The frequency and severity of periodontal disease in Australians with type-2 diabetes mellitus and diabetic complication: A pilot study.

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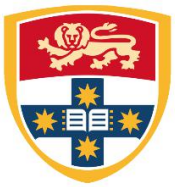
A thesis submitted to fulfill requirements for the degree of
Master of Philosophy



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Declaration

This is to certify that to the best of my knowledge the content of this thesis is my own work. This thesis has not been submitted for any degree or other purposes. I solemnly certify that the intellectual content of this thesis is the product of my own work and that all the assistance received in preparing this thesis and sources have been acknowledged. I certify that if my candidature is successful, this thesis will be lodged with the Director of University Libraries and made available for immediate use.

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For this publication, I designed the study, conducted the search, analyzed the data and wrote the drafts of the manuscript.

Nguyen Thi Mai Anh

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List of abbreviations:

Aa: *Aggregatibacter Actinomycetemcomitans*

AAP: American Association of Periodontology

ADA: American Diabetics Association

ADS: Australia Diabetes Society

AGEs: advanced glycation end-products

BOP: bleeding on probing

CAL: clinical attachment loss

CDC: Centre for Disease Control and Prevention

CEJ: cemento-enamel junction

CPITN: Community Periodontal Index of Treatment Needs

CrVD: cerebrovascular disease

CVD: cardiovascular disease

DM: diabetes mellitus

eGFR: estimated glomerular filtration rate

FBC: full blood count

HbA1c: hemoglobin A1c

HDL: high density lipoprotein

hsCRP: high-sensitivity C-reactive protein

OR: odd ratio

Pg: Porphyromonas gingivalis

PPD: probing pocket depth

PVD: peripheral vascular disease

ROS: reactive oxygen species

RPAH: Royal Prince Alfred Hospital

T2DM: type-2 diabetes mellitus

Td: Treponema denticola

Tf: Tannerella forsythia

WHO: World Health Organization

Abstract

Background: Periodontal diseases are chronic inflammatory processes affecting the tooth-supporting tissues in response to bacterial accumulation and are the main cause of tooth loss. Chronic oral infections and their consequent inflammatory responses have been demonstrated to have negative effects on multiple systemic disease, including type-2 diabetes mellitus.

Aim: The aim of the systematic review in Part B was to investigate the association between periodontal disease and diabetic complications. The study project in Part C aimed to record the frequency and severity of periodontal disease (gingivitis and periodontitis) in patients with diabetes, diabetic complications or high HbA1c levels. Another aim was to validate a diagnostic tool for the screening of periodontal disease by non-dental health care practitioners and evaluate a referral pathway between diabetes clinics and dental clinics.

Method: To conduct the systematic review, PubMed/MEDLINE was searched. For the research project, women and men aged 18 years to 75 years, with a proven diagnosis of type 2 diabetes were recruited from the Royal Prince Alfred Hospital Diabetes Centre. Consented participants then went through a structured procedures to have their periodontal conditions assessed, biological samples collected, and their intra-oral photos taken. Those participants who were diagnosed with periodontal disease at the RPAH Oral Health Clinic would receive a referral letter for further treatment. Three months after the assessment the participants were contacted by telephone to evaluate with a structured questionnaire the oral care they had received.

Results: Fourteen studies included in the systematic review consistently reported an increased risk for diabetic complications including microvascular, macrovascular and death in the presence of periodontal disease. High prevalence (77.8%) of chronic periodontitis was found among participants with type-2 diabetes mellitus included in the study project. Additionally, we found that bleeding on probing was positively correlated with HbA1c levels in the participants.

Conclusion: The limited evidence from this study suggests an association between type 2 diabetes mellitus and periodontal disease. We believe that patients with type 2

diabetes mellitus should be routinely screened for periodontal disease as part of their diabetes management.

Keywords: periodontitis, periodontal disease, type-2 diabetes mellitus, diabetic complications

PART A:

LITERATURE REVIEW

Chapter 1: Periodontal disease

1.1. An introduction to periodontal disease

Periodontal disease are series of inflammatory conditions affecting the tooth supporting tissues. Two most common forms of periodontal disease are gingivitis and periodontitis. Gingivitis is characterized by the inflammatory lesions that are contained in gingival tissues. Gingivitis is mainly induced by dental plaque biofilm and it was shown in studies that the morphological changes of gingivitis can be reversed by reducing local microorganisms in biofilm and improving oral hygiene. On the other hand, periodontitis is identified as a more severe chronic disease, causing the damage of periodontal tissues. By definition, periodontitis is "an inflammatory disease of the supporting tissues of the teeth caused by specific microorganisms or groups of specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone with increased probing depth formation, recession, or both" [1]. The aggressive host inflammatory response in periodontitis, along with the bacterial infection, results not only in periodontal tissue destruction but also has adverse systemic effects [2-4]. There are many general conditions in which the influences of periodontal disease are well-documented with substantial amount of evidences, including cardiovascular disease (CVD) and CVD-related events such as stroke, congestive heart failure, coronary artery disease [5,6]; diabetes mellitus (DM) and its complications [2,7]; preterm labor, low birth weight and preeclampsia [8]; and respiratory conditions [9].

1.2. Epidemiology of periodontal disease

Periodontal disease, including gingivitis and periodontitis, involve nearly 90% of the world's population and have become one of the major global health burdens in the past few decades [10]. Severe chronic periodontitis affects 538 million people worldwide, which is around 7.4% of the world population [11]. Periodontitis, along with caries, are the two most frequent oral disease and responsible for 90% of tooth loss in Australia [12]. It is estimated that chronic periodontitis affects approximately 23% of the Australian adult population [13]. A recent study that collected data from the National Survey of Adult Oral Health (NSAOH) 2017-18 concluded that three in ten Australian adults suffered from moderate to severe periodontitis [14]. The prevalence of people showing signs of periodontal disease was from 23.5% to 39.2%

among subjects aged from 15 to over 75. It is also pointed out that in comparison to the NSAOH 2004-06, higher prevalence of periodontitis tended to be found in people of the same age in NSAOH 2017-18. Regarding gingivitis, the youngest generation (aged 15-34) presented with the highest prevalence of 31.3% and the oldest (over 75) presented with the lowest of 20.9% [14].

1.3. Pathogenesis of periodontal disease

1.3.1. Features of healthy periodontal tissues

For better understanding of pathological changes of periodontal tissues in gingivitis and periodontitis, it is crucial to overview some anatomical, clinical and histological features of healthy tissues. The periodontal tissues consist of the gingiva, periodontal ligament, cementum and alveolar bones (Figure 1). Gingiva comprises of marginal gingiva and attached gingiva. Marginal gingival is the unattached border of the gingiva that surrounds the tooth and attached gingiva is bound to the underlying alveolar bones. The v-shaped space between the tooth surface and the epithelium of marginal gingiva is called gingival sulcus. In normal condition, gingival sulcus is around 0-3 mm deep [1].

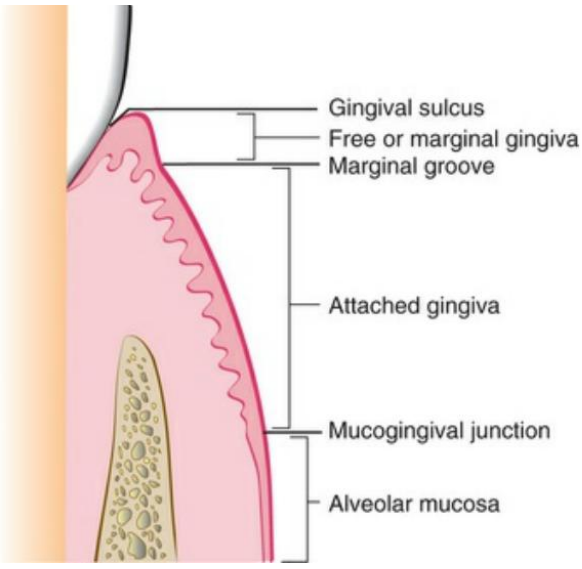

Figure 1: Anatomical features of periodontal tissues (Figure from Newman et al., 2018 [1])

Clinically healthy gingival tissues are normally pink in color, not inflamed or swollen, attached firmly to the underlying teeth and bones and appear with minimal bleeding on probing (Figure 2). Even in clinically healthy stages, gingival tissues contain at least some inflammatory cells, specifically neutrophils. Neutrophils

perpetually migrate from the connective tissues to reach the periodontal pockets (Figure 3). On the other hand, there is a constant fluid with flushing and diluting effects secreted from the periodontal pockets, called gingival crevicular fluid (GCF), which contributes in maintaining the equilibrium between low-grade inflammation and the continual presence of biofilm in the healthy periodontal tissues [1].

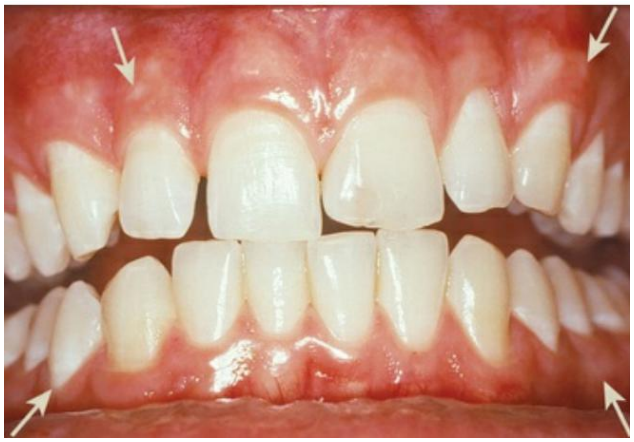

Figure 2: Clinical characteristics of healthy gingiva tissues. Clinically healthy gingival tissues are normally pink in color, not inflamed or swollen (white arrows) (Figure from Newman et al., 2018 [1])

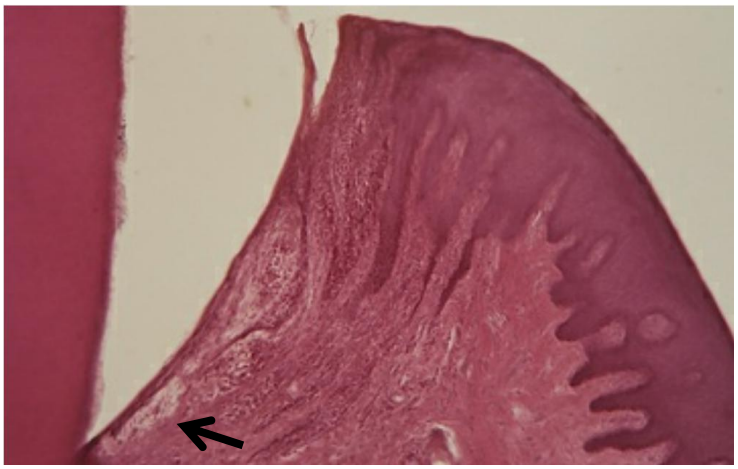

Figure 3: Histological features of clinically healthy gingival tissues with minor signs of inflammation at the base of the gingival sulcus (black arrow) (Figure from Newman et al., 2018 [1])

1.3.2. Pathogenesis of periodontal disease

The landmark studies of Page and Schroeder described the histologic changes in the gingival tissues during the progression of periodontal disease through 4 stages: the initial, early, established and advanced gingival lesions [15]. As the disease progress, vascular permeability, vasodilation and GCF flow significantly increase. There is a

shift of inflammatory cells in connective tissues from neutrophils to lymphocytes, plasma cells and macrophage with significantly higher loads. The accumulation of inflammatory cells shuffles the equilibrium stage in the periodontal tissues. As a result, the release of matrix metalloproteinase (MMP) and lysosomal contents from neutrophils elevates, which results in extracellular degradation of the connective tissue matrix [15].

Periodontal disease, especially periodontitis, results from a complicated interplay between the bacterial biofilm and the host immune system. Initial pathologic characteristics of gingivitis are local host responses to oral microorganisms on the tooth surface, especially along the gingival sulcus. The microflora in periodontitis are diverse, both inter-individually and within the same individual at different time point. It has been estimated that nearly 700 bacterial species existing in human biofilms. However, the presence of some specific bacterial complex in subgingival biofilms was found to play an important role in the initiation of periodontal disease [16]. Socransky et al. have classified the microbiota into complexes, representing bacterial groups that appear to occur together and are associated with different stages of periodontal disease (Figure 4) [17]. Among those complexes, the red complex appears later in biofilm development and are considered fundamental pathogens of the disease, including *Porphyromonas gingivalis (Pg), Tannerella forsythia (Tf), Treponema denticola (Td).* These bacteria can produce trypsin-like enzymes which have the potential to demolish both collagenous and non-collagenous matrix proteins [1,18].

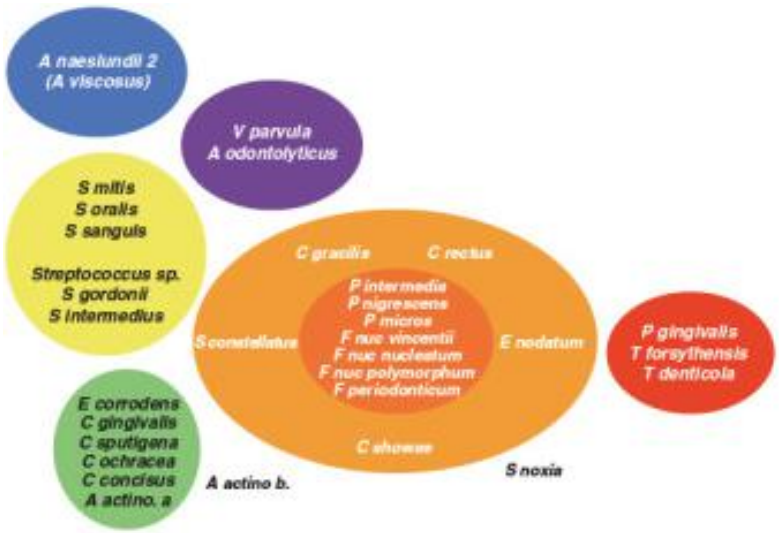

Figure 4: Periodontal bacterial complexes (Figure from Socransky et al., 1998 [17])

Pathogenic bacteria are responsible for periodontitis, but they are insufficient to cause the disease by themselves. The bacteria are essential to initiate the disease and maintain the inflammatory levels, however, the major of periodontal tissue destruction is due to the host immune response process. Mediators release during the inflammatory response exacerbate the breakdown of periodontal connective tissues, specifically, via fibroblast functions. Some molecules associated with periodontal fibroblasts are prostaglandin E_2 (PGE_2), interleukin-1 beta (IL-1β) and tissue necrosis factor alpha (TNF-α). These mediators can disturb with the regulation of several cytokines, thereby, activate fibroblasts and osteoclasts to produce enzymes that damage periodontal tissues and alveolar bones [19,20].

Furthermore, host modifying factors are influential in assessing periodontal disease progression, susceptibility and response to treatment. Common modifying factors that have been widely accepted to worsen periodontal disease are tobacco smoking [21], puberty, pregnancy, menopause [22] and diabetes mellitus [3,4]. These factors can impair the functions of pro-inflammatory cells and related mediators, by that, exacerbating inflammatory periodontal damage and decreasing response to treatment [1,21,22]. Additionally, tobacco smoking can also mask the signs of inflammation, therefore, prolonging the time span from disease onset to diagnosis. Consequently, silent tissue destruction continues to progress in patients and is not discovered until it becomes significantly severe [21]. Therefore, it is crucial that the clinicians identify relevant factors prior to the treatment to come up with the optimal approach of periodontal therapy for each individual.

1.4. Diagnosis of periodontal disease

The description of the histologic changes in periodontal tissues mentioned above, however, is not the criteria for diagnosis of the disease. Periodontal disease are usually diagnosed based on clinical parameters. The most common clinical feature of periodontal disease is periodontal pocket formation. Periodontal pocket is defined as a pathologically deepened gingival sulcus.

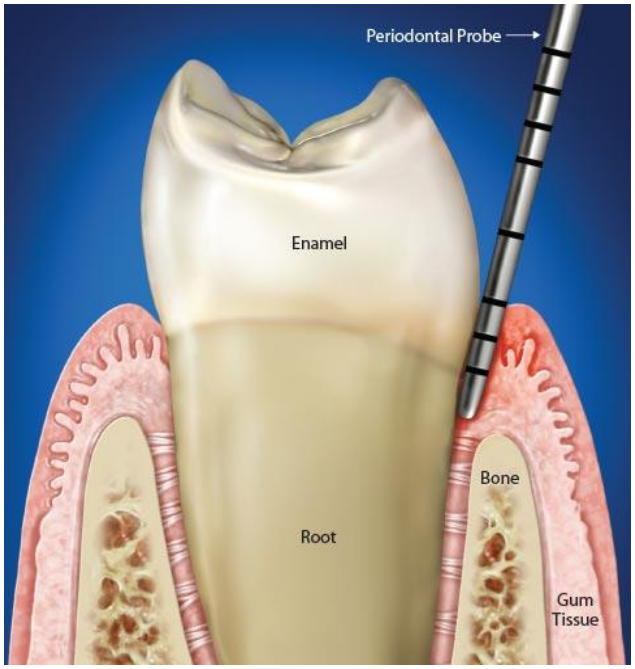

Figure 5: Periodontal probing (Figure from Newman et al., 2018 [1])

Periodontal pockets show destruction of the supporting periodontal tissues which might lead to tooth mobility and possible tooth loss. To evaluate a periodontal pocket, some periodontal clinical parameters are commonly used, including periodontal probing depth (PPD), clinical attachment loss/level (CAL) and bleeding on probing (BOP). In order to do this assessing procedure, an instrument called the periodontal probe will be used (Figure 5). This probe will go into the sulcus between the tooth surface and the gingival margin and go down until the tip of the periodontal probe touch the bottom of the periodontal pocket to record the depth of that pocket. Therefore, PPD is defined as the distance between the gingival margin and the tip of the periodontal probe. PPD equal or below 3 mm is considered healthy and PPD above 3 mm is considered sign of periodontitis. Another important clinical parameter is CAL. CAL is defined as the distant from the cemento-enamel junction (CEJ) to the apical extent of the probe tip during the periodontal probing. The distant from CEJ to the gingival margin presents the gingival recession. The main difference between PPD and CAL is that they use different reference point: PPD's reference point is on the soft tissue (gingiva) and CAL's is on the hard tissue (teeth) (Figure 6). Another parameter that is frequently used is BOP which records the bleeding status of the pocket during the probing procedure. BOP signifies local gingival inflammation, which is a crucial parameter to diagnose gingivitis.

