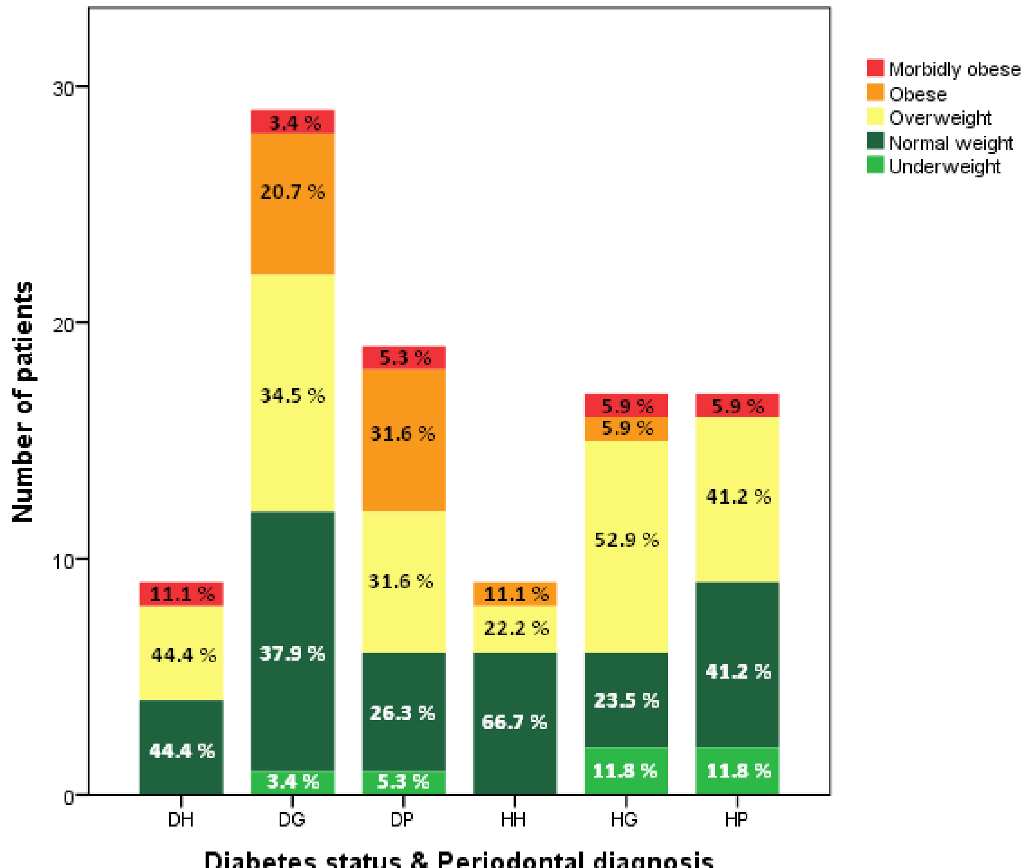
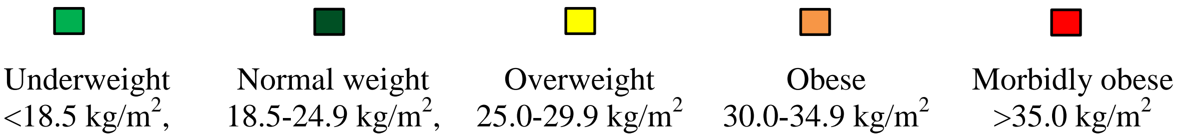


Figure 3.2: BMI category of T1DM and non-T1DM patients based on periodontal diagnosis.

DH patients (underweight n=0, normal weight n=4, overweight n=4, obese n=0, morbidly obese n=1), DG patients (underweight n=1, normal weight n=11, overweight n=10, obese n=6, morbidly obese n=1) DP patients (underweight n=1, normal weight n=5, overweight n=6, obese n=6, morbidly obese n=1), HH patients (underweight n=0, normal weight n=6, overweight n=2, obese n=1, morbidly obese n=0), HG patients (underweight n=2, normal weight n=4, overweight n=9, obese n=1, morbidly obese n=1) and HP patients (underweight n=2, normal weight n=7, overweight n=7, obese n=0, morbidly obese n=1). DH; T1DM, periodontal health, DG; T1DM, gingivitis, DP; T1DM, periodontitis, HH; non-T1DM, periodontal health, HG; non-T1DM, gingivitis, HP; non-T1DM, periodontitis.



Underweight	Normal weight	Overweight	Obese	Morbidly obese
<18.5 kg/m^2,	18.5-24.9 kg/m^2,	25.0-29.9 kg/m^2	30.0-34.9 kg/m^2	>35.0 kg/m^2

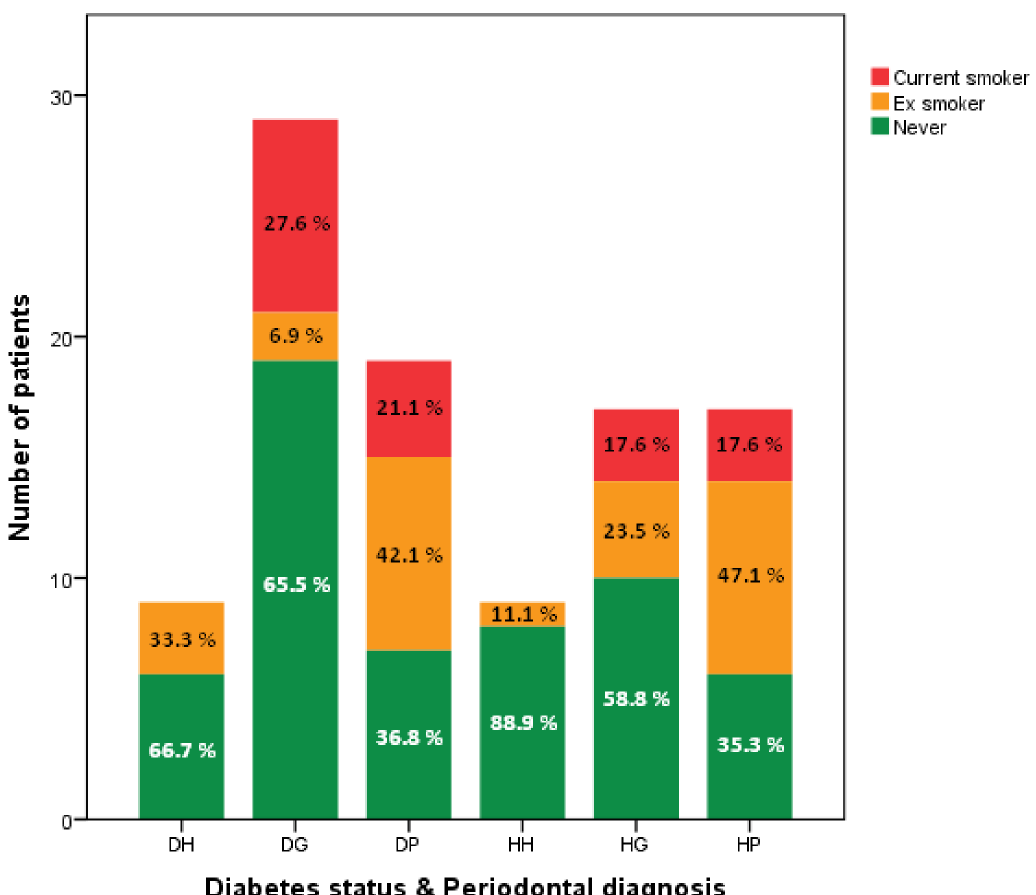
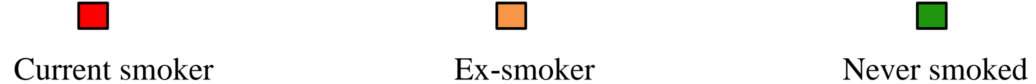


Figure 3.3: Smoking status of T1DM and non-T1DM patients based on periodontal diagnosis.

DH patients (current smoker n=0, ex- smoker n=3, never smoked n=6), DG patients (current smoker n=8, ex-smoker n=2, never smoked n=19), DP patients (current smoker n=4, ex-smoker n=8, never smoked n=7), HH patients (current smoker n=0, ex-smoker n=1, never smoked n=9), HG patients (current smoker n=3, ex-smoker n=4, never smoked n=10) and HP patients (current smoker n=3, ex-smoker n=8, never smoked n=6). DH; T1DM, periodontal health, DG; T1DM, gingivitis, DP; T1DM, periodontitis, HH; non-T1DM, periodontal health, HG; non-T1DM, gingivitis, HP; non-T1DM, periodontitis.



Current smoker Ex-smoker Never smoked

3.2.2 Diabetes care

Table 3.4 presents diabetes care data and demonstrates that of the 57 patients with T1DM, 52.6% gave a family history of diabetes, and the mean duration since diagnosis of T1DM was 14.0±7.47 years. With reference to method of diabetes control, a majority of patients (87.7%) controlled their diabetes by a combination of diet modification, physical exercise and drug therapy. Only a small proportion of patients (12.3%) were maintaining their glycaemic control with drug therapy alone. With reference to type of drug therapy used, a majority of patients (86%) were taking only insulin therapy and a smaller proportion of patients (14%) were taking a combination therapy of oral glucose lowering drugs, lipid lowering drugs and/or antihypertensive drugs in addition to insulin therapy.

With reference to diabetes-related complications, 22.8% of the T1DM patients presented with at least one self-reported diabetes complication, not including periodontitis. Of these, 5.3% reported having neuropathy, 3.50% reported having nephropathy, 8.80% reported having eye damage and 5.30% reported having all three of these microvascular complications. None of the patients reported having any macrovascular complication such as heart disease, stroke or peripheral vascular disease. With reference to screening and ruling out diabetes-related complications within the past 12 months, 5.30% of the T1DM patients had only their eyes screened and 94.7% received a combination of diabetes-related education and examination of their eyes and feet.

Based on glycaemic control category, a majority of the T1DM patients (n=24, 42.1%) were categorised as having poor glycaemic control (i.e. HbA1c >8.5 % / >69 mmol/mol), followed by 40.4% (n=23) of the T1DM patients having moderate glycaemic control (i.e. HbA1c 7.0-8.5 % / 53–69 mmol/mol) and only a small proportion of these patients [n (%) 6 (10.5%)] had good glycaemic control (i.e. HbA1c <7.0 % / <53 mmol/mol) (WHO 1999).

Table 3.5 and 3.6 presents diabetes care data following categorisation of the T1DM patients based on periodontal diagnosis. With reference to duration of diabetes, the DP patients presented with a significantly longer history of diabetes (17.5±8.32 years) compared to the DG (12.5±6.86 years) and the DH (11.7±5.12 years) patients, (*P*<0.05). With reference to diabetes management regimen, no statistically significant differences were found based on periodontal diagnosis, (*P*>0.05). All the DH patients managed their diabetes by a combination of diet modification, physical exercise and drug therapy, followed by 93.1% of

the DG and 73.7% of the DP patients who manged their diabetes by combination therapy. A lower proportion of the DP patients (26.3%) followed by the DG patients (6.90%) managed their diabetes with drug therapy alone. With reference to type of drug therapy used, no statistically significant differences were found based on periodontal diagnosis, ($P>0.05$). All the DH patients were taking insulin therapy only, followed by 84.2% of the DP and 82.8% of the DG patients who were on insulin therapy only. A lower proportion of the DG (17.2%) and DP (15.8%) patients were taking a combination therapy of oral glucose lowering drugs, lipid lowering drugs and/or antihypertensive drugs in addition to insulin therapy. With reference to glycaemic control category, no statistically significant differences were found based on periodontal diagnosis, ($P>0.05$). Interestingly, a majority of the DP patients (n=10, 52.6%) had poor glycaemic control whereas a majority of the DG (n=13, 44.8%) and the DH (n=5, 55.6%) patients had moderate glycaemic control.

With reference to diabetes-related complications, no statistically significant differences were found based on periodontal diagnosis, ($P>0.05$). Only 2 DH patients reported having neuropathy. Within the DG group, 2 patients reported having neuropathy, 1 patient reported having eye damage and 2 patients reported having a combination of these complications. Within the DP group, 1 patient reported having neuropathy, 2 patients reported having nephropathy, 2 patients reported having eye damage and 1 patient reported having a combination all three microvascular complications. With reference to screening for diabetes complications, no statistically significant differences were found based on periodontal diagnosis ($P>0.05$). A majority of the DH (88.9%), DG (96.6%) and DP (94.7%) patients had received a combination of examination of eyes and feet, and were given diabetes-related education or information within the last 12 months. Only a smaller proportion of these patients received examination of their eyes only.

	T1DM (n=57)
History of diabetes (years)	14.0 ± 7.50
Family history [n (%)]	30 (52.6)
Methods of diabetes control [n (%)]	
Drug therapy only	7 (12.3)
Combination: Diet/ Physical exercise/ Drug therapy	50 (87.7)
Current drug therapy [n (%)]	49 (86.0)
Insulin	8 (14.0)
Combination: Oral glucose lowering drug/ Insulin/ Lipid lowering drug/ Anti-hypertensive/ Other	
Glycaemic control categories [n (%)]	
Good (<7.0%)	6 (10.5)
Moderate (7.0-8.5%)	23 (40.4)
Poor (>8.5%)	24 (42.1)
Patient perception of glycaemic control [n (%)]	
Good	15 (26.3)
Moderate	36 (63.2)
Poor	6 (10.5)
Diabetes complications	
Microvascular complications [n (%)]	
None	44 (77.2)
Neuropathy	3 (5.30)
Nephropathy	2 (3.50)
Eye damage	5 (8.80)
Combination	3 (5.30)
Macrovascular complications [n (%)]	
None	57 (100)
Heart disease/ Stroke/ Peripheral vascular disease/ Combination	0 (0.00)
Screening for complications [n (%)]	
Eye screened	3 (5.30)
Combination: Eyes/ Education given/ Foot examined	54 (94.7)

Table 3.4: Diabetes care data for T1DM patients.

Mean ± SD presented for parametric data and n (%) presented for discrete variables. For glycaemic control categories: T1DM n=53.

T1DM patients (n=57)	Health (n=9)	Gingivitis (n=29)	Periodontitis (n=19)	*P**
History of diabetes (years)	11.7 ± 5.12	12.5 ± 6.86	17.5 ± 8.32 *, †	< 0.05
Family history [n (%)]	5 (55.6)	15 (51.7)	10 (52.6)	NS
Methods of diabetes control [n (%)]				
Drug therapy only	0 (0.00)	2 (6.90)	5 (26.3)	NS
Combination: Diet/ Physical exercise/ Drug therapy	9 (100)	27 (93.1)	14 (73.7)	
Current drug therapy [n (%)]				
Insulin	9 (100)	24 (82.8)	16 (84.2)	NS
Combination: Oral glucose lowering drug/ Insulin/ Lipid lowering drug/ Anti-hypertensive/ Other	0 (0.00)	5 (17.2)	3 (15.8)	
Glycaemic control categories [n (%)]				
Good (<7.0%)	1 (11.1)	2 (6.90)	3 (15.8)	NS
Moderate (7.0-8.5%)	5 (55.6)	13 (44.8)	5 (26.3)	
Poor (>8.5%)	3 (33.3)	11 (37.9)	10 (52.6)	
Patient perception of glycaemic control [n (%)]				
Good	3 (33.3)	7 (24.1)	5 (26.3)	NS
Moderate	5 (55.6)	21 (72.4)	10 (52.6)	
Poor	1 (11.1)	1 (3.40)	4 (21.1)	

Table 3.5: Diabetes care data for T1DM patients based on periodontal diagnosis.

Mean ± SD presented for parametric data and n (%) presented for discrete variables. P-values determined using chi-square test for discrete variables, one way ANOVA test with post-hoc independent t-test for continuous parametric variables. *$P<0.05$ indicates statistically significant differences compared to health and †$P<0.05$ indicates statistically significant differences compared to gingivitis within the T1DM group. *P** indicates overall p-value comparing across periodontal categories within the T1DM group. NS; not significant.

T1DM patients (n=57)	Health (n=9)	Gingivitis (n=29)	Periodontitis (n=19)	*P**
Diabetes complications				
Microvascular complications [n (%)]				
None	7 (77.8)	24 (82.8)	13 (68.4)	
Neuropathy	2 (22.2)	2 (6.90)	1 (5.30)	
Nephropathy	0 (0.00)	0 (0.00)	2 (10.5)	NS
Eye damage	0 (0.00)	1 (3.40)	2 (10.5)	
Combination	0 (0.00)	2 (6.90)	1 (5.30)	
Macrovascular complications [n (%)]				
None	9 (100)	29 (100)	19 (100)	NS
Heart disease/ Stroke/ Peripheral vascular disease/ Combination	0 (0.00)	0 (0.00)	0 (0.00)	
Screening for complications [n (%)]				
Eye screened	1 (11.1)	1 (3.40)	1 (5.30)	NS
Combination: Eyes/ Education given/ Foot examined	8 (88.9)	28 (96.6)	18 (94.7)	

Table 3.6: Diabetes care data for T1DM patients based on periodontal diagnosis.

N (%) presented for discrete variables. P-values determined using chi-square test for discrete variables. *P** indicates overall p-value comparing across periodontal categories within the T1DM group. NS; not significant.

3.2.3 Oral and dental

Oral and dental findings comparing T1DM and non-T1DM patients are presented in Table 3.7. When comparing the two groups, significant differences were found relating to the number of teeth present and the number of sound and unrestored teeth. The number of teeth present was significantly higher in T1DM patients (27.5±2.78) compared to the non-T1DM patients (26.0±2.91), (*P*<0.01). Also the T1DM patients had a significantly higher number of sound and unrestored teeth (22.5±6.84) compared to the non-T1DM patients (18.8±5.62), (*P*<0.01). Additionally, the T1DM patients had a significantly lower number of restored teeth (1–3 surfaces) (3.33±3.34 and n=40, 70.2%) compared to the non-T1DM patients (6.19±4.14 and n=39, 90.7%), (*P*<0.001).

No statistically significant differences were found between the two groups for abnormal soft tissue findings, dry mouth (clinically assessed or patient reported), pain in the past one month, removable prosthesis, teeth with restoration including 4 or more surfaces, teeth with caries into dentine, broken down teeth with pulpal involvement, endodontically treated teeth and teeth with periapical radiolucencies, (*P*>0.05).

Table 3.8 and 3.9 presents oral and dental data following further categorisation of T1DM and non-T1DM patients based on periodontal diagnosis. With reference to the number of teeth present, the DH patients had a significantly greater number of teeth present (29.1±1.83) compared to the HH patients (27.0±1.87), (*P*<0.05). Also the DG patients had a significantly higher number of teeth present (28.2±2.39) compared to the HG patients (24.2±2.83), (*P*<0.001). Within the T1DM group, a significantly higher number of teeth was present in the DH (29.1±1.83) and the DG (28.2±2.39) patients compared to the DP patients (25.7±2.88), (*P*<0.01). Within the non-T1DM group, the HH (27.0±1.87) and the HP (27.2±2.59) patients had a significantly higher number of teeth compared to the HG patients (24.2±2.83), (*P*<0.05 and *P*<0.01, respectively). With reference to the number of sound and unrestored teeth, the DH patients had significantly more sound teeth compared to the HH patients (19.9±5.23), (*P*<0.01). Also the DG patients had significantly more sound teeth (23.1±7.02) compared to the HG patients (16.0±5.49), (*P*<0.001). Within the T1DM group, the DH (27.7±2.60) and the DG (23.1±7.02) patients had significantly more sound teeth compared to the DP patients (19.2±6.33), (*P*<0.001 and *P*<0.05 respectively). However, within the non-T1DM group, the HP patients had significantly more sound teeth (21.0±5.00) compared to the HG patients (16.0±5.49), (*P*<0.01).

With reference to the number of restored teeth (1-3 surfaces), the HH patients had significantly more restored teeth (5.78±4.92 and n=8, 88.9%) compared to the DH patients (1.44±1.74 and n=5, 55.6%), (*P*<0.05). Similarly, the HG patients had a significantly greater number of restored teeth (7.18±4.45 and n=15, 88.2%) compared to the DG patients (3.45±3.57 and n=20, 69%), (*P*<0.01). Within the T1DM group, the DG (3.45±3.57 and n=20, 69%) and the DP (4.05±3.34 and n=15, 78.9%) patients had a significantly higher number of restored teeth compared to the DH patients (1.44±1.74 and n=5, 55.6%), (*P*<0.05 and *P*<0.01 respectively).

Of note, no other statistically significant differences for oral and dental data were found between the two groups based on periodontal diagnosis.

	T1DM (n=57)	Non-T1DM (n=43)	*P*
Abnormal soft tissue findings [n (%)]	2 (3.50)	2 (4.70)	NS
Clinician assessed dry mouth [n (%)]	0 (0.00)	1 (2.30)	NS
Patient reported dry mouth [n (%)]	2 (3.50)	2 (4.70)	NS
Pain in previous month [n (%)]	8 (14.0)	4 (9.30)	NS
Removable prosthesis [n (%)]			
None	54 (94.7)	41 (95.3)	NS
Acrylic	3 (5.30)	2 (4.70)	
Chrome	0 (0.00)	0 (0.00)	
Teeth present	27.5 ± 2.78	26.0 ± 2.91	< 0.01
Sound and unrestored teeth	22.5 ± 6.84	18.8 ± 5.62	< 0.01
Restored teeth (1-3 surfaces)	3.33 ± 3.34	6.19 ± 4.14	< 0.001
[n (%) with at least one]	40 (70.2%)	39 (90.7%)	
Restored teeth (+4 surfaces)	0.65 ± 2.86	0.63 ± 1.54	NS
[n (%) with at least one]	9 (15.8%)	11 (25.6%)	
Teeth with caries into dentine	0.75 ± 1.87	0.26 ± 0.93	NS
[n (%) with at least one]	13 (22.8%)	4 (9.30%)	
Broken down teeth	0.23 ± 1.00	0.23 ± 0.15	NS
[n (%) with at least one]	5 (8.80%)	1 (2.30%)	
Endodontically treated teeth	0.02 ± 0.13	0.07 ± 0.34	NS
[n (%) with at least one]	1 (1.80%)	2 (4.60%)	
Periapical radiolucencies	0.19 ± 0.74	0.05 ± 0.21	NS
[n (%) with at least one]	6 (10.5%)	2 (4.70%)	

Table 3.7: Oral and dental data comparing T1DM and non-T1DM patients.

Mean ± SD presented for parametric data and n (%) presented for discrete variables. P-values determined using Independent t-test for continuous parametric variables and chi-squared test for discrete variables. *P* indicates significant difference between T1DM and non-T1DM patients. NS; not significant.

		Health (DH n=9) (HH n=9)	Gingivitis (DG n=29) (HG n=17)	Periodontitis (DP n=19) (HP n=17)	*P**
Abnormal soft tissue findings [n (%)]	**T1DM**	0 (0.00)	2 (6.90)	0 (0.00)	NS
	Non-T1DM	0 (0.00)	1 (5.90)	1 (5.90)	NS
	P	NS	NS	NS	
Clinician assessed dry mouth [n (%)]	**T1DM**	0 (0.00)	0 (0.00)	0 (0.00)	NS
	Non-T1DM	0 (0.00)	1 (5.90)	0 (0.00)	NS
	P	NS	NS	NS	
Patient reported dry mouth [n (%)]	**T1DM**	0 (0.00)	2 (6.90)	0 (0.00)	NS
	Non-T1DM	0 (0.00)	1 (5.90)	1 (5.90)	NS
	P	NS	NS	NS	
Pain in previous month [n (%)]	**T1DM**	0 (0.00)	4 (13.8)	4 (21.1)	NS
	Non-T1DM	0 (0.00)	2 (11.8)	2 (11.8)	NS
	P	NS	NS	NS	
Removable prosthesis [n (%)]	**T1DM**				NS
	None	9 (100)	29 (100)	16 (84.2) †	
	Acrylic	0 (0.00)	0 (0.00)	3 (15.8)	
	Chrome	0 (0.00)	0 (0.00)	0 (0.00)	
	Non-T1DM				NS
	None	8 (88.9)	16 (94.1)	17 (100)	
	Acrylic	1 (11.1)	1 (5.90)	0 (0.00)	
	Chrome	0 (0.00)	0 (0.00)	0 (0.00)	
	P	NS	NS	NS	
Teeth present	**T1DM**	29.1 ± 1.83	28.2 ± 2.39	25.7 ± 2.88 **, ††	< 0.001
	Non-T1DM	27.0 ± 1.87	24.2 ± 2.83 *	27.2 ± 2.59 ††	< 0.01
	P	< 0.05	< 0.001	NS	
Sound & unrestored teeth	**T1DM**	27.7 ± 2.60	23.1 ± 7.02	19.2 ± 6.33 ***, †	< 0.01
	Non-T1DM	19.9 ± 5.23	16.0 ± 5.49	21.0 ± 5.00 ††	< 0.05
	P	< 0.01	< 0.001	NS	

Table 3.8: Oral and dental data comparing T1DM and non-T1DM patients based on periodontal diagnosis.

Mean ± SD presented for parametric data and n (%) presented for discrete variables. P-values determined using chi-squared test for discrete variables, and One way ANOVA test with post-hoc Independent t-test for continuous parametric variables. *P** indicates overall p-value comparing across periodontal categories within T1DM or non-T1DM groups. P-values under columns (*P*) relate to comparisons between T1DM and non-T1DM groups. *$P<0.05$, **$P<0.01$, ***$P<0.001$ indicates statistically significant differences compared to health and †$P<0.05$, ††$P<0.01$ indicates statistically significant differences compared to gingivitis within the T1DM and non-T1DM groups. DH; T1DM, periodontal health, DG; T1DM, gingivitis, DP; T1DM, periodontitis, HH; non-T1DM, periodontal health, HG; non-T1DM, gingivitis, HP; non-T1DM, periodontitis, NS; not significant.

		Health (DH n=9) (HH n=9)	Gingivitis (DG n=29) (HG n=17)	Periodontitis (DP n=19) (HP n=17)	*P**
Restored teeth (1-3 surfaces) [n (%) with at least one]	**T1DM**	1.44 ± 1.74 5 (55.6)	3.45 ± 3.57 * 20 (69.0)	4.05 ± 3.34 ** 15 (78.9)	NS
	Non-T1DM	5.78 ± 4.92 8 (88.9)	7.18 ± 4.45 15 (88.2)	5.41 ± 3.36 16 (94.1)	NS
	P	< 0.05	< 0.01	NS	
Restored teeth (+4 surfaces) [n (%) with at least one]	**T1DM**	- 0 (0.00)	1.00 ± 3.92 5 (17.2)	0.42 ± 1.01 4 (21.1)	NS
	Non-T1DM	1.33 ± 2.69 3 (33.3)	0.29 ± 0.59 4 (23.5)	0.59 ± 1.37 4 (23.5)	NS
	P	NS	NS	NS	
Teeth with caries into dentine [n (%) with at least one]	**T1DM**	- 0 (0.00)	0.52 ± 1.45 5 (17.2)	1.47 ± 2.59 * 8 (42.1)	NS
	Non-T1DM	- 0 (0.00)	0.41 ± 1.28 2 (11.8)	0.24 ± 0.75 2 (11.8)	NS
	P	NS	NS	NS	
Broken down teeth [n (%) with at least one]	**T1DM**	- 0 (0.00)	0.10 ± 0.41 2 (6.90)	0.53 ± 1.65 3 (15.8)	NS
	Non-T1DM	- 0 (0.00)	0.06 ± 0.24 1 (5.90)	- 0 (0.00)	NS
	P	NS	NS	NS	
Endodontically treated teeth [n (%) with at least one]	**T1DM**	- 0 (0.00)	0.03 ± 0.19 1 (3.40)	- 0 (0.00)	NS
	Non-T1DM	- 0 (0.00)	- 0 (0.00)	0.18 ± 0.53 2 (11.8)	NS
	P	NS	NS	NS	
Periapical radiolucencies [n (%) with at least one]	**T1DM**	- 0 (0.00)	0.07 ± 0.26 2 (6.9)	0.47 ± 1.22 4 (21.1)	NS
	Non-T1DM	- 0 (0.00)	- 0 (0.00)	0.12 ± 0.33 2 (11.8)	NS
	P	NS	NS	NS	

Table 3.9: Oral and dental data comparing T1DM and non-T1DM patients based on periodontal diagnosis.

Mean ± SD presented for parametric data. P-values were determined using One way ANOVA test with post-hoc Independent t-test for continuous parametric variables. *P** indicates overall p-value comparing across periodontal categories within T1DM or non-T1DM groups. P-values under columns relate to comparisons between T1DM and non-T1DM groups. **P*<0.05, ***P*<0.01 indicates statistically significant differences compared to health within the T1DM group. DH; T1DM, periodontal health, DG; T1DM, gingivitis, DP; T1DM, periodontitis, HH; non-T1DM, periodontal health, HG; non-T1DM, gingivitis, HP; non-T1DM, periodontitis, NS; not significant.

3.2.4 Oral health behaviour

Table 3.10 summarises oral health behaviour data for T1DM and non-T1DM patients. Of note, when comparing the two groups, no statistically significant differences were found in the proportions of patients for all the oral health behaviours (attends GDP regularly, attended GDP within the last 12 months, reason for last visit at the GDP, and frequency of tooth brushing), except for frequency of interproximal teeth cleaning. A significantly higher proportion of T1DM patients (n=40, 70.2%) reported to have never performed interproximal teeth cleaning compared to non-T1DM patients (n=15, 34.9%), (P<0.001). On the other hand, a significantly higher proportion of the non-T1DM patients reported performing interproximal teeth cleaning once per week (n=8, 18.6%) and ≥ three times per week (n=20, 46.5%) compared to the T1DM patients (once per week: n=7, 12.3%; and ≥ three times per week: n=8, 14%), respectively, (P<0.001).

Table 3.11, Figures 3.4, 3.5 and 3.6 summarize oral health behaviour data comparing T1DM and non-T1DM patients based on periodontal diagnosis. Within the non-T1DM group, a significantly higher proportion of the HP patients (n=15, 88.2%) reported to regularly visit their GDP compared to the HG patients (n=8, 47%), (P<0.05). With reference to self-reported interproximal teeth cleaning, a significantly higher proportion of the DH patients reported to have never performed or were not currently performing interproximal teeth cleaning (n=5, 55.6%) or carried out interproximal cleaning once per week (n=3, 33.3%) compared to HH patients (n=1, 11.1%) and (n=2, 22.2%) respectively, (P<0.05). On the other hand, a significantly higher proportion of the HH patients (n=6, 66.7%) reported performing interproximal teeth cleaning ≥ three times per week compared to the DH patients (n=1, 11.1%), (P<0.05). Within non-T1DM group, a significantly higher proportion of the HG patients reported to never have performed interproximal teeth cleaning (n=10, 58.8%) or carried out interproximal cleaning once per week (n=4, 23.5%) compared to the HH patients (n=1, 11.1%) and (n=2, 22.2%) respectively, (P<0.05). Interestingly a higher proportion of the HP patients reported performing interproximal teeth cleaning ≥ three times per week (n=11, 64.7%) compared to the HG patients (n=3, 17.6%), (P<0.05). Conversely, a significantly higher proportion of HG patients reported to have never performed interproximal teeth cleaning (n=10, 58.8%) or carried out interproximal cleaning once per week (n=4, 23.5%) compared to the HP patients (n=4, 23.5%) and (n=2, 11.8%) respectively, (P<0.05).

	T1DM (n=57)	**Non-T1DM (n=43)**	***P***
Attends GDP regularly [n (%)]	39 (68.4)	29 (67.4)	NS
Attended GDP within 12 months [n (%)]	43 (75.4)	33 (76.7)	NS
Reason for last visit [n (%)]			
N/A	12 (21.1)	6 (14.0)	
Check-up	27 (47.4)	28 (65.1)	
Emergency	8 (14.0)	2 (4.70)	NS
Restoration	5 (8.80)	2 (4.70)	
Periodontal therapy	1 (1.80)	0 (0.00)	
Other	2 (3.50)	1 (2.30)	
Frequency of tooth brushing [n (%)]			
<1/day	2 (3.50)	1 (2.30)	
1/day	11 (19.3)	2 (4.70)	
2/day	38 (66.7)	36 (83.7)	NS
>2/day	4 (7.00)	4 (9.30)	
Frequency of interproximal cleaning [n (%)]			
Never	40 (70.2)	15 (34.9)	
1/week	7 (12.3)	8 (18.6)	< 0.001
>3/week	8 (14.0)	20 (46.5)	

Table 3.10: Oral health behaviour data comparing T1DM and non-T1DM patients.

N (%) presented for discrete variables. P-values were determined using chi-squared test for discrete variables. *P* indicates significant difference between T1DM and non-T1DM patients. GDP; general dental practitioner, N/A; not applicable, NS; not significant.

		Health (DH n=9) (HH n=9)	Gingivitis (DG n=29) (HG n=17)	Periodontitis (DP n=19) (HP n=17)	*P**
Attends GDP regularly [n (%)]	**T1DM**	6 (66.7)	19 (65.5)	14 (73.7)	NS
	Non-T1DM	6 (66.7)	8 (47.1)	15 (88.2) †	NS
	P	NS	NS	NS	
Attended GDP within 12 months [n (%)]	**T1DM**	6 (66.7)	21 (72.4)	16 (84.2)	NS
	Non-T1DM	7 (77.8)	11 (64.7)	15 (88.2)	NS
	P	NS	NS	NS	
Reason for last visit [n (%)]	**T1DM**				
	N/A	3 (33.3)	6 (20.7)	3 (15.8)	
	Check-up	5 (55.6)	13 (44.8)	9 (47.4)	
	Emergency	0 (0.00)	4 (13.8)	4 (21.1)	NS
	Restoration	1 (11.1)	3 (10.3)	1 (5.30)	
	Periodontal therapy	0 (0.00)	0 (0.00)	1 (5.30)	
	Other	0 (0.00)	1 (3.40)	1 (5.30)	
	Non-T1DM				
	N/A	1 (11.1)	4 (23.5)	1 (5.90)	
	Check-up	7 (77.8)	5 (29.4)	16 (94.1)	
	Emergency	0 (0.00)	2 (11.8)	0 (0.00)	NS
	Restoration	0 (0.00)	2 (11.8)	0 (0.00)	
	Periodontal therapy	0 (0.00)	0 (0.00)	0 (0.00)	
	Other	0 (0.00)	1 (5.90)	0 (0.00)	
	P	NS	NS	NS	
Frequency of tooth brushing [n (%)]	**T1DM**				
	<1/day	0 (0.00)	0 (0.00)	2 (10.5)	
	1/day	1 (11.1)	7 (24.1)	3 (15.8)	NS
	2/day	8 (88.9)	17 (58.6)	13 (68.4)	
	>2/day	0 (0.00)	3 (10.3)	1 (5.30)	
	Non-T1DM				
	<1/day	1 (11.1)	0 (0.00)	0 (0.00)	
	1/day	0 (0.00)	1 (5.90)	1 (5.90)	NS
	2/day	7 (77.8)	16 (94.1)	13 (76.5)	
	>2/day	1 (11.1)	0 (0.00)	3 (17.6)	
	P	NS	NS	NS	
Frequency of interproximal cleaning [n (%)]	**T1DM**				
	Never	5 (55.6)	23 (79.3)	12 (63.2)	
	1/week	3 (33.3)	2 (6.90)	2 (10.5)	NS
	>3/week	1 (11.1)	2 (6.90)	5 (26.3)	
	Non-T1DM				
	Never	1 (11.1)	10 (58.8) *	4 (23.5) †	NS
	1/week	2 (22.2)	4 (23.5) *	2 (11.8) †	
	>3/week	6 (66.7)	3 (17.6) *	11 (64.7) †	
	P	< 0.05	NS	NS	

Table 3.11: Oral health behaviour data comparing T1DM and non-T1DM patients based on periodontal diagnosis.

N (%) presented for discrete variables. P-values were determined using chi-squared test for discrete variables. *P** indicates overall p-value comparing across periodontal categories within T1DM or non-T1DM groups. P-values under columns relate to comparisons between T1DM and non-T1DM groups. *$P<0.05$ indicates statistically significant differences compared to health and †$P<0.05$ indicates statistically significant differences compared to gingivitis within the non-T1DM group. DH; T1DM, periodontal health, DG; T1DM, gingivitis, DP; T1DM, periodontitis, HH; non-T1DM, periodontal health, HG; non-T1DM, gingivitis, HP; non-T1DM, periodontitis, GDP; general dental practitioner, N/A; not applicable, NS; not significant.

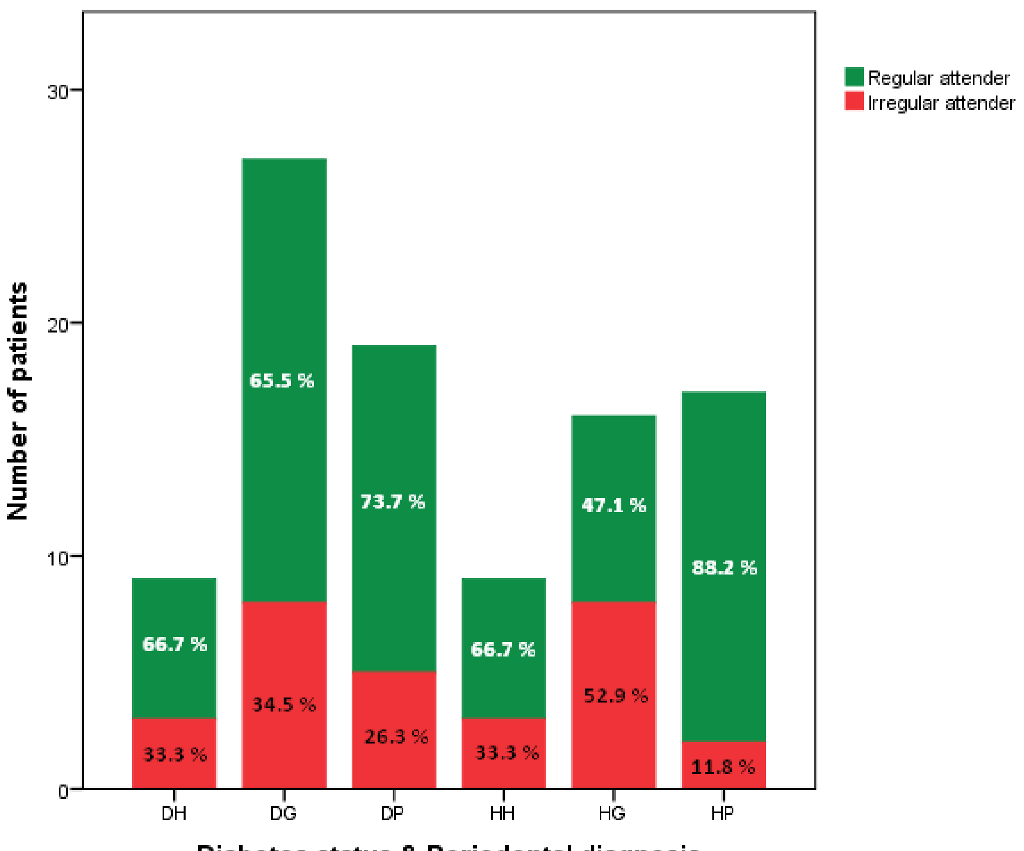


Figure 3.4: Attendance at GDP of T1DM and non-T1DM patients based on periodontal diagnosis.

DH patients (regular attender n=6, irregular attender n=3), DG patients (regular attender n=19, irregular attender n=10), DP patients (regular attender n=14, irregular attender n=5), HH patients (regular attender n=6, irregular attender n=3), HG patients (regular attender n=8, irregular attender n=9) and HP patients (regular attender n=15, irregular attender n=2). DH; T1DM, periodontal health, DG; T1DM, gingivitis, DP; T1DM, periodontitis, HH; non-T1DM, periodontal health, HG; non-T1DM, gingivitis, HP; non-T1DM, periodontitis.

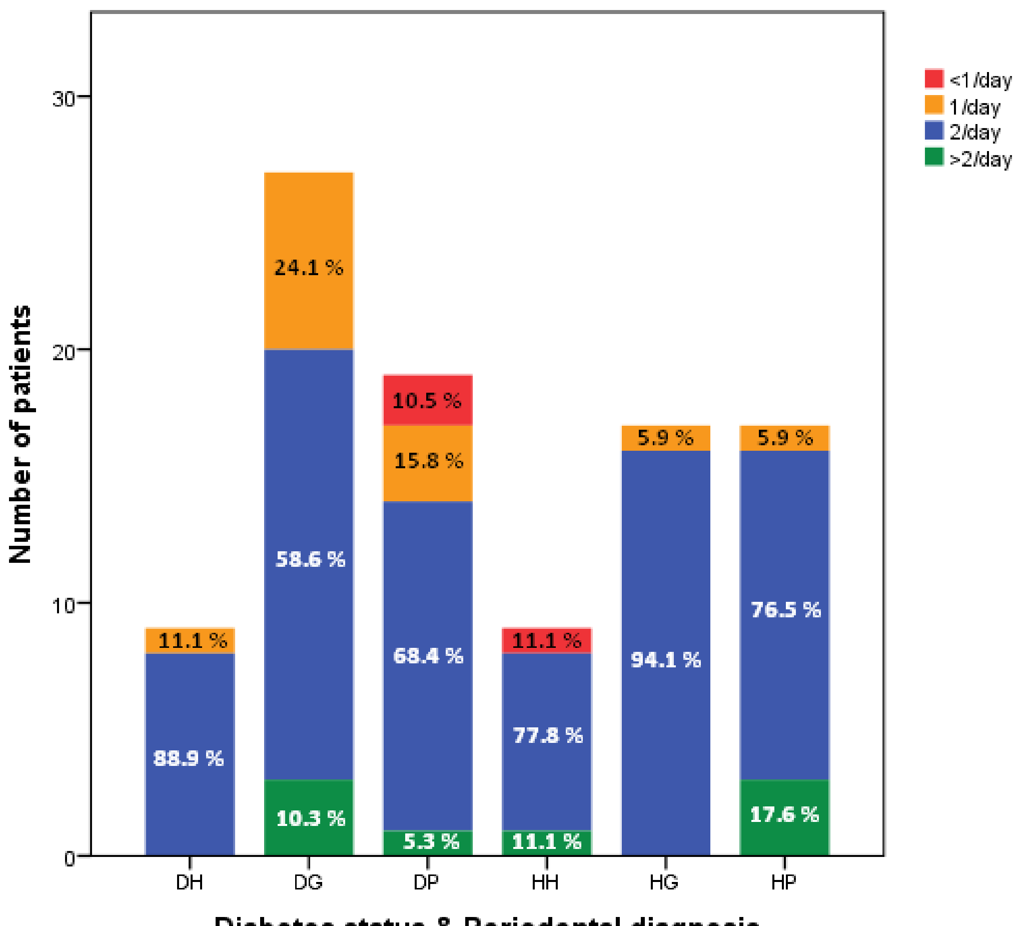
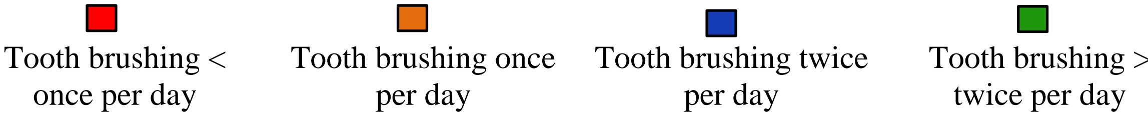


Figure 3.5: Frequency of tooth brushing of T1DM and non-T1DM patients based on periodontal diagnosis.

DH patients (< once per day n=0, once per day n=1, twice per day n=8, > twice per day n=0), DG patients (< once per day n=0, once per day n=7, twice per day n=17, > twice per day n=3), DP patients (< once per day n=2, once per day n=3, twice per day n=13, > twice per day n=1), HH patients (< once per day n=1, once per day n=0, twice per day n=7, > twice per day n=1), HG patients (< once per day n=0, once per day n=1, twice per day n=16, > twice per day n=0) and HP patients (< once per day n=0, once per day n=1, twice per day n=13, > twice per day n=3). DH; T1DM, periodontal health, DG; T1DM, gingivitis, DP; T1DM, periodontitis, HH; non-T1DM, periodontal health, HG; non-T1DM, gingivitis, HP; non-T1DM, periodontitis.



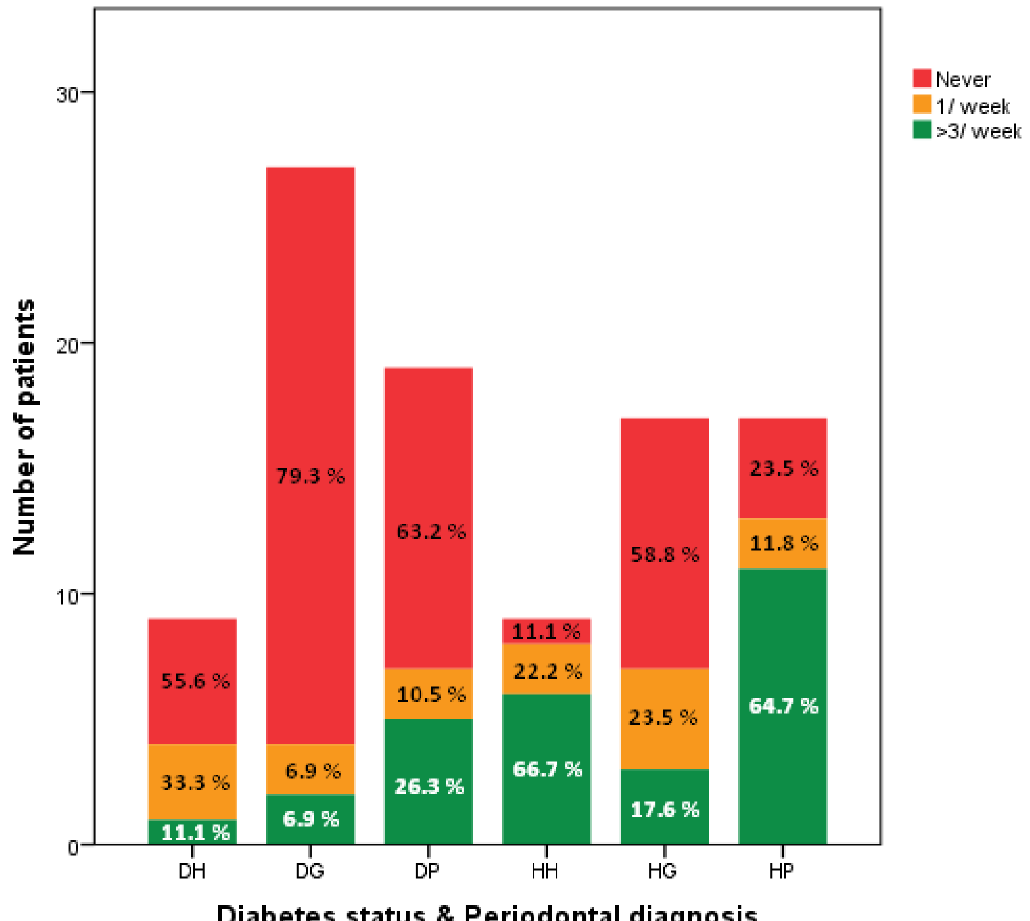


Figure 3.6: Frequency of interproximal teeth cleaning of T1DM and non-T1DM patients based on periodontal diagnosis.

DH patients (never n=5, once per week n=3, > thrice per week n=1), DG patients (never n=23, once per week n=2, > thrice per week n=2), DP patients (never n=12, once per week n=2, > thrice per week n=5), HH patients (never n=1, once per week n=2, > thrice per week n=6), HG patients (never n=10, once per week n=4, > thrice per week n=3) and HP patients (never n=4, once per week n=2, > thrice per week n=11). DH; T1DM, periodontal health, DG; T1DM, gingivitis, DP; T1DM, periodontitis, HH; non-T1DM, periodontal health, HG; non-T1DM, gingivitis, HP; non-T1DM, periodontitis.

3.2.5 Pre-treatment clinical biochemistry parameters

Table 3.12 summarises pre-treatment clinical biochemistry data for T1DM and non-T1DM patients. Of note, when comparing the two groups, statistically significant differences were found for HbA1c, non-HDL and cholesterol levels. As would be expected, the T1DM patients had a significantly higher HbA1c (%)/ (mmol/mol) [8.30 (7.60–9.35) %/ 67 (60-79) mmol/mol] compared to the non-T1DM patients [5.40 (5.30–5.60) %/ 36 (34-38) mmol/mol], (P<0.001). Levels of hsCRP also appeared to be higher in patients with T1DM [1.70 (0.68–5.58) mg/L] compared to the non-T1DM patients [1.00 (0.50–3.40) mg/L], however the difference was not statistically significant, (P>0.05). On the other hand, T1DM patients had significantly lower levels of non-HDL [2.90 (2.50–3.53) mmol/L] compared to the non-T1DM patients [3.50 (2.40–4.30) mmol/L], (P<0.05). Similarly, T1DM patients had significantly lower levels of cholesterol (4.50±0.74 mmol/L) compared to the non-T1DM patients (5.10±0.93 mmol/L), (P<0.001). Levels of triglycerides and HDL were higher in non-T1DM patients [1.40 (0.80–1.90) mmol/L and 1.60 (1.30–1.80) mmol/L] compared to the T1DM patients [1.30 (0.78–1.60) mmol/L and 1.40 (1.20–1.63) mmol/L], however, the differences were not statistically significant, (P>0.05).

Table 3.13 summarises pre-treatment clinical biochemistry data for T1DM and non-T1DM patients based on periodontal diagnosis. Interestingly, within the T1DM group, the HbA1c level appeared highest in the DP patients [8.95 (8.03-9.65) %/ 75 (64-83) mmol/mol] compared to the DG [8.25 (7.65-10.0) %/ 67 (61-86) mmol/mol] and DH [7.90 (7.30-8.58) %/ 63 (56-70) mmol/mol] patients, however these differences were not statistically significant, (P>0.05). No statistically significant differences were found within the non-T1DM group for HbA1c levels based on periodontal diagnosis (P>0.05). Considering lipid profile, HDL levels were significantly higher in the HH patients [1.80 (1.60–2.05) mmol/L] compared to the DH patients [1.40 (1.13–1.65) mmol/L], (P<0.05). Cholesterol levels were significantly higher in the HG (5.11±1.14 mmol/L) and the HP (5.18±0.86 mmol/L) patients compared to the DG (4.49±0.73 mmol/L) and the DP (4.52±0.83 mmol/L) patients, (P<0.05). Levels of hsCRP were significantly higher in the DH patients [1.40 (0.73–4.03) mg/L] compared to the HH patients [0.60 (0.20–1.10) mg/L], (P<0.05). Within the T1DM group, triglyceride levels were significantly higher in the DG patients [1.35 (0.95-1.75) mmol/L] compared to the DH patients [0.80 (0.70-1.28) mmol/L], (P<0.05). Similarly within the non-T1DM group, the HG patients had significantly higher triglyceride levels [1.70 (1.30-1.90)

mmol/L] compared to the HH patients [0.90 (0.20-1.05) mmol/L], (P<0.05). With reference to HDL levels, within the non-T1DM group, the HH patients had significantly higher HDL levels [1.80 (1.60-2.05) mmol/L] compared to the HG patients [1.50 (1.35-1.73) mmol/L], (P<0.05). With reference to hsCRP levels, within the non-T1DM group, the HG patients had a significantly higher hsCRP levels [2.85 (0.73-5.45) mg/L] compared to the HH patients [0.60 (0.20–1.10) mg/L], (P<0.05).

	T1DM (n=57)	**Non-T1DM (n=43)**	***P***
HbA1c (%)	8.30 (7.60-9.35)	5.40 (5.30-5.60)	< 0.001
(mmol/mol)	67 (60-79)	36 (34-38)	
Triglycerides (mmol/L)	1.30 (0.78–1.60)	1.40 (0.80-1.90)	NS
HDL (mmol/L)	1.40 (1.20-1.63)	1.60 (1.30-1.80)	NS
Non-HDL (mmol/L)	2.90 (2.50–3.53)	3.50 (2.40-4.30)	< 0.05
Cholesterol (mmol/L)	4.50 ± 0.74	5.10 ± 0.93	< 0.001
hsCRP (mg/L)	1.70 (0.68-5.58)	1.00 (0.50-3.40)	NS

Table 3.12: Pre-treatment clinical biochemistry data comparing T1DM and non-T1DM patients.

Mean ± SD presented for parametric data and median (IQR) presented for non-parametric data. P-values determined using Mann Whitney-U tests for continuous non-parametric variables and Independent t-test for continuous parametric variables. *P* indicates significant difference between T1DM and non-T1DM patients. HbA1c; glycated haemoglobin, HDL; high density lipoprotein, non-HDL; non-high density lipoprotein, hsCRP; high-sensitivity C-reactive protein, NS; not significant.

		Health (DH n=9) (HH n=9)	Gingivitis (DG n=26) (HG n=14)	Periodontitis (DP n=18) (HP n=16)	*P**
HbA1c (% & mmol/mol)	**T1DM**				
	%	7.90 (7.30-8.58)	8.25 (7.65-10.0)	8.95 (8.03-9.65)	NS
	mmol/mol	63 (56-70)	67 (61-86)	75 (64-83)	
	Non-T1DM				
	%	5.50 (5.30-5.60)	5.40 (5.28-5.45)	5.40 (5.10-5.68)	NS
	mmol/mol	37 (34-38)	36 (34-37)	36 (32-39)	
	P	< 0.01	< 0.01	< 0.01	
Triglycerides (mmol/L)	**T1DM**	0.80 (0.70-1.28)	1.35 (0.95-1.75) *	1.30 (0.75-1.80)	NS
	Non-T1DM	0.90 (0.70-1.05)	1.70 (1.30-1.90) *	1.35 (0.75-1.98)	< 0.05
	P	NS	NS	NS	
HDL (mmol/L)	**T1DM**	1.40 (1.13-1.65)	1.45 (1.20-1.70)	1.35 (1.23-1.60)	NS
	Non-T1DM	1.80 (1.60-2.05)	1.50 (1.35-1.73) *	1.40 (1.13-1.75)	< 0.05
	P	< 0.05	NS	NS	
Non-HDL (mmol/L)	**T1DM**	2.90 (2.58-3.50)	2.90 (2.50-3.45)	3.20 (2.30-3.88)	NS
	Non-T1DM	3.20 (2.40-3.90)	3.85 (2.28-4.65)	3.55 (2.68-4.20)	NS
	P	NS	NS	NS	
Cholesterol (mmol/L)	**T1DM**	4.47 ± 0.69	4.49 ± 0.73	4.52 ± 0.83	NS
	Non-T1DM	4.97 ± 0.76	5.11 ± 1.14	5.18 ± 0.86	NS
	P	NS	< 0.05	< 0.05	
hsCRP (mg/L)	**T1DM**	1.40 (0.73-4.03)	2.20 (0.55-5.85)	1.80 (1.23-7.75)	NS
	Non-T1DM	0.60 (0.20-1.10)	2.85 (0.73-5.45) *	1.50 (0.60-3.20)	NS
	P	< 0.05	NS	NS	

Table 3.13: Pre-treatment clinical biochemistry data comparing T1DM and non-T1DM patients based on periodontal diagnosis.

Mean ± SD presented for parametric data and median (IQR) presented for non-parametric data. P-values determined using One-way ANOVA test with post-hoc independent t-test for continuous parametric variables and Kruskal-Wallis with Mann-Whitney U post hoc tests for continuous non-parametric variables. *P** indicates overall p-value comparing across periodontal categories within T1DM or non-T1DM groups. P-values under columns (*P*) relate to comparisons between T1DM and non-T1DM groups. **P*<0.05 indicates statistically significant differences compared to health within the T1DM and non-T1DM groups. DH; T1DM, periodontal health, DG; T1DM, gingivitis, DP; T1DM, periodontitis, HH; non-T1DM, periodontal health, HG; non-T1DM, gingivitis, HP; non-T1DM, periodontitis. HbA1c; glycated haemoglobin, HDL; high density lipoprotein, non-HDL; non-high density lipoprotein, hsCRP; high-sensitivity C-reactive protein, NS; not significant.

3.2.6 Pre-treatment clinical periodontal parameters

Table 3.14 summarises pre-treatment clinical periodontal data for T1DM and non-T1DM patients. While comparing the two groups, the T1DM patients had a significantly higher PI score (0.86±0.49) compared to the non-T1DM patients (0.56±0.31), (*P*<0.001). Although % BOP was higher in the T1DM patients (36.9±19.3 %) compared to non-T1DM patients (33.8±25.3 %), this difference was not statistically significant, (*P*>0.05). Of note, no statistically significant differences were found between the two groups for mGI score, mean PD, mean recession, mean LOA, the number (%) of PD sites 5mm or greater and the number (%) of PD sites 4mm or less.

Table 3.15 presents pre-treatment clinical periodontal data for T1DM and non-T1DM patients based on periodontal diagnosis. A number of significant differences were found between the groups based on periodontal diagnosis, while comparing across the two groups and while comparing within each group. For ease of understanding, based on periodontal diagnosis first the differences between the T1DM and non-T1DM groups will be discussed, followed by the differences within the T1DM group and the non-T1DM group.

Considering the amount of plaque present, the PI score was significantly higher in the DG patients (0.94±0.40) compared to the HG patients (0.66±0.23), (*P*<0.01). Similarly, the DP patients had significantly higher PI score (0.98±0.54) compared to the HP patients (0.66±0.29), (*P*<0.05). When considering PD measurements, the mean PD was significantly higher in the DH patients (1.73±0.20 mm), compared to the HH patients (1.57±0.11 mm), (*P*<0.05). No statistically significant differences were found for mean PD between T1DM and non-T1DM gingivitis and periodontitis patients. While considering LOA, no statistically significant differences were found for LOA measurements between T1DM and non-T1DM patients with periodontitis. While considering BOP scores, % BOP was significantly higher in the DH patients (9.88±5.67 %) compared to the HH patients (0.83±1.17 %), (*P*<0.001). No statistically significant differences were found for % BOP scores between T1DM and non-T1DM gingivitis and periodontitis patients. While considering PD sites measuring 5 mm or greater, the HP patients had a significantly higher number (%) of PD sites measuring 5 mm or greater [37.4±25.2 (23.7±15.5 %)] compared to the DP patients [20.3±21.7 (14.7±16.4 %)], (*P*<0.05). No statistically significant differences were found for PD sites measuring 5mm or greater between T1DM and non-T1DM healthy tissue and gingivitis patients. Of

note, no statistically significant differences were found for mGI, mean recession, and PD sites measuring 4mm or less, between the two groups based on periodontal diagnosis.

Comparing pre-treatment periodontal data within the T1DM group, significant differences were found between patients for all periodontal parameters assessed. The mGI score was significantly higher in the DG (1.61±0.40) and the DP (1.96±0.51) patients compared to the DH patients (0.66±0.55), (*P*<0.001). Also, the mGI score was significantly higher in the DP patients (1.96±0.51) compared to the DG patients (1.61±0.40), (*P*<0.01). The PI score was significantly higher in the DG (0.94±0.40) and the DP (0.98±0.54) patients compared to the DH patients (0.32±0.25), (*P*<0.001). While considering PD measurements, the DG (2.16±0.23 mm) and the DP (3.02±0.81 mm) patients had significantly higher mean PD compared to the DH patients (1.73±0.20 mm), (*P*<0.001). Also, the DP patients (3.02±0.81 mm) had a significantly higher mean PD compared to the DG patients (2.16±0.23 mm), (*P*<0.001). While considering gingival recession, the DP patients had a significantly higher amount of recession (0.43±0.64 mm) compared to the DG (0.08±0.26 mm) and the DH (0.09±0.15 mm) patients, (*P*<0.05). While considering LOA, the DH and the DG patients had no LOA (0.00±0.00 mm) and the DP patients had significantly more LOA (3.45±1.18 mm) compared to the DH and DG patients, (*P*<0.001). While considering BOP scores, the DP patients had significantly higher % BOP (52.7±17.4 %) compared to the DG (35.0±11.5 %) and the DH (9.88±5.67 %) patients, (*P*<0.001). Also, the DG patients had a significantly higher % BOP (35.0±11.5 %) compared to the DH patients (9.87±5.67 %), (*P*<0.001). With reference to PD sites measuring 5 mm or greater, the DP patients had a significantly higher number (%) of sites measuring 5mm or more [20.3±21.7 (14.7±16.4 %)] compared to the DG [0.76±1.35 (0.47±0.85 %)] and the DH [0.22±0.67 (0.13±0.4 %)] patients, (*P*<0.001). With reference to PD sites measuring 4mm or less, the DH patients had significantly higher number (%) of sites measuring 4mm or less [162.4±6.58 (99.9±0.40 %)] compared to the DP patients [122.5±29.4 (85.3±16.4 %)], (*P*<0.001). Also, the DG patients had a significantly higher number of sites measuring 4mm or less [158.8±9.87 (99.5±9.87 %)] compared to the DP patients [122.5±29.4 (85.3±16.4 %)], (*P*<0.001).

Comparing pre-treatment periodontal data within the non-T1DM group, the HP patients had a significantly higher mGI score (2.29±0.53) compared to the HG (1.50±0.57) and the HH (0.56±0.61) patients, (*P*<0.001). Also, the HG patients (1.50±0.57) had a significantly higher mGI score compared to the HH patients (0.56±0.61), (*P*<0.001). While considering PI score, the HG (0.66±0.23) and the HP (0.66±0.29) patients had a significantly higher levels of

plaque compared to the DH patients (0.20±0.19), (*P*<0.001). With reference to PD measurements, the HP patients had a significantly higher mean PD (3.31±0.75 mm) compared to the HG (2.04±0.24 mm) and the HH (1.57±0.11 mm) patients, (*P*<0.001). Also, the HG patients had a significantly higher mean PD (2.04±0.24 mm) compared to the HH patients (1.57±0.11 mm), (*P*<0.001). While considering LOA, the HH and the HG patients had no LOA (0.00±0.00 mm) and the HP patients had significantly higher mean LOA (3.55±0.75 mm) compared to the HH and HG patients, (*P*<0.001). While considering BOP scores, the HP patients had significantly higher % BOP (53.9±21.1 %) compared to the HG (31.3±13.6 %) and the HH (0.83±1.17 %) patients, (*P*<0.001). Also, the HG patients (31.3±13.6 %) had a significantly higher % BOP compared to the HH patients (0.83±1.17 %), (*P*<0.001). With reference to PD sites measuring 5mm or greater, the HH patients had no sites measuring 5mm or greater, and the HP patients had a significantly higher number (%) of sites measuring 5mm or more [37.4±25.2 (23.7±15.5 %)] compared to the HG patients [0.94±1.81 (0.64±1.26 %)], (*P*<0.001). With reference to PD sites measuring 4mm or less, the HH patients had a significantly higher number (%) of sites measuring 4mm or less [161.3±10.6 (100%)] compared to the HG [143.4±16.1 (99.4±1.26 %)] and the HP [119.0±25.1 (74.7±17.7 %)] patients, (*P*<0.01 and *P*<0.001 respectively). Also the HG patients had a significantly higher number of sites measuring 4mm or less [143.4±16.1 (99.4±1.26 %)] compared to the HP patients [119.0±25.1 (74.7±17.7 %)], (*P*<0.01).

