Association between α-MMP-8 Chairside Test for Chronic Periodontitis and Selected Reproductive Health Parameters

Solomon Olusegun Nwhator

Department of Oral and Maxillofacial Diseases &

Doctoral program in Oral Sciences,

Faculty of Medicine,

University of Helsinki

ACADEMIC DISSERTATION

To be presented with the permission of the Faculty of Medicine of the University of Helsinki, for public examination in Lecture Hall 2, Biomedicum Helsinki 1,

Haartmaninkatu 8, Helsinki on June 15, 2018

Helsinki 2018

Supervised by

Associate Professor Anna Maria Heikkinen

Department of Oral and Maxillofacial Diseases,

University of Helsinki, Finland

Professor Timo Sorsa

Department of Oral and Maxillofacial Diseases,

University of Helsinki, Finland

Department of Oral and Maxillofacial Diseases,

Helsinki University Hospital, Finland and

Division of Periodontology, Department of Dental Medicine,

Karolinska Institute, Huddinge, Sweden

Reviewed by

Professor Nicole Arweiler

Department of Periodontology, University of Marburg, Germany

Ass Prof Kimmo Suomalainen

Unit for Oral and Maxillofacial Diseases, Tampere University Hospital

Opponent

Professor Arzu Tezvergil-Mutluay DDS, Ph.D. FADM,

Specialist in Prosthodontics and Clinical Dentistry

Professor and Chair, Department of Restorative Dentistry and Cariology

Institute of Dentistry, University of Turku

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This dissertation is based on the following original studies thereafter referred to in the text by Roman numerals I-IV.

I.   Nwhator, S.O., Ayanbadejo, P.O., Umeizudike, K.A., Opeodu, O.I., Agbelusi, G.A., Olamijulo, J.A., Arowojolu, M., Sorsa, T., Babajide, B.S and Opedun, D.O. (2014). Clinical Correlates of A Lateral Flow Immunoassay Oral Risk Indicator. *Journal of periodontol*ogy, 85(1):188-94.

II. Heikkinen, A.M., Nwhator, S.O., Rathnayake, N., Mäntylä, P., Vatanen, P and Sorsa, T.A. (2015). Pilot Study on Oral Health Status as Assessed by an Active Matrix Metalloproteinase-8 (aMMP-8) Chair-Side Mouthrinse Test in Adolescents. *Journal of periodontology*, 2:1-9.

III. Nwhator, S.O., Umeizudike, K.A., Ayanbadejo, P.O., Agbelusi, G.A., Arowojolu, M., Sorsa, T., Opeodu, O.I., Olamijulo, J.A., Akaba, G.O. and Ekele B.A. (2014). Black Women's Predisposition to Preterm Birth; Could We Be Near The Answer*? International Journal of Tropical Disease & Health*, 4(2):194-203.

IV. Nwhator, S.O., Opeodu, O. I., Ayanbadejo, P.O., Umeizudike, K.A., Olamijulo, J.A., Alade, G.O., Agbelusi, G.A., Arowojolu, M.O. and Sorsa, T. (2014). Could periodontitis affect time to conception? *Annals of Medical Health Sciences Research*, 4:817-22.

LIST OF ABBREVIATIONS

aMMP   Active Matrix metalloproteinase

BOP   Bleeding on probing

CMV   Cytomegalovirus

CP   Chronic periodontitis

CRP   C-reactive protein

EBV   Epstein Barr Virus

ED   Erectile dysfunction

TNF   Tumor necrosis factor

MI   Myocardial infarction

OHIS   Oral hygiene index score

PPD   Probing pocket depth

TIMP   Tissue inhibitor of metalloproteinase

VPI   Visible plaque index

ABSTRACT

There are many established measures of chronic periodontitis but with many limitations. The problem with these established measures is that they assess past disease rather than the present pathology of periodontal tissues. To overcome these limitations, scientists conceived the idea of a novel system to assess chronic periodontitis accurately and predictably. A novel system is sought that will favorably compare with established measures, while overcoming their limitations, and also providing high validity, sensitivity, specificity, reliability, and reproducibility. To investigate how a potential candidate for this system works, a series of studies were conducted in Nigeria and Helsinki. The goal was to assess how well a novel point-of-care test (aMMP-8 chairside test) measures up to these desirable objectives. The series of studies were conducted to investigate the validity in terms of sensitivity and specificity for chronic periodontitis.

To achieve our objectives, in study (I), we assessed chronic periodontitis using a novel aMMP-8 test -neutrophil collagenase-2 lateral flow immunoassay. This test qualitatively detects the presence of matrix metalloproteinase-8. Its correlation with established measures of CP, like bleeding on probing (BOP) and probing pocket depth (PPD), was then assessed. The neutrophil collagenase-2 immunoassay was 96% sensitive for poor oral hygiene, 95% sensitive for chronic periodontitis, and 83% sensitive for BOP. In all instances, chronic periodontitis was regarded as two sites with PPD or BOP. The reproducibility of the novel aMMP-8 chairside test was investigated in study (II), where oral health and periodontal assessment was performed on adolescents at the Kotka Health Center in Finland. The sensitivity of the aMMP-8 test for at least 2 sites with PPD $\geq$ 4 mm was 63.5% and specificity 100%, and for more than 2 sites the sensitivity was 76.5 % and specificity 96.7%. Thus, the test is effective in recognizing oral inflammatory burden in adults and adolescents with early signs of periodontitis.

Bacterial biofilm has been established as the primary etiological agent for chronic periodontitis. Similarly, the oral manifestations of systemic diseases and conditions

that predispose to chronic periodontitis are well-reported in the literature. The impact of chronic periodontitis on systemic health, however, has been under-reported until a few decades ago. Thus, the validity, sensitivity, specificity, reliability and reproducibility of the aMMP-8 chairside test were first established. After this, its applicability was assessed in the primary health care setting, as it applies to dentistry as well as general health. The role of chronic periodontitis (CP) on fertility-related events was of a particular interest in this investigation.

In study III, the levels of MMP-8 were assessed in pregnant women and a widespread (87.3%) elevation of active matrixmetalloproteinase-8 was evident among the black pregnant Nigerians. Independent of demographics, educational level, and trimester, positive test results were more abundant among the study subjects than what could be anticipated from previous studies performed on proteolytic enzymes in saliva.

Finally, the link between chronic periodontitis and time to conception (TTC) was investigated in study IV using the aMMP-8 test. Through a cross-sectional hospital survey involving 58 fertility clinic attendees and 70 pregnant controls, the odds of increased conception were higher with the aMMP-8-test-assessed periodontitis risk (OR 0.157, 95% CI 0.041-0.600, $P < 0.01$).

1. INTRODUCTION

Dental biofilm is a complex association of micro-colonies of bacteria existing in slime complexes with special characteristics. It remains the primary etiologic agent for chronic gingival and periodontal inflammation, however, it does not fully explain the prevalence and severity of chronic periodontitis. The literature has established a causal relationship between dental biofilm and chronic gingivitis but not with chronic periodontitis (Loe 1965; Niederman, 2013). Therefore, while the quantity of plaque correlates with the severity of chronic gingivitis, the quality of plaque tells a more detailed story of chronic periodontitis (Genco, 1996; Hajishengallis et al. 2011; Hajishengallis et al. 2012).

Chronic inflammatory periodontal diseases consist of gingivitis and periodontitis, which are the clinical results of a sub-cellular chain of events which begin as non-specific inflammation (Theilade, 1986). This chain of events is no different from acute inflammation anywhere else in the body. The initially gram-positive 85% aerobic environment of the mouth slowly changes from a 25-45% anaerobic state in chronic gingivitis to a 75% anerobic environment in chronic periodontitis (Slots, 1979).

There is a gradual paradigm shift in periodontics with dwindling emphasis on the identity and characterization of periodontopathic organisms. Emphasis is now on evidence-based concepts of genetically-determined inflammatory processes, rather than the old-school, erroneous view of chronic periodontitis as a mere infection. Current evidence, however, suggests that it is in fact predominantly inflammatory (Pihlstrom and Tabak, 2005). The desire to understand these inflammatory mediators has opened new frontiers in periodontal research.

What we see clinically as the periodontal pocket represents the end product of a series of events that culminate in the apical migration of the junctional epithelium. As bacterial lipopolysaccharide attaches to the tissues, it stimulates interleukin-8 expression that initiate these events. Interleukin releases signals that initiate

neutrophil chemotaxis, representing the stage of acute inflammation-- the body's first attempt at containing the microbial assault. If this succeeds, visible inflammation does not develop. If this fails, however, the tissues mobilize other cells like monocytes, lymphocytes, and plasma cells. The release of pro-inflammatory mediators follows—this is the stage of chronic inflammation (Cekici et al.2014).

Eventually, there is a release of pro-inflammatory mediators including cytokines and complement. The cytokines released include the interleukins (Il-1, 2, 6, 8), tumor necrosis factor (TNF)–α, and several enzymes (Cekici et. al. 2014), such as metalloproteinases, trypsinase, elastase, and neuraminidase. With this advancement of pro-inflammatory agents, the body acts to protect itself from a potential bacteremia. This protection is rendered by apical migration of the junctional epithelium interpreted as periodontal pocket formation.

The periodontal pockets thus formed are a rich reservoir of pro-inflammatory mediators and chronic endotoxemia which have the capacity to exert influences on distant body tissues and organs. This has led to the view that the periodontal pocket acts like an endocrine source of continual chronic endotoxemia with far-reaching effects (Hajishengallis, 2015).

The ever-increasing evidence of periodontitis-systemic interactions are of great interest, particularly the early work of Offenbacher and colleagues (Offenbacher et al. 1996). Their work has stimulated research to better understand these complex interactions and fuelled the departure from the overly simplistic view of chronic periodontitis as a mere infection. Their work has also provoked a flurry of research activity to further explore and explain other potential associations with conflicting reports in the literature.

This conflict is natural with any new scientific claim. To explain away the observations, scientists resort to using statistical jargon like 'differences in study settings' and 'inadequate elimination of confounders'. However, it is possible that

all these conflicts may actually be due to our limited understanding of the mediators of chronic inflammation.

Over-simplistic attempts at explaining the associations between periodontology and general health may be the real reason for the medical community's delay in accepting these novel associations. Despite the controversies, the desire remains—to characterize and better understand the inflammatory mediators behind chronic periodontitis. It has been suggested that the inflamed periodontal pocket serves as an endocrine source of pro-inflammatory agents thus explaining some of the systemic association (Hajishengallis, 2015).

On a positive note, it is now possible to measure levels of inflammatory agents in a non- invasive manner due to recent advances in saliva diagnostics (Kinane et al. 2017). Of particular interest and relevance to periodontics, however, are the cytokines and metalloproteinases. Among the cytokines, Tumor necrosis factor - alpha (TNF-α), Interleukinn-1 beta (IL-1 β), Interleukin- 6, and Interleukin -8 are worthy of note.

The metalloproteinases have increased in prominence due to increasing evidence of their role in the etiopathogenesis of chronic periodontitis. Of the 28 known human metalloproteinases, just a couple are of direct relevance and interest to periodontics. Of these, the most abundant and probably most important is the matrix metalloproteinase-8 (MMP-8). MMP-8 is the most abundant matrix metalloproteinase produced by gingival and periodontal tissues (Sorsa et al. 2006). In its active form, it plays an important role in the pathogenesis of chronic periodontitis with MMPs 9 and 13. Levels of active MMP-8 (aMMP-8) correlate with the severity of chronic periodontitis (Sorsa et al. 2004, Sorsa et al. 2006, Akbari et al. 2015). Interest in aMMP-8 is growing given increasing concerns that chronic periodontitis may impact an increasing number of general health parameters.