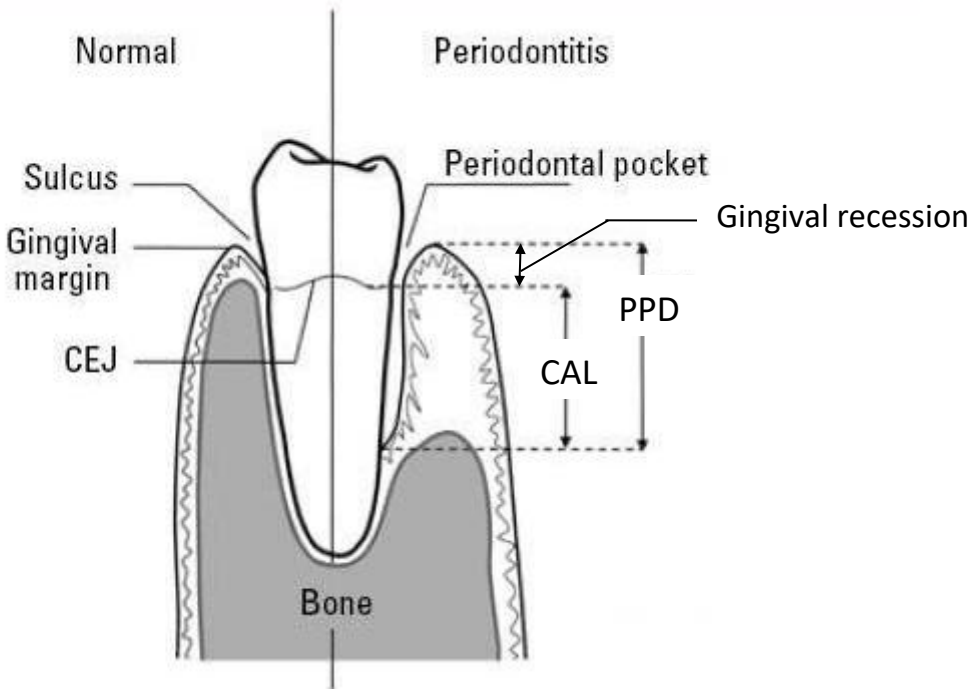

Figure 6: Definition of PPD and CAL (Figure from Newman et al., 2018 [1])

1.5. Treatment of periodontal disease

The main purpose of periodontal treatment is to reduce local inflammation by removing plaque and preventing its further accumulation. After eliminating other contributing factors, gingivitis, which is localized in the gingiva, might be easily treated by scaling and root planing procedure. On the other hand, periodontitis treatment can be slightly more complicated. Periodontal therapy can be done either non-surgically or more invasively by some specific surgical procedures. The reduction in BOP and/or PPD and/or the stability or gain in CAL are commonly regarded as an indicative of successful periodontal therapy [23,24]. In the past few decades, more and more supplementary elements were added to periodontal therapy to augment the success rate of the procedure. One of those is the use of antimicrobial agents. It is demonstrated in multiple studies that the use of adjunctive irrigation of antimicrobial agents such as chlorhexidine can significantly improve the outcomes of both non-surgical and surgical periodontal treatment [24,25]. It is important to note here is that periodontal disease can not be treated after one therapy. Due to the chronic nature of the disease, periodontal treatment required a life-long period of management and prevention. Patients diagnosed with periodontal disease are recommend to see a

general dentist or periodontist 2 to 4 times a year for routine check-up, scaling, root planing and follow-up on the local inflammatory response. Besides in-chair dental treatment, patients' personal oral hygiene plays an integral part in the management of the disease. Successful treatment is usually accompanied by daily toothbrushing, flossing/interdental brushing and antibacterial mouthwash use [26,27].

Chapter 2: Type-2 diabetes mellitus and its complications

2.1. Type-2 diabetes mellitus

2.1.1. An introduction to type-2 diabetes mellitus

Insulin is a peptide hormone secreted by the pancreatic beta cells that regulates glucose uptake, storage and release. Diabetes mellitus (DM) is characterized as a combination of different metabolic conditions in which the body fails to produce insulin or efficiently use the produced insulin. The lack of insulin or inability of insulin usage may lead to hyperglycemia, which eventually commonly results in serious life-threatening complications [24,28]. There are 2 main types of DM: type-1 DM (T1DM) and type-2 (T2DM). T2DM is the most common form of DM and responsible for 80-90% of cases worldwide [28,29].

2.1.2. Epidemiology of type-2 diabetes mellitus

T2DM has become one of the major global medical burden and requires intense medical attention. In 2010, it was estimated that 285 million people worldwide have diabetes, of whom 80% live in less developed countries and areas [28]. In Australia, there is evidence that the prevalence of diabetes has increased over the past few decades [30-32]. According to the Australian Diabetes, Obesity and Lifestyle Study (AusDiab) in 2000, the overall prevalence of diabetes in Australians aged ≥ 25 years was 7.6%, ranging from 2.5% in subjects from 25 to 44 years old to 24% among those over 75 years old [30]. Using data from the AusDiab study, it has been estimated that the prevalence of diabetes is likely to rise from 7.6% in 2000 to 11.4% by 2025 [31]. The Fremantle Diabetes Study Phase II (FDS2) reported in 2018 that 1.1 millions Australian had DM in 2011-2012, of whom 85.8% had T2DM [32].

2.1.3. Pathogenesis of type-2 diabetes mellitus

T2DM is a heterogeneous disorder resulted from a combination of genetic and environmental factors in which β-cell function and tissue insulin sensitivity were impaired. One of the key features of the pathogenesis of T2DM is insulin resistance. Insulin resistance can be defined as the inability of the body to use insulin for its usual biologic actions when it is secreted in an efficient concentration in normal subjects. Since the body cells can not use the produced insulin, pancreatic β-cells work increasingly harder to release enough insulin to compensate the body resistance level

in order to maintain blood glucose levels within normal range. Overtime, the β-cell insulin secretion capacity can no longer overcome the insulin resistance of the tissues. Without efficient insulin to regulate, blood glucose levels increase, which results in the development of T2DM. The exact causes of insulin resistance are not fully understood, however, there are some factors contributing to it such as obesity, pregnancy, alcohol intake, stress and aging [29,33,34].

Another crucial feature of T2DM pathophysiology is β-cell dysfunction. It has been debated over a long period about whether β-cell failure is the primary cause that precedes insulin resistance in the pathogenesis of T2DM, or the other way around. Nevertheless, it is commonly recognized that both insulin resistance and β-cell dysfunction play integral roles in the pathogenesis of T2DM [34,35]. A substantial amount of studies have demonstrated that in subjects with T2DM, reduction in both β-cell mass and insulin secretory granules were found. The contribution of reduced β-cell mass in the onset of hyperglycemia is still controversial as some cases reported patients can still maintain normal glycaemic levels after receiving partial pancreatectomy. For that reason, functional defects such as abnormal pulsatility of basal insulin secretion and loss of first-phase insulin release in response to a glucose challenge were believed to be more critical in the development of T2DM [33,36].

2.1.4. Diagnosis of type-2 diabetes mellitus

Some characteristic symptoms of DM can be observed by individuals before a proper diagnosis, including thirst, polyuria, weight loss, recurrent infections and in some more severe cases, pre-coma. However, blood test is required to reach a definite diagnosis. World Health Organization (WHO) recommends the criteria for the diagnosis of DM is fasting plasma glucose ≥ 7.0mmol/l (126mg/dl) and/or 2-hour post-glucose load plasma glucose ≥ 11.1 mmol/l (200mg/dl). All values refer to venous plasma glucose. Capillary plasma glucose values would be the same for fasting glucose but 1 mmol/l (18mg/dl) higher than venous levels after the glucose load. The glucose load is 75g anhydrous glucose [37].

Plasma glucose measurement has been the “gold-standard” of DM diagnosis for over a century, however, it has some drawbacks. Plasma glucose levels can be variable within the same individual from day to day or even at different time point during the day, which can be unreliable when they are close to the threshold for diagnosis. It can also be affected by drugs or some coexisting conditions. Another

disadvantage is that the coefficient of variation of the blood test results from the labs can be as high as 20%, making it unsuitable for diagnostic purpose. Additionally, unless the blood sample is analyzed right after being taken, 5 to 20% of glucose can be lost, posing a high risk of imprecise results [38]. The introduction of glycated haemoglobin (HbA1c) which is a test to evaluate glycemic control has been a monumental milestone in the management of DM. HbA1c can be defined as a stable product of glucose and haemoglobin. HbA1c formation occurs dependently on plasma glucose levels and continuously during the whole lifetime of erythrocytes, which prolongs 120 days. Therefore, HbA1c reflects the weighted mean plasma glucose level over a period of 2-3 months [39]. In 2011, WHO recommended HbA1c level of 6.5% to be the cut point for diagnosing DM [40]. Advantages of using HbA1c for DM diagnosis over plasma glucose measurement are no need for fasting or glucose load before sampling; less likely to be affected by other factors (such as stress, diet, exercise); less day-to-day inconsistency; better pre-analytic stability; and closer associations with DM-related chronic complications [38-40].

2.1.5. Management of type-2 diabetes mellitus

T2DM is a chronic condition that requires lifelong management. Majority of T2DM management approach concentrates on reducing the risk of long-term complications through screening, maintaining adequate glycemic control, identifying and diminishing cardiovascular risk factors. Glycemic control and diabetic complications will be detailed in Section 2.2. It is been estimated that patients with T2DM spend the vast majority of their time managing their condition and only around 1% of their time with health care professionals [29]. Therefore, it is very important to improve their awareness and self-care via encouragement and education. Two common lifestyle interventions considered the underlying strategy throughout the management of T2DM are diet and exercise.

In the recent years, a large body of studies have demonstrated that dietary factors are associated with the incidence of T2DM [41]. Dietary guidelines for the management of T2DM have been similar in Europe and America. Those guidelines concentrate mostly on the composition of macronutrients (protein, fat, and especially carbohydrate), glycemic index and meal frequency [42]. A pooled estimates from a meta-analysis of 9 randomized controlled trials concluded that a low carbohydrate diet (LCD) had a significant effect on HbA1c levels in patients with T2DM. The

results from that study also showed that LCD also significantly reduced triglycerides and increased high density lipoprotein (HDL), demonstrating a reduction in cardiovascular risk factors [43]. A recent meta-analysis of prospective observational studies has shown the association for increased incidence of T2DM with higher intake of red meat, process meat, bacon and sweetened beverages [42]. Many prospective studies have found relation between fat intake and impaired glucose tolerance [41-44]. Therefore, dietary cholesterol, saturated and trans-unsaturated fatty acids are recommended to be restricted to reduce the risk of developing T2DM and the subsequent risk for cardiovascular disease [29,44]. Useful fat sources suggested for individuals with T2DM are monounsaturated fatty acids and oily fish rich in omega-3 polyunsaturated fatty acids. Vegetables, fruits and wholegrain foods are advised to be part of diet as they are rich in dietary fiber, micronutrients and proven to have an inverse association for T2DM incidence [29,41].

It is widely acknowledged that increase physical activity is associated with a decrease in the risk of T2DM development and in people already with T2DM, physical inactivity is associated with cardiovascular complications and mortality [45]. Regular exercise is proven to significantly improve glycemic control in patients with T2DM [29,46,47]. According to Van Dijk et al., a short 30-minute session of moderate-intensity endurance-type exercise remarkably reduces the prevalence of hyperglycemia throughout the subsequent day in patients with T2DM [48]. People with T2DM and moderate or high aerobic fitness have long-term mortality that is 50-60% lower than those with T2DM and low cardiorespiratory fitness [29]. Therefore, exercise advice should always be offered by health care professionals as a standard management of T2DM.

2.2. Hyperglycaemia and diabetic complications

2.2.1. An introduction to diabetic complications

Long-term hyperglycaemia is associated with vascular complications due to vascular endothelial cell damage, which can be classified as microvascular complications including retinopathy, neuropathy and nephropathy; or macrovascular complications such as cardiovascular disease, cerebrovascular disease and peripheral arterial disease. Beside those common vascular complications, DM also has some other complications and metabolic consequences such as non-alcoholic fatty live disease (NAFLD), impaired sexual functions, gastrointestinal manifestations, etc.

Diabetic complications can have an enormous adverse impact on a patients' physical and mental health [29]. Maintaining an adequate HbA1c level is considered an essential way to help reducing risk of diabetic complications developing. According to The United Kingdom Prospective Diabetes Study, a 1% decline in levels of HbA1c resulted in a 21% risk reduction for diabetes-related deaths and a 14% reduction for myocardial infarction and microvascular complications. Therefore, glycemic control is crucial for diabetic therapy and the control of diabetes-related complications, and to maintain and improve patient's quality of life [49].

2.2.2. Metabolic mechanism of diabetic complications

Diabetic vascular complications are the result of interactions between systemic metabolic abnormalities, genetic and epigenetic modulators, and local tissue responses to toxic metabolites. Hyperglycemia is considered the main risk factor for these diabetic micro- and macrovascular complications. Prolonged cell exposure to high glucose concentrations results in intracellular hyperglycemia, especially in endothelial vascular cells with a low glucose transport rate. Therefore, endothelial vascular cells become a main target of hyperglycemic damage. A result of intracellular hyperglycemia is mitochondrial production of reactive oxygen species (ROS) that may lead to cell damage [29,50]. Several potential consequences exist, including (1) exacerbating intracellular oxidative stress via the polyol pathway, (2) disturbing extracellular signals, (3) the production of toxic metabolites such as advanced glycation end-products (AGEs) and methylglyoxal and (4) altered gene expressions. Altered gene expression of pathogenic factors can result in cell signaling dysfunction, which is thought to eventually lead to the abnormal production of inflammatory cytokines and growth factors. For example, increased glucose levels increase the synthesis of diacylglycerol, a co-factor of protein kinase C, which leads to an up or down-regulation in the production of various cytokines including vascular endothelial growth factor (VEGF) and transforming growth factor β (TGF-β). This results in change of vascular permeability and angiogenesis, vasoconstriction, vascular occlusion and blood flow abnormalities (Figure 7). Thereby, diabetic vascular complications, through different inflammatory responses, may develop as a result of hyperglycaemia [29].

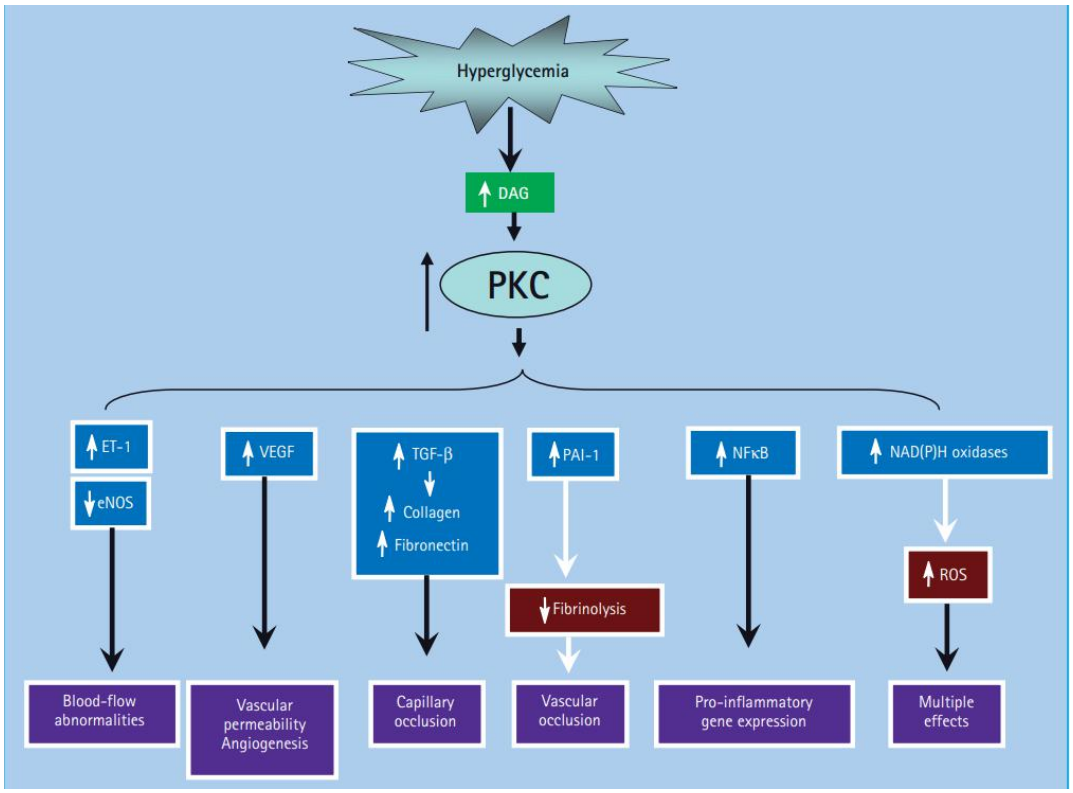

Figure 7: Activation of protein kinase C induced by hyperglycemia and some of its pathologic consequences. ET-1: endothelin 1; eNOS: endothelial NO synthase; VEGF: vascular endothelial growth factor; TGF-β: transforming growth factor β; PAI-1: plasminogen activator inhibitor 1; NFkB: nuclear factor kB; NAD(P)H: nicotinic acid adenine dinucleotide (phosphate); ROS: reactive oxygen species (Figure from Holt et al., 2017 [29])

2.2.3. Diabetic microvascular complications

Diabetic microvascular complication includes retinopathy, nephropathy and neuropathy. Diabetic retinopathy affects up to 34.6% of patients and in many countries is the most prevalent cause of working age blindness [51]. A person with DM has 25-fold higher risk of vision loss compared with one without DM. There are multiple stages of diabetic retinopathy. Classification of the relevant stage depends on the absence or presence of some clinical ocular biomarkers such as microaneurysms, hemorrhages, exudates, edema and neovascularization [52]. Fortunately, over the past 30 years, the incidence as well as the risk of progression of diabetic retinopathy have decreased significantly thanks to medical care improvement [53].