	T1DM (n=57)	Non-T1DM (n=43)	*P*
mGI	1.57 ± 0.63	1.62 ± 0.86	NS
PI	0.86 ± 0.49	0.56 ± 0.31	< 0.001
Mean PD (mm)	2.38 ± 0.69	2.45 ± 0.89	NS
Mean recession (mm)	0.20 ± 0.44	0.20 ± 0.34	NS
Mean LOA (mm)	2.04 ± 1.51	2.25 ± 1.43	NS
BOP (%)	36.9 ± 19.3	33.8 ± 25.3	NS
Sites 5 mm or greater [n (%)]	7.18 ± 15.5 (5.16 ± 11.5)	15.5 ± 24.2 (9.84 ± 15.1)	NS
Sites 4 mm or less [n (%)]	147.2 ± 25.4 (94.8 ± 11.5)	137.4 ± 25.4 (89.5 ± 16.6)	NS

Table 3.14: Pre-treatment clinical periodontal data comparing T1DM and non-T1DM patients.

Mean ± SD presented for parametric variables. P-values determined using Independent t-test for continuous parametric variables. mGI; modified gingival index, PI; plaque index, PD; probing depth, LOA; loss of attachment, BOP; bleeding on probing, NS; not significant.

		Health (DH n=9) (HH n=9)	Gingivitis (DG n=29) (HG n=17)	Periodontitis (DP n=19) (HP n=17)	*P**
mGI	**T1DM**	0.66 ± 0.55	1.61 ± 0.40 ***	1.96 ± 0.51 ***, ††	< 0.001
	Non-T1DM	0.56 ± 0.61	1.50 ± 0.57 ***	2.29 ± 0.53 ***, †††	< 0.001
	P	NS	NS	NS	
PI	**T1DM**	0.32 ± 0.25	0.94 ± 0.40 ***	0.98 ± 0.54 ***	< 0.001
	Non-T1DM	0.20 ± 0.19	0.66 ± 0.23 ***	0.66 ± 0.29 ***	< 0.001
	P	NS	< 0.01	< 0.05	
Mean PD (mm)	**T1DM**	1.73 ± 0.20	2.16 ± 0.23 ***	3.02 ± 0.81 ***, †††	< 0.001
	Non-T1DM	1.57 ± 0.11	2.04 ± 0.24 ***	3.31± 0.75 ***, †††	< 0.001
	P	< 0.05	NS	NS	
Mean recession (mm)	**T1DM**	0.09 ± 0.15	0.08 ± 0.26	0.43 ± 0.64 *, †	< 0.05
	Non-T1DM	0.29 ± 0.38	0.10 ± 0.17	0.24 ± 0.43	NS
	P	NS	NS	NS	
Mean LOA (mm)	**T1DM**	0.00 ± 0.00	0.00 ± 0.00	3.45 ± 1.18 ***, †††	< 0.001
	Non-T1DM	0.00 ± 0.00	0.00 ± 0.00	3.55 ± 0.75 ***, †††	< 0.001
	P	N/A	N/A	NS	
BOP (%)	**T1DM**	9.88 ± 5.67	35.0 ± 11.5 ***	52.7 ± 17.4 ***, †††	< 0.001
	Non-T1DM	0.83 ± 1.17	31.3 ± 13.6 ***	53.9 ± 21.1 ***, †††	< 0.001
	P	< 0.001	NS	NS	
Sites 5 mm or greater [n (%)]	**T1DM**	0.22 ± 0.67 (0.13 ± 0.40)	0.76 ± 1.35 *** (0.47 ± 0.85)	20.3 ± 21.7 ***, ††† (14.7 ± 16.4)	< 0.001
	Non-T1DM	0.00 ± 0.00 (0.00)	0.94 ± 1.81 (0.64 ± 1.26)	37.4 ± 25.2 ***, ††† (23.7 ± 15.5)	< 0.001
	P	NS	NS	< 0.05	
Sites 4 mm or less [n (%)]	**T1DM**	162.4 ± 6.86 (99.9 ± 0.40)	158.8 ± 9.87 (99.5 ± 0.85)	122.5 ± 29.4 ***, ††† (85.3 ± 16.4)	< 0.001
	Non-T1DM	161.3 ± 10.6 (100)	143.4 ± 16.1 ** (99.4 ± 1.26)	119.0 ± 25.1 ***, †† (74.7 ± 17.7)	< 0.001
	P	NS	NS	NS	

Table 3.15: Pre-treatment clinical periodontal data comparing T1DM and non-T1DM patients based on periodontal diagnosis.

Mean ± SD presented for parametric data. P-values were determined using One-way ANOVA with post-hoc Independent t-test for continuous parametric variables. *P** indicates overall p-value comparing across periodontal categories within T1DM or non-T1DM groups. P-values under columns relate to comparisons between T1DM and non-T1DM groups. **P*<0.05, ***P*<0.01, ****P*<0.001 indicates statistically significant differences compared to health and †*P*<0.05, ††*P*<0.01, †††*P*<0.001 indicates statistically significant differences compared to gingivitis within T1DM and non-T1DM groups. DH; T1DM, periodontal health, DG; T1DM, gingivitis, DP; T1DM, periodontitis, HH; non-T1DM, periodontal health, HG; non-T1DM, gingivitis, HP; non-T1DM, periodontitis. mGI; modified gingival index, PI; plaque index, PD; probing depth, LOA; loss of attachment, BOP; bleeding on probing, NS; not significant.

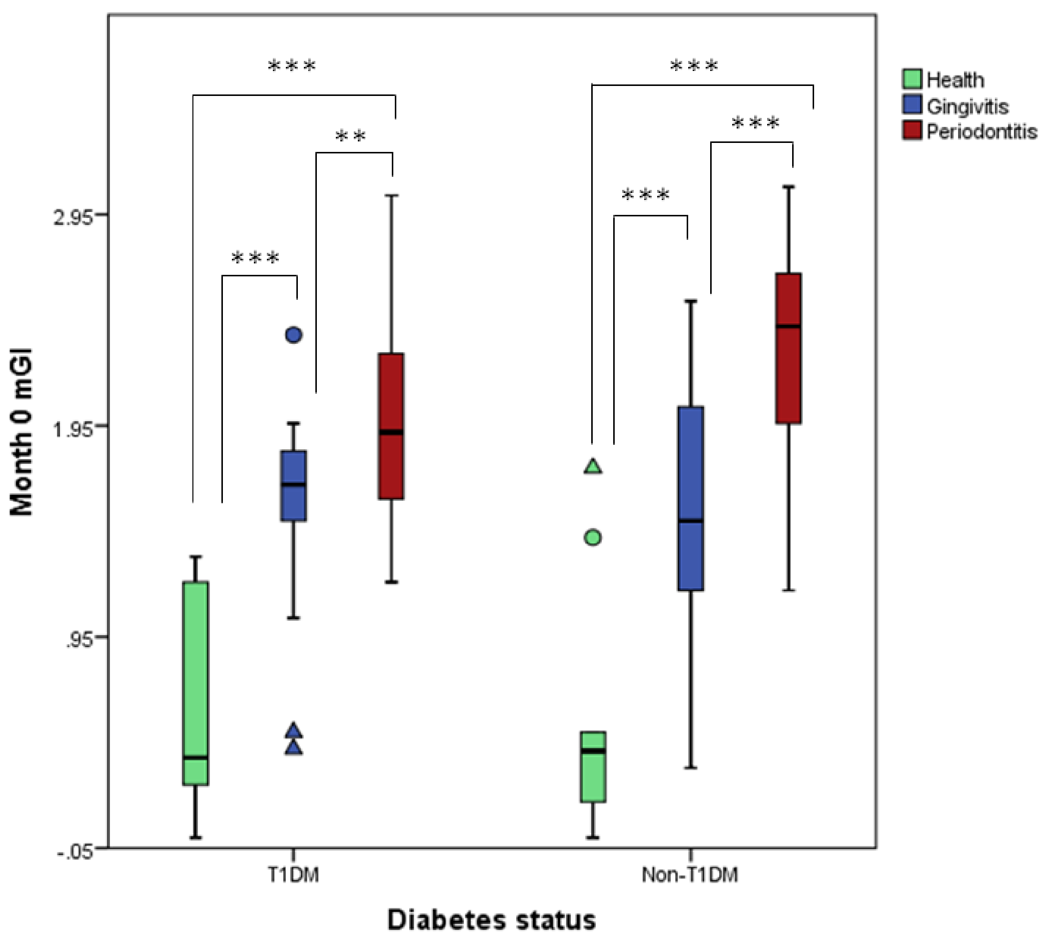


Figure 3.7: Pre-treatment mGI score comparing T1DM and non-T1DM patients based on periodontal diagnosis.

Box plot figure showing medians, interquartile ranges, standard errors and outliers for 57 T1DM patients (health n=9, gingivitis n=29, periodontitis n=19) and 43 non-T1DM patients (health n=9, gingivitis n=17, periodontitis n=17). Statistics: One-way ANOVA with post-hoc Independent t-test: ***P*<0.01, ****P*<0.001 (according to periodontal status within the T1DM or non-T1DM group). ● indicates outlier more than 1.5 but less than 3 times the IQR from the box boundaries, ▲ indicates outlier more than 3 times the IQR from the box boundaries.

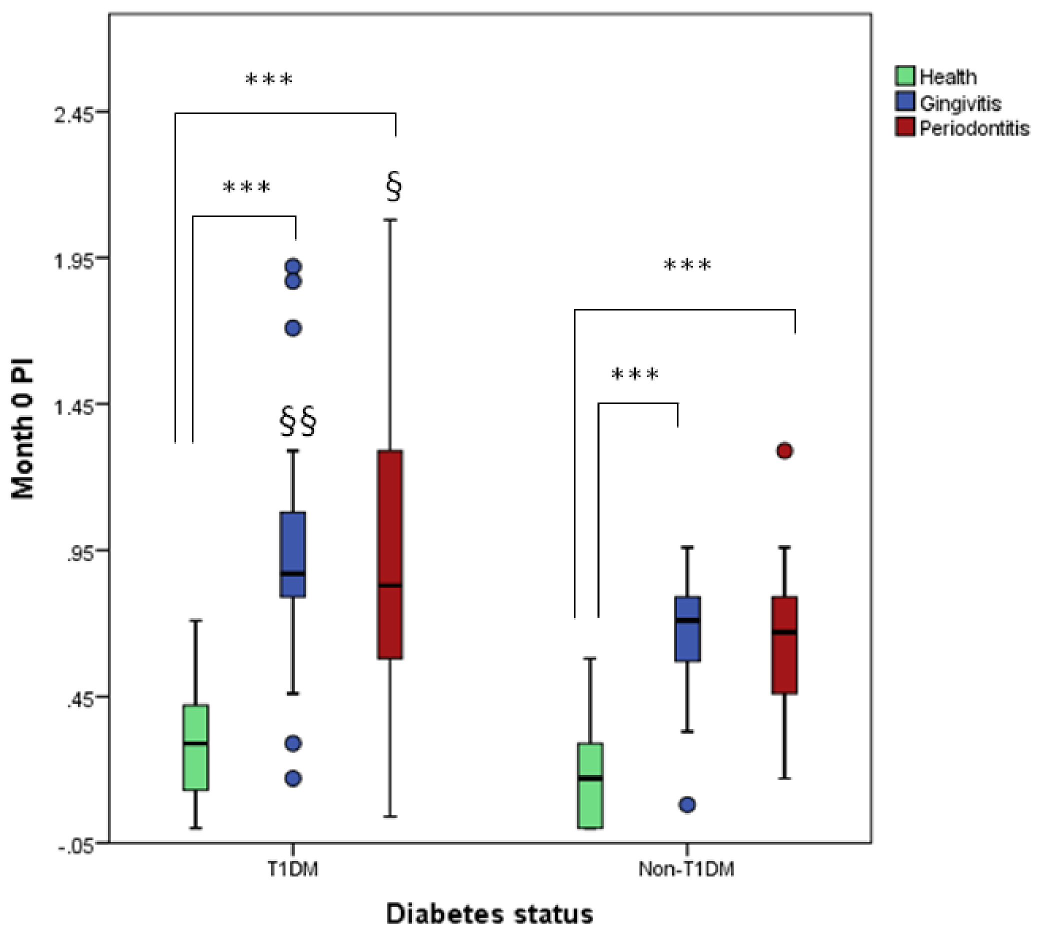


Figure 3.8: Pre-treatment PI score comparing T1DM and non-T1DM patients based on periodontal diagnosis.

Box plot figure showing medians, interquartile ranges, standard errors and outliers for 57 T1DM patients (health n=9, gingivitis n=29, periodontitis n=19) and 43 non-T1DM patients (health n=9, gingivitis n=17, periodontitis n=17). Statistics: One-way ANOVA with post-hoc Independent t-test: ****P*<0.001 (according to periodontal status within the T1DM or non-T1DM group); §*P*<0.05, §§*P*<0.01 (T1DM versus non-T1DM group within the corresponding periodontal status). ● indicates outlier more than 1.5 but less than 3 times the IQR from the box boundaries.

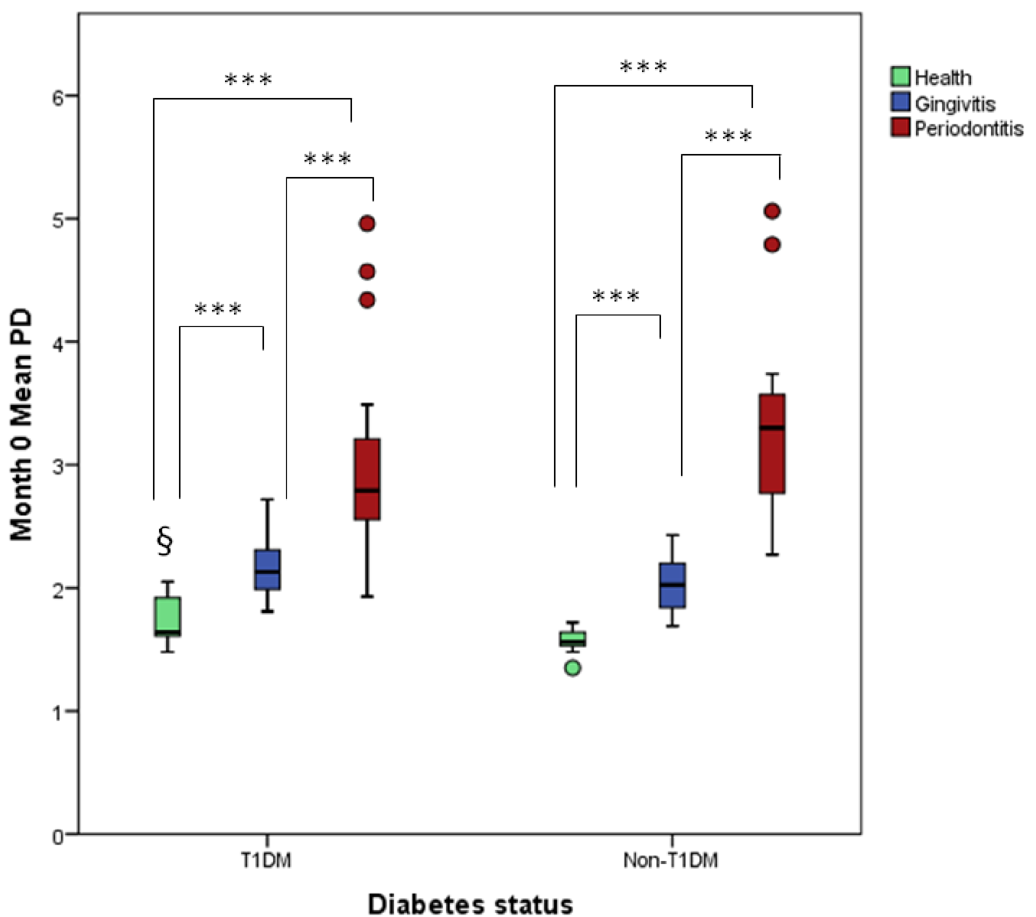


Figure 3.9: Pre-treatment mean PD comparing T1DM and non-T1DM patients based on periodontal diagnosis.

Box plot figure showing medians, interquartile ranges, standard errors and outliers for 57 T1DM patients (health n=9, gingivitis n=29, periodontitis n=19) and 43 non-T1DM patients (health n=9, gingivitis n=17, periodontitis n=17). Statistics: One-way ANOVA with post-hoc Independent t-test: ****P*<0.001 (according to periodontal status within the T1DM or non-T1DM group); §*P*<0.05 (T1DM versus non-T1DM group within the corresponding periodontal status). ● indicates outlier more than 1.5 but less than 3 times the IQR from the box boundaries.

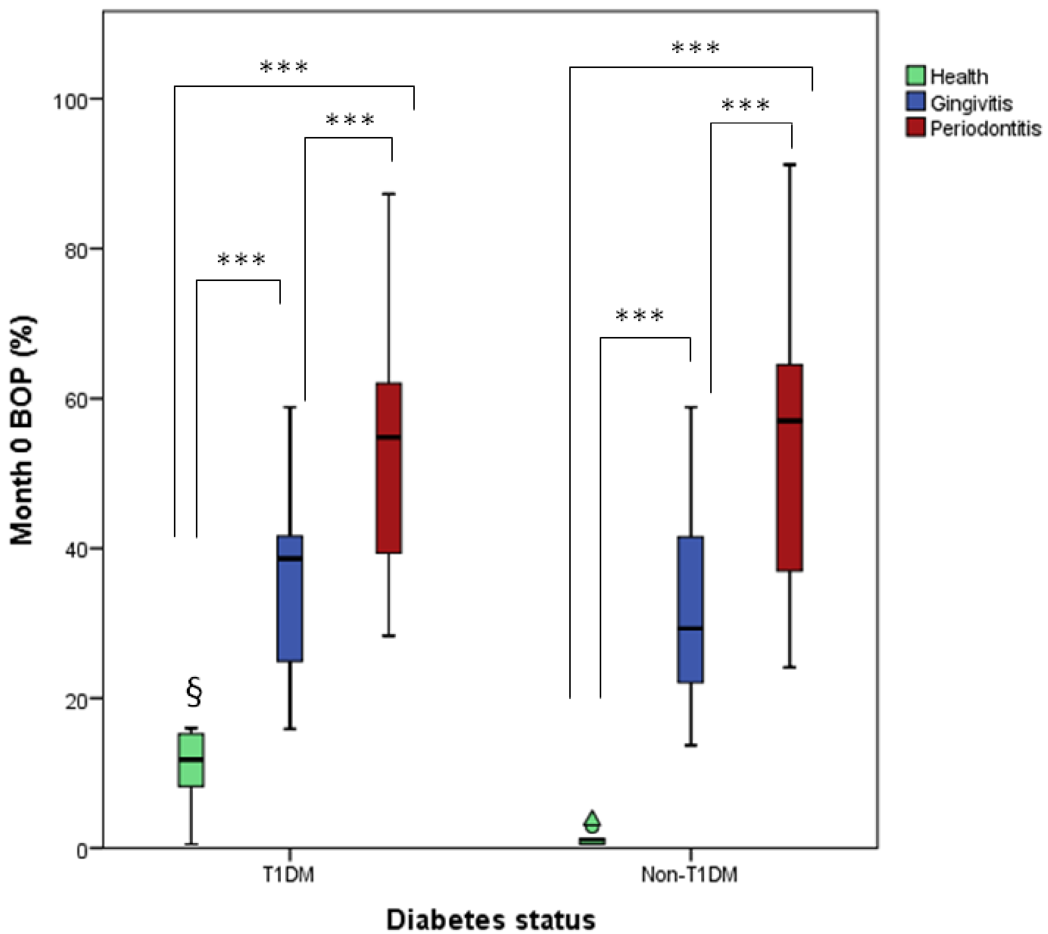


Figure 3.10: Pre-treatment % BOP comparing T1DM and non-T1DM patients based on periodontal diagnosis.

Box plot figure showing medians, interquartile ranges, standard errors and outliers for 57 T1DM patients (healthy periodontal tissues n=9, gingivitis n=29, periodontitis n=19) and 43 non-T1DM patients (healthy periodontal tissues n=9, gingivitis n=17, periodontitis n=17). Statistics: One-way ANOVA with post-hoc Independent t-test: ***P<0.001 (according to periodontal status within the T1DM or non-T1DM group); §P<0.05 (T1DM versus non-T1DM within the corresponding periodontal status). ● indicates outlier more than 1.5 but less than 3 times the IQR from the box boundaries, ▲ indicates outlier more than 3 times the IQR from the box boundaries.

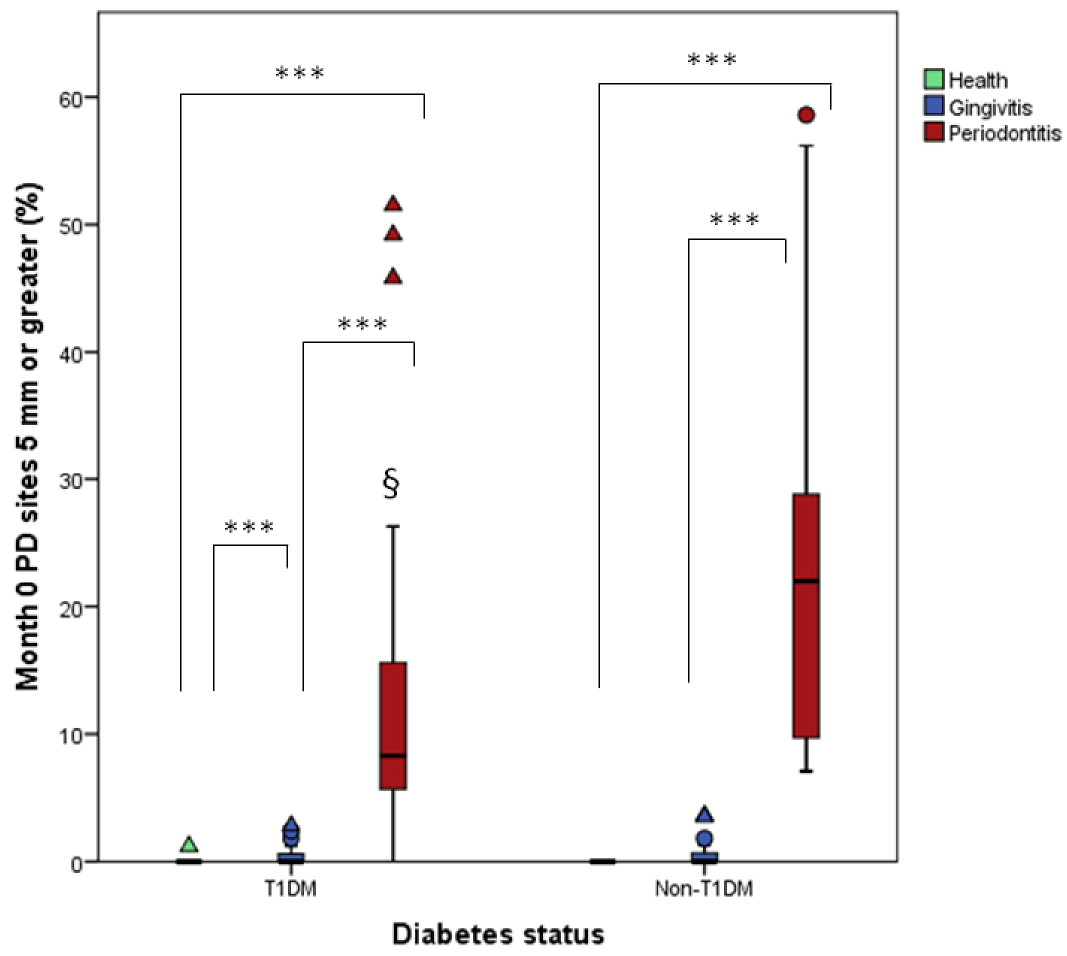


Figure 3.11: Pre-treatment % PD sites 5 mm or greater comparing T1DM and non-T1DM patients based on periodontal diagnosis.

Box plot figure showing medians, interquartile ranges, standard errors and outliers for 57 T1DM patients (health n=9, gingivitis n=29, periodontitis n=19) and 43 non-T1DM patients (health n=9, gingivitis n=17, periodontitis n=17). Statistics: One-way ANOVA with post-hoc Independent t-test: ****P*<0.001 (according to periodontal status within the T1DM or non-T1DM group); §*P*<0.05 (T1DM versus non-T1DM within the corresponding periodontal status). ● indicates outlier more than 1.5 but less than 3 times the IQR from the box boundaries, ▲ indicates outlier more than 3 times the IQR from the box boundaries.

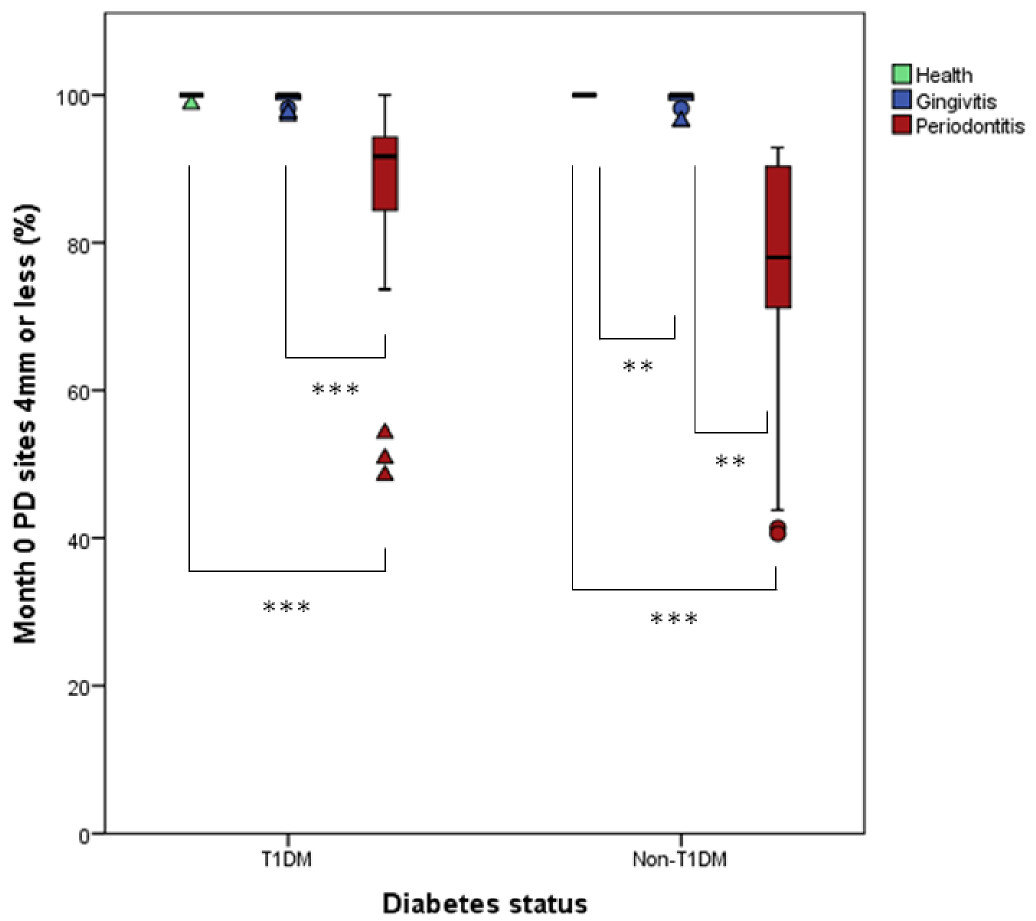


Figure 3.12: Pre-treatment % PD sites 4mm or less comparing T1DM and non-T1DM patients based on periodontal diagnosis.

Box plot figure showing medians, interquartile ranges, standard errors and outliers for 57 T1DM patients (health n=9, gingivitis n=29, periodontitis n=19) and 43 non-T1DM patients (health n=9, gingivitis n=17, periodontitis n=17). Statistics: One-way ANOVA with post-hoc Independent t-test: ** P<0.01, ***P<0.001 (according to periodontal status within the T1DM or non-T1DM group). ● indicates outlier more than 1.5 but less than 3 times the IQR from the box boundaries, ▲ indicates outlier more than 3 times the IQR from the box boundaries.

3.3 Discussion

In this chapter, the pre-treatment demographic, metabolic and periodontal data were compared between the T1DM and non-T1DM patients and also within these two groups based on periodontal diagnosis.

With reference to age, the non-T1DM patients were significantly older in age compared to the T1DM patients, ($P<0.001$) (Table 3.1). This was a limitation in this study as during the time of patient recruitment, the control patients were under-recruited (n=12) and due to time constraints, it was not possible to recruit more. Hence, in order to match the number of recruited T1DM patients (n=57), control patients for the T1DM study were recruited from the control group of another parallel study of T2DM patients, by matching for gender and periodontal status. Unfortunately, the age range for the T2DM patients was higher than the range for this T1DM study. A total of 31 control patients from the T2DM study were recruited into this T1DM study to achieve a total of 43 non-T1DM patients.

High levels of obesity, unhealthy diet, physical inactivity, poor BP control and smoking are strongly associated with deprivation. All these factors are inevitably associated with the risk of diabetes or the risk of developing serious diabetes-related complications. The National Diabetes Audit data suggest that individuals in the most deprived quantile are at a 1.5 times higher risk of diabetes than those in the least deprived quantile (NDA 2014). A variation in deprivation and diabetes is only seen in individuals with T2DM. Deprivation does not have an effect on the development of T1DM, as it is not lifestyle related (NDA 2014). This possibly explains the lack of significant difference seen for IMD rank scores comparing T1DM and non-T1DM patients, suggesting similar lifestyle experiences in both groups.

Smoking is recognised as a major risk factor in the development and progression of periodontal disease (Tomar and Asma 2000). In the present study, smoking habits were only self-reported by each patient. Unfortunately this must be considered as a potential flaw in the study design. Self-reported smoking status can lead to underestimation of smoking rates, especially because of the decreasing social acceptability of smoking (Gallus et al. 2011). It would be beneficial in future studies to validate self-reported smoking status by the use of biochemical measures such as, determining nicotine or its metabolite cotinine in saliva, serum, plasma or urine (Binnie et al. 2004), or measuring exhaled carbon monoxide levels (Middleton and Morice 2000). Nevertheless, when considering the results of self-reported smoking status in this study, the prevalence of current smokers was 21.1% in the T1DM

group and 14% in the non-T1DM group. The prevalence data for smoking in England at the time this study was initiated showed that 21% of the adult population were current smokers (Robinson and Dunstan 2011). Compared to the prevalence rate of the 2009 National Statistics, the prevalence of smoking in this study was almost equal for T1DM patients (21.1%); and markedly lower than 21% for non-T1DM patients (14%). However, the current prevalence rate for smoking in England is 19% (Niblett 2015) and compared to this value, the prevalence rate for T1DM current smokers was higher while in non-T1DM patients it was markedly lower. The importance of smoking status and development of periodontitis is highlighted in the current study. Interestingly, irrespective of diabetes status, patients with healthy periodontal tissues contained no current smokers and had the lowest pack years of smoking compared to the gingivitis and periodontitis patients. Also, patients with periodontitis in both groups contained a significantly higher percentage of ex-smokers compared to patients with gingivitis and healthy tissues. Although not statistically significant, pack years was highest in patients with periodontitis in both T1DM and non-T1DM groups.

With reference to BP values (Table 3.1 and 3.3), the non-T1DM patients had a significantly higher diastolic BP compared to the T1DM patients, ($P<0.05$). Additionally, based on periodontal diagnosis, the T1DM patients with healthy tissues had significantly higher systolic BP compared to the non-T1DM patients with healthy tissues, ($P<0.05$). And, diastolic BP was significantly higher in non-T1DM patients with gingivitis compared to T1DM patients with gingivitis, ($P<0.05$). Within the non-T1DM group, the gingivitis patients had significantly higher systolic BP compared to those with healthy tissues, ($P<0.05$). These differences in systolic and diastolic BP could possibly be a chance finding in this study, and the BP values of all groups were within limits of the recommended levels for patients with diabetes (≤140/80 mmHg) (NICE 2015).

With reference to duration of diabetes, it is interesting to note that T1DM patients with periodontitis had a significantly longer history of diabetes compared to those with gingivitis and healthy tissues, ($P<0.05$) (Table 3.5). This finding could likely be a manifestation of the patients who were recruited, as the periodontitis patients were significantly older in age compared to those with healthy tissues ($P<0.001$), and although not statistically significant, periodontitis patients were older in age compared to those with gingivitis (Table 3.2). It has been well established that age is a common confounding factor for periodontal disease, with

older people having experienced more periodontitis (Genco and Borgnakke 2013). A significantly longer duration of diabetes in the patients with periodontitis compared to those with gingivitis and healthy tissues, could also suggest that a longer duration of diabetes leads to the increased chances of individuals suffering from more severe periodontal disease. Our findings are in agreement with previous studies which found that a longer duration of diabetes is related to poorer periodontal health (Cianciola et al. 1982; Thorstensson and Hugoson 1993; Firatli et al. 1996; Silvestre et al. 2009). Silvestre *et al.'s* study found that patients with a longer history of T1DM had significantly higher bleeding index scores and periodontal attachment loss compared to those with a short duration of diabetes (Silvestre et al. 2009). Our findings are in contrast to other studies which showed that the duration of diabetes had no effect on the severity of periodontal disease (de Pommereau et al. 1992; Tervonen and Oliver 1993; Sandberg et al. 2000). Since the relationship between duration of diabetes and periodontal disease has been studied to a lesser extent and data are often conflicting, it would be advisable to carry out more research in this domain.

It has been established that T1DM has a genetic predisposition; this study found that 52.6% of the T1DM patients had a family history of diabetes. With reference to diabetes control, a majority of the T1DM patients controlled their diabetes with a combination of diet, physical exercise and drug therapy (87.7%). Only a small proportion of patients controlled their diabetes with drug therapy alone (12.3%). With reference to type of drug therapy, a majority of the patients were taking insulin therapy alone (86%), while only a minor proportion (14%) controlled their diabetes by taking a combination of insulin, oral glucose lowering drugs, lipid lowering drugs and/or anti-hypertensive medication.

In this study based on glycaemic control categories, a majority of the T1DM patients were categorised as having poor glycaemic control (i.e. HbA1c >8.5 % / >69 mmol/mol). Although not statistically significant, it is interesting to note that a majority of the periodontitis patients (52.6%) had poor glycaemic control, whereas a majority of the gingivitis (44.8%) and heathy tissue (55.6%) patients had moderate glycaemic control. This possibly supports evidence of a two-way relationship between diabetes and periodontal disease whereby poor glycaemic control increases the risk for severe periodontal disease and periodontal disease negatively affects glycaemic control (Preshaw et al. 2012).

In the current study, the T1DM patients were asked to self-report if they suffered from any diabetes-related complications other than periodontal disease. None of the patients suffered

from any macrovascular complications. However, of the 57 T1DM patients, 22.9% reported at least one microvascular complication. Interestingly, a higher proportion reporting diabetes-related microvascular complications were patients with periodontitis (31.6%) followed by healthy tissue (22.2%) and gingivitis (17.2%) patients, although these differences were not statistically significant. While considering patient care pathways within diabetes management, it is interesting to note that a majority of the T1DM patients (94.7%) had received examination of their eyes and feet and had also been educated on the importance of routine examinations for the betterment of their condition within the past 12 months. This clearly demonstrates that a robust patient care pathway does exist for screening of diabetic complications. Unfortunately, the same is not true for screening of oral complications of T1DM, with as many as 1/3 of the T1DM patients in this study reporting not being examined by a dentist in the past 12 months. Hence, an opportunity to regularly screen for oral complications in this disease-susceptible population is clearly being lost.

While considering oral and dental data (Table 3.7, 3.8 and 3.9), the T1DM patients were found to have a significantly higher number of unrestored teeth compared to the non-T1DM patients, ($P<0.01$). This suggests that the T1DM patients had a more sound and untreated dentition compared to the non-diabetic patients. The one possible reason for this difference could also be due to the difference in age between the two groups, with older patients having a higher possibility of having restored teeth. While comparing the two groups based on periodontal diagnosis, patients with healthy tissues and gingivitis had significantly more number of teeth present compared to those with periodontitis, ($P<0.01$), indicating a possible loss of a greater number of teeth in T1DM patients with periodontitis. However, in determining the likely cause of tooth loss, a distinction was not made between extractions due to, for example, orthodontic treatment and extraction due to dental diseases, which might be relevant information to take note of in further research related to periodontal disease. Due to the disturbed glucose metabolism, patients with diabetes are considered to be at a higher risk of developing dental caries (Siudikiene et al. 2005b). In the present study, the non-T1DM patients were found to have a significantly higher number restored teeth (1-3 surfaces) compared to the T1DM patients, ($P<0.001$). A previous study assessed dietary and oral hygiene habits in children with T1DM, and found that the pattern of food consumption differed between T1DM and non-diabetic children, with T1DM children consuming more main meals per day and less snacking throughout the day, while the diet of non-diabetic children was characterised by the frequent consumption of sweet snacks (Siudikiene et al.

2005b). This could also be a possible explanation for the significantly reduced number of restored teeth in T1DM patients compared to the non-T1DM patients in the current study. No statistically significant differences were found between the two groups for number of teeth with caries, broken down teeth, endodontically treated teeth and teeth with periapical radiolucencies. Our findings are similar to other research studies which also demonstrated no significant differences in dental findings between T1DM and non-diabetic patients (Faulconbridge et al. 1981; Tenovuo et al. 1986; Harrison and Bowen 1987b; Twetman et al. 1989; Swanljung et al. 1992; Edblad et al. 2001; Siudikiene et al. 2008; Tagelsir et al. 2011), but contrast to other studies which found both significantly higher caries prevalence (Albrecht et al. 1988; Jones et al. 1992; Moore et al. 2001b; Lopez et al. 2003; Miralles et al. 2006) and lower caries prevalence in T1DM patients compared to non-diabetic controls (Matsson and Koch 1975; Leeper et al. 1985; Kirk and Kinirons 1991; Siudikiene et al. 2006; Orbak et al. 2008).

Since oral health behaviour is directly related to the amount of plaque accumulation, it is reasonable to presume that the level of oral hygiene in addition to an individual's host response correlates with the prevalence and severity of periodontal disease and dental caries. In this study, no statistically significant differences were found in oral health behaviour (i.e. attendance at GDP, reason for last dental visit and frequency of tooth brushing) between T1DM and non-T1DM patients, suggesting that both groups had a similar outlook towards seeking and maintaining oral health. These findings are similar to those of a previous study which found that a majority of the T1DM and non-diabetic patients reported brushing their teeth once or twice a day and did not differ with respect to the number of dental visits during the past year (Siudikiene et al. 2005a). Interestingly, statistical differences were only found with regards to interproximal tooth cleaning, and the non-T1DM patients reported performing more frequent interproximal teeth cleaning compared to a majority of the T1DM patients who reported having never used interproximal cleaning aids (n=40, 70.2%), as opposed to a majority of the non-T1DM patients (n=20, 46.5%) who reported cleaning their teeth three or more times per week. It is also interesting to note that within the non-T1DM group (Table 3.11), a majority of the periodontitis patients (n=11, 64.7%) reported cleaning their teeth interproximally three or more times per week compared to the gingivitis and healthy tissue patients. This suggests that the possible awareness of the advanced periodontal disease might lead to increased vigilance and motivation in performing and maintaining oral health in this cohort of patients by using interproximal cleaning aids. It is also important to note that

irrespective of the diabetes status, a higher proportion of periodontitis patients reported having attended their GDP regularly within the past 12 months compared to patients with gingivitis and healthy tissues. This suggests that the presence and possible awareness of oral disease leads to patients with periodontitis seeking professional help for the betterment and maintenance of their oral health condition.

With reference to the clinical biochemistry analysis, in the current study, as expected the T1DM patients had a significantly higher %/ mmol/mol of HbA1c compared to the non-T1DM patients, ($P<0.001$) (Table 3.12). This study found higher HbA1c levels in T1DM patients with periodontitis and gingivitis compared to those with healthy tissues, suggesting a possible role for periodontal inflammation plays in elevating glycated haemoglobin levels in patients with gingivitis and periodontitis. However, these differences did not reach statistical significance (Table 3.13). The HbA1c level for patients with T1DM and periodontitis in this study was comparable to the HbA1c level reported in Lalla *et al.'s* study (8.49±1.74 %) (Lalla et al. 2007b) but higher than that in Silvestre *et al.'s* study (7.83±1.62 %) (Skaleric et al. 2004) and lower than the HbA1c levels reported in studies by Erhan Firatli (9.33±3.98 %), Moore *et al.* (11.0±0.1%) and Dakovic *et al.* (9.2±1.6 %) (Firatli 1997; Moore et al. 1999; Dakovic and Pavlovic 2008).

Further clinical biochemistry data analysis in this study revealed that compared to the non-T1DM patients, T1DM patients had significantly lower levels of non-HDL and cholesterol. Non-HDL and cholesterol are considered indicators for CVD, and are particularly useful parameters in predicting CVD risk in patients with diabetes (Lu et al. 2003). The non-T1DM patients also had higher levels of triglycerides and HDL compared to the T1DM patients, however these differences were not statistically significant (Table 3.12). These findings favourably reflect on the UK management guidelines for T1DM, which recommend the implementation of dietary advice and the annual screening for CVD risk factors for patients with T1DM to ensure that they have optimal lipid profile (including HDL, non-HDL, cholesterol and triglyceride) levels (NICE 2015). Therefore, it is reasonable to presume that the T1DM patients in this study were receiving more aggressive management and monitoring of CVD risk factors compared to the control patients. Also, 14% of the T1DM patients were taking lipid lowering and anti-hypertensive medication, however comparable data from non-T1DM patients were not collected to confirm the influence of medication on lipid profile levels.

In the present study, levels of hsCRP appeared higher in patients with T1DM compared to the non-T1DM patients; however the difference was not statistically significant (Table 3.12). Interestingly, when comparing the two groups based on periodontal diagnosis, the T1DM patients with healthy tissues had significantly higher hsCRP levels than the non-T1DM patients with healthy tissues, (P<0.05) (Table 3.13). Our findings are in support of an association of the increase in systemic inflammation in patients with T1DM (Devaraj et al. 2007; Snell-Bergeon et al. 2010). Similarly, a meta-analyses carried out on CRP levels in relation to periodontitis consistently found elevated hsCRP levels in patients with periodontitis compared to those with healthy tissues (Paraskevas et al. 2008). Likewise this was confirmed in this study, as non-T1DM patients with gingivitis had significantly higher levels of hsCRP compared to those with healthy tissues (P<0.05) (Table 3.13). However, within the T1DM group, similar differences were not found when comparing patients with gingivitis and those with healthy tissues. A possible explanation of this effect would be that the elevated background levels of hsCRP in patients with T1DM masks additional differences in hsCRP levels caused by inflammatory periodontal disease. It is interesting to note that irrespective of the diabetes status, the gingivitis patients had the highest hsCRP levels compared to patients with periodontitis and healthy tissues.

Previous epidemiological research studies related to T1DM and periodontal disease have reported an increased prevalence and severity of periodontal disease in T1DM patients compared to non-diabetic individuals. In the present study, when exploring pre-treatment periodontal status of the T1DM patients in comparison to those without diabetes, significantly higher levels of plaque were found in T1DM patients compared to the non-T1DM patients, (P<0.001) (Table 3.14). This finding could be explained by the fact that excessive glucose related to diabetes, enters the oral cavity through the GCF and saliva, a sugar-rich biofilm which forms will then, in general, enhance plaque growth. A lack of understanding and knowledge about oral hygiene and maintaining optimal oral health may be factors related to higher plaque scores in patients with diabetes (Hugoson et al. 1989). Our findings are similar to results of previous studies reporting significantly higher plaque scores in T1DM patients compared to non-diabetic individuals (Novaes et al. 1991; Aren et al. 2003; Lalla et al. 2006a). Our finding was contrary to previous studies which found similar levels of plaque in patients with and without T1DM (Bay et al. 1974; Bernick et al. 1975; Hugoson et al. 1989; Sandholm et al. 1989a; de Pommereau et al. 1992; Firatli et al. 1996; Firatli 1997; Tervonen and Karjalainen 1997; Lalla et al. 2006b). Similarly, when comparing the two

groups based on periodontal diagnosis, T1DM patients with gingivitis and periodontitis had significantly higher amounts of plaque compared to the non-T1DM patients with gingivitis and periodontitis, (*P*<0.01 and *P*<0.05 respectively) (Table 3.15 and Figure 3.8). Although T1DM patients with healthy tissues had higher plaque scores compared to non-T1DM patients with healthy tissues, this difference was not statistically significant. The results of this study in relation to plaque scores suggest that the T1DM patients had poorer oral hygiene compared to those without diabetes, which could also be due to the greater attention and importance placed by the T1DM patients in focusing and maintaining their systemic health and the necessary daily doses of insulin as opposed to maintaining optimal oral hygiene, which was found to be superior in the non-T1DM patients as measured by the Silness and Loe plaque index scoring system.

With reference to periodontal probing depths, no statistically significant differences were found for PD measurements between T1DM and non-T1DM patients (Table 3.14). Our findings are similar to previous studies which found similar PD measurements in patients with and without T1DM (Sandholm et al. 1989a; Novaes et al. 1991; Firatli 1997; Lalla et al. 2006b; Luczaj-Cepowicz et al. 2006; Kaur et al. 2009). The findings of the present study are in contrast to those of other studies which found deeper periodontal pockets in T1DM patients compared to non-diabetic controls (Hugoson et al. 1989; Dakovic and Pavlovic 2008; Silvestre et al. 2009; Hodge et al. 2012). Interestingly, the PD measurements for this study are comparable to PD data from a number of previous studies (Aren et al. 2003; Lalla et al. 2006b; Kaur et al. 2009) but higher than a longitudinal study which evaluated the clinical status of periodontal tissues in children with T1DM (Firatli 1997), indicating variations in the extent of periodontal disease between different research studies. However, it is important to note that most investigations of T1DM and periodontal disease included a cohort of a younger age group, which might limit the extent of periodontal disease and hence may not permit appropriate comparison to the results of this study. Nevertheless irrespective of the age range, a majority of the T1DM patients had comparable PD measurements compared to non-T1DM patients. In the current study, when comparing the two groups based on periodontal diagnosis, T1DM patients with healthy tissues had significantly higher mean PD compared to non-T1DM patients with healthy tissues, (*P*<0.05). However, no statistically significant differences were found for mean PD while comparing T1DM and non-T1DM patients with periodontitis (Table 3.15 and Figure 3.9).

In addition to PD measurements, a previous study calculated the % of PD sites with advanced periodontal disease, demonstrating that when considering the % of PD sites ≥5 mm, the differences between the T1DM patients (8.50±13.1 %) and the non-T1DM patients (5.30±9.00 %) was found to be statistically significant (Lalla et al. 2006b). It is very rare that periodontal disease would affect all parts of the periodontium equally, and the measurement of mean PD alone provides a crude description of the pocket depths found in each patient. Hence, the utilization of reporting mean PD, without additional data such as the % of sites with advanced periodontal disease, can be seen as a limitation of all studies in this field of research. In the present study, the % of PD sites measuring ≥5 mm was significantly lower in the T1DM patients with periodontitis compared to the non-T1DM patients with periodontitis ($P<0.05$), indicating the presence of more severe periodontal disease in non-T1DM patients compared to the T1DM patients (Table 3.15 and Figure 3.11). This may reflect on the differences in the recruitment strategy utilised for T1DM and non-T1DM patients in the current study. As previously described, the T1DM patients were recruited from medical databases of T1DM patients held in both primary and secondary care settings, whereas the non-T1DM patients were recruited from diagnostic or student treatment clinics within the School of Dental Sciences, who had been referred for periodontal diagnosis and care from their general dental practice. Although the T1DM and non-T1DM patients were matched based on their periodontal diagnosis, the extent of their periodontal disease was not considered in the process. This is a limitation in the present study, and highlights a need in future studies, to stratify periodontal case selection based on the extent and severity of periodontal disease to ensure a more meaningful and robust matching of groups with respect to the periodontal status of selected patients.

With reference to clinical LOA, no statistically significant difference was found for mean LOA while comparing patients with and without T1DM. Our findings are similar to a previous study which found no statistically significant difference in LOA levels between the two groups (Kaur et al. 2009), and contrast with those of previous studies which found significantly higher LOA in T1DM patients compared to non-diabetic controls (Firatli 1997; Lalla et al. 2006a; Silvestre et al. 2009; Hodge et al. 2012).

In the current study, exploring data related to gingival inflammation indicates a difference in patterns for T1DM compared to non-T1DM patients. The T1DM patients with healthy tissues had a significantly higher % BOP compared to the non-T1DM patients with healthy

tissues, ($P<0.001$) (Table 3.15 and Figure 3.10). Also, the T1DM patients with healthy tissues had higher mGI scores compared to the non-T1DM patients with healthy tissues, but this difference was not statistically significant (Table 3.15 and Figure 3.7). Our findings support data from past research studies that demonstrated significantly higher levels of gingival inflammation in T1DM patients compared to non-diabetic controls (Novaes et al. 1991; Aren et al. 2003; Lalla et al. 2006a; Dakovic and Pavlovic 2008; Orbak et al. 2008; Silvestre et al. 2009). A possible explanation for the presence of increased levels of gingival inflammation seen in T1DM patients is a manifestation of the upregulated diabetes-related systemic inflammation which presents itself even in patients diagnosed with healthy periodontal tissues. The similar pattern of significantly higher levels of gingival inflammation in T1DM patients compared to non-T1DM patients was not replicated in patients with gingivitis and periodontitis and no statistically significant differences in levels of % BOP and mGI were seen between patients with T1DM and gingivitis and periodontitis compared to non-T1DM patients with gingivitis and periodontitis (Table 3.15). It would not be unreasonable to presume that the more severe periodontal disease present in the non-T1DM patients compared to the T1DM patients may have masked the presence of greater background level of gingival tissue inflammation in T1DM patients with periodontitis compared to non-T1DM patients with periodontitis.

Summary of key findings from chapter 3

- The non-T1DM patients were significantly older compared to the T1DM patients.
- Diastolic BP was significantly higher in non-T1DM patients compared to the T1DM patients.
- Patients with periodontitis in both T1DM and non-T1DM groups contained a significantly higher % of ex-smokers compared to those with gingivitis and healthy tissues.
- The T1DM patients with periodontitis had a significantly longer duration of diabetes compared to those with gingivitis and healthy tissues.
- The T1DM patients with healthy tissues controlled their diabetes with insulin alone, whereas patients with gingivitis and periodontitis controlled their diabetes with insulin, oral glucose lowering drugs, lipid lowering drugs and anti-hypertensive drugs.

- 94.7% of the T1DM patients had received examination of their eyes and feet within the past 12 months. However, 1/3 of the T1DM patients in the study had not visited the dentist in the past 12 months.
- The T1DM patients had a significantly higher number of sound and unrestored teeth compared to the non-T1DM patients.
- The T1DM patients reported poorer oral health behaviours with regards to interproximal teeth cleaning compared to the non-T1DM patients.
- Levels of non-HDL and cholesterol were significantly lower in the T1DM patients compared to the non-T1DM patients.
- Levels of hsCRP appeared higher in patients with T1DM compared to non-T1DM patients however this difference was not statistically significant. Also, in non-T1DM patients, levels of hsCRP were significantly lower in patients with healthy tissues compared to those with gingivitis.
- Although not statistically significant, T1DM patients with periodontitis had higher HbA1c levels compared to T1DM patients with gingivitis and healthy tissues.
- No statistically significant differences were found for mean PD between T1DM and non-T1DM patients. However, the non-T1DM patients with periodontitis had a significantly higher % of PD sites measuring ≥5 mm compared to the T1DM patients with periodontitis.
- The T1DM patients with healthy tissues had a significantly higher % BOP compared to the non-T1DM patients with healthy periodontal tissues.

4 Chapter 4. Quantification of protein levels, and detection and analysis of pre-treatment local and systemic biomarker levels in patients with T1DM

4.1 Introduction

Clinical studies have demonstrated that T1DM is associated with an increase in circulating inflammatory mediators (AboElAsrar et al. 2012; Redondo et al. 2014). Diabetes gives rise to impaired macrophage and neutrophil functioning, altered collagen production and exaggerated collagenase activity (Lalla et al. 2000; Noack et al. 2000a; Mealey and Rose 2008b) and has the potential to lead to a heightened inflammatory state, as interactions of AGEs have been known to increase the production of pro-inflammatory mediators (Mealey and Rose 2008b).

The tissue destruction that occurs in periodontal disease results mainly from an upregulated immune-inflammatory response which is caused by prolonged exposure to subgingival plaque bacteria. The importance of the host immune response to dental plaque and the increase in local production of inflammatory mediators has been well established (Kornman et al. 1997). Research studies have demonstrated elevated levels of inflammatory biomarkers in serum, plasma, GCF, saliva and gingival tissues of patients with periodontitis (Makela et al. 1994; Maeso et al. 2007; Marcaccini et al. 2009b; Marcaccini et al. 2010; Lappin et al. 2011). These inflammatory mediators have the ability to activate osteoclasts leading to bone resorption, increase collagen breakdown and impair wound healing, leading to clinical signs of periodontal disease.

Over the last decade, the increasing recognition of the clinical and pathogenic association between periodontitis with general health and disease (including diabetes) has contributed to our understanding of periodontal pathogenesis with several potential clinical applications (Nassar et al. 2007; Lalla and Papapanou 2011; Preshaw et al. 2012). A dysregulated immune response is central to the pathogenesis of diabetes and its related complications (Taylor et al. 2013). Cytokines are critical in the development of T1DM and the modulation of cytokines is a likely therapeutic modality (Mandrup-Poulsen et al. 2010; Baumann et al. 2012). The cytokine literature related to T1DM patients with periodontitis is limited and the elevation of GCF IL-1β and PGE_2 in T1DM patients compared to systemically healthy individuals with similar levels of periodontal disease is the only consistent finding (Salvi et al. 1997b; Salvi et al. 2010). Other than cytokines, chemokines (IL-8 and ENA-78/CXCL5) and other mediators such as MMPs (MMP-9), have been detected in samples of GCF, saliva

and plasma and are possibly associated with the cross-susceptibility between T1DM and periodontal disease (Salvi et al. 2010; Dakovic et al. 2013; Lappin et al. 2015).

As research investigating the pathogenic mechanisms of T1DM and periodontal disease is notably lacking, the experiments presented in this chapter reports the analysis of pre-treatment inflammatory biomarkers in patients with T1DM compared to non-diabetic patients with and without periodontal disease.

4.2 Results

4.2.1 Quantification of protein

The samples collected for the present study were collected from 2006 to 2008. After collection, all samples were stored in a -80 °C freezer, until further analyses, which were performed in 2015. Hence, protein content of samples was measured to ensure that there has not been substantial protein degradation or loss in the stored samples Therefore, in order to assess protein levels in the samples, all baseline serum samples (n=96) were analysed using the Bradford assay protocol. The GCF samples were not analysed due to the limited volume of GCF available from the sampling procedure.

All patient and control samples were diluted (1:300) in distilled water. Table 4.1 presents protein concentration data for all baseline serum samples. The amount of protein present in the samples of all patients (T1DM and non-T1DM) was (mean±SD) 355.1±203.9 mg/ml. When analysing the samples based on diabetes status, no significant differences were found for protein levels in patients with T1DM (322.7±196.0 mg/ml) compared to non-T1DM patients (395.1±208.6 mg/ml), ($P>0.05$).

Table 4.2 presents protein concentration data for T1DM and non-T1DM patients based on periodontal diagnosis. Of note, no statistically significant differences were found for protein concentrations between the DH (424.4±287.4 mg/ml), DG (293.8±142.3 mg/ml) and DP (313.7±205.1 mg/ml) patients compared to the HH (459.0±174.2 mg/ml), HG (366.7±166.3 mg/ml) and HP (389.5±261.3 mg/ml) patients, ($P>0.05$). Similarly, no statistically significant differences were found within the T1DM and non-T1DM groups based on periodontal diagnosis, ($P>0.05$).

	All patients (n=96)	T1DM (n=53)	Non-T1DM (n=43)
Amount of protein (mg/ml)	355.1 ± 203.9	322.7 ± 196.0	395.1 ± 208.6

Table 4.1: Protein levels in baseline serum samples comparing T1DM and non-T1DM patients.

Mean ± SD presented for parametric data. P-values determined using Independent t-test for continuous parametric variables (no statistically significant differences were found).

		Health (DH n=9) (HH n=9)	Gingivitis (DG n=26) (HG n=17)	Periodontitis (DP n=18) (HP n=17)	*P**
Amount of protein (mg/ml)	**T1DM**	424.4 ± 287.4	293.8 ± 142.3	313.7 ± 205.1	NS
	Non-T1DM	459.0 ± 174.2	366.7 ± 166.3	389.5 ± 261.3	NS
	P	NS	NS	NS	

Table 4.2: Protein levels in baseline serum samples based on diabetes status and periodontal diagnosis.

Mean ± SD presented for parametric data. P-values were determined using One-way ANOVA with post-hoc Independent t-test for continuous parametric variables. *P** indicates overall p-value comparing across periodontal categories within T1DM or non-T1DM groups. P-values under columns (*P*) relate to comparisons between T1DM and non-T1DM group. DH; T1DM, periodontal health, DG; T1DM, gingivitis, DP; T1DM, periodontitis, HH; non-T1DM, periodontal health, HG; non-T1DM, gingivitis, HP; non-T1DM, periodontitis, NS; not significant.

4.2.2 Investigation of candidate biomarkers using cytokine arrays

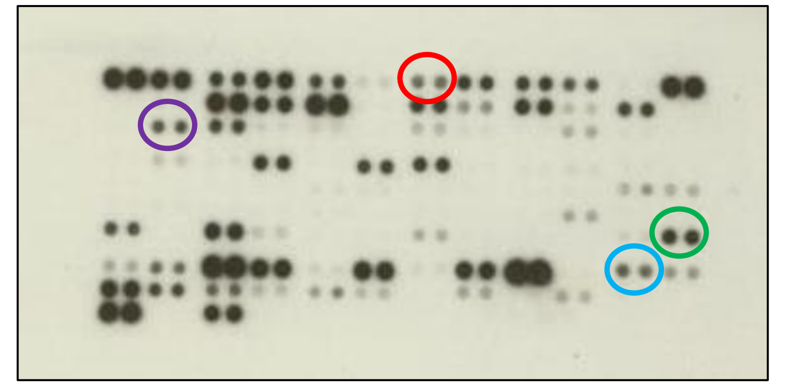
To investigate the biomarkers present in the samples collected, a cytokine array experiment was carried out using the Proteome Profiler Human XL Cytokine Array Kit (R&D Systems). Only serum samples were analysed using this technique. The cytokine array experiment involved pooling three serum samples from each diabetes status and periodontal diagnosis category: DH, HH, DP and HP and incubating 4 separate nitrocellulose membranes (which constitute the antibody arrays) with these samples. It was considered important to match the clinical periodontal parameters of the DH and HH group and the DP and HP group as closely as possible so that any differences in the results of the array experiment would be a manifestation of the diabetes status and not the periodontal condition. Therefore, 3 patients from each group (DH, HH, DP and HP) were selected after matching for clinical periodontal parameters. Table 4.3 presents the demographic (age) and clinical periodontal data of the T1DM and non-T1DM patients selected for the cytokine array analysis.

Figure 4.1 shows radiographic images of the 4 nitrocellulose membranes as follows: DP (membrane 1), DH (membrane 2), HP (membrane 3), and HH (membrane 4). The intensity of the spots (signals) on all 4 membranes, were compared to determine differences in biomarker levels between the four groups. The radiograph was also cross-checked with a template provided in the kit, to rule out any false positive signals or any referred signals from a neighbouring spot. Radiograph intensities were determined by densitometry and graphs were plotted to compare data from of all four groups.

		DH (n=3)	HH (n=3)	DP (n=3)	HP (n=3)
Age (years)	Patient 1	28	40	32	35
	Patient 2	28	38	34	35
	Patient 3	28	34	29	36
mGI	Patient 1	0.38	0.17	2.42	2.67
	Patient 2	0.00	0.25	2.04	2.83
	Patient 3	0.33	0.50	1.21	3.08
PI	Patient 1	0.13	0.13	0.54	0.88
	Patient 2	0.00	0.29	1.25	0.96
	Patient 3	0.25	0.00	0.54	1.29
Mean PD (mm)	Patient 1	1.61	1.72	4.96	5.06
	Patient 2	1.48	1.35	3.46	3.57
	Patient 3	1.68	1.64	4.57	4.79
Mean recession (mm)	Patient 1	0.00	0.11	0.62	-0.05
	Patient 2	0.11	0.13	0.12	-0.36
	Patient 3	0.47	0.11	1.16	-0.23
Mean LOA (mm)	Patient 1	0.00	0.00	5.58	5.01
	Patient 2	0.00	0.00	3.58	3.19
	Patient 3	0.00	0.00	5.73	4.48
BOP (%)	Patient 1	14.7	2.40	59.8	90.7
	Patient 2	14.3	0.00	57.7	89.3
	Patient 3	2.70	0.00	45.0	88.3

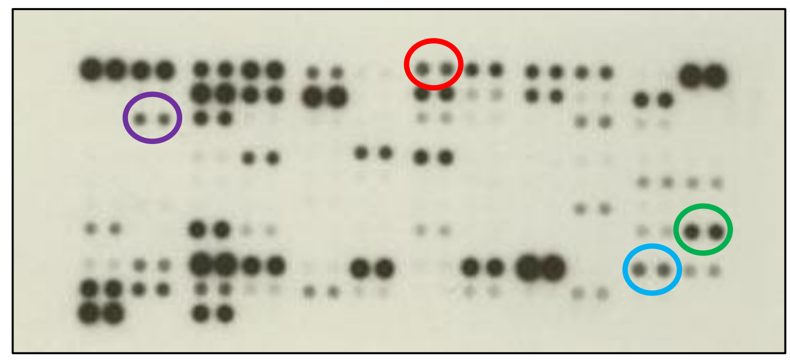
Table 4.3: Demographic and clinical periodontal data of the T1DM and non-T1DM patients selected for the cytokine array analysis.