For any test to be useful to periodontologists, it must possess a high level of specificity and sensitivity. Since a-MMP-8 is the most abundant MMP in saliva/gingival crevicular fluid, it is prudent to measure its salivary level in a fast, non-invasive manner using saliva. The MMP-8 chairside test makes it possible to accurately detect periodontal inflammation among pregnant women, men undergoing seminal fluid analysis, stroke patients, renal patients, and others.

The 'traditionalist' may find periodontal diagnosis without using established parameters somewhat unsettling. Thus, the study has provided some evidence to support traditional views. As such, the established measures were compared to novel measurement systems. Interestingly, the results showed a strong correlation between the novel qualitative salivary assessment of aMMP-8 and established periodontal diagnostic criteria.

With the validity of the novel test established, the focus of this study was infertility and the racial predisposition of black women to preterm birth. Preliminary work in Nigeria and Helsinki shows early evidence of a relationship between some reproductive health issues with CP using established diagnostic indices like bleeding on probing and pocket depth against a simple point-of-care test that does not require the expertise of an oral healthcare professional.

2. REVIEW OF THE LITERATURE

2.1 Periodontal health, general considerations

The normal periodontium consists of 4 major components: the gingiva, periodontal ligament, cementum, and alveolar bone. In light of emerging evidence of the two-way relationship between periodontal and general health, periodontal health should no longer be the sole concern of the periodontist. Kavoussi and co-workers have linked chronic periodontitis with endometriosis with a suggested principle of generalized immune deregulation (Kavoussi et al. 2009) and myocardial infarction (Rydén et al. 2016). Despite the recent controversies surrounding this association, the report found that 'the risk of a first MI was increased in patients with PD even after adjustment for confounding factors' (Rydén et al. 2016). CP is the sixth complication of poorly-controlled diabetes mellitus. The association between CP and diabetes mellitus relates to the prolonged release of reactive oxygen species and altered inflammatory/immune response to plaque (Patil et al. 2016). The emerging phenomenon, yet unknown to most endocrinologists, however, is the fact that chronic periodontitis worsens glycemic control, while periodontal therapy improves it (Li et al. 2015). Other systemic conditions linked with CP include renal insufficiency, (Grubbs et al. 2015), Alzheimer's disease (Uppoor et al. 2013), and respiratory tract infections (Pedersen et al. 2016).

The association between chronic periodontitis reproductive health is of particular interest to this study. Oğuz and co-workers reported a significant high association between erectile dysfunction (ED) and chronic periodontitis (Oğuz et al. 2013), and the link is vasculogenic (Sharma et al. 2011). Chronic periodontitis also affects sperm motility (Klinger et al. 2011), sperm count (Nwhator et al. 2014), conception (Hart et al. 2012; Nwhator et al. 2015), and preterm birth (Jeffcoat et al. 2001; Offenbacher et al. 1996; Usin et al. 2016).

2.2 The healthy periodontium – traditional view and emerging paradigm

The view of periodontal health as the absence of periodontal diseases is erroneous. Current evidence suggests that periodontal health is a mere absence of visible inflammation, however this view appears to be changing given the constant presence of inflammatory cells in so-called clinically 'healthy' patients (Vasconcelos et al. 2016). Inflammatory mediators being present in apparently healthy gingival and periodontal tissues supports this new thinking (Rahnama et al. 2014). It appears therefore, that a completely healthy periodontium might be non-existent. This means that healthy periodontal tissues with different levels of clinically-undetectable periodontal inflammation, is the best scenario possible. This position agrees with the state of equilibrium existing between bacteria and host defences on one hand, and between pro-inflammatory and anti-inflammatory mediators on the other. The literature alludes to this relationship in different ways such as 'active dynamic equilibrium' (Galgut, 1994), 'dynamic equilibrium' (Lang & Tonetti, 2003),'biologic equilibrium' (Nield-Gehrig & Willmann, 2007), 'homeostatic equilibrium' (Newman et al. 2011), and 'host-bacterial equilibrium'.

2.3 Beyond the red complex organisms

The red complex pathogens (notably *P. gingivalis*) remain important, but the view of a mere handful of periopathogens and a series of color-coded complexes is becoming archaic. *P. gingivalis* suppresses nitric oxide-dependent intracellular killing in macrophages through the induction of cAMP-dependent activation of protein kinase A (Wang et al. 2010). Over 700 periopathogens can now be identified due to recent advances in molecular-based microbe detection. Of this number, 200 can be present in a single individual and 50 in a single site. (Aas et al. 2005, Dewhirst et al. 2010, Griffen et al. 2011). Contrary to established teaching in periodontology, organisms identified by quantitative clonal analysis were mostly gram-positive, adding to the complexity of the pathogenesis of chronic periodontitis (Kumar et al. 2005).

2.4 Novel model of periodontal pathogenesis- polymicrobial synergy and dysbiosis- 'PSD'

There has been a metamorphosis of ideas starting from the specific plaque hypothesis, and later the keystone-pathogen concept (Hajishengallis et al. 2011). The keystone pathogen emphasis, *P. gingivalis,* has also come under subtle criticism. A report has suggested that *P. gingivalis* has been over-studied because it is the easiest red complex organism to grow and genetically manipulate. The so-called 'keystone-pathogen status' of the organism might therefore be the result of extensive preferential study of the organism (Hajishengallis & Lamont, 2012). From the emerging body of evidence therefore, it has become inevitable to explore other explanations for the pathogenesis of this complex entity. It wasn't until the last few years that proponents of a new model of periodontal pathogenesis suggested a paradigm shift. They affirm that a synergistic and dysbiotic microbial community initiates periodontitis thus de-emphasizing the idea of 'select periopathogens' such as the 'red complex'.

This model called 'polymicrobial synergy and dysbiosis (PSD)' originates from Hajishengallis and Lamont. (Hajishengallis and Lamont 2012). Dysbiosis is 'the concept that some diseases are due to a decrease in the number of beneficial symbionts and an increase in the number of pathogens' (Nath and Raveendran 2013). This new model is no surprise given that supporting evidence was already being developed several years earlier. For instance, in support of the proposed dsysbiosis and synergism, an earlier report had found that Epstein–Barr virus (EBV) and Human Cytomegalovirus CMV synergistically interact in the pathogenesis of chronic periodontitis (Kubar et al. 2005). The ability of *P. gingivalis* to 'incite' other microorganisms against the host garnered its keystone pathogen status is linked to its ability to induce 'dysbiotic microbial communities' (Hajishengallis et al. 2012). The keystone pathogen hypothesis is part of the metamorphosis towards the PSD model.
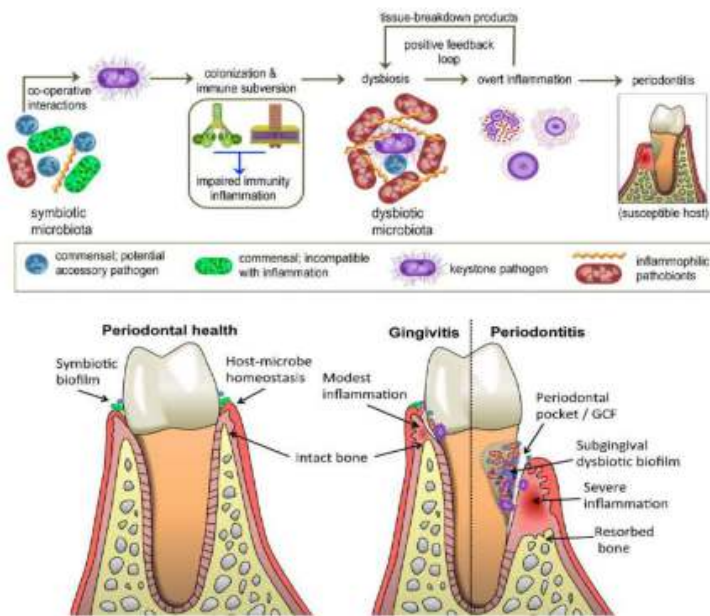
Figure 1

NOVEL MODEL FOR CHRONIC PERIODONTITIS- POLYMICROBIAL SYNERGY & DYSBIOSIS. Reprinted with permission from Macmillan Publishers Ltd: [Nature Reviews Immunology] (Hajishengallis G. Periodontitis: from microbial immune subversion to systemic inflammation. *Nature Reviews Immunology.* 2015. 15(1):30-44), copyright (2015).

2.5 Host response; a double-edged sword?

The relationship between pathogenic bacteria and host defence mechanisms is well-known (*vide infra*). This relationship and its inherent dynamism form the basis of

current thinking in periodontal pathogenesis. The natural sequence should naturally be further consideration of the role of these host defences in the pathogenesis of periodontal diseases.

Emphasis on the role of bacteria dates to the early 1800s (Darveau, 2010). Nonetheless, the emphasis placed on this role has changed, as current evidence points to a more important role being played by host immunity and defence. This paradigm shift is strongly reflected in a recent study, which suggests that bacteria are the 'microbial factor contributing to initiation of the disease and immunological factor of the host propagating the disease' (Nath & Raveendran, 2013).

The periodontal environment harbours an ecologically-balanced biofilm, which when maintained ensures periodontal tissue homeostasis. The process of gradual dysbiosis disrupts the original, controlled immuno-inflammatory state that maintains the host–microbe homeostasis in the periodontium (Darveau, 2014). This appears to be commensalism, but this is debatable because periopathogens elicit an immune response contrary to the original concepts of commensalism. Williams had excluded commensals from eliciting an immune response (Williams, 1973), but more recent work claims that 'commensals may trigger an immune response. The immune response thus triggered can damage tissues and contribute to chronic inflammatory diseases' (Grainger et al. 2013).

Darveau proposed that maintaining an environment of controlled immuno-inflammatory state is a pre-requisite for periodontal health (Darveau,2014). Chronic periodontitis, however, is now regarded as a state of dysbiosis and immune dysregulation. Metalloproteinase-induced immune-dysregulation also leads to cancer (Godefroy & Bhardwaj, 2012). The immediate fears arising from the new model relate not to its complete novelty but rather its inherent complexity. The model is not completely novel considering the long-established fact that organisms behave differently in micro-colonies or biofilms. The real fear is that the complexity and the immune subversion are thought to result from two possible mechanisms: 1) direct immune subversion by the dysbiotic polymicrobial community itself, and 2)

host immunoregulatory issues. The latter renders host defences 'ineffective to restrain bacterial outgrowth and overt pathogenicity' (Hajishengallis & Lamont, 2012). The potential synergy between dysbiosis and inflammation forms a 'pathogenic cycle and a positive feedback loop' which ensures that the periodontal pathogenic process becomes self-perpetuating (Hajishengallis, 2015). Matrix metalloproteinases and their inhibitors play an important role in trying to prevent this self-perpetuating periodontal tissue homeostasis. This they do through 'balanced and regulated degradation of extracellular matrix (ECM) protein' which depends on metalloproteinases and their tissue inhibitors (Hernández et al. 2012).