For 60-70% of affected individuals, neuropathy becomes the most common diabetic microvascular complication, represented by peripheral neuropathy and

proneness to foot ulceration as well as cardiac autonomic neuropathy. These conditions can lead to physical disability, amputations and cardiac interventions, and pose a colossal burden on the public health system [54]. Diabetic neuropathies can remain asymptomatic for a long period before having any clinical manifestation [55]. Clinical presentation of diabetic neuropathies can be various from pain, numbness to ulcerations, muscle weakness and limb deformities depending on the effected parts of the nervous system. There is no preventative treatment for any forms of diabetic neuropathy. For that reason, intensive glycemic control and early diagnosis become crucial in preventing and managing the complications [54,55].

Diabetic nephropathy is characterized by the slow rising of urine albumin excretion (UAE) over many years, together with gradually increased blood pressure and reduced glomerular filtration rate (GFR) [29]. Diabetic nephropathy is the most common single aetiologic factor for end-stage renal disease (ESRD), which has the highest prevalence in patients with diabetes and tremendously reduces quality of life. Due to its association with ESRD and cardiovascular diseases, diabetic nephropathy is one of the primary causes of disability and death in patients with diabetes [56]. Therefore, screening for diabetic nephropathy is recommended to be performed every year for patients with DM by evaluating urine albumin : creatinine ratio and estimated GFR [29].

2.2.4. Diabetic macrovascular complications

Diabetic macrovascular complications are disease that affect the heart and large blood vessels in the body, which can include cardiovascular disease, cerebrovascular disease (stroke) and peripheral vascular disease. Atherosclerotic cardiovascular complications remain the principal cause of morbidity and mortality among individuals with DM. Approximately two-thirds of deaths in patients with DM result from cardiovascular diseases, in which, about 40% are due to ischemic heart disease, 10% due to stroke and 10% due to congestive heart failure [57]. A meta-analysis of 102 prospective studies in 2010 estimated that DM was accounted for 2-fold increase risk of coronary heart disease and stroke [58]. The association between glycemic control and macrovascular complications is not as well-established as that with microvascular complications, although there are strong in vitro and in vivo evidences supporting that plasma glucose exerts direct and indirect toxic effects on the vascular

systems. Moreover, some specific insulin resistance pathways were shown to have contribution in atherogenesis in patients with DM [59].

Two most common cardiovascular risk factors associated with T2DM are hypertension and dyslipidemia. The prevalence of hypertension among patients with DM is more than 50%, and even higher in patients with T2DM with the prevalence of 60-80%. Patients that have both hypertension and DM have 4-times higher risk of developing cardiovascular disease compared with normotensive nondiabetic individuals. Dyslipidemia is present in more than 90% patients with DM, with a characteristic pattern of increased triglycerides and decline in HDL [60]. There are substantial evidences suggesting that both hypertension and dyslipidemia are associated with insulin resistance and other DM-related metabolic features via oxidative stress and the state of low-level, chronic inflammation of DM [60,61].

Chapter 3: The two-way relationship between periodontal disease and type-2 diabetes mellitus

3.1. An introduction to the two-way relationship between periodontal disease and type-2 diabetes mellitus

Several studies have identified a two-way relationship between periodontitis and DM [4]. In particular, the chronic immune response and the concordant release of pro-inflammatory mediators into the blood in patients with periodontal disease interferes with glycemic control in people with DM [62,63]. And the other way around, patients struggling to control their glucose levels are more likely to develop periodontitis compared to those with good glycemic control and those without DM [4]. There have been hundreds of publications including reviews, animal studies, cohort studies and randomized controlled trials addressing the topic. These studies mostly concentrated in investigating how (1) more severe periodontal disease were developed in patients with DM, (2) periodontal disease affect glycemic control and (3) periodontal treatment can improve glycemic control in patients with DM [3].

3.2. More severe periodontal disease in patients with DM

3.2.1. Scientific evidence

DM has been commonly accepted as a major risk factor for periodontal disease. The risk of developing periodontitis is 2-3 times higher in people with DM compared to those without [4]. In fact, periodontitis was sometimes referred as “the sixth complication of diabetes” in the early 1990s. Like other diabetic complications, the risk of developing periodontitis is closely associated with poor glycemic control [4]. According to a systematic review in 2013, 27 in 29 studies included in the review suggested the adverse effects of DM on periodontal health [64]. However, the presumption that DM is a risk factor for periodontitis had mostly been based on results from cross-sectional and animal studies. A meta-analysis of 13 longitudinal studies in 2018 concluded that DM increased the risk of incidence and progression of periodontitis by 86%, showing protracted impacts of DM on periodontal status over the patients’ lifetime [65].

3.2.2. Metabolic mechanism

The pathogenic processes linking DM and periodontal disease mostly surround the upregulated inflammatory responses arising from each condition that adversely affect the other. Possible pathway explaining how patients with uncontrolled DM are more likely to develop periodontal disease are summarized in Figure 8. Related factors can be classified as (1) microbial factors, (2) cytokines and adipokines, (3) immune cell functions, (4) advanced glycation end products (AGEs) and their receptor (RAGE) and (5) alveolar bone homeostasis [3,29].

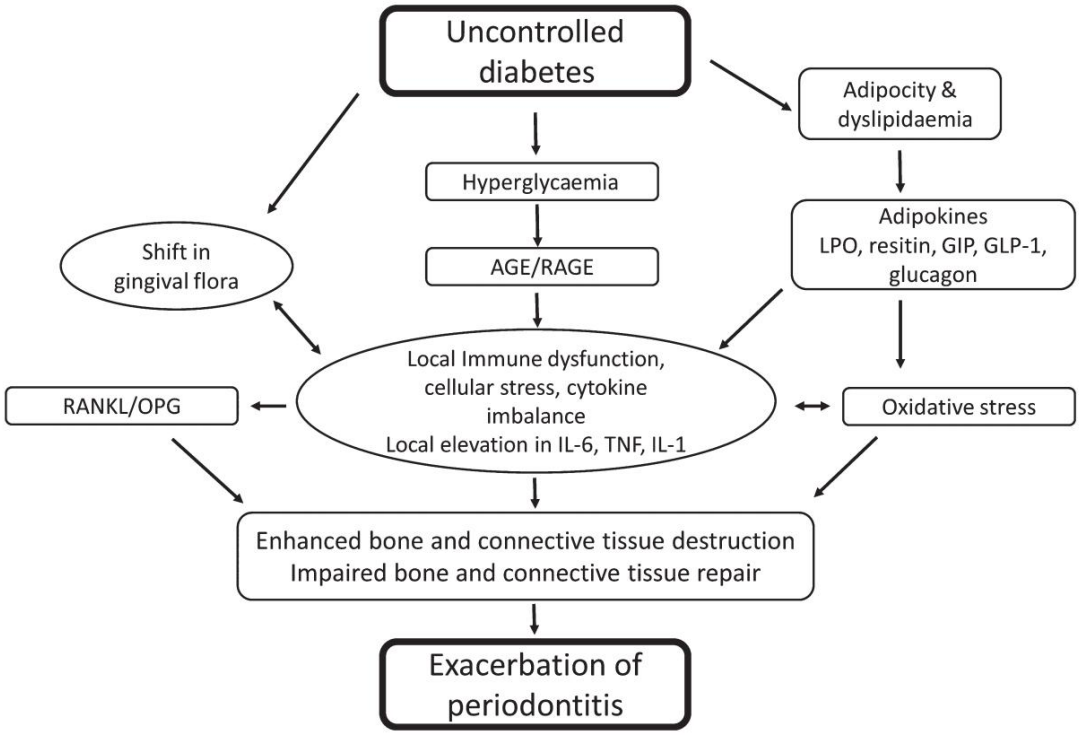

Figure 8: Potential mechanism how uncontrolled DM exacerbate periodontal disease (From Polak et al. 2018 [3])

In patients with severe and prolonged hyperglycemia, there is an increased deposition of AGEs and interactions between AGEs and their receptors RAGE. This results in the activation of multiple local immune cells and inflammatory responses. These upregulated functions lead to an elevated secretion of interleukin-1β (IL-1β), IL-6 and tumor necrosis factor-α (TNF-α). This also exacerbates oxidative stress and disrupts the receptor activator of NF-kB ligand/osteoprotegerin (RANKL/OPG). All of these disruptions result in enhanced bone and connective tissue damage and impaired tissue repair, thereby, worsening patient's periodontal status. It is also been shown in multiple studies that dyslipidemia, as a diabetes metabolic consequence, also contributed in the cytokine imbalance and local immune dysfunctions via abnormal regulation of pro-inflammatory adipokines [3,4]. The association between

the presence of DM in patients and the composition of their periodontal microflora is still controversial. Taylor et al. concluded in their review that DM had no significant impacts on the supra- and subgingival microbiota. However, multiple recent studies have proven otherwise. They have shown that there is a shift in the microbial composition in patients with DM [66,67]. However, these evidences are insufficient for the causal relationship between uncontrolled DM and periodontal microbial dysbiosis.

3.3. Periodontal disease affecting glycemic control in patients with DM

3.3.1. Scientific evidence

Substantial evidence has been supported that periodontal disease has an adverse effect on glycemic control in patients with DM [10]. A small body of evidence showed that in healthy subjects, the presence of periodontitis significantly is associated with elevated glucose level and increase the risk of developing DM by 19-33% [4]. In those subjects, periodontal disease is associated with lower insulin sensitivity and poorer β cell functions. Among people with type-2 DM, 3 cohort studies with a total of 786 subjects with type-2 DM concluded that periodontitis was significantly associated with poorer glycemic control as measured by HbA1c. The risk was more remarkable in those with poorer glycemic control at baseline. On the contrary, 2 case-control studies reported an insignificant difference in HbA1c levels in patients with type-2 DM with periodontal disease in comparison with those without periodontal disease [2,68]. Moreover, a large body of evidence have shown that periodontal disease effects not only glycemic control in patients with DM but also have an enormous impact on diabetic complications. A recent systematic review of 14 studies consistently reported an increased risk for diabetic complications including microvascular, macrovascular and death in the presence of periodontal disease. Higher risks for diabetic retinopathy (odds ratios: 2.8 - 8.7), neuropathy (3.2 - 6.6), nephropathy (1.9 - 8.5), cardiovascular complications (1.28 - 17.7) and mortality (2.3 - 8.5) were reported for people with diabetes with periodontitis compared to those with diabetes who have no periodontitis [7].

3.3.2. Metabolic mechanism

Multiple human and animal laboratory studies have been conducted to establish a possible metabolic mechanism illustrating how periodontal disease affect glycemic

control in patients with DM [3]. The pathway mostly surrounded the fact that periodontal-related bacteria and their products, along with inflammatory cytokines are produced locally in periodontal tissues, released into bloodstream and contributed to the upregulation of systemic inflammation (Figure 9). Those factors includes periodontal pathogens such as *Porphyromonas gingivalis (Pg), Treponema denticola (Td), Aggregatibacter Actinomycetemcomitans (Aa)* and their bacterial antigens; elevated circulating levels of pro-inflammatory cytokines and mediators: IL-6, TNF-α, C-reactive protein (CRP) and oxygen radicals. The increased level of these factors can lead to impaired insulin signaling and as a result, insulin resistance. Worsening of insulin resistance results in poorer glycemic control in patients with DM and eventually a rising risk of developing diabetic complications [4].

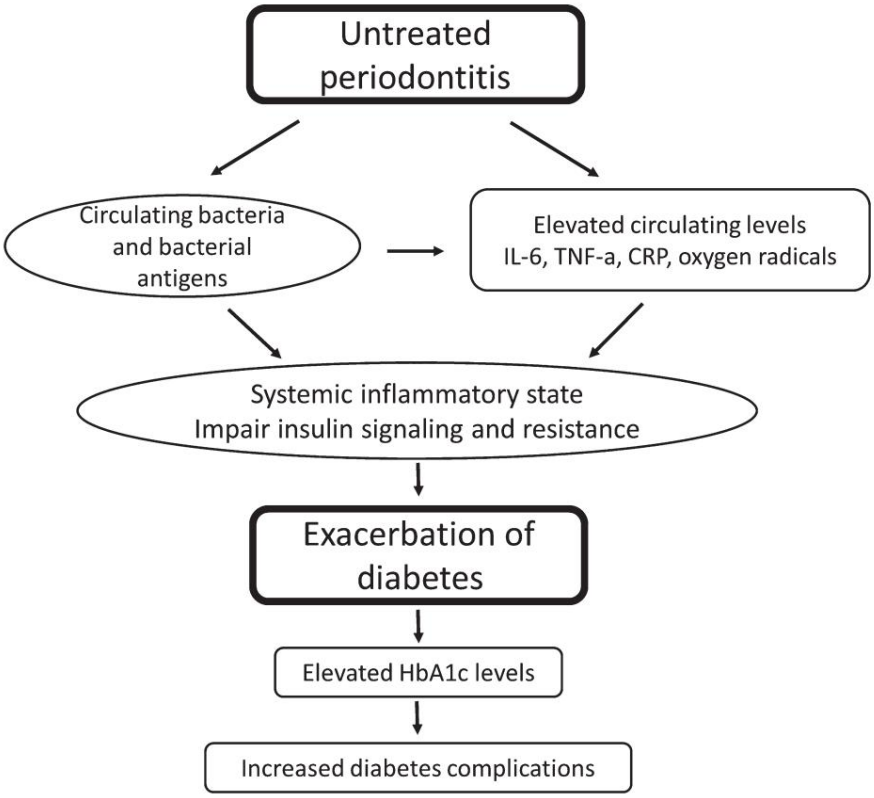

Figure 9: Potential mechanism how periodontal disease effect DM and diabetic complications (From Polak et al., 2018 [3])

3.4. Periodontal treatment improving glycemic control in patients with DM

3.4.1. Scientific evidence

Six systematic reviews and meta-analyses have been done from 2014 to 2016 to investigate the role of periodontal treatment in improving glycemic control in patients

with DM. Among them, four provided consistent evidence that therapy of periodontal disease can significantly improve HbA1c levels in patients with DM after 3-4 months of treatment. HbA1c reduction levels from those meta-analyses ranges from 0.27% to 0.48% at 3-4 months after periodontal therapy [2,69]. A Cochran systematic review in 2015 concluded that periodontal treatment by scaling and root planing helped reduce HbA1c level by the mean reduction of 0.29% at 3-4 months. However, evidence demonstrating that the effect can be maintained after 4 months is insufficient [24]. A well-executed randomized controlled trial in 2018 with 12-month follow-up period suggested that after 12 months of intensive periodontal treatment, mean HbA1c level of patients in the intervention group was 0.6% lower than that of the control group, fortifying the hypothesis that periodontal therapy has not only short-term effect but also prolonged benefit on DM patients' glycemic control [23]. Additionally, periodontal therapy also have some remarkable benefits in improving DM patients' lipid profile. It has been demonstrated that 3 months after periodontal treatment, there is a significant reduction of cholesterol and triglycerides in the intervention arm in comparison with the control arm among patients with type-2 DM [60].

3.4.2. Metabolic mechanism

The main goal of periodontal treatment is to reduce local periodontal inflammation. By reducing local bacterial load in subgingival biofilm via scaling and root planing, periodontal therapy also decreases circulating bacteria load and their antigen products in the patients' system. Moreover, periodontal treatment was also proven to have beneficial effects in reducing systemic levels of pro-inflammatory cytokine and mediator levels such as CRP and TNF-α. However, the detailed mechanism that explains how periodontal treatment in patients with DM is followed by the improved glycemic control and HbA1c reduction is not fully understood. It is widely presumed to result from the combination of decreased systemic inflammation and reduced bacterial challenge. This eventually leads to the improvement in insulin signaling and insulin resistance, which contributes in improved glycemic control and the reduced risk of developing diabetic complications (Figure 10) [3,4].

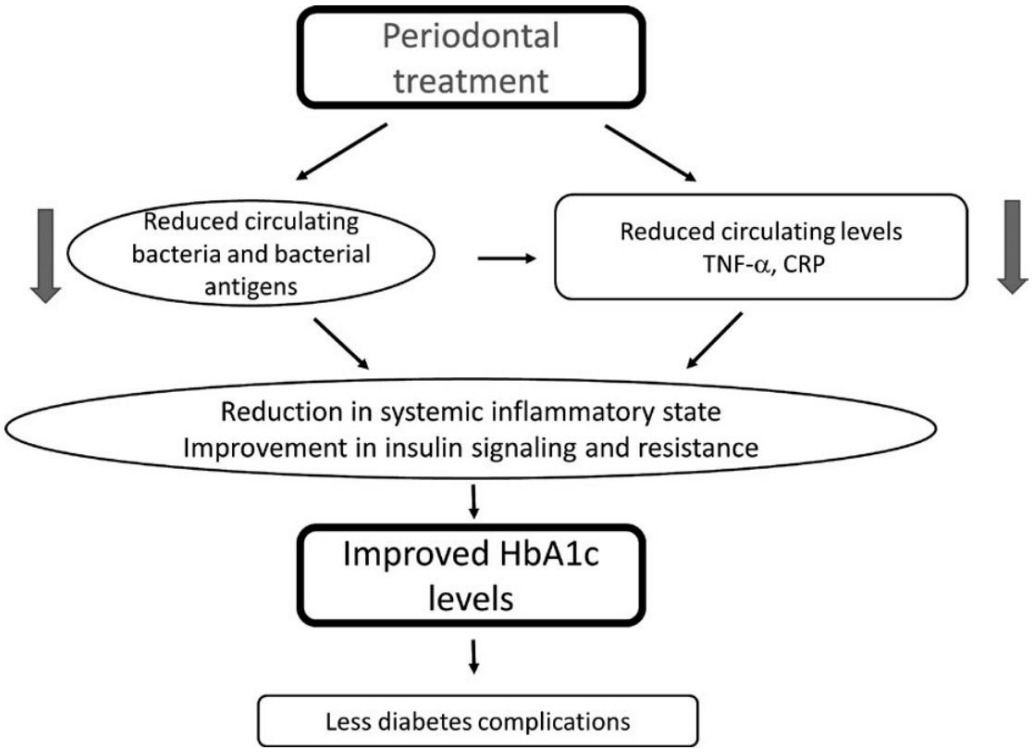

Figure 10: Potential mechanism how periodontal treatment can improve glycemic control in patients with DM (From Polak et al., 2018 [3])

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PART B:
SYSTEMATIC REVIEW

The association of periodontal disease with the complications of diabetes mellitus. A systematic review.