This table presents the age and clinical periodontal data of the T1DM and non-T1DM patients selected for the cytokine array analysis. Three patients from each diabetes status and periodontal diagnosis group (DH, HH, DP and HP) were selected after matching for clinical periodontal status. Selection was made by comparing data of the DH and HH patients (**in blue**) and the DP and HP patients (**in red**). DH; T1DM, periodontal health, HH; non-T1DM, periodontal health, DP; T1DM, periodontitis, HP; non-T1DM, periodontitis, mGI; modified gingival index, PI; plaque index, PD; probing depth, LOA; loss of attachment, BOP; bleeding on probing.

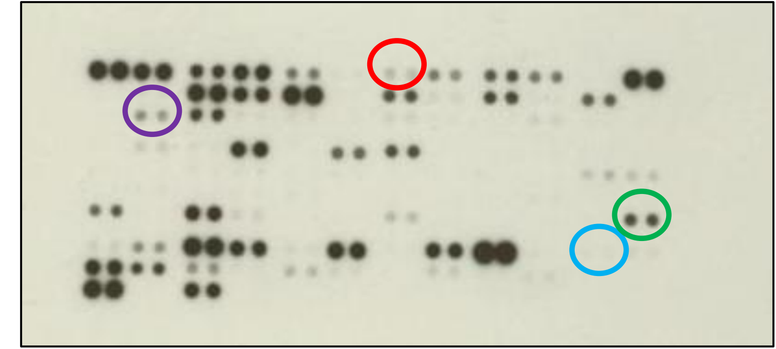


Membrane 1: T1DM, periodontitis

Membrane 2: T1DM, periodontal health



Membrane 3: Non-T1DM, periodontitis



Membrane 4: Non-T1DM, periodontal health

Figure 4.1: Radiographic images of the four nitrocellulose membranes highlighting selected candidate biomarkers.

In all four membranes: (green circle) indicates MMP-9, (red circle) indicates BAFF, (blue circle) indicates resistin and (purple circle) indicates ENA-78/CXCL5.

Table 4.4 presents results of the cytokine array experiment, with intensity values presented for biomarkers found present in all the four groups of diabetes status and periodontal diagnosis. The intensity values of different biomarkers showed either differences in intensity values or similar intensity values while comparing the four groups (DP, HP, DH and HH). The decision to select a candidate biomarker was based on finding greater differences in intensity values and preferably higher intensity values in the periodontitis groups (with or without diabetes) compared to the healthy periodontal tissue groups. Based on these results and after consideration of the relevant literature it was decided to further analyse the following 4 candidate biomarkers in serum samples: MMP-9, BAFF, resistin and ENA-78/CXCL5. Figure 4.2 shows bar graphs comparing intensities of biomarker MMP-9, BAFF, resistin and ENA-78/CXCL5 (intensity values highlighted in orange and bold in Table 4.4).

The levels of MMP-9 [as assessed by relative intensity (RI) of the radiograph dots] were found to be highest in the DP group (RI 10427.5), followed by the HP group (RI 9185), the DH group (RI 8993) and was found least in the HH group (RI 6112). Comparing the intensity of the signals, the intensity of MMP-9 was 1.16-fold higher in the DP group (RI 10427.5) compared to the DH group (RI 8993). The intensity of MMP-9 was 1.50-fold higher in the HP group (RI 9185) compared to the HH group (RI 6112). The intensity of MMP-9 was 1.14-fold higher in the DP group (RI 10427.5) compared to the HP group (RI 9185). And the intensity of MMP-9 was 1.47-fold higher in the DH group (RI 8993) compared to the HH group (RI 6112). Since MMP-9 was found highest in the DP group compared to the HP group, MMP-9 was selected for further analysis (Table 4.4, Figure 4.1 and 4.2).

The intensity of BAFF, was found to be highest in the DP group (RI 6807.5), followed by the HP group (RI 6318.5), the DH group (RI 3957) and was found least in the HH group (RI 1318.5). Comparing the intensity of the signals, the intensity of BAFF was 1.72-fold higher in the DP group (RI 6807.5), compared to the DH group (RI 3957). The intensity of BAFF was 4.79-fold higher in the HP group (RI 6318.5) compared to the HH group (RI 1318.5). The intensity of BAFF was 1.08-fold higher in the DP group (RI 6807.5) compared to the HP group (RI 6318.5). And the intensity of BAFF was 3-fold higher in the DH group (RI 3957) compared to the HH group (RI 1318.5). Since BAFF was found highest in the DP group compared to the HP group, BAFF was selected for further analysis (Table 4.4, Figure 4.1 and 4.2).

The apparent levels of resistin were highest in the DP group (RI 6215), followed by the HP group (RI 6125.5), the DH group (RI 4069.5) and lowest of all in the HH group (RI 491.5). Comparing the intensity of membrane signals, the intensity of resistin was 1.53-fold higher in the DP group (RI 6215) compared to the DH group (RI 4069.5). The intensity of resistin was 12.5-fold higher in the HP group (RI 6125.5) compared to the HH group (RI 491.5). The intensity of resistin was 1.01-fold higher in the DP group (RI 6215) compared to the HP group (RI 6125.5). The intensity of resistin was 8.28-fold higher in the DH group (RI 4069.5) compared to the HH group (RI 491.5). Since resistin was found highest in the DP group compared to the HP group, resistin was selected for further analysis (Table 4.4, Figure 4.1 and 4.2).

The intensity of ENA-78/CXCL5, was found to be highest in the DP group (RI 6062), followed by the DH group (RI 5523.5), the HP group (RI 4783) and was found least in the HH group (RI 2640). Comparing the intensity of signals, the intensity of ENA-78/CXCL5 was 1.10-fold higher in the DP group (RI 6062) compared to the DH group (RI 5523.5). The intensity of ENA-78/CXCL5 was 1.81-fold higher in the HP group (RI 4783) compared to the HH group (RI 2640). The intensity of ENA-78/CXCL5 was 1.27-fold higher in the DP group (RI 6062) compared to the HP group (RI 4783). And the intensity of ENA-78/CXCL5 was 2.09-fold higher in the DH group (RI 5523.5) compared to the HH group (RI 2640). Since ENA-78/CXCL5 was found highest in the DP group compared to the HP group, ENA-78/CXCL5 was selected for further analysis (Table 4.4, Figure 4.1 and 4.2).

Biomarker	DP (n=3)	HP (n=3)	DH (n=3)	HH (n=3)
Adiponectin	16066.5	14154	15104	12677.5
Aggrecan	14736.5	12080	12850.5	8755
Angiogenin	16147.5	14609	15430.5	12413
Angiopoietin-1	10675.5	5141	9737.5	3981
BAFF	**6807.5**	**6318.5**	**3957**	**1318.5**
BDNF	9406.5	7395.5	7436	3179
Complement Component C5/C5a	10073	7643.5	8350.5	5887.5
CD14	6606	6508.5	5612	3889
CD40 ligand	2168.5	2374	2041.5	3712
Chitinase 3-like 1	20223.5	20485.5	20669	17482.5
Complement Factor D	14234.5	14427.5	15119	14457
C-Reactive Protein	20508.5	18408.5	20290.5	18360.5
Cystatin C	10242.5	9440.5	7812.5	6369
Dkk-1	3890	2362.5	2667.5	570.5
DPPIV	11692	7704	10147.5	7092
EGF	2260	1890	2271.5	784.5
EMMPRIN	8829	10101	8219	5864.5
ENA-78/CXCL5	**6062**	**4783**	**5523.5**	**2640**
Endoglin	7637.5	8666	8923	8594
Fas Ligand	958.5	683	1491.5	1081.5
FGF basic	1505.5	1423.5	2157.5	2073.5
FGF-19	1785	1605.5	1366.5	293.5
GDF-15	2226	3874	2065.5	507.5
ICAM-1	9634.5	5924	10882	9864
IGFBP-2	7298	6326.5	6755.5	4055.5
IGFBP-3	8510	8064.5	7245	5608.5
IL-17A	2710	3321.5	2440.5	1384.5

Table 4.4: Intensity values of biomarkers present.

Intensity values for biomarkers detected in all groups of diabetes status and periodontal diagnosis. Highlighted in orange and bold are the selected candidate biomarkers. DP; T1DM, periodontitis, HP; non-T1DM, periodontitis, DH; T1DM, periodontal health, HH; non-T1DM, periodontal health.

Biomarker	DP (n=3)	HP (n=3)	DH (n=3)	HH (n=3)
IL-18 Bpa	2429	3077	2343	1301.5
IP-10	2431	2924.5	2271	130
Leptin	6402.5	2748.5	3662.5	4149
Lipocalin-2	13355	12979	12816.5	10546.5
MCP-1	1361	1520.5	1702	279.5
MIF	1740	1386	1563.5	739
MIP-3β	1409	2210.5	741	355.5
MMP-9	**10427.5**	**9185**	**8993**	**6112**
Myeloperoxidase	2713	2503	1983	417.5
Osteopontin	5382.5	5685.5	4582.5	2664
PDGF-AA	23259	23276	21004.5	16240
PDGF-AB/BB	14782.5	14937.5	13350.5	10148.5
PF4	13194.5	15113.5	13677.5	12191.5
RANTES	14634.5	14033.5	11443.5	11617.5
RBP4	21215.5	24127.5	24175.5	20151
Resistin	**6215**	**6125.5**	**4069.5**	**491.5**
SDF-1α	3667	3301.5	2142	528
Serpin E1	16549.5	18098.5	15833.5	12952.5
ST2	12286	9628	10458.5	7266.5
TARC	5999.5	2081	3558	3306
TFF3	2891	2750	1197.5	1406
TfR	7213.5	1907.5	2099	4327.5
Thrombospondin-1	6673.5	2236	4864.5	3068
UPAR	2773.5	3230.5	3025	937
Vitamin D BP	13811.5	11402	14343	10838.5

Table 4.4: Intensity values of biomarkers present.

Intensity values for biomarkers detected in all groups of diabetes status and periodontal diagnosis. Highlighted in orange and bold are the selected candidate biomarkers. DP; T1DM, periodontitis, HP; non-T1DM, periodontitis, DH; T1DM, periodontal health, HH; non-T1DM, periodontal health.

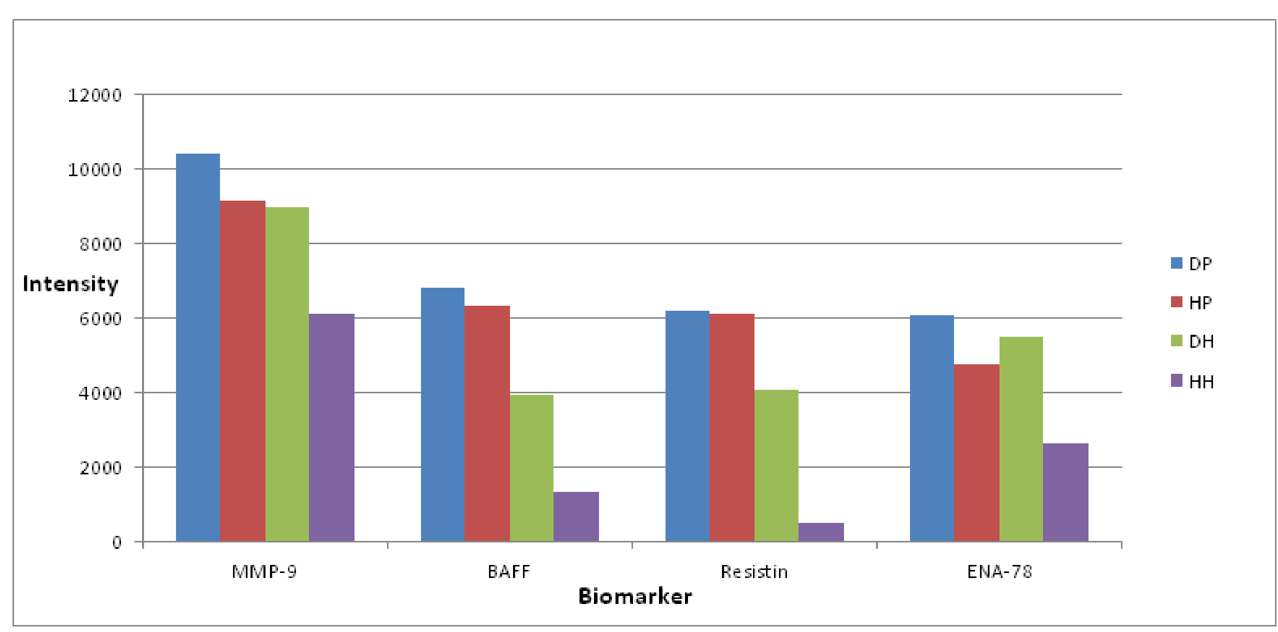


Figure 4.2: Bar graph presenting intensity values for selected candidate biomarkers.

X-axis represents the intensity of the signal produced on the nitrocellulose membrane and Y-axis represents selected biomarkers. DP; T1DM, periodontitis, HP; non-T1DM, periodontitis, DH; T1DM, periodontal health, HH; non-T1DM, periodontal health, MMP-9; matrix metalloproteinase-9, BAFF; B-cell activating factor, ENA-78; epithelial neutrophil activating peptide-78/CXCL5.

4.2.3 Verification methods

The accurate quantification of biomarkers in biological samples is largely dependent on the analytical technique used. The intra-assay precision, inter-assay precision, recovery and sensitivity data for serum samples for selected candidate biomarkers are quoted from the R&D systems product datasheet for each biomarker and are presented in Table 4.5, 4.6, 4.7 and 4.8. Recovery experiments were performed for resistin, as the recovery value for serum was not provided in the resistin product datasheet.

Intra-assay precision

To test the precision within an assay, three samples of known concentration were tested 20 times on one plate to determine intra-assay precision. The intra-assay variations for the calculated lower limit of detection (LLOD) for candidate biomarkers in serum are presented in Table 4.5.

	MMP-9 (ng/ml)	**BAFF (pg/ml)**	**Resistin (ng/ml)**	**ENA-78/CXCL5 (pg/ml)**	**IL-8 (pg/ml)**
n	20	20	20	20	20
Mean	0.833	433	0.60	113	168
SD	0.017	28.2	0.03	9.4	9.4
CV (%)	2.0	6.5	5.0	8.3	5.6

Table 4.5: Intra-assay variation of LLOD for Human Quantikine ELISA.

MMP-9; matrix metalloproteinase-9, BAFF; B-cell activating factor, ENA-78/CXCL5; epithelial neutrophil activating peptide-78, IL-8; interleukin-8, SD; standard deviation, CV; coefficient of variation.

Inter-assay precision

To test the precision between assays, three samples of known concentration were tested in 40 separate assays to determine inter-assay precision. The inter-assay variations for the calculated LLOD for candidate biomarkers in serum are presented in Table 4.6.

	MMP-9 (ng/ml)	BAFF (pg/ml)	Resistin (ng/ml)	ENA-78/CXCL5 (pg/ml)	IL-8 (pg/ml)
n	40	20	40	40	20
Mean	0.972	474	0.61	109	196
SD	0.077	46.8	0.05	10.1	14.5
CV (%)	7.9	9.9	8.2	9.3	7.4

Table 4.6: Inter-assay variation of LLOD for Human Quantikine ELISA.

MMP-9; matrix metalloproteinase-9, BAFF; B-cell activating factor, ENA-78/CXCL5; epithelial neutrophil activating peptide-78, IL-8; interleukin-8, SD; standard deviation, CV; coefficient of variation.

Recovery

The recovery experiment is important for analysing the accuracy of the ELISA for particular sample types. The resulting “recovery” of the spiked sample or the resulting concentration, demonstrates is the expected value can be accurately measured. The recovery experiment is important for analysing the accuracy of the ELISA for particular sample types. To determine the recovery for each assay for human serum samples, a serum sample is spiked with the human recombinant protein provided as standard in each ELISA kit and the % recovery of the protein is calculated in reference to an unspiked, neat serum sample run in the same assay. If the serum samples require dilution to fit within the range of the standard curve, the dilutions are treated as the new “neat” samples. A spiked reagent diluent is used as a control (control spike). The % recovery is calculated by using the formula: (assay result for spiked sample – assay result for neat sample) / (amount spiked) x 100 (Jaedicke et al. 2012). The recovery data for the selected candidate biomarkers are presented in Table 4.7.

	MMP-9	BAFF	Resistin	ENA-78/CXCL5	IL-8
n	5	4	1	5	5
Range (%)	91-99	84-106	-	93-109	88-106
Mean (%)	95	93	110	101	98

Table 4.7: Recovery for candidate biomarkers in serum.

The R&D systems recommend an acceptable recovery range of 80-120%. MMP-9; matrix metalloproteinase-9, BAFF; B-cell activating factor, ENA-78/CXCL5; epithelial neutrophil activating peptide-78, IL-8; interleukin-8.

Sensitivity

Sensitivity represents the smallest amount of substance in a sample which can accurately be measured by an assay (Saah and Hoover 1997), and which is statistically not equal to zero. To determine assay sensitivity, 20 zero standard replicates are run in one assay and sensitivity is calculated from the standard curve. Sensitivity is defined as the mean of the assay result for the 20 zero standard replicates + 2 standard deviations of the means (Jaedicke et al. 2012). The sensitivity data for the selected candidate biomarkers are presented in Table 4.8.

	MMP-9 (ng/ml)	BAFF (pg/ml)	Resistin (ng/ml)	ENA-78/CXCL5 (pg/ml)	IL-8 (pg/ml)
Range	-	1.01-6.44	0.010-0.055	-	1.5-7.5
Mean	< 0.156	2.68	0.026	< 15	3.5

Table 4.8: The minimum detectable dose of candidate biomarkers.

MMP-9; matrix metalloproteinase-9, BAFF; B-cell activating factor, ENA-78/CXCL5; epithelial neutrophil activating peptide-78, IL-8; interleukin-8.

4.2.4 Pre-treatment serum biomarker levels

Table 4.9 summarises pre-treatment serum levels of candidate biomarkers (MMP-9, BAFF, resistin and ENA-78/CXCL5) comparing T1DM and non-T1DM patients. The levels of MMP-9, resistin and ENA-78/CXCL5 were significantly higher in the T1DM patients (882.3±577.1 ng/ml, 11.3±4.78 ng/ml and 1664.0±886.8 pg/ml) compared to the non-T1DM patients (483.8±277.5 ng/ml, 8.98±3.25 ng/ml and 1296.3±805.6 pg/ml), ($P<0.001$, $P<0.01$ and $P<0.05$ respectively) (Figure 4.3, 4.4 and 4.5). The level of BAFF was higher in the

T1DM patients (1179.2±290.7 pg/ml) compared to the non-T1DM patients (1155.0±286.7 pg/ml), however this difference was not statistically significant ($P>0.05$).

Table 4.10 summarises pre-treatment serum candidate biomarker levels following further categorization of T1DM and non-T1DM patients based on periodontal diagnosis. While considering MMP-9 levels, the DP patients had significantly higher serum MMP-9 levels (1052.3±489.4 ng/ml) compared to the HP patients (502.2±272.2 ng/ml), ($P<0.001$). However, there were no statistically significant differences in serum MMP-9 levels in those with healthy tissues or gingivitis when comparing T1DM and non-T1DM patients. Within the T1DM group, although the DP patients had higher serum MMP-9 levels (1052.3±489.4 ng/ml) compared to the DG (796.1±652.7 ng/ml) and the DH (791.3±475.6 ng/ml) patients, this difference was not statistically significant, ($P>0.05$). Likewise, serum MMP-9 levels showed no statistically significant differences between the DG (796.1±652.7 ng/ml) and the DH (791.3±475.6 ng/ml) patients, ($P>0.05$). Similar findings were found within the non-T1DM group, where serum MMP-9 levels showed no statistically significant differences between the HH (437.5±233.0 ng/ml), HG (490.0±315.1 ng/ml) and HP (502.2±272.2 ng/ml) patients, ($P>0.05$) (Table 4.10 and Figure 4.6).

When considering serum BAFF levels, no statistically significant differences were found between T1DM and non-T1DM patients based on periodontal diagnosis. Within the T1DM group, no statistically significant differences were found between the DH (1089.0±143.8 pg/ml), DG (1199.7±279.0 pg/ml) and DP (1194.8±359.4 pg/ml) patients, ($P>0.05$). Likewise within the non-T1DM group, serum BAFF levels showed no statistically significant differences between the HH (1259.5±514.6 pg/ml), HG (1103.9±224.9 pg/ml) and HP (1150.7±154.3 pg/ml) patients, ($P>0.05$) (Table 4.10).

When considering serum resistin, the levels were significantly higher in the DG patients (11.1±5.37 ng/ml) compared to the HG patients (8.06±2.58 ng/ml), ($P<0.05$). Within the non-T1DM group, serum resistin levels were significantly higher in the HP patients (10.5±3.55 ng/ml) compared to the HG patients (8.06±2.58 ng/ml), ($P<0.05$). No statistically significant difference was found for serum resistin levels between the HH (7.88±3.01 ng/ml), HG (8.06±2.58 ng/ml) and HP (10.5±3.55 ng/ml) patients, ($P>0.05$). Within the T1DM group, serum resistin levels showed no statistically significant differences between the DH (11.0±4.70 ng/ml), DG (11.1±5.37 ng/ml) and DP (11.7±4.09 ng/ml) patients, ($P>0.05$) (Table 4.10 and Figure 4.7).

While considering serum ENA-78/CXCL5, the levels were significantly higher in the DG patients (1607.3±754.0 pg/ml) compared to the HG patients (1140.3±696.8 pg/ml), ($P<0.05$). Although the DH (1859.1±970.4 pg/ml) and DP (1648.4±1049.2 pg/ml) patients had higher levels of ENA-78/CXCL5 compared to the HH (1216.5±513.7 pg/ml) and HP (1494.5±1007.4 pg/ml) patients, these differences were not statistically significant, ($P>0.05$). Within the T1DM group, serum ENA-78/CXCL5 levels showed no statistically significant differences between the DH (1859.1±970.4 pg/ml), DG (1607.3±754.0 pg/ml) and DP (1648.4±1049.2 pg/ml) patients, ($P>0.05$). Similarly, within the non-T1DM group, serum ENA-78/CXCL5 levels showed no statistically significant differences between the HH (1216.5±513.7 pg/ml), HG (1140.3±696.8 pg/ml) and HP (1494.5±1007.4 pg/ml) patients, ($P>0.05$) (Table 4.10 and Figure 4.8).

	T1DM (n=53)	Non-T1DM (n=43)	*P*
Serum MMP-9 (ng/ml)	882.3 ± 577.1	483.8 ± 277.5	< 0.001
Serum BAFF (pg/ml)	1179.2 ± 290.7	1155.0 ± 286.7	NS
Serum resistin (ng/ml)	11.3 ± 4.78	8.98 ± 3.25	< 0.01
Serum ENA-78/CXCL5 (pg/ml)	1664.0 ± 886.8	1296.3 ± 805.6	< 0.05

Table 4.9: Pre-treatment serum biomarker levels comparing T1DM and non-T1DM patients.

Mean ± SD presented for parametric data. P-values determined using Independent t-test for continuous parametric variables. *P* indicates significant difference between T1DM and non-T1DM patients. MMP-9; matrix metalloproteinase-9, BAFF; B-cell activating factor, ENA-78/CXCL5; epithelial neutrophil activating peptide-78, NS; not significant.

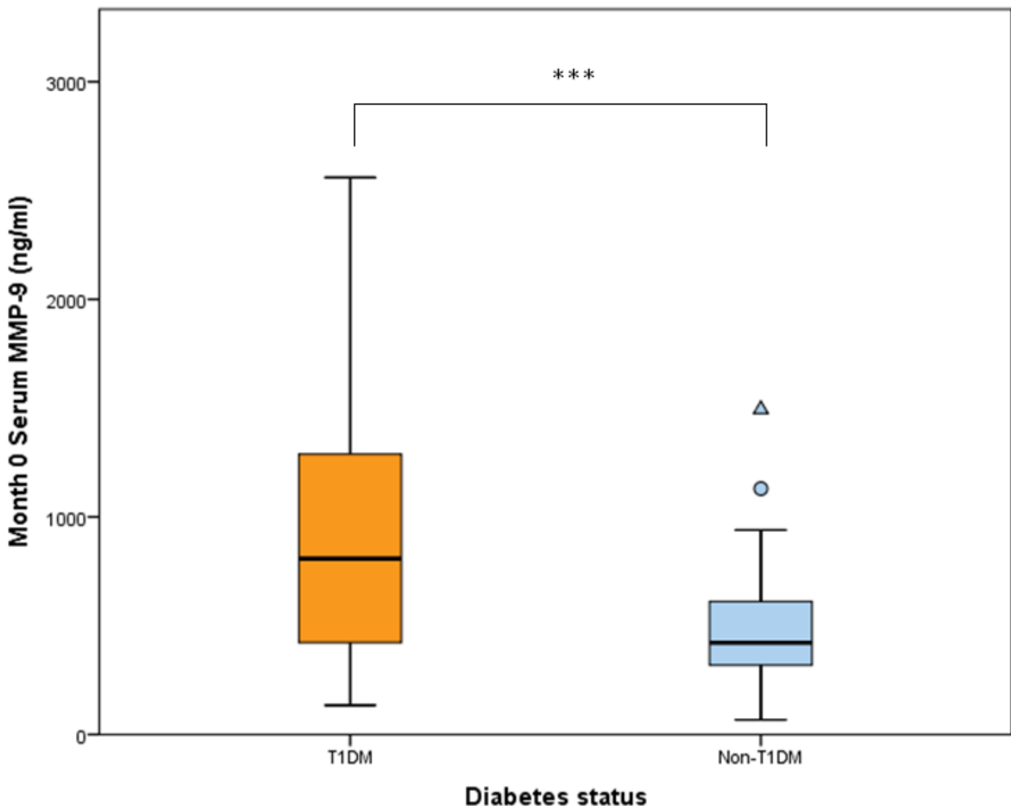


Figure 4.3: Pre-treatment serum MMP-9 levels comparing T1DM and non-T1DM patients.

Box plot figure showing medians, interquartile ranges, standard errors and outliers for T1DM (n=53) and non-T1DM (n=43) patients. Statistics: Independent t-test: ***P<0.001. ● indicates outlier more than 1.5 but less than 3 times the IQR from the box boundaries, ▲ indicates outlier more than 3 times the IQR from the box boundaries.

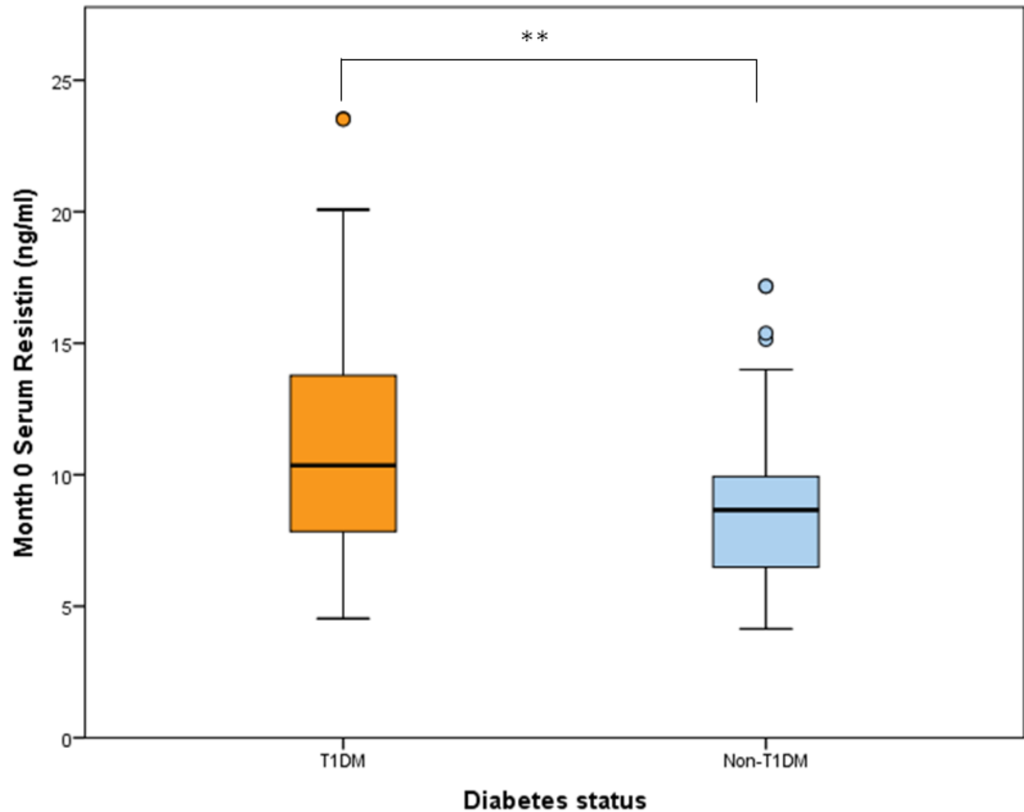


Figure 4.4: Pre-treatment serum resistin levels comparing T1DM and non-T1DM patients.

Box plot figure showing medians, interquartile ranges, standard errors and outliers for T1DM (n=53) and non-T1DM (n=43) patients. Statistics: Independent t-test: ***P*<0.01.
● indicates outlier more than 1.5 but less than 3 times the IQR from the box boundaries.

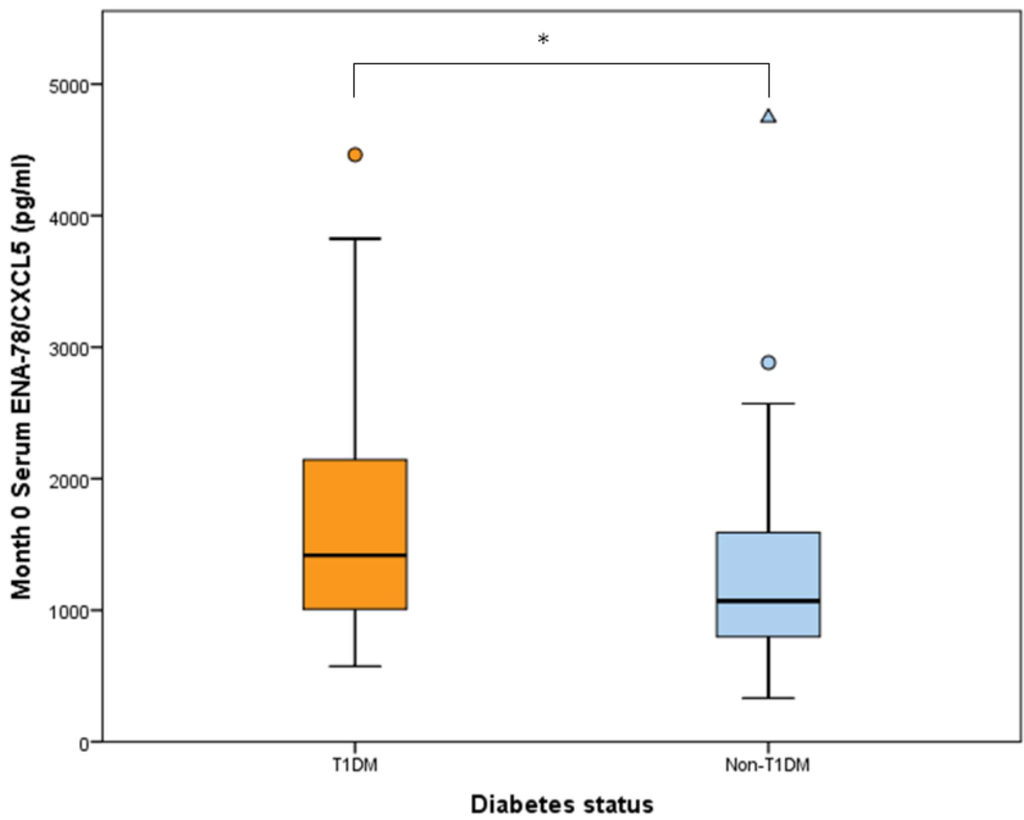


Figure 4.5: Pre-treatment serum ENA-78/CXCL5 levels comparing T1DM and non-T1DM patients.

Box plot figure showing medians, interquartile ranges, standard errors and outliers for T1DM (n=53) and non-T1DM (n=43) patients. Statistics: Independent t-test: *P<0.05. ● indicates outlier more than 1.5 but less than 3 times the IQR from the box boundaries, ▲ indicates outlier more than 3 times the IQR from the box boundaries.

		Health (DH n=9) (HH n=9)	**Gingivitis (DG n=26) (HG n=17)**	**Periodontitis (DP n=18) (HP n=17)**	***P****
Serum MMP-9 (ng/ml)	**T1DM**	791.3 ± 475.6	796.1 ± 652.7	1052.3 ± 489.4	NS
	Non-T1DM	437.5 ± 233.0	490.0 ± 315.1	502.2 ± 272.2	NS
	P	NS	NS	< 0.001	
Serum BAFF (pg/ml)	**T1DM**	1089.0 ± 143.8	1199.7 ± 279.0	1194.8 ± 359.4	NS
	Non-T1DM	1259.5 ± 514.6	1103.9 ± 224.9	1150.7 ± 154.3	NS
	P	NS	NS	NS	
Serum resistin (ng/ml)	**T1DM**	11.0 ± 4.70	11.1 ± 5.37	11.7 ± 4.09	NS
	Non-T1DM	7.88 ± 3.01	8.06 ± 2.58	10.5 ± 3.55 †	< 0.05
	P	NS	< 0.05	NS	
Serum ENA-78/CXCL5 (pg/ml)	**T1DM**	1859.1 ± 970.4	1607.3 ± 754.0	1648.4 ± 1049.2	NS
	Non-T1DM	1216.5 ± 513.7	1140.3 ± 696.8	1494.5 ± 1007.4	NS
	P	NS	< 0.05	NS	

Table 4.10: Pre-treatment serum biomarker levels comparing T1DM and non-T1DM groups based on periodontal diagnosis.

Mean ± SD presented for parametric data. P-values determined using One-way ANOVA with post-hoc Independent t-test for continuous parametric variables. *P** indicates overall p-value comparing across periodontal categories within T1DM or non-T1DM groups. P-values under columns (*P*) relate to comparisons between T1DM and non-T1DM groups. †*P*<0.05 indicates statistically significant differences compared to gingivitis within the non-T1DM group. DH; T1DM, periodontal health, DG; T1DM, gingivitis, DP; T1DM, periodontitis, HH; non-T1DM, periodontal health, HG; non-T1DM, gingivitis, HP; non-T1DM, periodontitis. MMP-9; matrix metalloproteinase-9, BAFF; B-cell activating factor, ENA-78/CXCL5; epithelial neutrophil activating peptide-78, NS; not significant.

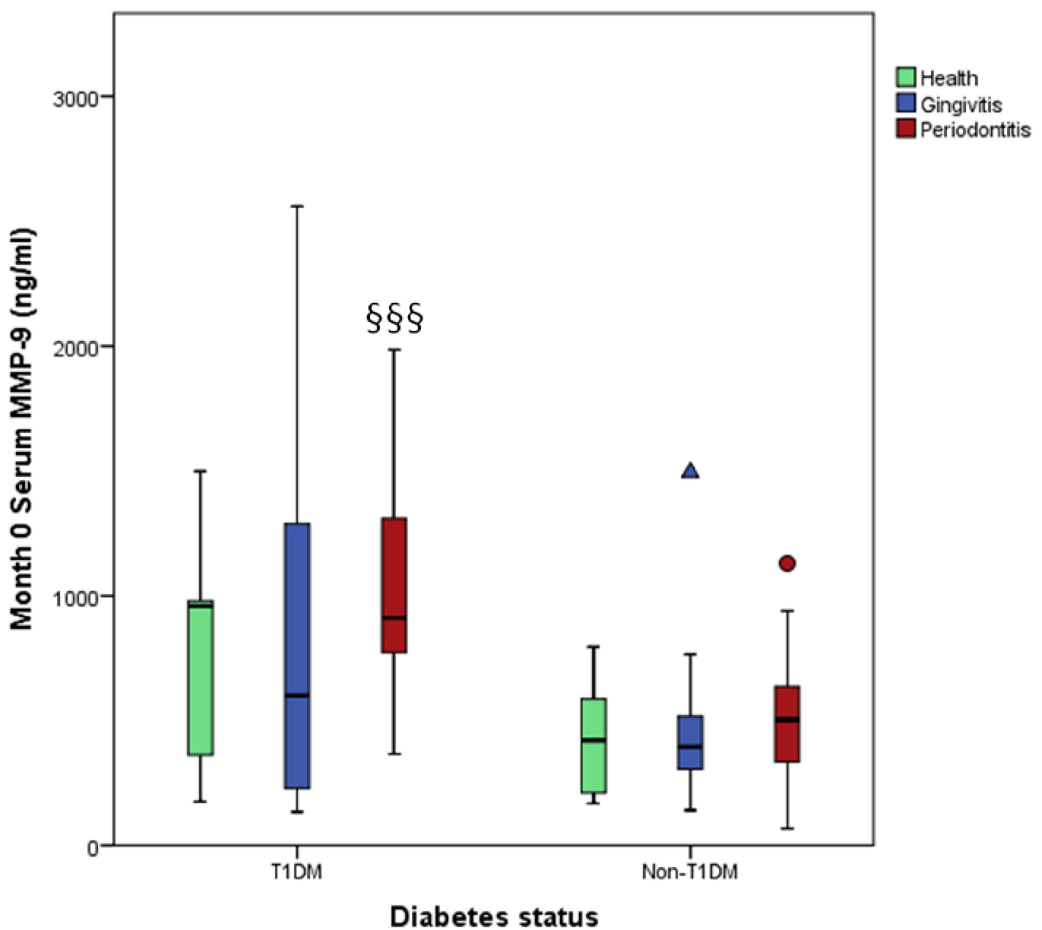


Figure 4.6: Pre-treatment serum MMP-9 levels comparing T1DM and non-T1DM patients based on periodontal diagnosis.

Box plot figure showing medians, interquartile ranges, standard errors and outliers for 53 T1DM patients (health n=9, gingivitis n=26 and periodontitis n=18) and 43 non-T1DM patients (health n=9, gingivitis n=17 and periodontitis n=17). Statistics: One-way ANOVA with post-hoc Independent t-test: §§§P<0.001 (T1DM versus non-T1DM group within the corresponding periodontal status). ● indicates outlier more than 1.5 but less than 3 times the IQR from the box boundaries, ▲ indicates outlier more than 3 times the IQR from the box boundaries.

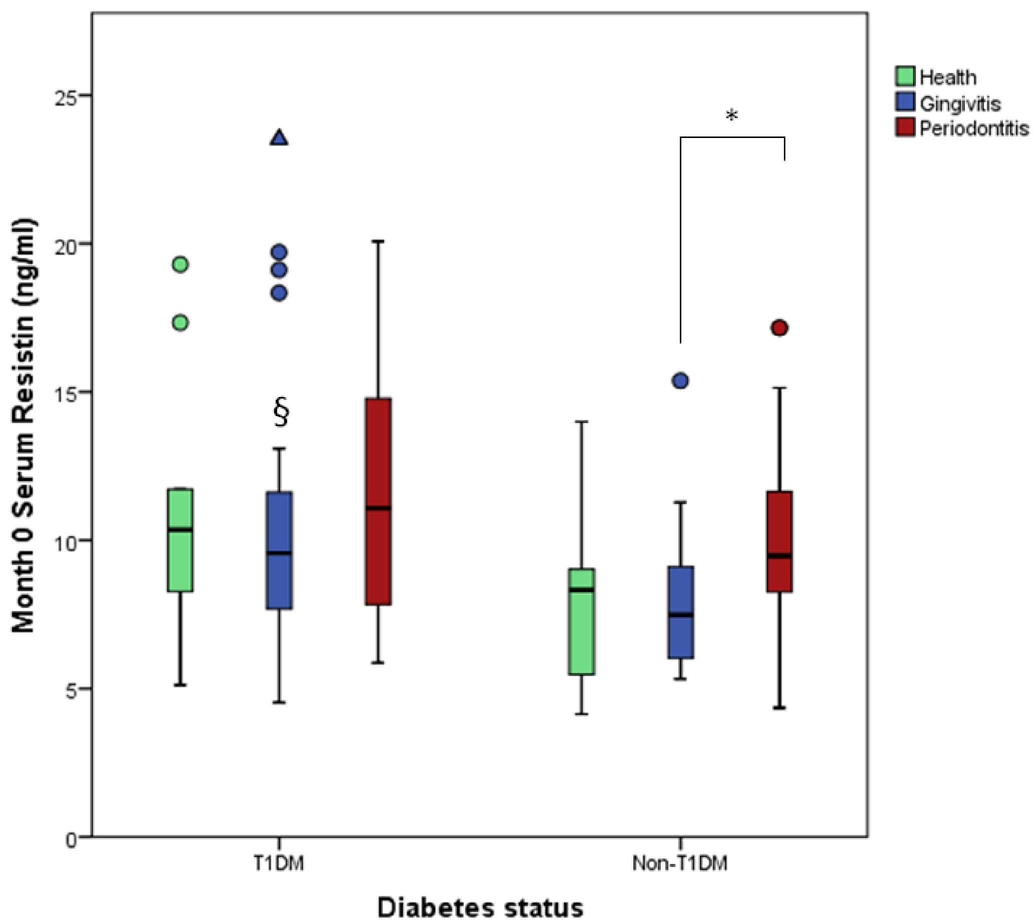


Figure 4.7: Pre-treatment serum resistin levels comparing T1DM and non-T1DM patients based on periodontal diagnosis.

Box plot figure showing medians, interquartile ranges, standard errors and outliers for 53 T1DM patients (health n=9, gingivitis n=26 and periodontitis n=18) and 43 non-T1DM patients (health n=9, gingivitis n=17 and periodontitis n=17). Statistics: One-way ANOVA with post-hoc Independent t-test: **P*<0.05 (according to periodontal status within the non-T1DM group); §*P*<0.05 (T1DM versus non-T1DM group within the corresponding periodontal status). ● indicates outlier more than 1.5 but less than 3 times the IQR from the box boundaries, ▲ indicates outlier more than 3 times the IQR from the box boundaries.

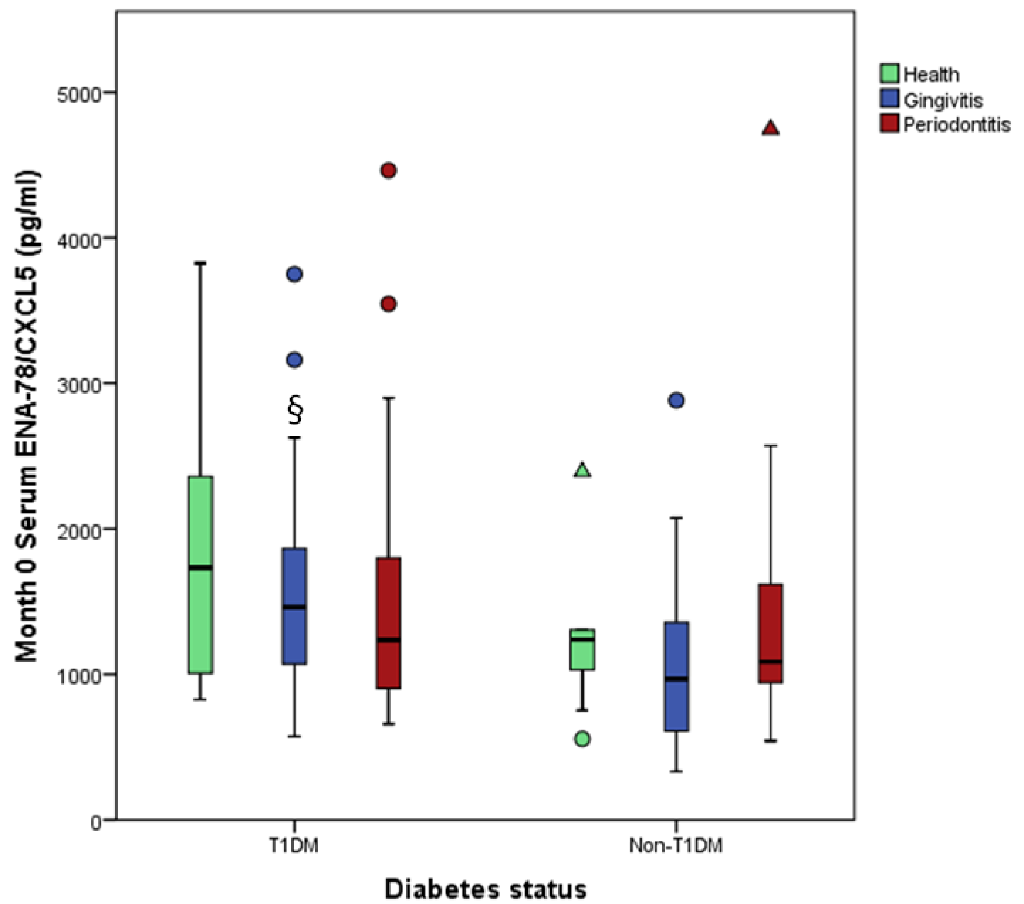


Figure 4.8: Pre-treatment serum ENA-78/CXCL5 levels comparing T1DM and non-T1DM patients based on periodontal diagnosis.

Box plot figure showing medians, interquartile ranges, standard errors and outliers for 53 T1DM patients (health n=9, gingivitis n=26 and periodontitis n=18) and 43 non-T1DM patients (health n=9, gingivitis n=17 and periodontitis n=17). Statistics: One-way ANOVA with post-hoc Independent t-test: §P<0.05 (T1DM versus non-T1DM group within the corresponding periodontal status). ● indicates outlier more than 1.5 but less than 3 times the IQR from the box boundaries, ▲ indicates outlier more than 3 times the IQR from the box boundaries.

Based on the ELISA results of the candidate biomarker analysis in pre-treatment serum samples, there is some evidence for differences in the levels of these potential biomarkers: MMP-9, resistin and ENA-78/CXCL5 between the clinical groups. Although, serum resistin was found to be significantly higher in the T1DM patients compared to the non-T1DM patients, when correlations were performed, serum resistin levels had a significant positive correlation with the BMI of T1DM patients (Spearman's rho=0.36, $P<0.01$), suggesting that higher the BMI value, higher the levels of resistin. Interestingly, serum resistin levels were not significantly correlated with the BMI in non-T1DM patients (Spearman's rho=-0.08, $P>0.05$). Therefore, resistin may not be an ideal biomarker in establishing the link between T1DM and periodontal disease, as obesity is not a cardinal finding in patients with T1DM. Due to the limited volume of GCF samples available and considering the serum resistin results in this study, it was decided not to further analyse resistin in the GCF samples. The analysis of serum BAFF failed to provide any evidence to suggest any differences in levels of BAFF between the clinical groups that were certainly significant. Hence, it was decided to not analyse BAFF in the GCF samples collected in the study as there was a limited volume of GCF available.

Following the cytokine array analysis, a screening ELISA experiment was carried out for ENA-78/CXCL5 and IL-8 using serum and GCF samples from 20 selected patients based on diabetes status and periodontal diagnosis (DP n=5, HP n=5, DH n=5 and HH n=5 samples). IL-8 is a chemokine with a similar function to ENA-78/CXCL5 and has been associated with various inflammatory conditions such as periodontal disease and T1DM. Interestingly, ENA-78/CXCL5 was detected only in the serum samples and not in the GCF samples. Also, IL-8 was detected only in the GCF samples and not in the serum samples. This possibly explains why IL-8 was not detected during the cytokine array experiment. The analysis of the IL-8 ELISA experiment revealed higher GCF IL-8 levels in the DP patients compared to the HP patients. Hence, it was decided to further analyse the GCF samples only for IL-8 and MMP-9 levels. The verification methods for IL-8 are presented in Table 4.5 to 4.8.

4.2.5 Pre-treatment GCF biomarker levels

Table 4.11 summarises pre-treatment levels of candidate biomarkers MMP-9 and IL-8 in GCF samples and GCF volume data for patients with and without T1DM. GCF MMP-9 levels were higher in T1DM patients (189.0±146.2 ng/ml) compared to MMP-9 levels in non-T1DM patients (175.8±167.8 ng/ml), however, this difference was not statistically

significant, (*P*>0.05). Similarly, GCF IL-8 levels were higher in T1DM patients (323.7±224.4 pg/ml) compared to IL-8 levels in non-T1DM patients (286.1±296.8 pg/ml), however, this difference was not statistically significant, (*P*>0.05). With reference to GCF volume, no statistically significant difference was found for the amount of GCF collected in T1DM patients (0.48±0.24 µl) compared to non-T1DM patients (0.40±0.22 µl), (*P*>0.05).

Table 4.12 presents pre-treatment GCF candidate biomarker levels and GCF volume following further categorization of T1DM and non-T1DM patients based on periodontal diagnosis. With reference to GCF MMP-9 levels, the DH patients had significantly higher MMP-9 levels (126.2±76.6 ng/ml) compared to the HH patients (63.1±36.0 ng/ml), (*P*<0.05). The DG patients had higher MMP-9 levels (130.6±65.2 ng/ml) compared to the HG patients (108.3±60.3 ng/ml), however, this difference was not statistically significant, (*P*>0.05). Similarly, no statistically significant differences were found for GCF MMP-9 levels between the DP patients (304.8±186.8 ng/ml) and the HP patients (303.0±201.1 ng/ml), (*P*>0.05). Within the T1DM group, the DP patients had significantly higher GCF MMP-9 levels (304.8±186.8 ng/ml) compared to the DH (126.2±76.6 ng/ml), and the DG (130.6±65.2 ng/ml) patients, (*P*<0.001). Although the DG patients (130.6±65.2 ng/ml) had higher GCF MMP-9 levels compared to the DH patients (126.2±76.6 ng/ml), this difference was not statistically significant, (*P*>0.05). Within the non-T1DM group, the HG patients had significantly higher GCF MMP-9 levels (108.3±60.3 ng/ml) compared to the HH patients (63.1±36.0 ng/ml), (*P*<0.05). Also the HP patients had significantly higher GCF MMP-9 levels (303.0±201.1 ng/ml) compared to the HH (63.1±36.0 ng/ml) and HG (108.3±60.3 ng/ml) patients, (*P*<0.001) (Table 4.12 and Figure 4.9).

With reference to GCF IL-8 levels, the DH patients had significantly higher GCF IL-8 levels (235.1±157.7 pg/ml) compared to the HH patients (102.7±68.2 pg/ml), (*P*<0.05). The DG patients had higher GCF IL-8 levels (320.7±245.9 pg/ml) compared to the HG patients (226.4±167.0 pg/ml), however, this difference was not statistically significant, (*P*>0.05). Although, the HP patients had higher GCF IL-8 levels (442.9±390.2 pg/ml) compared to the DP patients (370.1±214.2 pg/ml) this difference was not statistically significant, (*P*>0.05). Within the T1DM group, the DP patients had higher GCF IL-8 levels (370.1±214.2 pg/ml) compared to the DG (320.7±245.9 pg/ml) and DH (235.1±157.7 pg/ml) patients, however these differences were not statistically significant, (*P*>0.05). Within the non-T1DM group, the HP patients had significantly higher GCF IL-8 levels (442.9±390.2 pg/ml) compared to the HH (102.7±68.2 pg/ml) and HG (226.4±167.0 pg/ml) patients, (*P*<0.01 and *P*<0.05

respectively). Also the HG patients had significantly higher GCF IL-8 levels (226.4±167.0 pg/ml) compared to the HH patients (102.7±68.2 pg/ml), (*P*<0.01) (Table 4.12 and Figure 4.10).

With reference to GCF volume, although the DH (0.35±0.12 µl), DG (0.40±0.16 µl) and DP (0.66±0.26 µl) patients had higher GCF volume compared to the HH (0.19±0.08 µl), HG (0.32±0.16 µl) and HP (0.60±0.16 µl) patients, these differences were not statistically significant, (*P*>0.05). Within the T1DM group, the DP patients (0.66±0.26 µl) had significantly higher GCF volume compared to the DG (0.40±0.16 µl) and DH (0.35±0.12 µl) patients, (*P*<0.001). Similar findings were seen within the non-T1DM group, the HP patients (0.60±0.16 µl) had significantly higher GCF volume compared to the HG (0.32±0.16 µl) and HH (0.19±0.08 µl) patients, (*P*<0.001). Additionally, the HG patients (0.32±0.16 µl) had significantly higher GCF volume compared to the HH patients (0.19±0.08 µl), (*P*<0.01) (Table 4.12 and Figure 4.11).

	T1DM (n=56)	**Non-T1DM (n=43)**	***P***
GCF MMP-9 (ng/ml)	189.0 ± 146.2	175.8 ± 167.8	NS
GCF IL-8 (pg/ml)	323.7 ± 224.4	286.1 ± 296.8	NS
GCF volume (µl)	0.48 ± 0.24	0.40 ± 0.22	NS

Table 4.11: Pre-treatment GCF biomarker levels and GCF volume comparing T1DM and non- T1DM patients.

Mean ± SD presented for parametric data. P-values determined using Independent t-test for continuous parametric variables. *P* indicates significant difference between T1DM and non-T1DM patients. GCF; gingival crevicular fluid, MMP-9; matrix metalloproteinase-9, IL-8; interleukin-8, NS; not significant.

		Health (DH n=9) (HH n=9)	Gingivitis (DG n=28) (HG n=17)	Periodontitis (DP n=19) (HP n=17)	*P**
GCF MMP-9 (ng/ml)	**T1DM**	126.2 ± 76.6	130.6 ± 65.2	304.8 ± 186.8 ***, †††	< 0.001
	Non-T1DM	63.1 ± 36.0	108.3 ± 60.3 *	303.0 ± 201.1 ***, †††	< 0.001
	P	< 0.05	NS	NS	
GCF IL-8 (pg/ml)	**T1DM**	235.1 ± 157.7	320.7 ± 245.9	370.1 ± 214.2	NS
	Non-T1DM	102.7 ± 68.2	226.4 ± 167.0 **	442.9 ± 390.2 **, †	< 0.01
	P	< 0.05	NS	NS	
GCF volume (µl)	**T1DM**	0.35 ± 0.12	0.40 ± 0.16	0.66 ± 0.26 ***, †††	< 0.001
	Non-T1DM	0.19 ± 0.08	0.32 ± 0.16 **	0.60 ± 0.16 ***, †††	< 0.001
	P	NS	NS	NS	

Table 4.12: Pre-treatment GCF biomarker levels and GCF volume comparing T1DM and non-T1DM groups based on periodontal diagnosis.

Mean ± SD presented for parametric data. P-values determined using One-way ANOVA with post-hoc Independent t-test for continuous parametric variables. *P** indicates overall p-value comparing across periodontal categories within T1DM or non-T1DM groups. P-values under columns (*P*) relate to comparisons between T1DM and non-T1DM group. *$P<0.05$, **$P<0.01$, ***$P<0.001$ indicates statistically significant differences compared to health, and †$P<0.05$, †††$P<0.001$ indicates statistically significant differences compared to gingivitis within the T1DM and non-T1DM groups. DH; T1DM, periodontal health, DG; T1DM, gingivitis, DP; T1DM, periodontitis, HH; non-T1DM, periodontal health, HG; non-T1DM, gingivitis, HP; non-T1DM, periodontitis. GCF; gingival crevicular fluid, MMP-9; matrix metalloproteinase-9, IL-8; interleukin-8, NS; not significant.

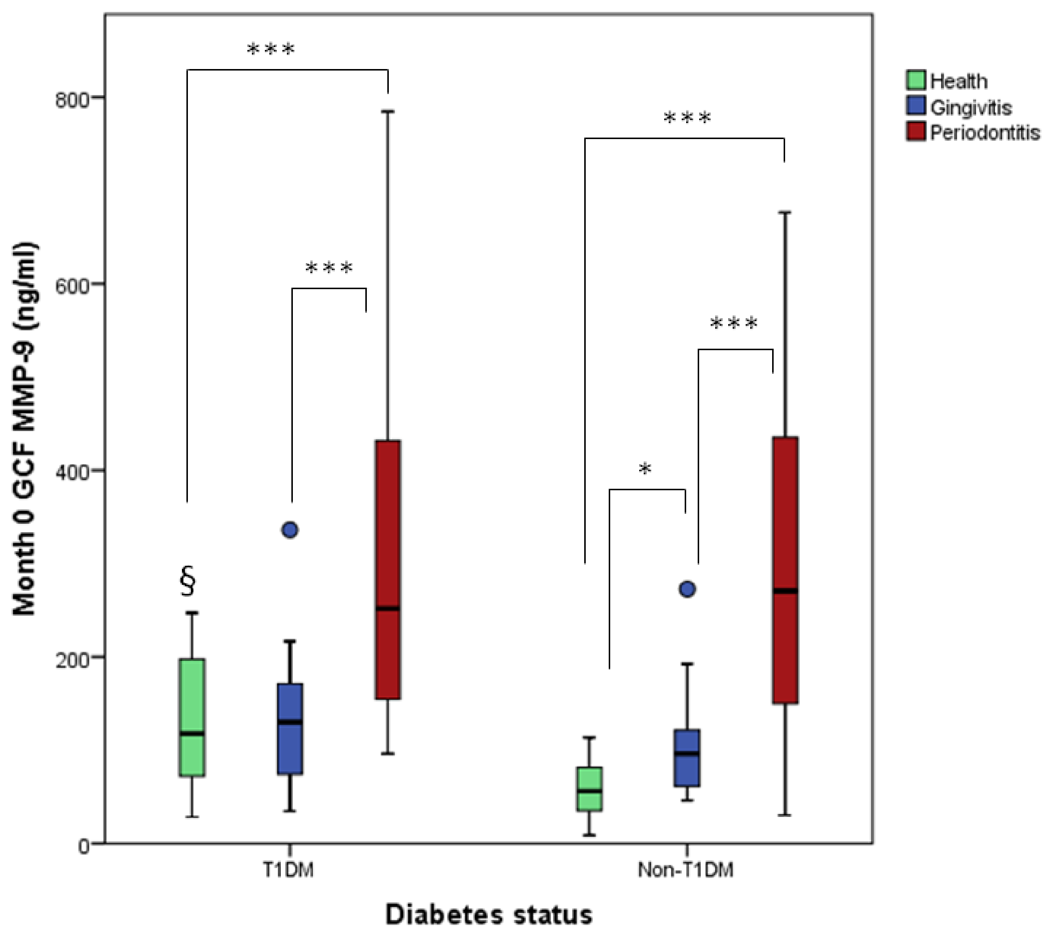


Figure 4.9: Pre-treatment GCF MMP-9 levels comparing T1DM and non-T1DM patients based on periodontal diagnosis.

Box plot figure showing medians, interquartile ranges, standard errors and outliers for 53 T1DM patients (health n=9, gingivitis n=26 and periodontitis n=18) and 43 non-T1DM patients (health n=9, gingivitis n=17 and periodontitis n=17). Statistics: One-way ANOVA with post-hoc Independent t-test: **P*<0.05, ****P*<0.001 (according to periodontal status within the T1DM or non-T1DM group); §*P*<0.05 (T1DM versus non-T1DM group within the corresponding periodontal status). ● indicates outlier more than 1.5 but less than 3 times the IQR from the box boundaries.

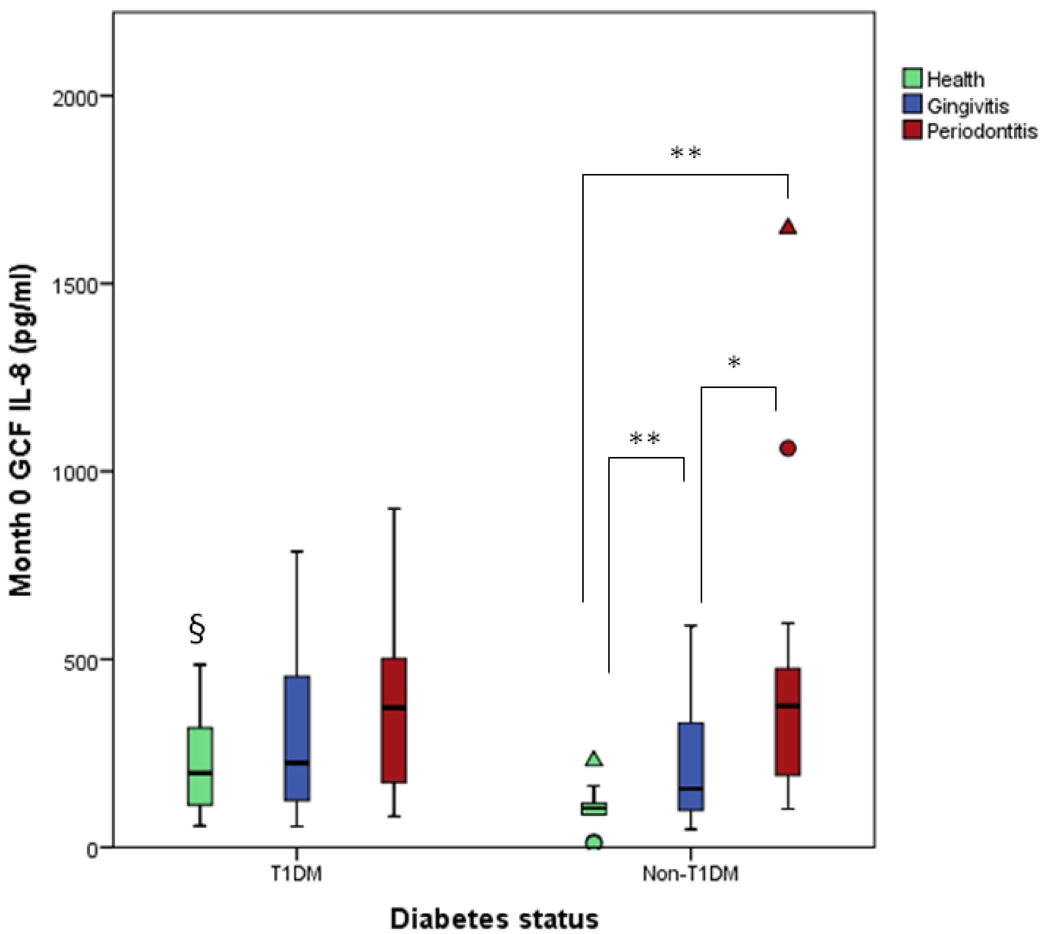


Figure 4.10: Pre-treatment GCF IL-8 levels comparing T1DM and non-T1DM patients based on periodontal diagnosis.