2.6 Matrix metalloproteinases (MMPs)

From the current evidence on the role of MMPs, 'An unbalance in favor [SIC] of collagenous matrix degradation will result in the loss of periodontal supporting tissue, the hallmark of chronic periodontitis' (Reynolds & Meikle, 1997). Matrix metalloproteinases are a family of structurally related, but genetically distinct, zinc-dependent proteolytic enzymes. They are capable of destroying virtually all the components of the extracellular matrix and basement membrane, acting in synergy to regulate several processes, including inflammatory responses (McQuibban et al. 2002). The number of categorized human MMPS has increased over the years from 23 to about 28. The classes (based on their structure and susceptible substrates) are collagenases (MMP-1, -8, -13), membrane-type MMPs (MT-MMPs, MMP-14, -15, -16, -17, -24, -25) gelatinases (MMP-9, -2) and other MMPs (Folgueras et al. 2004).

2.61 Matrix metalloproteinase-8 and chronic periodontitis

Only a few of the 28 known human metalloproteinases are relevant to chronic periodontitis as currently understood. Of these, the most abundant and perhaps most important to periodontics is the matrix metalloproteinase- 8 (MMP-8). It is the most abundant MMP produced by gingival and periodontal tissues. MMP-8 exists in three forms: a latent form, an inactive pro-enzyme/zymogene form, and an active

form after cleavage of a C-terminal peptide. The active form plays an important role in the pathogenesis of chronic periodontitis alongside MMPs 9 and 13.

Researchers established the scientific basis for the use of MMP-8 in monitoring periodontal health more than a decade ago. Some researchers have affirmed that 'MMPs, especially collagenase-2 (MMP-8), are key mediators of irreversible tissue destruction associated with periodontitis and peri-implantitis. MMP8 exists in elevated amounts in its active form in the gingival crevicular fluid (GCF) and peri-implant sulcular fluid (PISF)' (Sorsa et al. 1999). In a later work, the authors stated authoritatively that based on available evidence, 'the role of collagenase, namely MMP-8, in periodontitis and peri-implantitis is the best-known example of the unwanted tissue destruction related to increased presence and activity of MMPs at the site of disease' (Sorsa et al. 2004).

The fact that active MMP-8 levels correlate with the severity of chronic periodontitis and is effective for its early diagnosis further corroborates the special role of MMP-8 (Akbari et al. 2015, Heikkinen et al. 2010, Heikkinen et al. 2016b). As stated in a study 'when periodontal disease MMP synthesis and secretion is dysregulated, the balance of MMPs and TIMPs is altered, and the levels of neutrophil MMPs such as MMP-8 and MMP-9 are elevated' (Uitto et al. 1990). The reason for this is immune-dysregulation 'likely due to greatly increased numbers of neutrophils emigrating into the periodontitis lesion and the GCF as the result of the inflammatory process' (Uitto et al. 1990). It is therefore undisputed that MMP-8 remains the most important matrix metalloproteinase detectable in gingival crevicular fluid for periodontopathic processes (Birkedal-Hansen, 1998; Sorsa et al. 2006).

2.7 Chronic periodontitis and reproductive health

The plausibility of the idea that periodontal health could have an impact on systemic health is not far-fetched. The fact that 'the periodontium is chronically exposed to a dysbiotic microbial community easily explains this' (Hajishengallis, 2015). The author further opined that 'periodontal bacteria can subvert host-

signaling pathways to instigate chronic inflammation in extra oral sites' (Hajishengallis, 2015). It is thus no surprise that chronic periodontitis is linked with erectile dysfunction (Oguz et al. 2013), preterm birth (Offenbacher et al. 1996), and endometriosis (Kavoussi et al. 2009), but the mechanisms remain unclear.

2.71 Chronic periodontitis and libido

The mechanism behind the proposed association between chronic periodontitis and reduced libido is unclear because testosterone may have an inhibitory effect on gingival inflammation (Daltaban et al. 2006). Possible mechanisms yet unexplored might, however, include the arginine link. Achieving and maintaining penile erection requires arginine—a direct nitrous oxide precursor. There is an association between arginine and increased libido in both males and females (English, 2011; Thompson, 2003). *P. gingivalis* enhances arginase activity and possesses an arginine-specific protease (Holden et al. 2014; Lourbakos et al. 2001). Arginase short-circuits a biochemical pathway that is critical to male sexual arousal (Kim et al. 2001). The fact that periodontal therapy reduces salivary arginase activity of patients with chronic periodontitis further corroborates this proposed mechanism (Gheren et al. 2008).

2.72 Chronic periodontitis and sperm count

In an Israeli pilot survey, Klinger and co-workers reported a positive association between chronic periodontitis and sperm sub-motility. They found 'no significant association between periodontitis/gingivitis and infertility based on the observed sperm counts' (Klinger et al. 2011). Camejo and colleagues, however, found an association between raised interleukin-6 levels and lipid peroxidation (Camejo et al. 2001). Lipid peroxidation and decreasing total antioxidant capacity are linked with low sperm count (Colagar et al. 2013).

2.73 Chronic periodontitis, conception, pregnancy, and its outcomes

There is a paucity of data on the link between chronic periodontitis and conception (getting pregnant) but chronic periodontitis is linked with known causes of infertility such as endometriosis (Kavoussi et al. 2009) and pelvic inflammatory disease (McKinnon et al. 2013). The possibility that chronic periodontitis could affect time to conception was first suggested by Hart and co-workers (Hart et al. 2012). Post conception, current evidence supports a link between chronic periodontitis and pregnancy outcomes. Two plausible mechanisms explain the link between chronic periodontitis and unfavourable pregnancy outcome. Periopathogens may cross the placenta into the foetal circulation and amniotic fluid, or through the release of acute-phase response. Locally-produced periodontal inflammatory mediators gaining access into the systemic circulation induce this response (Hajishengallis, 2015).

F. nucleatum is an organism of interest in this process, despite being described as 'a potential accessory pathogen that facilitates the colonization of periodontitis-associated bacteria' (Hajishengallis, 2015). *F. nucleatum* translates into an 'overt pathogen' if it translocates to extraoral sites (Socransky & Haffajee, 2005). Isolation of *F. nucleatum* clones from the subgingival biofilm of a still-born infant whose mother had pregnancy-associated gingivitis suggests a hematogenous spread (Han et al. 2010). *F. nucleatum* crossed the endothelium and colonizes the fetal–placental compartment using the E-cadherin-binding FadA adhesin in experimental models. (Liu et al. 2007). The observed effects are a result of TLR4-dependent necroinflammatory responses (Liu et al. 2007). Another organism, *P. gingivalis,* induces cardiolipin-specific antibody production which causes foetal loss. (Han, et al. 2014; Schenkein et al. 2013).

2.8 Racial differences in inflammatory response and matrix metalloproteinases

Africans possess higher baseline levels of inflammatory markers in a study conducted by Fergusson and co-workers. Paradoxically, they reported lower levels of evoked inflammation in response to endotoxin in persons of African ancestry than those of European ancestry. They concluded that 'levels of inflammatory biomarkers measured in epidemiological settings might not predict the degree of acute stress-response or risk of diseases characterized by activation of innate immunity' (Ferguson et al. 2013). Their observation may be due to 'chronic conditioning or priming of innate immunity' in persons of African ancestry 'resulting in an attenuated responsiveness during inflammation' (Morris & Li, 2012).

Interpreting the results warrants caution as even the authors suggested that the differences might apply to acute inflammatory disorders rather than real-life chronic endotoxemia which applies to chronic periodontitis. The need for caution is predominantly based on their observation that 'baseline levels of inflammatory biomarkers may reflect an on-going low-grade inflammation' (Ferguson et al. 2013).

Another important aspect of racial difference is in the prevalence of pre-term birth. Anum and co-workers (2009) observed that 'genes, particularly the pro-inflammatory cytokine genes and their receptors, are linked to matrix metabolism since these cytokines increase expression of matrix degrading metalloproteinases'. They however concluded that 'the role that genetic variants that are different between populations play in preterm birth cannot yet be quantified' (Anum et al. 2009).

Greater oxidative stress and inflammation, both *in vivo* and *in vitro*, are more prevalent among Africans than Caucasians (Feairheller et al. 2011). Significantly higher nitrous oxide expression is evident in African American human umbilical vein endothelial cells (HUVECs) than Caucasian HUVECs. Heightened expression exists in primary cultures from African Americans just as it does *in vivo*.

(Feairheller et al. 2011) Another study published in Ethnicity and Disease reported higher levels of inflammation among blacks than whites based on IL-6 levels, but failed to demonstrate differences in TNF-α, C-reactive protein, and Interleukin -10 (Paalani et al. 2011). IL-6 is a potent stimulator of MMPs from mononuclear cells (Li et al. 2015) and MMP activity increases in the pre-partum period. (Athayde et al. 1998) This is not surprising since it is well-known that 'fetal [SIC] membranes are composed primarily of collagen and matrix metalloproteinases are enzymes capable of degrading extracellular matrix macromolecules, including collagens' (Athayde et al. 1998).

2.9 Future of metalloproteinase inhibitors and reproductive health parameters.

Links have been reported between increased levels of tissue inhibitor of metalloproteinase-1 (TIMP-1) and successful conception after *in vitro* fertilization. (Shibahara et al. 2005). The over-expression of matrilysin, a member of the stromelysin subfamily of MMPs, leads to premature mammary gland differentiation and male infertility (Rudolph-Owen et al. 1998). Scholars already know that 'an early event in premature labour [SIC] and premature rupture of membranes is the activation of the metalloproteinases' (Speroff & Fritz, 2005). The authors hope that scholars will replicate these studies in relation to chronic periodontitis and metalloproteinase-8 across racial lines. This could help to further understand the links between chronic periodontitis and preterm birth vis-à-vis MMP-8 levels.

3. AIMS OF THE STUDY

The Sorsa group has reported on their novel chair-side point-of-care tool for the rapid assessment of MMP-8 levels in saliva. Since this invention, there have been studies to validate the reproducibility of their findings in other climes. The simplicity of the method makes it a useful tool in the assessment of chronic periodontitis in association with various systemic entities. How chronic periodontitis affects selected reproductive health parameters is the focus of this study. The application of the Sorsa group's novel tool requires comparison with established, well-known indices to confirm sensitivity and specificity. This was the basis of an earlier study with promising results. Having established an excellent correlation with existing tools, it is proposed that the application of the chair-side point-of-care tool to a varied population of subjects will help to quickly assess the effects of chronic periodontitis on selected reproductive health parameters.

The specific aims of the study were;

1. To assess the validity of a rapid aMMP-8 chairside test in the detection and prediction of CP among Africans and Caucasians.
2. To assess the sensitivity and specificity of a rapid aMMP-8 chairside test in the detection and prediction of CP among Africans and Caucasians.
3. To investigate how chronic periodontitis measured by a rapid aMMP-8 chairside test affects time to conception.

4. SUBJECTS, MATERIAL AND METHODS

4.1.   Participants, Inclusion Criteria, Examinations, and Measurements

Study I

This study was part of a bigger study investigating the link between CP and subfertility. It was conducted as a multicentre study in two urban teaching hospitals in Lagos and Ibadan-Nigeria. The participants were all patients for whom a seminal fluid analysis had been ordered for various reasons. A total of 86 males requiring seminal fluid analysis were serially recruited. Of this number, 76 were included in the study because a few subjects had incomplete data. Inclusion criteria included being male, since the study related to sperm count, and being a non-smoker. However, all but five of the participants were non-smokers.