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Highlights:

- This review summarizes epidemiological studies that have consistently reported higher risk for diabetes complications of micro- and macrovascular origin in the presence of periodontal disease
- This systematic review of complications in diabetes is an important addition to the existing evidence linking periodontitis with poor glycemic control in diabetes.
- The observed limitations of included studies warrant well-designed clinical trials to further investigate how periodontitis prevention or treatment modulates diabetes complications.

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Summary

This review investigated the association of periodontal disease with diabetes mellitus (DM) and diabetic complications. PubMed/MEDLINE was searched including search terms “periodontal” OR “periodontitis” AND “diabetic complications” OR “diabetic retinopathy” OR “diabetic nephropathy” OR “diabetic neuropathy” OR “cardiovascular disease diabetes” OR “myocardial infarction diabetes” OR “cerebrovascular disease diabetes” OR “stroke diabetes” OR “peripheral vascular disease diabetes”. Fourteen studies included in this review consistently reported an increased risk for diabetic complications including microvascular, macrovascular and death in the presence of periodontal disease. Higher risks for diabetic retinopathy (odds ratios: 2.8 - 8.7), neuropathy (3.2 - 6.6), nephropathy (1.9 - 8.5), cardiovascular complications (1.28 - 17.7) and mortality (2.3 - 8.5) were reported for people with diabetes with periodontitis compared to those with diabetes who have no periodontitis. This novel review summarizes current data providing further evidence of a link between poor oral health and DM and its complications. It has also drawn attention to major limitations of the available data linking periodontal disease and diabetic complications.

Keywords: diabetic complications, periodontal disease, oral health, periodontitis, microvascular complications, macrovascular complications

Chapter 4: Introduction

Diabetes mellitus (DM) affects ~5% of the world's population and requires intense and costly medical treatment [1]. Long-term hyperglycemia may be associated with micro- and/or macrovascular complications with severe negative impact on the patients' physical and mental health [2]. Therefore, adequate glycaemic control is crucial to reduce the risk of diabetes-related complications and to maintain quality of life in people with diabetes [3].

Periodontal disease represents a series of inflammatory conditions of the tooth supporting tissues in response to bacterial accumulation. Constantly forming bacterial deposits on the teeth cause a chronic inflammatory response with many stages ranging from reversible low-level inflammatory gingivitis to irreversible higher-level inflammatory periodontitis that, if left untreated, causes tooth loss [4]. Approximately 10% of the global population is affected by severe periodontitis and this prevalence may raise to 15% in people with DM [5,6]. The inflammatory host response in periodontal tissues and the concordant release of pro-inflammatory mediators into blood can lead to adverse systemic effects including in glycaemic control of people with DM [7-9]. The increased levels of HbA1c in patients with DM who have periodontitis may increase the risk of diabetes complications.

While a number of original epidemiologic studies have investigated the relationship between periodontitis and particular diabetes complications, to date no studies have assembled and examined the entire published evidence across the spectrum of traditional end-organ diabetes complications. The aim of this review was to systematically assess current evidence of the association between periodontal disease and diabetes complications, so to inform dental and medical professions and to provide direction for future research aimed to improve the delivery of health care.

Chapter 5: Materials and methods

5.1. Criteria for considering studies for this review

5.1.1. Types of studies

Observational and clinical studies in human including cross-sectional, case-control, cohort studies and clinical trials investigating the association between periodontal disease (periodontitis, gingivitis) and micro- or macrovascular complications including diabetic retinopathy, nephropathy, neuropathy, cardiovascular disease and death. Animal studies and studies involving periodontal interventions were excluded.

5.1.2. Types of participants

Studies and trials reporting participants with type-1 or type-2 DM and periodontal disease (gingivitis or periodontitis) were eligible. No restrictions with respect to age, gender, or race were applied.

5.1.3. Types of outcome measures

The main outcomes were measures quantifying the risk (odds ratio, standardized beta coefficient) of patients with periodontal disease and DM to develop diabetic complications compared to patients who had DM but no periodontal disease. This comprised presence of diabetic microvascular complications including retinopathy, nephropathy and neuropathy; and macrovascular complications including cardiovascular complications and death.

5.2. Search methods for identification of studies

An electronic search was conducted in PubMed/MEDLINE from 1966 to March 2019 using the search terms “periodontal” OR “periodontitis” AND “diabetic complications” OR “diabetic retinopathy” OR “diabetic nephropathy” OR “diabetic neuropathy” OR “cardiovascular disease diabetes” OR “myocardial infarction diabetes” OR “cerebrovascular disease diabetes” OR “stroke diabetes” OR

"peripheral vascular disease diabetes". Full text articles were manually searched to identify eligible studies. No restrictions were placed on the date of publication and only publications in English were selected.

5.3. Data collection

5.3.1. Selection of studies

Initially, titles and abstracts were screened, and full-text publications were assessed from included abstracts. The decision for in- or excluding of studies was done by two independent reviewers (A.N and S.G) who were blinded for the outcomes of the other reviewer. Any disagreement was discussed between the two reviewers and a third person (JE).

5.3.2. Data extraction and management

Investigators extracted relevant data using a standardized form including the year of publication, authors, title, type of study, country where the study took part, number of patients (gender distribution and mean age), DM duration and mean HbA1c levels if available, diagnosis of DM, diagnosis of diabetic complications, dental examination and main outcomes. The authors were contacted in cases relevant information was missing.

5.3.3. Assessment of quality of selected studies

The quality of included studies was independently evaluated by 2 investigators (AN and SG) using the Study Quality Assessment Tools of the National Heart, Lung and Blood Institute of the National Institutes of Health. For longitudinal and cross-sectional studies, the "Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies" and for case-control studies, the "Quality Assessment of Case-Control Studies" was used (Appendix 1) [10].

5.4. Data synthesis

After completing the data extraction, descriptive statistics were calculated for demographic characteristics and odd ratios.

Chapter 6: Results

6.1. Description of studies

6.1.1. Search results

The search initially identified 2481 records and 2430 articles were eliminated based on the title due to one of the following reasons: non-human/primate/animal study, study involved periodontal treatment, periodontal status not reported or diabetic complications not studied. Thirty-three articles were selected based on the titles and abstracts for the assessment of the full-text publications. Nineteen references were eliminated because they did not report diabetic complications and 14 full-text articles were further analyzed (Figure 11).

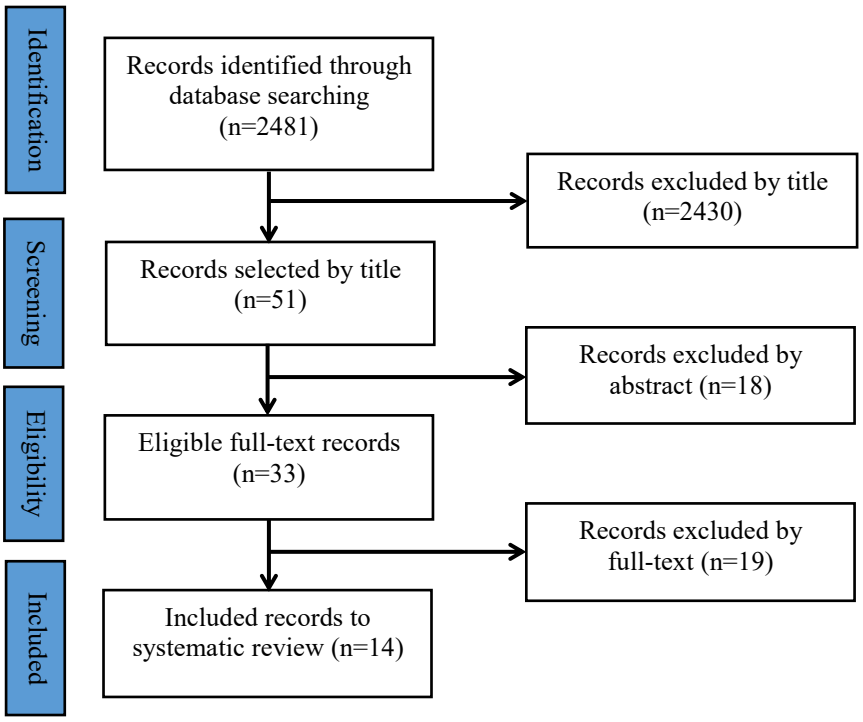

Figure 11: Study selection flow diagram

6.1.2. Characteristics of included studies

Characteristics of the included studies are summarized in Table 1. Of the 14 included studies, 9 reported data from cross-sectional studies [11-19], 4 publications reported data from longitudinal studies [20-23] and 1 reported data of a case-control study [24]. The studies were conducted in Asia [14,16,18], the Middle East [12], Europe [13,20,21,24], South America [11,17] and North America [15,19,22,23].

Table 1. A summary table of included studies

Year	Author	Title	Type of study	Country	Number of patients	DM duration (years)	Mean HbA1c level (%)	Diagnosis of DM	Diabetic complications - How to diagnose?	Dental examination	Main outcomes
2010	Abrao et al.	Periodontal disease and risk for neuropathic foot ulceration in type 2 diabetes.	Cross-sectional study	Brazil	122 patients (53 M, 69 F), mean age: 60.5	14.5	8.9	ADA criteria	Neuropathic foot ulceration - Foot examinations, classified according to presence or absence of risk for neuropathic foot ulceration (DM-NFUR), defined as insensitivity to the 10-g Semmes-Weinstein monofilament (SW) at four or more of ten sites (nine plantar, one dorsal) on either foot.	CPITN, PI, dental health habits, dental records, number of missing teeth	After adjustment for sex, age, diabetes duration, dental health care and education, risk for neuropathic foot ulceration was higher in the group with moderate/severe periodontal diseases, odds ratio was 6.6 (p<=0.001), and in the edentulism group, odds ratio was 4.9 (p<=0.01).
2014	Amiri et al.	Relationship between Type 2 Diabetic Retinopathy and Periodontal Disease in Iranian Adults.	Cross-sectional study	Iran	286 patients (122 M, 164 F), mean age: 50.8	11.9	n/r	Not specified	Retinopathy - Fundus examination, documented by fundus fluorescein angiography. Retinopathy was defined by the presence of characteristic changes, including hemorrhages, exudates, laser marks, and fibrous proliferation, detected by ophthalmoscopy through dilated pupils. DR is classified either as nonproliferative DR (NPDR) or PDR.	PI, CPITN, PD and CAL. Periodontitis was defined as presence of any sites exhibiting probing depth (PD) ≥ 4 mm or clinical attachment loss (CAL) ≥ 4 mm.	The severity of periodontal disease was significantly correlated with the severity of diabetic retinopathy, and the risk of proliferative diabetic retinopathy was significantly higher in the presence of periodontal disease (OR = 2.80, p<0.029).
2000	Collin et al.	Oral symptoms and signs in elderly patients with type 2 diabetes mellitus. A focus on diabetic neuropathy.	Longitudinal study	Kuopio, Finland	122 patients (64 M, 58 F), mean age: 67.4	n/r	8.8	FPG level >=126mg/dL	Peripheral and autonomic neuropathy - The subjects were considered to have peripheral neuropathy when more than 3 abnormal values were seen. The criteria for parasympathetic neuropathy were an E/I ratio ≤ 1.10 and for sympathetic neuropathy a systolic blood pressure decrease ≥30 mm Hg in the orthostatic test	Tooth number, type of dentition, mucosal lesions, TMJ examination	Frequency of parasympathetic neuropathy is significantly higher (p=0.008) in patients with <6 teeth (70%) compared to patients with more than 6 teeth (25%, data taken from graph).
2010	Franek et al.	Blood pressure and left ventricular mass in subjects with type 2 diabetes and gingivitis or chronic periodontitis.	Cross-sectional study	Poland	155 patients (88 M, 67 F) mean age: 61.1	12.4	7.6	ADA criteria	Left ventricular mass (LVM) measured by echocardiography, and central blood pressure (CBP).	BOP, PD	Periodontitis and Gingivitis subjects had higher LVM (238.6 and 222.8g) and LVMI (95.2 and 87.8g/m2) versus Periodontally Healthy subjects (170.3g; 63.7g/m2) (p<0.05). Periodontitis subjects had higher central and systemic systolic and diastolic blood pressure than subjects from Gingivitis and Periodontally Healthy groups (p<0.05).
2015	Han et al.	Association of Periodontitis With Urinary Albumin Excretion in Korean Adults With Diabetes	Cross-sectional study	Korea	547 patients (266 M, 281 F), mean age: 58.4	4.6	7.3	FPG level >= 126 mg/dL, HbA1c level >= 6.5%	Albuminuria - Albuminuria was defined as a urinary albumin to creatinine ratio (UACR) of 30 mg/g	CPI, frequency of tooth brushing and use of secondary oral products. Periodontitis is diagnosed when CPI ≥3	The mean UACR in patients with periodontitis (15.4 mg/g) was significantly higher than that in patients without periodontitis (11.0 mg/g) (p = 0.02). The prevalence of albuminuria (defined as UACR >30 mg/g) was significantly higher in patients with periodontitis (27.1%) compared with patients without periodontitis (16.6%) (p = 0.02).
2010	Hujoel et al.	Retinal and gingival hemorrhaging and chronic hyperglycemia	Cross-sectional study	USA	Not mentioned	n/r	n/r	Not specified	Retinal hemorrhaging	Dental hemorrhaging	Individuals in whom one or more in five gingival sites was hemorrhaging had a 57% increased odds for retinal hemorrhaging.
2016	Napora et al.	Prospective Analysis of the Relationship Between the State of Periodontal Tissues and Changes in Selected Cardiovascular Parameters in Patients with Type 2 Diabetes	Longitudinal study	Poland	119 patients (67 M, 52 F), age 39-82, median age: 62	8 (median)	n/r	Not specified	Cardiovascular diseases - The intima-media thickness (IMT) and the left ventricular mass index (LVMI) were determined by means of ultrasonography. After one year the examinations were repeated.	PI, PD, CAL, BOP	A significant positive correlation between the intima-media wall thickness (IMT) and the number of deep periodontal pockets (correlation coefficient (r) = 0.21, p=0.05) and a negative correlation between the IMT and the number of retained teeth (r = -0.26, p<0.05) .A significant correlation between the mean clinical attachment loss (CAL), changes of the IMT and the left ventricular mass index (LVMI) over a one year observation period (r = 0.25, p=0.011, respectively, r = 0.21, p=0.05)

2004	Noma et al.	Relationship between periodontal disease and diabetic retinopathy.	Cross-sectional study	Japan	73 patients	14.3	7.5	Not specified	Retinopathy - The severity of diabetic retinopathy was quantified according to the modified Early Treatment Diabetic Retinopathy Study (ETDRS) retinopathy severity scale	The severity of periodontal disease was quantified according to bone loss and then graded and evaluated	The risk of proliferative diabetic retinopathy was significantly higher in the presence of periodontal disease (odds ratio 2.80, p=0.036)
2016	Oliveira et al.	Self-Reported Periodontitis and Complications in Type 1 Diabetes Patients: A Brazilian Nationwide Survey	Cross-sectional study	Brazil	3,591 patients (1581 M, 2010 F), mean age: 21.2	9.6	9.2	Not specified	Retinopathy, Neuropathy, Nephropathy - Screenings for retinopathy, using fundoscopy; nephropathy, according to microalbuminuria; and foot examinations in patients with diabetes duration equal or greater than five years were noted when these procedures were performed within one year of the study assessment.	Periodontitis was evaluated through self-report after giving a brief explanation about what periodontitis is. The following question was asked to all patients ' Do you think you have gum disease?' Whenever available, medical records were checked for a possible diagnosis of periodontitis	Periodontitis patients presented ORs of 2.43 and 2.48 for microvascular and micro/macrovascular chronic complications, respectively. Regarding hospitalization, periodontitis patients showed increased ORs for hyperglycemia (2.76) and diabetic ketoacidosis (2.72) in comparison with patients with no periodontitis.
2005	Saremi et al.	Periodontal disease and mortality in type 2 diabetes.	Longitudinal study	Arizona, USA	628 patients (222 M, 406 F), mean age: 51	10.2	8.5	FPG level >= 200 mg/dl 2h after a 75-g oral glucose load	Mortality - Follow-up every 2 years. For all deaths, the underlying and contributing causes were assessed by review of clinical reports and reports of autopsy, medical examiner findings, and death certificates	Evaluation of the oral mucous membranes by visual and digital examination, an evaluation of alveolar bone loss score from a panoramic radiograph of all existing teeth, probing attachment levels.	The age- and sex-adjusted death rates for all natural causes expressed as the number of deaths per 1,000 person-years of follow-up were 3.7 for no or mild periodontal disease, 19.6 for moderate periodontal disease, and 28.4 for severe periodontal disease (p<0.01). In subjects with severe periodontal disease, the death rate from IHD was 2.3 (0.9–5.8) times as high (p=0.04) and the death rate from diabetic nephropathy was 8.5 (1.1– 65.0) times as high (p<0.01) as in those with less severe periodontal disease (no or mild and moderate periodontal disease combined), after adjustment for age, sex, and duration of diabetes. After adjustment for age, sex, duration of diabetes, HbA1c, macroalbuminuria, BMI, serum cholesterol concentration, hypertension, electrocardiographic abnormalities, and current smoking, subjects with severe periodontal disease had 3.5 times the risk of cardiorenal mortality (IHD and diabetic nephropathy combined) compared with those with less severe periodontal disease.
2007	Shultis et al.	Effect of periodontitis on overt nephropathy and end-stage renal disease in type 2 diabetes.	Longitudinal study	Arizona, USA	529 patients (168 M and 361 F), mean age: 45.1	6.2	7.9	FPG level >= 200 mg/dl 2h after a 75-g oral glucose load	Nephropathy - Urinary albumin was measured by nephelometric immunoassay. Urinary and serum creatinine were measured by a modification of the Jaffereaction. Serum creatinine values were calibrated to the Modification of Dietin Renal Disease (MDRD). ESRD was defined as the requirement for renal replacement therapy due to diabetes or death from diabetic nephropathy.	Alveolar bone loss was determined by scoring percent bone loss from the cementoenamel junction to the apex at the deepest point on the mesial or distal surfaces of each tooth present, excluding third molars. Periodontitis was classified in order of severity by the number of missing teeth and percent alveolar bone loss.	After adjustment for age, sex, diabetes duration, BMI, and smoking, the incidences of macroalbuminuria were 2.0, 2.1, and 2.6 times as high in individuals with moderate or severe periodontitis or those who were edentulous, respectively, compared with those with none/mild periodontitis (p=0.01). Incidences of end-stage renal disease in individuals with moderate or severe periodontitis or in those who were edentulous were 2.3, 3.5, and 4.9 times as high, respectively, compared with those with none/mild periodontitis (p=0.02).