Box plot figure showing medians, interquartile ranges, standard errors and outliers for 53 T1DM patients (health n=9, gingivitis n=26 and periodontitis n=18) and 43 non-T1DM patients (health n=9, gingivitis n=17 and periodontitis n=17). Statistics: One-way ANOVA with post-hoc Independent t-test: *P<0.05, **P<0.01 (according to periodontal status within the non-T1DM group); §P<0.05 (T1DM versus non-T1DM group within the corresponding periodontal status). ● indicates outlier more than 1.5 but less than 3 times the IQR from the box boundaries, ▲ indicates outlier more than 3 times the IQR from the box boundaries.

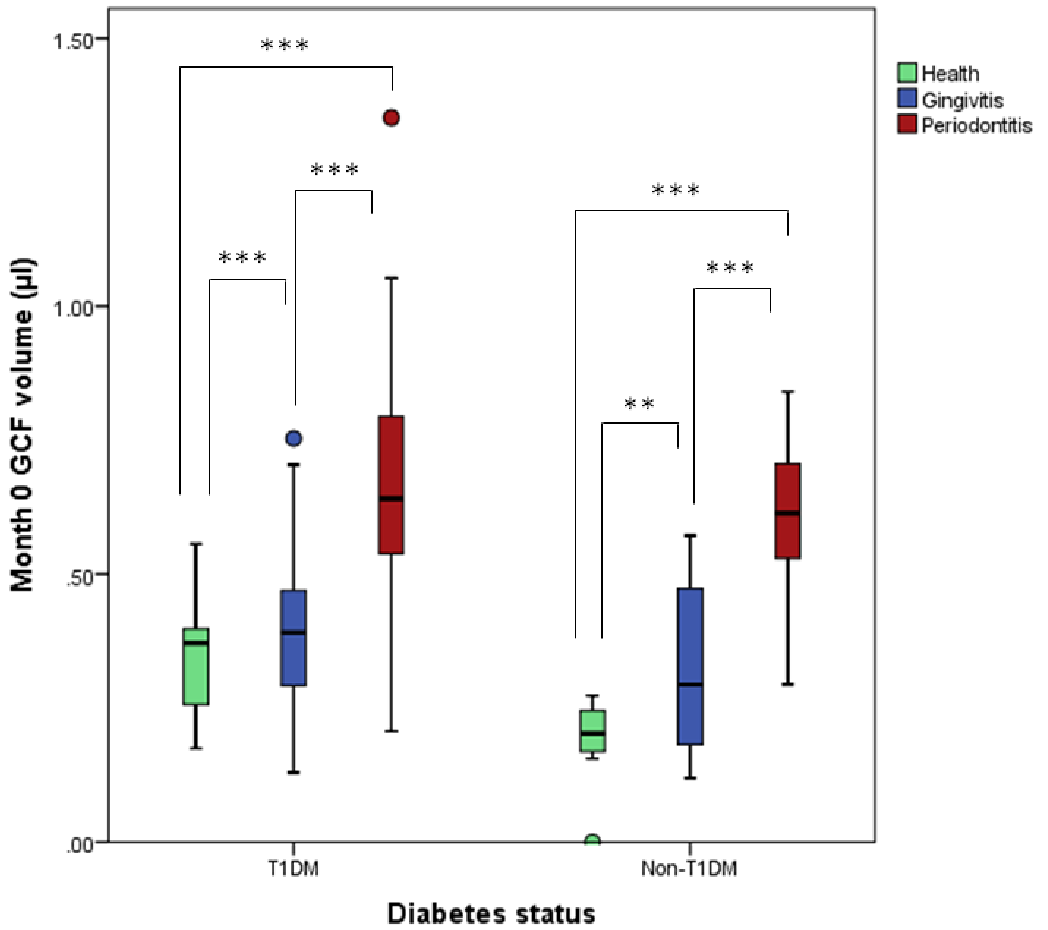


Figure 4.11: Pre-treatment GCF volume comparing T1DM and non-T1DM patients based on periodontal diagnosis.

Box plot figure showing medians, interquartile ranges, standard errors and outliers for 53 T1DM patients (health n=9, gingivitis n=28 and periodontitis n=19) and 43 non-T1DM patients (health n=9, gingivitis n=16 and periodontitis n=16. Statistics: One-way ANOVA with post-hoc Independent t-test: **P<0.01, ***P<0.001 (according to periodontal status within the T1DM or non-T1DM group). ● indicates outlier more than 1.5 but less than 3 times the IQR from the box boundaries.

4.2.6 Exploration of the association between clinical periodontal parameters, biomarker levels and clinical markers of diabetes control and inflammation

All Spearman's rank correlation coefficients (rho) were first undertaken using data for all patients (T1DM and non-T1DM), and were then repeated taking diabetes status into account.

Association between HbA1c and hsCRP and clinical periodontal parameters

While considering all patients (T1DM and non-T1DM), a significant positive (medium) correlation was demonstrated between HbA1c levels and PI (Spearman's rho=0.34, $P<0.01$) (Cohen 1988). All other correlations between HbA1c levels and mGI, % BOP and mean PD were not statistically significant (Table 4.13). When the data were split according to diabetes status, in T1DM patients, a significant positive (medium) correlation was demonstrated between HbA1c levels and PI (Spearman's rho=0.30, $P<0.05$). All other correlations for T1DM patients were not statistically significant (Table 4.14). In non-T1DM patients, no statistically significant correlations were found between HbA1c levels and mGI, PI, % BOP and mean PD (Table 4.15).

While considering all patients and when data were split according to diabetes status, no statistically significant correlations were found between hsCRP levels and mGI, PI, % BOP and mean PD (Table 4.13, 4.14 and 4.15).

	Spearman's Rank Correlation Coefficient	
	HbA1c (% & mmol/mol)	hsCRP (mg/mL)
mGI	-0.00	0.13
PI	0.34 ***	0.13
BOP (%)	0.07	0.12
Mean PD (mm)	0.05	0.11

Table 4.13: Correlations between HbA1c and hsCRP levels and clinical periodontal parameters for all patients.

	Spearman's Rank Correlation Coefficient	
	HbA1c (% & mmol/mol)	hsCRP (mg/mL)
mGI	0.26	0.03
PI	0.30 *	0.02
BOP (%)	0.21	0.02
Mean PD (mm)	0.25	0.03

Table 4.14: Correlations between HbA1c and hsCRP levels and clinical periodontal parameters for T1DM patients.

	Spearman's Rank Correlation Coefficient	
	HbA1c (% & mmol/mol)	hsCRP (mg/mL)
mGI	-0.20	0.25
PI	-0.08	0.24
BOP (%)	-0.20	0.24
Mean PD (mm)	-0.14	0.20

Table 4.15: Correlations between HbA1c and hsCRP levels and clinical periodontal parameters for non-T1DM patients.

Tables 4.13, 4.14 and 4.15 showing Spearman's rho highlighted with colour indicates strength of correlation: small (r=0.10 to 0.29), medium (r=0.30 to 0.49) and large (r=0.50 to 1.00) (Cohen 1988). Significant correlation between clinical parameters and biochemistry parameters: $*P<0.05$ and $***P<0.001$. mGI; modified gingival index, PI; plaque index, BOP; bleeding on probing, PD; probing depth, HbA1c; glycated haemoglobin, hsCRP; high-sensitivity C-reactive protein.

Association between HbA1c and hsCRP levels and candidate biomarker levels in serum and GCF

While considering all patients (T1DM and non-T1DM), a significant positive (small) correlation was demonstrated between HbA1c and serum MMP-9 (Spearman's rho=0.23, $P<0.05$) and ENA-78/CXCL5 (Spearman's rho=0.27, $P<0.01$) levels. All other correlations between HbA1c and serum biomarker levels were not statistically significant (Table 4.16). When the data were split according to diabetes status, in T1DM patients, no statistically significant correlations were found between HbA1c and serum MMP-9, BAFF, resistin and ENA-78/CXCL5 levels (Table 4.17). In non-T1DM patients, a significant positive (medium) correlation was demonstrated between HbA1c and serum BAFF levels (Spearman's rho=0.33, $P<0.05$). All other correlations between HbA1c and serum biomarker levels were not statistically significant (Table 4.18). Additionally, while considering all patients and T1DM patients, no statistically significant correlations were found between HbA1c and GCF MMP-9 and IL-8 levels (Table 4.16 and 4.17). In non-T1DM patients, a significant negative (medium) correlation was demonstrated between HbA1c and GCF MMP-9 levels (Spearman's rho=-0.34, $P<0.05$) (Table 4.18).

While considering all patients (T1DM and non-T1DM), a significant positive (medium) correlation was demonstrated between hsCRP and serum MMP-9 levels (Spearman's rho=0.30, $P<0.01$). All other correlations between hsCRP and serum biomarker levels were not statistically significant (Table 4.16). When the data were split according to diabetes status, in T1DM patients, a significant positive (medium) correlation was demonstrated between hsCRP and serum MMP-9 levels (Spearman's rho=0.36, $P<0.01$). All other correlations between hsCRP and serum biomarker levels in T1DM patients were not statistically significant (Table 4.17). In non-T1DM patients, no statistically significant correlations were found between hsCRP and serum biomarker levels (Table 4.18).

Additionally, while considering all patients and when data were split according to diabetes status no statistically significant correlations were found between hsCRP and GCF MMP-9 and IL-8 levels (Table 4.16, 4.17 and 4.18).

	Spearman's Rank Correlation Coefficient					
	Serum biomarkers				GCF biomarkers	
	MMP-9	BAFF	Resistin	ENA-78	MMP-9	IL-8
HbA1c (% & mmol/mol)	0.23 *	0.11	0.19	0.27 **	0.06	0.10
hsCRP (mg/L)	0.30 **	0.21	0.16	-0.08	0.12	0.10

Table 4.16: Correlations of HbA1c and hsCRP levels and biomarker levels in serum and GCF for all patients.

	Spearman's Rank Correlation Coefficient					
	Serum biomarkers				GCF biomarkers	
	MMP-9	BAFF	Resistin	ENA-78	MMP-9	IL-8
HbA1c (% & mmol/mol)	-0.25	0.04	-0.07	0.10	0.09	0.03
hsCRP (mg/L)	0.36 *	0.22	0.27	-0.18	-0.01	-0.02

Table 4.17: Correlations of HbA1c and hsCRP levels and biomarker levels in serum and GCF for T1DM patients.

	Spearman's Rank Correlation Coefficient					
	Serum biomarkers				GCF biomarkers	
	MMP-9	BAFF	Resistin	ENA-78	MMP-9	IL-8
HbA1c (% & mmol/mol)	-0.07	0.33 *	-0.07	0.21	-0.34 *	-0.20
hsCRP (mg/L)	0.07	0.20	-0.03	-0.11	-0.28	0.25

Table 4.18: Correlations of HbA1c and hsCRP levels and biomarker levels in serum and GCF for non-T1DM patients.

Tables 4.16, 4.17 and 4.18 showing Spearman's rho with colour indicates strength of correlation: small (r=0.10 to 0.29), medium (r=0.30 to 0.49) and large (r=0.50 to 1.00) (Cohen 1988). Significant correlation between biochemistry parameters and biomarkers: *P<0.05 and **P<0.01. HbA1c; glycated haemoglobin, hsCRP; high-sensitivity C-reactive protein, MMP-9; matrix metalloproteinase-9, BAFF; B-cell activating factor, ENA-78/CXCL5; epithelial neutrophil activating peptide-78, IL-8; interleukin-8, GCF; gingival crevicular fluid.

Association between candidate biomarker levels in serum and GCF and clinical periodontal parameters

Whether considering all patients or when data were split according to diabetes status, no statistically significant correlations were found between serum MMP-9, BAFF, resistin and ENA-78/CXCL5 levels and any of the clinical periodontal parameters (Table 4.19, 4.20 and 4.21).

While considering all patients (T1DM and non-T1DM), a significant positive correlation was demonstrated between GCF MMP-9 levels and mGI (Spearman's rho=0.42, $P<0.01$, medium correlation), % BOP (Spearman's rho=0.51, $P<0.01$, large correlation) and mean PD (Spearman's rho=0.53, $P<0.01$, large correlation) (Table 4.19 and Figure 4.12). No statistically significant correlation was found between GCF MMP-9 levels and PI for all patients. A significant positive correlation was found between GCF IL-8 levels and mGI (Spearman's rho=0.34, $P<0.01$, medium correlation), PI (Spearman's rho=0.20, $P<0.05$, small correlation), % BOP (Spearman's rho=0.37, $P<0.01$, medium correlation) and mean PD (Spearman's rho=0.33, $P<0.01$, medium correlation) in all patients (Table 4.19 and Figure 4.13). When the data were split according to diabetes status, in T1DM patients a significant positive correlation was demonstrated between GCF MMP-9 levels and % BOP (Spearman's rho=0.42, $P<0.01$, medium correlation) and mean PD (Spearman's rho=0.37, $P<0.01$, medium correlation). No statistically significant correlation was found between GCF MMP-9 levels and mGI and PI for T1DM patients. A significant positive (small) correlation was demonstrated between GCF IL-8 levels and % BOP (Spearman's rho = 0.29, $P<0.05$). No statistically significant correlation was found between GCF IL-8 levels and mGI, PI and mean PD for T1DM patients (Table 4.20). In non-T1DM patients, a significant positive correlation was demonstrated between GCF MMP-9 levels and mGI (Spearman's rho=0.67, $P<0.01$, large correlation), PI (Spearman's rho=0.38, $P<0.05$, medium correlation), % BOP (Spearman's rho=0.56, $P<0.01$, medium correlation) and mean PD (Spearman's rho=0.61, $P<0.01$, large correlation). Likewise, a significant positive correlation was demonstrated between GCF IL-8 levels and mGI (Spearman's rho=0.52, $P<0.01$, large correlation), PI (Spearman's rho=0.32, $P<0.05$, medium correlation), % BOP (Spearman's rho=0.48, $P<0.01$, medium correlation) and mean PD (Spearman's rho=0.56, $P<0.01$, large correlation) in non-T1DM patients (Table 4.21).

	Spearman's Rank Correlation Coefficient					
	Serum biomarkers				GCF biomarkers	
	MMP-9	BAFF	Resistin	ENA-78	MMP-9	IL-8
mGI	0.06	0.14	0.16	0.06	0.42 **	0.34 **
PI	0.10	0.17	0.15	0.09	0.18	0.20 *
BOP (%)	0.13	0.00	0.18	0.02	0.51 **	0.37 **
Mean PD (mm)	0.11	-0.03	0.13	-0.00	0.53 **	0.33 **

Table 4.19: Correlations of clinical parameters and biomarker levels in serum and GCF for all patients.

	Spearman's Rank Correlation Coefficient					
	Serum biomarkers				GCF biomarkers	
	MMP-9	BAFF	Resistin	ENA-78	MMP-9	IL-8
mGI	0.06	0.15	0.17	-0.03	0.22	0.18
PI	0.01	0.26	0.20	0.05	0.01	0.10
BOP (%)	0.11	-0.04	0.04	0.02	0.42 **	0.29 *
Mean PD (mm)	0.13	-0.04	0.05	-0.11	0.37 **	0.13

Table 4.20: Correlations of clinical parameters and biomarker levels in serum and GCF for T1DM patients.

	Spearman's Rank Correlation Coefficient					
	Serum biomarkers				GCF biomarkers	
	MMP-9	BAFF	Resistin	ENA-78	MMP-9	IL-8
mGI	0.21	0.10	0.20	0.14	0.67 **	0.52 **
PI	0.06	0.02	-0.00	-0.00	0.38 *	0.32 *
BOP (%)	0.11	0.03	0.29	-0.01	0.56 **	0.48 **
Mean PD (mm)	0.13	0.01	0.28	0.13	0.61 **	0.56 **

Table 4.21: Correlations of clinical parameters and biomarker levels in serum and GCF for non-T1DM patients.

Tables 4.19, 4.20 and 4.21 showing Spearman's rho with colour indicates strength of correlation: small (r=0.10 to 0.29), medium (r=0.30 to 0.49) and large (r=0.50 to 1.00) (Cohen 1988). Significant correlation between clinical parameters and biomarkers: *$P<0.05$ and **$P<0.01$. mGI; modified gingival index, PI; plaque index, BOP; bleeding on probing, PD; probing depth, MMP-9; matrix metalloproteinase-9, BAFF; B-cell activating factor, ENA-78/CXCL5; epithelial neutrophil activating peptide-78, IL-8; interleukin-8, GCF; gingival crevicular fluid.

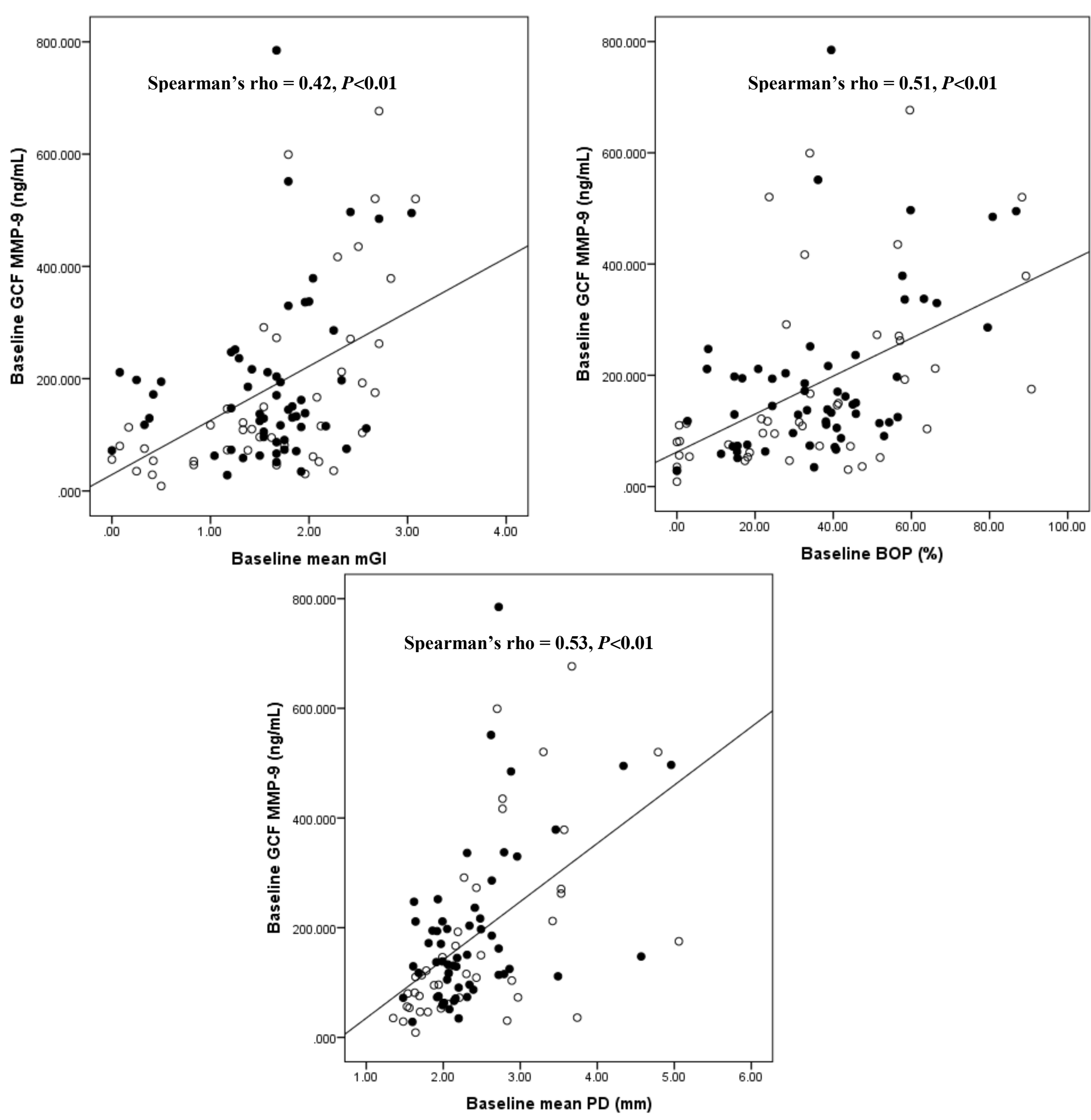


Figure 4.12: Association between pre-treatment GCF MMP-9 levels and clinical periodontal parameters in all patients.

Figures show Spearman's Rank Correlation Coefficient (rho) GCF MMP-9 levels with mGI, PI, BOP and PD at baseline.● indicates T1DM patients (n=57) and ○ indicates non-T1DM patients (n=43). The addition of a trend-line demonstrates the presence of a significant correlation.

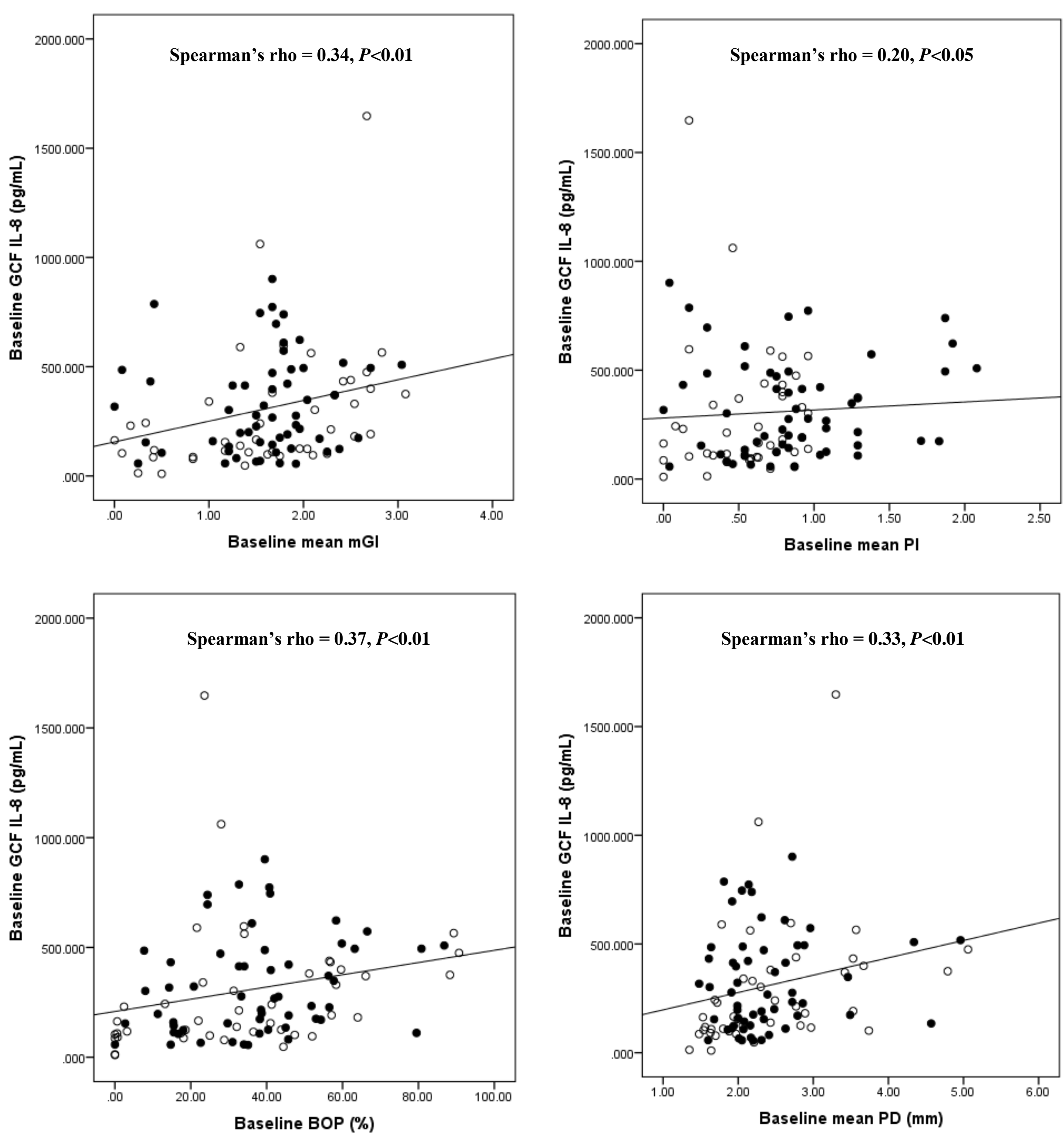


Figure 4.13: Association between pre-treatment GCF IL-8 levels and clinical periodontal parameters in all patients.

Figures show Spearman's Rank Correlation Coefficient (rho) GCF IL-8 levels with mGI, PI, BOP and PD at baseline. ● indicates T1DM patients (n=57) and ○ indicates non-T1DM patients (n=43). The addition of a trend-line demonstrates the presence of a significant correlation.

4.3 Discussion

In this chapter, the protein levels in serum were quantified, cytokine array analysis was carried out to determine biomarkers present in serum, and pre-treatment candidate biomarker levels in serum and GCF were compared between the T1DM and non-T1DM groups and according to periodontal category within groups.

Quantification of protein

The Bradford assay is considered practical, rapid, readily automated and relatively easy to perform (Bradford 1976; Okutucu et al. 2007). This technique relies on the binding of dye to protein present in the sample to form a dye-protein complex with increase in molar absorbance (Bradford 1976), and has been proven to be more sensitive than other methods for protein quantification (Okutucu et al. 2007). The results of the Bradford assay in this study revealed a protein content of 355.1±203.9 mg/ml in all baseline serum samples. When dividing the serum samples based on diabetes status, no significant differences were found for protein levels between T1DM and non-T1DM patients. Also, no significant differences were found between the two groups based on periodontal diagnosis (Table 4.1 and 4.2). A previous study compared the Bradford technique to a new high-sensitivity protein assay based on decreased light scattering of zwitterionic gemini surfactant to determine protein levels in 3 serum samples. The results of their Bradford assay revealed a serum protein concentration of 67.1±1.00 mg/ml, 72.5±0.85 mg/ml and 74.6±1.70 mg/ml, respectively (Chen et al. 2009). Another previous study analysed protein concentration in pooled plasma samples, comparing different protein quantification techniques including the Bradford assay. Additionally standards of BSA and human serum albumin (HSA) were used for calibration. The authors reported a protein concentration of 95.1±3.3 mg/ml in plasma, 89.5±3.1 mg/ml in BSA and 72.5±2.5 mg/ml in HSA (Okutucu et al. 2007). Serum contains a total protein concentration of 60-80 mg/ml (Adkins et al. 2002). However, in this study the levels detected seemed to be higher, a possible explanation for this could be the variations in Bradford assay protocols followed in various studies. As per the Bradford protocol followed in this study the serum samples were found to be viable and the lack of significant differences in protein levels based on diabetes status and periodontal diagnosis provides further assurance that any differences found between groups during biomarker analysis using ELISA, were not attributed to variations in the protein levels of the serum samples being tested. Only the baseline serum samples were tested using the Bradford assay protocol, and this provided

sufficient assurance regarding the viability of all serum samples collected. Due to the limited volume of GCF samples obtained during sample collection and the amount needed to perform protein analysis, it was considered not feasible to analyse the studies' GCF samples, which were collected during the same time period and stored in a similar manner as the serum samples.

Serum is that component of blood which lacks clotting factors. Serum and plasma are similar as both contain glucose, hormones, antibodies, electrolytes, antigens, nutrients and other particles except clotting factors which are present only in plasma. Hence, one can say that serum is plasma minus clotting factors (Guyton and Hall 2016). Both these terms have been used interchangeably in past literature and hence it is reasonable to assume that serum and plasma are two similar liquids and therefore, while making comparisons to the findings of this study, it has been considered that serum and plasma are similar.

Cytokine array

Based on the results of the cytokine array analysis and a review of the literature, it was decided to further analyse candidate biomarkers MMP-9, BAFF, resistin and ENA-78/CXCL5 in the serum samples collected for the study.

Matrix metalloproteinase-9

MMPs are a family of zinc-dependent endopeptidases that degrade extracellular matrix, basement membrane, regulate fibrosis formation and are known to play a crucial role in the regulation of periodontal tissue turnover in disease and health (Uitto et al. 2003; Sorsa et al. 2004; Sorsa et al. 2006; Li et al. 2012; Salazar et al. 2013). MMPs can be divided into 5 groups: collagenases (MMPs 1, 8 and 13); gelatinases (MMPs 2 and 9); stromelysins (MMPs 3, 10 and 11); membrane-type MMPs (MMPs 14, 15, 16 and 17); and others (Sorsa et al. 2004; Sorsa et al. 2006). The main collagenase in periodontitis is MMP-8 followed by MMP-9 (Sorsa et al. 1995). MMP-8, MMP-9 and MMP-13 are the most widely reported MMPs in GCF sites with active periodontal disease (Lee et al. 1995; Choi et al. 2004; Tuter et al. 2005; Beklen et al. 2006; Kumar et al. 2006; Soder et al. 2006). The MMPs released from the inflamed periodontal tissues may have an impact on systemic health, as past research suggests that inflammatory markers may enter the circulation and stimulate inflammation in other parts of the body (Moutsopoulos and Madianos 2006). An experimental gingivitis study found elevated MMP-9 levels after 7-14 days in patients with

T1DM compared to systemically healthy controls (Salvi et al. 2010). Li *et al.'s* study in systemically healthy patients with and without chronic periodontitis confirmed a significant upregulation of serum MMP-9 concentrations in chronic periodontitis patients in comparison to healthy controls (Li et al. 2012). Levels of MMP-9 have been found to be elevated in T1DM patients with other diabetes-related complications besides periodontitis. Previous research related to T1DM and retinopathy found significantly higher serum MMP-9 and TIMP-1 levels in T1DM patients with retinopathy compared to those with T1DM alone and non-diabetic controls; also T1DM patients had higher serum MMP-9 and TIMP-1 levels compared to systemically healthy controls (Jacqueminet et al. 2006). Similarly, significantly elevated serum MMP-2 and MMP-9 levels were found in T1DM patients with nephropathy compared to healthy controls (Gharagozlian et al. 2009). Recent research studies have related several immune, hormonal or connective tissue impairments to periodontal disease. A recent study by Silosi *et al.* investigated the association between inflammatory rheumatoid arthritis and chronic periodontitis, by quantifying MMP-9 levels in serum and GCF (Silosi et al. 2015). The authors reported significantly elevated serum and GCF MMP-9 levels in patients with chronic periodontitis, rheumatoid arthritis and those with both rheumatoid arthritis and chronic periodontitis compared to the periodontally and systemically healthy controls. Additionally, patients with both rheumatoid arthritis and chronic periodontitis were found to have the highest MMP-9 levels, suggesting that the periodontitis-associated rheumatoid arthritis was a reflection of the underlying MMP-9 local and systemic inflammatory response in their study (Silosi et al. 2015).

Research related to MMP-9 levels in diabetes and periodontal disease has been carried out separately. However, research related to serum and GCF MMP-9 levels in patients with T1DM and periodontal disease is lacking hence it was of interest to carry out further investigations in context to these two conditions. Additionally, based on the results of the cytokine array experiment, MMP-9 was found to be highest in the T1DM with periodontitis group compared to the non-T1DM periodontitis group therefore, MMP-9 was selected for further analysis in serum samples using ELISA (Table 4.4 and Figure 4.2).

B-cell activating factor

BAFF is a member of the TNF superfamily, best known for its role in the survival and maturation of B cells. BAFF is produced by several tissue and cell types including spleen, bone marrow, lymph node, macrophages, monocytes, dendritic cells, neutrophils, T

lymphocytes and epithelial cells (Mackay and Schneider 2009). BAFF plays a role in human autoimmune disorders and has been found to be elevated in serum samples of patients with systemic lupus erythematosus and Sjögren's syndrome (Zhang et al. 2001; Varin et al. 2010). BAFF is produced locally in the joints of patients with rheumatoid arthritis and serum levels correlate with antibody titres in Sjögren's syndrome and rheumatoid arthritis (Nakajima et al. 2007; Varin et al. 2010). B cells play a pathogenic role as antigen-presenting cells and autoantibody secretors in the lead up to T cell-mediated autoimmune destruction of the insulin-producing β-cells of the pancreas in T1DM (Marino et al. 2011). Likewise, advanced periodontal lesions have been associated with infiltration of B lymphocytes in patients with periodontitis, which when activated induce osteoclastic differentiation. B cells in periodontitis have been found to be partly mediated by salivary BAFF in patients with Sjögren's syndrome (Pers et al. 2005). Research related to BAFF levels in patients with T1DM and periodontal disease is lacking, hence it was considered of interest to carry out further investigations in the context of these two inflammatory conditions. Additionally, based on the results of the cytokine array experiment, BAFF was found to be highest in the T1DM with periodontitis group compared to the non-T1DM periodontitis group; therefore, BAFF was selected for further analysis in serum samples using ELISA (Table 4.4 and Figure 4.2).

Resistin

Resistin is an adipokine, belonging to the cysteine-rich secretory protein family, known as resistin-like molecules (Bokarewa et al. 2005). The most significant source of resistin is the mononuclear blood cells, but some human studies suggest that resistin is also expressed by adipose tissue. Additionally, resistin is expressed by pre-adipocytes, pancreatic islets, placenta and primary leukaemia cells. Resistin is well known for its potential role in linking obesity, insulin resistance and T2DM. Recent studies have reported that resistin plays a role in inflammatory conditions such as rheumatoid arthritis, atherosclerosis and inflammatory bowel disease (Bokarewa et al. 2005; Reilly et al. 2005; Konrad et al. 2007). T1DM is characterised by increases in inflammation independent of glycaemic control and adiposity (Geyikli et al. 2013). A recent study by Geyikli and colleagues found significantly higher serum resistin levels in children and adolescents with T1DM compared to healthy controls (Geyikli et al. 2013). The authors suggested that resistin may possibly play a role in inflammation and in the pathophysiology of T1DM (Geyikli et al. 2013). Knowledge about

the relationship between resistin, T1DM and periodontal disease is limited, hence it was considered interesting to investigate the levels of this biomarker in relation to these two conditions. Additionally, based on the results of the cytokine array experiment, resistin was found to be highest in the T1DM with periodontitis group compared to the non-T1DM periodontitis group; therefore, resistin was selected for further analysis in serum samples using ELISA (Table 4.4 and Figure 4.2).

Epithelial neutrophil activating peptide-78/CXCL5

Neutrophils are fundamentally essential in contributing to host defence against bacterial infections and a defect or loss in neutrophil function significantly predisposes individuals to inflammatory conditions such as periodontitis (Nussbaum and Shapira 2011). ENA-78 is a C-X-C chemokine (CXCL5) and is a major neutrophil chemoattractant and activator. ENA-78/CXCL5 is primarily expressed by epithelial cells, monocytes and platelets and, has also been detected in neutrophils and macrophages (Walz et al. 1997; Damas et al. 2000). An increase in expression of ENA-78/CXCL5 has been associated with neutrophil influx in inflammatory conditions such as rheumatoid arthritis, adult respiratory distress syndrome, inflammatory bowel diseases and chronic pancreatitis (Walz et al. 1997). A previous study by Lappin and colleagues, demonstrated significantly higher plasma ENA-78/CXCL5 levels in patients with periodontitis compared to those with healthy periodontal tissues (Lappin et al. 2011). Additionally, elevated plasma ENA-78/CXCL5 levels correlated with PD measurements, and ENA-78/CXCL5 was found to be a good systemic indicator of disease severity and inflammatory processes in patients with periodontitis (Lappin et al. 2011). Very little is known about the role that ENA-78/CXCL5 plays in the pathogenesis of periodontal disease, T1DM and the cross-susceptibility between the two diseases. A recent study by Lappin and colleagues demonstrated significantly higher plasma ENA-78/CXCL5 levels in patients with T1DM and periodontitis and T1DM alone compared to non-diabetic patients with healthy periodontal tissues (Lappin et al. 2015). However, the authors found no statistically significant differences between T1DM patients with healthy tissues and those with T1DM and periodontitis (Lappin et al. 2015).

In the current study, an ELISA experiment carried out following the cytokine array analysis on 20 serum and 20 GCF samples of the study, detected ENA-78/CXCL5 only in the serum samples. ENA-78/CXCL5 was not detected in the GCF samples during the ELISA experiment. Given the key role ENA-78/CXCL5 plays in various inflammatory conditions,

and in order to increase our knowledge and the evidence of circulating ENA-78/CXCL5 in patients with T1DM and periodontal disease, it was considered interesting to analyse this biomarker in the serum samples of the current study. Additionally, based on the results of the cytokine array experiment, ENA-78/CXCL5 was found to be highest in the T1DM with periodontitis group compared to the non-T1DM periodontitis group therefore, ENA-78/CXCL5 was selected for further analysis in serum samples using ELISA (Table 4.4 and Figure 4.2).

Interleukin-8

Another major neutrophil and lymphocyte chemoattractant is IL-8 which is also known as CXCL8. IL-8 belongs to the CXC chemokine family, and is known for its important role in the induction and maintenance of inflammation (Li et al. 2012). IL-8 is mainly produced by epithelial cells and macrophages upon inflammatory stimulation (Harada et al. 1994; Okada and Murakami 1998). Elevated circulating levels of IL-8 have been associated with poor clinical outcomes in patients with T1DM (AboElAsrar et al. 2012). Additionally, IL-8 levels have been tightly linked to increased susceptibility for periodontitis (Figueredo and Gustafsson 2000). IL-8 levels in GCF (Salvi et al. 2010) and saliva (Dakovic et al. 2013) have been linked to the cross-susceptibility between periodontal disease and T1DM. The other major neutrophil chemoattractant ENA-78/CXCL5, shares 22% of its amino acid sequence identity with IL-8, additionally ENA-78/CXCL5 activity can be mediated through the IL-8 receptor system (Walz et al. 1997). Similar to ENA-78/CXCL5, IL-8 has also been found to be increased in inflammatory conditions such as, rheumatoid arthritis, adult respiratory distress syndrome, inflammatory bowel diseases and chronic pancreatitis (Walz et al. 1997). A recent study by Lappin and colleagues concluded that elevated plasma IL-8 levels potentially contribute to the cross-susceptibility between T1DM and periodontitis (Lappin et al. 2015). Engebretson *et al.'s* study in chronic periodontitis patients with and without T2DM, found significantly lower GCF IL-8 levels in T2DM patients compared to non-diabetic patients with similar periodontal status (Engebretson et al. 2006). Other studies related to T2DM found no statistically significant differences between T2DM and non-diabetic patients with periodontitis while analysing of IL-8 levels in gingival tissues (Duarte et al. 2007) and serum samples (Longo et al. 2014).

In the current study, IL-8 was not detected in serum samples during the cytokine array experiment. The ELISA experiment carried out following the cytokine array analysis on 20

serum and 20 GCF samples of the study, detected IL-8 detected only in the GCF samples. IL-8 was once again not detected in serum samples during the ELISA experiment. Since IL-8 shares a common identity and inflammatory role with ENA-78/CXCL5, is known for its role in periodontal disease and T1DM, and was detected in the GCF samples of the current study, it was considered interesting to further analyse this biomarker in the GCF samples of the current study.

Pre-treatment biomarker levels in serum

The current study found significantly higher serum MMP-9, resistin and ENA-78/CXCL5 levels in T1DM patients compared to non-T1DM patients (Table 4.9, Figure 4.3, 4.4 and 4.5). Based on periodontal diagnosis, serum MMP-9 levels were significantly higher in T1DM patients with periodontitis compared to non-T1DM patients with periodontitis ($P<0.001$) (Table 4.10 and Figure 4.6). Currently no research has been carried out related to serum MMP-9 levels in patients with T1DM and periodontal disease and hence, it is has not been possible to make comparisons with the findings of other studies. With the non-T1DM group, in the current study, serum MMP-9 levels were higher in the periodontitis patients compared to those with gingivitis and healthy tissues; however these differences were not statistically significant. A previous study analysed serum MMP-9 levels in systemically healthy patients with (n=122) and without (n=532) chronic periodontitis (aged 21-52 years) and found significantly higher MMP-9 levels in patients with chronic periodontitis (11.65±2.17 ng/ml) compared to the healthy controls (2.17±1.91 ng/ml), ($P<0.001$) (Li et al. 2012). The authors suggested that serum concentrations of MMP-9 indicated the potential clinical significance of serum measurements in patients with chronic periodontitis (Li et al. 2012). Another study evaluated plasma MMP-9 levels in patients with and without chronic periodontitis and found significantly elevated plasma MMP-9 levels in patients with chronic periodontitis compared to those with healthy tissues (Marcaccini et al. 2009b). However, the authors presented the data only graphically and no numerical values were given, which limits the ability to directly compare their findings with those of the current study.

In the current study, serum ENA-78/CXCL5 levels were significantly higher in T1DM patients with gingivitis (1607.3±754.0 pg/ml) compared to non-T1DM patients with gingivitis (1140.3±696.8 pg/ml), ($P<0.05$) (Table 4.10 and Figure 4.8). A recently published study reported plasma ENA-78/CXCL5 levels in patients with T1DM and periodontal disease (Lappin et al. 2015). T1DM patients with healthy periodontal tissues (n=28) had

significantly higher plasma ENA-78/CXCL5 levels [approximate levels from presented graph: 500 (100-1,800) pg/ml] compared to non-diabetic patients with healthy tissues (n=19) [approximate levels from presented graph: 200 (50-500) pg/ml], ($P<0.01$) (Lappin et al. 2015). In the current study, within the T1DM group, no statistically significant differences were found between serum ENA-78/CXCL5 levels in patients with healthy tissues, gingivitis and periodontitis. Our findings are similar to Lappin *et al.*'s study, which showed no statistically significant difference in plasma ENA-78/CXCL5 levels between T1DM patients with healthy tissues (n=28) [approximate levels from presented graph: 500 (100-1,800) pg/ml] and T1DM patients with periodontitis (n=34) [approximate levels from presented graph: 700 (100-2,000) pg/ml], ($P>0.05$) (Lappin et al. 2015). In the current study, within the non-T1DM group although higher serum ENA-78/CXCL5 levels were found in periodontitis patients compared to those with gingivitis and healthy tissues, the differences between the groups were not statistically significant. The recent study by Lappin *et al.* found similar but significantly higher plasma ENA-78/CXCL5 levels in non-diabetic patients with periodontitis (n=23) [approximate levels from presented graph: 300 (150-1,000) pg/ml] compared to those with healthy periodontal tissues (n=19) [approximate levels from presented graph: 200 (50-500) pg/ml], ($P<0.1$) (Lappin et al. 2015).

In the current study, while considering serum resistin levels, within the non-T1DM group serum resistin levels were significantly higher in periodontitis patients (10.5±3.55 ng/ml) compared to those with gingivitis (8.06±2.58 ng/ml), ($P<0.05$) (Table 4.10 and Figure 4.7). The findings of this study are similar to a previous study which found significantly higher serum resistin levels in women with periodontitis (9.9±5.0 ng/ml) compared to those with gingivitis (8.0±5.2 ng/ml), ($P<0.05$) (Saito et al. 2008). The authors also reported that women with periodontitis had significantly higher BMI compared to women with gingivitis (Saito et al. 2008). On performing correlations, in the current study, serum resistin levels in the T1DM patients were significantly positively correlated with BMI (Spearman's rho=-0.08, $P<0.05$), suggesting that T1DM patients with higher BMI scores had elevated serum resistin levels. T1DM is not typically associated with obesity, and hence the findings in this study may demonstrate that resistin may not be an ideal biomarker to establish the mechanistic link between T1DM and periodontal disease.

In the current study, the ELISA results of BAFF in serum revealed no statistically significant difference in levels between T1DM and non-T1DM patients and also found no differences

between the groups based on periodontal diagnosis. Hence, it was decided not to further analyse this biomarker in the GCF samples. The findings of the ELISA experiment for serum BAFF levels did not replicate the findings of the cytokine array analysis, which revealed the highest intensity of serum BAFF in the T1DM patients with periodontitis and the lowest intensity in the non-T1DM patients with healthy tissues. The cytokine array experiment involved the pooling of three serum samples for each diagnosis, whereas the ELISA experiment involved all serum samples collected for the study. The findings of the BAFF results were confirmed and quantified using ELISA. It is evident from the ELISA results that the three selected samples from each group may not have been fully representative of all the patients in the study. It might be beneficial in future research to pool more than three samples for array experiments. Overall, these findings emphasize that, caution must be used in interpreting the outcomes of array experiments and the results must always be followed up and confirmed by ELISA.

Pre-treatment biomarker levels in GCF

The local host response in periodontal disease has been investigated by biochemical analysis of GCF samples (Chung et al. 1997). In the current study, although GCF MMP-9 and IL-8 levels were higher in T1DM patients compared to non-T1DM patients, the differences were not statistically significant (Table 4.11). So far, no research studies have evaluated the GCF levels of MMP-9 or IL-8 in patients with T1DM and periodontal disease and hence, it is has not been possible to make comparisons to the findings of other studies.

Looking at past literature related to T2DM, a previous study in chronic periodontitis patients with and without T2DM, found significantly lower GCF IL-8 levels in T2DM patients (33.0±33.4 pg/sample) compared to non-diabetic patients with similar periodontal disease status (90.8±83.2 pg/sample), ($P<0.00001$) (Engebretson et al. 2006). Also, GCF IL-8 levels significantly correlated with PD measurements but not with HbA1c levels. The authors reported that their findings were unexpected as diabetes is often associated with an increase in inflammatory mediators (Engebretson et al. 2006). Duarte *et al.* investigated the expression of IL-8, IL-6, IL-1β and interferon-γ in the gingival tissues of T2DM patients with and without periodontitis, and concluded that T2DM was associated with elevated expression of IL-1β and IL-6 in patients with periodontitis compared to systemically healthy controls with comparable periodontitis, however no such differences were observed for IL-8 levels between periodontitis patients with and without T2DM (Duarte et al. 2007). The authors

stated that although IL-8 is locally delivered in the gingival tissues during host defence mechanism in periodontitis, IL-8 may not directly be involved in the mechanism by which alveolar bone destruction is modulated in T2DM (Duarte et al. 2007). A recent study analysed serum IL-8 levels in T2DM patients with and without periodontal disease (Longo et al. 2014). The authors reported significantly more severe periodontal disease in the T2DM inadequate- and adequate-glycaemic control patient groups, compared to the non-diabetic patients with periodontitis, however despite this, no statistically significant differences were found for serum IL-8 levels between groups (Longo et al. 2014). The findings of the above studies were in T2DM patients, based on glycaemic control categories and in gingival tissue and serum samples, hence may not be an ideal comparison to the IL-8 results of the present study.

In the current study, based on periodontal diagnosis, GCF MMP-9 and IL-8 levels were significantly higher in T1DM patients with healthy tissues compared to non-T1DM patients with healthy tissues, ($P<0.05$) (Table 4.12, Figure 4.9 and 4.10). This is an interesting finding, as even in the absence of inflammatory periodontal disease, elevated GCF MMP-9 and IL-8 levels were present in T1DM patients compared to non-diabetic controls, possibly suggesting the manifestation of diabetes-related inflammation in the GCF of patients with T1DM. Additionally, these findings are in accordance with the studies' pre-treatment clinical periodontal data findings, where significantly higher levels of gingival inflammation (indicated by % BOP) was present in T1DM patients with healthy tissues (9.87±5.67 %) compared to non-T1DM patients with healthy tissues (0.83±1.17 %), ($P<0.001$) (Table 3.15 and Figure 3.10). No statistically significant differences in GCF MMP-9 and IL-8 levels were found between T1DM and non-T1DM patients with gingivitis and periodontitis, though drawing from conclusions in this regard is difficult because the non-T1DM patients with periodontitis presented with more severe periodontal disease than the T1DM patients with periodontitis (Table 3.15 and Figure 3.11).

Currently, studies related to GCF biomarker levels in T1DM and periodontal disease are extremely limited, and no studies have evaluated biomarker levels based on a patient's periodontal diagnosis as was considered in this study. A previous experimental gingivitis study in T1DM patients found that GCF MMP-9 levels were highest at day 21 following oral hygiene cessation, for both T1DM and non-diabetic patients, and were also significantly higher in T1DM patients compared to non-diabetic patients with gingivitis (Salvi et al. 2010).

However, as the authors provided only graphical presentation of their data and numerical values were not reported, it is difficult to compare their findings directly to the results of the current study. A study of IL-8 levels in saliva, found that T1DM patients with periodontally healthy tissues had significantly higher salivary IL-8 levels compared to non-diabetic controls (Dakovic et al. 2013). Also, no statistically significant differences were found for salivary IL-8 levels between periodontitis patients with and without T1DM (Dakovic et al. 2013). The use of saliva (not GCF) prevents direct comparison to the IL-8 levels in the current study. Additionally, the patients included in the Dakovic study were aged 7-18 years, and hence their data might not be an ideal comparison to the findings of the current study. A study by Li *et al.* analysed serum IL-8 levels in systemically healthy patients with (n=122) and without (n=532) chronic periodontitis (aged 21-52 years) (Li et al. 2012). Their analysis revealed significantly higher serum IL-8 levels in chronic periodontitis patients (155.28±57.20 pg/ml) compared to the periodontally healthy controls (58.69±6.70 pg/ml), ($P<0.001$) (Li et al. 2012). The authors suggested that serum concentrations of IL-8 indicated the potential clinical significance of serum measurements in patients with chronic periodontitis. GCF is a serum exudate which contains all the key molecular and cellular components of the immune response required to prevent tissue invasion by subgingival bacteria (Taylor and Preshaw 2016), however, in the serum samples of the current study, the ELISA experiment carried out revealed no IL-8, which was found to be present only in the GCF samples. Another recent study found significantly higher plasma IL-8 levels in T1DM patients with periodontitis (n=34) [approximate levels from presented graph: 30 (20-80) pg/ml] compared to systemically healthy patients with periodontitis (n=23) [approximate levels from presented graph: 15 (10-30) pg/ml], ($P<0.1$) (Lappin et al. 2015). However, the use of plasma (not GCF) limits the comparison of their results to those of the present study.

Within the T1DM group, in the present study, significantly higher GCF MMP-9 levels were found in the periodontitis patients compared to those with gingivitis and healthy tissues, ($P<0.001$) (Table 4.12 and Figure 4.9). These findings are in accordance with the clinical periodontal data of this study which revealed significantly higher gingival inflammation (indicated by % BOP), PD measurements and severity of periodontal disease in T1DM patients with periodontitis compared to those with gingivitis and healthy tissues (Table 3.15). Within the non-T1DM group, significantly higher GCF MMP-9 levels were found in the periodontitis patients compared to those with gingivitis and healthy tissues, ($P<0.001$). Also, significantly higher GCF MMP-9 levels were found in the gingivitis patients compared to

those with healthy tissues, (P<0.05) (Table 4.12 and Figure 4.9). The findings of this study are in agreement with previous research, which found elevated MMP-9 levels in GCF, whole saliva and mouth rinse samples in patients with periodontitis compared to those with healthy tissues (Makela et al. 1994). The researchers utilised a gelatin zymogram to evaluate MMP-9 levels and reported that gelatinase activity in the oral cavity was mainly due to MMP-9 (Makela et al. 1994). The utilization of gelatin zymography for biomarker quantification and not ELISA, limits the comparison of their findings to the data of the current study. The GCF MMP-9 data from the non-T1DM patients in the present study are in contrast to those of a previous study in systemically healthy patients which compared GCF MMP-9 levels between those with healthy tissues (3607±2381 pg/ml), gingivitis (3999±7715 pg/ml) and periodontitis (4637±4581 pg/ml) (Maeso et al. 2007). The authors reported that although GCF MMP-9 levels were higher in periodontitis patients compared to those with gingivitis and healthy tissues, the differences were not statistically significant (Maeso et al. 2007). The findings of the present study are contrast to another study, which reported no differences in GCF MMP-9 levels in systemically healthy patients with periodontitis (n=27) [approximate levels from presented graph: 1,400 (100-3,500) ng/site in 30s] compared to healthy controls (n=15) [approximate levels from presented graph: 1,100 (400-2,000) ng/site in 30s] (P>0.05) (Marcaccini et al. 2010). Patients with periodontitis had significantly higher PD and CAL (4.50±1.84 mm and 4.8±1.9 mm) compared to those with healthy tissues (2.24±0.68 mm and 2.3±0.6 mm) (P<0.0001), and no statistically significant differences in BOP scores [periodontitis: n (%), 21 (77.78 %) and healthy tissues: n (%), 8 (53.34 %)]. In the current study, along with PD and LOA measurements the non-T1DM patients with periodontitis had significantly higher % BOP (53.9±21.1 %) compared to those with healthy tissues (0.83±1.17) (P<0.001), which was not seen in Marcaccini *et al.'s* study which could probably lead us to assume that an increase in gingival inflammation could attribute to an increases in GCF MMP-9 levels in patients with periodontitis. Additionally, Marcaccini *et al.'s* study collected and analysed only a single GCF sample per patient and reported MMP-9 levels as ng/site in 30s, limits the comparison of their results to those of the current study, which collected and analysed four GCF samples per patient and reported MMP-9 levels in ng/ml.

Within the T1DM group, in the current study, although GCF IL-8 levels were higher in periodontitis patients compared to those with gingivitis and healthy tissues, the differences were not statistically significant. Likewise, in Lappin *et al.'s* recent study although higher plasma IL-8 levels were found in the T1DM patients with periodontitis (n=34) [approximate

levels from presented graph: 30 (20-80) pg/ml] compared to the T1DM patients with healthy tissues (n=28) [approximate levels from presented graph: 25 (15-35) pg/ml], these differences were not statistically significant, (P>0.05) (Lappin et al. 2015). The use of plasma (not GCF) limits the comparison to the data of the current study. In the current study, within the non-T1DM group, GCF IL-8 levels were significantly higher in the periodontitis patients compared to those with gingivitis and healthy tissues, (P<0.01 and P<0.05, respectively) (Table 4.12 and Figure 4.10). The findings of the present study are in agreement with those of a previous small study which reported significantly elevated GCF IL-8 levels in periodontitis patients (n=16, 203.8±136.3 ng/ml) compared to those with healthy tissues (n=5, 205.9±115.4 ng/ml), (P>0.05) (Tsai et al. 1995). Our findings are in contrast to another study which found significantly higher GCF IL-8 levels in patients with healthy tissues (n=14, 2902.3±432.5 pg/µl) compared to those with periodontitis (n=30, 1471.5±231.4 pg/µl) (Chung et al. 1997). The authors also reported that clinical periodontal data were significantly higher in periodontitis patients compared to those with healthy tissues, however, in spite this GCF IL-8 levels were higher in patients with healthy tissues. The authors suggested that their findings demonstrated that there is an inverse relationship between PMN recruitment and IL-8 activity (Chung et al. 1997), consistent with Tonetti *et al.'s* study which demonstrated that IL-8 transcripts were more consistently detected in the gingival tissue biopsies from healthy tissues compared to biopsies from patients with periodontitis (Tonetti et al. 1993). The findings of the current study are in agreement to Lappin *et al.'s* recent findings in systemically healthy patients, which found significantly higher plasma IL-8 levels in periodontitis patients (n=23) [approximate levels from presented graph: 15 (10-30) pg/ml] compared to those with healthy tissues (n=19) [approximate levels from presented graph: 5 (0-10) pg/ml], (P<0.001) (Lappin et al. 2015). The use of plasma (not GCF) limits the comparison of their findings to the data from the present study.

The heterogeneity in methodology used between research studies is a possible explanation for the variations in GCF biomarker levels seen in the published literature and in the present study. These differences could arise from variations in case definitions of periodontitis, analytical techniques, GCF sampling methods, techniques used for eluting GCF from Periopapers and storage of samples. While considering methods used for eluting GCF from Periopapers, a variety of protocols were used within studies, for example, in Tsai *et al.'s* study following GCF collection the Periopaper strip was eluted twice in 100 µl of Hank's balanced salt solution and centrifugation 3000 x g at 4 °C for 15 minutes before storing the

sample at -70 °C (Tsai et al. 1995). Chung *et al.'s* study used pre-cut methylcellulose filter paper strips for GCF collection, and GCF was eluted by adding 50 µl of PBS with 0.05% Tween 20 and centrifuging for 3 times following which the eluted GCF was frozen at -20 °C (Chung et al. 1997). Gamonal *et al.'s* study placed 3 GCF Periopapers in vials containing 50 µl of PBS with 0.05% Tween 20 and centrifuged at 10,000 g for 5 minutes at 4 °C and this was repeated 3 times (Gamonal et al. 2000). Salvi *et al.'s* study, collected 4 GCF samples per quadrant and pooled them together, eluting each strip with 5 wash-centrifugation cycles with a buffer containing 200 mM phenylmethylsulfonyl fluoride in methanol, 1 mg/ml Aprotinin, 30% HSA, and PBS, following which the eluted GCF was frozen at -80 °C (Salvi et al. 2010). By comparison, Marcaccini *et al.'s* study collected a single GCF sample and placed it in a vial containing 100 µl buffer for 30 minutes at 4 °C, after which the GCF was centrifuged at 13,000 g for 10 minutes at 4 °C and stored at -70 °C (Marcaccini et al. 2010). A lack of consistency in the literature in the methodologies used for GCF collection and elution will have likely increased inter-study variations in GCF biomarker levels and hence, prevent clear conclusions from being made regarding the role that GCF biomarkers play in patients with T1DM and periodontal disease.

With reference to GCF volume, in the present study no statistically significant differences were found between T1DM and non-T1DM patients for the volume of GCF collected. However, based on periodontal diagnosis, in both T1DM and non-T1DM groups significantly higher GCF volumes were recorded in the periodontitis patients compared to those with gingivitis and healthy tissues, ($P<0.001$). In only the non-T1DM group a significantly higher GCF volume was recorded in the gingivitis patients compared to those with healthy tissues, ($P<0.01$) (Table 4.12). The findings of this study related to non-T1DM patients are in agreement to a previous study of systemically healthy patients, which found significantly higher GCF volume in patients with periodontitis (n=25, 1.11±0.39 µl) compared to those with gingivitis (n=18, 0.92±0.33 µl) and healthy tissues (n=16, 0.48±0.29 µl), ($P<0.05$ and $P<0.001$, respectively) (Maeso et al. 2007). Also, patients with gingivitis (0.92±0.33 µl) had significantly higher GCF volume compared to those with healthy tissues (0.48±0.29 µl), ($P<0.001$) (Maeso et al. 2007). Similarly, another small study found significantly higher GCF volume in patients with periodontitis (n=16, 0.46±0.39 µl) compared to those with healthy tissues (n=5, 0.07±0.06 µl), ($P<0.001$) (Tsai et al. 1995). The GCF volume collected is the sum of two components i.e. the GCF void volume (the GCF resting volume which is independent of flow) and the GCF flow contribution (which is dependent upon the flow rate

and collection time). The GCF resting void volume is dependent upon the pocket depth, as pocket depth increases from 3 mm to 6 mm there is a 50% increase in the GCF resting void volume (Barros et al. 2016). In the current study, irrespective of diabetes status the periodontitis patients had significantly higher mean PD compared to those with heathy tissues ($P<0.001$) (Table 3.15). The findings of the current study and those of past literature strengthened the observations that more severe periodontal disease leads to an increase in GCF volume.

Correlations

Association between HbA1c and hsCRP levels and clinical periodontal parameters

A positive correlation between severity of periodontal disease and glycaemic control has been reported in literature (Tanwir and Tariq 2012; Costa et al. 2013). In the current study, when considering all patients, the only positive significant correlation was found between PI and HbA1c levels. However, no statistically significant correlations were found between HbA1c and mGI, % BOP and mean PD. While categorising the patients based on diabetes status, in T1DM patients the only positive correlation was found between PI and HbA1c levels (Table 4.14). The findings of this study are in agreement with a study by Aren and colleagues in T1DM and non-diabetic children which found a significant correlation between PI scores and HbA1c levels, (Pearson's $r=0.31$, $P=0.03$) (Aren et al. 2003). On the other hand, our findings are in contrast to Firatli's study of T1DM patients and non-diabetic controls which found no statistically significant correlation between clinical periodontal parameters (PI, mGI, PD and LOA) and laboratory parameters (glucose, fructosamine and HbA1c) (Firatli 1997). Lappin *et al.'s* recent study of T1DM patients and non-diabetic controls (with and without periodontitis), found a significant positive correlation between % BOP and HbA1c levels, ($P<0.001$) (Lappin et al. 2015). Our findings suggest that higher HbA1c levels are associated with an increase in the amount of plaque in patients with T1DM, and can be supported by the concept that excessive diabetes-related glucose enters the oral cavity through the GCF and saliva, a sugar-rich biofilm which forms will then, in general, enhance plaque growth. A lack of knowledge about oral hygiene and maintaining optimal oral health in patients with diabetes plus increases in HbA1c levels could collectively lead to increases in plaque accumulation (Hugoson et al. 1989). But, clearly, further work is needed to study the relationship between HbA1c and clinical periodontal parameters.

Association between HbA1c, hsCRP levels and inflammatory biomarkers

In the current study, while considering all patients, a significant positive correlation was found between HbA1c and serum MMP-9 and ENA-78/CXCL5 levels (Table 4.16). Similar correlations were demonstrated in Lappin *et al.'s* study in which plasma IL-8 and ENA-78/CXCL5 levels were significantly correlated with and HbA1c levels (P=0.001 and P=0.02) in T1DM and non-diabetic patients (with and without periodontitis) (Lappin et al. 2015). The finding of their study and ours, suggest that an increase in the systemic inflammatory burden is associated with an increase in HbA1c levels irrespective of diabetes status.

In the current study, when considering all patients, a significant positive correlation was found between hsCRP and serum MMP-9 levels. Similarly, when patients were split according to diabetes status, a significant positive correlation was found between hsCRP and serum MMP-9 levels in T1DM patients (Table 4.16 and 4.17). The positive association observed between serum MMP-9 and hsCRP in this study, indicates that MMP-9 may reflect the chronic inflammatory state which is typical of periodontitis. Our findings suggest a clear systemic association between hsCRP and MMP-9 levels particularly in patients with T1DM.

Association between clinical periodontal parameters and inflammatory biomarkers

While considering serum biomarker levels, in the current study, no statistically significant correlations were found for serum MMP-9, BAFF, resistin and ENA-78/CXCL5 levels and mGI, PI, % BOP and mean PD when considering all patients and when splitting the patients based on diabetes status. Our findings are in contrast to Lappin *et al.'s* study of T1DM and non-diabetic controls (with and without periodontitis), which found a significant positive correlation between plasma ENA-78/CXCL5 levels and PD (P=0.02) and LOA (P=0.03) (Lappin et al. 2015). Our findings are also in contrast to Lappin *et al's* previous study in systemically healthy patients with and without periodontitis, which found a significant correlation between LOA and plasma ENA-78/CXCL5 levels (P=0.003) (Lappin et al. 2011). Clearly more research is required with regards to systemic inflammation and clinical periodontal status. However, the findings of this study demonstrated that periodontal status had no association with the systemic inflammatory state.