A basic intra-oral dental and periodontal examination was conducted on each patient under natural light. The Community Periodontal Index of Treatment Needs (CPITN) probe was used for periodontal examination status and treatment need codes assigned following the recognized CPITN scores. Oral hygiene status was assigned to each participant through summing up of debris and calculus index scores. Oral hygiene status was assigned to each participant as either 'good', 'fair', or 'poor' oral hygiene categories based on the Simplified Oral Hygiene Index Score of Greene & Vermillion (1960), (OHIS). Bleeding on gentle probing (BOP) was assessed with the ball-ended tip of the CPITN probe. Periodontitis was also assessed using the established pocket probing as well as the novel lateral flow of neutrophil collagenase-2 immunoassay, which measures levels of matrix metalloproteinase-8.

Study II

This was a cross-sectional study which was carried out at the Kotka Health Center, Finland. Of the participants, 15- to 17-year-old male and female adolescents, altogether 47 participated in this study. All were volunteers and gave permission to participate in this study. Five were smokers, two positive and three negative aMMP-8 tests were recorded. Smoking did not interfere with the test significantly.

All participants (adolescents) had their full-mouth checked with detailed intra-oral examination. Full-mouth clinical parameters of oral health were assessed for all the participants, including the state of the oral mucosa, dental caries, and periodontal health status for 4 sites. The study also involved the use of the novel aMMP-8 chairside mouthrinse test was performed on 47 enrolled individuals before full-mouth examination. The measured results of the novel aMMP-8 chairside mouthrinse test were based on a colour change within 5 minutes.

Study III

The study was a cross-sectional survey among attendees of the antenatal clinic of the University of Abuja Teaching Hospital in Abuja, Nigeria. The participants were pregnant women with a mean age of 30.6 years (SD 4.5) recruited through a purposive, non-random sampling technique; they were sequentially recruited after giving informed consent.

Inclusion criteria included being a black pregnant woman, being systemically healthy and being a non-smoker except for one participant. Clinical examinations and measurements were performed by two dental therapists calibrated to recognize the colour change under the supervision of a single consultant periodontologist who also performed all oral/periodontal examinations. The percentage agreement among examiners was found to be 100 percent due to the clarity of the blue colour change. A 30-second rinse with distilled water was quickly followed by the collection of saliva expectorated into a small sterile dish of which 3 ml was drawn up. Three to four drops of the saliva were placed in the immunoassay dish following which the

results were read-off as a colour change within 5 minutes, but not beyond 10 minutes from starting time. One blue line was taken to indicate a negative test while two blue lines indicated a positive test. This is shown in the original study (III, Figure 1)

Study IV

This was a cross-sectional study involving a non-random serial recruitment of women aged 23 to 48 years with a mean age of 33.9 years (SD 5.04). Altogether 70 pregnant women were recruited with a mean age of 32.8 years (SD 4.81), and 58 women with a mean age of 35.3 years (SD 5.00). The 58 women were not yet pregnant but were attending the fertility clinic whilst attempting to get pregnant.

The inclusion criteria were being pregnant or attempting to get pregnant with a history of regular sexual intercourse and being clinically healthy. Patients on fertility medications and those who got pregnant through fertility medications were included as fertility clinic attendees. Fertility was based on gravidity rather than parity so that a woman who suffered an abortion was considered fertile despite not having a baby.

Full mouth and periodontal examination was performed using natural illumination and all parameters including oral hygiene index score (OHIS), and community periodontal index (CPI) were recorded. Periodontitis risk score as assessed by the novel chairside active matrix metalloproteinase-8 (aMMP-8) (neutrophil collagenase-2) was also recorded. Based on the depth of colour change of the chairside aMMP-8 test, the participants were categorized into a negative result which meant no risk, and two blue lines for a positive result meaning increased risk. Oral hygiene was also categorized good, fair, or poor, based on the simplified Oral Hygiene Index Score (OHIS) of Greene and Vermillion (1960), ['Good' (0-1.2), 'Fair' (1.3-3.0), and 'Poor' (3.1-6.0)].

In summary therefore, the study participants included men (study I), women (studies III, IV), and adolescents (study II). The participants had to be systemically

healthy to be included. All women who participated in the study had to be pregnant or attempting to get pregnant. Women on fertility medications and those who got pregnant through fertility medications were included under the category of fertility clinic attendees. Fertility was based on gravidity rather than parity, therefore a woman who suffered an abortion was considered fertile despite not having a baby. Based on these criteria, 58 fertility clinic attendees and 70 pregnant controls were included in the studies involving women. Women not trying to get pregnant or with a history of not having regular sexual intercourse were excluded from the study. Inclusion criteria for the male participants was being a male requiring seminal fluid analysis and being the spouse of a woman undergoing investigation for infertility. All participants had to be systemically healthy to be included in the studies. Smokers were excluded from the study except a single woman who was found to be a smoker.

4.2 Clinical examinations

Periodontal examination was performed under natural light. The composite score consisting of a calculus index score and a debris index score were summed up to arrive at individual oral hygiene index scores (OHIS). Participants were classified into 'good', 'fair', and, 'poor' oral hygiene categories using recommended cut off OHIS expressed as good (0 to 1.2), fair (1.3 to 3.0), and poor (3.1 to 6.0)(Green & Vermillion, 1960). Periodontal evaluation was based on both the novel aMMP-8 test and established periodontal scores of bleeding on gentle probing (BOP). BOP was assessed with the ball-ended tip of the CPITN probe. CPITN code 3 formed the basis of periodontal pocket assessment.

4.3 Immunoassay procedure

The immunoassay test was performed following the manufacturer's instructions. The procedure commenced with a 30-second pre-rinse with tap water which was followed by a 30-second wait and a subsequent 30-second rinse with the test liquid. Patients were then instructed to pour the resultant mouthrinse into a collection cup accompanying the test kit. Following this, 3ml of the rinse was drawn up into a syringe and a filter was placed through which a maximum of four drops was placed in a lateral-flow immunoassay system. The result was read as a colour change within 5 minutes with the immunoassay system showing a single blue line for a negative result which meant no risk and two blue lines for a positive result meaning increased risk (Figure 2).

Figure 2: Two blue lines shown indicate a positive result meaning increased periodontitis risk.

4.4 Case definitions for periodontitis

A participant was diagnosed with periodontitis when they had either two sites with periodontal pockets or one site with a periodontal pocket. The values for single site or at least two sites with periodontal pockets were tabulated against immunoassay results. A similar procedure and comparison was repeated with participants with single site BOP or at least two sites with BOP. For adolescents, full-mouth clinical parameters (visible plaque index [VPI], bleeding on probing index [BOP] (sites for cut point 20% were positive), with probing depth [PD] 4 mm, were evaluated. Those with more than one PD 4 mm were assessed positive (Heikkinen, 2011). The chairside test could detect initial periodontitis among young patients with a predisposing genetic background (Heikkinen et al. 2016b). Since CPI does not have 1mm graduations nor does it measure recession, clinical attachment, loss, and periodontal status were not assessed.

4.5 Statistical analyses

SPSS (PASW statistics, IBM) was used for all study analyses and for all studies .In study I, Data were analysed using statistical software (SPSS). Sensitivity was expressed as the proportion of positive cases detected among the total number of cases. Specificity was not assessed because of the claims that the marker could detect even cases that were not overt/ obvious. Because there were 2 x 2 tables, Fisher exact statistic was used to estimate significance at 95% confidence level. In study II, results were analysed with software by cross tabulation and Fisher exact test.; the confidence level of the test was set at 95% therefore p-values yielding results ≤ 0.05 were accepted as being significant. In study III, Chi-square statistic was applied using cross-tabulations to examine the relationship between the outcome variable (aMMP-8 test result) and explanatory variables. In study IV, TTC was recoded into two groups - <1 year and >1 year following acceptable standards for infertility. OHIS was recoded into good (0-1.2), fair (1.3-3.0), and poor (>3.1). Chi-square statistic, Z statistic, and exploration of the strength of associations between the oral hygiene and TTC were made using Pearson's correlation statistics.

Variables not normally distributed by Shapiro-Wilk's test ($P < 0.05$) were analysed with a Spearman's rank order correlation statistic as a non-parametric alternative after preliminary analysis showed the relationship to be monotonic. For the same reasons, a Spearman's rank order correlation test was performed as a non-parametric alternative to assess the relationship between OHIS and time lapsed without conception.

Logistic regression was performed to explore the relative contribution of the various independent variables to the occurrence of the dependent variable (TTC).

Cross-tabulations and Chi-square statistics were used to measure strength of association between the outcome and explanatory variables. Adjunctive statistical analysis was performed using t-test (Epi-Info) to evaluate the association between two continuous variables and ANOVA used to evaluate associations between the means of three or more dependent variables.

5. RESULTS

This investigator set out to determine the ability of a novel chairside test which claimed to detect and even predict the occurrence of occult periodontitis. We were also interested in assessing the effect of the measurements on selected reproductive health parameters. Despite the interest in reproductive health parameters, it was important to first establish the validity of the novel test and how it correlates with recognized established tests. To ascertain the validity of the aMMP-8 chairside test, the clinical correlates were studied and the sensitivity was found to be 95% for periodontitis, 82.6% for bleeding on gentle probing, and 96%, for poor oral hygiene (Study I). Although the test kit was more sensitive to two sites with PPD/BOP than single site PPD/BOP (Study I).

5.1 Clinical correlates of the novel aMMP-8 chairside test) kit (I)

When the clinical correlates of the novel lateral-flow immunoassay aMMP-8 rapid test kit were studied, very strong correlations between the novel kit and established measures of oral hygiene and chronic periodontitis were found. Having either two sites with BOP or a periodontal pocket was associated with positive immunoassay results. The proportions of participants with positive immunoassay results rose from 38% among those with good oral hygiene, to 96% among those with poor oral hygiene (Table 1, 2).

Table 1: Oral hygiene and aMMP-8 immunoassay result

Category	Immunoassay Negative	Immunoassay Positive	Total	P value
Good	8	5 (38.5%)	13	
Fair	15	23 (60.5%)	38	
Poor	1	24 (96%)	25	
Total	24	52	76	0.000

There was a direct relationship between oral hygiene and immunoassay result, with 96% of those with poor oral hygiene being immunoassay positive.

Table 2: Pockets, BOP and aMMP-8 immunoassay results

Number	Immunoassay Positive	Immunoassay Negative	Total	P Value
≥ 2 pockets	18	1	19	
< 2 pockets	34	23	57	
Total	52	24	76	0.003
≥ 2 sites with BOP	19	4	23	0.000
< 2 sites with BOP	6	15	21	
Total	25	19	44	

There was a strong association between immunoassay result and the presence of two periodontal pockets and two sites with BOP. (BOP = bleeding on Probing).

In summary, in study I utilizing a neutrophil collagenase-2 immunoassay, the novel aMMP-8 test kit was 96% specific for poor oral hygiene, 95% sensitive for chronic periodontitis (defined as at least two sites with periodontal pockets), and 82.6% sensitive for at least two sites with BOP.

In study II, because the validity of the novel chairside test kit was established, deeper investigation was pursued. The results from study I showed that the novel chairside test correlated/compared very favourably with established measures of chronic periodontitis, however, these finding were among adults. For this reason, the validity of the novel chairside test was assessed among younger people/adolescents. The results were similar those of the adults in study I, with sensitivity and specificity of 71.8% and 77.5% respectively. The greater sensitivity for two sites with PD was similar as described below.

5.2 Sensitivity of aMMP-8 test kit among adolescents (II)

The sensitivity and specificity of the aMMP-8 test kit for BOP among adolescents were 71.8% and 77.5% respectively. The test kit also showed higher sensitivity for two sites with PD 63.6% versus 48.3% while the specificity was 100% with hardly any false-positive results identified.