2017	Song et al.	Association between the number of natural teeth and diabetic retinopathy among type 2 diabetes mellitus: The Korea national health and nutrition examination survey	Cross-sectional study	Korea	2078 patients (1140 M, 938 F), mean age: 58.7	5.7	7.4	FPG level was > 126mg/dL	Diabetic retinopathy was identified as defined by the ETDRS severity scale according to the presence of microaneurysms (MAs), hemorrhages, hard exudates (HEs), cotton wool spots, intraretinal microvascular abnormalities, venous beading, and retinal new vessels. A diabetic retinopathy severity score was assigned to each eye according to the modification of the Airlie House Classification system. The level of retinopathy was graded based on the worse eye.	Tooth count, frequency of tooth brushing per day, use of secondary oral products	Type 2 diabetes with fewer teeth were more likely to have diabetic retinopathy ($p<0.001$). After adjusting for age, sex, BMI, smoking, drinking, exercise, hypertension, diabetes duration, and HbA1c, patients with type 2 diabetes with <20 teeth had an 8.7-fold risk of having vision-threatening diabetic retinopathy when compared with those with ≥28 teeth (95% CI: 2.69–28.3).
2012	Southerland et al.	Periodontitis and diabetes associations with measures of atherosclerosis and CHD	Cross-sectional study	USA	6048 patients, aged 52-74	n/r	n/r	FPG level >= 126 mg/dl, a non-fasting level >= 200 mg/dl	Atherosclerosis (IMT and shadowing) and CHD - Sub-clinical atherosclerosis was defined as mean carotid artery intimal–medial wall thickness of at least one millimeter (IMT ≥ 1.0 mm), as assessed by B-mode ultrasound. Presence and number of arterial plaque lesions were also evaluated by B mode ultrasonography along with scoring the presence of calcification by acoustic shadowing which is indicative of more advanced atherosclerotic lesions. Prevalent coronary heart disease (CHD) was defined as a prior myocardial infarction by electrocardiogram, a history of physician diagnosed myocardial infarction, or a prior coronary reperfusion procedure at either visit 1 or visit 2.	PD, CAL. Periodontitis was defined as extent of PD ≥ 4 mm and CAL ≥ 3 mm	Individuals with diabetes and with severe periodontitis were found to be significantly more likely to have IMT > 1 mm [OR = 2.2], acoustic shadowing [OR = 2.5], and CHD [OR = 2.6] compared to those without diabetes or periodontal disease ($p<0.001$). (All models were adjusted for gender, age, race/center, LDL and HDL cholesterol, BMI, triglycerides, hypertension, smoking, income and education).
1996	Thorstensson et al.	Medical status and complications in relation to periodontal disease experience in insulin-dependent diabetics.	Case control study	Sweden	78 patients (39 case-control pairs) (42 M, 36 F), mean age: 55.8	24	n/r	Not specified	Urine proteinuria, Retinopathy (using ophthalmoscopy or photography of fundus), Cardiovascular abnormalities, Foot ulcer, Death	A full-mouth clinical and radiological dental examination	In the follow-up study, 21 cases and 11 controls had proteinuria of varying severity, and the difference between 2 groups was significant ($p<0.05$). Number of cardiovascular complications developed during the follow-up study in the case group was significantly higher than that of the control group ($p<0.05$).

Among the 14 articles, 8 studies investigated microvascular complications [11,12,14-16,18,20,23] and 3 reported macrovascular complications [13,19,21]. Oliveira et al. and Thorstensson et al. investigated both micro- and macrovascular complications and Saremi et al. used mortality as a primary outcome for diabetic complication [17,22,24]. Among the 8 articles related to microvascular complications, 4 reported diabetic retinopathy [12,15,16,18], 2 reported diabetic nephropathy [14,23] and 2 reported diabetic neuropathy [11,20].

6.1.3. Characteristics of patients

A total of 8,969 patients with DM were included in 13 studies, ranging from 73 to 6048 participants (Hujoel et al. did not report the number of participants) [11-24]. The majority of the publications presented the mean age and one study reported the median age of the study population [21]. Three studies did not report age [15,16,19]. The calculated mean age of participants was 55.8 years (range 35 to 82 years), 46.2% of the participants were male and 53.8% female (Hujoel et al. and Southerland et al. did not report gender distribution) [15,19].

Patients included in the studies were diagnosed with type 1 (n=3591) or type 2 (n=5378) DM. The majority of studies used the American Diabetics Association (ADA) or World Health Organization (WHO) criteria for the diagnosis of DM, which refer to a fasting blood glucose level of ≥ 126 mg/dL [14,18,20], or a plasma glucose concentration ≥ 200 mg/dl two hours after oral glucose load [22,23], or both [11,13,19]. Six studies did not report the diagnostic criteria of DM and enrolled patients currently presented for DM treatment at a medical clinic or were previously diagnosed with DM by a physician [12,15-17,21,24]. Han et al. included patients with HbA1c levels ≥ 6.5% in the data analysis [14]. The duration of DM ranged from 4.6 to 24 years and the mean HbA1c levels across studies from 7.3 - 9.2%.

The periodontal status of patients was evaluated by the Community Periodontal Index of Treatment Needs (CPITN) [11,12,14] or the case definition of the American Association of Periodontology - Centre for Disease Control and Prevention (AAP-CDC) [13,19,21]. The number of teeth as a surrogate for the diagnosis of periodontitis was used by Song et al. and Collin et al. [18,20]. A combination of the number of teeth and radiographic measurements of the alveolar bone level was used by Saremi et al., Shultis et al., Noma et al. and Thorstensson et al. [16,22-24]. Oliveira et al. used a self-reported questionnaire for the diagnosis of periodontal

conditions [17] and Hujoel et al. considered “dental hemorrhaging” as a sign of gingivitis [15].

6.1.4. Quality of included studies

Nine studies were rated as of good quality with a low risk of bias (Table 2) and provided adequate information about the study objectives, population and how the exposures and outcomes were measured [11,12,14,18,19,21-24]. The quality of 3 publications was rated as fair (Collin et al., 2000, Franek et al., 2010, Oliveira et al., 2016) [13,17,20] and 2 publications were rated as poor [15,16]. Reasons were failed to report methods and to blind assessors to the exposure status of participants.

6.2. Association of diabetic complications and periodontal disease

A summary of reported outcomes is presented in Table 3. In 3 studies involving 2437 participants, the risk (odds ratio) for diabetic retinopathy in patients with periodontitis was reported to be 2.8-8.7 times higher compared to patients with DM and no periodontitis [12,16,18]. The odds ratio of 8.7 was calculated by Song et al.

	Question number according to NIH Quality Assessment Tool (Appendix 1)															
Author (Year)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total	Rate
Abrao et al., 2010	Y	Y	Y	Y	N	N	N	Y	Y	Y	Y	NR	NA	Y	9	GOOD
Amiri et al., 2014	Y	Y	Y	Y	Y	N	N	Y	N	NR	Y	NR	NA	Y	8	GOOD
Collin et al., 2000	Y	Y	N	Y	N	Y	Y	N	NR	NR	Y	NR	N	Y	7	FAIR
Franek et al., 2010	Y	Y	Y	Y	N	N	N	Y	Y	NR	Y	NR	NA	N	7	FAIR
Han et al., 2015	Y	Y	Y	Y	N	N	N	Y	Y	NR	Y	NR	NA	Y	8	GOOD
Hujoel et al., 2010	Y	NR	Y	Y	N	N	N	N	NR	NR	NR	NR	NA	N	3	POOR
Napora et al., 2016	Y	Y	Y	Y	N	N	Y	N	Y	Y	Y	NR	N	Y	9	GOOD
Noma et al., 2004	Y	NR	Y	NR	N	N	N	N	Y	NR	Y	NR	NA	N	4	POOR
Oliveira et al., 2016	Y	Y	Y	Y	Y	N	N	N	Y	N	Y	NR	NA	N	7	FAIR
Saremi et al., 2005	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	13	GOOD
Shultis et al., 2007	Y	Y	Y	Y	N	Y	Y	Y	Y	NR	Y	NR	Y	Y	10	GOOD
Song et al., 2017	Y	Y	Y	Y	N	N	N	Y	Y	NR	Y	NR	NA	Y	8	GOOD
Southerland et al., 2012	Y	Y	Y	Y	N	N	N	Y	Y	NR	Y	NR	NA	Y	8	GOOD
Thorstensson et al., 1996	Y	Y	N	N	Y	Y	NR	N	Y	Y	NR	Y			7	GOOD
Y: Yes; N: No; NR: Not Reported; NA: Not Applicable																

Table 2: Quality assessment of included studies

Complication	Publication	Total number of participants	Risk	ORs
Retinopathy	Amiri et al. (2014), Noma et al. (2004), Song et al. (2017),	2437	Periodontitis, tooth loss	2.8 - 8.7
Retinal hemorraging	Hujoel et al. (2010)	n/r	Gingivitis	1.57
Neuropathy	Abrao et al. (2010), Collins et al (2000)	244	Periodontis, tooth loss	3.2 - 6.6
Nephropathy	Han et al. (2015), Saremi et al. (2005), Shultis et al. (2007), Thorstensson et al. (1996)	1782	Periodontitis	1.9 - 8.5
Macrovascular complications	Napora et al. (2016), Saremi et al. (2005), Southerland et al. (2012), Thorstensson et al. (1996)	6873	Periodontitis	1.28 - 17.7
Micro/macrovascular complications	Oliveira et al. (2016)	3591	Periodontitis	2.43 - 2.48
Death	Saremi et al. (2005)	628	Periodontitis	2.3 - 8.5

Table 3: Summary of odd ratios of diabetic complications in included studies

who used tooth loss as a diagnostic criteria for periodontitis and Amiri et al. and Noma et al. used probing pocket depth to determine the presence of periodontitis [12,16,18]. In two studies with a total of 244 patients the calculated odds ratio for diabetic neuropathy in patients with periodontitis ranged from 3.2 to 6.6 compared to patients with DM and no periodontitis [11,20]. Patients with periodontitis and diabetes (n=1782) carried a 1.9 to 8.5 higher risk for developing diabetic nephropathy compared to patients with diabetes and no periodontitis [14,22-24]. Several studies demonstrated that not only the presence but also the severity of periodontal disease was associated with the risk of developing diabetic complications and patients' glycaemic control. According to Shultis et al., the incidences of macroalbuminuria were 2.0, 2.1, and 2.6 times higher in individuals with moderate, severe periodontitis and those who were edentulous, respectively, compared with those with no or mild periodontitis (p=0.01)) [23].

For cardiovascular complications an odds ratio of 1.3 to 2.6 was reported for patients with periodontitis compared to patients without periodontitis [19,21,22]. Thorstensson et al. gave an odds ratio of 17.7 over a 6-year follow-up period for

cardiovascular complications in patients with periodontitis compared to those without periodontitis [24]. Franek et al. reported that patients with DM and periodontitis had a higher left ventricular mass (LVM) (238.6g) and left ventricular mass index (LVMI) (95.2g/m^2) compared with patients who did not suffer from periodontal disease (170.3g and 63.7g/m^2, respectively) [13]. Oliveira et al. reported 2.4 - 2.5-fold risk of developing any micro- and/or macrovascular complications in patients with DM and periodontitis compared to patients with DM and no periodontitis [17].

Saremi et al. studied 628 patients and calculated a death rate of 3.7 per 1000 person-years for no or mild periodontal disease, of 19.6 for moderate periodontal disease, and of 28.4 for severe periodontal disease. In patients with severe periodontal disease death rates per 1000 person-years were 2.3 times higher for ischemic heart disease, 8.5 times higher for diabetic nephropathy and 3.5 times higher for cardiorenal mortality compared to patients with moderate or mild forms of periodontal disease [22].

Chapter 7: Discussion

Although the included studies showed considerable heterogeneity, they consistently reported a higher risk for diabetic complications in patients with periodontitis compared to periodontal healthy patients. In addition, to the evidence from observational studies and trials that demonstrated the association between periodontitis and higher HbA1c levels, we conclude from this review that patients with DM should be deliberately screened and referred to therapy if necessary, to reduce the risk for diabetic complications.

Periodontal disease is characterized by an immune reaction in response to accumulating bacterial pathogens on tooth surfaces and therefore, share the same inflammatory pathology as many chronic diseases including DM [25]. Although the observed association between diabetic complications and periodontitis may be multifactorial, hyperglycemia is likely the main mechanism. Periodontitis is a causal factor for elevated HbA1c levels in patients with diabetes and these levels can be reduced by an anti-infective periodontitis therapy [26,27]. Prolonged cell exposure to high glucose concentrations results in intracellular hyperglycemia, especially in endothelial vascular cells with low glucose transport rates. Therefore, endothelial vascular cells become a main target for hyperglycaemic damage by the increased production of reactive oxygen species and altered gene expression that may lead to pathological changes characteristic for microvascular complications [2,28]. For macrovascular complications periodontal bacteria and their products, together with inflammatory cytokines produced locally in the inflamed periodontal tissues, may contribute to the upregulation of systemic inflammation [29]. By continuously exposing the vascular walls with bacteria and inflammatory cytokines, periodontitis contributes to atherogenesis, thereby exposing patients with a higher risk of cardiovascular events including myocardial infarction, peripheral artery disease, stroke and heart failure [30,31].

Overall studies included in this systematic review provided evidence for higher risks for diabetes complications in patients with periodontal disease compared to patients with diabetes who lacked periodontal disease. However, the observational studies included in this review showed considerable heterogeneity with respect to the number of patients and diagnostic criteria used for the classification of periodontitis and diabetes cases. The diagnostic criteria for periodontitis included the number of

teeth, clinical measurements of periodontal health or radiography techniques. For future studies the coherent reporting of diagnostic criteria and relevant medical information is mandatory. The various periods from DM diagnosis to data sampling ranging between 4.6 and 24 years is likely to affect the reported outcomes, as the onset and severity of periodontal disease over this period was not recorded in these epidemiological studies and may change during the course of DM. The participants in studies included in this review were from a variety of different ethnic backgrounds, hence the results are likely to be generalized to global populations. Although, barriers appear high with respect to follow-up times, number of patients included and drop-outs, high-quality trials with clinical endpoints are required to confirm the observed associations between periodontitis and diabetic complications. Therefore, for future studies, a coherent reporting of diagnostic criteria, relevant medical information as well as well-planned randomized clinical trials are mandatory to further understand the link between periodontitis and diabetic complications.

Multiple recent reviews summarized the evidence for the association between periodontitis and DM establishing a strong association and causal link between periodontitis and DM, in which DM increases the risk for periodontitis and periodontitis negatively affects glycaemic control [6]. Periodontal bacteria and their products, together with inflammatory cytokines and other mediators produced locally in inflamed periodontal tissues, enter the circulation and augment the systemic inflammatory burden [29]. The continuous exposure of the vascular endothelium with bacteria and inflammatory cytokines progress atherogenesis and thereby increasing the risk for cardiovascular events including myocardial infarction, peripheral artery disease, stroke and heart failure [30,31]. In this regard, it has been shown that individuals with periodontitis and diabetes have elevated levels of circulating tumor necrosis factor-α, C-reactive protein and markers of oxidative stress, which were significantly reduced following periodontal treatment [32]. In people who do not have diabetes, the presence of severe periodontitis is associated with a 19-33% increased risk of developing diabetes compared to people who are periodontally healthy [33]. Results from large-sample studies demonstrated that periodontitis was significantly associated with poorer glycaemic control as measured by HbA1c levels. A number of trials that have been conducted over the last decades investigating the impact of periodontal treatment on glycaemic control in people with diabetes found that periodontitis treament resulted in a significant reduction of HbA1c levels of roughly

0.3 to 0.4% in the short term (3-4 months) [34-36]. In contrast to those recent reviews summarizing the link between periodontitis and DM, this current review highlighted relevant associations between oral health and diabetic complications and has also drawn attention to major limitations of the available data linking periodontal disease and diabetic complications.

Chapter 8: Conclusion

This review systematically addressed current epidemiological data providing evidence that periodontitis is associated with a higher risk for developing diabetic complications compared to patients with no periodontitis. Despite current knowledge gaps this review adds to the current evidence demonstrating a link between poor oral health and diabetes mellitus and proposes oral health care to be an essential component of diabetes management. We suggest, well-planned intervention trials aimed to investigate the impact of periodontal treatment on the development of diabetic complications to potentially reduce micro- and macrovascular complications in people with DM.

Declarations of interest

No potential conflicts of interest relevant to this article were reported.

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Authors' contribution

All authors contributed to the study conception and design. Data collection and analysis were performed by AN, SG and JE. The first draft of the manuscript was written by AN and all authors commented and critically revised on the previous versions of the manuscript. All authors read and approved the final manuscript.