While considering GCF biomarker levels, in the current study, a significant positive correlation was found between GCF MMP-9 levels and mGI, % BOP and mean PD in all

patients. Based on diabetes status, a significant positive correlation was found between GCF MMP-9 levels and % BOP and mean PD in T1DM patients. In non-T1DM patients, a significant positive correlation was found between GCF MMP-9 levels and mGI, PI, % BOP and mean PD. With reference to GCF IL-8 levels, a significant positive correlation was found between GCF IL-8 levels and mGI, PI, % BOP and mean PD in all patients. Based on diabetes status, a similar significant positive correlation was found between GCF IL-8 levels and mGI, PI, % BOP and mean PD in non-T1DM patients. However, in T1DM patients, a significant positive correlation was only found between GCF IL-8 levels and % BOP (Table 4.19, 4.20 and 4.21). Our findings demonstrate that poorer periodontal health is associated with increases in local inflammatory state which reflected in GCF. Similar findings were seen in other studies, for example, Salvi *et al.'s* experimental gingivitis study in T1DM and non-diabetic patients found that in both groups, mGI correlated with GCF MMP-8, MMP-9 and IL-8 levels at day 21, also in T1DM patients there was a significant correlation between mGI and GCF MMP-9 levels at day 0 (Salvi et al. 2010). In non-diabetic patients a significant correlation was found between PI and GCF MMP-9 levels at day 0. In T1DM patients, PI was significantly correlated with GCF MMP-9 levels at day 0 (Salvi et al. 2010). Lappin *et al.'s* study of T1DM and non-diabetic controls (with and without periodontitis), found a significant positive correlation between plasma IL-8 levels and PD (P=0.001) and LOA (P=0.005) (Lappin et al. 2015). Marcaccini *et al.'s* study in systemically healthy patients with and without periodontitis, found a significant correlation between total GCF MMP-9 levels and PD (Spearman's rho=0.67, P<0.001) (Marcaccini et al. 2010). However, Marcaccini *et al.'s* correlations were related to total GCF MMP-9 levels and hence, may not be an ideal comparison to the GCF MMP-9 concentration levels in the current study. Tsai *et al's.* study in systemically healthy patients with and without periodontitis, found no statistically significant differences in GCF IL-8 concentration levels between the two groups, and found a significant negative correlations between GCF IL-8 concentration levels and clinical periodontal parameters: mGI (Spearman's rho=-0.37, P<0.01),
PD (Spearman's rho=-0.42, P<0.01), LOA (Spearman's rho=-0.44, P<0.01) and tooth mobility (Spearman's rho=-0.43, P<0.01) (Tsai et al. 1995). Their results suggest that a better periodontal condition is associated with elevated GCF IL-8 levels (Tsai et al. 1995). Gamonal *et al.'s* study of systemically healthy patients with and without moderate to advanced periodontitis found weak and non-significant correlations between total GCF IL-8 levels and clinical parameters PI (Spearman's rho=0.12), BOP (Spearman's rho=0.01) and PD (Spearman's rho=0.05), (P>0.05) (Gamonal et al. 2000). Also, a significant positive

correlation was found between total GCF IL-8 and RANTES (regulated on activation, normal T cell expressed and secreted) and GCF volume (Spearman's rho=0.71 and 0.74, $P<0.05$) (Gamonal et al. 2000). However, these correlations were related to total GCF IL-8 levels and hence, may not be an ideal comparison to the GCF IL-8 concentration levels in the current study.

The findings of the current study and a review of previous literature related to GCF biomarker levels in patients with periodontal disease with and without T1DM indicate in broad terms that an increase in periodontal disease leads to elevated levels of MMP-9 and IL-8 in GCF. However, in the current study, a similar association was not found for serum biomarker levels and periodontal parameters. Based on the evidence it would be reasonable to suggest that GCF is a good local indicator for periodontal inflammatory status due to its local production within the periodontal tissues. The findings of this study related to serum biomarker levels found significantly elevated MMP-9, resistin and ENA-78/CXCL5 levels in T1DM patients compared to controls. Furthermore, serum MMP-9 levels were significantly correlated with hsCRP levels suggesting that serum has potential of a systemic indicator for the inflammatory disease status in patients with periodontal disease and T1DM.

Summary of key findings from chapter 4

- Serum levels of MMP-9, resistin and ENA-78/CXCL5 were significantly higher in T1DM patients compared to non-T1DM patients. Furthermore, serum MMP-9 levels were significantly higher in the T1DM patients with periodontitis compared to the non-T1DM patients with periodontitis.
- Although serum levels of resistin and ENA-78/CXCL5 were apparently higher in the T1DM patients with periodontitis compared to the non-T1DM patients with periodontitis, these differences were not statistically significant.
- Serum resistin and ENA-78/CXCL5 levels were significantly higher in the T1DM patients with gingivitis compared to the non-T1DM patients with gingivitis. Also, within the non-T1DM group, serum resistin levels were significantly higher in the periodontitis patients compared to those with gingivitis.
- Although the levels of MMP-9, resistin and ENA-78/CXCL5 in serum were apparently higher in the T1DM patients with healthy tissues compared to the non-T1DM patients with healthy tissues, these differences were not statistically significant.

- Serum resistin levels were significantly correlated with BMI in T1DM patients.
- Although GCF MMP-9 and IL-8 levels were higher in the T1DM patients compared to non-T1DM patients, these differences were not statistically significant.
- In both T1DM and non-T1DM patients, GCF MMP-9 levels were significantly higher in the periodontitis patients compared to those with healthy tissues. Also, MMP-9 levels in GCF were significantly higher in periodontitis patients compared to those with gingivitis. Within the non-T1DM group, GCF MMP-9 levels were significantly higher in the gingivitis patients compared to those with healthy tissues.
- Interestingly, T1DM patients with healthy tissues had significantly higher GCF MMP-9 and IL-8 levels compared to non-T1DM patients with healthy tissues, possibly indicating upregulated periodontal inflammation unmasked by periodontal status in patients with T1DM.
- In non-T1DM patients, significantly higher GCF IL-8 levels were found in the periodontitis patients compared to those with healthy tissues and gingivitis. Also, patients with gingivitis had significantly higher GCF IL-8 levels compared to those with healthy tissues.
- Considering all patients, significant positive correlations were found between GCF MMP-9 levels and mGI, % BOP and mean PD. Also, a significant positive correlation was found between GCF IL-8 levels and mGI, PI, % BOP and mean PD. No statistically significant correlations were demonstrated between serum levels of MMP-9, BAFF, resistin and ENA-78/CXCL5 and any of the clinical periodontal parameters.
- In patients with T1DM, significant positive correlations were found between serum MMP-9 and hsCRP levels and between PI score and HbA1c levels.

5 Chapter 5. Impact of non-surgical periodontal management on clinical periodontal status, markers of diabetes control and local and systemic biomarker levels in patients with T1DM

5.1 Introduction

The impact of diabetes on periodontal disease has been well established but whether there is any influence of periodontal treatment on diabetes control is less clear. Clinical data supporting the existence of an effect of periodontal treatment on diabetes is limited. Some publications have suggested that improvement in clinical periodontal status improves glycaemic control in diabetes (Miller et al. 1992; Grossi et al. 1996; Taylor et al. 1996; Grossi et al. 1997; Iwamoto et al. 2001; Stewart et al. 2001; Rodrigues et al. 2003; Kiran et al. 2005), while other studies have found no such relationship between periodontal treatment and diabetes (Seppala et al. 1993; Seppala and Ainamo 1994; Aldridge et al. 1995; Smith et al. 1996; Firatli 1997; Christgau et al. 1998; Jones et al. 2007; Llambes et al. 2008). Additionally, it has been reported that periodontal treatment can improve glycaemic control with reductions in HbA1c of up to 0.4% (Simpson et al. 2010; Teeuw et al. 2010; Liew et al. 2013).

A positive impact of NSM on clinical outcomes in patients with T1DM has been demonstrated in a small number of studies (Bay et al. 1974; Grossi et al. 1996; Smith et al. 1996; Westfelt et al. 1996; Martorelli de Lima et al. 2004; Llambes et al. 2005). NSM has been found to reduce MMP-9 and IL-8 levels in GCF in systemically healthy individuals (Marcaccini et al. 2010). However, research related to the effect of NSM on circulating inflammatory biomarkers in patients with T1DM is notably lacking. Hence, the aim of this chapter is to report on the effect of NSM on the clinical periodontal status, metabolic control and levels of inflammatory biomarkers in patients with T1DM.

5.2 Results

5.2.1 BMI, BP and medical history following NSM

Table 5.1 summarises pre- and post-treatment (month 0 and 6) BMI data for T1DM and non-T1DM patients with periodontitis. Of note, no statistically significant differences were found for BMI values between T1DM and non-T1DM patients at month 0 (27.0±4.80 kg/m^2 and 24.7±4.91 kg/m^2) and month 6 (26.9±3.87 kg/m^2 and 24.9±2.40 kg/m^2), (P>0.05). The BMI of the T1DM and non-T1DM patients did not significantly change from month 0 to month 6, (P>0.05).

Table 5.2 summarises pre- and post-treatment (month 0 and 6) BP data for T1DM and non-T1DM patients with periodontitis. Of note, no statistically significant differences were found between T1DM and non-T1DM patients at month 0 and month 6, ($P>0.05$). In T1DM patients, compared to pre-treatment [125 (113-138) mmHg], there was a significant increase in systolic BP at month 6 [132.5 (118-148.8) mmHg] following NSM, ($P<0.05$). No statistically significant changes were observed for diastolic BP over the same timescale. In non-T1DM patients, compared to pre-treatment [124 (118-143) mmHg], there was an increase in systolic BP levels at month 6 [136 (129-154) mmHg] following NSM, however, this was not statistically significant, ($P>0.05$). No statistically significant difference was found for diastolic BP levels following NSM.

With reference to the patient's medical history, from the data collected at month 6, it appeared that there were minimal changes to the medical care for all patients over the study period. Out of the 12 T1DM patients and 13 non-T1DM patients seen at month 6, medical history data was collected for 12 T1DM and 9 non-T1DM patients. Of these, change in medical history was reported in 4 (33.3%) T1DM patients and the non-T1DM patients reported no change in medical history at month 6.

		Month 0 (n=19) (n=17)	Month 6 (n=12) (n=13)
BMI (kg/m^2)	**T1DM**	27.0 ± 4.80	26.9 ± 3.87
	Non-T1DM	24.7 ± 4.91	24.9 ± 2.40
	P	NS	NS
BMI category [n (%)]	**T1DM**		
	Underweight (<18.5 kg/m^2)	1 (5.30)	-
	Normal weight (18.5-24.9 kg/m^2)	5 (26.3)	3 (25.0)
	Overweight (25.0-29.9 kg/m^2)	6 (31.6)	7 (58.3)
	Obese (30.0-34.9 kg/m^2)	6 (31.6)	2 (16.7)
	Morbidly obese (>35.0 kg/m^2)	1 (5.30)	-
	Non-T1DM	2 (11.8)	-
	Underweight (<18.5 kg/m^2)	7 (41.2)	4 (44.4)
	Normal weight (18.5-24.9 kg/m^2)	7 (41.2)	5 (55.6)
	Overweight (25.0-29.9 kg/m^2)	0 (0.00)	-
	Obese (30.0-34.9 kg/m^2)	1 (5.90)	-
	Morbidly obese (>35.0 kg/m^2)		
	P	NS	NS

Table 5.1: BMI data for T1DM and non-T1DM patients with periodontitis pre- and post-NSM.

Mean ± SD presented for parametric data and n (%) presented for discrete variables. For comparison between T1DM and non-T1DM patients, p-values determined using independent t-test for continuous parametric variables and p-values determined using chi-squared test for discrete variables. For longitudinal comparisons, p-values determined using paired t-test for parametric variables (no statistically significant differences found). P-values under columns (*P*) relate to comparisons between T1DM and non-T1DM group at that particular time point. BMI; body mass index, NS; not significant.

		Month 0 (n=19) (n=17)	Month 6 (n=12) (n=13)
T1DM	**Systolic BP (mmHg)**	125 (113-138)	132.5 (118-148.8) *
	Diastolic BP (mmHg)	72 (66.0-80.0)	73 (64.5-77.8)
Non-T1DM	**Systolic BP (mmHg)**	124 (118-143)	136 (129-154)
	Diastolic BP (mmHg)	80 (69.0-89.5)	77 (75.0-83.0)

Table 5.2: BP data for T1DM and non-T1DM patients with periodontitis pre- and post-NSM.

Median (IQR) presented for non-parametric data. For comparison between T1DM and non-T1DM patients, p-values determined using Mann-Whitney U test for continuous non-parametric variables (no statistically significant differences found). For longitudinal comparisons, p-values determined using Friedman test with post-hoc Wilcoxon signed rank test for continuous non-parametric variables: significant difference from month 0 *$P<0.05$.

5.2.2 Clinical biochemistry parameters following NSM

Table 5.3 and Figures 5.1 to 5.4 summarise pre- and post-treatment clinical biochemistry data for T1DM and non-T1DM patients with periodontitis. As expected, at each time point, the T1DM patients had a significantly higher HbA1c (%/ mmol/mol) compared to non-T1DM patients, (*P*<0.001). In T1DM patients, pre-treatment HbA1c levels [8.95 (8.03-9.65) %/ 75 (64-83) mmol/mol] showed a reduction at 3 months [8.50 (6.60-9.60) %/ 69 (49-81) mmol/mol], and 6 months [8.05 (6.95-10.1) %/ 64 (53-87) mmol/mol] following NSM, which is a reduction of 0.45% and 0.90% respectively. However, these reductions were not statistically significant, (*P*>0.05) (Table 5.3 and Figure 5.1). Figure 5.2 shows a line and scatter plot of HbA1c levels for individual patients with T1DM and periodontitis at pre-treatment and month 6, with the lines depicting variations in changes in HbA1c levels over this time period. With reference to glycaemic control categories, no statistically significant changes were found between pre- and post-NSM glycaemic control categories (Table 5.4).

When considering non-HDL levels, the non-T1DM patients had significantly higher non-HDL levels at month 3 [4.05 (3.18-4.45) mmol/L] compared to the T1DM patients [3.00 (2.60-3.80) mmol/L], (*P*<0.05). No statistically significant differences were found between the two groups at month 0 and 6. Within the T1DM and non-T1DM groups, compared to pre-treatment, non-HDL levels showed no statistically significant changes following NSM (Table 5.3 and Figure 5.3).

When considering cholesterol levels, the non-T1DM patients had significantly higher levels of cholesterol at pre-treatment (5.18±0.86 mmol/L) compared to the T1DM patients (4.52±0.83 mmol/L), (*P*<0.05). However, no statistically significant differences were found for cholesterol levels between the two groups following NSM at months 3 and 6. Within the T1DM and non-T1DM groups, compared to pre-treatment no significant changes in cholesterol levels were found following NSM (Table 5.3 and Figure 5.4).

When considering levels of triglycerides, HDL and hsCRP, no statistically significant differences were found at any time point between T1DM and non-T1DM patients, (*P*>0.05). Compared to pre-treatment, triglycerides and HDL levels showed no statistically significant changes following NSM in either T1DM or non-T1DM patients at any time point (Table 5.3).

		Month 0 (n=18) (n=16)	Month 3 (n=11) (n=14)	Month 6 (n=12) (n=13)
HbA1c	**T1DM**			
	%	8.95 (8.03-9.65)	8.50 (6.60-9.60)	8.05 (6.95-10.1)
	mmol/mol	75 (64-83)	69 (49-81)	64 (53-87)
	Non-T1DM			
	%	5.40 (5.10-5.68)	5.40 (5.25-5.60)	5.40 (5.25-5.50)
	mmol/mol	36 (32-39)	36 (34-38)	36 (34-37)
	P	< 0.001	< 0.001	< 0.001
Triglycerides (mmol/L)	**T1DM**	1.30 (0.75-1.80)	0.90(0.70-1.70)	1.20 (0.73-1.58)
	Non-T1DM	1.35 (0.75-1.98)	1.55 (0.98-1.93)	1.40 (1.10-2.13)
	P	NS	NS	NS
HDL (mmol/L)	**T1DM**	1.35 (1.23-1.60)	1.60 (1.20-2.10)	1.30 (1.03-1.80)
	Non-T1DM	1.40 (1.13-1.75)	1.40 (1.28-1.93)	1.40 (1.23-1.80)
	P	NS	NS	NS
Non-HDL (mmol/L)	**T1DM**	3.20 (2.30-3.88)	3.00 (2.60-3.80)	2.85 (1.98-3.88)
	Non-T1DM	3.55 (2.68-4.20)	4.05 (3.18-4.45)	4.00 (3.13-4.58)
	P	NS	< 0.05	NS
Cholesterol (mmol/L)	**T1DM**	4.52 ± 0.83	4.66 ± 0.90	4.64 ± 1.49
	Non-T1DM	5.18 ± 0.86	5.27 ± 0.78	5.43 ± 0.91
	P	< 0.05	NS	NS
hsCRP (mg/L)	**T1DM**	1.80 (1.23-7.75)	1.60 (0.50-6.00)	1.90 (0.33-5.28)
	Non-T1DM	1.50 (0.60-3.20)	0.85 (0.55-2.45)	1.60 (0.60-3.80)
	P	NS	NS	NS

Table 5.3: Clinical biochemistry data for T1DM and non-T1DM patients with periodontitis pre- and post-NSM.

Mean ± SD presented for parametric data. For comparison between T1DM and non-T1DM patients, p-values determined using Independent t-test for continuous parametric variable and using Mann-Whitney U test for continuous non-parametric variables. For longitudinal comparisons, p-values determined using Paired t-test for parametric variables and using Friedman test with post-hoc Wilcoxon signed rank test for continuous non-parametric variables (no statistically significant differences found). P-values under columns (*P*) relate to comparisons between T1DM and non-T1DM group at that particular time point. HbA1c; glycated haemoglobin, HDL; high density lipoprotein, non-HDL; non-high density lipoprotein, hsCRP; high-sensitivity C-reactive protein, NS; not significant. At month 6: HbA1c (T1DM n=12 and non-T1DM n=13); triglycerides, HDL, non-HDL and cholesterol (T1DM n=12 and non-T1DM n=12); hsCRP (T1DM n=12 and non-T1DM n=11).

	Month 0 (n=19)	Month 3 (n=11)	Month 6 (n=12)
Glycaemic control categories [n (%)]			
Good (<7.0 %)	3 (15.8)	3 (27.3)	3 (25.0)
Moderate (7.0-8.5 %)	5 (26.3)	2 (18.2)	4 (33.3)
Poor (>8.5 %)	10 (52.6)	6 (54.5)	5 (41.7)

Table 5.4: Glycaemic control category of T1DM patients with periodontitis pre- and post-NSM.

N (%) presented for discrete variables. P-values determined using chi-squared test for discrete variables (no statistically significant differences found between time points).

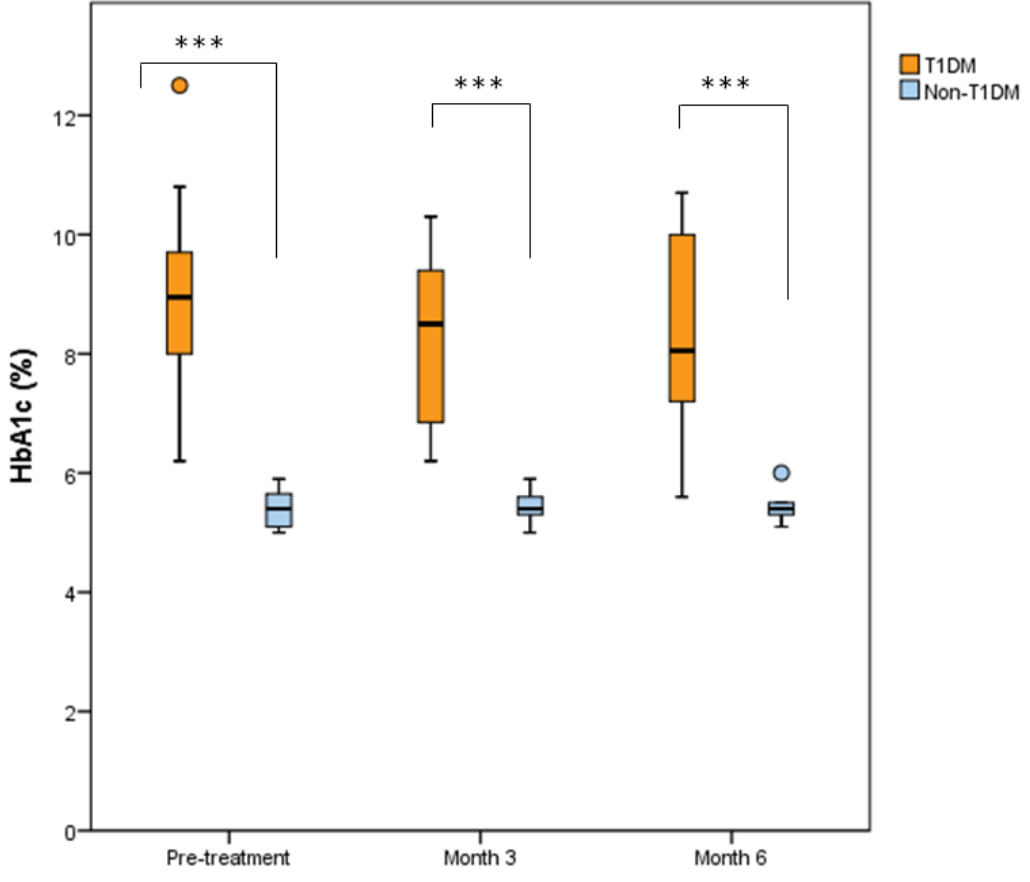


Figure 5.1: HbA1c levels in T1DM and non-T1DM patients with periodontitis pre- and post-NSM.

Box plot figure showing medians, interquartile ranges, standard errors and outliers for T1DM patients (pre-treatment n=18, month 3 n=11, month 6 n=12) and non-T1DM patients (pre-treatment n=16, month 3 n=14, month 6 n=13). Statistics: Friedman test with post-hoc Wilcoxon signed rank test for longitudinal comparisons according to time point within T1DM or non-T1DM group (no statistically significant differences found); Mann-Whitney U test for T1DM versus non-T1DM group at each time point: ****P*<0.001. ● indicates outlier more than 1.5 but less than 3 times the IQR from the box boundaries.

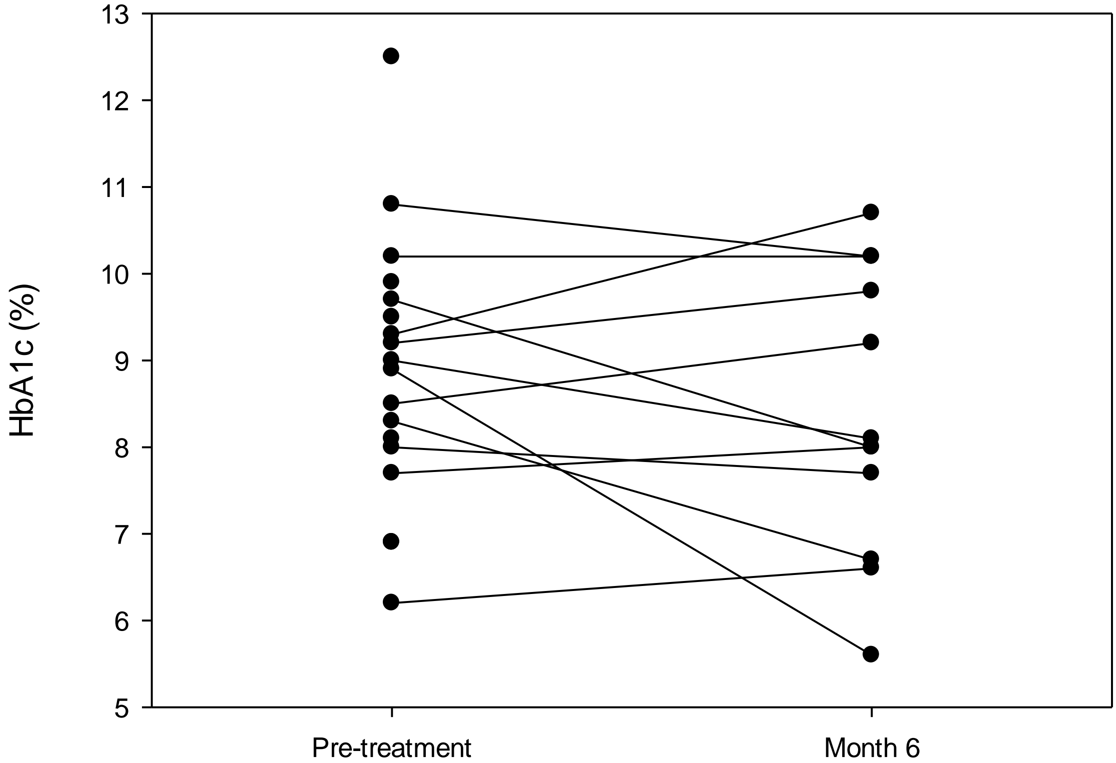


Figure 5.2: HbA1c levels in individual patients with periodontitis and T1DM pre-treatment and month 6.

Line and scatter plot of HbA1c levels for individual patients with T1DM and periodontitis at pre-treatment (n=18) and month 6 (n=12). Lines highlight the direction of change in HbA1c.

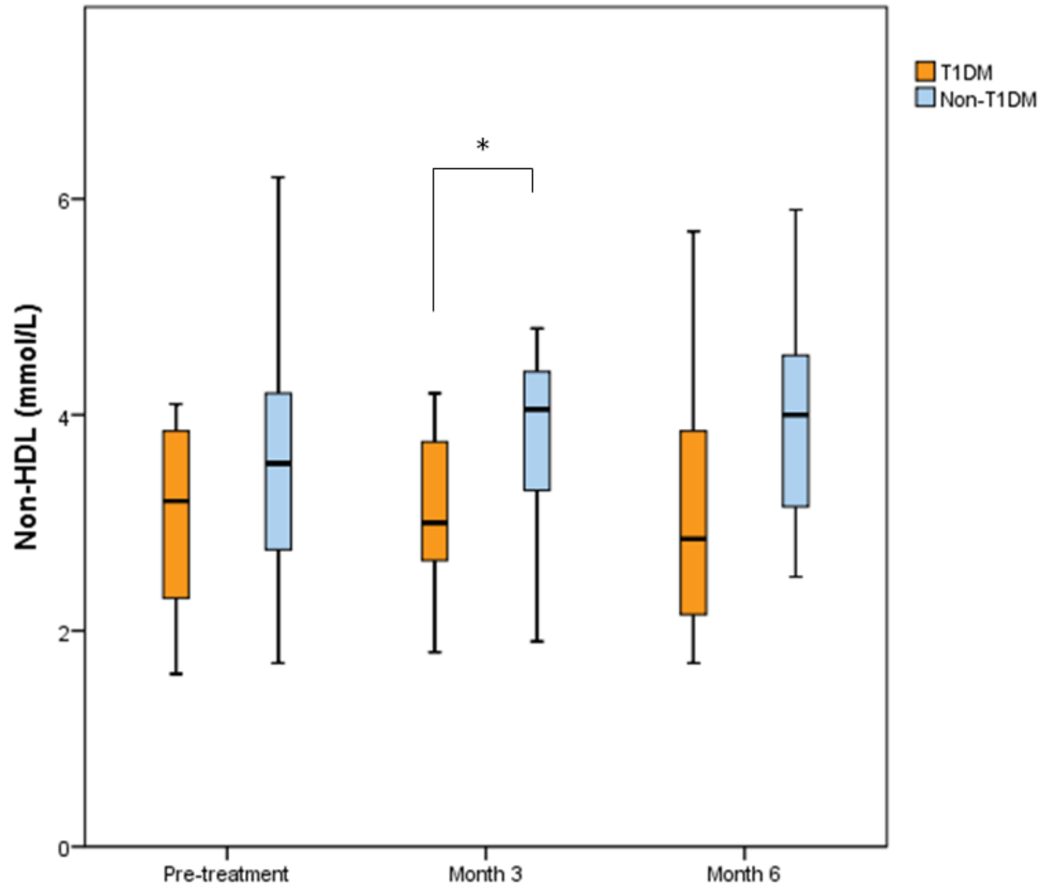


Figure 5.3: Non-HDL levels in T1DM and non-T1DM patients with periodontitis pre- and post-NSM.

Box plot figure showing medians, interquartile ranges, standard errors and outliers for T1DM patients (pre-treatment n=18, month 3 n=11, month 6 n=12) and non-T1DM patients (pre-treatment n=16, month 3 n=14, month 6 n=13). Statistics: Friedman test with post-hoc Wilcoxon signed rank test for longitudinal comparisons according to time point within T1DM or non-T1DM group (no statistically significant differences found); Mann-Whitney U test: *$P<0.05$ (T1DM versus non-T1DM group at each time point).

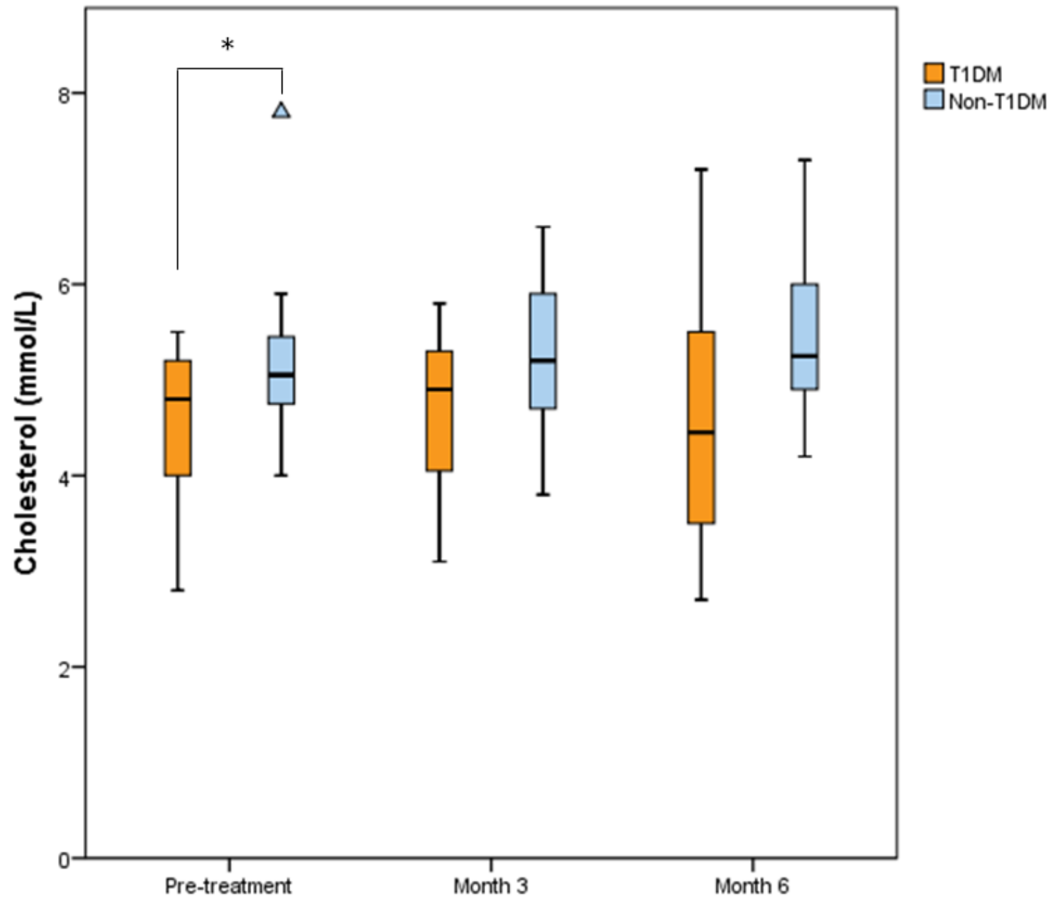


Figure 5.4: Cholesterol levels in T1DM and non-T1DM patients with periodontitis pre- and post-NSM.

Box plot figure showing medians, interquartile ranges, standard errors and outliers for T1DM patients (pre-treatment n=18, month 3 n=11, month 6 n=12) and non-T1DM patients (pre-treatment n=16, month 3 n=14, month 6 n=13). Statistics: Paired t-test for longitudinal comparisons according to time point within T1DM or non-T1DM group (no statistically significant differences found); Independent t-test: *$P<0.05$ (T1DM versus non-T1DM group at each time point). ▲ indicates outlier more than 3 times the IQR from the box boundaries.

5.2.3 Clinical periodontal parameters following NSM

Table 5.5 and Figures 5.5 to 5.12 summarise pre- and post-treatment clinical periodontal data for T1DM and non-T1DM patients with periodontitis. When considering gingival inflammation, no statistically significant differences in mGI scores were found between T1DM and non-T1DM patients at any time point, ($P>0.05$). In patients with T1DM, compared to pre-treatment (1.96±0.51) mGI score showed significant reduction (improvement) following NSM at 3 months (0.87±0.53) and 6 months (1.12±0.76), ($P<0.001$). Similarly in non-T1DM patients, compared to pre-treatment (2.29±0.53) mGI score showed significant reduction following NSM at 3 months (1.25±0.63) and 6 months (1.27±0.61), ($P<0.001$) (Table 5.5 and Figure 5.5).

When considering plaque levels, no statistically significant differences in PI scores were found between T1DM and non-T1DM patients at any time point, ($P>0.05$). In patients with T1DM, compared to pre-treatment levels (0.98±0.54), PI score showed significant reduction (improvement) following NSM at 3 months (0.44±0.23) and 6 months (0.50±0.24), ($P<0.01$). Similarly in non-T1DM patients, compared to pre-treatment levels (0.66±0.29) PI score showed significant reduction following NSM at 3 months (0.28±0.25) and 6 months (0.32±0.26), ($P<0.001$ and $P<0.01$ respectively) (Table 5.5 and Figure 5.6).

When considering PD measurements, no statistically significant differences in mean PD were found between T1DM and non-T1DM patients at pre-treatment and following NSM at months 3 and 6, ($P>0.05$). In patients with T1DM, compared to pre-treatment (3.02±0.81 mm), mean PD showed significant reduction (improvement) following NSM at 3 months (2.40±0.81 mm) and 6 months (2.42±0.53 mm), ($P<0.01$ and $P<0.001$ respectively). Similarly in non-T1DM patients, compared to pre-treatment (3.31±0.75 mm), mean PD showed significant reduction following NSM at 3 months (2.70±0.37 mm) and 6 months (2.65±0.49 mm), ($P<0.001$ and $P<0.01$ respectively) (Table 5.5 and Figure 5.7).

When considering gingival recession, no statistically significant differences were found between T1DM and non-T1DM patients at any time point, ($P>0.05$). In patients with T1DM, compared to pre-treatment (0.43±0.64 mm), mean recession showed a significant increase following NSM at 6 months (0.55±0.61 mm), ($P<0.01$). In non-T1DM patients, compared to pre-treatment (0.24±0.43 mm), mean recession showed a significant increase following NSM at 3 months (0.54±0.55 mm) and 6 months (0.61±0.66 mm), ($P<0.001$) (Table 5.5 and Figure 5.8).

When considering clinical LOA, no statistically significant differences were found for mean LOA between T1DM and non-T1DM patients at any time point, ($P>0.05$). In patients with T1DM, compared to pre-treatment (3.45±1.18 mm), mean LOA showed a significant reduction following NSM at 3 months (2.74±1.13 mm) and 6 months (2.97±0.94 mm), ($P<0.05$). Similarly in non-T1DM patients, compared to pre-treatment (3.55±0.75 mm), mean LOA showed significant reductions following NSM at 3 months (3.24±0.50 mm) and 6 months (3.26±0.54 mm), ($P<0.01$) (Table 5.5 and Figure 5.9).

When considering BOP scores, no statistically significant differences were found between the T1DM and non-T1DM patients at any time point, ($P>0.05$). In patients with T1DM, compared to pre-treatment scores (52.7±17.4 %), % BOP showed a significant reduction following NSM at 3 months (26.2±20.1 %), and 6 months (29.2±17.7 %), ($P<0.01$ and $P<0.001$ respectively). Similarly in non-T1DM patients, compared to pre-treatment scores (53.9±21.1 %), % BOP showed significant reduction following NSM at 3 months (22.1±15.1 %) and 6 months (20.6±16.2 %), ($P<0.001$) (Table 5.5 and Figure 5.10).

For PD sites measuring ≥5 mm, the non-T1DM patients had significantly higher number (%) of PD sites ≥5 mm at pre-treatment [37.4±25.2 (23.7±15.5 %)] compared to T1DM patients [20.3±21.7 (14.7±16.4 %)], ($P<0.05$). However, no statistically significant differences were found between the two groups at months 3 and 6, ($P>0.05$). In patients with T1DM, compared to pre-treatment [20.3±21.7 (14.7±16.4 %)], there was a significant reduction (improvement) in number (%) of PD sites ≥5 mm following NSM at 3 months [11.0±17.5 (7.94±13.8 %)] and 6 months [9.69±13.9 (6.97±10.9 %)], ($P<0.01$). Similarly in non-T1DM patients, compared to pre-treatment [37.4±25.2 (23.7±15.5 %)] there was a significant reduction in number (%) of PD sites ≥5 mm following NSM at 3 months [20.6±13.1 (12.8±7.59 %)] and 6 months [18.7±15.6 (12.6±9.29 %)], ($P<0.001$ and $P<0.01$ respectively) (Table 5.5 and Figure 5.11).

For PD sites measuring ≤4 mm, no statistically significant differences were found between T1DM and non-T1DM patients at any time point, ($P>0.05$). In patients with T1DM, compared to pre-treatment [122.5±29.4 (85.3±16.4 %)], there was a significant increase (improvement) in number (%) of PD sites ≤4 mm following NSM at 3 months [136.3±25.2 (92.1±13.8 %)] and 6 months [136.6±20.7 (93.0±10.9 %)], ($P<0.01$). Similarly in non-T1DM patients, compared to pre-treatment [119.0±25.1 (74.7±17.7 %)], there was a significant increase in number (%) of PD sites ≤4 mm following NSM at 3 months

[135.8±14.4 (82.3±19.3 %)] and 6 months [136.8±15.7 (84.1±17.1 %)], ($P<0.01$) (Table 5.5 and Figure 5.12).

Table 5.6 and Figures 5.13 and 5.14 summarises the number (%) of sites displaying increase and decrease in PD from pre-treatment to month 3 and 6. Compared to pre-treatment mean PD, at month 3 the number (%) of sites with ≥2 mm PD increase in T1DM patients [4.18±5.25 (3.04±4.18 %)] and non-T1DM patients [3.83±2.41 (2.45±1.50 %)] were not statistically significantly different, ($P>0.05$). Similarly, compared to pre-treatment mean PD, at month 6 the number (%) of sites with ≥2 mm PD increase in T1DM patients [4.31±4.05 (3.08±3.12 %)] and non-T1DM patients [3.09±2.74 (1.98±1.67 %)] were not statistically significantly different, ($P>0.05$).

Compared to pre-treatment mean PD, at month 3 the number (%) of sites with 0+/-1 mm PD reduction in T1DM patients [123.2±20.2 (83.8±13.3 %)] and non-T1DM patients [120.4±23.8 (74.2±15.6 %)] were not statistically significantly different, ($P>0.05$). However compared to pre-treatment, at month 6 the number (%) of sites with 0+/-1 mm PD reduction in T1DM patients [124.2±16.9 (84.9±9.83 %)] was significantly higher compared to non-T1DM patients [113.7±26.1 (73.2±15.9 %)], ($P<0.05$) (Table 5.6 and Figure 5.13).

Compared to pre-treatment mean PD, at month 3 the number (%) of sites with ≥2 mm PD reduction in T1DM [19.9±19.4 (13.2±12.5 %)] and non-T1DM patients [32.7±22.9 (20.8±14.1 %)] were not statistically significantly different, ($P>0.05$). However compared to pre-treatment, at month 6 the number (%) of sites with ≥2 mm PD reduction in non-T1DM patients [39.0±26.7 (24.9±16.4 %)] was significantly higher compared to T1DM patients [17.5±12.5 (12.1±8.44 %)], ($P<0.05$) (Table 5.6 and Figure 5.14).

Of note, in both T1DM and non-T1DM patients a majority of PD sites had a reduction of 0+/-1 mm, followed by a reduction of ≥2 mm and whereas only a small proportion of sites had an increase of PD ≥2 mm following NSM.

		Month 0 (n=19) (n=17)	Month 3 (n=11) (n=15)	Month 6 (n=13) (n=13)
mGI	**T1DM**	1.96 ± 0.51	0.87 ± 0.53 ***	1.12 ± 0.76 ***
	Non-T1DM	2.29 ± 0.53	1.25 ± 0.63 ***	1.27 ± 0.61 ***
	P	NS	NS	NS
PI	**T1DM**	0.98 ± 0.54	0.44 ± 0.23 **	0.50 ± 0.24 **
	Non-T1DM	0.66 ± 0.29	0.28 ± 0.25 ***	0.32 ± 0.26 **
	P	NS	NS	NS
Mean PD (mm)	**T1DM**	3.02 ± 0.81	2.40 ± 0.81 **	2.42 ± 0.53 ***
	Non-T1DM	3.31 ± 0.75	2.70 ± 0.37 ***	2.65 ± 0.49 **
	P	< 0.05	NS	NS
Mean recession (mm)	**T1DM**	0.43 ± 0.64	0.34 ± 0.52	0.55 ± 0.61 **
	Non-T1DM	0.24 ± 0.43	0.54 ± 0.55 ***	0.61 ± 0.66 ***
	P	NS	NS	NS
Mean LOA (mm)	**T1DM**	3.45 ± 1.18	2.74 ± 1.13 *	2.97 ± 0.94 *
	Non-T1DM	3.55 ± 0.75	3.24 ± 0.50 **	3.26 ± 0.54 **
	P	NS	NS	NS
BOP (%)	**T1DM**	52.7 ± 17.4	26.2 ± 20.1 **	29.2 ± 17.7 ***
	Non-T1DM	53.9 ± 21.1	22.1 ± 15.1 ***	20.6 ± 16.2 ***
	P	NS	NS	NS
Sites 5 mm or greater [n (%)]	**T1DM**	20.3 ± 21.7 (14.7 ± 16.4)	11.0 ± 17.5 ** (7.94 ± 13.8)	9.69 ± 13.9 ** (6.97 ± 10.9)
	Non-T1DM	37.4 ± 25.2 (23.7 ± 15.5)	20.6 ± 13.1 *** (12.8 ± 7.59)	18.7 ± 15.6 ** (12.6 ± 9.29)
	P	< 0.05	NS	NS
Sites 4 mm or less [n (%)]	**T1DM**	122.5 ± 29.4 (85.3 ± 16.4)	136.3 ± 25.2 ** (92.1 ± 13.8)	136.6 ± 20.7 ** (93.0 ± 10.9)
	Non-T1DM	119.0 ± 25.1 (74.7 ± 17.7)	135.8 ± 14.4 ** (82.3 ± 19.3)	136.8 ± 15.7 ** (84.1 ± 17.1)
	P	NS	NS	NS

Table 5.5: Clinical periodontal data for T1DM and non-T1DM patients with periodontitis pre- and post-NSM.

Mean ± SD presented for parametric variables. For comparison between T1DM and non-T1DM patients, p-values determined using Independent t-test for continuous parametric variables. For longitudinal comparisons, p-values determined using Paired t-test for parametric variables: significant difference from baseline *P<0.05, **P<0.01 and ***P<0.001. P-values under columns (*P*) relate to comparisons between T1DM and non-T1DM group at that particular time point. mGI; modified gingival index, PI; plaque index, PD; probing depth, LOA; loss of attachment, BOP; bleeding on probing, NS; not significant.

		Month 0-Month 3 **(n=11)** **(n=14)**	**Month 0-Month 6** **(n=13)** **(n=12)**
Sites 2 mm or greater PD increase [n (%)]	**T1DM**	4.18 ± 5.25 (3.04 ± 4.18)	4.31 ± 4.05 (3.08 ± 3.12)
	Non-T1DM	3.83 ± 2.41 (2.45 ± 1.50)	3.09 ± 2.74 (1.98 ± 1.67)
	P	NS	NS
Sites 0 +/- 1 mm PD reduction [n (%)]	**T1DM**	123.2 ± 20.2 (83.8 ± 13.3)	124.2 ± 16.9 (84.9 ± 9.83)
	Non-T1DM	120.4 ± 23.8 (74.2 ± 15.6)	113.7 ± 26.1 (73.2 ± 15.9)
	P	NS	< 0.05
Sites 2 mm or greater PD reduction [n (%)]	**T1DM**	19.9 ± 19.4 (13.2 ± 12.5)	17.5 ± 12.5 (12.1 ± 8.44)
	Non-T1DM	32.7 ±22.9 (20.8 ± 14.1)	39.0 ± 26.7 (24.9 ± 16.4)
	P	NS	< 0.05

Table 5.6: Changes in PD in T1DM and non-T1DM patients with periodontitis pre- and post-NSM.

Mean ± SD presented for parametric variables. For comparison between T1DM and non-T1DM patients, p-values determined using Independent t-test for continuous parametric variables. For longitudinal comparisons, p-values determined using Paired t-test for parametric variables (no statistically significant differences found). *P* represents the significant difference between T1DM and non-T1DM patients at each time point. PD; probing depth, NS; not significant.

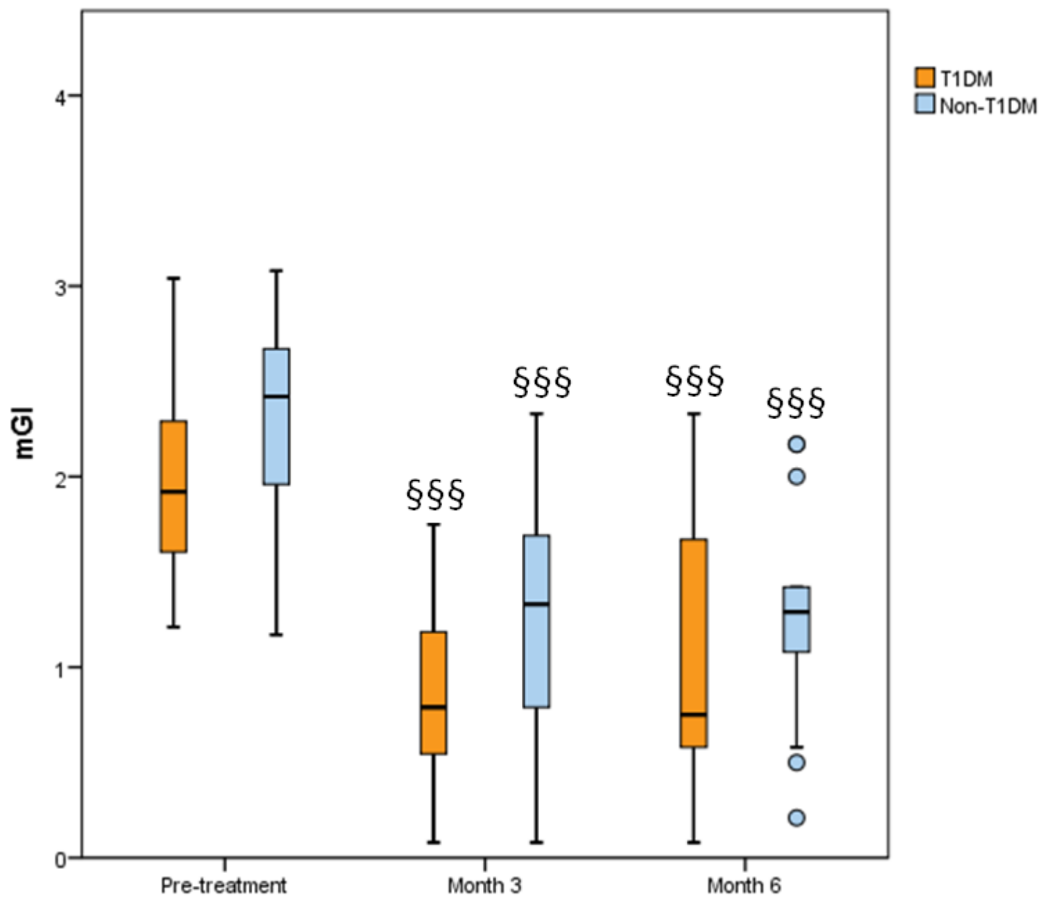


Figure 5.5: mGI scores in T1DM and non-T1DM patients with periodontitis pre- and post-NSM.

Box plot figure showing medians, interquartile ranges, standard errors and outliers for T1DM patients (pre-treatment n=19, month 3 n=11, month 6 n=13) and non-T1DM patients (pre-treatment n=17, month 3 n=15, month 6 n=13). Statistics: Paired t-test for longitudinal comparisons: §§§$P<0.001$ (within the T1DM or non-T1DM group indicating significant differences from pre-treatment at each time point); Independent t-test for T1DM versus non-T1DM group at each time point (no statistically significant differences found). ● indicates outlier more than 1.5 but less than 3 times the IQR from the box boundaries.

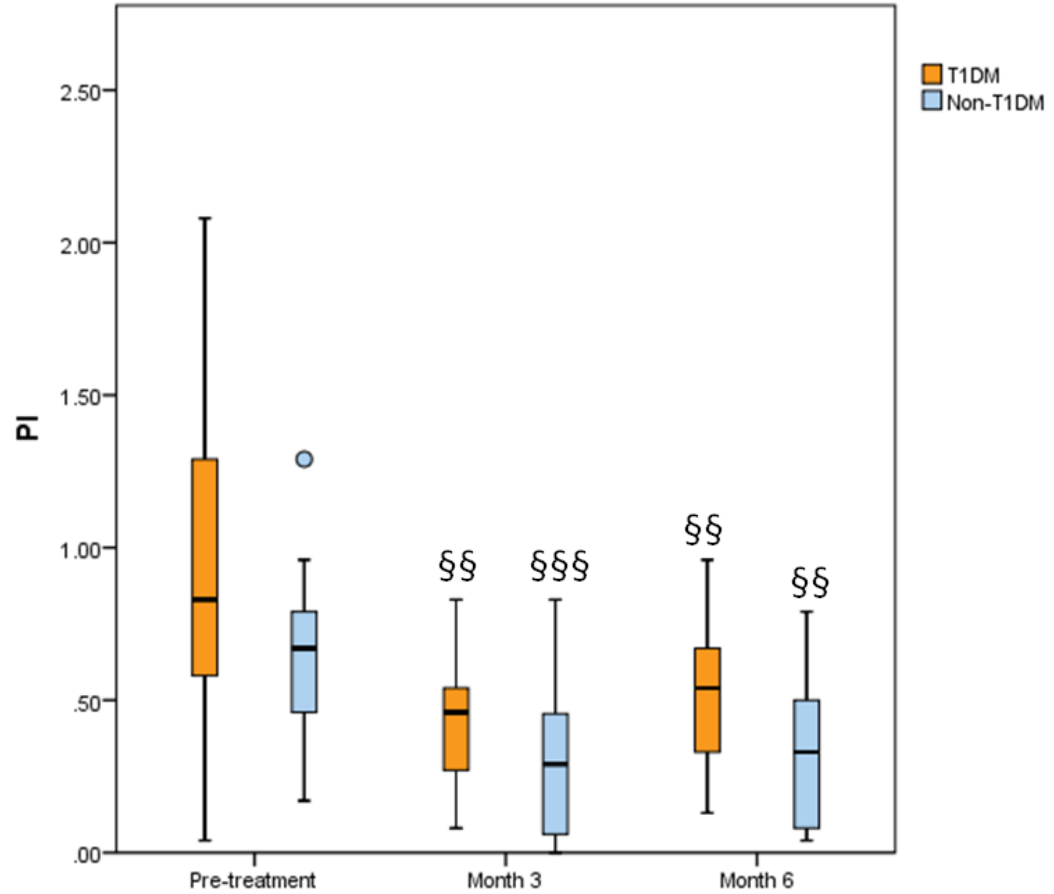


Figure 5.6: PI scores in T1DM and non-T1DM patients with periodontitis pre- and post-NSM.

Box plot figure showing medians, interquartile ranges, standard errors and outliers for T1DM patients (pre-treatment n=19, month 3 n=11, month 6 n=13) and non-T1DM patients (pre-treatment n=17, month 3 n=15, month 6 n=13). Statistics: Paired t-test for longitudinal comparisons: §§$P<0.01$, §§§$P<0.001$ (within the T1DM or non-T1DM group indicating significant differences from pre-treatment at each time point); Independent t-test for T1DM versus non-T1DM group at each time point (no statistically significant differences found).
● indicates outlier more than 1.5 but less than 3 times the IQR from the box boundaries.

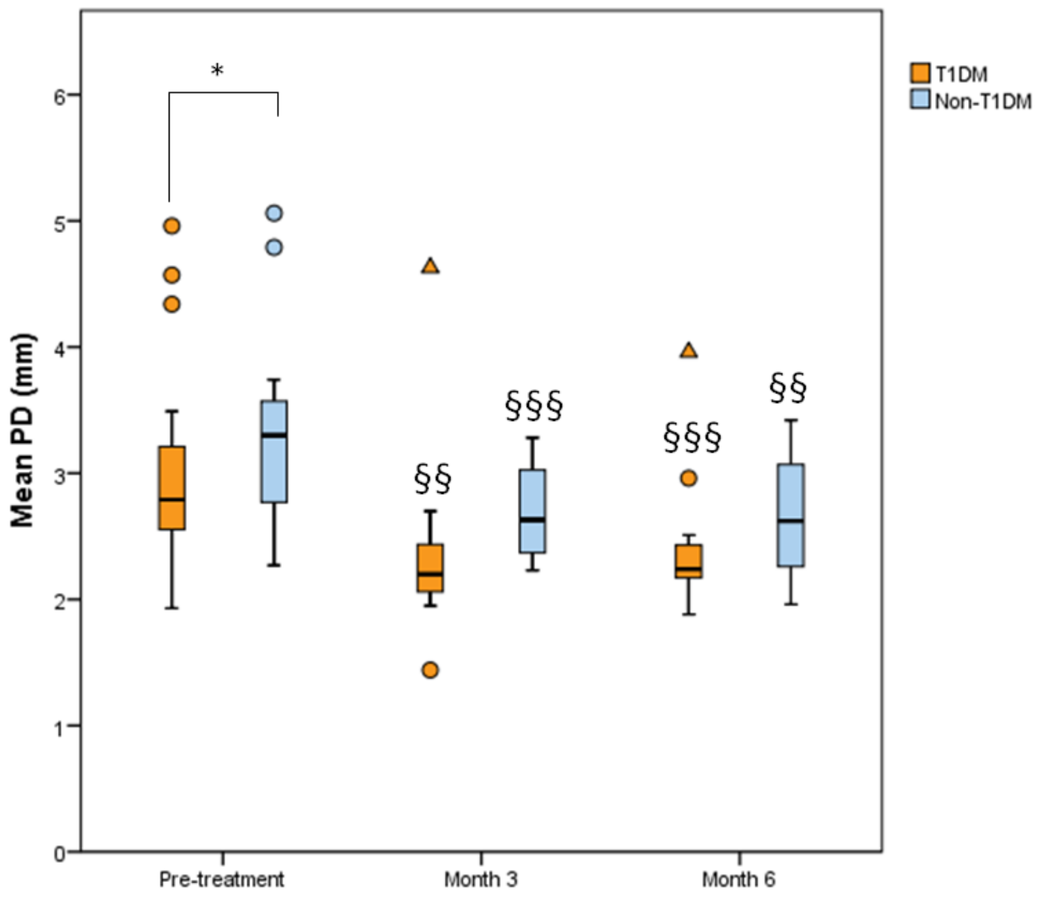


Figure 5.7: Mean PD in T1DM and non-T1DM patients with periodontitis pre- and post-NSM.

Box plot figure showing medians, interquartile ranges, standard errors and outliers for T1DM patients (pre-treatment n=19, month 3 n=11, month 6 n=13) and non-T1DM patients (pre-treatment n=17, month 3 n=15, month 6 n=13). Statistics: Paired t-test for longitudinal comparisons: §§P<0.01, §§§P<0.001 (within the T1DM or non-T1DM group indicating significant differences from pre-treatment at each time point); Independent t-test: *P<0.05 (T1DM versus non-T1DM group at each time point). ● indicates outlier more than 1.5 but less than 3 times the IQR from the box boundaries, ▲ indicates outlier more than 3 times the IQR from the box boundaries.

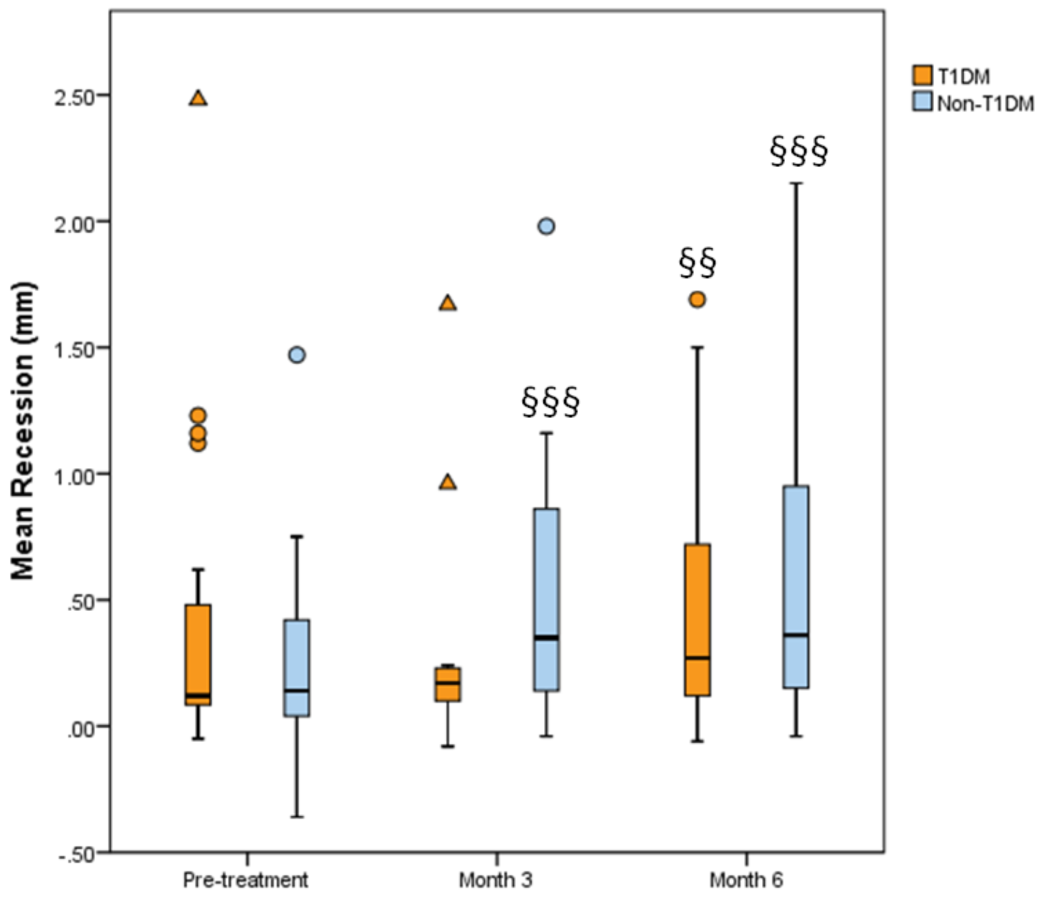


Figure 5.8: Mean recession in T1DM and non-T1DM patients with periodontitis pre- and post-NSM.

Box plot figure showing medians, interquartile ranges, standard errors and outliers for T1DM patients (pre-treatment n=19, month 3 n=11, month 6 n=13) and non-T1DM patients (pre-treatment n=17, month 3 n=15, month 6 n=13). Statistics: Paired t-test for longitudinal comparisons: §§*P*<0.01, §§§*P*<0.001 (within the T1DM or non-T1DM group indicating significant differences from pre-treatment at each time point); Independent t-test for T1DM versus non-T1DM group at each time point (no statistically significant differences found).
● indicates outlier more than 1.5 but less than 3 times the IQR from the box boundaries,
▲ indicates outlier more than 3 times the IQR from the box boundaries.

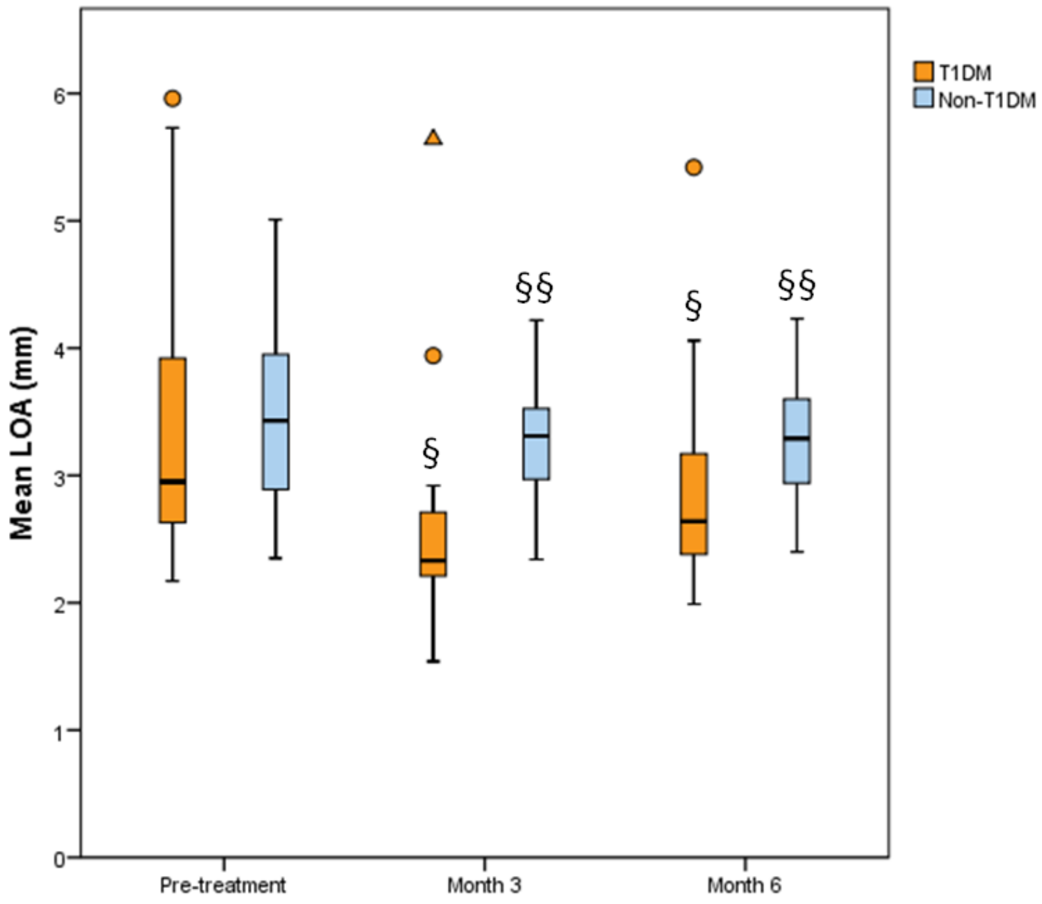


Figure 5.9: Mean LOA in T1DM and non-T1DM patients with periodontitis pre- and post-NSM.