In study III, having established the validity of the novel test kit, objective 3 was investigated. This was to determine how the aMMP-8 chairside test results affect time to conception, specifically for black women. Altogether 87% of the pregnant women recruited for the study had elevated aMMP-8 levels measured with the novel aMMP-8 chairside test kit. This finding was independent of demographics, educational level, and trimester as detailed below.

5.3 Elevated MMP-8 levels measured with novel aMMP-8 chairside test kit in 87% of pregnant women (III)

Using the novel aMMP-8 chairside test/kit, 117 of the 134 women had elevated aMMP-8 levels across all age groups, BMIs, and pregnancy trimesters. Levels of aMMP-8 increased with trimester though the differences were not significant (Table 3).

Table 3: Elevated a-MMP-8 levels by age, trimester, and BMI (Study III)

	a-MMP-8 level Normal		Elevated		P-value
	n	%	n	%	
20 to 25 years	2	11.8	15	88.2	
26 to 31 years	9	13.8	56	86.2	
> 31 years	6	11.5	46	88.5	
Total	17	12.7	117	87.3	0.93
1st trimester	4	15.4	22	84.6	
2nd trimester	10	13.2	66	86.8	
3rd trimester	3	9.4	29	90.6	
Total	17	12.7	117	87.3	0.62

In Table 3 above, a-MMP-8 levels were elevated in participants across all age groups, although the differences were not statistically significant ($X^2 (2) = 0.154$, P = 0.93). The a-MMP-8 levels were directly proportional to trimester, although differences did not attain statistical significance ($X^2(2) = 0.503$, P = 0.78).

In study IV, with the widespread elevation of aMMP-8 found in study III, the effects on time to conception (TTC) were investigated. Established oral hygiene

and periodontitis were assessed, and the novel chairside test for aMMP-8 was performed. Poor oral hygiene and calculus was observed to increase time to conception (TTC). Chronic periodontitis was also detected with the novel aMMP-8 chairside test kit associated with an increased time to conception. Participants with good oral hygiene had greater odds of conception within 1 year as detailed below.

5.4.1 Greater calculus deposits among women who were trying to get pregnant (IV)

Non-pregnant fertility clinic attendees had higher calculus deposits than their pregnant counterparts (P = 0.05).

5.4.2 Oral hygiene status and time to conception (IV)

The odds of conception within 1 year were greater in participants with good oral hygiene, OR 0.79 (95% CI 0.3210-1.3771) than fair oral hygiene, OR 0.56 (95% CI 0.3210-1.3771), which translated approximately to 44% versus 36%, but the difference failed to achieve statistical significance (Z statistic = 1.099, P = 0.27). A weak positive correlation existed between oral hygiene and TTC among pregnant ANC attendees, albeit insignificant (rs [69] = 0.201, P = 0.09).

5.4.3 Periodontitis assessed with novel aMMP-8 kit test and time to conception (TTC) (IV)

Using the novel aMMP-8 chairside test, a positive association was found between elevated aMMP-8 levels and increased TTC. Those women with elevated aMMP-8 levels took longer to conceive. This correlated closely with increased TTC among women with higher CPI scores. A significant positive correlation was found between oral hygiene and waiting time without pregnancy (rs [39] =0. 327, P = 0.04). 'rs = 1= perfect positive and -1 = perfect negative correlation in spearman's rank order correlation.' The oral hygiene of the test and control group did not differ as shown by a students' t-test and age-matched Z-statistic which found no

significant differences in oral hygiene scores between test and control groups (Z statistic = −0.6124).

5.4.4 Logistic regression to assess the relative strengths of the various factors (IV)

A logistic regression analysis was performed to assess the relative influence of the various explanatory variables on time to conception. The results showed that oral hygiene became irrelevant to TTC (P = 0.47) but the odds of increased TTC were higher with CPI (OR: 0.482 (95% CI), P = 0.021), periodontitis risk assessed with the novel aMMP-8 chairside test, (OR: 0.157 (95% CI), P = 0.007), and age (OR: 0.842 (95% CI), P = 0.02)(Study IV).

The combined results demonstrate that chronic periodontitis measured with the novel aMMP-8 chairside test might affect fertility/reproductive health parameters in women. It was considered prudent to investigate if these findings were applicable to men and the results were compared with traditional measures of CP.

6. DISCUSSION

6.1 Validity of the aMMP-8 chairside test as a measure of chronic periodontitis.

There has been an increased understanding of the pathogenesis of chronic periodontitis and the limitations of established measures of the disease. It became important to develop a novel test which would be both valid and reliable. For this reason, the aim for performing these studies was to assess the validity of the aMMP-8 chairside test as a measure of chronic periodontitis. This was achieved through the measurement of sensitivity and specificity which are recognized measures of validity. It is interesting to note that the aMMP-8 chairside test was found to have high validity for the measurement of chronic periodontitis, being 95% sensitive for chronic periodontitis among adults. It was also 100% specific for chronic periodontitis among adolescents with hardly any false-positive results identified. Since bleeding on probing is a notable sign among periodontologists for assessing active chronic periodontitis, it was important to measure how closely this novel aMMP-8 chairside test compares with BOP.

6.2 Comparison between the novel aMMP-8 test results and established measures of chronic periodontitis

Our findings show that the novel aMMP-8 chairside test aMMP-8 test to be have a high validity with 77.5% specificity and 71.8% sensitivity for BOP among adolescents, coupled with 82.6% sensitivity for BOP among adult participants. This was important because most dentists and periodontologists still rely on established measures of chronic periodontitis, such as bleeding on probing, periodontal pocket depth, and attachment loss. For this reason, the comparison between the novel aMMP-8 test results and established measures will be of great interest to periodontologists and other dentists. The sensitivity of the aMMP-8 test kit was found to be 95%, 82.6%, and 96% for periodontitis, BOP, and poor oral hygiene respectively. The sensitivity was true when periodontitis and BOP were regarded as

two sites with periodontal pockets or bleeding on probing respectively. The difference in sensitivity for sites with one PPD or BOP was significant, with a strong association between immunoassay result and the presence of two at least 4mm periodontal pockets and two sites with BOP. The test results of adults and adolescents also show notable similarity. This shows the repeatability of the study findings.

6.3 Predictive ability of the rapid aMMP-8 chairside test

The novel aMMP-8 chairside test has great predictive ability because it is capable of predicting the likelihood of periodontitis among participants (Sorsa et al. 2010). The risk for periodontitis is detected as a colour change just as active cases with periodontal pockets also appear as a similar colour change. The difference appears too close for meaningful clinical patient classification and treatment planning. The predictive ability of this novel chair-side kit lies in its ability to detect periodontitis even ahead of obvious clinical signs of periodontal breakdown, such as attachment loss. The prediction here is not statistical, but clinical. It simply refers to the ability of the test to detect chronic periodontitis even ahead of obvious clinical tissue destruction. It is unclear though, how the biomarker is able to detect six apparently healthy sites but miss some sites with at least one site with periodontal pocket. The predictive ability of aMMP-8 also applies to treatment outcome. Leppi and co-workers found that persistently high aMMP-8 levels predict poorer post-treatment prognosis and strongly predict MMP-8 levels during the maintenance period (Leppilahti et al, 2014, 2015). Findings from our study support the call for a paradigm shift to consider periodontitis in terms of inflammatory mediators that rather immunoassay measures rather than a mere measurement of random periodontal pockets.

The strong association between the aMMP-8 test and BOP is quite important because BOP is not exclusive to gingival inflammation. It is a recognized risk indicator for attachment loss hence an effective tool in the diagnosis and monitoring of active periodontal inflammation (Claffey & Egelberg, 1995; Leppilahti et al.

2011). Eliciting BOP in a pocket on consecutive appointments is also a recognized strong predictor of periodontal disease activity (Biyikoglu et al. 2009). Also, significantly more inflammation is found in sites that show BOP than when BOP is absent, thus it is an established sign of periodontal stability (Lang et al. 1990). Recently, Heikkinen and co-workers reported that that the aMMP-8 chairside test could detect initial periodontitis among young patients with a predisposing genetic background (Heikkinen et al. 2016a; Heikkinen et al. 2016b).

6.4 Usefulness of the novel aMMP-8 chairside test in the assessment of periodontitis in patients with reproductive health issues and applications for primary health care

Having established the validity of the novel aMMP-8 chairside test, the results were examined vis-à-vis the other objectives relating to reproductive health. Using the test, chronic periodontitis was detected, which affects time to conception. Time to conception is a reproductive health parameter. The high validity of the test showed in its usefulness in assessing periodontitis in patients with reproductive health issues.

This is not surprising, however, because just like extracellular matrices anywhere else in the body, foetal membranes are susceptible to the effect of matrix metalloproteinases. It is therefore reasonable to expect that any mechanism that prematurely degrades these membranes would facilitate delivery ahead of the normal time. This role is played by metalloproteinases hence their implication in the chain of events leading to preterm birth. (Nien et al. 2006) This role makes it even more important to use the aMMP-8 chairside test in a primary health care setting for the detection of women at risk for preterm birth hence the importance of our studies. The ability to pick early genetic predisposing as reported by Heikkinen adds value to this test among Africans where aggressive (Heikkinen et al. 2016a; Heikkinen et al. 2016b) periodontitis is quite common.

6.5 Implications of study findings for the reproductive health of black women

The studies under discussion were carried out among exclusively black women. The final pathway in the pathogenesis of preterm birth is through the breakdown of these delicate membranes and this action performed by matrix metalloproteinases. There have been early concerns of a possible role of chronic periodontitis in fertility-related events. From the focal infection theory of 1909, Berger's focal allergy theory of 1939, and Slauk's focal toxicosis theory of the 1940s. Much later in 1982, Linossier and co-workers isolated sperm immobilizing factor thereby raising new questions. The association between CP and antibiotic-resistant bacteriospermia reported by Bieniek and Riedel in 1986 awakened interest in the link between CP and fertility-pregnancy-related events. The evidence has grown stronger with the remarkable work by Offenbacher and colleagues linking preterm birth in 1996. Kavoussi et al. in 2009 also reported links between CP and endometriosis, while Kligner and colleagues reported links with reduced sperm motility in 2011. Of interest is the link with delayed conception by Hart in 2012 and erectile dysfunction by Grant in 2013. The mechanisms are not yet fully understand, but some of the possible mechanisms have been investigated herein. The study has stumbled upon a very important finding never previously reported in the literature, an almost wholesale rise in the values of alpha-metrixmetalloproteinase-8 which is a direct opposite of what was found by Gursoy and colleagues, who reported that MMP-8 concentrations were much lower than postpartum concentrations (Gürsoy et al. 2010). Also, the elevated aMMP-8 levels found in the present study were independent of age, educational qualification, gestational age, duration of last child birth, and BMI which had been identified as a major risk factor for preterm birth in a previous Nigerian study (Mokuolu et al. 2002).

The literature remains equivocal as to why preterm birth remains an essentially black man's problem. In order to fully rule out the possible influence of race on the results of the current study, all participants were of the same race, adult blacks and

Caucasians. Racial differences in sensitivity to LPS have been documented in the literature. While Caucasians showed greater sensitivity to LPS, their African-American counterparts were twice as sensitive to *E.coli* (Peltier et al. 2012). An attempt to explain the racial differences through a genetic basis has also been inconclusive using the mutations in the human gene CARD15 of the NOD1/APAF1 gene family. It has also been suggested though, that TLR4 variant alleles play a protective role, but no racial difference between the expression of these mutant genes variant alleles have been found between black Americans and Caucasians (Ferrand, Fujimoto, et al. 2002). It simply implies therefore that the pathogenesis of this differential prevalence is either not genetic or that the genetic pathway is yet-to-be identified. It is also possible that the dilemma lies in the quantitative dynamics of matrix metalloproteinases with MMP-8 being of interest.