Appendix 1. Questions for NIH Quality Assessment Tool

Questions for Observational Cohort and Cross-sectional studies	
1	Was the research question or objective in this paper clearly stated?
2	Was the study population clearly specified and defined?
3	Was the participation rate of eligible persons at least 50%?
4	Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?
5	Was a sample size justification, power description, or variance and effect estimates provided?
6	For the analyses in this paper, were the exposures of interest measured prior to outcomes being measured?
7	Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?
8	For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome?
9	Were the exposure measures clearly defined, valid, reliable, and implemented consistently across all study participants?
10	Were the exposures assessed more than once over time?
11	Were the outcome measures clearly defined, valid, reliable, and implemented consistently across all study participants?
12	Were the outcome assessors blinded to the exposure status of participants?
13	Was loss to follow-up after baseline 20% or less?
14	Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposures and outcomes?
Questions for Case-Control Studies	
1	Was the research question or objective in this paper clearly stated and appropriate?
2	Was the study population clearly specified and defined?
3	Did the author include a sample size justification?
4	Were controls selected or recruited from the same or similar population that gave rise to the cases (including the same timeframe)?
5	Were the definitions, inclusion and exclusion criteria, algorithms or processes used to identify or select cases and controls valid, reliable and implemented consistent across all study participants?
6	Were the cases clearly defined and differentiated from controls?
7	If less than 100% of eligible cases and/or controls were selected for the study, were the cases and/or controls selected randomly from those eligible?
8	Was there use of concurrent controls?
9	Were the investigators able to confirm that the exposure/risk occurred prior to the development of the condition or event that defined a participant as a case?
10	Were the measures of exposure/risk clearly defined, valid, reliable, and implemented consistently across all study participants?
11	Were the assessors of exposure/risk blinded to the case or control status of participants?
12	Were the key potential confounding variables measured and adjusted statistically in the analyses? If matching was used, did the investigators account for matching during study analysis?

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PART C:

RESEARCH PROJECT

Chapter 9: Experimental design

9.1. Rationale of the research project

In the recent decades, effects of chronic local inflammatory diseases on systemic conditions have caught the attention of medical practitioners because of their enormous impacts on the management of various conditions. Gingivitis and periodontitis, which belong to the spectrum of periodontal diseases, are in the spotlight of research interest due to their chronic nature and high frequency among the world population. Periodontal disease is initiated by the pathogenic bacterial accumulation in dental plaque biofilm. Constantly forming bacterial deposits on the tooth surface lead to microbial dysbiosis and eventually cause chronic inflammatory responses with various levels ranging from a reversible stage of gingivitis to a more destructive irreversible stage of periodontitis [1,2]. Periodontitis has a prevalence of 45-50% in adults and the frequency rises higher and higher in older population. Severe periodontitis is the dominant cause of tooth loss, altered speech, nutritional compromise and severely decrease people's life quality [1].

Multiple studies have demonstrated the significant association between periodontitis and various chronic non-communicable systemic diseases such as respiratory diseases, immune-deficiencies, cardiovascular disorders, and type-2 diabetes mellitus [1-5]. A two-way relationship between type-2 diabetes mellitus and periodontitis has been well-reported in the literature. More severe periodontal tissue destruction is found in those with type-2 diabetes mellitus in comparison with those without and the other way around, individuals with type-2 diabetes mellitus tend to have poorer glycaemic control in the presence of periodontitis [1,2]. The details of the two-way relationship were demonstrated in Part A of this thesis. Periodontitis and type-2 diabetes mellitus are 2 non-communicable chronic conditions that effect the course and outcomes of each other and their interaction can contribute in increasing the risk of the development of multiple ailments and complications. Severe forms of periodontal disease have been proven to be associated with poor glycaemic presented with higher HbA1c levels, and the increased risk of developing diabetic complications [6]. The systematic review in Part B have demonstrated that the presence as well as the severity of periodontal disease significantly raised the risk of the development of micro- and macrovascular complications in patients with type-2 diabetes mellitus [7]. Periodontitis was also recently shown as an independent risk factor for increased

HbA1c levels in people with type-2 diabetes mellitus undertaking a physical activity lifestyle intervention [8].

Despite the well-established and significant association between periodontal disease and type-2 diabetes mellitus, epidemiological data for the prevalence and severity of periodontal diseases in Australian people with type-2 diabetes mellitus is lacking. As far as our knowledge up to date, there is no data in Australian population on the frequency of gingivitis in people with type-2 diabetes mellitus. Representative epidemiological data is mandatory to address public health relevance in this country. High recognition and awareness of the negative effects of periodontal diseases on diabetes conditions among medical practitioners as well as people with type-2 diabetes mellitus could contribute in improving treatment and managing outcomes and quality of life for diabetic patients. Moreover, the usage of current referral pathways between diabetes clinics and dental clinics and their potential restraints needs to be addressed. This study project hopes to provide the necessary data and help establish a practical and feasible referral pathway in order to improve diabetes care in Australia. The study is a prospective design that involves a single group of patients who will undertake specific tests for the study which will be analyzed in conjunction with their existing clinical outcome data.

9.2. Aims of the research project

This research project aimed to determine the severity and frequency of periodontal disease, which include periodontitis and gingivitis, among people with type-2 diabetes mellitus and diabetic complications. In particular, we aimed to:

- Record the frequency and severity of periodontal disease (gingivitis and periodontitis) in patients with diabetes
- Record the frequency and severity of periodontal disease (gingivitis and periodontitis) in patients with diabetic complications or high HbA1c levels
- Validate a diagnostic tool for the screening of periodontal disease by non-dental health care practitioners
- Establish and evaluate a referral pathway between diabetes clinics and dental clinics

9.3. Hypotheses of the research project

We hypothesize a high prevalence of periodontal disease exists in individuals with diabetes in Australia and especially in those with higher HbA1c levels.

Chapter 10: Materials and Methods

10.1. Ethics

This research study titled "Frequency and severity of periodontal disease in Australians with type 2 diabetes mellitus: Building representative epidemiological data and validation of an oral health screening tool for non-dental personnel" was approved by the Ethics Review Committee (Royal Prince Alfred Hospital Zone) of Sydney Local Health District (SLHD) under protocol number X17-0351 and HREC2019/ETH07425. The conduct of this study at Royal Prince Alfred Hospital (RPAH) has been authorized by SLHD.

Informed written consents of the study participants were obtained prior to any procedures. Participants were aware that they could withdraw from the study at any time and were ensured that they are not obligated to be part of the study and that their on-going treatment would not be affected if they choose not to participate.

10.2. Subjects

We recruited participants for the study from the RPAH Diabetes Centre with the following criteria.

Inclusion criteria:

- A proven diagnosis of T2DM based on Australian Diabetes Society (ADS) criteria [9]:
  - HbA1c ≥ 6.5% (48 mmol/mol)
  - Fasting glucose ≥ 7.0 mmol/l
  - Random glucose ≥ 11.1 mmol/l
  - On a 75g oral glucose tolerance test: fasting glucose ≥ 7.0 mmol/l or 2 hour glucose ≥ 11.1 mmol/l
- Being treated at RPAH Diabetes Centre over a period of at least 10 months by their treating clinician at the Diabetes Centre
- Having more than 10 teeth

Exclusion criteria:

- Patients with prosthetic cardiac valve or prosthetic material for cardiac valve repair; history of infective endocarditis; congenital heart disease; cardiac transplantation with development of valvulopathy; rheumatic heart disease (Aboriginal and Torres Strait Islanders only)

- Patients unable to provide informed consent

After patients had signed their informed consent forms, an oral assessment appointment were scheduled at the RPAH Oral Health Clinic, which located on Level 4 of Queen Elizabeth II Building, RPAH.

10.3. Laboratory assessment

Patients at RPA Diabetes Centre were screened routinely for diabetic complications during a single 3-hour visit [10]. The screening process was conducted by an endocrinologist and 2 diabetes specialist nurses. The procedures included tests for retinopathy, nephropathy, peripheral neuropathy, autonomic neuropathy, foot problems and peripheral artery disease.

To diagnose diabetic retinopathy, the clinicians examined:

- pinhole visual acuity
- intraocular pressure
- direct ophthalmoscopy with pupils dilated

For nephropathy, they examined:

- dipstick detection of proteinuria
- measurement of microalbuminuria or proteinuria by a 2.5-hour urine collection when 300 ml of water is drunk after an initial 30-minute rest
- measurement of blood pressure

The examination for neuropathy and foot problems included:

- recording of symptoms
- testing of ankle reflexes
- examination of feet for high risk characteristics
- measurement of vibration-perception threshold with a biothesiometer
- measurement of thermal discrimination threshold by a thermothesiometer

The examination for peripheral vascular disease included:

- recording of symptoms
- examination of foot pulse

For autonomic neuropathy, these examinations were conducted:

- postural changes in blood pressure
- heart-rate variation in response to deep breathing
- heart-rate variation during Valsalva maneuver.

Other related complications were recorded based on patients' self-record, symptoms and their previous medical records. These included any cardiovascular abnormalities and events such as stroke, heart failure, coronary heart disease, hypertensive heart disease, peripheral artery disease, thromboembolic disease, etc; cerebrovascular disease, and erectile dysfunction (if male). Evidence of non-alcoholic fatty liver disease (NAFLD) was identified based on ultrasound or liver enzyme profiles in the absence of excessive alcohol intake history.

All diabetes patients attending to RPAH Diabetes Centre had a comprehensive medical history record including their diabetic complication status. An electronic dataset was created for each participant including: the patient's MRN, a unique identifier, age, gender, diabetes duration, most recent HbA1c level and date, documented diabetes complications (retinopathy, nephropathy, neuropathy, CVD (cardiovascular disease), cerebrovascular disease (CrVD), peripheral vascular disease (PVD), erectile dysfunction (if male) and NAFLD), documented comorbidities and any current medications.

At RPAH Diabetes Centre, the patients were routinely assessed for HbA1c, fasting lipids and subsets, urea, electrolytes and creatinine, estimated glomerular filtration rate (eGFR), full blood count (FBC), and liver function and enzyme tests. This information was obtained from the patient's medical records, along with information on main medical therapies (statins, ACEi/ARBs, and insulin therapy).

10.4. Periodontal examination

Approximately 25 minutes per patient were required for the periodontal examination by a qualified dental practitioner, including filling out the questionaires, periodontal parameter measurements and dental plaque sample collections. The oral assessment took place at RPAH Oral Health Clinic. A reclining dental chair and disposable dental instrumentation was used for patient comfort during the examination. An electronic Florida Probe system (Florida Probe Co, USA) was used to record periodontal measurements directly to a computer file.

To formally assess periodontal conditions, the dental practitioner recorded periodontal probing depth (PPD), clinical attachment loss (CAL) and bleeding on probing (BOP) in each subject. The periodontal measurements were done using a pressure calibrated periodontal probe (Florida Probe-System) at 6 sites per tooth. For comparability, the existence of 'no or mild' or 'moderate' or 'severe' periodontal

conditions were determined by the clinical classification of periodontitis introduced by the Centres for Disease Control and Prevention and the American Academy of Periodontology (CDC-AAP) [11]. 'No or mild' periodontitis was defined if neither 'moderate' nor 'severe' periodontitis was found at the time of examination. Subjects were classified as having 'moderate' periodontitis if they had ≥ 2 interproximal sites with CAL ≥ 4 mm, or ≥ 2 interproximal sites with PPD ≥ 5 mm (not at the same tooth). Patients were rated as having 'severe' periodontitis if they had ≥ 2 interproximal sites with CAL ≥ 6 mm and ≥ 1 interproximal site with PPD ≥5 mm (not at the same tooth). The presence of gingivitis was noted in people with diabetes classified as having no periodontitis yet showing bleeding on probing positive sites, signifying local gingival inflammation.

Oral plaque deposits were also collected from the tooth surfaces for future analysis. Dental plaque samples were collected from the supra-gingival and sub-gingival surfaces of the tooth with the deepest periodontal pocket and then from the corresponding contralateral tooth. For supra-gingival plaque collection, the dental practitioner used the dental probe, scraped along the supra-gingival surface of the tooth to gather the plaque, then wiped it onto a sterile paper-point and dropped into a labelled sterile sealable eppendorf tube. To collect sub-gingival plaque, the examiner first thoroughly wiped the supra-gingival surface of the tooth with gauze to remove supra-gingival plaque. They then inserted the dental probe into the deepest pocket of the tooth and moved it along the pocket to collect the sample. This plaque sample was also wiped on a sterile paper-point and placed into an eppendorf tube until analysis.

10.5. Validation of a non-dental workforce diagnostic tool

Two questionnaires were given to the patients. The first one was a routine dental history questionnaire based on WHO Oral Health Questionnaire for Adults, which included questions pertaining to the dental treatment history, oral health, oral-related habits and diets of the subject. The second one was the "Parodontitis Selbsttest (Periodontitis Self-test)" introduced by "Deutsche Gesellschaft für Parodontologie (German Society for Periodontology)". It was based on a series of 9 questions related to clinical signs of periodontal disease. These questions included the participant's perception of their overall oral health as well as specific questions about their oral health habits. The likelihood of periodontitis by this screening survey was calculated by adding the number of positive answers. This questionnaire was used in the present

study for a screening and a preliminary diagnosis of periodontal conditions in patients with diabetes. It would suggest the individual to attend to a dental clinic for a full oral health examination if signs and symptoms of periodontal disease were detected. Analysis of the relationship/association between the questionnaire and the gold standard dental examination were undertaken, to determine its sensitivity and specificity in an Australian context.

10.6. Intra-oral photography

Intra-oral photos of each participant's mouth were taken by a clinician with the patient's lips and cheeks retracted. A total of 4 photos were taken: (1) The participant closed their upper and lower jaws, one photo were taken with a high-quality digital camera, the second photo were taken with a compact camera (optional to the participant's comfort, the camera was purchased for the study and had no internet connectivity). (2) For the second photograph, the participant brushed their front teeth with a new toothbrush for 5 seconds and immediately afterwards the two photos were taken (one with high-quality digital camera and one with a compact camera). This photography setting was selected to provoke bleeding of the gums, which is an early sign of gum disease and may be a good indicator of disease in the following computer-assisted diagnostic procedures. Photos taken by the compact camera was to simulate a low resolution picture taken with a low cost camera that may be available in general practitioner offices or in remote areas. The photos were used to validate another possible diagnostic tool that could be utilized by non-dental personnel. This preliminary diagnostic tool was an image processing application that can classify intra-oral images as being 'healthy' or 'unhealthy'. This image-processing application could be combined with the survey and algorithm to develop an efficient periodontal diagnostic tool, to be used by non-dental personnel, and validated with the clinical data recordings from this study.

Only the unique identifier were used for the study subjects with all information provided. All those information were collected via a case report form, which were securely kept in in enclosed cabinet at the Oral Health Clinic RPAH. Participant information were then transferred and stored in REDCAP.

10.7. Referral pathway

After the periodontal assessment, if the patients were diagnosed with periodontal disease (including gingivitis and periodontitis), they would be given a written letter of referral along with a copy of the periodontal charting by the Florida Probe in order to seek for appropriate treatment. The dental examiner would also briefly explain to them about their conditions and answer to their main concerns. They might then pursue to get the treatment at a dental clinic of their choice. Three months after the assessment, the participants were contacted by telephone to evaluate with a structured questionnaire the oral care they had received. In case the participant hadn't received any treatment, she or he would be contacted for a second interview in three months.

Although these oral health assessment tasks in no way constitute as an oral examination, urgent oral issues or conditions may be noticed during the tasks and should be referred to a dental practice for appropriate management. If urgent issues, such as questionable lesions or abscess, are noticed, the dental practitioner would provide the participant with a written letter of referral and briefly explain the concern. All other non-urgent oral issues noticed should be verbally explained to participant, advising they seek a professional dental examination.

10.8. Statistical analysis

The proportions of periodontal disease and diabetes complications in the population were calculated and logistic regression analysis was used to analyse independent samples. The test validity was calculated by comparing scores from the survey with the periodontal clinical parameter recorded by the Florida Probe (gold-standard), to determine specificity and sensitivity. Additionally, positive and negative predictive values in the populations were studied. Univariate and multivariable analysis was undertaken using NCSS/SPSS.

Chapter 11: Results

11.1. Descriptive characteristics of included participants

Nine patients (8 male, 1 female; 88.9% male) with an average age of 67.2 ± 5.7 (ranging from 57 to 74) participated in the study. Their demographic characteristics are depicted in Table 4. The average BMI of the participants was 32.4 ± 5.8 kg/m^2, ranging from 26.3 to 46.5 kg/m^2. Their mean time since being diagnosed with type-2 diabetes mellitus was 17.8 ± 11.0 years, ranging from 5 to 40 years. Seven (77.8%) participants reported that they finished college/university, while 2 (22.2%) finished a postgraduate degree.

Table 4. Demographic characteristics of included participants (n=9)

	Mean ± SD	
Age, years	67.2 ± 5.7	
Gender, male (%)	8 (88.9%)	
BMI, kg/m2	32.4 ± 5.8	
Diabetes duration, years	17.8 ± 11.0	
Educational level (%)	***Finished college***	***Finished postgraduate degree***
	7 (77.8%)	2 (22.2%)

Participants' diabetic complications are described in Table 5. Among the 9 participants included in the study, 5 (55.6%) were diagnosed with diabetic nephropathy, 4 (44.4%) with diabetic neuropathy, 2 (22.2%) with retinopathy, 2 (22.2%) with cataract, 3 (33.3%) with any kind of cardiovascular/ischemic heart disease, 1 (11.1%) with cerebrovascular disease and 1 (11.1%) with erectile dysfunction. The distribution of the number of diabetic complications per participants is presented in Figure 12. A mean HbA1c level of 8.3 ± 1.1 (%), ranging from 6.7 to 10.1%, was measured in participants' blood samples. Other laboratory parameters measured are depicted in Table 6.

Table 5. Diabetic complications among 9 included participants. Neuro: neuropathy; Retino: retinopathy; Nephro: nephropathy; IHD: ischemic heart disease; CVD: cardiovascular disease; CrVD: cerebrovascular disease; PVD: peripheral vascular disease; ED; erectile dysfunction; NAFLD: non-alcoholic fatty liver disease.