Box plot figure showing medians, interquartile ranges, standard errors and outliers for T1DM patients (pre-treatment n=19, month 3 n=11, month 6 n=13) and non-T1DM patients (pre-treatment n=17, month 3 n=15, month 6 n=13). Statistics: Paired t-test for longitudinal comparisons: §$P<0.05$, §§$P<0.01$ (within the T1DM or non-T1DM group indicating significant differences from pre-treatment at each time point); Independent t-test for T1DM versus non-T1DM group at each time point (no statistically significant differences found).
● indicates outlier more than 1.5 but less than 3 times the IQR from the box boundaries,
▲ indicates outlier more than 3 times the IQR from the box boundaries.

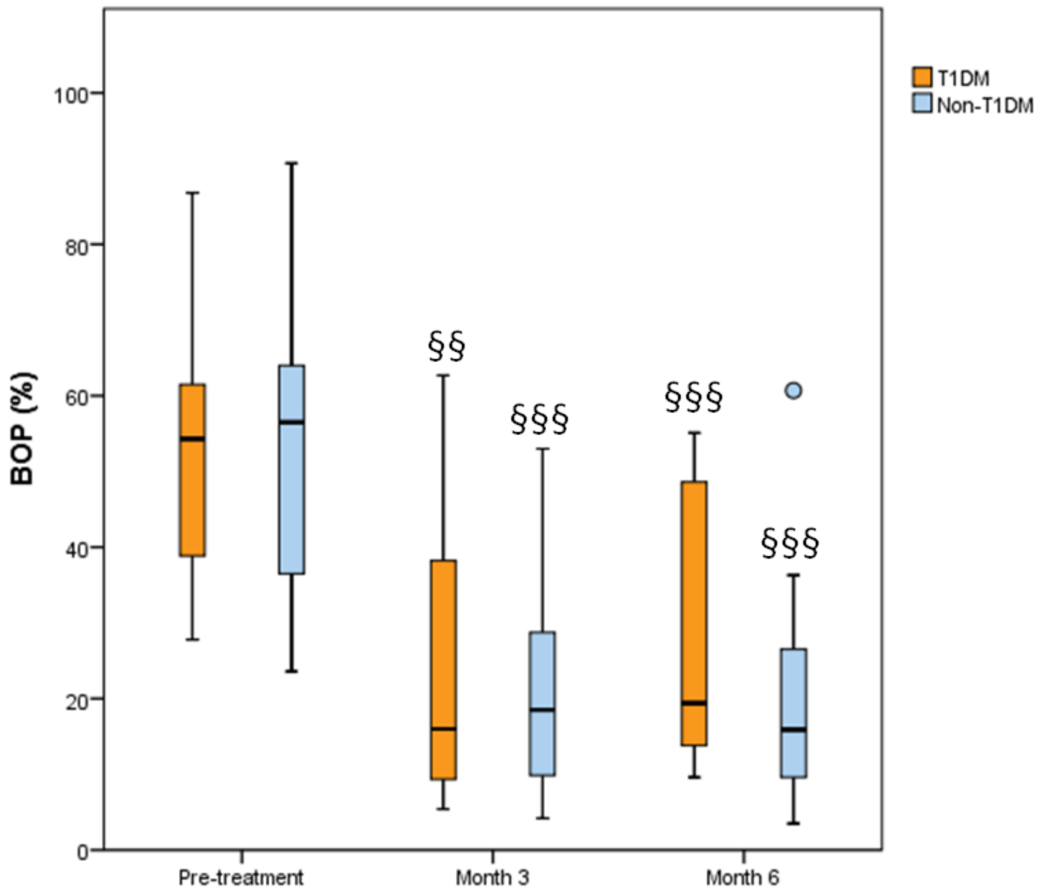


Figure 5.10: BOP (%) in T1DM and non-T1DM patients with periodontitis pre- and post-NSM.

Box plot figure showing medians, interquartile ranges, standard errors and outliers for T1DM patients (pre-treatment n=19, month 3 n=11, month 6 n=13) and non-T1DM patients (pre-treatment n=17, month 3 n=15, month 6 n=13). Statistics: Paired t-test for longitudinal comparisons: §§P<0.01, §§§P<0.001 (within the T1DM or non-T1DM group indicating significant differences from pre-treatment at each time point); Independent t-test for T1DM versus non-T1DM group at each time point (no statistically significant differences found).
● indicates outlier more than 1.5 but less than 3 times the IQR from the box boundaries.

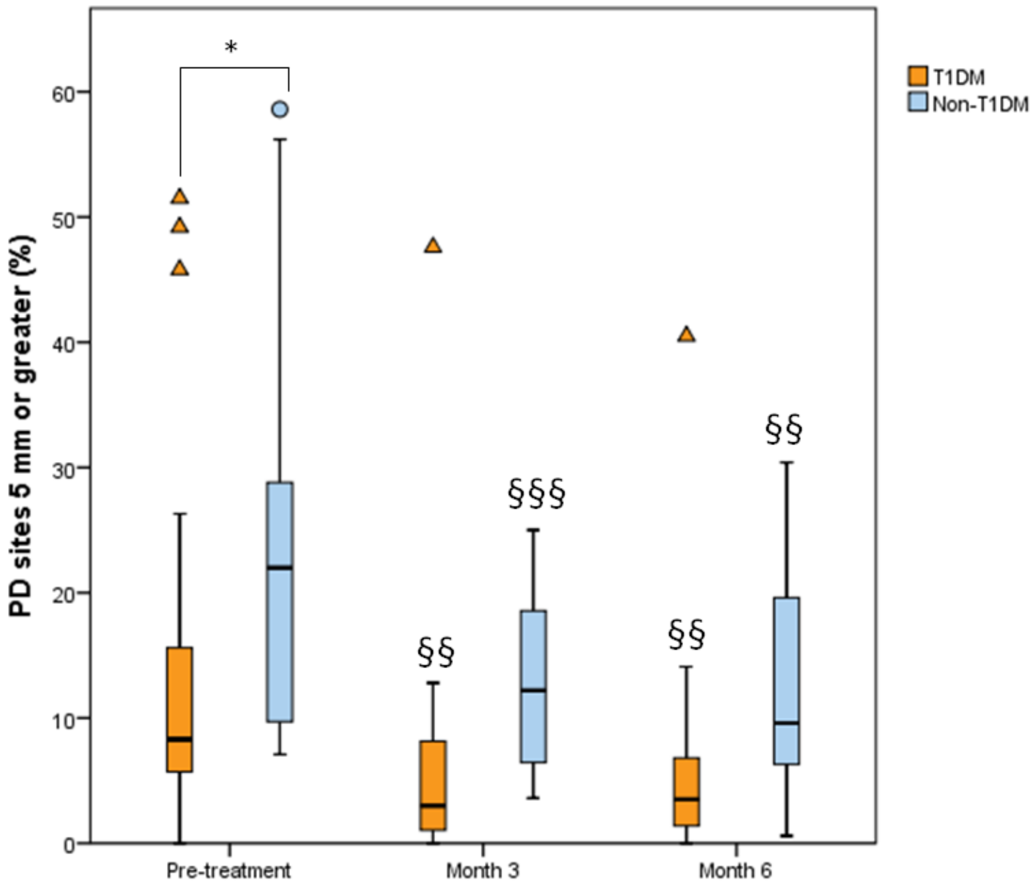


Figure 5.11: PD sites 5 mm or greater (%) in T1DM and non-T1DM patients with periodontitis pre- and post-NSM.

Box plot figure showing medians, interquartile ranges, standard errors and outliers for T1DM patients (pre-treatment n=19, month 3 n=11, month 6 n=13) and non-T1DM patients (pre-treatment n=17, month 3 n=15, month 6 n=13). Statistics: Paired t-test for longitudinal comparisons: §§*P*<0.01, §§§*P*<0.001 (within the T1DM or non-T1DM group indicating significant differences from pre-treatment at each time point); Independent t-test: **P*<0.05 (T1DM versus non-T1DM group at each time point). ● indicates outlier more than 1.5 but less than 3 times the IQR from the box boundaries, ▲ indicates outlier more than 3 times the IQR from the box boundaries.

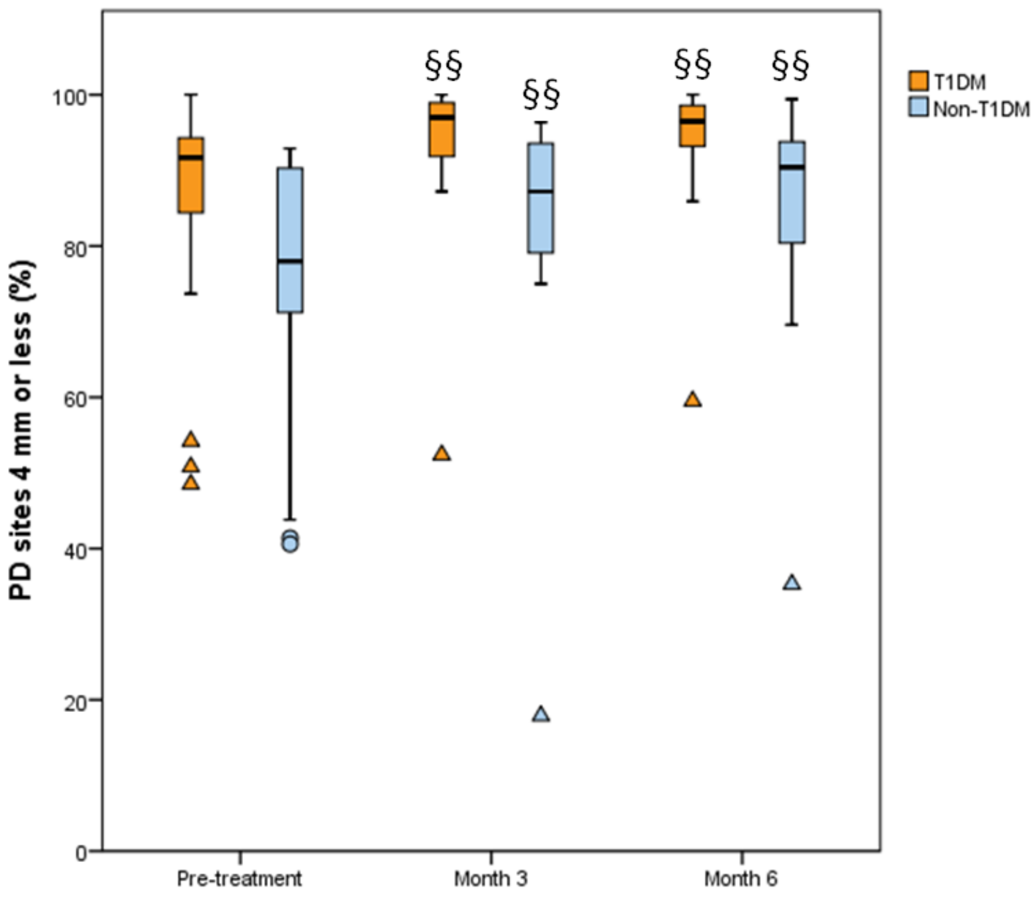


Figure 5.12: PD sites 4 mm or less (%) in T1DM and non-T1DM patients with periodontitis pre- and post-NSM.

Box plot figure showing medians, interquartile ranges, standard errors and outliers for T1DM patients (pre-treatment n=19, month 3 n=11, month 6 n=13) and non-T1DM patients (pre-treatment n=17, month 3 n=15, month 6 n=13). Statistics: Paired t-test for longitudinal comparisons: §§$P<0.01$ (within the T1DM or non-T1DM group indicating significant differences from pre-treatment at each time point); Independent t-test for T1DM versus non-T1DM group at each time point (no statistically significant differences found). ● indicates outlier more than 1.5 but less than 3 times the IQR from the box boundaries, ▲ indicates outlier more than 3 times the IQR from the box boundaries.

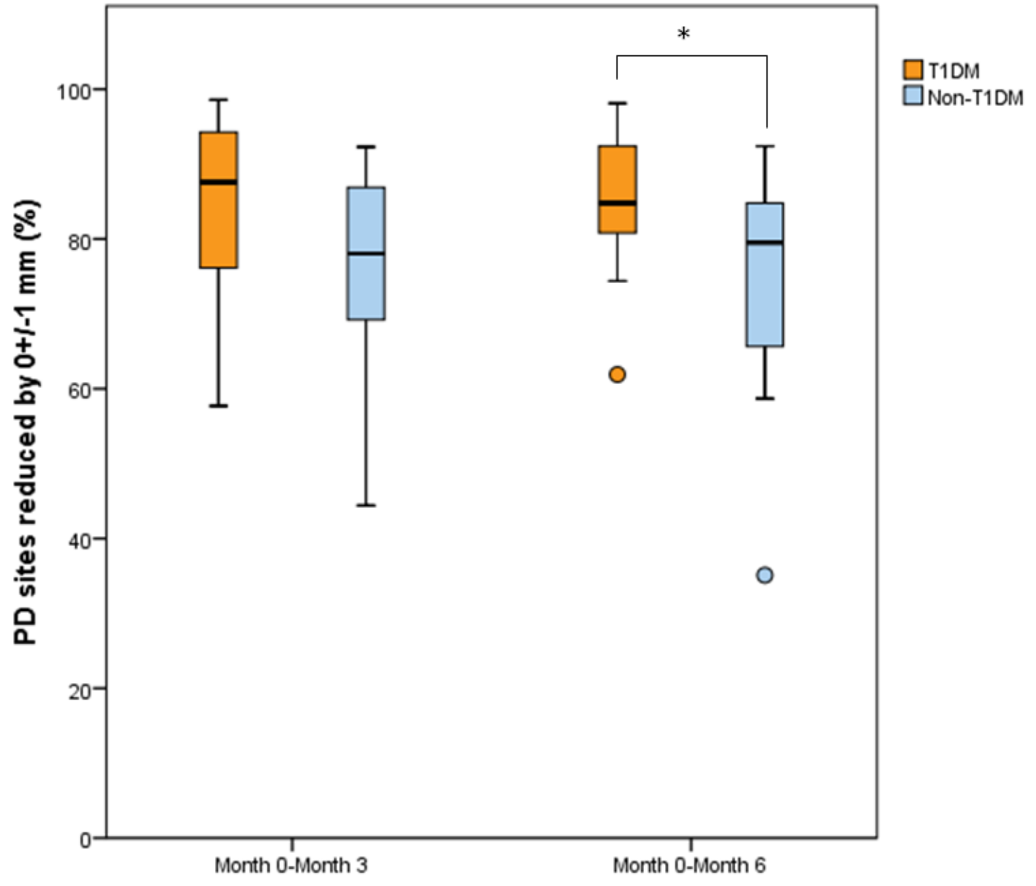


Figure 5.13: PD sites reduced by 0+/-1 mm (%) in T1DM and non-T1DM patients with periodontitis post-NSM.

Box plot figure showing medians, interquartile ranges, standard errors and outliers for T1DM patients (month 0-month 3 n=11, month 0-month 6 n=13) and non-T1DM patients (month 0-month 3 n=14, month 0-month 6 n=12). Statistics: Paired t-test for longitudinal comparisons within T1DM or non-T1DM group (no statistically significant differences found); Independent t-test: $*P<0.05$ (T1DM versus non-T1DM group at each time point). ● indicates outlier more than 1.5 but less than 3 times the IQR from the box boundaries.

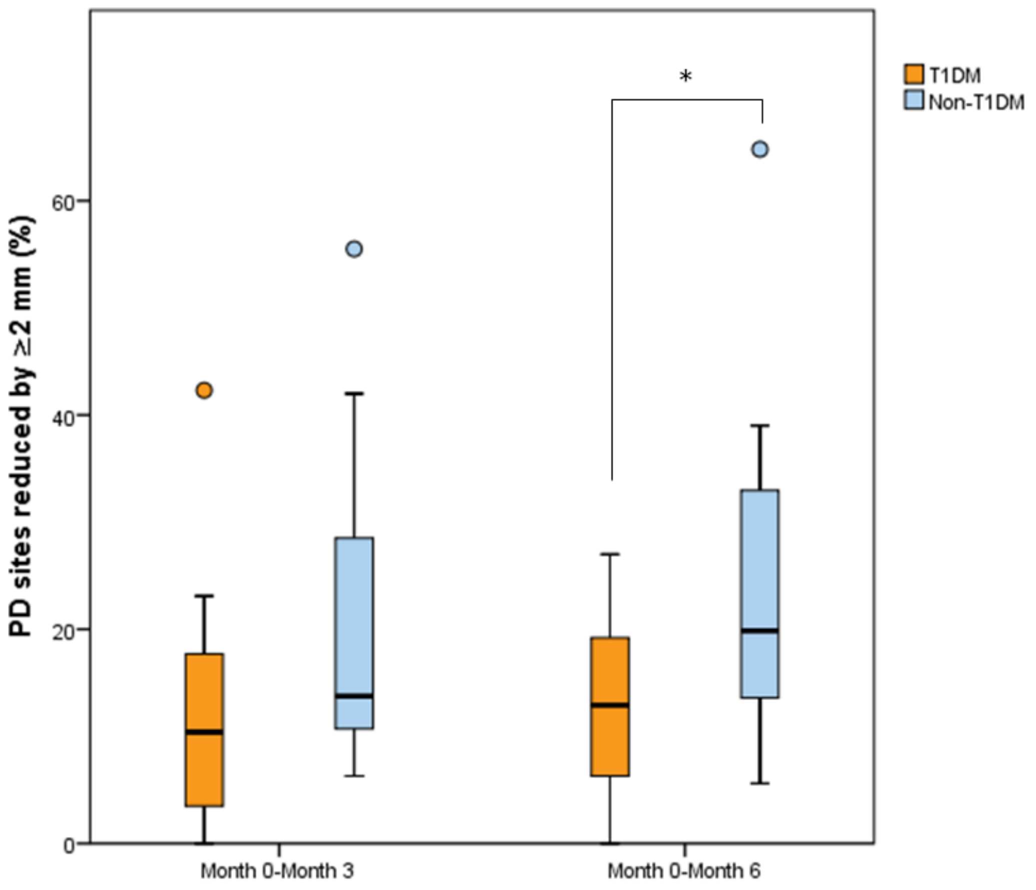


Figure 5.14: PD sites reduced by ≥2 mm (%) in T1DM and non-T1DM patients with periodontitis post-NSM.

Box plot figure showing medians, interquartile ranges, standard errors and outliers for T1DM patients (month 0-month 3 n=11, month 0-month 6 n=13) and non-T1DM patients (month 0-month 3 n=14, month 0-month 6 n=12) with periodontitis. Statistics: Paired t-test for longitudinal comparisons within T1DM or non-T1DM group (no statistically significant differences found); Independent t-test: *P<0.05 (T1DM versus non-T1DM group at each time point). ● indicates outlier more than 1.5 but less than 3 times the IQR from the box boundaries.

5.2.4 Local and systemic biomarker levels following NSM

Serum biomarker levels following NSM

Table 5.7 and Figure 5.15 to 5.18 summarise pre- and post-treatment candidate biomarker data in serum samples for T1DM and non-T1DM patients with periodontitis. When considering levels of MMP-9 in serum, T1DM patients had significantly higher pre-treatment levels (1052.3±489.4 ng/ml) compared to non-T1DM patients (502.2±272.2 ng/ml), (P<0.001). However, no statistically significant differences were found for serum MMP-9 levels between T1DM and non-T1DM patients at months 3 and 6 following NSM, (P>0.05). In patients with T1DM, compared to pre-treatment (1052.3±489.4 ng/ml), serum MMP-9 levels showed reductions following NSM at 3 months (620.9±404.4 ng/ml) and 6 months (732.7±448.8 ng/ml), however these reductions were not statistically significant, (P>0.05). Similarly in non-T1DM patients, compared to pre-treatment (502.2±272.2 ng/ml) serum MMP-9 levels showed reductions at 3 months (461.1±296.5 ng/ml) and 6 months (472.9±259.9 ng/ml), however these reductions were not statistically significant, (P>0.05) (Table 5.7 and Figure 5.15).

When considering levels of BAFF in serum, no statistically significant differences were found between T1DM and non-T1DM patients at any time point, (P>0.05). In T1DM patients, no statistically significant differences were found between pre-treatment and month 3, and between pre-treatment and month 6 serum BAFF levels, (P>0.05). In non-T1DM patients compared to pre-treatment (1150.7±154.3 pg/ml), serum BAFF levels showed significant reductions following NSM at month 3 (1011.5±219.1 pg/ml), (P<0.05). No statistically significant differences were found in serum BAFF levels between pre-treatment and month 6, in non-T1DM patients, (P>0.05) (Table 5.7 and Figure 5.16).

When considering levels of resistin in serum, no statistically significant differences were found between T1DM and non-T1DM patients at any time point, (P>0.05). In T1DM patients, compared to pre-treatment (11.7±4.09 ng/ml), serum resistin levels showed reductions following NSM at month 6 (9.08±2.83 ng/ml), however this difference was not statistically significant, (P>0.05). Also no statistically significant difference was found in serum resistin levels between pre-treatment and month 3 in T1DM patients, (P>0.05). In non-T1DM patients, compared to pre-treatment (10.5±3.55 ng/ml), serum resistin levels showed significant reductions at month 6 (8.59±2.79 ng/ml), (P<0.05). However, no

statistically significant differences were found in serum resistin levels between pre-treatment and month 3 in non-T1DM patients following NSM, (*P*>0.05) (Table 5.7 and Figure 5.17).

When considering levels of ENA-78/CXCL5 in serum, no statistically significant differences were found between T1DM and non-T1DM patients at any time point, (*P*>0.05). In T1DM patients, compared to pre-treatment (1648.4±1049.2 pg/ml), serum ENA-78/CXCL5 levels showed reductions following NSM at 3 months (1461.3±964.3 pg/ml) and 6 months (1630.7±925.8 pg/ml), however these reductions were not statistically significant (*P*<0.05). Similarly, in non-T1DM patients, compared to pre-treatment (1494.5±1007.4 pg/ml), serum ENA-78/CXCL5 levels showed no significant changes at month 3 (1533.6±1160.9 pg/ml) and month 6 (1302.5±680.0 pg/ml) following NSM (Table 5.7 and Figure 5.18).

		Month 0 (n=18) (n=17)	Month 3 (n=12) (n=13)	Month 6 (n=12) (n=12)
Serum MMP-9 (ng/ml)	**T1DM**	1052.3 ± 489.4	620.9 ± 404.4	732.7 ± 448.8
	Non-T1DM	502.2 ± 272.2	461.1 ± 296.5	472.9 ± 259.9
	P	< 0.001	NS	NS
Serum BAFF (pg/ml)	**T1DM**	1194.8 ± 359.4	1215.2 ± 313.1	1119.5 ± 240.3
	Non-T1DM	1150.7 ± 154.3	1011.5 ± 219.1 *	1092.5 ± 461.5
	P	NS	NS	NS
Serum resistin (ng/ml)	**T1DM**	11.7 ± 4.09	11.9 ± 4.83	9.08 ± 2.83
	Non-T1DM	10.5 ± 3.55	9.80 ± 3.11	8.59 ± 2.79 *
	P	NS	NS	NS
Serum ENA-78/CXCL5 (pg/ml)	**T1DM**	1648.4 ± 1049.2	1461.3 ± 964.8	1630.7 ± 925.8
	Non-T1DM	1494.5 ± 1007.4	1533.6 ± 1160.9	1302.5 ± 680.0
	P	NS	NS	NS

Table 5.7: Serum biomarker levels comparing T1DM and non-T1DM patients with periodontitis pre- and post-NSM.

Mean ± SD presented for parametric variables. For comparison between T1DM and non-T1DM patients, p-values determined using Independent t-test for continuous parametric variables. For longitudinal comparisons, p-values determined using Paired t-test for parametric variables: significant difference from baseline *$P<0.05$ and ***$P<0.001$. P-values under columns (*P*) relate to comparisons between T1DM and non-T1DM group at that particular time point. MMP-9; matrix metalloproteinase-9, BAFF; B-cell activating factor, ENA-78/CXCL5; epithelial neutrophil activating peptide-78, NS; not significant.

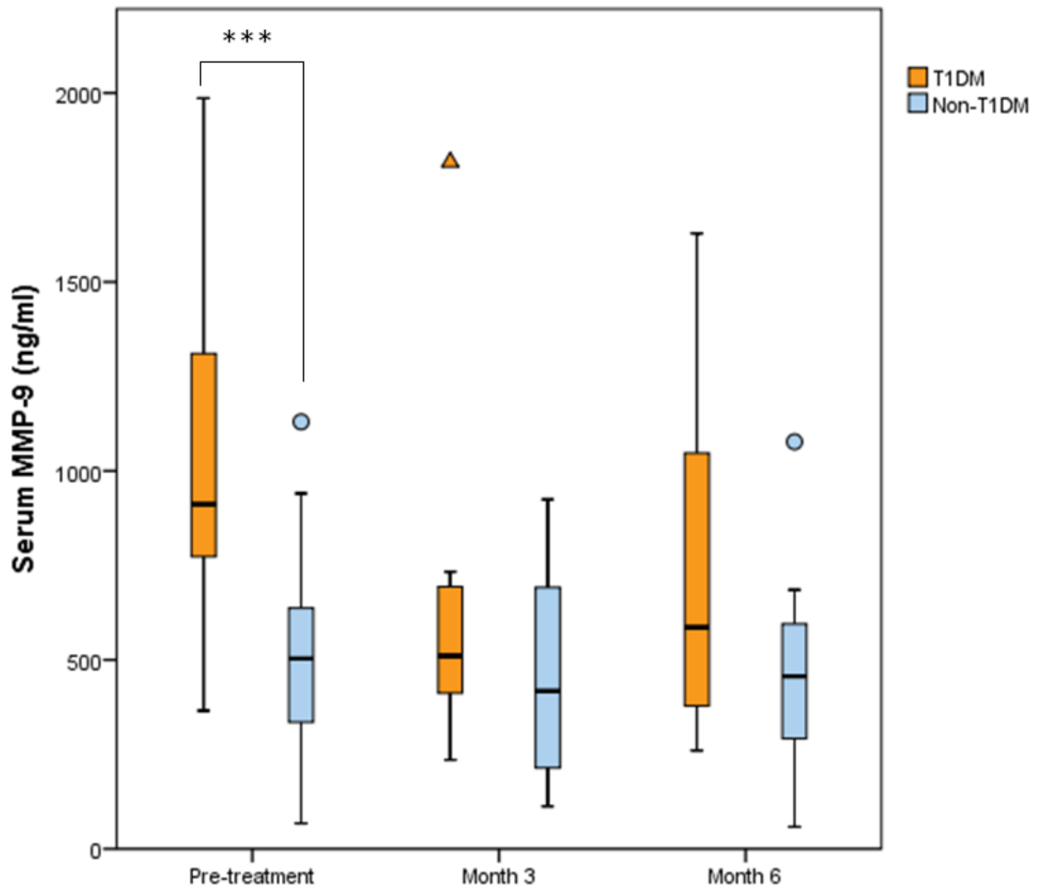


Figure 5.15: Serum MMP-9 levels in T1DM and non-T1DM patients with periodontitis pre- and post-NSM.

Box plot figure showing medians, interquartile ranges, standard errors and outliers for T1DM patients (pre-treatment n=18, month 3 n=12, month 6 n=12) and non-T1DM patients (pre-treatment n=17, month 3 n=13, month 6 n=12). Statistics: Paired t-test for longitudinal comparisons within T1DM or non-T1DM group indicating significant differences from pre-treatment at each time point (no statistically significant differences found); Independent t-test: ****P*<0.001 (T1DM versus non-T1DM group at each time point). ● indicates outlier more than 1.5 but less than 3 times the IQR from the box boundaries.

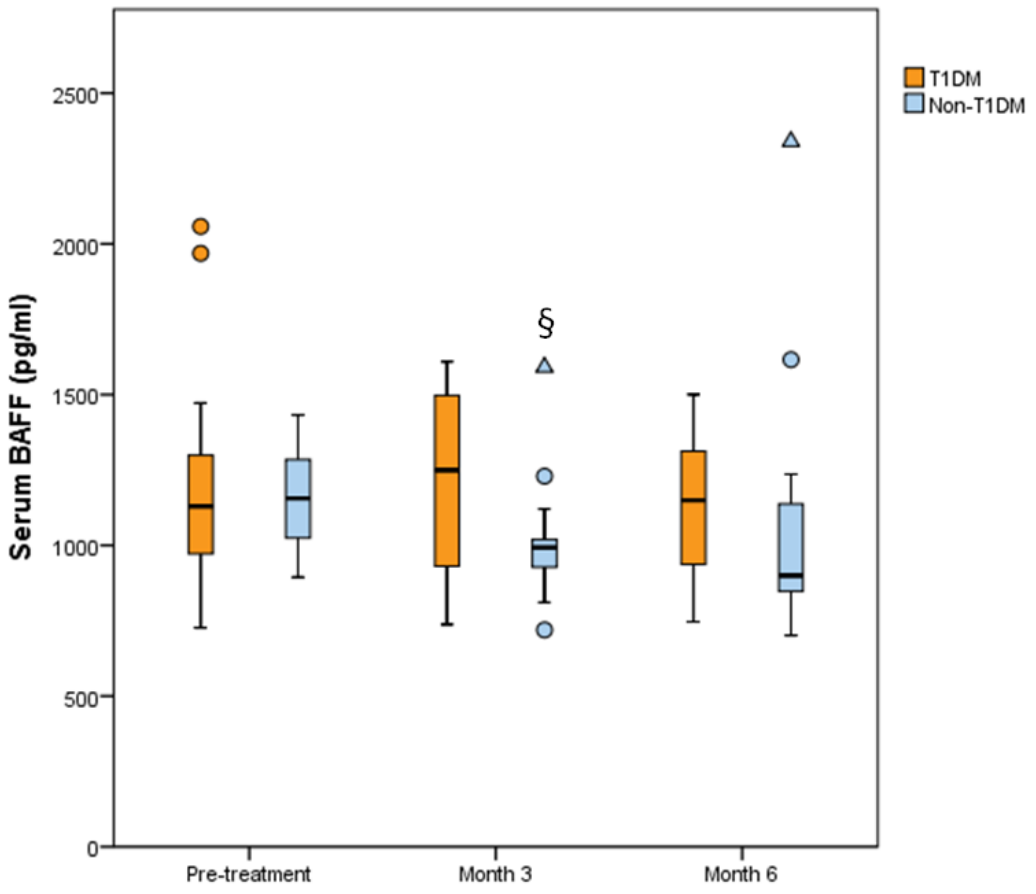


Figure 5.16: Serum BAFF levels in T1DM and non-T1DM patients with periodontitis pre- and post-NSM.

Box plot figure showing medians, interquartile ranges, standard errors and outliers for T1DM patients (pre-treatment n=18, month 3 n=12, month 6 n=12) and non-T1DM patients (pre-treatment n=17, month 3 n=13, month 6 n=12). Statistics: Paired t-test for longitudinal comparisons: §$P<0.05$ (within the T1DM or non-T1DM group indicating significant differences from pre-treatment at each time point); Independent t-test for T1DM versus non-T1DM group at each time point (no statistically significant differences found). ● indicates outlier more than 1.5 but less than 3 times the IQR from the box boundaries, ▲ indicates outlier more than 3 times the IQR from the box boundaries.

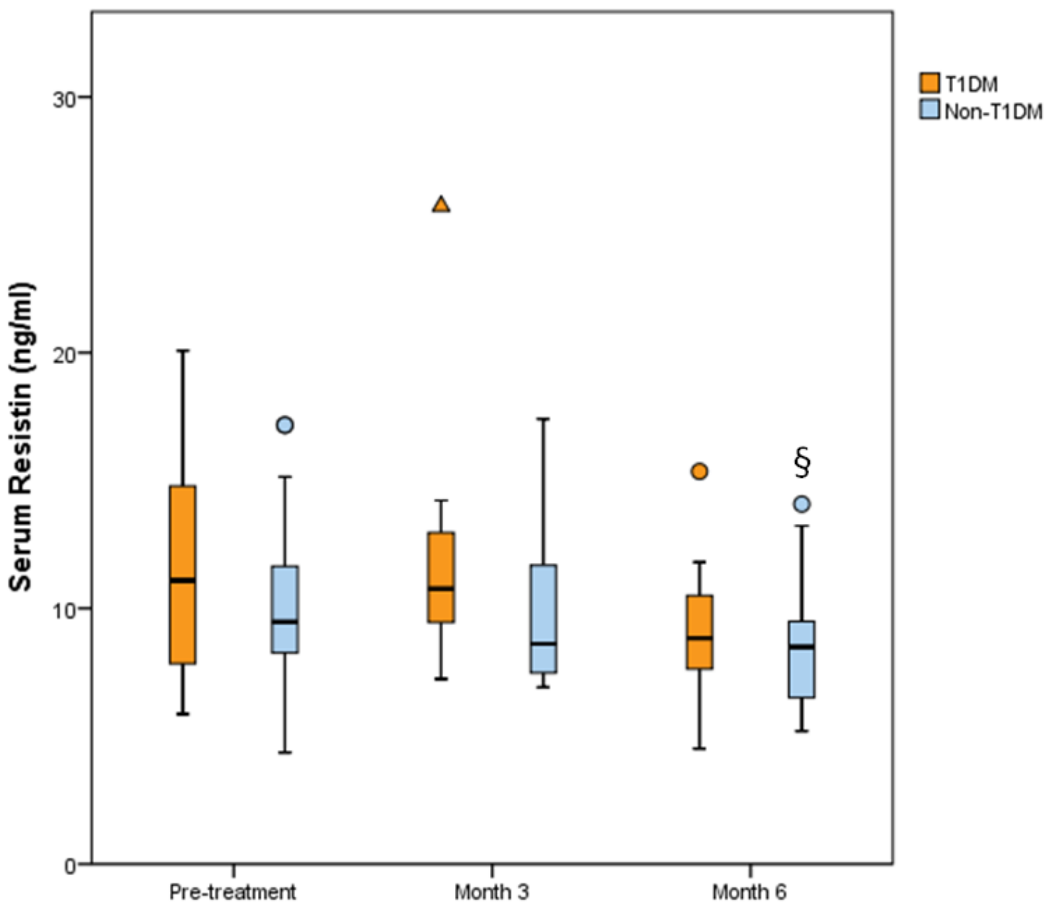


Figure 5.17: Serum resistin levels in T1DM and non-T1DM patients with periodontitis pre- and post-NSM.

Box plot figure showing medians, interquartile ranges, standard errors and outliers for T1DM patients (pre-treatment n=18, month 3 n=12, month 6 n=12) and non-T1DM patients (pre-treatment n=17, month 3 n=13, month 6 n=12). Statistics: Paired t-test for longitudinal comparisons: §$P<0.05$ (within the T1DM or non-T1DM group indicating significant differences from pre-treatment at each time point); Independent t-test for T1DM versus non-T1DM group at each time point (no statistically significant differences found). ● indicates outlier more than 1.5 but less than 3 times the IQR from the box boundaries, ▲ indicates outlier more than 3 times the IQR from the box boundaries.

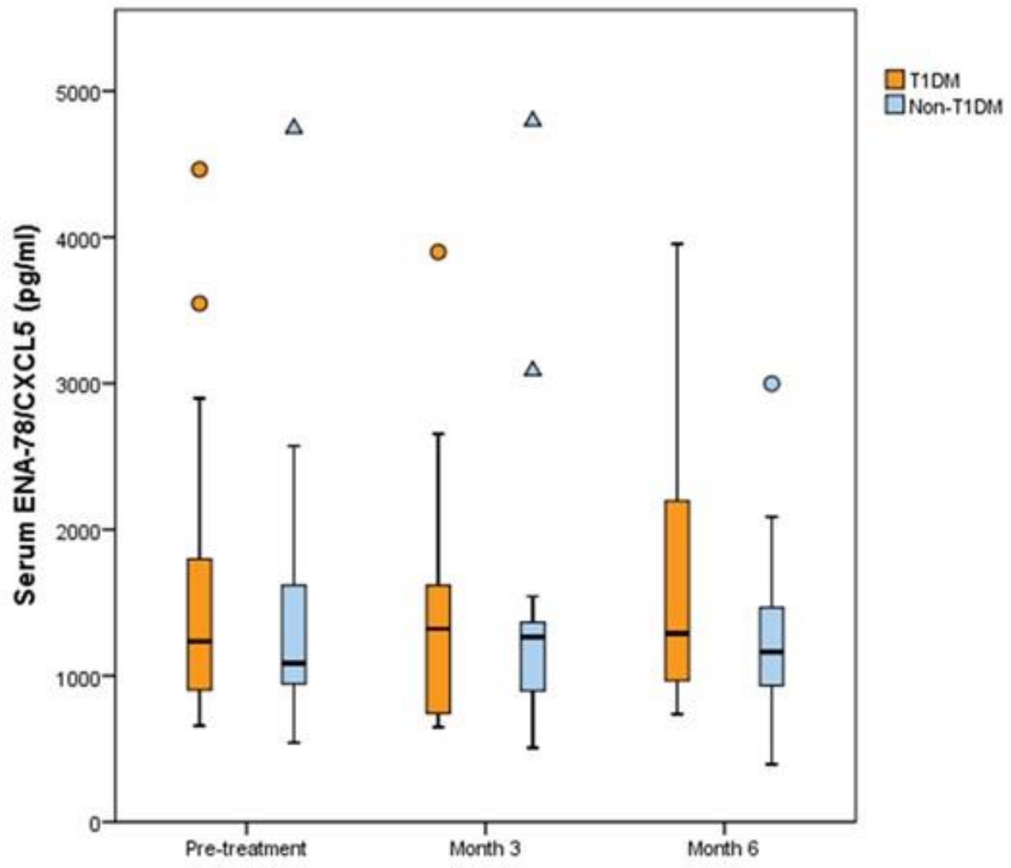


Figure 5.18: Serum ENA-78/CXCL5 levels in T1DM and non-T1DM patients with periodontitis pre- and post-NSM.

Box plot figure showing medians, interquartile ranges, standard errors and outliers for T1DM patients (pre-treatment n=18, month 3 n=12, month 6 n=12) and non-T1DM patients (pre-treatment n=17, month 3 n=13, month 6 n=12). Statistics: Paired t-test for longitudinal comparisons within T1DM or non-T1DM group indicating significant differences from pre-treatment at each time point (no statistically significant differences found); Independent t-test for T1DM versus non-T1DM group at each time point (no statistically significant differences found). ● indicates outlier more than 1.5 but less than 3 times the IQR from the box boundaries, ▲ indicates outlier more than 3 times the IQR from the box boundaries.

GCF biomarker levels following NSM

Table 5.8 and Figure 5.19 to 5.21 summarise pre- and post-NSM candidate biomarker data in GCF samples and GCF volume for T1DM and non-T1DM patients with periodontitis. When considering GCF MMP-9 levels, no statistically significant differences were found between T1DM and non-T1DM patients at any time point, ($P>0.05$). In T1DM patients, compared to pre-treatment (304.8±186.8 ng/ml), GCF MMP-9 levels showed significant reduction following NSM at 3 months (135.4±130.4 ng/ml) and 6 months (225.2±113.7 ng/ml), ($P<0.01$ and $P<0.05$ respectively). In non-T1DM patients, compared to pre-treatment (303.0±201.1 ng/ml) GCF MMP-9 levels showed a reduction at month 3 (209.8±243.8 ng/ml), however this difference was not statistically significant, ($P>0.05$). Also, there was a significant reduction in GCF MMP-9 levels in non-T1DM patients at month 6 (179.0±140.7 ng/ml) compared to pre-treatment levels (303.0±201.1 ng/ml), ($P<0.05$) (Table 5.8 and Figure 5.19).

When considering GCF IL-8 levels, no statistically significant differences were found between T1DM and non-T1DM patients at any time point, ($P>0.05$). In T1DM patients, compared to pre-treatment (370.1±214.2 pg/ml) there was a significant reduction in GCF IL-8 levels following NSM at 3 months (146.6±111.1 pg/ml) and 6 months (249.7±192.7 pg/ml), ($P<0.001$ and $P<0.05$ respectively). In non-T1DM patients, compared to pre-treatment (442.9±390.2 pg/ml) there was a significant reduction in GCF IL-8 levels following NSM at month 3 (318.3±284.1 pg/ml), ($P<0.05$). Although there was a reduction in GCF IL-8 levels at month 6 (328.4±323.5 pg/ml) compared to pre-treatment (442.9±390.2 pg/ml), this reduction was not statistically significant, ($P>0.05$) (Table 5.8 and Figure 5.20).

With reference to the GCF volume, in T1DM patients, compared to pre-treatment (0.66±0.26 μl) there was a significant reduction in GCF volume recorded at month 3 (0.34±0.14 μl) following NSM, ($P<0.01$). Although, there was a reduction in GCF volume at month 6 (0.54±0.30 μl) following NSM compared to pre-treatment (0.66±0.26 μl), this reduction was not statistically significant, ($P>0.05$). In non-T1DM patients, compared to pre-treatment (0.60±0.16 μl) there was a reduction in GCF volume at month 3 (0.57±0.30 μl) and month 6 (0.41±0.21 μl) following NSM, however, this reduction was not statistically significant, ($P>0.05$). Only at month 3, the non-T1DM patients had significantly higher GCF volume (0.57±0.30 μl) compared to the T1DM patients (0.34±0.14 μl), ($P<0.05$) (Table 5.8 and Figure 5.21).

		Month 0 (n=19) (n=17)	Month 3 (n=12) (n=12)	Month 6 (n=12) (n=9)
GCF MMP-9 (ng/ml)	**T1DM**	304.8 ± 186.8	135.4 ± 130.4 **	225.2 ± 113.7 *
	Non-T1DM	303.0 ± 201.1	209.8 ± 243.8	179.0 ± 140.7 *
	P	NS	NS	NS
GCF IL-8 (pg/ml)	**T1DM**	370.1 ± 214.2	146.6 ± 111.1 ***	249.7 ± 192.7 *
	Non-T1DM	442.9 ± 390.2	318.3 ± 284.1 *	328.4 ± 323.5
	P	NS	NS	NS
GCF volume (µl)	**T1DM**	0.66 ± 0.26	0.34 ± 0.14 **	0.54 ± 0.30
	Non-T1DM	0.60 ± 0.16	0.57 ± 0.30	0.41 ± 0.21
	P	NS	< 0.05	NS

Table 5.8: GCF biomarker levels in T1DM and non-T1DM patients with periodontitis pre- and post-NSM.

Mean ± SD presented for parametric variables. For comparison between T1DM and non-T1DM patients, p-values determined using Independent t-test for continuous parametric variable. For longitudinal comparisons, p-values determined using Paired t-test for parametric variables: significant difference from baseline *$P<0.05$, **$P<0.01$ and ***$P<0.001$. P-values under columns (*P*) relate to comparisons between T1DM and non-T1DM group at that particular time point. GCF; gingival crevicular fluid, MMP-9; matrix metalloproteinase-9, IL-8; interleukin-8, NS; not significant. At month 3: GCF volume (T1DM n=11and non-T1DM n=15); at month 6: GCF volume (T1DM n=13 and non-T1DM n=13).

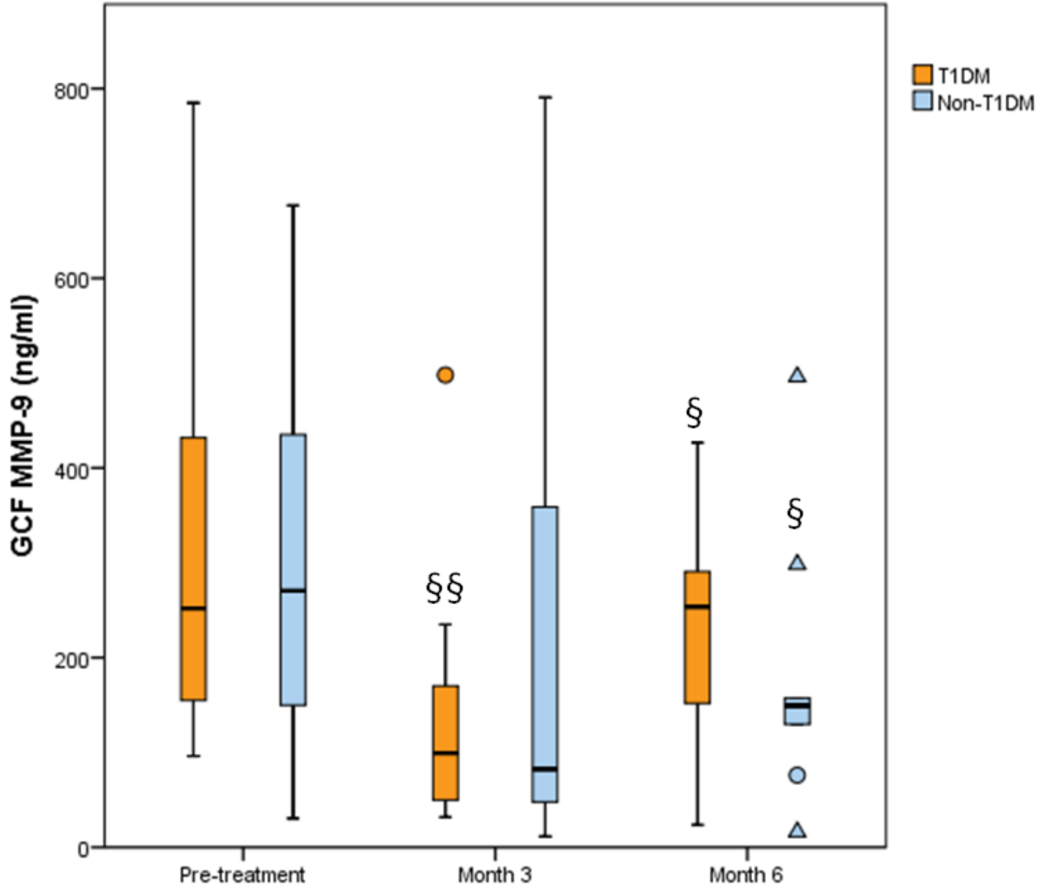


Figure 5.19: GCF MMP-9 levels in T1DM and non-T1DM patients with periodontitis pre- and post-NSM.

Box plot figure showing medians, interquartile ranges, standard errors and outliers for T1DM patients (pre-treatment n=19, month 3 n=12, month 6 n=12) and non-T1DM patients (pre-treatment n=17, month 3 n=12, month 6 n=9). Statistics: Paired t-test for longitudinal comparisons: §P<0.05, §§P<0.01 (within the T1DM or non-T1DM group indicating significant differences from pre-treatment at each time point); Independent t-test for T1DM versus non-T1DM group at each time point (no statistically significant differences found).
● indicates outlier more than 1.5 but less than 3 times the IQR from the box boundaries,
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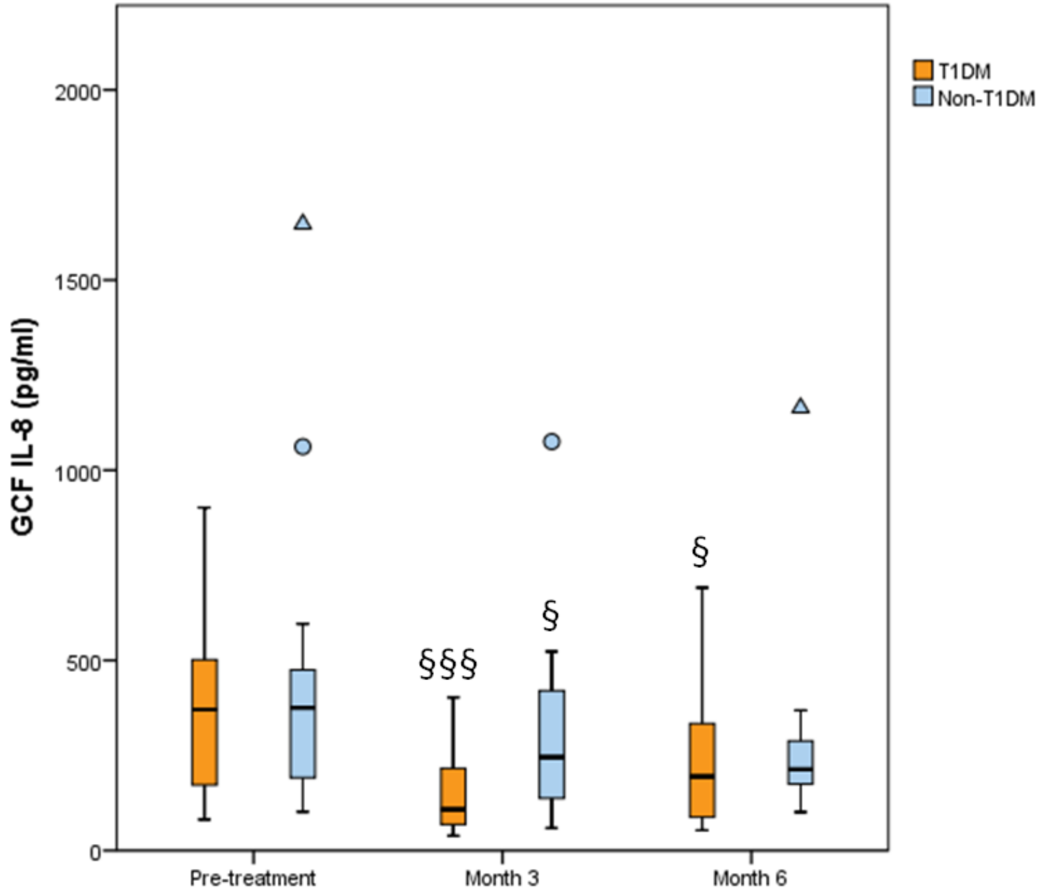

Figure 5.20: GCF IL-8 levels in T1DM and non-T1DM patients with periodontitis pre- and post-NSM.

Box plot figure showing medians, interquartile ranges, standard errors and outliers for T1DM patients (pre-treatment n=19, month 3 n=12, month 6 n=12) and non-T1DM patients (pre-treatment n=17, month 3 n=12, month 6 n=9). Statistics: Paired t-test for longitudinal comparisons: §$P<0.05$, §§§$P<0.001$ (within the T1DM or non-T1DM group indicating significant differences from pre-treatment at each time point); Independent t-test for T1DM versus non-T1DM group at each time point (no statistically significant differences found).
● indicates outlier more than 1.5 but less than 3 times the IQR from the box boundaries,
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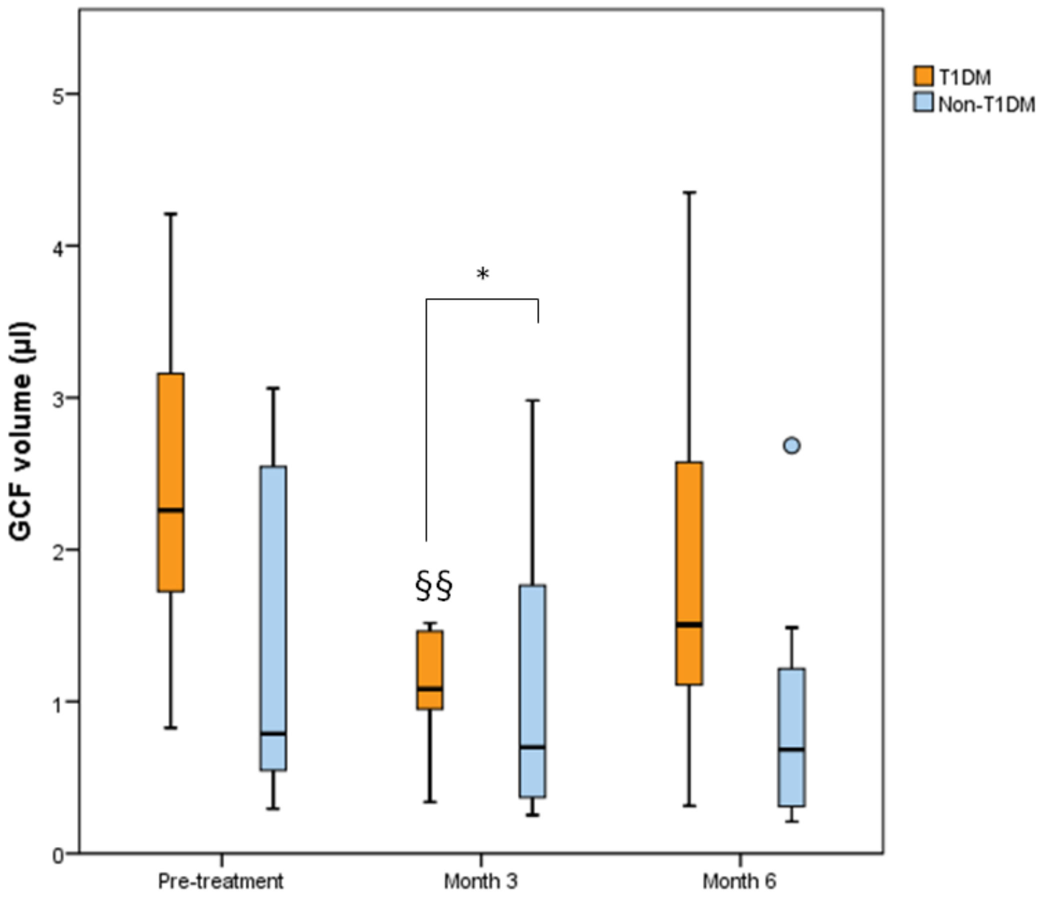


Figure 5.21: GCF volume in T1DM and non-T1DM patients with periodontitis pre- and post-NSM.

Box plot figure showing medians, interquartile ranges, standard errors and outliers for T1DM patients (pre-treatment n=19, month 3 n=12, month 6 n=12) and non-T1DM patients (pre-treatment n=17, month 3 n=12, month 6 n=9). Statistics: Paired t-test for longitudinal comparisons: §§P<0.01 (within the T1DM or non-T1DM group indicating significant differences from pre-treatment at each time point); Independent t-test: *P<0.05 (T1DM versus non-T1DM group at each time point). ● indicates outlier more than 1.5 but less than 3 times the IQR from the box boundaries, ▲ indicates outlier more than 3 times the IQR from the box boundaries.

5.3 Discussion

In this chapter, the effect of periodontal therapy on demographic, metabolic, periodontal and candidate biomarker levels was assessed. Comparisons were made between the T1DM and non-T1DM patients with periodontitis to assess response of treatment in both patient groups.

BP levels following NSM

The current study found a significant increase in systolic BP in T1DM patients at month 6 following NSM, ($P<0.05$) (Table 5.2). This significant increase in systolic BP could possibly be a chance finding, nevertheless the BP values for all T1DM patients recorded pre- and post-NSM were within limits of the recommended levels for patients with diabetes (≤140/80 mmHg) (NICE 2015).

Clinical biochemistry parameters following NSM

In the current study, pre-treatment HbA1c levels in T1DM patients showed a reduction at months 3 and 6 following NSM, which was a reduction of 0.45% and 0.90% respectively, however these reductions were not statistically significant (Table 5.3 and Figure 5.1). Our findings are similar to those of a study which found a 0.43% non-significant reduction in HbA1c levels in T1DM patients 6 months following treatment with NSM plus adjunctive doxycycline therapy (Miller et al. 1992). Our findings are also in line with previous studies which found no statistically significant improvement in HbA1c levels following periodontal treatment in patients with T1DM (Miller et al. 1992; Aldridge et al. 1995; Llambes et al. 2008).

An RCT investigated the effect of NSM with or without adjunctive doxycycline, on the metabolic control of 60 T1DM patients (Llambes et al. 2008). Both treatment groups showed a good response to periodontal therapy indicated by improved PI, BOP, PD and LOA measurements (Llambes et al. 2005). In the group receiving NSM and doxycycline, there was no statistically significant change in HbA1c from pre-treatment levels (7.64±1.81 %) compared to 3 months following NSM (7.71±1.74 %). Likewise, in the group receiving only NSM, compared to pre-treatment HbA1c levels (7.51±1.36 %) there was no statistically significant change 3 months after NSM (7.45±1.29 %) (Llambes et al. 2008). These findings are similar to the current study which found no improvement in metabolic control as measured by HbA1c levels, following NSM in T1DM patients at the 3 month follow-up

appointment. Aldridge and colleagues carried out 2 single-blinded clinical trials to determine the effect of periodontal treatment on metabolic control in T1DM patients (Aldridge et al. 1995). The first trial included 31 T1DM patients with gingivitis (aged 16-40 years) and the second included 22 T1DM patients with advanced periodontitis (aged 20-60 years). In both trials, the authors reported no statistically significant improvement in HbA1c levels 2 months following periodontal therapy (Aldridge et al. 1995). Another small study of 9 T1DM patients with moderate-to-severe periodontitis demonstrated a reduction in pre-treatment HbA1c from 9.4% to 9.0% at 2 months following NSM, however this reduction was not statistically significant (Miller et al. 1992). Additionally, the authors reported that the T1DM patients having improvements in BOP scores were found to have a significant reduction in pre-treatment HbA1c levels from 8.7% to 7.8% at 2 months following NSM, whereas those who showed no improvement in BOP scores showed no improvement in post-treatment HbA1c levels (Miller et al. 1992).

In a previous study, changes in HbA1c levels following periodontal treatment were assessed separately in well-controlled and poorly-controlled T1DM patients (Seppala and Ainamo 1994). T1DM patients (aged 35-56 years) were assessed based on the level of glycaemic control [well-controlled (n=6) and poorly-controlled (n=16)] (Seppala and Ainamo 1994). An improvement in HbA1c levels in both groups was found at the 2-year follow-up appointment, with a reduction in the HbA1c level in poorly-controlled patients from 9.9% to 9.6% and from 9.5% to 7.6% in well-controlled patients, however these reductions were not statistically significant (Seppala and Ainamo 1994). A study of 18 well-controlled T1DM patients with advanced periodontitis (aged 26-57 years) reported no statistically significant change in HbA1c level from baseline to 2 months following successful NSM (Smith et al. 1996). The stratification of patients based on HbA1c levels was not incorporated into the recruitment strategy for the present study and to avoid low numbers in each group, the data were not subsequently stratified during data analyses.

A study including both T1DM and T2DM patients (n=91), performed a limited comparison of metabolic control in 46 patients based on if their periodontal condition did (n=23) and did not (n=23) improve following periodontal treatment (Wolf 1977). The authors found that patients who responded well to periodontal treatment, with decreases in inflammation and improvement in periodontal parameters had a statistically significant improvement in metabolic control, measured by decreased blood glucose levels, insulin dose and urinary

glucose levels 8-12 months following periodontal therapy (Wolf 1977). A longitudinal study of T1DM or T2DM patients (n=20) and non-diabetic controls (n=20) received NSM prior to the baseline examination 3 months later, found no statistically significant changes in HbA1c levels over a 5-year period (Westfelt et al. 1996). The patients were followed up every 3 months for 5 years during which they were provided necessary periodontal maintenance care. At each time-point, HbA1c levels were measured and there were no statistically significant changes in HbA1c levels in diabetic patients from baseline-to-24 months and from 24 months-to-60 months (Westfelt et al. 1996). Another longitudinal study included 20 well-controlled T1DM (n=7) and T2DM (n=13) patients (aged 30-66 years) and 20 non-diabetic controls with moderate-to-advanced periodontitis (Christgau et al. 1998). Following NSM, although both diabetic and non-diabetic patients showed statistically significant improvements in periodontal parameters, no statistically significant improvements were found in HbA1c levels at the 4 month follow-up (Christgau et al. 1998). It is difficult to compare the findings of the current study to these studies due to the differences in study design. Furthermore, the patients in the present study were only T1DM patients and were not categorised and compared based on success of periodontal treatment or glycaemic control categories.

From the review of literature on the effects of periodontal treatment on glycaemic control in patients with T1DM, overall it appears that periodontal treatment does improve glycaemic control but the findings are not statistically significant. Similar results were found in the current study where, even though HbA1c reduced following NSM by around 0.90% at 6 months, the difference was not statistically significant. One must bear in mind the heterogeneity among the studies such as, diabetes-related factors (type of diabetes, diabetes duration, baseline glycaemic control and type of diabetes treatment), periodontal-related factors (baseline periodontal disease status, methods utilised to assess periodontal status and periodontal treatment protocols), sample size and power to detect differences in metabolic and periodontal response, follow-up time frames for glycaemic control and periodontal status evaluation, inclusion of control groups and specific hypothesis tested. Despite the variations and limitations, and though the evidence is not unequivocal there is evidence that supports the concept that periodontal disease contributes to poor glycaemic control in patients with diabetes and the treatment of periodontal disease can have a beneficial effect in patients with T1DM (Taylor 2003). There is a need to further investigate the effect of preventing and

treating periodontal diseases with an aim to contribute to glycaemic control especially in patients with T1DM as this group of patients has been under-researched.

Data from the current study showed that, non-HDL levels were higher in the non-T1DM patients compared to the T1DM patients at each time point, however this difference was statistically significant only at month 3 following NSM ($P<0.05$). Similarly, cholesterol levels were higher in the non-T1DM patients compared to the T1DM patients at each time point, however this difference was statistically significant only at pre-treatment ($P<0.05$) (Table 5.3, Figures 5.3 and 5.4). This is not surprising, given the key priority within the national management guidelines for T1DM involves the control of serum lipid levels (NICE 2015). Therefore, it is reasonable to presume that the T1DM patients in this study were receiving more aggressive management and monitoring of CVD risk factors compared to the control patients. Also, pre-treatment diabetes care data showed that 15.8% of the T1DM patients with periodontitis were taking lipid lowering and anti-hypertensive medication; however comparable data from non-T1DM patients were not collected to confirm the influence of medication on lipid profile levels.