Is it possible that black women are selectively predisposed to preterm birth because they produce more metalloproteinases than their Caucasian counterparts? If this is established through quantitative analyses, the genetic angle could then be explored in an attempt to explain why black women are more predisposed to pre-term birth. This position enjoys biologic plausibility, since LPS levels correlate with infection. The greater prevalence of amniotic fluid markers of inflammation further supports this position (Diaz-Cueto et al. 2006; Guinn et al. 1995). The infections angle is further supported by reports of elevated matrix metalloproteinases among women suffering from bacterial vaginosis compared with healthy controls (Diaz-Cueto et al. 2006). Fiscella declared that 'significantly higher rates of bacterial vaginosis among black women may account for nearly 30% of the racial gap in preterm births'. They cautioned, however, that 'these findings are limited by the reliability of published estimates of prevalence and relative risk for these infections' (Fiscella, 1996). Unfortunately, in a Nigerian study, Adesiji and colleagues found no association between bacterial vaginosis and preterm birth (Adesiji et al. 2007). The closest link was the association between preterm birth and malaria (Omole-Ohonsi & Attah, 2012).

If the genetic angle and infections are inconclusive, differential levels of expressed matrix metalloproteinases during pregnancy could alone play a major role in determining the predisposition of black women to preterm premature rupture of membranes and subsequent preterm birth. The idea that differential metalloproteinase expressions during pregnancy hold the key has some support. A report which cited a polymorphism in the MMP-9 promoter as being associated with an increased risk of preterm birth among African-Americans provides such support (Ferrandet al. 2002). Fujimoto and colleagues further corroborate this position with the report that an increased risk of preterm birth was attributable to a single nucleotide polymorphism in MMP-1 (Fujimoto et al. 2002).

6.6 Implications of study findings to selected reproductive health parameters

The studies also explored the possible association between chronic periodontitis and time to conception (TTC). This was assessed using both established measures and the novel aMMP-8 test. Most of the analyses were considered separately for pregnant antenatal clinic and non-pregnant fertility clinic attendees. The odds of conception within 1-year among women with good oral hygiene in this study failed to attain statistical significance, but warrants further investigation using a larger sample size. The difficulty encountered in the research is the absence of a basis for comparison, since the pioneer work failed to consider oral hygiene. The significant correlation between oral hygiene and increasing waiting time among non-pregnant fertility clinic attendees in this study also warrants further investigation.

The research presented here shows that fertility clinic attendees had more calculus than pregnant controls which is surprising, because calculus is believed to be incapable of inducing inflammation without plaque, though this position is now controversial. The inability to clearly differentiate effects of calculus versus 'plaque on calculus' reflects the current equivocal nature of the observation (White, 1997). Interestingly, the assessment of periodontitis in the present study did not depend on CPI code 3 alone. The study also employed the use of a modern rapid chair-side MMP-8 immunoassay kit. Although initial analysis detected differences limited to

the 38-42 years age group, a regression analysis showed that periodontitis was strongly associated with increased TTC corroborating the replicated study (Klinger et al. 2011). Is it possible that periodontitis affects TTC, just as TTC is affected by other inflammatory conditions such as endometriosis, polycystic ovarian syndrome, and hydrosalpinges? (Barnhart et al. 2002; Hart & Norman, 2006; Johnson et al. 2004). This view appears strengthened by the observation that periodontitis exerts an 'endometrial effect'. This effect has been likened to those exerted by endometriosis, polycystic ovarian syndrome and hydrosalpinges (Barnhart et al. 2002; Hart & Norman, 2006; Johnson et al. 2004; Weiss et al. 2009). This possibly explains our findings but despite these preliminary findings, the pathogenesis of the effects of periodontitis on fertility and TTC remains unclear and warrants further investigation.

Since the report of the isolation of a 'sperm immobilizing factor' from E. coli isolated from necrotic dental pulp (Slauk, 1940), there has been a lull in research into this unusual association. Kligner and co-workers (Klinger et al. 2011) reopened research efforts into this unexpected yet possible association. They found an association between periodontitis and sperm submotility (Klinger et al. 2011). Being a strong indicator of male fertility, adequate progressive motility is one of the very important in the fertilization process. TNF-alpha levels rise with chronic periodontitis and play a mediating role in systemic diseases associated with periodontitis. The effects of anti-TNF therapy in treatment of sufferers of rheumatic diseases as well as the beneficial effects on sperm motility have been documented. (Lachter et al. 2004; Perdichizzi et al. 2007; Ponchietti et al. 2009).

Elevated levels of TNF-alpha levels reflecting the persistent low grade systemic inflammation probably hold, and may explain the link between chronic periodontitis and reduced sperm submotility. Nwhator and co-workers (Nwhator et al. 2014) found no association between chronic periodontitis and sperm submotility. This may be due to differences in methodology between their work and the work done by Klinger et al. (2011).

In our study, we used analysis of sextants to overcome the loss of details occasioned by recordings limited to the worst score per sextant as used in the literature. (Nwhator, 2005; Nwhator et al. 2014; Umeizudike et al. 2016) Despite this, we found no association between chronic periodontitis and subnormal count and submotility for Codes 0 to 2. However, the analysis of Code 3 sextants was significant ($P < 0.05$) corroborating a previous report (Klinger et al. 2011).

For the first time, we demonstrated an association between oral hygiene status and sperm count. There was a significant association between poor oral hygiene and oligospermia and azoospermia when considered together as subnormal sperm count ($P < 0.05$). The association between poor oral hygiene and low sperm count, while being difficult to explain, cannot be ignored given that 21 of 25 (84%) of participants with poor oral hygiene were found to suffer from subnormal sperm counts.

Based on this finding, two possible hypotheses may be considered for the link between poor oral hygiene and low sperm count. One possible link is between poor oral hygiene and chronic periodontitis through elevated TNF-alpha levels. Though biologically plausible, this fails to explain reduced sperm counts except if TNF can cause testicular dysfunction. The remote possibility is that TNF-alpha is associated with varicocele-induced testicular dysfunction with consequent apoptosis and low sperm count through the TNF-related apoptosis-inducing ligand (Celik et al. 2013). Rise in TNF alpha levels occasioned by chronic periodontitis could therefore explain the reduced sperm count through TNF-induced testicular dysfunction.

A more plausible explanation lies in the similarity between oral microflora and microbial in spermiograms, as demonstrated in a previous report (Bieniek & Riedel, 1993). It is biologically plausible that poor oral hygiene and increased bacterial load cause bacteriospermia in the subjects. From recent reports, certain oral organisms like Fusobacterium nucleatum together with aMMP-8 to act as 'gate openers' for other periodontopathic organisms. It is possible that these combine to act as gate openers for more microorganisms to access the systemic circulation. This they

effect by disrupting endothelial integrity (Fardini et al. 2011; Sorsa et al. 2010). *E. coli* failed to penetrate the endothelium into the systemic circulation in the absence of F. nucleatum. The documented spermatozoa inhibitory factor expressed by *E. coli* would also have no effect without the help of *F. nucleatum*—both findings emphasize the important role of *F. nucleatum* (Paulson & Polakoski, 1977; Prabha et al. 2010).

Therefore future studies should evaluate TNF-alpha and aMMP-8 levels in subjects and controls. F. nucleatum and E. coli counts in the mouths of subfertile males should also be assessed to explore these possible links.

Since all the studies before now were among adults, the tests were repeated among adolescents and found similar results. A pilot study among adolescents with early signs of periodontitis was conducted and it was found that the novel aMMP-8 chairside mouthrinse test showed promising results in recognizing oral inflammatory burden within 5 minutes.

In summary, these findings showed a 96% sensitivity of the novel aMMP-8 test for poor oral hygiene, 95% sensitivity for chronic periodontitis and 82.6% sensitivity for BOP, The novel aMMP-8 chairside test therefore proved to be a valid and reliable instrument for the measurement of chronic periodontitis both among adults and adolescents, in different ethnic populations. Its sensitivity and specificity positions it as a promising test for use in the primary health care sector for rapid assessment of MMP-8 levels in relevant diseases. This is important within and outside periodontology and dentistry, and would be a useful tool in primary health care where elevated levels of MMP are considered important.

7. CONCLUSIONS

1. The sensitivity of the aMMP-8 test kit was 95% for periodontitis, 82.6% for bleeding on probing, and 96% for poor oral hygiene.

2. The sensitivity values of the aMMP-8 test kit were for two sites with PPD/BOP; single site PPD/BOP recorded less sensitivity.

3. Among adolescents, the sensitivity of the aMMP-8 test for 2 sites with PPD ≥ 4 mm was 63.6% and specificity 100% and for more than 2 sites the sensitivity was 76.5 % and specificity 96.7%.

4. Eighty-seven percent (87%) of pregnant subjects had elevated aMMP-8 levels measured with a novel aMMP-8 chairside test kit independent of demographics, educational level, and trimester.

5. Chronic periodontitis, detected also with a novel aMMP-8 chairside test kit, was associated with increased time to conception.

8.   ACKNOWLEDGEMENTS

REFERENCES

AAP. (2012). Men's Sexual Health May be Linked to Periodontal Health.   Retrieved from https://www.perio.org/consumer/erectile_dysfunction

Aas, J. A., Paster, B. J., Stokes, L. N., Olsen, I., & Dewhirst, F. E. (2005). Defining the normal bacterial flora of the oral cavity. *Journal of Clinical Microbiology, 43*(11), 5721-5732.

Adesiji, Y., Taiwo, S., Adekanle, D., Oboro, V., Fayemiwo, S., & Opaleye, O. (2007). Bacteria vaginosis and pregnancy outcome in Osogbo, Nigeria. *Research Journal of Medical Sciences, 1*(4), 195-198.

Akbari, G., Prabhuji, M. L. V., Karthikeyan, B. V., Raghunatha, K., & Narayanan, R. (2015). Analysis of matrix metalloproteinase-8 levels in gingival crevicular fluid and whole mouth fluid among smokers and nonsmokers using enzyme-linked immune-sorbent assay and a novel chair-side test. *Journal of Indian Society of Periodontology, 19*(5), 525.

Amar, S., Gokce, N., Morgan, S., Loukideli, M., Van Dyke, T. E., & Vita, J. A. (2003). Periodontal disease is associated with brachial artery endothelial dysfunction and systemic inflammation. *Arteriosclerosis, Thrombosis, and Vascular Biology, 23*(7), 1245-1249.

Anum, E. A., Springel, E. H., Shriver, M. D., & Strauss, J. F. (2009). Genetic contributions to disparities in preterm birth. *Pediatric Research, 65*(1), 1-9.

ASHA. (2016). Erectile dysfuntion.   Retrieved from http://www.ashasexualhealth.org/ sexual-health/mens -health/erectile-dysfunction/

Athayde, N., Edwin, S. S., Romero, R., Gomez, R., Maymon, E., Pacora, P., & Menon, R. (1998). A role for matrix metalloproteinase-9 in spontaneous rupture of the fetal membranes. *American Journal of Obstetrics and Gynecology, 179*(5), 1248-1253.