Patient	Neuro	Retino	Nephro	Cataract	IHD/ CVD	CrVD	PVD	ED	NAFLD	Total number of diabetic complications
1			x		x	x		x		4
2										0
3		x	x							2
4	x									1
5		x	x		x					3
6	x			x						2
7	x		x		x					3
8				x						1
9	x		x							2
Total	4	2	5	2	3	1	0	1	0	18
%	44.4	22.2	55.6	22.2	33.3	11.1	0	11.1	0	

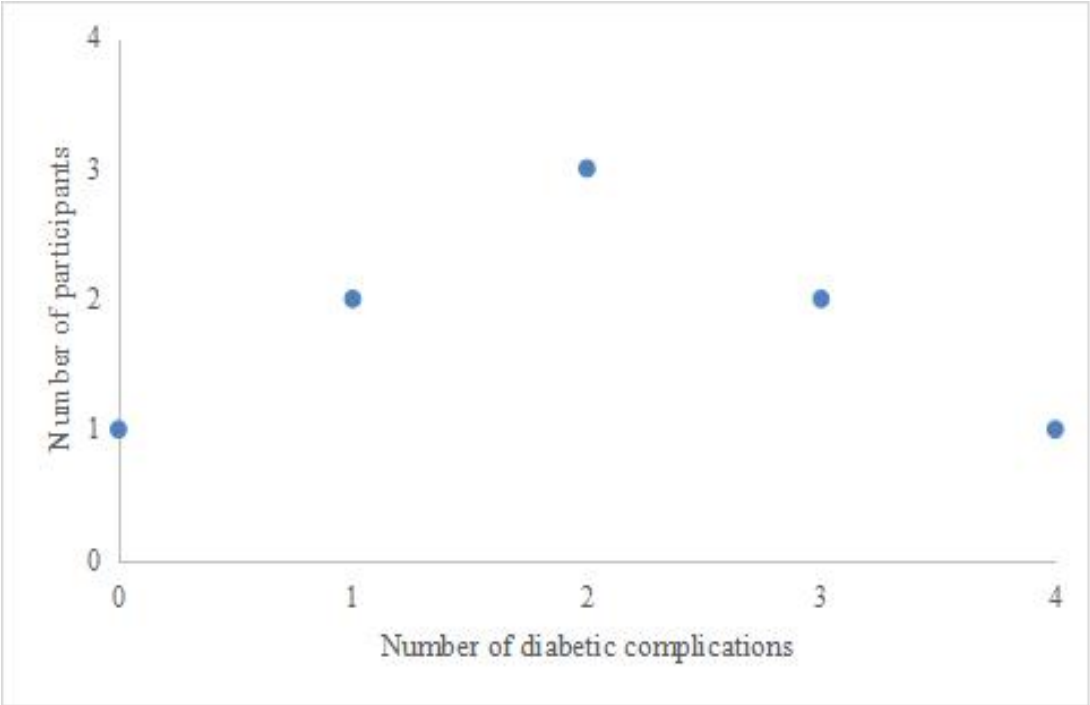

Figure 12: Distribution of participants based on their number of diabetic complications

Table 6. Laboratory parameters of included participants (n=9). HbA1c: hemoglobin A1C; HDL: high-density lipoprotein; LDL: low-density lipoprotein; ALP: alkaline phosphatase; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma-glutamyl transferase; RBC: red blood cell; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean cell hemoglobin concentration; RDW: red cell distribution width; WBC: white blood cell. All data presented as mean ± SD. *Data from 7 participants.

HbA1c level, %	8.3 ± 1.1	**Bilirubin (µmol/l)**	10.2 ± 3.0
Total cholesterol, mmol/l	3.6 ± 1.5	**Globulin (g/l)**	28.1 ± 3.9
Triglycerides, mmol/l	2.1 ± 1.6	**Albumin (g/l)**	43.2 ± 4.7
HDL, mmol/l	1.1 ± 0.2	**Hemoglobin (g/l)***	140.1 ± 25.0
LDL, mmol/l	1.4 ± 0.5	**RBC ($x10^{12}$/l)***	4.9 ± 0.8
Urea, mmol/l	9.5 ± 3.6	**Hematocrit***	0.4 ± 0.1
eGFR, ml/min/1.73 m^2	60.7 ± 18.0	**MCV (fl)***	87.9 ± 5.6
Microalbuminuria (mg/dl)	100.2 ± 90.7	**MCH (pg)***	28.7 ± 2.4
Creatinine, µmol/l	112.4 ± 33.7	**MCHC (g/l)***	326.4 ± 10.7
Albumin Creatinin ratio	13.3 ± 16.3	**RDW***	14.6 ± 1.9
Sodium, mmol/l	140.6 ± 5.2	**WBC ($x10^9$/l)***	8.0 ± 2.4
Potassium, mmol/l	4.8 ± 0.6	**Lymphocytes ($x10^9$/l)***	2.6 ± 1.2
Bicarbonate (mmol/l)	23.9 ± 3.8	**Neutrophils ($x10^9$/l)***	4.3 ± 0.9
Chloride (mmol/l)	104.2 ± 2.8	**Monocytes ($x10^9$/l)***	0.6 ± 0.2
Total Protein (g/l)	71.3 ± 4.5	**Basophils ($x10^9$/l)***	0.1 ± 0.04
ALP (U/l)	74.3 ± 23.0	**Eosinophils ($x10^9$/l)***	0.5 ± 0.6
AST (U/l)	27.7 ± 10.8	**Platelet count ($x10^9$/l)***	238.7 ± 70.2
ALT (U/l)	34.2 ± 26.5		
GGT (U/l)	58.8 ± 76.5		

Participants' oral hygiene and dental-related habits are shown in Table 7. Eight participants (88.9%) attended to a dental clinic within the last 6 months and 6 of them visited the dentist for a routine check-up and clean. According to the periodontal charting using the Florida Probe, 2 (22.2%) participants had no or mild periodontitis, 6 (66.7%) had moderate periodontitis and 1 (11.1%) had severe periodontitis. Their periodontal parameters are presented in Table 8. The median number of missing teeth among participants is 6, ranging from 2 to 13 teeth. Three participants (33.3%) presented with at least 1 site with PPD ≥ 5mm and 8 (88.9%) presented with at least 1 site with CAL ≥ 5mm. The mean percentage of bleeding sites was 19.3%, ranging from 11.1% to 41.7%.

Table 7. Oral hygiene and dental-related habit questionnaire of participants (n=9)

During the past 12 months, did your teeth or mouth cause any pain or discomfort?	***Yes***	***No***				
	2 (22.2%)	7 (77.8%)				
Do you have any removable dentures?	***Partial denture***	***No***				
	2 (22.2%)	7 (77.8%)				
How often do you clean your teeth?	***Once a day***	***Twice or more a day***				
	2 (22.2%)	7 (77.8%)				
The use of different oral hygiene instruments	***Toothbrush***	***Wooden toothpick***	***Plastic toothpick***	***Dental floss***	***Charcoal***	***Interdental toothbrush***
	9 (100%)	3 (33.3%)	1 (11.1%)	6 (66.7%)	1 (11.1%)	1 (11.1%)
Do you use toothpaste?	***Yes***					
	9 (100%)					
Does your toothpaste contain fluoride?	***Yes***	***Don't know***				
	7 (77.8%)	2 (22.2%)				
How long is it since your last dental visit?	***Less than 6 months ago***	***2-5 years ago***				
	8 (88.9%)	1 (11.1%)				
What was the reason of your last dental visit?	***Routine check up and clean***	***Pain-related***	***Consultation***			
	6 (66.7%)	2 (22.2%)	1 (11.1%)			
Because of the state of your teeth and mouth, how often did you experience these following problems during the last 12 months?						
	Never	***Sometimes***	***Fairly often***	***Very often***		
1. Difficulty in biting food	6 (66.7%)	3 (33.3%)				
2. Difficulty in chewing food	6 (66.7%)	3 (33.3%)				
3. Difficulty with speech/trouble pronoucing words	7 (77.8%)	2 (22.2%)				
4. Dry mouth	4 (44.4%)	4 (44.4%)	1 (11.1%)			
5. Felt embarrassed due to appearance of teeth	5 (55.6%)	4 (44.4%)				
6. Felt tense because of problem with teeth and mouth	8 (88.9%)	1 (11.1%)				
7. Have avoided smiling because of teeth	7 (77.8%)	2 (22.2%)				

8. Had sleep that often interrupted	5 (55.6%)	3 (33.3%)	1 (11.1%)			
9. Have taken days off work	7 (77.8%)	2 (22.2%)				
10. Difficulty doing usual activities	9 (100%)					
11. Felt less tolerant of spouse or people who are close to you	7 (77.8%)	2 (22.2%)				
12. Have reduced participation in social activities	9 (100%)					
How often do you eat or drink these following food?						
	Never	***Several times a month***	***Once a week***	***Several times a week***	***Everyday***	
1. Fresh fruits			1 (11.1%)	2 (22.2%)	6 (66.7%)	
2. Biscuits, cakes, cream cakes		2 (22.2%)	2 (22.2%)	4 (44.4%)	1 (11.1%)	
3. Sweet pies, buns	5 (55.6%)	2 (22.2%)	2 (22.2%)			
4. Jam or honey	3 (33.3%)	2 (22.2%)	2 (22.2%)	1 (11.1%)	1 (11.1%)	
5. Chewing gums containing sugar	7 (77.8%)	1 (11.1%)	1 (11.1%)			
6. Sweets or candies	3 (33.3%)	4 (44.4%)	2 (22.2%)			
7. Lemonade, Coca Cola or other soft drinks	3 (33.3%)	3 (33.3%)	1 (11.1%)	1 (11.1%)	1 (11.1%)	
8. Tea with sugar	6 (66.7%)	1 (11.1%)	1 (11.1%)	1 (11.1%)		
9. Coffee with sugar	5 (55.6%)			2 (22.2%)	2 (22.2%)	
How often do you smoke cigarettes?	***Never***	***Everyday***				
	8 (88.9%)	1 (11.1%)				
During the last 30 days, on the day you drank alcohol, how many drinks do you drink per day?	***Do not drink***	***Less than 1 drink***	***1 drink***	***2 drinks***	***3 drinks***	***5 or more drinks***
	2 (22.2%)	3 (33.3%)	1 (11.1%)	1 (11.1%)	1 (11.1%)	1 (11.1%)

Table 8. Periodontal parameters of included participants (n=9). *Data presented as median and interquartile range. **Data presented as mean ± SD.

Number of missing teeth*	6 [3;7]
Mean PPD, mm**	1.9 ± 0.3
Mean CAL, mm**	2.6 ± 0.7
Bleeding site, %**	19.3 ± 10.7
Number of sites with PPD ≥ 5mm**	0.9 ± 1.7

% sites with PPD ≥ 5mm**	0.8 ± 1.8		
Number of sites with CAL ≥ 5mm**	11.8 ± 10.0		
% sites with CAL ≥ 5mm**	9.9 ± 9.8		
Periodontitis Diagnosis	***No or Mild***	***Moderate***	***Severe***
	2 (22.2%)	6 (66.7%)	1 (11.1%)

11.2. Follow-up questionnaire to investigate the utilization of the referral pathway

Table 9. Follow-up questionnaire about the referral pathway (n=9). *These questions only applied for participants that answered "Yes" to the first question (n=6)

Did you go to the dentist after the oral assessment?	***Yes***	***No***			
	6 (66.7%)	3 (33.3%)			
Why did you decide to go to the dentist?*	***I think that it's important to take care of my teeth***	***I regularly go to the dentist***	***Because I was asked by the study personnel***	***Dental is covered by my insurance***	***Because it's beneficial for my diabetes condition***
	6 (100%)	5 (83.3%)	1 (16.7%)	3 (50%)	2 (33.3%)
Did you go to a private or public clinic?*	***Private***	***Public***			
	5 (83.3%)	1 (16.7%)			
Who provided your dental treatment?*	***Specialist***	***General dentist***	***Hygienist***		
	2 (33.3%)	4 (66.7%)	1 (16.7%)		
What kind of dental treatment did you receive?*	***Routine check up and clean***	***Deep clean***	***Fillings***	***Extraction***	***Others***
	6 (100%)	2 (33.3%)	1 (16.7%)	1 (16.7%)	1 (16.7%)
What do you think about the information and advice you received at our oral assessment?	***Increase awareness about dental condition***	***Increase awareness about relationship between oral health and diabetes***	***Provide motivation for better oral care***	***Provide useful information***	
	9 (100%)	6 (66.7%)	7 (77.8%)	9 (100%)	

When being contacted for the telephone follow-up questionnaire, 66.7% patients went to the dentist after the examination and 33.3% did not. When asked about the reasons why they didn't go to the dentist to get the treatment proposed by the dental professional, participants presented with different responses. Two participants answered that "it was not necessary" and "can not afford financially". One participant

hesitated to go to the dentist because he was afraid of the COVID-19 situation in New South Wales at that moment. When asked about the consultation with the dental practitioner during their diabetes appointment, all participants responded that it "increased their awareness about their dental condition" and "provided helpful information". Information about the dental treatment received is presented in Table 9.

11.3. Correlation between periodontal parameter and diabetes-related metabolic parameter

Table 10 presents the analyses of the relationship between HbA1c levels, the number of diabetic complications and periodontal parameters, including number of missing teeth, mean PPD, mean CAL, proportion of bleeding sites, proportion of sites with PPD ≥ 5mm and percentage of sites with CAL ≥ 5mm. A significant positive correlation between HbA1c level and percentage of bleeding sites ($r = 0.70$, $p = 0.03$) was observed (Figure 13).

Table 10. Results of a univariate analysis (Pearson's correlation test) of the correlation between periodontal parameters and HbA1c levels and number of diabetic complications. *Significance level of 0.05.

	HbA1c (%)	**Number of diabetic compications**
Missing teeth (Florida Probe)	r = 0.47	r = -0.27
	p = 0.21	p = 0.49
Mean PPD (mm)	r = 0.21	r = -0.31
	p = 0.58	p = 0.41
Mean CAL (mm)	r = 0.50	r = -0.29
	p = 0.17	p = 0.45
% Bleeding site	r = 0.70*	r = 0.31
	p = 0.03*	p = 0.43
% PPD ≥ 5mm	r = 0.16	r = -0.39
	p = 0.69	p = 0.30
% CAL ≥ 5mm	r = 0.52	r = -0.35
	p = 0.15	p = 0.35

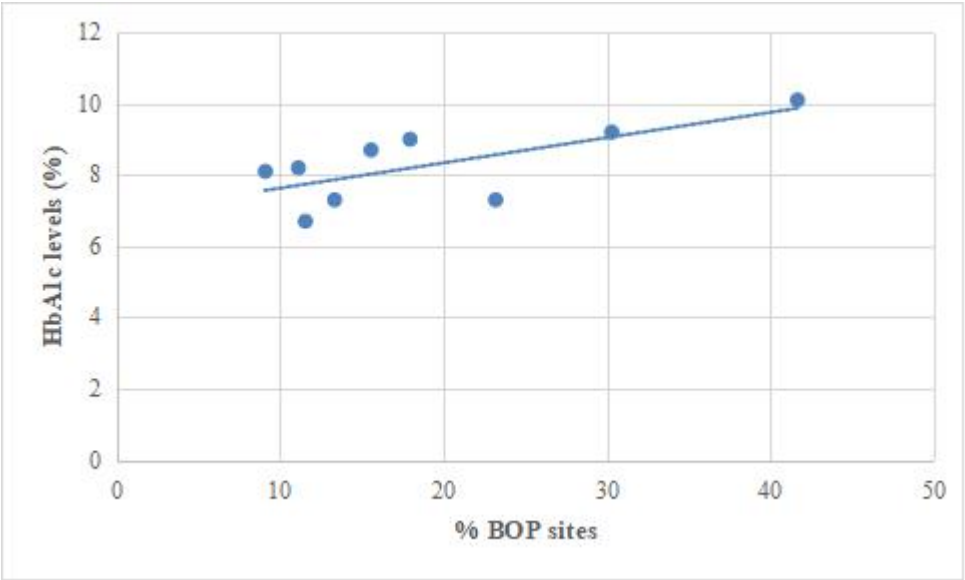

Figure 13: Linear correlation between HbA1c levels and proportion of sites with positive bleeding on probing (r = 0.70, p = 0.03)

Chapter 12: Discussion

The first objective of this study was to identify the relationship between periodontal disease and type 2 diabetes mellitus and diabetic complications. It was hypothesized that a high prevalence of periodontal disease existed in individuals with type 2 diabetes, and especially in those with higher HbA1c levels. Overall, the study showed that a large number of participants in the study presented with moderate to severe periodontitis and HbA1c levels was associated with periodontal conditions. We involved only patients with type-2 DM in study because the association between periodontal disease and type 1 diabetes is less relevant, as inflammation is currently not considered part of the mechanism [1,3].

According to this present study, 77.8% of the participants showed signs of moderate to severe periodontitis. This prevalence is slightly higher than ones published in previous studies. Factors contributing to the difference could be different criteria of periodontitis diagnosis, different ethnic groups, different socio-economic status, different age groups of the study population. Another possible reason is that our study had an underpower sample size, therefore, our study sample might not be representative of the whole population of people with type 2 diabetes. A cross-sectional study by Singh et al. in 427 North Indians between 30 and 65 years of age (mean age: 49.13 years old) reported that the prevalence of moderate to severe periodontitis was 69.1% among participants with type-2 diabetes mellitus [12]. In this study, participants were divided into subgroups based on their glycaemic control: good glycaemic control (HbA1c levels: 6-7%), moderate control (HbA1c levels: 7-8%) and poor control (HbA1c levels >8%). In our present study, most participants have their HbA1c levels higher than 7%. Therefore, in comparison with this study by Singh et al., if we only consider participants in the moderately and poorly controlled HbA1c level groups only, the frequency of moderate to severe periodontitis was 76.7%. That number is interestingly very close to our reported prevalence although our study populations were derived from different ethnic groups [12]. A community-based longitudinal study in Taiwan in 2015 reported that the cumulative incidence rates of periodontal disease in people with type 2 diabetes mellitus for 5-year-follow-up period was up to 62% [13]. The study population in that study was adults aged from 35 to 44 years, which was younger than the age range in our present study (mean age: 67.2 years old, ranging from 57 to 74 years old). That could be one possible

explanation why our reported prevalence of periodontal disease is higher as the frequency of periodontal disease tends to rise higher when people get older [1]. In a multicentered observational cross-sectional study in the USA and the UK in 2017, the frequency of periodontitis was published as 54.2% among people with type-2 diabetes mellitus [14]. In this study, the criteria of periodontitis was different from our study's. In our study, participants were classified as having moderate periodontitis if they have ≥ 2 interproximal sites with CAL ≥ 4 mm, or ≥ 2 interproximal sites with PPD ≥ 5 mm, while in this study, the diagnostic criteria was ≥ 1 site with PPD ≥ 5 mm. By considering PPD as well as CAL, we lowered the threshold of diagnosing periodontitis and were able to identify not only patients with active periodontitis with ≥ 5 mm pocket depth but also those who have a history of periodontitis with attachment loss. The lower diagnostic threshold might also contribute in our higher reported prevalence of periodontitis among participants.