While considering hsCRP levels, in the current study pre-treatment hsCRP levels in T1DM and non-T1DM periodontitis patients showed no statistically significant improvement following NSM (Table 5.3). Our findings are similar to a meta-analysis which concluded that it is highly unlikely that periodontal treatment could modulate systemic hsCRP levels in patients with severe periodontal disease (Ioannidou et al. 2006). Our findings are in contrast to studies which found a decrease in hsCRP levels after periodontal therapy (Ide et al. 2003; D'Aiuto et al. 2005; Marcaccini et al. 2009a; Marcaccini et al. 2009b). An RCT which investigated the impact of periodontal therapy on serum inflammatory markers and cholesterol, in systemically healthy individuals with severe periodontitis using a three-arm intervention strategy, found a significant reduction in serum hsCRP levels 2 months following periodontal treatment (D'Aiuto et al. 2005). The periodontal treatment groups comprised an untreated control group (n=24), NSM only group (n=21) and NSM and adjunctive local antibiotic group (n=20). The NSM plus antibiotic groups showed a reduction from pre-treatment hsCRP levels [2.0±1.1 (1.5-2.5) mg/L] to month 2 follow-up [1.6±0.9 (1.2-2.0) mg/L] compared to the untreated controls in which hsCRP levels at baseline [2.4±1.6 (1.8-3.1) mg/L] and at 2 months [2.5±1.7 (1.8-3.2) mg/L] remained unchanged. The authors suggested that periodontal treatment does have a positive impact in

reducing the systemic inflammatory burden as assessed by hsCRP levels. Similar to the lipid profile findings in the current study, their study found insignificant changes in total cholesterol, HDL, non-HDL and triglyceride levels following periodontal therapy in both treatment groups (D'Aiuto et al. 2005). In the current study, it is also worth noting that the pre-treatment hsCRP levels in both T1DM and non-T1DM patients were not very high and thus these values may not be conducive to a large improvement with periodontal treatment. Additionally, the relatively small sample size at months 3 and 6 may have led to finding no significant effect following NSM (Table 5.3).

Clinical periodontal parameters following NSM

In the current study, in both T1DM and non-T1DM patients compared to pre-treatment there was a significant improvement seen at months 3 and 6 in all periodontal parameters: PI, mGI, mean PD, mean recession, mean LOA and % BOP measurements following NSM, (P<0.05) (Table 5.5 and Figures 5.5 to 5.10). A significant increase in mean recession at months 3 and 6 indicates a resolution of inflammation and improvement in periodontal status following periodontal therapy. Additionally, in both T1DM and non-T1DM patients there was a significant reduction in the number of PD sites measuring ≥5 mm from pre-treatment to month 3 (P<0.01 and P<0.001 respectively) and month 6 (P<0.01), indicating a reduction in the severity of periodontal disease in both groups. Prior to periodontal treatment, non-T1DM patients were found to have more severe periodontal disease compared to T1DM patients possibly due to the differences in recruitment strategy utilised for the two groups in the current study. It is interesting to note that following NSM unlike pre-treatment, no statistically significant differences were found in severity of periodontal disease (PD sites measuring ≥5 mm) between T1DM and non-T1DM patients (Table 5.5 and Figure 5.11), indicating an improvement in periodontal status in this cohort of T1DM patients. To confirm this, in both patient groups there was a significant increase in the number of PD sites measuring ≤4 mm at month 3 and 6 following NSM, (P<0.01) (Table 5.5 and Figure 5.12). While considering the amount of PD reduction, compared to pre-treatment, at month 6 the non-T1DM patients had significantly higher number of PD sites with a reduction ≥2 mm compared to the T1DM patients (P<0.05), these differences could possibly be a reflection of the fact that the non-T1DM patients had significantly deeper sites at the outset of the study compared to T1DM patients (Table 5.6 and Figure 5.14).

The findings of this study confirm that the NSM was successful in both T1DM and non-T1DM patients, showing improvements in periodontal parameters with a reduction in inflammation and severity of periodontal disease following treatment. Our findings are similar to those of other studies in T1DM patients which found significant improvements in periodontal indices following periodontal therapy (Bay et al. 1974; Smith et al. 1996; Martorelli de Lima et al. 2004; Llambes et al. 2005). Additionally, in this study the positive outcome of periodontal treatment was demonstrated in both T1DM and non-T1DM patients and both groups responded similarly to NSM. Our findings are similar to previous research which found that patients with T1DM have a good response to periodontal management, and the short- and long-term response to periodontal treatment is similar to that seen in non-diabetic patients (Bay et al. 1974; Westfelt et al. 1996; Christgau et al. 1998).

Serum biomarker levels following NSM

In the current study, compared to pre-treatment, serum BAFF levels in non-T1DM patients showed a significant reduction only at month 3 following NSM ($P<0.05$), and pre-treatment serum resistin levels showed a significant reduction only at month 6 following NSM ($P<0.05$) (Table 5.7 Figure 5.16 and 5.17). In the literature, no study has evaluated the effect of NSM on levels of BAFF in serum. From the results of this study, BAFF may not be an ideal biomarker to determine the severity of periodontal disease and the benefits of periodontal treatment in patients with or without T1DM.

To the best of our knowledge, this is the first study to investigate the effect of periodontal treatment on serum resistin levels in patients with T1DM. A previous study, assessed serum resistin levels in T2DM patients with periodontal disease (aged 35-75 years) and reported no significant reductions 6 months following periodontal therapy with adjunctive local antibiotics (Bharti et al. 2013). The T2DM patients were allocated to either periodontal therapy with local antibiotics (intervention group n=28) or non-periodontal treatment (control group n=8). In the T2DM intervention group, compared to pre-treatment periodontal measurements (PD 2.8±0.7 mm and BOP 32.4±21.8 %) there was a significant improvement seen at month 2 (PD 2.0±0.3 mm and BOP 7.0±5.0 %) and month 6 (PD 1.9±0.3 mm and BOP 7.9±5.2 %) following NSM. Also, compared to pre-treatment HbA1c levels (7.1±0.8 %) there was a significant reduction seen at month 6 (6.8±0.6 %) following NSM. However, compared to pre-treatment serum resistin levels (12.5±10.6 ng/ml), no statistically significant improvement was seen at month 2 (11.2±7.8 ng/ml) and month 6 (14.4±13.2 ng/ml)

following treatment. Similarly, no statistically significant changes were seen in serum TNF-α, IL-6 and leptin levels in the T2DM patients following NSM with adjunctive antibiotics (Bharti et al. 2013). Their study involved T2DM patients and had a different treatment strategy compared to the current study and hence the comparison to these findings is questionable. In the current study, the post-treatment findings related to serum resistin levels in non-T1DM patients at month 3 are similar to a study in 40 systemically healthy patients (aged 20-50 years), with chronic periodontitis (n=20) and healthy periodontal tissues (n=20), which found no statistically significant reduction in serum resistin levels 6-8 weeks following NSM (Devanoorkar et al. 2012). In patients with chronic periodontitis significant improvements in clinical periodontal parameters were seen pre- to post-NSM, additionally compared to pre-treatment (1.89±1.83 ng/ml) serum resistin levels decreased following NSM (1.59±1.01 ng/ml), but this decrease was not statistically significant (Devanoorkar et al. 2012). In the current study, in non-T1DM patients, serum resistin levels had a significant reduction at only month 6 following NSM (Table 5.7 and Figure 5.17).

In the current study, at pre-treatment despite non-T1DM patients having more severe periodontal disease compared to T1DM patients, pre-treatment serum MMP-9 levels were significantly higher in the T1DM patients compared to the non-T1DM patients with periodontitis, (P<0.001) (Table 5.7 and Figure 5.15). The possible explanation for this would be that circulating MMP-9 levels are elevated in patients with T1DM (Maxwell et al. 2001). Diabetes-associated pathophysiological processes such as oxidative stress, possibly enhances MMP-9 activity and production (Uemura et al. 2001). MMP-9 has been demonstrated to be a potentially useful biomarker in serum, for T1DM patients at risk of progression to chronic kidney disease (Gharagozlian et al. 2009). Results of T1DM studies and other diabetes-related complications suggest that serum MMP-9 may contribute to the chronic inflammatory process inherent to diabetic retinopathy (Maxwell et al. 2001; Jacqueminet et al. 2006). Circulating MMP-9 levels have been found to be increased in T2DM patients with coronary artery disease, and elevated MMP-9 levels in serum have been linked to premature coronary atherosclerosis (Noji et al. 2001). Circulating MMP-9 levels are raised in treated hypertensive patients with T2DM compared to normotensive control patients (Tayebjee et al. 2004). It has also been suggested that increases in serum MMP-9 levels occur prior to the development of microvascular renal complications in T2DM patients (Ebihara et al. 1998). In the current study, the T1DM patients with periodontitis having significantly higher serum MMP-9 levels compared to then non-T1DM patients with periodontitis is a key finding, and

from the literature and the results of this study one could conclude that this significant increase could possibly be due to increases in MMP-9 serum levels found routinely in T1DM patients. Despite having less severe periodontal disease, increased diabetes-related inflammation may have led to increases in MMP-9 levels in serum.

From the results of the current study, it is noteworthy that although not statistically significant, serum MMP-9 levels in both T1DM and non-T1DM patients were found to reduce following NSM. It might be useful in future research studies to include larger sample sizes to investigate this further in both patient groups. The reduction in serum MMP-9 levels following NSM could possibly be a reflection of the significant improvement in periodontal status and reduction in inflammation and severity of periodontal disease seen in both T1DM and non-T1DM patients. Interestingly, post-treatment (month 6) serum MMP-9 levels in T1DM and non-T1DM patients with periodontitis were similar to the pre-treatment serum MMP-9 levels observed in T1DM and non-T1DM patients with healthy periodontal tissues (Table 4.9 and 5.7). Until now, no studies have evaluated the effect of periodontal treatment on circulating serum MMP-9 levels in T1DM patients with periodontitis. A study of systemically healthy patients with chronic periodontitis (n=28) and periodontally healthy controls (n=22) (aged 35-55 years) found a significant decrease in circulating plasma MMP-9 concentrations and proteolytic activity in chronic periodontitis patients 3 months after effective NSM ($P<0.01$) (Marcaccini et al. 2009b). However, the authors reporting post-treatment data only graphically, limits the comparison to the findings of the current study.

In the current study, pre-treatment serum ENA-78/CXCL5 levels in the T1DM and non-T1DM patients showed no statistically significant changes at months 3 and 6 following NSM ($P>0.05$) (Table 5.7 and Figure 5.18). Until now, no studies have the evaluated the effect of periodontal treatment on serum ENA-78/CXCL5 levels in periodontitis patients with and without T1DM. The lack of any statistically significant differences in serum MMP-9 and ENA-78/CXCL5 levels following NSM between T1DM and non-T1DM patients could be due to the lack of statistically significant differences between the two groups with regards to clinical periodontal parameters, as both groups were found to have similar periodontal measurements and severity of periodontal disease following NSM. The findings of this study relating to serum MMP-9 and ENA-78/CXCL5 levels indicate the possible role MMP-9 and ENA-78/CXCL5 may play in the two-way relation linking T1DM and periodontal disease.

GCF biomarker levels following NSM

In the current study, pre-treatment GCF MMP-9 levels in T1DM patients significantly reduced at months 3 and 6 following NSM ($P<0.01$ and $P<0.05$, respectively). In non-T1DM patients, pre-treatment GCF MMP-9 levels had a non-significant reduction at month 3 and a significant reduction at month 6 ($P<0.05$) following NSM (Table 5.8 and Figure 5.19). In T1DM patients, pre-treatment GCF IL-8 levels significantly reduced at months 3 and 6 following NSM ($P<0.001$ and $P<0.05$, respectively). In non-T1DM patients pre-treatment GCF IL-8 levels significantly reduced only at month 3 ($P<0.05$), and although there was a reduction in GCF IL-8 levels month 6 this was not statistically significant (Table 5.8 and Figure 5.20). Overall, in both T1DM and non-T1DM patients, reductions in GCF levels of these two candidate pro-inflammatory biomarkers appeared to mirror the improvement in periodontal status seen within this study following successful treatment.

Successful periodontal therapy is known to significantly reduce GCF volume. In the current study while considering GCF volume in T1DM patients, compared to pre-treatment volume there was a significant reduction at month 3 ($P<0.01$) and a non-significant reduction at month 6 following NSM. In non-T1DM patients, compared to pre-treatment although there was a reduction in GCF volume at months 3 and 6, this reduction was not statistically significant (Table 5.8 and Figure 5.21). A longitudinal study of systemically healthy patients with chronic periodontitis (n=27) and periodontally healthy controls (n=15) found a significant reduction in pre-treatment total MMP-9 GCF levels [approximate levels from presented graph - chronic periodontitis: 1,400 (100-3,500) ng/site in 30s and periodontally healthy controls: 1,100 (400-2,000) ng/site in 30s] following periodontal treatment at month 3 follow-up [approximate levels from presented graph - chronic periodontitis: 950 (50-2,000) ng/site in 30s and periodontally healthy controls: 500 (100-1,100) ng/site in 30s], ($P<0.0001$ and $P=0.0006$) respectively (Marcaccini et al. 2010). The authors also reported no statistically significant reductions in pre-treatment GCF volume in chronic periodontitis and control patients prior to (0.7 ± 0.41 μl and 0.43 ± 0.35 μl) and following periodontal treatment (0.6 ± 0.41 μl and 0.42 ± 0.27 μl) (Marcaccini et al. 2010). Their findings for GCF volume are in agreement with the current study which found no statistically significant reduction in GCF volume in non-diabetic patients 3 months following NSM.

Another longitudinal follow-up study of systemically healthy patients with moderate to advanced periodontitis (n=6) and periodontally healthy controls (n=6) demonstrated that

NSM significantly reduced GCF IL-8 levels and that IL-10 and RANTES levels were undetectable following NSM (Gamonal et al. 2000). Also, compared to pre-treatment GCF volume (0.72±0.3 μl) there was a significant reduction in GCF volume 2 months (0.38±0.1 μl) following NSM. Additionally compared to pre-treatment GCF IL-8 concentration levels (316.7±209 pg/μl) there was a non-significant reduction at 2 months (241.4±163 pg/μl) following NSM. However, total GCF IL-8 levels significantly reduced from pre-treatment (212.5±133 pg) to 2 months (85.4±49.0 pg) following NSM, (P<0.01). The authors also reported that IL-8 levels were significantly elevated in PD sites measuring >6 mm compared to PD sites <6 mm but following NSM, PD sites showed significantly reduced levels of IL-8 (Gamonal et al. 2000). Their findings for GCF volume and IL-8 concentration levels are in contrast to the results of the current study for non-diabetic patients. The relatively small sample size and the differences in GCF elution method in their study, limits the comparison to the findings of the present study.

A longitudinal, split-mouth interventional study in systemically healthy patients with moderate to advanced periodontitis (aged 35-75 years) reported increases in GCF IL-8 concentration levels and a reduction in total GCF IL-8 levels following periodontal therapy (Goutoudi et al. 2012). Two quadrants from either the maxillary or mandibular arch were randomly selected in each patient and a total of 72 diseased sites and 24 non-diseased sites were examined and treated. One half of the mouth was treated with OHI and NSM and the other half was treated with NSM and surgical periodontal therapy. In the diseased sites, following periodontal treatment irrespective of the treatment modality used, compared to pre-treatment GCF volume (0.19±0.04 μl) there was a significant reduction in GCF volume at 6 weeks (0.05±0.01 μl), 4 months (0.03±0.01 μl) and 8 months (0.04±0.01 μl), (P<0.05). In the diseased sites, compared to pre-treatment GCF concentration IL-8 levels (1103.8±498.2 pg/μl) there was a significant increase at 6 weeks (2085.3±664.0 pg/μl) (P<0.05) and a non-significant increase at month 4 (3243.4±2271.1 pg/μl) and month 8 (3290.5±609.8 pg/μl) following periodontal therapy. For total GCF IL-8 levels, compared to pre-treatment (95.5±40.0 pg/ 30s) there was a non-significant reduction seen at 6 weeks (59.7±24.8 pg/ 30s), 4 months (62.6±17.9 pg/ 30s) and 8 months (71.3±14.6 pg/ 30s) following periodontal treatment. The authors discussed that the increase in concentration of IL-8 following periodontal therapy could be due to the reduction in GCF volume following successful treatment (Goutoudi et al. 2012).

To the best of our knowledge, the current study is the first to investigate the effect of periodontal treatment on GCF MMP-9 and IL-8 levels in patients with T1DM. From the results of this study, it can be concluded that MMP-9 and IL-8 are good local biomarkers to determine severity of periodontal disease and the benefit effective periodontal treatment has in patients with T1DM.

Summary of key findings from chapter 5

- In T1DM patients with periodontitis, HbA1c levels reduced from 8.95 (8.03-9.65) %/ 75 (64-83) mmol/mol at baseline to 8.50 (6.60-9.60) %/ 69 (49-81) mmol/mol at month 3 and 8.05 (6.95-10.1) %/ 64 (53-87) mmol/mol at month 6. HbA1c levels showed 0.45% and 0.90% reduction at month 3 and month 6 respectively following NSM, although these reductions were not statistically significant.
- In both T1DM and non-T1DM patients, significant reductions in PI, mGI, mean PD, mean LOA and % BOP were found at months 3 and 6 after NSM indicating a good response to periodontal treatment. Furthermore, PI, mGI, mean PD, mean recession, mean LOA and % BOP showed no statistically significant difference between T1DM and non-T1DM patients at months 3 and 6 following NSM, suggesting that periodontal treatment outcomes were similar in both groups.
- In both T1DM and non-T1DM patients a significant reduction in % of PD sites measuring ≥5 mm was seen at months 3 and 6 after NSM. Also, taking into account the pre-treatment difference in % of PD sites ≥5 mm, the differences between T1DM and non-T1DM patients at months 3 and 6 were not statistically significant.
- The % of PD sites that reduced by ≥2 mm was significantly higher in non-T1DM patients compared to T1DM patients at month 6 following NSM.
- At pre-treatment, serum MMP-9 levels were significantly higher in T1DM patients with periodontitis compared to non-T1DM patients with periodontitis.
- In both T1DM and non-T1DM patients, there was a reduction in serum MMP-9 and ENA-78/CXCL5 following NSM, however, this reduction was not statistically significant. Also, serum MMP-9 levels between T1DM and non-T1DM patients were not significantly different following NSM at months 3 and 6.
- Although there was a reduction in serum resistin levels in both T1DM and non-T1DM patients at month 6 after NSM, this reduction was only statistically significant in the non-T1DM patients.

- In both T1DM and non-T1DM patients there was a significant reduction in GCF MMP-9 levels at month 6 following NSM.
- In T1DM patients there was a significant reduction in GCF IL-8 levels at months 3 and 6 following NSM. In non-T1DM patients there was also a reduction in GCF IL-8 levels following NSM at months 3 and 6 however the reduction was statistically significant only at month 3.

6 Chapter 6. Impact of T1DM and periodontal status on quality of life

6.1 Introduction

Chronic periodontitis is a chronic inflammatory condition exhibiting clinical signs such as periodontal pockets which are usually painless (Cunha-Cruz et al. 2007). As this condition is typically asymptomatic, especially in its initial stages, individuals might be unware of their periodontal condition (Gilbert and Nuttall 1999; Pitiphat et al. 2002; Dietrich et al. 2005) and hence may underestimate the need for treatment (Tervonen and Knuuttila 1988). As periodontal disease progresses, it demonstrates a number of signs and symptoms that can be readily perceived by individuals, such as tooth mobility, eating difficulties, pain, discomfort and compromised aesthetics (Cunha-Cruz et al. 2007). Clinicians usually document disease severity based on clinical parameters such as BOP, increased PD and LOA. The signs and symptoms associated with periodontal disease are highly relevant from an individual's point of view and those diagnosed with this condition often report a considerable negative impact on their daily lives. Patients with chronic periodontal diseases are known to experience, in particular functional, psychological and social impacts on their QoL as a result of their oral health status (Brennan et al. 2007; O'Dowd et al. 2010; Durham et al. 2013). Thus patients with periodontal diseases experience a worse OHRQoL, but this impact can partly be ameliorated by effective periodontal treatment (Jowett et al. 2009). Investigations have demonstrated that periodontal treatment has a positive impact and may contribute to changing their perceptions from a negative effect on OHRQoL to a more positive one (Aslund et al. 2008; Jowett et al. 2009; Saito et al. 2010).

Periodontal disease has also been associated with diabetes, and both T1DM and T2DM are associated with an increased risk of developing macrovascular and microvascular complications, which have been found to affect HRQoL in diabetic patients (Wandell 2005). Diabetes also increases the prevalence of oral disorders such as xerostomia, sialosis, taste impairment, oral candidiasis and lichen planus (Manfredi et al. 2004). Studies have indicated that patients with diabetes are 2 to 3 times more likely to develop periodontal disease (Seppala et al. 1993; Lalla et al. 2004; Campus et al. 2005). There is emerging evidence which supports the two-way relation between diabetes and periodontal disease, with periodontal inflammation negatively affecting glycaemic control and diabetes increasing the risk for severe periodontal disease (Preshaw et al. 2012). Other known risk factors related to

periodontal disease include smoking and psychological conditions such as impaired coping abilities and stress (Genco et al. 1999b).

With regards to the impact of diabetes on QoL, the prevalence of depression is known to be approximately three times higher in patients with diabetes compared to the general population (Gavard et al. 1993; Peyrot and Rubin 1997). Psychological conditions such as depression and anxiety not only negatively impact QoL of patients with diabetes, but can also adversely affect their adherence to treatment and glycaemic control (Rubin and Peyrot 1992; Lustman et al. 1998). Patients suffering from psychological conditions can be effectively treated, but the accurate recognition of these conditions in clinical practice is often misdiagnosed. Hence, in order to facilitate the recognition of serious psychological problems it is important to use standardised and reliable psychological questionnaires in routine clinical practice (Pouwer et al. 1999).

In order to establish patient perception of QoL related to their diabetes and periodontal condition in this study, QoL of T1DM patients with periodontal disease was assessed using the W-BQ12 and the ADDQoL-19 questionnaire before and after NSM. For the QoL data collected, cross-sectional comparisons were made for all T1DM patients based on periodontal diagnosis at baseline and longitudinal comparisons were made only for T1DM patients with periodontitis.

6.2 Results

At baseline, a total of 57 T1DM patients were recruited into the study: 29 (50.9%) females and 28 (49.1%) males. Each patient manually self-completed the W-BQ12 and the ADDQoL-19 questionnaire. Of these, 1 patient's questionnaires were misplaced during data collection (n=1), hence the final sample size comprised 56 T1DM patients, 29 females (51.8%) and 27 males (48.2%). At baseline there were: 9 DH, 28 DG and 19 DP patients who completed both questionnaires. Unfortunately, due to loss to follow-up, at months 3 and 6 there were 10 T1DM patients with periodontitis who remained in the study and completed both questionnaires.

6.2.1 Analysis of the W-BQ12

Table 6.1 summarises the subscale and overall W-BQ12 scores and Figure 6.1 shows the W-BQ12 subscale scores for all T1DM patients, at baseline.

	T1DM (n=56)
Negative well-being	1.93 ± 1.94
Energy	6.13 ± 1.93
Positive well-being	7.59 ± 2.15
General well-being	23.8 ± 4.78

Table 6.1: The W-BQ12 scores for all T1DM patients at baseline.

Mean ± SD presented for all scores.

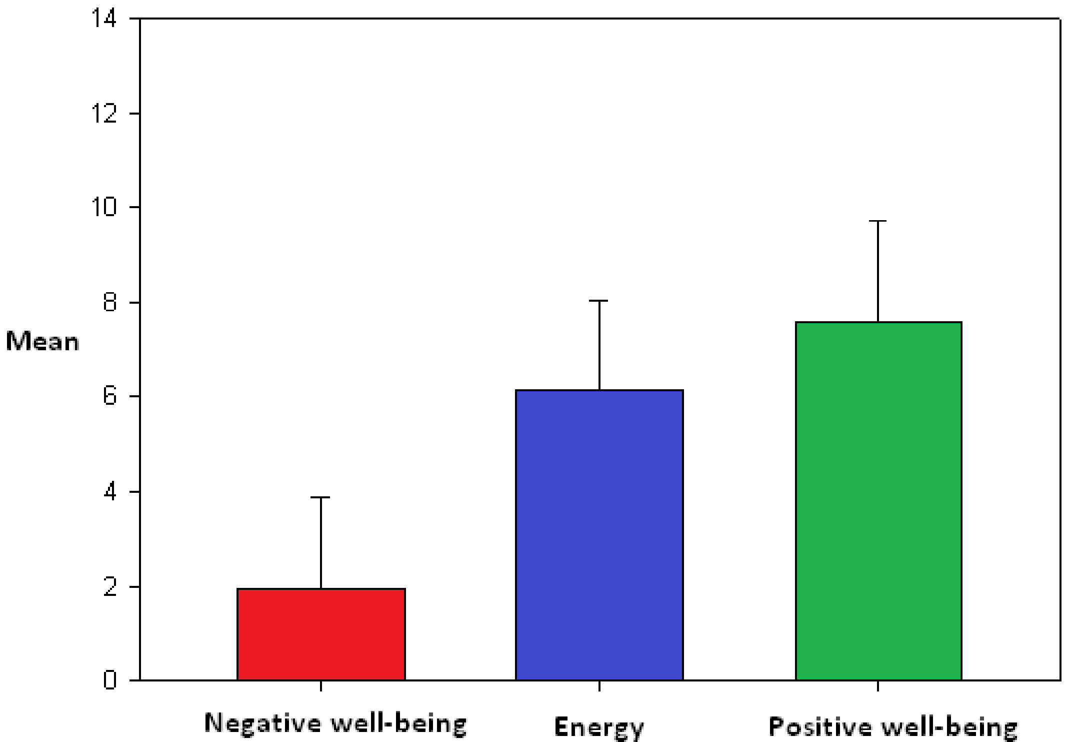
Table 6.2 summarises baseline W-BQ12 scores based on periodontal diagnosis. No statistically significant differences between any subscale scores (negative well-being, energy or positive well-being) were detected based on periodontal diagnosis, (P>0.05). Although not significant, the DP patients had higher (poorer QoL) negative well-being score (2.42±2.09) compared to the DH (1.44±1.24) and the DG (1.75±2.01) patients. Also, the DG patients had a higher (poorer QoL) negative well-being score (1.75±2.01) compared to the DH patients (1.44±1.24). A higher score in the negative subscale possibly indicates a higher negative impact of diabetes and periodontal disease on QoL in these patient groups.

With reference to the baseline general well-being score, the DH patients had a score of 24.6±2.19, the DG patients had a score of 24.1±5.14 and the DP patients had a score of 23.0±5.19. The DH patients had a higher (better QoL) general well-being score compared to the DG and DP patients. A higher general well-being score indicates a better QoL. However, none of these observations were statistically significant, (P>0.05).

	Health (n=9)	**Gingivitis (n=28)**	**Periodontitis (n=19)**	***P****
Negative well-being	1.44 ± 1.24	1.75 ± 2.01	2.42 ± 2.09	NS
Energy	6.56 ± 1.74	6.18 ± 1.93	5.84 ± 2.06	NS
Positive well-being	7.44 ± 1.33	7.64 ± 2.57	7.58 ± 1.84	NS
General well-being	24.6 ± 2.19	24.1 ± 5.14	23.0 ± 5.19	NS

Table 6.2: Comparing the W-BQ12 scores in T1DM patients based on periodontal diagnosis.

Mean ± SD presented for all scores. P-values determined using ANOVA with post-hoc Bonferoni. *P** indicates overall p-value comparing within the T1DM group. NS; not significant.



X-axis presents the mean ± SD scores and Y-axis indicates the subscales of the W-BQ12: negative well-being, energy and positive well-being.

Figure 6.1: The W-BQ12 subscale scores for all T1DM patients at baseline.

For patients with healthy periodontal tissues and gingivitis, their participation in the study was restricted to baseline (month 0). Only patients diagnosed with periodontitis completed the W-BQ12 at months 3 and 6. Table 6.3 presents the W-BQ12 scores in T1DM patients with periodontitis pre- and post-NSM. The general well-being score at month 3 (25.7±5.85) was significantly higher (better QoL) than the general well-being score at pre-treatment (23.0±5.19), ($P<0.05$). With regards to subscale scores, the energy subscale score at month 3 score (6.60±2.17) was significantly higher (better QoL) than the pre-treatment energy subscale score (5.84±2.06), ($P<0.05$).

Analysis of the baseline W-BQ12 scores based on gender (reporting only statistically significant findings), revealed that males had a significantly better QoL compared to females as indicated by higher general well-being score in males (25.2±4.06) compared to females (22.5±5.07), ($P<0.05$). Females had a significantly higher negative impact on QoL as indicated by higher negative well-being subscale score in females (2.59±2.18) compared to males (1.22±1.37), ($P<0.01$).

No statistically significant correlations were found between W-BQ12 scores and age, duration of diabetes, diabetic complications, HbA1c levels and mean PD (Spearman's correlation $P>0.05$).

	Month 0 (n=19)	**Month 3 (n=10)**	**Month 6 (n=10)**
Negative well-being	2.50 ± 2.27	1.60 ± 2.22	1.90 ± 2.47
Energy	5.10 ± 1.73	6.60 ± 2.17 *	6.00 ± 2.79
Positive well-being	7.50 ± 1.72	8.70 ± 2.06	7.10 ± 1.97
General well-being	22.1 ± 5.11	25.7 ± 5.85 *	23.2 ± 5.94

Table 6.3: The W-BQ12 scores in T1DM patients with periodontitis pre- and post-NSM.

Mean ± SD presented for all scores. For longitudinal comparisons, p-values determined using Paired t-test: significant difference from baseline *$P<0.05$.

6.2.2 Analysis of the ADDQoL-19

Table 6.4 summarises the ADDQoL-19 scores for all T1DM patients at baseline. The result of overview item 1 (generic assessment of QoL) was 1.45±0.76, indicating that the patients experienced a "good" to "very good" overall QoL. The result of overview item 2 (diabetes assessment of QoL) was -1.23±0.87, indicating that the patients felt their QoL would have been a "little better" or "much better" if they did not have diabetes.

The ADDQoL-19 score for all T1DM patients ranged from -6.68 to 0.00. The ADDQoL-19 score was -1.81±1.40 (Table 6.4, Figure 6.2). The median ADDQoL-19 score was calculated at -1.37, lower quartile cut off was calculated at -2.59. Based on the cut off strategy, 42 (75%) T1DM patients reported an ADDQoL-19 score of -2.59 or greater and only 14 (25%) T1DM patients had an ADDQoL-19 score of less than -2.59 (lower QoL). Only 1 (1.8%) patient reported an ADDQoL-19 score of 0, suggesting that their QoL was not affected by diabetes at all.

The 'impact rating' signifies the impact of the particular life aspect on QoL and, the 'importance rating' signifies the importance of that particular life aspect on the individual's QoL. In this cohort of T1DM patients, diabetes had the greatest impact on "freedom to eat" (impact rating -1.83±1.03) and the least impact on "living conditions" (impact rating -0.21±0.53). "Family life" was rated as the most important (importance rating 2.76±1.47) and "journeys" was rated as the least important (importance rating 1.60±1.08) by the T1DM patients. After considering weighting (weighted impact score) (Figure 6.3), the most negative impact of diabetes was on "freedom to eat" (weighted impact score -3.77±3.00) and the least negative impact of diabetes was on "financial situation" (weighted impact score -0.46±-1.33) domains of the ADDQoL-19.

	Impact rating (n=56)	Importance rating (n=56)	Weighted impact score (n=56)
Overview item 1			1.45 ± 0.76
Overview item 2			-1.23 ± 0.87
Leisure activities	-1.16 ± 1.04	1.91 ± 0.77	-2.43 ± 2.56
Working life	-0.86 ± 0.97	2.40 ± 0.76	-2.22 ± 2.72
Journeys	-1.00 ± 1.01	1.60 ± 1.08	-2.04 ± 2.61
Holidays	-1.19 ± 0.96	2.36 ± 0.74	-2.79 ± 2.59
Physical health	-1.04 ± 0.92	2.20 ± 0.68	-2.28 ± 2.36
Family life	-0.55 ± 0.85	2.76 ± 0.47	-1.49 ± 2.47
Friendship and social life	-0.73 ± 0.90	2.61 ± 0.53	-1.96 ± 2.57
Personal relationships	-0.55 ± 0.77	2.69 ± 0.63	-1.42 ± 2.13
Sex life	-0.40 ± 0.68	2.39 ± 0.65	-0.85 ± 1.58
Physical appearance	-0.64 ± 0.90	2.05 ± 0.88	-1.55 ± 2.49
Self confidence	-0.64 ± 0.82	2.21 ± 0.73	-1.46 ± 2.21
Motivation	-0.66 ± 0.90	2.14 ± 0.72	-1.52 ± 2.40
People's reaction	-0.30 ± 0.63	1.75 ± 0.94	-0.64 ± 1.59
Feelings about the future	-1.41 ± 1.14	2.18 ± 0.77	-3.09 ± 3.07
Financial situation	-0.25 ± 0.69	2.16 ± 0.71	-0.46 ± 1.33
Living conditions	-0.21 ± 0.53	2.29 ± 0.62	-0.54 ± 1.40
Dependence on others	-0.58 ± 0.80	2.00 ± 0.83	-1.36 ± 2.20
Freedom to eat	-1.83 ± 1.03	1.92 ± 0.81	-3.77 ± 3.00
Freedom to drink	-1.72 ± 1.10	1.64 ± 1.04	-3.28 ± 3.29
ADDQoL-19 score (mean ± SD)			-1.81 ± 1.40
[median (IQR)]			-1.37 (-2.59 - -0.75)

Table 6.4: The ADDQoL-19 scores for all T1DM patients at baseline.

Mean ± SD presented for overview 1, overview 2, impact rating, importance rating, weighted impact scores of all domains, and mean ± SD and median (IQR) presented for the overall ADDQoL-19 score.

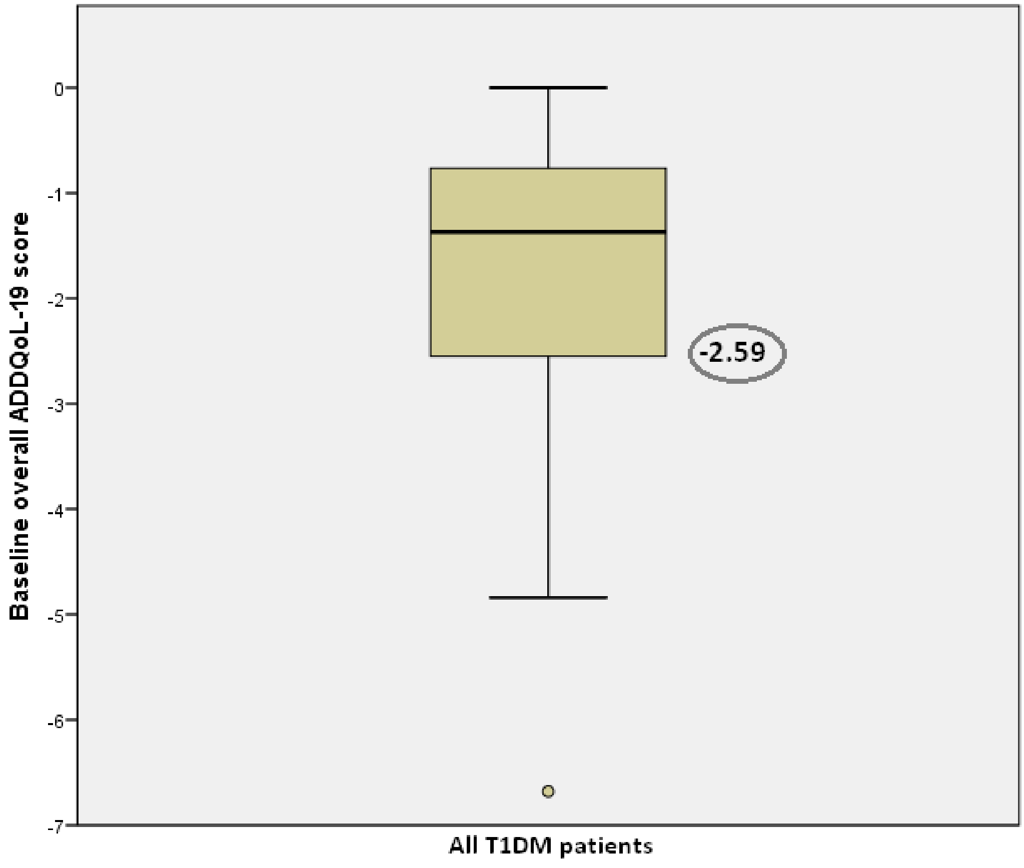


Figure 6.2: The overall ADDQoL-19 score for all T1DM patients at baseline.

Box plot figure showing medians, interquartile ranges, standard errors and outliers for 56 T1DM patients. The lower quartile was calculated at -2.59. Based on the cut off strategy 75% of the T1DM patients reported an ADDQoL-19 score above the lower quartile and 25% of the T1DM patients reported an ADDQoL-19 score below the lower quartile (poorer QoL). ● indicates outlier more than 1.5 but less than 3 times the IQR from the box boundaries.

Impact of T1DM on individual life domains

Financial situation
Living conditions
People's reaction
Sex life
Dependence on others
Motivation
Family life
Self-confidence
Personal relationship
Physical appearance
Friendship and social life
Journeys
Physical health
Working life
Leisure activities
Holidays
Feelings about future
Freedom to drink
Freedom to eat

-4 -3 -2 -1 0

Mean impact

Figure 6.3: The weighted impact scores of the ADDQoL-19 domains for all T1DM patients.

The most negative impact of T1DM on QoL was on the “freedom to eat” domain and the least negative impact of T1DM on QoL was on the “financial situation” domain.

Table 6.5 summarises the baseline ADDQoL-19 scores based on periodontal diagnosis. For the DH group, the overview item 1 score was 1.56±0.53 and overview item 2 was -1.11±0.60. For the DG group, the overview item 1 score was 1.50±0.75 and overview item 2 score was -1.14±0.85. For the DP group, the overview item 1 score was 1.32±0.89 and overview item 2 score was -1.42±1.01. No statistically significant differences were detected for the overview item 1 and 2 scores based on periodontal diagnosis, ($P>0.05$).

The ADDQoL-19 score for the DH group was -1.58±0.92, for the DG group was -1.79±1.35 and for the DP group was -1.94±1.68. Of note, no statistically significant differences were found for the ADDQoL-19 score based on periodontal diagnosis, ($P>0.05$). Interpreting the ADDQoL-19 score further based on quartiles, for the DH group, the median ADDQoL-19 score was calculated at -1.39 and lower quartile cut off was calculated at -1.93 (Table 6.5 and Figure 6.4). Based on the cut off strategy, 7 (77.8%) DH patients reported an ADDQoL-19 score of -1.93 or more and only 2 (22.2%) DH patients had an ADDQoL-19 score of less than -1.93 (lower QoL). For the DG group, the median ADDQoL-19 score was calculated at -1.55, lower quartile cut off was calculated at -2.59 (Table 6.5 and Figure 6.4). Based on the cut off strategy, 21 (75%) DG patients reported an ADDQoL-19 score of -2.59 or more and only 7 (25%) DG patients had an ADDQoL-19 score of less than -2.59 (lower QoL). For the DP group, the median ADDQoL-19 score was calculated at -1.26, lower quartile cut off was calculated at -2.94 (Table 6.5 and Figure 6.4). Based on the cut off strategy, 5 (26.3%) DP patients reported an ADDQoL-19 score of -2.94 or more, and 14 (73.7%) DP patients had an ADDQoL-19 score of less than -2.94 (lower QoL).

Considering baseline ADDQoL-19 weighted impact scores, for the DH group, T1DM had the most negative impact on "freedom to eat" (weighted impact score -3.00±2.35) and the least negative impact on "financial situation" (weighted impact score 0.00±0.00) domains, respectively. For the DG group, T1DM had the most negative impact on "freedom to eat" (weighted impact score -3.93±3.17) and the least negative impact on "financial situation" (weighted impact score -0.29±-1.12) domains, respectively. For the DP group, T1DM had the most negative impact on "freedom to eat" (weighted impact score -3.94±3.13) and the least negative impact on "people's reaction" (weighted impact score -0.58±-1.22) domains (Table 6.5).

With reference to the individual domain scores, at baseline, in the 'holidays' domain, the DG group had a significantly higher (lesser effect of diabetes on the holiday domain) score

(weighted impact score -2.12±1.97) compared to the DP group (weighted impact score -3.72±3.30), (P<0.05) (Table 6.5). Of note, no other statistically significant differences were based on periodontal diagnosis, (P>0.05).

	Health (n=9)	**Gingivitis (n=28)**	**Periodontitis (n=19)**
Overview item 1	1.56 ± 0.53	1.50 ± 0.75	1.32 ± 0.89
Overview item 2	-1.11 ± 0.60	-1.14 ± 0.85	-1.42 ± 1.01
Leisure activities	-2.11 ± 2.15	-2.43 ± 2.71	-2.58 ± 2.63
Working life	-1.25 ± 1.49	-2.13 ± 2.79	-2.78 ± 3.02
Journeys	-2.00 ± 2.06	-2.15 ± 2.98	-1.89 ± 2.38
Holidays	-2.89 ± 2.20	-2.12 ± 1.97	-3.72 ± 3.30 †
Physical health	-2.22 ± 1.79	-2.38 ± 2.61	-2.16 ± 2.34
Family life	-1.22 ± 2.99	-1.37 ± 2.37	-1.79 ± 2.46
Friendship and social life	-1.78 ± 2.05	-2.39 ± 2.85	-1.42 ± 2.36
Personal relationships	-0.78 ± 1.30	-1.64 ± 2.06	-1.39 ± 2.57
Sex life	-1.13 ± 1.64	-0.79 ± 1.45	-0.84 ± 1.80
Physical appearance	-1.33 ± 2.18	-1.46 ± 2.59	-1.79 ± 2.57
Self confidence	-0.89 ± 1.17	-1.64 ± 2.60	-1.47 ± 1.98
Motivation	-1.67 ± 2.55	-1.29 ± 2.31	-1.79 ± 2.55
People's reaction	-0.44 ± 1.33	-0.75 ± 1.90	-0.58 ± 1.22
Feelings about the future	-3.67 ± 2.65	-2.64 ± 2.83	-3.47 ± 3.61
Financial situation	0.00 ± 0.00	-0.29 ± 1.12	-0.95 ± 1.78
Living conditions	-0.22 ± 0.67	-0.43 ± 1.00	-0.84 ± 2.03
Dependence on others	-1.11 ± 1.36	-1.30 ± 2.09	-1.59 ± 2.79
Freedom to eat	-3.00 ± 2.35	-3.93 ± 3.17	-3.94 ± 3.13
Freedom to drink	-2.22 ± 2.22	-3.67 ± 3.43	-3.24 ± 3.56
ADDQoL-19 score (mean ± SD) [median (IQR)]	-1.58 ± 0.92 -1.39 (-1.93 - -1.24)	-1.79 ± 1.35 -1.55 (-2.59 - -0.74)	-1.94 ± 1.68 -1.26 (-2.94 - -0.82)

Table 6.5: Comparing the ADDQoL-19 scores in T1DM patients based on periodontal diagnosis.

Mean ± SD presented for overview 1, overview 2, weighted impact scores of all domains, and mean ± SD and median (IQR) presented for the ADDQoL-19 score. P-values determined using ANOVA with post-hoc Bonferoni. †*P*<0.05 indicates statistically significant difference compared to gingivitis within T1DM group.

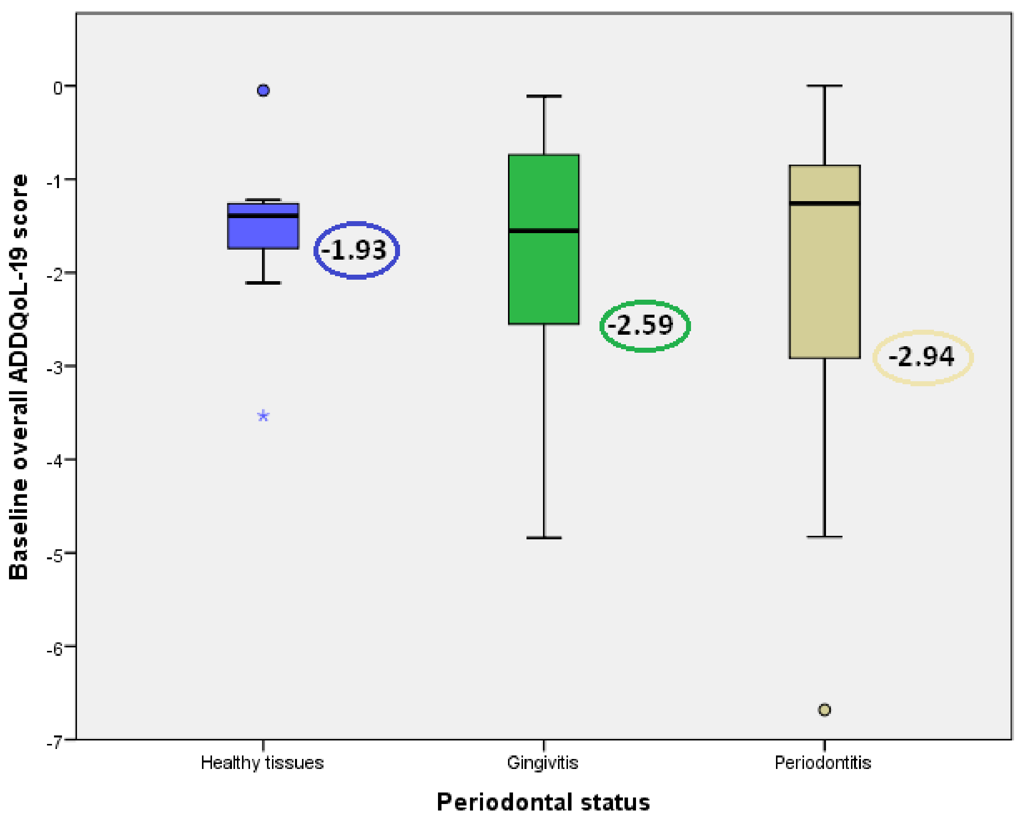


Figure 6.4: The ADDQoL-19 score for T1DM patients based on periodontal diagnosis.

Box plot figure showing medians, interquartile ranges, standard errors and outliers for T1DM patients based on periodontal diagnosis. For healthy tissue patients, the lower quartile was calculated at -1.93, 77.8% of these patients (n=9) reported an ADDQoL-19 score above -1.93 and 22.2% had a score below -1.93 (poorer QoL). For gingivitis patients, the lower quartile was calculated at -2.59, 75% of these patients (n=28) reported an ADDQoL-19 score above -2.59 and 25% had a score below -2.59 (poorer QoL). For periodontitis patients, the lower quartile was calculated at -2.94, 26.3% of these patients (n=19) reported an ADDQoL-19 score above -2.94 and 73.7% reported a score below -2.94 (poorer QoL). ● indicates outlier more than 1.5 but less than 3 times the IQR from the box boundaries, ★ indicates outlier more than 3 times the IQR from the box boundaries.

Table 6.6 summarises the baseline impact and importance ratings of the ADDQoL-19 domains based on periodontal diagnosis. In the DH group, diabetes had the greatest impact on “feelings about the future” (impact rating -1.67±1.00) and least impact on “financial situation” (impact rating 0.00±0.00). “Family life” was rated as the most important (importance rating 2.78±0.44) and “people’s reaction” was rated as the least important (importance rating 1.44±0.53). In the DG group, diabetes had the greatest impact on “freedom to eat” (impact rating -1.93±1.00) and least impact on “living conditions” (impact rating -0.18±0.39). “Family life” was rated as the most important (importance rating 2.74±0.53) and “journeys” was rated as the least important (importance rating 1.44±1.12). In the DP group, diabetes had the greatest impact on “freedom to drink” (impact rating -1.88±1.22) and least impact on “living conditions” (impact rating -0.32±0.75). “Family life” was rated as the most important (importance rating 2.79±0.42) and “journeys” was rated as the least important (importance rating 1.63±1.07).

Of note, no statistically significant differences were found between impact and importance ratings based on periodontal diagnosis, (P>0.05).

	Health (n=9)	Gingivitis (n=28)	Periodontitis (n=19)
Leisure activities			
Impact rating	-1.00 ± 0.87	-1.11 ± 1.03	-1.32 ± 1.16
Importance rating	1.89 ± 0.60	2.07 ± 0.86	1.68 ± 0.67
Working life			
Impact rating	-0.50 ± 0.53	-0.79 ± 0.93	-1.11 ± 1.13
Importance rating	2.38 ± 0.92	2.38 ± 0.71	2.44 ± 0.78
Journeys			
Impact rating	-1.00 ± 0.76	-0.96 ± 1.06	-1.05 ± 1.08
Importance rating	2.00 ± 1.00	1.44 ± 1.12	1.63 ± 1.07
Holidays			
Impact rating	-1.11 ± 0.78	-1.04 ± 0.92	-1.44 ± 1.10
Importance rating	2.67 ± 0.50	2.19 ± 0.80	2.44 ± 0.70
Physical health			
Impact rating	-0.89 ± 0.60	-1.07 ± 0.96	-1.05 ± 1.03
Importance rating	2.22 ± 0.67	2.12 ± 0.65	2.32 ± 0.75
Family life			
Impact rating	-0.44 ± 1.01	-0.54 ± 0.84	-0.63 ± 0.83
Importance rating	2.78 ± 0.44	2.74 ± 0.53	2.79 ± 0.42
Friendship & social life			
Impact rating	-0.67 ± 0.71	-0.86 ± 0.97	-0.58 ± 0.90
Importance rating	2.67 ± 0.50	2.68 ± 0.55	2.47 ± 0.51
Personal relationships			
Impact rating	-0.33 ± 0.50	-0.61 ± 0.74	-0.56 ± 0.92
Importance rating	2.67 ± 0.71	2.68 ± 0.67	2.72 ± 0.57
Sex life			
Impact rating	-0.50 ± 0.76	-0.39 ± 0.63	-0.37 ± 0.76
Importance rating	2.63 ± 0.52	2.34 ± 0.67	2.37 ± 0.68
Physical appearance			
Impact rating	-0.44 ± 0.73	-0.61 ± 0.92	-0.79 ± 0.98
Importance rating	2.00 ± 1.12	2.14 ± 0.80	1.95 ± 0.91
Self confidence			
Impact rating	-0.44 ± 0.53	-0.71 ± 0.98	-0.63 ± 0.68
Importance rating	2.33 ± 0.71	2.07 ± 0.60	2.37 ± 0.90
Motivation			
Impact rating	-0.67 ± 0.87	-0.54 ± 0.84	-0.84 ± 1.01
Importance rating	2.33 ± 0.87	2.04 ± 0.69	2.21 ± 0.71
People's reaction			
Impact rating	-0.22 ± 0.67	-0.29 ± 0.66	-0.37 ± 0.60
Importance rating	1.44 ± 0.53	1.64 ± 1.03	2.05 ± 0.91
Feelings about the future			
Impact rating	-1.67 ± 1.00	1.32 ± 1.09	-1.42 ± 1.30
Importance rating	2.11 ± 0.33	2.07 ± 0.86	2.37 ± 0.76
Financial situation			
Impact rating	0.00 ± 0.00	-0.21 ± 0.69	-0.42 ± 0.84
Importance rating	2.22 ± 0.83	2.07 ± 0.66	2.26 ± 0.73
Living conditions			
Impact rating	-0.11 ± 0.33	-0.18 ± 0.39	-0.32 ± 0.75
Importance rating	2.22 ± 0.67	2.21 ± 0.63	2.42 ± 0.61
Dependence on others			
Impact rating	-0.67 ± 0.71	-0.56 ± 0.75	-0.59 ± 0.94
Importance rating	2.00 ± 0.71	1.89 ± 0.75	2.18 ± 1.01
Freedom to eat			
Impact rating	-1.56 ± 1.01	-1.93 ± 1.00	-1.82 ± 1.13
Importance rating	1.78 ± 0.67	1.81 ± 0.88	2.18 ± 0.73
Freedom to drink			
Impact rating	-1.33 ± 1.00	-1.74 ± 1.06	-1.88 ± 1.22
Importance rating	1.56 ± 0.53	1.63 ± 1.11	1.71 ± 1.60

Table 6.6: Comparisons of the impact and importance ratings of the ADDQoL-19 in T1DM patients based on periodontal diagnosis.

Mean ± SD presented for impact, impact rating and importance rating of the ADDQoL-19 domains. P-values determined using ANOVA with post-hoc Bonferoni (no statistically significant differences found). Values in **blue** indicate impact rating scores and values in **red** indicate importance rating scores.

Table 6.7 summarises pre- and post-treatment ADDQoL-19 scores in patients with periodontitis. At pre-treatment, overview item 1 score was 1.60±0.84 and overview item 2 score was -1.20±1.03. At month 3, the overview item 1 score was 1.40±0.97 and overview item 2 score was -1.30±1.16. At month 6, the overview item 1 score was 1.70±1.16 and overview item 2 score was -1.20±0.92. No statistically significant differences were found for overview item 1 and 2 scores pre- and post-NSM, ($P>0.05$).

At pre-treatment, the ADDQoL-19 score for the DP group (n=19) was -1.94±1.68 (Table 6.7 and Figure 6.5). The median ADDQoL-19 score was calculated at -1.26, and the lower quartile cut off was calculated at -2.94. Based on the cut off strategy, 5 (26.3%) DP patients had an ADDQoL-19 score of -2.94 or more, and 14 (73.7%) DP patients had an ADDQoL-19 score of less than -2.94 (poorer QoL). At month 3, the ADDQoL-19 score for the DP group (n=10), was -1.89±1.81 (Table 6.7 and Figure 6.5). The median ADDQoL-19 score was calculated at -1.44, and the lower quartile cut off was calculated at -2.54. Based on the cut off strategy, 8 (80%) DP patients reported an ADDQoL-19 score of -2.54 or more, and 2 (20%) DP patients had an ADDQoL-19 score of less than -2.54 (lower QoL). At month 6, the ADDQoL-19 score for the DP group (n=10), was -2.22±2.11 (Table 6.7 and Figure 6.5). The median ADDQoL-19 score was calculated at -1.29, and the lower quartile cut off was calculated at -3.82. Based on the cut off strategy, 8 (80%) DP patients reported an ADDQoL-19 score of -3.82 or more, and 2 (20%) patients had an ADDQoL-19 score of less than -3.82 (lower QoL). No statistically significant differences were found for the ADDQoL-19 scores pre- and post-NSM, ($P>0.05$).

The weighted impact score for the 'working life' domain score at month 6 (-1.29±2.36) was significantly higher than the pre-treatment score (-3.29±3.25), ($P<0.05$). Also, the 'feelings about the future' weighted impact score at month 3 (-2.40±3.13) was significantly higher than the score at month 0 (-3.90±3.92) ($P<0.05$). No other statistically significant findings were found in domain scores pre- and post-NSM, ($P>0.05$).

	Month 0 (n=19)	Month 3 (n=10)	Month 6 (n=10)
Overview item 1	1.60 ± 0.84	1.40 ± 0.97	1.70 ± 1.16
Overview item 2	-1.20 ± 1.03	-1.30 ± 1.16	-1.20 ± 0.92
Leisure activities	-3.56 ± 3.00	-3.78 ± 3.35	-2.90 ± 3.03
Working life	-3.00 ± 3.21	-3.25 ± 2.55	-1.29 ± 2.36 **
Journeys	-2.10 ± 3.11	-2.10 ± 3.11	-2.60 ± 3.63
Holidays	-3.56 ± 3.64	-3.67 ± 2.96	-2.78 ± 3.03
Physical health	-2.33 ± 2.55	-3.67 ± 2.78	-3.10 ± 3.31
Family life	-2.00 ± 3.00	-2.00 ± 3.00	-1.00 ± 1.50
Friendship and social life	-1.44 ± 2.96	-1.33 ± 2.18	-2.10 ± 2.88
Personal relationships	-1.90 ± 3.28	-1.20 ± 2.90	-0.89 ± 2.03
Sex life	-1.00 ± 2.16	-0.30 ± 0.95	-0.33 ± 1.00
Physical appearance	-1.60 ± 1.90	-1.70 ± 1.57	-2.50 ± 2.88
Self confidence	-1.90 ± 2.42	-1.30 ± 2.98	-2.30 ± 2.98
Motivation	-1.90 ± 3.28	-1.90 ± 3.14	-2.30 ± 3.13
People's reaction	-0.90 ± 1.52	-0.30 ± 0.95	-0.90 ± 1.52
Feelings about the future	-3.90 ± 3.92	-2.40 ± 3.13 *	-2.60 ± 3.24
Financial situation	-0.80 ± 1.32	-0.60 ± 1.26	-1.50 ± 2.92
Living conditions	-0.60 ± 1.90	-0.30 ± 0.95	-0.90 ± 2.85
Dependence on others	-1.38 ± 2.20	-1.25 ± 1.39	-0.83 ± 1.33
Freedom to eat	-2.88 ± 2.10	-3.13 ± 3.72	-4.50 ± 4.04
Freedom to drink	-2.38 ± 3.42	-3.13 ± 3.87	-3.83 ± 4.07
ADDQoL-19 score (mean ± SD)	-1.94 ± 1.68	-1.89 ± 1.81	-2.22 ± 2.11
[median (IQR)]	-1.26 (-2.94 - -0.82)	-1.44 (-2.54 - -0.71)	-1.29 (-3.82 - -0.66)

Table 6.7: The ADDQoL-19 scores in T1DM patients with periodontitis pre- and post-NSM.

Mean ± SD presented for overview 1, overview 2, weighted impact scores for all domains, and mean ± SD and median (IQR) presented for the overall ADDQoL-19 scores. For longitudinal comparisons, p-values determined using Paired t-test: significant difference from baseline *P<0.05 and **P<0.01.

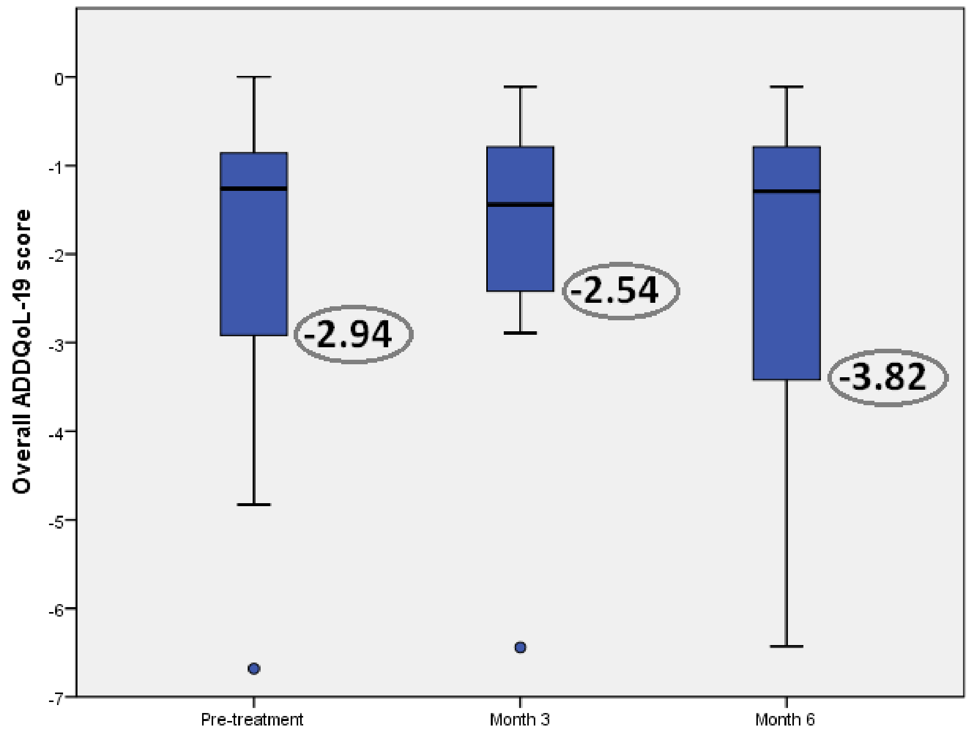


Figure 6.5: The ADDQoL-19 score in T1DM patients with periodontitis pre- and post-NSM.

Box plot figure showing medians, interquartile ranges, standard errors and outliers for T1DM patients with periodontitis. At pre-treatment the lower quartile was calculated at -2.94, 26.3% of patients (n=19) reported an ADDQoL-19 score above -2.94 and 73.7% reported a score below -2.94 (poorer QoL). At month 3, the lower quartile was calculated at -2.54, 80% of patients (n=10) reported an ADDQoL-19 score above -2.54 and 20% reported a score below -2.54 (poorer QoL). At month 6, the lower quartile was calculated at -3.82, 80% of patients (n=10) reported an ADDQoL-19 score above -3.82 and 20% reported a score below -3.82 (poorer QoL). ● indicates outlier more than 1.5 but less than 3 times the IQR from the box boundaries,★ indicates outlier more than 3 times the IQR from the box boundaries.

Table 6.8 summarises the impact and importance ratings of the ADDQoL-19 domains for T1DM patients with periodontitis pre- and post-NSM. At pre-treatment, in the DP group, diabetes had the greatest impact on "freedom to drink" (impact rating -2.00±1.20) and least impact on "living conditions" (impact rating -0.20±0.63). "Family life" was rated as the most important (importance rating 2.89±0.33) and "journeys" was rated as the least important (importance rating 1.90±1.10). At month 3, diabetes had the greatest impact on "freedom to eat" (impact rating -1.56±1.24) and least impact on "sex life" (impact rating -0.10±0.32) and "living conditions" (impact rating -0.10±0.32). "Family life" was rated as the most important (importance rating 2.89±0.33) and "freedom to drink" was rated as the least important (importance rating 1.67±1.00). At month 6, diabetes had the greatest impact on "freedom to eat" (impact rating -1.86±1.21) and least impact on "sex life" (impact rating -0.11±0.33). "Family life" was rated as the most important (importance rating 3.00±0.00) and "journeys" was rated as the least important (importance rating 1.70±0.95).

At pre-treatment, diabetes had a significantly greater impact on "working life" (impact rating -1.14±1.07), compared to month 6 (impact rating -0.43±0.79) following NSM, ($P<0.05$). Also, at pre-treatment, the DP group had a significantly greater impact on "feelings about the future" (impact rating -1.50±1.35) compared to month 3 (impact rating -0.90±1.10) following NSM, ($P<0.05$). At month 3, the DP patients reported a significantly greater importance on "physical appearance" (importance rating 2.40±0.52) compared to pre-treatment (importance rating 2.00±0.47), ($P<0.05$).