Barnhart, K., Dunsmoor-Su, R., & Coutifaris, C. (2002). Effect of endometriosis on in vitro fertilization. *Fertility and Sterility, 77*(6), 1148-1155.

Bieniek, K., & Riedel, H. H. (1993). Bacterial foci in the teeth, oral cavity, and jaw— secondary effects (remote action) of bacterial colonies with respect to bacteriospermia and subfertility in males. *Andrologia, 25*(3), 159-162.

Birkedal-Hansen, H. (1998). Links between microbial colonization, inflammatory response and tissue destruction. *Oral Biology at the Turn of the Century: Misconceptions, Truths, Challenges and Prospects*, 170-178.

Biyikoglu, B., Buduneli, N., Kardesler, L., Aksu, K., Pitkala, M., & Sorsa, T. (2009). Gingival crevicular fluid MMP-8 and-13 and TIMP-1 levels in patients with rheumatoid arthritis and inflammatory periodontal disease. *Journal of Periodontology, 80*(8), 1307-1314.

Blum, A., Kryuger, K., Eizenberg, M. M., Tatour, S., Vigder, F., Laster, Z., & Front, E. (2007). Periodontal care may improve endothelial function. *European Journal of Internal Medicine, 18*(4), 295-298.

Camejo, M., Segnini, A., & Proverbio, F. (2001). Interleukin-6 (IL-6) in seminal plasma of infertile men, and lipid peroxidation of their sperm. *Archives of Andrology, 47*(2), 97-101.

Cekici, A., Kantarci, A., Hasturk, H. and Van- Dyke, T.E. Inflammatory and immune pathways

in the pathogenesis of periodontal disease.(2014). *Periodontology* 2000, 64(1):57-80.

Celik, O., Kutlu, O., Tekcan, M., Celik-Ozenci, C., & Koksal, I. T. (2013). Role of TNF-related apoptosis-inducing ligand (TRAIL) in the pathogenesis of varicocele-induced testicular dysfunction. *Asian Journal of Andrology, 15*(2), 269.

Claffey, N., & Egelberg, J. (1995). Clinical indicators of probing attachment loss following initial periodontal treatment in advanced periodontitis patients. *Journal of Clinical Periodontology, 22*(9), 690-696.

Colagar, A. H., Karimi, F., & Jorsaraei, S. G. A. (2013). Correlation of sperm parameters with semen lipid peroxidation and total antioxidants levels in astheno-and oligoasheno-teratospermic men. *Iranian Red Crescent Medical Journal, 15*(9), 780-785.

Daltaban, Ö., Saygun, I., & Bolu, E. (2006). Periodontal status in men with hypergonadotropic hypogonadism: effects of testosterone deficiency. *Journal of Periodontology, 77*(7), 1179-1183.

Darveau, R. P. (2010). Periodontitis: a polymicrobial disruption of host homeostasis. *Nature Reviews Microbiology, 8*(7), 481-490.

Darveau, R. P. (2014). Porphyromonas gingivalis neutrophil manipulation: risk factor for periodontitis? *Trends in Microbiology, 22*(8), 428-429.

De Nardin, E. (2001). The role of inflammatory and immunological mediators in periodontitis and cardiovascular disease. *Annals of Periodontology, 6*(1), 30-40.

Dewhirst, F. E., Chen, T., Izard, J., Paster, B. J., Tanner, A. C., Yu, W.-H., Wade, W. G. (2010). The human oral microbiome. *Journal of Bacteriology, 192*(19), 5002-5017.

Diaz-Cueto, L., Cuica-Flores, A., Ziga-Cordero, F., Ayala-Mendez, J. A., Tena-Alavez, G., Dominguez-Lopez, P., Arechavaleta-Velasco, F. (2006). Vaginal matrix metalloproteinase levels in pregnant women with bacterial vaginosis. *Journal of the Society for Gynecologic Investigation, 13*(6), 430-434.

English, J. (2011). Arginine and Select Phytonutrients Enhance Libido. *Accessed online at https://nutritionreview.org/2013/04/arginine-select-phytonutrients-enhance-libido/ September, 26*, 2017.

Fardini, Y., Wang, X., Témoin, S., Nithianantham, S., Lee, D., Shoham, M., & Han, Y. W. (2011). Fusobacterium nucleatum adhesin FadA binds vascular endothelial cadherin and alters endothelial integrity. *Molecular Microbiology, 82*(6), 1468-1480.

Feairheller, D. L., Park, J. Y., Sturgeon, K. M., Williamson, S. T., Diaz, K. M., Veerabhadrappa, P., & Brown, M. D. (2011). Racial differences in oxidative stress and inflammation: in vitro and in vivo. *Clinical and Translational Science, 4*(1), 32-37.

Ferguson, J. F., Patel, P. N., Shah, R. Y., Mulvey, C. K., Gadi, R., Nijjar, P. S., Master, S. R. (2013). Race and gender variation in response to evoked inflammation. *Journal of Translational Medicine, 11*(1), 63.

Ferrand, P. E., Fujimoto, T., Chennathukuzhi, V., Parry, S., Macones, G. A., Sammel, M., Strauss, J. F. (2002). The CARD15 2936insC mutation and TLR4 896 A> G polymorphism in African Americans and risk of preterm premature rupture of membranes (PPROM). *Molecular Human Reproduction, 8*(11), 1031-1034.

Ferrand, P. E., Parry, S., Sammel, M., Macones, G. A., Kuivaniemi, H., Romero, R., & Strauss, J. F. (2002). A polymorphism in the matrix metalloproteinase-9 promoter is associated with increased risk of preterm premature rupture of membranes in African Americans. *Molecular Human Reproduction, 8*(5), 494-501.

Fiscella, K. (1996). Racial disparities in preterm births. The role of urogenital infections. *Public Health Reports, 111*(2), 104.

Folgueras, A. R., Pendás, A. M., Sánchez, L. M., & López-Otín, C. (2004). Matrix metalloproteinases in cancer: from new functions to improved inhibition strategies. *International Journal of Developmental Biology, 48*, 411-424.

Fujimoto, T., Parry, S., Urbanek, M., Sammel, M., Macones, G., Kuivaniemi, H., Strauss, J. F. (2002). A single nucleotide polymorphism in the matrix metalloproteinase-1 (MMP-1) promoter influences amnion cell MMP-1 expression and risk for preterm premature rupture of the fetal membranes. *Journal of Biological Chemistry, 277*(8), 6296-6302.

Galgut, P. (1994). Observations on the recurrence of destructive periodontal disease during a period of four years after treatment. Paper presented at the Journal of the western society of periodontology/periodontal abstracts.

Genco, R., Kornman, K., Williams, R. et al. (1996). Consensus report: periodontal diseases: pathogenesis and microbial factors. *Annals of Periodontology*. 1:926.

Gheren, L., Cortelli, J., Rodrigues, E., Holzhausen, M., & Saad, W. (2008). Periodontal therapy reduces arginase activity in saliva of patients with chronic periodontitis. *Clinical Oral Investigations, 12*(1), 67-72.

Godefroy, E., & Bhardwaj, N. (2012). Dysregulation of anti-tumor immunity by the matrix metalloproteinase-2. *Oncoimmunology, 1*(1), 109-111.

Grainger, J. R., Wohlfert, E. A., Fuss, I. J., Bouladoux, N., Askenase, M. H., Legrand, F., Belkaid, Y. (2013). Inflammatory monocytes regulate pathologic responses to commensals during acute gastrointestinal infection. *Nature Medicine, 19*(6), 713-721.

Green, J., & Vermillion, J. (1960). The oral hygiene index: a method for classifying oral hygiene status. *Journal of American Dental Association. 61*(172), 10.

Griffen, A. L., Beall, C. J., Firestone, N. D., Gross, E. L., DiFranco, J. M., Hardman, J. H., Leys, E. J. (2011). CORE: a phylogenetically-curated 16S rDNA database of the core oral microbiome. *PLoS ONE, 6*(4), e19051.

Grubbs, V., Vittinghoff, E., Taylor, G., Kritz-Silverstein, D., Powe, N., Bibbins-Domingo, K., Cummings, S. R. (2015). The association of periodontal disease with kidney function decline: a longitudinal retrospective analysis of the MrOS dental study. *Nephrology Dialysis Transplantation*, gfv312.

Guinn, D. A., Goldenberg, R. L., Hauth, J. C., Andrews, W. W., Thom, E., & Romero, R. (1995). Risk factors for the development of preterm premature rupture of the membranes after arrest of preterm labor. *American Journal of Obstetrics and Gynecology, 173*(4), 1310-1315.

Gürsoy, M., Könönen, E., Tervahartiala, T., Gürsoy, U., Pajukanta, R., & Sorsa, T. (2010). Longitudinal study of salivary proteinases during pregnancy and postpartum. *Journal of Periodontal Research, 45*(4), 496-503.

Hajishengallis, G. (2015). Periodontitis: from microbial immune subversion to systemic inflammation. *Nature Reviews Immunology, 15*(1), 30-44.

Hajishengallis, G., Darveau, R. P., & Curtis, M. A. (2012). The keystone-pathogen hypothesis. *Nature Reviews Microbiology, 10*(10), 717-725.

Hajishengallis, G., & Lamont, R. J. (2012). Beyond the red complex and into more complexity: the polymicrobial synergy and dysbiosis (PSD) model of periodontal disease etiology. *Molecular Oral Microbiology, 27*(6), 409-419.

Hajishengallis, G., Liang, S., Payne, M. A., Hashim, A., Jotwani, R., Eskan, M. A., Lambris, J. D. (2011). Low-abundance biofilm species orchestrates inflammatory periodontal disease through the commensal microbiota and complement. *Cell Host & Microbe, 10*(5), 497-506.

Han, Y., Houcken, W., Loos, B., Schenkein, H., & Tezal, M. (2014). Periodontal disease, atherosclerosis, adverse pregnancy outcomes, and head-and-neck cancer. *Advances in Dental Research, 26*(1), 47-55.

Han, Y. W., Fardini, Y., Chen, C., Iacampo, K. G., Peraino, V. A., Shamonki, J. M., & Redline, R. W. (2010). Term stillbirth caused by oral Fusobacterium nucleatum. *Obstetrics and Gynecology, 115*(2 Pt 2), 442.

Hart, R., Doherty, D. A., Pennell, C. E., Newnham, I. A., & Newnham, J. P. (2012). Periodontal disease: a potential modifiable risk factor limiting conception. *Human Reproduction*, des034.

Hart, R., & Norman, R. (2006). Polycystic ovarian syndrome–prognosis and outcomes. *Best Practice & Research Clinical Obstetrics & Gynaecology, 20*(5), 751-778.

Heikkinen AM, Pajukanta R, Pitkäniemi J, Broms U, Sorsa T, Koskenvuo M, et al. (2008). The effect of smoking on periodontal health of 15- to 16-year-old adolescents. *J Periodontol.* ;79:2042-47.

Heikkinen AM, Sorsa T, Pitkäniemi J, Tervahartiala T, Kari K, Broms U, Koskenvuo M, Meurman JH. (2010). Smoking affects   diagnostic salivary periodontal disease biomarker levels in adolescents. *J Periodontol* : 81:1299-307.

Heikkinen AM. (2011). Oral health, smoking and adolescence. University of Helsinki, Faculty of Medicine, Institute of Dentistry. Available at: http://urn.fi URN:ISBN:978-952-10-7250-5. Accessed October 21, 2011. 39.