Clinical periodontal parameter including PPD, CAL and BOP reported by our study was consistent with data presented by other previous studies on periodontal status of patients with type 2 diabetes mellitus [15-17]. Mean PPD reported from a study among Gullah African Americans aged from 34 to 77 with type-2 diabetes mellitus was 1.9mm, which is similar to our average reported PPD. However, this study reported a lower CAL of 1.9mm, compared to our CAL of 2.6mm [16]. This discrepancy might be due to different ethnic groups of the 2 population. Another possible explanation is that the participants in our study have longer diabetes duration. Our participants' average diabetes duration was 17.8 years, and the person with the longest one was 40 years, compared to the mean diabetes duration of 10.7 years reported by Bandyopadhyay et al. [16]. Therefore, the participants in our study might have longer history of having periodontal disease demonstrated by attachment loss in periodontal tissues. Another study in a similar Caucasian diabetic population with similar age groups also reported a comparable mean CAL of 2.4mm [17].

Eighty-nine percent of the participants presented with at least 1 diabetic complications. This is higher than the prevalence published by Oliveira et al. in 2016 (20.2%) [18]. Possible explanations for this dissimilarity are the difference in diabetes duration between 2 studies (17.8 years in our present study versus 9.6 years in the study by Oliveira et al.); different ethnic groups; different screening procedures for diabetic micro- and macrovascular complications; patients attending to RPAH Diabetes Centre are those with moderate to severe diabetes conditions that need more

intensive care. Among various diabetic complications, diabetic nephropathy affects our participants the most with 55.6% of them presented with signs and symptoms of abnormal renal functions. This is consistent with the prevalence reported by Shultis et al. in 2007 among an Indian community with type-2 diabetes mellitus [19]. According to literature, neuropathy is the most common diabetic microvascular complication with 60-70% affected individuals [20]. In our present study, a relatively high number of 44.4% of the participants were diagnosed with neuropathy. Our frequency is slightly lower which might be due to our small sample size that did not represent the whole type-2 diabetes mellitus population. The prevalence of other diabetic complications as well as participants' metabolic parameter are similar with previous studies and are expected to be observe in patients with type-2 diabetes mellitus [21,22].

Among 9 participants, 6 of them (66.7%) visited the dentist for their routine check-up and clean within 6 months prior to the study periodontal assessment. This is a relatively high number, compared to 29% reported by Amarasena et al. in a study on dental attendance among an Aboriginal Australian population in 2016, showing good oral health awareness among patients with type-2 diabetes mellitus at RPAH Diabetes Centre [23]. Two other participants also attended to a dental clinic within 6 months, however, it was an appointment related to pain/discomfort of the oral cavity. One patient reported that he hadn't been to the dentist for 5 years. Looking into this patient's periodontal status, we can see that he was diagnosed with moderate periodontitis and severe gingivitis with the highest percentage of bleeding on probing sites (50%) among all participants. Hence, routine dental check-up with scaling and root planing every 6 months plays an important role in maintaining good oral health, especially for those with type-2 diabetes mellitus. Participants in this study are patients that attended to RPAH Diabetes Centre for at least 10 months. For that reason, they received adequate medical attention from the endocrinologists, dietitians and other medical practitioners from the hospital. This reflects in the results from the dental questionnaires showing that the study participants overall have quite well-controlled diet with moderate sugar consumption. Only 1 of the participants is smoker. This participant was also diagnosed with moderate periodontitis, which is corresponding with the fact that cigarette smoking is a contributing factor of periodontal disease [24].

All participants in this study reported that they brushed their teeth at least once a day and 77.8% brushed twice or more daily. Our participants also presented with high frequency of interproximal surface cleaning with 66.7% of them used dental floss and/or interdental toothbrush daily. This showed good oral self-care behaviors among our participants, in comparison with previous studies on dental self-care patterns in patients with type-2 diabetes mellitus [25,26]. Studies on the effects of good oral self-care on glycaemic control in patients with type-2 diabetes mellitus are lacking, however, there are substantial evidence on the positive impacts of good oral self-care and usual attendance to dental clinic on cardiovascular health. A Scottish Health Survey and a population-based study in Korea both showed that subjects that rarely or never brushed their teeth had a significantly higher risk of atherosclerotic cardiovascular disease [27,28]. Individuals who had more frequent dental visits have also been shown to have a decreased ischaemic stroke risk [29]. The reduction of residual inflammatory factor seems to be the main reason for this. Thus, with the association between systemic inflammation and glycaemic control demonstrated in Part A, we have reasons to believe that patients with type-2 diabetes mellitus can benefit from decent oral self-care.

Despite good oral home care and usual dental attendance, periodontal disease still existed in our participants with high prevalence. This fact poses some notable questions: 1) Is the patients' oral home care technique really effective?; 2) Is the professional care that patients with type-2 diabetes mellitus received at the dental clinic sufficient?; 3) Should some more intensive dental care be applied on patients with type-2 diabetes? Either way, the importance of oral self-care and routine dental check up and clean is undeniable, especially for those with type-2 diabetes mellitus. Therefore, it is crucial that not only the dental practitioners but also diabetes clinicians to encourage their patients to have good dental behaviour, including brushing at least twice a day, cleaning in between their teeth with dental floss or interdental toothbrush at least once a day, using antibacterial mouthwash if required and having regular dental examination and dental prophylaxis at least once every 6 months.

In this present study, we found a positive correlation between HbA1c levels and percentage of bleeding sites. This finding is similar to ones reported by previous studies [30,31]. In the study by Qureshi et al. in a Pakistan population of adults with type-2 diabetes mellitus and mean age of 51.7 years old, the authors not only reported the positive correlation between HbA1c levels and percentage of bleeding sites but

additional positive correlations between HbA1c levels and CAL and PPD were also observed [31]. Isola et al. also reported positive correlation between HbA1c levels with other periodontal and metabolic parameter including hsCRP levels, CAL, PPD, periodontal index (PI) and a negative correlation between HbA1c and the number of teeth [30]. We could not observe those correlation in our study possibly due to small sample size. Interestingly, few studies presented with contradictory results [32,33]. In a study in Puerto Rico in 2017, the authors demonstrated that BOP was associated with pre-diabetes status, impaired fasting glucose, impaired 1-hour plasma glucose, impaired glucose tolerance and resistance but there was no association between HbA1c and BOP [32]. Islam et al. also stated there was no association between HbA1c levels and periodontal status [33]. One potential reason is that these 2 studies are on pre-diabetes patients with impaired glucose levels, yet not a definite diagnosis of type-2 diabetes mellitus. It was suggested by Perez et al. from their preliminary analysis that in comparison to plasma glucose criteria, the HbA1c test has relatively lower sensitivity and specificity, in particular for pre-diabetes diagnosis [32]. Further studies are required to ascertain the association between HbA1c levels and periodontitis among pre-diabetes patients as periodontal condition can be affected by impaired glucose levels even before diabetes onset.

Bleeding on probing during periodontal charting demonstrates local inflammation in periodontal tissues. It was shown in previous study that people harbouring any serotype of *Aggregatibacter Actinomycetemcomitans* had significantly higher BOP sites that those who don't [34,35]. Therefore, bleeding on probing is believed to be associated with active periodontal bacteria. Periodontal-related bacteria and their bacterial antigens circulated in the bloodstream and contributed to the upregulation of systemic inflammation. The exacerbation of systemic inflammatory state can interfere with diabetes patients' glycemic control, resulting in elevated HbA1c levels [2,36]. This could be a possible explanation about the association between BOP and HbA1c levels. This study is a cross-sectional study, so we can not be certain about the direction of the relationship. Thus, the relationship could also be interpreted in the other way around. Patients presented with higher HbA1c levels are likely to be exposed with more severe and prolonged hyperglycaemia. This can lead to abnormal cell functions, disregulation of multiple inflammatory cytokines and mediators, which can worsen oxidative stress, enhance periodontal tissue destruction and defective tissue repair [2,36]. There are also multiple findings in the shift of microflora

composition between patients with diabetes mellitus and those who don't [37,38]. As a result, local inflammatory state of periodontal tissues was exacerbated, contributing in higher bleeding on probing sites.

One of the initial aims of this study was to validate the periodontal disease screening tools for non-dental personnel. We have the data of both survey questionnaire and the photography screening application for each participant, however, due to the small number of participants, it is impossible to properly evaluate the sensitivity and specificity of the screening tools. For that reason, we did not report those results in this thesis. Another aim that we were unable to fulfill due to the small sample size is to assess and establish the referral pathway between the diabetes clinic and the dental clinic. However, we still contacted all the participants 3 months after the study dental examination to follow up on their reaction upon the referral that they got regarding their periodontal health. Sixty-seven percent of the patients went to the dentist after the oral examination. All of them answered to the follow-up questionnaires that they went to the dentist because they thought it was important to take care of their teeth. This is consistent with the findings mentioned earlier about the high oral health awareness among the participants in this study. However, only 33.3% said that they thought it would be beneficial for their diabetes conditions. Although the participants have adequate understanding about the importance of dental care, one of our main goal in this study is to help patients understand that good oral health care also have good impact on their diabetes conditions. This is a noteworthy concern among literature as another survey in 102 Swedes with type-2 diabetes mellitus also reported that 85% of the participants had never received any information about the link between periodontal disease and diabetes mellitus [39]. For that reason, awareness about the association between oral health and diabetes management needs to be raised among patients with type-2 diabetes mellitus. Among patients that didn't go to the dentist, when asked about the reason, 2 of them said that they didn't think that it was important. One of them answered that he couldn't go to the dentist because of the COVID-19 situation at that moment and he was planning to go next year when the pandemic cools down. Therefore, it is important to note that COVID-19 also effected on the patients' decision to seek for periodontal treatment and has become an influential confounder in this study. When asked about the dental appointment and the oral assessment of our study, most patients found it to be helpful, increase their awareness between oral health and diabetes and provide useful information. Although

periodontal disease was considered “the sixth complication of diabetes mellitus”, oral care and screening was usually overlooked in multiple diabetes guidelines and management plan [40]. This information contributed in encouraging the idea that oral health care should be an essential component of diabetes management.

Our study had a number of methodological strengths. We used the Florida Probe in the periodontal assessment, which is found to be more accurate than manual probe [41]. Using the Florida Probe also assured that the procedures were calibrated and standardized across participants. All of the participants are recruited at the same site, which is Royal Prince Alfred Hospital (RPAH) Diabetes Centre. At the Diabetes Centre, every patient has a standardized data set and comprehensive medical records including age, gender, diabetes duration, metabolic parameter, related test results, documented diabetes complications, documented comorbidities and any current medications. Furthermore, we had a thorough and standardized questionnaires from WHO to examine participants’ oral and dental related habits. These helped control any confounding factors that can affect the results of the study. Another advantage of this study is that we used PPD, CAL and BOP to evaluate participants’ periodontal status. It was pointed out in Nascimento’s systematic review and meta-analysis that more than half of the selected studies used community periodontal index (CPI) to assess periodontal disease, which is considered as a crucial limitation in chronic periodontitis diagnosis [42].

Nonetheless, there were some weaknesses of the study that need to identified. The main disadvantage is the underpower sample size. Due to the COVID-19, our study recruitment was postponed and as a result, we were not able to recruit enough participants as planned. Consequently, as an underpower study, we would not be able to state any definite conclusion about the association between periodontal disease and type 2 diabetes mellitus. Another disadvantage of this study is that this was a cross-sectional study while both periodontal disease and type 2 diabetes mellitus are chronic disease that need lifelong follow-up time period. Therefore, a cross-sectional point of view might not be able to give a comprehensive picture on the relationship between periodontal disease and type 2 diabetes mellitus. Furthermore, participants were recruited at diabetes clinic only, as the target population in this study was patients with DM and diabetic complications. For that reason, the results from this study might not be generalized for all patients.

Despite of the disadvantages, our study has some interesting result that can contribute in the future clinical diabetes and dental practice guidelines. Even though it was an underpower study, our study was the first step of building representative epidemiological data of periodontal disease in Australian population which is lacking at the moment. Oral health care is often ignored by diabetes clinicians during consultation about diabetes management. Our study showed a high prevalence and concerning severity of periodontal disease among patients with type-2 diabetes mellitus, hence demonstrating that oral health screening should play a crucial role in diabetes management. Although we were not able to validate the specificity and sensitivity, our study has introduced of 2 non-dental personnel screening tools of periodontal disease, that can contribute in establishing the referral pathway between diabetes clinic and dental clinic. Further studies with a larger sample size and possibly follow-up time to build the epidemiological data and validate the screening tool would shed more light on this specific field.

Chapter 13: Conclusion

This study was designed to evaluate the prevalence and severity of periodontal disease in patients with type 2 diabetes mellitus. We found a high prevalence of periodontal disease among participants in contrast to self-reported high levels of oral self-care behaviours and the regular attendance at a dental practitioner. Another significant finding from this study is that bleeding on probing was positively correlated with HbA1c levels, indicating that active inflammation of the gums may be a good predictor of glycaemic control. The limited evidence from this study supported the two-way relationship between periodontitis and type-2 diabetes mellitus. Patients with type 2 diabetes mellitus should be routinely screened for periodontal disease as part of their diabetes management. In the light of the limited number of participants included in this study any conclusion should be drawn with uttermost caution. This study is the initial step of a more coherent project to build representative epidemiological data of periodontal disease in adults with type 2 diabetes mellitus and establish a referral pathway between diabetes clinic and dental clinic.

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PART D:
LEARNING OUTCOMES

Learning outcomes of the author of this thesis's Master of Philosophy (in the field of Dentistry) degree can be summarized into 3 sections: 1) learnings from conducting the Systematic review in Part B and another one not included in this thesis; 2) learnings from the research project in Part C; and finally; 3) experience and knowledge on the association between oral health and systemic condition and its impact on research and daily dental practice.

During the time of my Master of Philosophy journey, I was able to publish 2 systematic reviews:

1) Nguyen AT, Akhter R, Garde S, Scott C, Twigg SM, Colagiuri S, Ajwani S, Eberhard J. The association of periodontal disease with the complications of diabetes mellitus. A systematic review. Diabetes Research and Clinical Practice. 2020 July 1; 165 - As first author.

And 2) Garde S, Akhter R, Nguyen MA, Chow CK, Eberhard J. Periodontal therapy for improving lipid profiles in patients with type 2 diabetes mellitus: a systematic review and meta-analysis. International journal of molecular sciences. 2019 Jan; 20(15): 3826 - As a co-investigator and contributing author.

Both systematic reviews investigated on the relationship of periodontal disease/periodontal treatment on patients with type-2 diabetes melltius's conditions. We observed interesting findings from 2 systematic reviews and provided useful indications for further research on this relationship. Another significant learning outcome was that I have learned so much from the process of conducting these 2 systematic reviews, including database searching method, screening with exclusion/inclusion criteria for the appropriate publications to include in the review, risk of bias assessment, data synthesis, performing the meta-analysis, and finally critically appraise the findings. After this, I can confidently said that I have substantial experience in carrying out systematic review and meta-analysis. In fact, I'm currently doing another systematic review with a fellow PhD student on the effects of macronutrients on periodontits in rodents as a co-investigator. The manuscript is under preparation and we hope that we can publish that soon as well.

Regarding the research project in Part C, I spent the first 6 months of my Master of Philosophy period finalizing the study protocol and preparing for the study recruitment. I applied for the protocol amendment and got approved by Ethics. I also prepared some other necessary documents for the recruitment process such as the case report form, the participants' information sheet, the referral letter, the brochure

provided for patients at the RPAH Diabetes Centre. As I'm an overseas dentist, I could not perform any examination or treatment on the patients here in Australia. Therefore, I applied for the AHPRA's limited registration for postgraduate training and got granted. With that registration, I was able to do the periodontal assessment on the participants at RPAH Oral Health Clinic. The recruitment was unfortunately postponed due to COVID-19, however, during our short time recruiting patients, I was able to apprehend much from the dental practitioners, diabetes clinicians and even the patients that I was working with. Prior to this, I have not had the opportunity to be a part of such a big research team like this. Additionally, thanks to this research project, I was trained to use the Florida Probe, which I have never had the chance to use before, so that was also a very interesting experience for me.

Last of all, this journey has given me so much experience and knowledge on the association between oral health and systemic conditions. Beside my main project on the relationship between periodontal disease and type-2 diabetes mellitus, I had an amazing opportunity to participate in a side project on the effects of tooth loss on mental health. The manuscript is under preparation and hopefully I can have another publication from that. Although the association between periodontal disease and type-2 diabetes mellitus was quite well-established across literature, I have never had a thorough understanding about the metabolic mechanisms of the relationship until I started my Master of Philosophy journey. In order to do my literature review as well as the systematic review, I have read multiple publications in this field, which remarkably enriched my knowledge. As a dental practitioner, being a part of a research project like this has certainly introduced me to a greater appreciation of the important of research on daily dental practice. I finally understood the saying that "If you want to be a great dentist, you must start from your head, then your heart, and finally your hands". The great amount of knowledge that I absorbed during my Master of Philosophy journey has definitely gave me more confidence in my general dental practice.