	Month 0 (n=19)	Month 3 (n=28)	Month 6 (n=19)
Leisure activities			
Impact rating	-1.50 ± 1.27	-1.50 ± 1.18	-1.30 ± 0.95
Importance rating	1.89 ± 0.78	1.89 ± 0.78	2.00 ± 0.94
Working life			
Impact rating	-1.00 ± 1.07	-1.13 ± 0.83	-0.43 ± 0.79 *
Importance rating	3.00 ± 0.00	2.88 ± 0.35	2.29 ± 0.76
Journeys			
Impact rating	-0.90 ± 0.99	-0.80 ± 1.03	-1.00 ± 1.25
Importance rating	1.90 ± 1.10	1.90 ± 1.10	1.70 ± 0.95
Holidays			
Impact rating	-1.22 ± 1.20	-1.33 ± 1.00	-1.00 ± 1.00
Importance rating	2.56 ± 0.73	2.44 ± 0.73	2.56 ± 0.53
Physical health			
Impact rating	-1.00 ± 1.00	-1.33 ± 0.87	-1.20 ± 1.03
Importance rating	2.56 ± 0.73	2.56 ± 0.53	2.30 ± 0.82
Family life			
Impact rating	-0.67 ± 1.00	-0.67 ± 1.00	-0.33 ± 0.50
Importance rating	2.89 ± 0.33	2.89 ± 0.33	3.00 ± 0.00
Friendship & social life			
Impact rating	-0.56 ± 1.01	-0.44 ± 0.73	-0.80 ± 1.03
Importance rating	2.56 ± 0.53	2.67 ± 0.50	2.60 ± 0.52
Personal relationships			
Impact rating	-0.70 ± 1.16	-0.40 ± 0.97	-0.33 ± 0.71
Importance rating	2.80 ± 0.42	2.80 ± 0.42	2.44 ± 0.73
Sex life			
Impact rating	-0.40 ± 0.84	-0.10 ± 0.32	-0.11 ± 0.33
Importance rating	2.60 ± 0.52	2.60 ± 0.52	2.44 ± 0.73
Physical appearance			
Impact rating	-0.80 ± 0.92	-0.70 ± 0.67	-1.10 ± 0.99
Importance rating	2.00 ± 0.47	2.40 ± 0.52 *	2.10 ± 0.74
Self confidence			
Impact rating	-0.70 ± 0.82	-0.50 ± 1.08	-0.90 ± 0.99
Importance rating	2.50 ± 0.71	2.20 ± 0.63	2.30 ± 0.67
Motivation			
Impact rating	-0.70 ± 1.16	-0.70 ± 1.06	-0.90 ± 1.10
Importance rating	2.40 ± 0.70	2.30 ± 0.67	2.20 ± 0.63
People's reaction			
Impact rating	-0.50 ± 0.71	-0.20 ± 0.42	-0.40 ± 0.70
Importance rating	2.10 ± 0.99	1.90 ± 0.99	1.80 ± 0.79
Feelings about the future			
Impact rating	-1.50 ± 1.35	-0.90 ± 1.10 *	-1.10 ± 1.10
Importance rating	2.60 ± 0.52	2.30 ± 0.67	2.00 ± 1.15
Financial situation			
Impact rating	-0.30 ± 0.48	-0.20 ± 0.42	-0.50 ± 0.97
Importance rating	2.30 ± 0.82	2.20 ± 0.79	2.20 ± 0.92
Living conditions			
Impact rating	-0.20 ± 0.63	-0.10 ± 0.32	-0.30 ± 0.95
Importance rating	2.50 ± 0.53	2.50 ± 0.53	2.20 ± 0.92
Dependence on others			
Impact rating	-0.50 ± 0.76	-0.50 ± 0.53	-0.33 ± 0.52
Importance rating	2.13 ± 0.99	2.13 ± 0.83	2.67 ± 0.52
Freedom to eat			
Impact rating	-1.63 ± 1.06	-1.38 ± 1.19	-1.67 ± 1.21
Importance rating	2.00 ± 0.76	2.00 ± 0.93	2.17 ± 0.98
Freedom to drink			
Impact rating	-2.00 ± 1.20	-1.50 ± 1.20	-1.67 ± 1.21
Importance rating	1.25 ± 1.28	1.75 ± 1.04	1.83 ± 0.98

Table 6.8: The impact and importance ratings of the ADDQoL-19 in T1DM patients with periodontitis pre- and post-NSM.

Mean ± SD presented for impact and importance rating of the ADDQoL-19 domains. For longitudinal comparisons, p-values determined using Paired t-test: significant difference from baseline **P*<0.05. Values in **blue** indicate impact rating scores and values in **red** indicate importance rating scores.

Interpreting the baseline ADDQoL-19 scores based on gender (reporting only statistically significant findings), females had a significantly higher impact of T1DM on their physical appearance (weighted impact score -2.45±2.84) compared to males (weighted impact score -0.59±0.64), ($P<0.01$). Females had a significantly higher impact of T1DM on their dependence on others (weighted impact score -2.00±2.78) compared to males (weighted impact score -0.64±0.95) ($P<0.05$), and females also had a significantly higher impact of T1DM on their freedom to eat (weighted impact score -4.54±3.34) compared to males (weighted impact score -2.92±2.22), ($P<0.05$).

Of note, no statistically significant correlations were found between ADDQoL-19 scores and age, duration of diabetes, diabetic complications, HbA1c levels and mean PD (Spearman's correlation $P>0.05$).

6.3 Discussion

In this study, QoL was assessed in patients with T1DM and periodontal disease pre-and post-periodontal treatment using two validated QoL instruments routinely used to assess the impact of diabetes on QoL, the W-BQ12 and the ADDQoL-19 questionnaire. Also, in this cohort of T1DM patients, QoL was further assessed based on periodontal diagnosis at baseline prior to any periodontal intervention.

The baseline findings of the clinical periodontal data suggest that the T1DM patients with periodontitis had significantly higher PI, mGI, BOP and mean PD compared to the T1DM patients with healthy tissues (Table 3.15). Longitudinal comparisons of the T1DM patients with periodontitis showed significant improvements in all clinical periodontal parameters (Table 5.5), thus indicating that periodontal treatment had a positive effect upon the clinical aspects of the condition, and the patients benefited from the treatment they received at the dental hospital. There were no statistically significant differences or improvement in glycaemic control in T1DM patients with periodontitis following NSM, and HbA1c levels remained similar to those recorded at pre-treatment.

The W-BQ12 and ADDQoL-19 questionnaires were analysed at baseline and, for the T1DM patients with periodontitis at months 3 and 6 following NSM. Analysis of the baseline W-BQ12 scores, comparing T1DM patients based on periodontal diagnosis revealed no statistically significant differences in any subscale or general well-being scores, suggesting

that all T1DM groups (healthy periodontal tissues, gingivitis and periodontitis) had a similar perception of QoL as assessed by the W-BQ12.

Analysis of post-treatment W-BQ12 scores in T1DM patients with periodontitis revealed that QoL did improve following NSM, as indicated by significantly higher (better QoL) post-treatment (month 3) general well-being score compared to the pre-treatment general well-being score. This suggests that successful NSM has a short-term positive impact on QoL in T1DM patients with periodontitis. The results of the current study are similar to previous research which showed that successful NSM had a positive impact and improves an individual's QoL (Aslund et al. 2008; Jowett et al. 2009; Saito et al. 2010; Shanbhag et al. 2012). However, no statistically significant differences were found in the general well-being score between pre-treatment and month 6, suggesting that there was perhaps an initial improvement in QoL at month 3 following the first periodontal treatment appointment, which stabilised during the course of the study and was not evidently identified at month 6 following NSM.

Analysis of the ADDQoL-19 questionnaire for all T1DM patients at baseline revealed that the patients experienced a "good to very good" overall general QoL, as revealed by the generic assessment overview item 1. However, assessment of the diabetes-specific overview item 2 revealed that the T1DM patients felt that their QoL would have been "a little better or much better" if they did not have diabetes. No statistically significant differences were found between both overview item scores based on periodontal diagnosis suggesting that patients with healthy periodontal tissues, gingivitis and periodontitis had a similar perception of QoL as related to their diabetes, and perceived a "good to very good" overall QoL and felt that their life would have been "a little better or much better" if they did not have diabetes. The analysis of the baseline ADDQoL-19 scores further revealed that for all T1DM patients, the greatest impact of diabetes on QoL was on their "freedom to eat", whereas their "living conditions" had the least impact. "Family life" for this cohort of patients was the most important and the least important was their "journeys" or the ability to travel.

Comparing the T1DM patients based on periodontal diagnosis, revealed no statistically significant differences in the overall ADDQoL-19 score. This suggests that all groups of periodontal diagnosis had a similar perception of the impact of diabetes on their QoL prior to periodontal treatment as assessed by the ADDQoL-19 questionnaire. Interestingly, interpreting the ADDQoL-19 score based on quartiles revealed that a majority of the

periodontitis patients (73.7%) experienced a poorer QoL in contrast to a majority of the healthy tissue (77.8%) and gingivitis (75%) patients who experienced a better QoL.

In all groups of periodontal diagnosis, “freedom to eat” had the highest weighted impact. T1DM patients with periodontitis had significantly higher impact on their “holidays” domain compared to those with gingivitis. The T1DM patients with healthy tissues had the greatest impact on their “feelings about the future”, the gingivitis patients had their greatest impact on their “freedom to eat”, whereas in periodontitis patients the greatest impact was on their “freedom to drink”. All groups of periodontal diagnosis placed greatest importance on their “family life”.

Analysis of the post-treatment overall ADDQoL-19 scores in T1DM patients with periodontitis revealed no statistically significant differences between pre-treatment, month 3 and month 6 ADDQoL-19 (overview 1 and 2, weighted impact, overall ADDQoL-19) scores. This suggests that the QoL remained the same pre- and post-NSM in this group of patients as assessed by the ADDQoL-19 questionnaire. Interestingly, at pre-treatment, 73.7% of the 19 DP patients reported ADDQoL-19 scores below the lower QoL cut off compared to only 20% of the 10 DP patients who reported ADDQoL-19 score below the lower QoL cut off at months 3 and 6, suggesting that following NSM fewer patients experienced a poorer QoL compared to pre-treatment. While considering impact and importance ratings in T1DM patients with periodontitis, at pre-treatment the greatest impact was on their “freedom to drink”, at months 3 and 6 the greatest impact was on their “freedom to eat”. At all three time points, the greatest importance was their “family life”. At pre-treatment, periodontitis patients had a significantly greater impact on their “feelings about the future” and “working life” compared to the impact in these domains at months 3 and 6, respectively. At month 3 periodontitis patients had a significantly greater impact on their “physical appearance” compared to pre-treatment.

We found that T1DM patients in all groups of periodontal diagnosis, placed great importance on their “freedom to eat and drink” domains as assessed by the ADDQoL-19 questionnaire. The findings of the current study are consistent with those of previous studies (Bradley and Speight 2002; Trief et al. 2003; Costa et al. 2006; Holmanova and Ziakova 2009; Turk et al. 2013), which found the greatest negative impact of diabetes on the domain “freedom to eat”, indicating a strong influence of dietary restrictions on QoL in this group of patients. Patients with T1DM have to prioritise their dietary intake in order to prevent diabetes-related

complications (Daneman 2006). Their nutritional intake has to be tailored to their age, stage of development, weight, culture, lifestyle and personal preferences (Franz 2004; Evert et al. 2013). Their daily diet is usually regulated by the intake of their medications and the time of day, and hence we found in this group of T1DM patients, their freedom to eat and drink had a major impact and importance on their daily lives.

In the current study, males had significantly better QoL compared to females, and females had a significantly higher negative impact on QoL compared to males as assessed by the W-BQ12. Females also had a significantly higher impact on their physical appearance, dependence on others and their freedom to eat as assessed by the ADDQoL-19 questionnaire. The findings of the present study are consistent with previous research that suggests QoL is better among diabetic males than diabetic females (Rubin and Peyrot 1992; Unden et al. 2008). Our findings are also consistent with reported gender differences in HRQoL in the general population, which suggest that males have a better perception of QoL compared to females (Hagedoorn et al. 2000; Riedinger et al. 2001; Emery et al. 2004; Mrus et al. 2005).

Our study has some limitations. Firstly, we did not analyse QoL in our control non-T1DM patients, hence we cannot compare QoL findings of the T1DM patients to the control patients. Secondly, we under-recruited T1DM patients according to our *a priori* power calculation due to difficulties in recruitment. Thirdly, the T1DM patients were recruited from a diabetes clinic which might be a disadvantage, as these patients are often at an extreme end of the disease spectrum and they may not be representative of the whole T1DM population. Lastly, the W-BQ12 is a general health assessment questionnaire and the ADDQoL-19 is a diabetes-specific questionnaire, specifically designed to asses QoL in patients with diabetes, hence, these measures might not to a full extent have analysed OHRQoL, the effect of periodontal disease and its treatment in this cohort of patients, as they contain limited oral-health related questions.

In conclusion, analyses of the W-BQ12 and the ADDQoL-19 questionnaire revealed that T1DM did impact on certain life aspects in this cohort of patients. However, T1DM did not have an impact on QoL based on periodontal diagnosis, and that patients with healthy tissues, gingivitis and periodontitis had a similar perception of QoL, suggesting that the severity of their periodontal disease did not reveal any additional negative effects on their QoL as assessed by these questionnaires. Although T1DM patients with periodontitis showed statistically significant improvements in their clinical periodontal condition following NSM,

the W-BQ12 and the ADDQoL-19 questionnaire did not appear to be useful in capturing the impact of periodontal disease and the positive outcomes of its treatment, in this cohort of patients. This suggests that although these validated QoL measures are ideal for assessing HRQoL in patients with diabetes, they but might not be beneficial in assessing OHRQoL in patients with periodontal disease and T1DM.

Summary of key findings from chapter 6

- At baseline, T1DM patients with periodontitis had higher (poorer QoL) negative well-being scores compared to those with healthy tissues and gingivitis. However, these differences were not statistically significant.
- The T1DM patients perceived that their QoL would be "a little better or much better" if they did not have diabetes.
- For all T1DM patients the most negative impact of diabetes on QoL was on their "freedom to eat" followed by their "freedom to drink".
- At baseline, no statistically significant differences were found for the W-BQ12 and the ADDQoL-19 scores based on periodontal diagnosis, suggesting that T1DM patients with healthy tissues, gingivitis and periodontitis had a similar perception of QoL.
- Following successful NSM, no statistically significant improvements were seen in the W-BQ12 and ADDQoL-19 scores in T1DM patients with periodontitis.
- Interestingly compared to pre-treatment, where a majority 73.7% of T1DM patients experienced a poorer QoL, only 20% of the patients experienced a poorer QoL following NSM.
- Based on gender, males had significantly better perception of QoL than females, and females reported to have a greater negative impact of diabetes and periodontal disease on their QoL. Females also had a higher negative impact of diabetes compared to males in terms of physical appearance, dependence on others and freedom to eat.

7 Chapter 7. Discussion

Diabetes is one of the largest global health emergencies of the 21st century. Every year more and more people are diagnosed with this condition that results in life-threatening complications. The estimated global prevalence of diabetes for adults for 2015 was 415 million, and it is predicted to affect 642 million individuals by 2040 (International Diabetes Federation 2015). Diabetes can pose a threat to oral health. There is an increased risk for inflammatory periodontal disease in patients with poor glycaemic control. It is important to effectively manage periodontal disease in diabetic patients as optimal oral hygiene is the key to prevent tooth loss, promote a healthy diet and improve metabolic control (International Diabetes Federation 2015). Since the prevalence of periodontal and oral diseases in patients with T1DM is largely under-researched, this study aimed to investigate the prevalence and severity of periodontal and oral diseases in T1DM patients, to establish the inflammatory links between T1DM and periodontal disease by investigating local and systemic markers of inflammation in biological samples and to further investigate the effect of periodontal treatment on clinical and biological parameters. Furthermore, while establishing scientific links between the two diseases it was considered important to assess QoL in patients with diabetes and periodontal disease, as both these inflammatory conditions are known to have a profound impact on QoL in terms of physical, psychological and social well-being (Glasgow et al. 1997; O'Dowd et al. 2010; Durham et al. 2013; Desai et al. 2014).

Analysis of the demographic data in the present study revealed that the patients with T1DM were significantly lower in age [28.0 (23.0-32.5) years] compared to the non-T1DM patients [40.0 (35.0-47.0) years]. This difference in age reflects the recruitment pattern of controls from a parallel T2DM study which included a higher age range than the current study, and unfortunately must be regarded as a weakness in this study. The under-recruitment of control patients during the time period granted for the study, and in order to match the number of recruited T1DM patients, 31 control patients were selected from the T2DM study based on periodontal diagnosis and matching as closely as possible for clinical periodontal parameters. Case definitions used to define healthy periodontal tissues, gingivitis and periodontitis were exactly the same in both studies. To be precise, the selection of controls included, 9 patients with healthy periodontal tissues, 13 patients with gingivitis and 9 patients with periodontitis. Nevertheless, it is not unreasonable to presume that irrespective of age, patients with healthy periodontal tissues did not have or never had periodontal disease and patients with gingivitis

and periodontitis were recruited with exactly the same case definition criteria set in both studies.

With reference to oral and dental findings, the current study found that patients with T1DM had significantly more sound and unrestored teeth (22.5±6.84) compared to the non-T1DM patients (18.8±5.62). Based on periodontal diagnosis, T1DM patients with periodontitis had less sound and unrestored teeth (19.2±6.33) compared to those with gingivitis (23.1±7.02) and healthy tissues (27.7±2.60). This finding could be a manifestation of age, as T1DM patients with periodontitis were significantly higher in age compared to those with gingivitis and healthy tissues. As age progresses, patients are more likely to have a greater number of restored teeth, which was also reflected in our study with non-T1DM patients having a significantly higher number of restored teeth compared to the T1DM patients, and T1DM patients with periodontitis having a significantly higher number of restored teeth compared to the T1DM patients with gingivitis and healthy tissues. The prevalence of caries in patients with T1DM compared to non-diabetic controls has been an area of research with inconsistent findings. In the current study, although in T1DM patients, a higher proportion of teeth had caries into dentine compared to the non-T1DM patients, this difference was not statistically significant, suggesting no difference in caries experience between the two groups. Our findings are similar to those of other studies which found similar caries experience in patients with and without T1DM (Faulconbridge et al. 1981; Tenovuo et al. 1986; Harrison and Bowen 1987b; Twetman et al. 1989; Swanljung et al. 1992; Edblad et al. 2001; Siudikiene et al. 2008; Tagelsir et al. 2011), and are in contrast to other studies which reported either a higher caries prevalence (Albrecht et al. 1988; Jones et al. 1992; Moore et al. 2001b; Lopez et al. 2003; Miralles et al. 2006) and lower caries prevalence (Matsson and Koch 1975; Leeper et al. 1985; Kirk and Kinirons 1991; Siudikiene et al. 2006; Orbak et al. 2008) in patients with T1DM compared to non-diabetic controls. The similar caries experience in patients with and without T1DM in the current study could be supported by the fact that modern management of diabetes, characterised by flexibility of insulin treatment and blood glucose monitoring, allows for less rigid meal planning and reduces the significance of dietary factors as an indicator for possible variations in caries development (Twetman et al. 2002; Siudikiene et al. 2006). Additionally, patients with T1DM were known to have more daily main meals and fewer snacks than non-diabetic children, who had fewer main meals per day but consumed more snacks (Siudikiene et al. 2006), which could possibly explain the similar caries experience in both groups in the present study. Lower salivary flow rates and self-

reported xerostomia have been frequently seen in patient with diabetes (Sreebny et al. 1992; Ben-Aryeh et al. 1993; Moore et al. 2001b; Siudikiene et al. 2006), especially if poorly-controlled (Harrison and Bowen 1987a; Harrison and Bowen 1987b). Diminished salivary flow has been linked to high caries prevalence in diabetic patients (Twetman et al. 1992; Karjalainen et al. 1997; Moore et al. 2001a; Siudikiene et al. 2006). In the current study, no statistically significant differences were found between T1DM and non-T1DM patients for both clinically assessed and patient-reported xerostomia. Only 2 patients with T1DM (3.50%) and 2 non-diabetic patients (4.70%) self-reported having xerostomia. This finding could also possibly explain the low caries prevalence in this cohort of patients and lack of significant differences in caries experience between the diabetic and non-diabetic in the present study.

In the current study, T1DM patients with periodontitis presented with a significantly longer history of diabetes (17.5±8.32 years) compared to those with healthy tissues (11.7±5.12 years). Although not statistically significant, periodontitis patients presented with a longer history of T1DM compared to those with gingivitis (12.5±6.86 years). Our findings could be a manifestation of the patients who were recruited, as the periodontitis patients were significantly older compared to those with healthy tissues and non-significantly older compared to those with gingivitis. It has been well established that age is a common confounding factor for periodontal disease, and older individuals may present with more severe periodontal disease (Genco and Borgnakke 2013). Additionally the greater duration of diabetes in the patients with periodontitis compared to those with gingivitis and healthy tissues could suggest that a longer history of diabetes increases the chances of individuals experiencing more severe periodontal disease. Data from previous studies also demonstrate that a longer duration of diabetes is related to poorer periodontal health (Cianciola et al. 1982; Thorstensson and Hugoson 1993; Firatli et al. 1996; Silvestre et al. 2009). However, our findings are in contrast to other studies which found no influence of duration of diabetes on periodontal health (de Pommereau et al. 1992; Tervonen and Oliver 1993; Sandberg et al. 2000).

In the present study, 42.1% of the T1DM patients were categorised as having poor glycaemic control, and although the differences in glycaemic control categories were not statistically significant, it is interesting to note that 52.6% of the periodontitis patients had poorly-controlled T1DM, whereas 44.8% of the gingivitis patients and 55.6% of the healthy tissue

patients had moderately-controlled T1DM. These findings possibly support the concept of a two-way relationship between diabetes and periodontal disease, in which poor glycaemic control increases the risk for severe periodontal disease and severe periodontal disease negatively affects glycaemic control (Preshaw et al. 2012). The baseline HbA1c level for all T1DM patients was 8.30 (7.60-9.35) % / 67 (60-79) mmol/mol, which was categorised as a moderate metabolic control. Further comparing HbA1c levels based on periodontal status, T1DM patients with periodontitis had higher HbA1c levels [8.95 (8.03-9.65) %/ 75 (64-83) mmol/mol] compared to the gingivitis [8.25 (7.65-10.0) %/ 67 (61-86) mmol/mol] and healthy tissue [7.90 (7.30-8.58) %/ 63 (56-70) mmol/mol] patients, suggesting a possible role of periodontal inflammation in elevating HbA1c levels in patients with gingivitis and periodontitis, however these differences were not statistically significant. Following NSM, in the current study the HbA1c levels of T1DM patients with periodontitis reduced by 0.45% and 0.90% at months 3 and 6, respectively, although these reductions were not statistically significant. Our findings are similar to those of a study which found a 0.43% non-significant reduction in HbA1c levels in T1DM patients 6 months following treatment with NSM plus adjunctive doxycycline therapy (Miller et al. 1992). The findings of the current study are also in agreement with those of previous studies in T1DM patients, which found no significant improvement in HbA1c levels following periodontal treatment (Miller et al. 1992; Aldridge et al. 1995; Llambes et al. 2008). From a review of the literature it appears that overall periodontal treatment does improve glycaemic control in patients with T1DM, however the findings are not statistically significant (Miller et al. 1992; Seppala et al. 1993; Aldridge et al. 1995; Smith et al. 1996; Llambes et al. 2008). One must bear in mind the heterogeneity among the studies such as diabetes-related and periodontal-related factors, sample sizes and power to detect differences in metabolic and periodontal response, follow-up time frames for glycaemic control and periodontal status evaluation, inclusion of control groups and specific hypothesis tested. Despite such variations and although the evidence is equivocal there is evidence that supports the concept that periodontal disease contributes to poor glycaemic control and the treatment of periodontal disease has a beneficial effect in patients with T1DM (Taylor 2003). There is need to further investigate the effect of treating periodontal disease on glycaemic control especially in T1DM patients, as this cohort of patients has been under-researched.

In the current study, levels of hsCRP appeared higher in T1DM patients compared to non-T1DM patients, although this difference was not statistically significant. Interestingly, when

patients were further categorised based on periodontal status, patients with T1DM and healthy tissues had significantly higher hsCRP levels [1.40 (0.73-4.03) mg/L] compared to non-T1DM patients with healthy tissues [0.60 (0.20-1.10) mg/L]. These findings may reflect the diabetes-associated inflammation present in T1DM patients, where even in the absence of inflammatory periodontal disease, higher hsCRP levels were detected. Additionally, hsCRP levels were significantly lower in non-T1DM patients with healthy tissues [0.60 (0.20-1.10) mg/L] compared to those with gingivitis [2.85 (0.73-5.45) mg/L], and although non-T1DM patients with healthy tissues had lower hsCRP levels compared to patients with periodontitis this difference was not statistically significant. Similar findings were seen in the T1DM group, where periodontitis and gingivitis patients had higher hsCRP levels compared to those with healthy tissues; however these differences were also not statistically significant. In patients with diabetes, clinical interventional trials have shown a significant reduction of acute phase protein levels, such as fibrinogen (Christgau et al. 1998), and CRP (Lalla et al. 2007a) following periodontal therapy. However, in the current study no statistically significant improvements were seen in hsCRP levels in both T1DM and non-T1DM patients following NSM. Our findings are similar to those of a meta-analysis which concluded that it is highly unlikely that periodontal treatment could modulate systemic hsCRP levels in patients with severe periodontal disease (Ioannidou et al. 2006), and are in contrast to studies which found a decrease in hsCRP levels after periodontal therapy (Ide et al. 2003; D'Aiuto et al. 2005; Marcaccini et al. 2009a; Marcaccini et al. 2009b).

Data from the current study show that cholesterol levels were significantly higher in non-T1DM patients than T1DM patients. Also, although triglyceride, HDL and non-HDL were higher in non-T1DM patients compared to T1DM patients, these differences were not statistically significant. Following NSM, cholesterol was higher in non-T1DM patients at each time point; however this difference was statistically significant only at pre-treatment. This is not surprising, given that a key priority within the national management guidelines for T1DM involves the control of serum lipid levels (NICE 2015). Therefore, it is reasonable to presume that the T1DM patients in this study were receiving more aggressive management and monitoring of CVD risk factors compared to the control patients. Additionally, in the present study, NSM had no significant influence on triglyceride, HDL, non-HDL and cholesterol levels in both T1DM and non-T1DM patients

The prevalence and severity of periodontal diseases are higher in patients with diabetes compared to healthy individuals (Grossi et al. 1997; Poplawska-Kita et al. 2014). Patients with diabetes are at a three-fold increased risk of developing periodontitis compared to non-diabetic patients (Mealey and Oates 2006). In the current study, when analysing pre-treatment periodontal data, no significant differences were found for mGI scores, mean PD, mean recession, mean LOA and % BOP between patients with T1DM and periodontitis and non-T1DM patients with periodontitis. However T1DM patients with gingivitis and periodontitis had significantly higher amounts of plaque (0.94±0.40 and 0.98±0.54, respectively) compared to the non-T1DM patients with gingivitis and periodontitis (0.66±0.23 and 0.66±0.29, respectively). Although not statistically significant, T1DM patients with healthy tissues had higher amounts of plaque compared to the non-T1DM patients with healthy tissues. Our findings are in agreement with previous research which showed significantly higher plaque scores in T1DM patients compared to non-diabetic controls (Novaes et al. 1991; Aren et al. 2003; Lalla et al. 2006a). The significantly higher amounts of plaque in diabetic patients can be explained by the fact that excessive glucose in diabetes enters the oral cavity via saliva and GCF, the sugar-rich biofilm which forms will then, in general, enhance plaque accumulation. The lack of understanding and knowledge about oral hygiene and maintenance of optimal oral health in patients with diabetes, may be factors related to the higher plaque scores seen in these patients (Hugoson et al. 1989). Our findings are in contrast to previous studies which found similar levels of plaque in patients with and without T1DM (Bay et al. 1974; Bernick et al. 1975; Hugoson et al. 1989; Sandholm et al. 1989a; de Pommereau et al. 1992; Firatli et al. 1996; Firatli 1997; Tervonen and Karjalainen 1997; Lalla et al. 2006b). The poorer oral hygiene found in T1DM patients compared to non-diabetic patients could also be due to the greater attention and importance placed by T1DM patients in maintaining their systemic health with daily doses of insulin, as opposed to maintaining optimal oral hygiene, which was found to be superior in the non-diabetic patients. A previous study assessed the attitudes and awareness of the risk for periodontal disease in patients with diabetes (n=101) and highlighted that only 33% of the patients were aware of their increased risk for periodontal disease, 43% had attended a dentist within the past year, 34% had not attended a dentist for >5 years, 37% of the patients attended the dentist for treatment once a year, while 63% attended only when they had dental problems (Allen et al. 2008). In the present study, while considering patient care pathways within diabetes management, a majority (94.7%) of the T1DM patients had received

examination of their eyes and feet and were educated on the importance of routine examinations for the betterment of their condition within the past 12 months. This clearly demonstrates that a robust patient care pathway does exist for screening of diabetic complications. Unfortunately, the same is not true for screening of oral complications of T1DM, with as many as 1/3 of the T1DM patients in this study reporting not being examined by a dentist in the past 12 months. Hence, an opportunity to regularly screen for oral complications in this disease-susceptible population is clearly being lost. This finding could also possibly explain the poorer oral hygiene experienced by T1DM patients compared to non-diabetic patients and needs to be addressed and included in the overall management of patients with diabetes.

It is extremely rare that periodontal disease would affect all parts of the periodontium equally, and measuring mean PD solely provides a crude description of the PD found in each patient. Hence, the utilization of reporting mean PD, without the % of sites with advanced periodontal disease, can be seen as a limitation of studies carried out in this research field. In the current study, based on severity of periodontal disease at pre-treatment, the % of PD sites ≥5 mm was significantly lower in patients with T1DM and periodontitis (14.7±46.4 %) compared to non-T1DM patients with periodontitis (23.7±15.5 %), indicating that more severe periodontal disease was present in non-T1DM patients compared to T1DM patients. This finding likely reflects the differences in the recruitment strategy utilized for both patient groups. The T1DM patients were recruited from medical databases of T1DM patients held in both primary and secondary care settings, whereas the non-T1DM patients were recruited from diagnostic or student treatment clinics within the School of Dental Sciences, Newcastle University who were referred in for periodontal diagnosis and care by their general dental practice. Although both groups were matched for periodontal diagnosis, the extent of their periodontal disease was not considered in the process. This is a limitation in the current study, and highlights a need in future studies to stratify periodontal case selection based on the extent and severity of periodontal disease to establish a more meaningful and robust matching of groups by periodontal status for the selected patients. With reference to the amount of gingival inflammation present, in the current study, interestingly the T1DM patients with healthy periodontal tissues had significantly higher % BOP (9.88±5.67 %) compared to the non-T1DM patients with healthy periodontal tissues (0.83±1.17 %). Our findings support data from previous studies that demonstrated significantly higher levels of gingival inflammation in patients with diabetes compared to non-diabetic controls (Aren et al.

2003; Dakovic and Pavlovic 2008). The possible explanation for the increase in gingival inflammation seen in patients with T1DM could be a manifestation of the upregulated diabetes-related systemic inflammation which manifests itself even in T1DM patients with healthy tissues. The similar pattern of significantly higher levels of gingival inflammation in T1DM patients compared to non-diabetic patients was not replicated in those with gingivitis and periodontitis. It is not unreasonable to conclude that the more severe periodontal disease present in non-T1DM patients compared to patients with T1DM may have masked the presence of greater background level of gingival tissue inflammation in T1DM patients with gingivitis and periodontitis.

Patients with T1DM have been found to have a good response to appropriate periodontal therapy and their response to therapy is similar to that seen in non-diabetic controls (Westfelt et al. 1996; Christgau et al. 1998). In the current study, following NSM both T1DM and non-T1DM patients demonstrated significant improvements in PI, mGI, mean PD, mean LOA and % BOP at months 3 and 6 following NSM. Compared to pre-treatment, a statistically significant increase in mean recession was seen in T1DM patients at month 6 and in non-T1DM patients at months 3 and 6 following NSM. A significant increase in mean recession following periodontal therapy indicates a resolution in inflammation and improvement in the periodontal condition. Both T1DM and non-T1DM patients also showed significant reductions in % of PD sites ≥5 mm at months 3 and 6 following NSM, and taking into account the pre-treatment differences in % of PD sites ≥5 mm, the differences between T1DM and non-T1DM patients with periodontitis at months 3 and 6 following NSM were not statistically significant. Our findings indicate a reduction in the severity of periodontal disease, and the lack of differences between the two groups following NSM indicates an improvement in the periodontal status of both patient groups. The significantly higher % of PD sites reducing by ≥2 mm seen in non-T1DM patients most probably reflects the greater pre-treatment severity of periodontal disease seen in the non-T1DM patients. The results of the current study confirm that NSM was successful in both patient groups, with significant reductions in inflammation and severity of periodontal disease following treatment. Our findings are similar to those of other studies in T1DM patients which showed significant improvements in periodontal parameters following periodontal therapy (Bay et al. 1974; Seppala and Ainamo 1994; Smith et al. 1996; Westfelt et al. 1996; Christgau et al. 1998; Martorelli de Lima et al. 2004; Llambes et al. 2005). Additionally, in the present study both T1DM and non-T1DM patients responded similarly to the periodontal treatment provided,

and our findings are similar to those of other studies which found that patients with T1DM had a good response to periodontal treatment, and their short- and long-term response to treatment was similar to that seen in non-diabetic patients (Bay et al. 1974; Westfelt et al. 1996; Christgau et al. 1998).

While considering biomarker levels in serum, in the present study, significantly higher pre-treatment serum MMP-9, resistin and ENA-78/CXCL5 levels were detected in patients with T1DM compared to non-T1DM patients, and significantly higher serum MMP-9 levels were found in T1DM patients with periodontitis compared to non-T1DM patients with periodontitis. Although higher serum levels of resistin and ENA-78/CXCL5 were seen in T1DM patients with periodontitis compared to non-T1DM patients with periodontitis, these differences were not statistically significant. Significantly higher serum resistin and ENA-78/CXCL5 levels were seen in T1DM patients with gingivitis compared to non-T1DM patients with gingivitis, and although higher serum MMP-9 levels were seen in T1DM patients with gingivitis compared to non-T1DM with gingivitis, this difference was not statistically significant. It is interesting to note that, although not statistically significant, serum MMP-9, resistin and ENA-78/CXCL5 levels were higher in the T1DM patients with healthy periodontal tissues compared to then non-T1DM patients with healthy tissues. To the best of our knowledge, this is the first study to analyse levels of MMP-9 in serum in T1DM patients with periodontal disease, hence it has not been possible to make comparisons with findings of other studies. It is a key finding in this study that despite non-T1DM patients having more severe periodontal disease compared to the T1DM patients (indicated by % of PD sites ≥5 mm), pre-treatment serum MMP-9 levels were significantly higher in T1DM patients with periodontitis compared to non-T1DM patients with periodontitis. The possible explanation for this could be that patients with T1DM have elevated circulating MMP-9 levels (Maxwell et al. 2001). Diabetes-associated pathophysiological processes such as oxidative stress could potentially enhance MMP-9 activity and production (Uemura et al. 2001). MMP-9 has proved to be a useful biomarker in serum in T1DM patients at a risk of progression of other diabetes-related complications, and chronic kidney disease (Gharagozlian et al. 2009), and serum MMP-9 levels possibly contribute to the inflammatory process inherent to diabetic retinopathy in T1DM patients (Maxwell et al. 2001; Jacqueminet et al. 2006). Studies involving T2DM patients have shown elevated serum MMP-9 levels in T2DM patients with coronary artery disease and premature atherosclerosis (Noji et al. 2001), and elevated circulating MMP-9 levels have been found in T2DM hypertensive patients

compared to normotensive patients (Tayebjee et al. 2004). Additionally, elevated serum MMP-9 levels occur prior to the onset of T2DM-associated renal complications (Ebihara et al. 1998). In the present study, patients with T1DM and periodontitis having significantly elevated serum MMP-9 levels compared to the non-diabetic patients with periodontitis is a key finding, and from the review of literature and the findings of this study, one can conclude that in the absence of more severe periodontal disease, the significant increase in serum MMP-9 levels in T1DM patients with periodontitis could likely be due to an increase in diabetes-related inflammation routinely observed in patients with T1DM. Our findings related to serum ENA-78/CXCL5 are similar to those of a recent study which reported significantly higher plasma ENA-78/CXCL5 in T1DM patients with healthy periodontal tissues compared to non-diabetic patients with healthy tissues (Lappin et al. 2015).

Following successful periodontal treatment, in the current study, compared to pre-treatment levels in both T1DM and non-T1DM patients, serum resistin levels reduced at month 6, however this reduction was statistically significant only in the non-T1DM patients. To the best of our knowledge, this is the first study to investigate the effect of periodontal treatment on resistin levels in serum in patients with T1DM. A study investigating the impact of periodontal therapy with adjunctive antibiotics in T2DM patients with periodontitis reported that despite significant improvements in clinical periodontal parameters and HbA1c levels in these patients, no statistically significant reduction in serum resistin levels was observed following treatment (Bharti et al. 2013). Similarly, a study in systemically healthy patients with periodontitis reported that despite significant improvements in clinical periodontal parameters, no statistically significant reduction was seen in serum resistin levels following periodontal treatment (Devanoorkar et al. 2012). A similar effect has been observed in the present study. On performing correlations, serum resistin levels were significantly correlated with BMI only in patients with T1DM and not in the non-diabetic patients. Therefore, resistin may not be an ideal biomarker in studying the link between T1DM and periodontal disease as obesity is not a cardinal finding in T1DM patients. Due to the limited volume of GCF samples available and considering our findings of resistin levels in serum, it was decided not to further analyse this biomarker in the GCF samples.

In the current study, compared to pre-treatment, both T1DM and non-T1DM patients with periodontitis showed non-significant reductions in serum ENA-78/CXCL5 levels at month 6 following NSM. To the best of our knowledge, this is the first study to investigate the effect

of periodontal therapy on levels of ENA-78/CXCL5 in serum in periodontitis patients with and without T1DM. As ENA-78/CXCL5 was only detected in serum samples of the study and not in GCF, this possibly suggests that this chemokine may potentially be a useful systemic indicator to study the effect of diabetes on periodontal status. It may be useful in future studies to ensure larger sample sizes to investigate the effects of periodontal therapy further in patients with and without T1DM.

In the current study, compared to pre-treatment, both T1DM and non-T1DM patients with periodontitis showed a reduction in serum MMP-9 levels at 3 and 6 months following NSM, however these differences were not statistically significant. It may be useful in future studies to ensure larger sample sizes to investigate the effects of periodontal therapy further in patients with and without T1DM. The reduction in serum MMP-9 levels in both patient groups following NSM could possibly be a reflection of the significant improvement in clinical periodontal status and reduction in inflammation and severity of periodontal disease seen in both groups. A study of systemically healthy patients with periodontitis showed a significant reduction in circulating plasma MMP-9 concentration and proteolytic activity 3 months following effective NSM (Marcaccini et al. 2009b). To the best of our knowledge, the current study is the first study to evaluate the effect of periodontal treatment on MMP-9 levels in serum in patients with T1DM and periodontitis. It is worth noting that post-treatment serum MMP-9 levels in T1DM and non-T1DM patients with periodontitis (732.7±448.8 ng/ml and 472.9±259.9 ng/ml, respectively) were comparable to the pre-treatment serum MMP-9 levels recorded in T1DM and non-T1DM patients with healthy periodontal tissues (791.3±475.6 ng/ml and 437.5±233.0 ng/ml, respectively). These findings could potentially suggest that a resolution in periodontal inflammation leads to a reduction in the burden of circulating inflammatory biomarker MMP-9 to the level observed in periodontal health.

While considering biomarker levels in GCF, the present study revealed higher pre-treatment GCF MMP-9 and IL-8 levels in patients with T1DM compared to non-T1DM patients; however these differences were not statistically significant. To the best of our knowledge this is the first study to analyse MMP-9 and IL-8 levels in GCF in patients with T1DM and periodontal disease, hence it has not been possible to make comparisons with findings of other studies. A previous study in T2DM patients unexpectedly reported significantly lower GCF IL-8 levels in T2DM patients with periodontitis compared to non-diabetic patients with

periodontitis (Engebretson et al. 2006). Other T2DM studies reported no statistically significant differences in expression of IL-8 in gingival tissues (Duarte et al. 2007) and IL-8 levels in serum (Longo et al. 2014) in periodontitis patients with and without T2DM. In the current study, significantly higher GCF MMP-9 and IL-8 levels were detected in T1DM patients with healthy periodontal tissues compared to non-T1DM patients with healthy tissues. The elevated inflammatory biomarker levels present in T1DM patients compared to non-diabetic patients is a key finding as it signifies that even in the absence of inflammatory periodontal disease, the manifestation of diabetes-related inflammation was presented in the GCF of patients with T1DM. The pre-treatment biomarker findings in GCF were in accordance with the pre-treatment clinical periodontal findings, in which significantly higher levels of gingival inflammation (indicated by % BOP) was present in T1DM patients with healthy periodontal tissues compared to non-T1DM patients with healthy tissues. Also, no statistically significant differences in GCF MMP-9 and IL-8 levels were found between T1DM and non-T1DM patients with gingivitis and periodontitis. It is not unreasonable to presume that the more severe periodontal disease (indicated by higher % of PD sites ≥5 mm) in the non-T1DM patients compared to T1DM patients, may have masked the presence of greater background levels of GCF MMP-9 and IL-8 in T1DM patients with gingivitis and periodontitis. Our findings related to GCF IL-8 levels are in agreement with a previous study which found significantly elevated salivary IL-8 levels in T1DM patients with healthy tissues compared to non-diabetic controls, and no statistically significant differences in salivary IL-8 levels between periodontitis patients with and without T1DM (Dakovic et al. 2013). The findings of this study are in contrast to those of a previous study which found significantly higher plasma IL-8 levels in patients with T1DM and periodontitis compared to non-diabetic patients with periodontitis (Lappin et al. 2011). Currently, research related to GCF biomarker levels in patients with T1DM and periodontal disease is extremely limited and no studies have evaluated biomarker levels based on periodontal diagnosis as was considered in this study. An experimental gingivitis study reported elevated GCF MMP-9 levels in both T1DM and non-diabetic patients, with significantly higher levels seen in T1DM patients with gingivitis compared to non-diabetic patients with gingivitis at the end of the study (Salvi et al. 2010).

In the current study, within the T1DM and non-T1DM group, significantly higher GCF MMP-9 levels were seen in periodontitis patients compared to those with gingivitis and healthy periodontal tissues. These findings are in accordance with the clinical periodontal

findings of the study which showed significantly greater gingival inflammation (indicated by % BOP), mean PD and severity of periodontitis disease in patients with periodontitis with and without T1DM compared to those with gingivitis and healthy periodontal tissues. A previous study reported significantly elevated MMP-9 levels in GCF, saliva and mouth rinse samples in periodontitis patients compared to those with healthy tissues (Makela et al. 1994). The results of the non-diabetic patients of the present study are in contrast with other studies which reported no significant differences in GCF MMP-9 levels while comparing patients with periodontitis, gingivitis and healthy periodontal tissues (Maeso et al. 2007), and while comparing patients with periodontitis and healthy periodontal tissues (Marcaccini et al. 2010). In the current study, within the T1DM and non-T1DM group, higher GCF IL-8 levels were seen in periodontitis patients compared to those with gingivitis and healthy periodontal tissues; however these differences were statistically significant only in the non-T1DM patients. The results of the non-T1DM patients are in agreement with other studies of systemically healthy individuals which reported significantly elevated IL-8 levels in GCF (Tsai et al. 1995), plasma (Lappin et al. 2011) and serum (Li et al. 2012) in periodontitis patients compared to those with healthy tissues, and in contrast to results of a study which reported that despite significantly greater periodontal disease severity in patients with periodontitis, patients with healthy periodontal tissues had significantly higher GCF IL-8 levels compared to those with periodontitis (Chung et al. 1997).

Following periodontal therapy, in the current study T1DM patients with periodontitis showed significant reductions in GCF MMP-9 and IL-8 levels at months 3 and 6 following NSM. Similarly in non-T1DM patients there was a reduction in GCF levels of MMP-9 and IL-8 at months 3 and 6 following NSM; however this was statistically significant for GCF MMP-9 only at month 6 and for GCF IL-8 only at month 3. Overall, in both T1DM and non-T1DM patients, reductions in GCF levels of these two pro-inflammatory biomarkers appeared to mirror the improvement in clinical periodontal status following NSM. The difficulty in comparing results of previous studies to the present study lies in the heterogeneity in methodologies used, and is a possible explanation for the variations in GCF biomarker levels seen in the published literature and the present study. The differences in results could arise from variations in case definitions of periodontitis, analytical techniques, GCF sampling methods, techniques used for eluting GCF and storage of samples. A review of past literature tells us that there is also a lack of consistency in the reporting of GCF data as some studies report biomarker concentration levels, while others report total biomarker levels and some

authors report both total and concentration levels of the biomarkers in GCF. All these factors collectively have increased inter-study variations in GCF biomarker levels, preventing clear conclusions from being made regarding the role biomarkers in GCF play in patients with T1DM and periodontal disease.

To the best of our knowledge, this is the first study to investigate in T1DM patients the effect of periodontal treatment on MMP-9 and IL-8 levels in GCF, and from the results of this study it can be concluded that MMP-9 and IL-8 are potentially good local biomarkers to determine severity of periodontal disease and the benefit effective periodontal treatment has in patients with T1DM. The lack of significant differences between inflammatory biomarkers in serum and GCF between T1DM and non-T1DM patients with periodontitis following NSM could possibly be due to the lack of significant differences between the two groups with regards to clinical periodontal status, as both groups were found to have no statistically significant differences in periodontal measurements and severity of periodontal disease following treatment.

A positive correlation between severity of periodontal disease and glycaemic control has been reported in literature (Tanwir and Tariq 2012; Costa et al. 2013). In the current study, the only positive significant correlation was found between PI score and HbA1c levels in all patients. No statistically significant correlations were found between HbA1c and other clinical periodontal parameters. While considering HbA1c levels and serum biomarker levels, a significant positive correlation was found between HbA1c and serum MMP-9 and ENA-78/CXCL5 levels in all patients, possibly suggesting that an increase in HbA1c levels is associated with an increase in the inflammatory burden in the serum, irrespective of diabetes status. A significant positive correlation was also found between hsCRP and serum MMP-9 levels in all patients and in patients with T1DM. This may indicate that MMP-9 may reflect the chronic inflammatory state which is typical of periodontitis and is indicative of the association between hsCRP and serum MMP-9 levels particularly in patients with T1DM. It is meaningful to note that serum biomarker levels did not statistically significantly correlate with clinical periodontal indices, whereas GCF MMP-9 and IL-8 levels showed a statistically significant positive correlation with clinical periodontal parameters. The findings of the current study related to GCF biomarker levels indicate, in broad terms, that an increase in periodontal disease leads to elevated levels of MMP-9 and IL-8 in GCF. However, a similar association was not found for serum biomarker levels and periodontal parameters. It would

be reasonable to suggest that GCF is a good local indicator for periodontal inflammatory status due to its local production within the periodontal tissues. Furthermore, the fact that serum MMP-9 levels significantly correlated with hsCRP levels implies that serum is potentially a good systemic indicator for the inflammatory disease status in patients with periodontal disease and T1DM.

With regards to QoL in patients with T1DM and periodontal disease, this study assessed QoL in patients with T1DM using two routinely used, validated questionnaires, the W-BQ12 and the ADDQoL-19. The pre-treatment analyses of the W-BQ12 revealed no statistically significant differences in any subscale scores (negative, energy and positive well-being) and overall general well-being score when comparing the T1DM patients based on periodontal diagnosis. Our findings suggest that despite having statistically significant differences in severity of periodontal disease, patients with healthy tissues, gingivitis and periodontitis had a similar perception of the effect their diabetes and periodontal condition had on their QoL. Analysis of the ADDQoL-19 questionnaire revealed that all T1DM patients experienced an overall “good to very good” general QoL, however while considering their diabetes status the patients felt that their QoL would have been “a little better or much better” if they did not have diabetes. Our findings captured the negative impact of T1DM on QoL in this study group. Based on periodontal diagnosis, no statistically significant differences were found for general and diabetes-specific overview items and overall ADDQoL-19 score suggesting that patients with healthy tissues, gingivitis and periodontitis had a similar perception of the effect their diabetes and periodontal condition had on their QoL. Our findings of the W-BQ12 and ADDQoL-19 are similar to those of a recent study investigating the impact of periodontal status and treatment on OHRQoL in patients with and without T2DM utilizing the OHIP-49 questionnaire (Irani et al. 2015). The OHIP-49 contains oral health-related questions, however despite this there was a lack of significant differences in OHIP-49 scores among T2DM patients with periodontitis, gingivitis and healthy tissues. The authors reported that this could possibly indicate that diabetic patients are less concerned about their oral health than they are about other health problems that they have to manage as part of their diabetes. However, within their non-diabetic group, patients with periodontitis and gingivitis had poorer OHRQoL compared to those with periodontal health (Irani et al. 2015). Potentially, systemically healthy patients might be more concerned about the signs and symptoms of periodontitis than diabetic patients who have to address other pressing health issues, and this might lead to lower expectations of oral health or better coping with the impact of

periodontitis (Irani et al. 2015). In the current study, interpreting the ADDQoL-19 scores further (based on quartiles) revealed that a majority (73.7%) of the periodontitis patients experienced a poorer QoL, while a majority of the gingivitis (75%) and healthy periodontal tissue (77.8%) patients experienced a better QoL. Our findings demonstrate that poorer periodontal health has a negative impact on QoL in people with T1DM.

Analysis of post-treatment W-BQ12 scores in T1DM patients with periodontitis revealed a significant improvement in QoL at month 3 following NSM, suggesting that successful NSM has a short-term positive impact by improving QoL in diabetic patients with periodontitis. Our findings are in agreement to those of previous studies which reported that effective NSM improves QoL (Aslund et al. 2008; Jowett et al. 2009; Saito et al. 2010; Shanbhag et al. 2012). However, compared to pre-treatment, no statistically significant differences were found in the W-BQ12 scores at month 6 following NSM, suggesting a possible initial improvement in QoL following the first periodontal treatment appointment, which stabilised during the course of the study and was not evidently identified at month 6. Analysis of the ADDQoL-19 scores at months 3 and 6 in T1DM patients with periodontitis revealed no statistically significant changes from pre- to post-treatment. Interestingly, interpreting scores based on quartiles revealed that compared to pre-treatment, where as 73.7% of the 19 T1DM patients with periodontitis reported a poorer QoL, only 20% of the 10 T1DM patients with periodontitis reported a poorer QoL at months 3 and 6 following NSM. Our findings could possibly suggest that effective periodontal treatment has the potential to change an individual's negative perception of QoL to positive one. In all groups of periodontal diagnosis, T1DM patients placed great importance and impact on their "freedom to eat and drink", these findings are consistent with those of previous studies which reported the greatest negative impact of diabetes is on "freedom to eat" (Bradley and Speight 2002; Trief et al. 2003; Costa et al. 2006; Holmanova and Ziakova 2009; Turk et al. 2013), which confirms the strong influence of dietary restrictions on QoL in patients with diabetes. Our findings are also similar to a previous study in patients with T2DM (aged ≥65 years) which utilised the ADDQoL-19 and highlighted that the greatest impact of diabetes on QoL was on their freedom to eat, family life and dependence on others (Turk et al. 2013). Patients with T1DM need to prioritise their dietary intake in order to prevent diabetes-related complications (Daneman 2006). Their daily diet is usually regulated by the intake of their medications and the time of day, and hence the current study also found that "freedom to eat and drink" had a major impact and importance on the daily lives in patients with T1DM.

In the current study, analysing QoL in patients with T1DM did reveal a number of meaningful findings, however QoL was not assessed in the non-diabetic patients, hence it was not possible to make comparisons between the two groups. Unfortunately, this must be regarded as a limitation in this study. The W-BQ12 is a general health assessment questionnaire and the ADDQoL-19 is a diabetes-specific questionnaire, specifically designed to evaluate QoL in patients with diabetes, and both questionnaires contain limited oral health-related questions. Although T1DM patients with periodontitis showed statistically significant improvements in clinical periodontal parameters following NSM, these questionnaires were unable to capture the positive outcomes of this treatment. Therefore, although these measures did provide some meaningful results, they were unable to capture to a full extent the effect of periodontal disease and the positive impact of treatment on QoL. It would be beneficial in future studies to assess QoL in both diabetic and non-diabetic patients simultaneously, utilising both generic questionnaires (for diabetic and non-diabetic patients) and diabetes-specific questionnaires (for diabetic patients only) in order to provide a robust assessment of oral health and systemic health-related QoL in patients with and without diabetes.

In conclusion, the findings of the present study contribute to the knowledge of the clinical and biological links between T1DM and periodontal disease and the response to periodontal therapy in both T1DM and non-diabetic patients. Furthermore, the present study highlights the importance of analysing pro-inflammatory biomarkers in both diabetes and periodontal disease, and more specifically, the pro-inflammatory enzyme MMP-9 and chemokines ENA-78/CXCL5 and IL-8 which show potential as contributors to the inflammatory mechanisms linking diabetes and periodontal disease, with a consideration of MMP-9 in serum and GCF, ENA-78/CXCL5 in serum and IL-8 in GCF as prognostic markers for periodontitis in patients with and without diabetes. Although not statistically significant, it is encouraging that a reduction in HbA1c levels was observed following successful periodontal treatment. Additional research is warranted to investigate these findings further, ensuring that the T1DM and non-diabetic groups are well matched, especially with regards to periodontal status.

8 Chapter 8. References

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9 Chapter 9. Appendices

9.1 *Appendix A: The W-BQ12*

Well-Being Questionnaire (W-BQ12)

Please circle one number on each scale, from 3 (all the time) to 0 (not at all), to indicate how often you feel each statement has applied to you in the past few weeks.

	all the time			not at all
1. I have crying spells or feel like it	3	2	1	0
2. I feel downhearted and blue	3	2	1	0
3. I feel afraid for no reason at all	3	2	1	0
4. I get upset easily or feel panicky	3	2	1	0
5. I feel energetic, active or vigorous	3	2	1	0
6. I feel dull or sluggish	3	2	1	0
7. I feel tired, worn out, used up or exhausted	3	2	1	0
8. I have been waking up feeling fresh and rested	3	2	1	0
9. I have been happy, satisfied, or pleased with my personal life	3	2	1	0
10. I have lived the kind of life I wanted to	3	2	1	0
11. I have felt eager to tackle my daily tasks or make new decisions	3	2	1	0
12. I have felt I could easily handle or cope with any serious problem or major change in my life	3	2	1	0

Please make sure that you have considered each of the 12 statements and have circled one number in response to each statement.

W-BQ12 © Prof Clare Bradley 6/96. Standard UK English (instructions rev. 31.1.02)
Health Psychology Research, Dept of Psychology, Royal Holloway, University of London, Egham, Surrey, TW20 0EX, UK.

9.2 *Appendix B: The ADDQoL-19*

ADDQoL

This questionnaire asks about your quality of life – in other words how good or bad you feel your life to be.

Please put an "X" in the box that best indicates your response for each item.

What we would like to know is how you feel about your life now.

I) In general, my present quality of life is:

☐	☐	☐	☐	☐	☐	☐
excellent	very good	good	neither good nor bad	bad	very bad	extremely bad

Now we would like to know how your quality of life is affected by your diabetes, its management and any complications you may have.

II) If I did *not* have diabetes, my quality of life would be:

☐	☐	☐	☐	☐
very much better	much better	a little better	the same	worse

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Please respond to the more specific questions on the following pages. For each aspect of life described:

For Part (a):	put an "X" in one box to show how diabetes affects this aspect of your life;
For Part (b):	put an "X" in one box to show how important this aspect of your life is to your quality of life.

1 (a)	**If I did *not* have diabetes, I would enjoy my leisure activities:**				
	☐ very much more	☐ much more	☐ a little more	☐ the same	☐ less
(b)	**My leisure activities are:**				
	☐ very important	☐ important	☐ somewhat important	☐ not at all important	

2	**Are you currently working, looking for work or would you like to work?**				
	Yes ☐ If *yes*, complete (a) and (b).				
	No ☐ If *no*, go straight to Question 3.				
(a)	**If I did *not* have diabetes, my working life would be:**				
	☐ very much better	☐ much better	☐ a little better	☐ the same	☐ worse
(b)	**For me, having a working life is:**				
	☐ very important	☐ important	☐ somewhat important	☐ not at all important	

3 (a)	**If I did *not* have diabetes, local or long distance journeys would be:**				
	☐ very much easier	☐ much easier	☐ a little easier	☐ the same	☐ more difficult
(b)	**For me, local or long distance journeys are:**				
	☐ very important	☐ important	☐ somewhat important	☐ not at all important	

4	Do you ever go on holiday or want to go on holiday? Yes ☐ If *yes*, complete (a) and (b). No ☐ If *no*, go straight to Question 5.				
(a)	**If I did *not* have diabetes, my holidays would be:**				
	☐ very much better	☐ much better	☐ a little better	☐ the same	☐ worse
(b)	**For me, holidays are:**				
	☐ very important	☐ important	☐ somewhat important	☐ not at all important	

5 (a)	**If I did *not* have diabetes, physically I could do:**				
	☐ very much more	☐ much more	☐ a little more	☐ the same	☐ less
(b)	**For me, how much I can do physically is:**				
	☐ very important	☐ important	☐ somewhat important	☐ not at all important	

6	Do you have family / relatives? Yes ☐ If *yes*, complete (a) and (b). No ☐ If *no*, go straight to Question 7.				
(a)	**If I did *not* have diabetes, my family life would be**				
	☐ very much better	☐ much better	☐ a little better	☐ the same	☐ worse
(b)	**My family life is:**				
	☐ very important	☐ important	☐ somewhat important	☐ not at all important	

7 (a)	**If I did *not* have diabetes, my friendships and social life would be:**				
	☐ very much better	☐ much better	☐ a little better	☐ the same	☐ worse
(b)	**My friendships and social life are:**				
	☐ very important	☐ important	☐ somewhat important	☐ not at all important	

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8	**Do you have or would you like to have a close personal relationship?**				
	Yes ☐ If *yes*, complete (a) and (b).				
	No ☐ If *no*, go straight to Question 9.				
(a)	**If I did *not* have diabetes, my closest personal relationship would be:**				
	☐ very much better	☐ much better	☐ a little better	☐ the same	☐ worse
(b)	**For me, having a close personal relationship is:**				
	☐ very important	☐ important	☐ somewhat important	☐ not at all important	

9	**Do you have or would you like to have a sex life?**				
	Yes ☐ If *yes*, complete (a) and (b).				
	No ☐ If *no*, go straight to Question 10.				
(a)	**If I did *not* have diabetes, my sex life would be:**				
	☐ very much better	☐ much better	☐ a little better	☐ the same	☐ worse
(b)	**For me, having a sex life is:**				
	☐ very important	☐ important	☐ somewhat important	☐ not at all important	

10 (a)	**If I did *not* have diabetes, my physical appearance would be:**				
	☐ very much better	☐ much better	☐ a little better	☐ the same	☐ worse
(b)	**My physical appearance is:**				
	☐ very important	☐ important	☐ somewhat important	☐ not at all important	

11 (a)	**If I did *not* have diabetes, my self-confidence would be:**				
	☐ very much greater	☐ much greater	☐ a little greater	☐ the same	☐ less
(b)	**My self-confidence is:**				
	☐ very important	☐ important	☐ somewhat important	☐ not at all important	

12 (a)	If I did *not* have diabetes, my motivation would be:				
	☐ very much greater	☐ much greater	☐ a little greater	☐ the same	☐ less
(b)	**My motivation is:**				
	☐ very important	☐ important	☐ somewhat important	☐ not at all important	

13 (a)	If I did *not* have diabetes, the way people in general react to me would be:				
	☐ very much better	☐ much better	☐ a little better	☐ the same	☐ worse
(b)	**The way people in general react to me is:**				
	☐ very important	☐ important	☐ somewhat important	☐ not at all important	

14 (a)	If I did *not* have diabetes, my feelings about the future (e.g. worries, hopes) would be:				
	☐ very much better	☐ much better	☐ a little better	☐ the same	☐ worse
(b)	**My feelings about the future are:**				
	☐ very important	☐ important	☐ somewhat important	☐ not at all important	

15 (a)	If I did *not* have diabetes, my financial situation would be:				
	☐ very much better	☐ much better	☐ a little better	☐ the same	☐ worse
(b)	**My financial situation is:**				
	☐ very important	☐ important	☐ somewhat important	☐ not at all important	

16 (a)	If I did *not* have diabetes, my living conditions would be:				
	☐ very much better	☐ much better	☐ a little better	☐ the same	☐ worse
(b)	**My living conditions are:**				
	☐ very important	☐ important	☐ somewhat important	☐ not at all important	

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17 (a)	If I did *not* have diabetes, I would have to depend on others when I do not want to: ☐ very much less ☐ much less ☐ a little less ☐ the same ☐ more
(b)	For me, not having to depend on others is: ☐ very important ☐ important ☐ somewhat important ☐ not at all important

18 (a)	If I did *not* have diabetes, my freedom to eat as I wish would be: ☐ very much greater ☐ much greater ☐ a little greater ☐ the same ☐ less
(b)	My freedom to eat as I wish is: ☐ very important ☐ important ☐ somewhat important ☐ not at all important

19 (a)	If I did *not* have diabetes, my freedom to drink as I wish (e.g. fruit juice, alcohol, sweetened hot and cold drinks) would be: ☐ very much greater ☐ much greater ☐ a little greater ☐ the same ☐ less
(b)	My freedom to drink as I wish is: ☐ very important ☐ important ☐ somewhat important ☐ not at all important

If there are any other ways in which diabetes, its management and any complications affect your quality of life, please say what they are below:

Thank you for completing this questionnaire.

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9.3 Appendix C: Related publications

Publication of Master's in Clinical Dentistry (MClinDent) in Restorative Dentistry, Newcastle University research study: Desai R, Durham J, Wassell RW, Preshaw PM. 2014. Does the mode of administration of the Oral Health Impact Profile-49 affect the outcome score? Journal of Dentistry 42(1):84-89.

Publication of abstract and oral presentation at the British Society for Oral and Dental Research (BSODR) conference, in Cardiff, UK September 2015, titled:
"Impact of type-1 diabetes and periodontal status on life quality".
Co-authors: Prof. Philip Preshaw, Dr. John Taylor and Dr. Giles McCracken.

Publication of abstract at International Association for Dental Research (IADR) conference, in Seoul, Korea June 2016, titled:
"Inflammatory mechanism linking type-1 diabetes and periodontal disease".
Co-authors: Prof. Philip Preshaw, Dr. John Taylor and Dr. Giles McCracken.

9.4 Appendix D: Personal achievement

I have successfully completed the Overseas Registration Examination parts 1 and 2 and registered with the General Dental Council, UK.