Heikkinen, A. M., Kettunen, K., Kovanen, L., Haukka, J., Elg, J., Husu, H., Sorsa, T. (2016a). Inflammatory mediator polymorphisms associate with initial periodontitis in adolescents. *Clinical and Experimental Dental Research, 2*(3), 208-215.

Heikkinen, A. M., Raivisto, T., Kettunen, K., Kovanen, L., Haukka, J., Pakbaznejad Esmaeili, E., Ruokonen, H. (2016b). Pilot Study on the Genetic Background of an Active Matrix Metalloproteinase (aMMP-8) Test in Finnish Adolescents. *Journal of Periodontology*(0), 1-10.

Hernández, M., Gamonal, J., Mäntylä, P., Vernal, R., Tervahartiala, T., & Sorsa, T. (2012). *The role of immuno-inflammatory response in the pathogenesis of chronic periodontitis and development of chair-side point of care diagnostics*: INTECH Open Access Publisher.

Holden, J. A., Attard, T. J., Laughton, K. M., Mansell, A., O'Brien-Simpson, N. M., & Reynolds, E. C. (2014). Porphyromonas gingivalis lipopolysaccharide weakly activates M1 and M2 polarized mouse macrophages but induces inflammatory cytokines. *Infection and Immunity, 82*(10), 4190-4203.

Holm, T., Aukrust, P., Andreassen, A. K., Ueland, T., Brosstad, F., Frøland, S. S., Gullestad, L. (2000). Peripheral endothelial dysfunction in heart transplant recipients: possible role of proinflammatory cytokines. *Clinical Transplantation, 14*(3), 218-225.

Jeffcoat, M. K., Geurs, N. C., Reddy, M. S., Cliver, S. P., Goldenberg, R. L., & Hauth, J. C. (2001). Periodontal infection and preterm birth: results of a prospective study. *The Journal of the American Dental Association, 132*(7), 875-880.

Johnson, N., van Voorst, S., Sowter, M. C., Strandell, A., & Mol, B. W. J. (2004). Surgical treatment for tubal disease in women due to undergo in vitro fertilisation. *The Cochrane Library.*

Kavoussi, S. K., West, B. T., Taylor, G. W., & Lebovic, D. I. (2009). Periodontal disease and endometriosis: analysis of the National Health and Nutrition Examination Survey. *Fertility and Sterility, 91*(2), 335-342.

Keller, J. J., Chung, S. D., & Lin, H. C. (2012). A nationwide population-based study on the association between chronic periodontitis and erectile dysfunction. *Journal of Clinical Periodontology, 39*(6), 507-512.

Kim, N. N., Cox, J. D., Baggio, R. F., Emig, F. A., Mistry, S. K., Harper, S. L., Traish, A. (2001). Probing Erectile Function: S-(2-Boronoethyl)-l-Cysteine Binds to Arginase as a Transition State Analogue and Enhances Smooth Muscle Relaxation in Human Penile Corpus Cavernosum. *Biochemistry, 40*(9), 2678-2688.

Kinane, D.F,, Stathopoulou, P.G. and Papapanou, P.N. (2017). Periodontal diseases. *Nature Review Disease Primers,* 22(3):17038

Klinger, A., Hain, B., Yaffe, H., & Schonberger, O. (2011). Periodontal status of males attending an in vitro fertilization clinic. *Journal of Clinical Periodontology, 38*(6), 542-546.

Kubar, A., Saygun, I., Ozdemir, A., Yapar, M., & Slots, J. (2005). Real-time polymerase chain reaction quantification of human cytomegalovirus and Epstein-Barr virus in periodontal pockets and the adjacent gingiva of periodontitis lesions. *Journal of Periodontal Research, 40*(2), 97-104.

Kumar, P. S., Griffen, A. L., Moeschberger, M. L., & Leys, E. J. (2005). Identification of candidate periodontal pathogens and beneficial species by quantitative 16S clonal analysis. *Journal of Clinical Microbiology, 43*(8), 3944-3955.

Lachter, J., Babich, J. P., Brookman, J. C., & Eliakim, R. (2004). Resolution of male infertility following anti-tumor necrosis factor (Infliximab): a case report. *International Journal of Gastroenterology, 3*(1).

Lang, N. P., Adler, R., Joss, A., & Nyman, S. (1990). Absence of bleeding on probing an indicator of periodontal stability. *Journal of Clinical Periodontology, 17*(10), 714-721.

Lang, N. P., & Tonetti, M. S. (2003). Periodontal risk assessment (PRA) for patients in supportive periodontal therapy (SPT). *Oral Health and Preventive Dentistry, 1*(1), 7-16.

Leppilahti, J., Ahonen, M. M., Hernández, M., Munjal, S., Netuschil, L., Uitto, V. J., Mäntylä, P. (2011). Oral rinse MMP-8 point-of-care immuno test identifies patients with strong periodontal inflammatory burden. *Oral Diseases, 17*(1), 115-122.

Leppilahti JM, Kallio MA, Tervahartiala T, Sorsa T, Mäntylä P. (2014). Gingival crevicular fluid matrix metalloproteinase-8 levels predict treatment outcome among smokers with chronic periodontitis. *Journal of Periodontology*, 85(2):250-60.

Leppilahti JM, Sorsa T, Kallio MA, Tervahartiala T, Emingil G, Han B, Mäntylä P. (2015). The utility of gingival crevicular fluid matrix metalloproteinase-8 response patterns

in prediction of site-level clinical treatment outcome. *Journal of Periodontology* 86(6):777-87.

Li, Q., Hao, S., Fang, J., Xie, J., Kong, X.-H., & Yang, J.-X. (2015). Effect of non-surgical periodontal treatment on glycemic control of patients with diabetes: a meta-analysis of randomized controlled trials. *Trials, 16*(1), 291.

Liu, H., Redline, R. W., & Han, Y. W. (2007). Fusobacterium nucleatum induces fetal death in mice via stimulation of TLR4-mediated placental inflammatory response. *The Journal of Immunology, 179*(4), 2501-2508.

Löe, H., Theilade, E. and Jensen SB. (1965). Experimental gingivitis in man. *Journal of Periodontology*, 36,177.

Lourbakos, A., Potempa, J., Travis, J., D'Andrea, M. R., Andrade-Gordon, P., Santulli, R., Pike, R. N. (2001). Arginine-Specific Protease fromPorphyromonas gingivalis Activates Protease-Activated Receptors on Human Oral Epithelial Cells and Induces Interleukin-6 Secretion. *Infection and Immunity, 69*(8), 5121-5130.

McKinnon, A., Black, A. Y., Lortie, K., & Fleming, N. A. (2013). A case of adolescent pelvic inflammatory disease caused by a rare bacterium: Fusobacterium nucleatum. *Journal of Pediatric Adolescent Gynecology, 26*(6), e113-115.

McQuibban, G. A., Gong, J.-H., Wong, J. P., Wallace, J. L., Clark-Lewis, I., & Overall, C. M. (2002). Matrix metalloproteinase processing of monocyte chemoattractant proteins generates CC chemokine receptor antagonists with anti-inflammatory properties in vivo. *Blood, 100*(4), 1160-1167.

Mokuolu, A., Abdul, I. F., & Adesiyun, O. (2002). Maternal factors associated with early spontaneous singleton preterm delivery in Nigeria. *Tropical Journal of Obstetrics and Gynaecology, 19*, 32-35.

Morris, M., & Li, L. (2012). Molecular mechanisms and pathological consequences of endotoxin tolerance and priming. *Archivum Immunologiae et Therapiae Experimentalis, 60*(1), 13-18.

Nath, S. G., & Raveendran, R. (2013). Microbial dysbiosis in periodontitis. *Journal of Indian Society of Periodontology, 17*(4), 543-545. doi:10.4103/0972-124x.118334

Navarro-Sanchez, A. B., Faria-Almeida, R., & Bascones-Martinez, A. (2007). Effect of non-surgical periodontal therapy on clinical and immunological response and glycaemic control in type 2 diabetic patients with moderate periodontitis. Journal of Clinical Periodontology, 34(10), 835-843.

Newman, M. G., Takei, H., Klokkevold, P. R., & Carranza, F. A. (2011). *Carranza's Clinical Periodontology*: Elsevier health sciences.

Niederman, R. (2013). Pregnancy gingivitis and causal inference. *Evidence Based Dentistry*, 14(4):107-8.

Nield-Gehrig, J. S., & Willmann, D. E. (2007). *Foundations of Periodontics for the Dental Hygienist*: Lippincott Williams & Wilkins.

Nien, J. K., Yoon, B. H., Espinoza, J., Kusanovic, J. P., Erez, O., Soto, E., Mazor, M. (2006). A rapid MMP-8 bedside test for the detection of intra-amniotic inflammation identifies patients at risk for imminent preterm delivery. *American Journal of Obstetrics and Gynecology, 195*(4), 1025-1030.

Nwhator, S. (2005). Periodontal disease in smokers: a study of factory workers in Lagos state [Dissertation]. *Nigeria, Faculty of Dental Surgery, National Postgraduate Medical College of Nigeria*.

Nwhator, S., Opeodu, O., Ayanbadejo, P., Umeizudike, K., Olamijulo, J., Alade, G., Sorsa, T. (2015). Could periodontitis affect time to conception? *Annals of Medical and Health Sciences Research, 4*(5), 817-822.

Nwhator, S. O., Umeizudike, K. A., Ayanbadejo, P. O., Opeodu, O. I., Olamijulo, J. A., & Sorsa, T. (2014). Another reason for impeccable oral hygiene: Oral hygiene-sperm count link. *The Journal of Contemporary Dental Practice, 15*(3), 352.

Offenbacher, S., Katz, V., Fertik, G., Collins, J., Boyd, D., Maynor, G., Beck, J. (1996). Periodontal infection as a possible risk factor for preterm low birth weight. *Journal of Periodontology, 67*(10s), 1103-1113.

Oğuz, F., Eltas, A., Beytur, A., Akdemir, E., Uslu, M. Ö., & Güneş, A. (2013). Is there a relationship between chronic periodontitis and erectile dysfunction? *The Journal of Sexual Medicine, 10*(3), 838-843.

Omole-Ohonsi, A., & Attah, R. (2012). Risk Factors of Preterm Deliveries at Aminu Kano Teaching Hospital, Kano, Nigeria. *South Asian Journal of Management Sciences, 1*, 3-10.

Paalani, M., Lee, J. W., Haddad, E., & Tonstad, S. (2011). Determinants of inflammatory markers in a bi-ethnic population. *Ethnicity & disease, 21*(2), 142.

Patil V., Patil, V. P., Gokhale, N., Acharya, A., & Kangokar, P. (2016). Chronic Periodontitis in Type 2 Diabetes Mellitus: Oxidative Stress as a Common Factor in Periodontal Tissue Injury. *Journal of Clinical & Diagnostic Research, 10*(4).

Paulson, J., & Polakoski, K. (1977). Isolation of a spermatozoal immobilization factor from Escherichia coli filtrates. *Fertility and Sterility, 28*(2), 182-185.

Pedersen, P. U., Larsen, P., & Håkonsen, S. J. (2016). The effectiveness of systematic perioperative oral hygiene in reduction of postoperative respiratory tract infections

after elective thoracic surgery in adults: a systematic review. *JBI Database of Systematic Reviews and Implementation Reports, 14*(1), 140-173.

Peltier, M. R., Drobek, C. O., Bhat, G., Saade, G., Fortunato, S. J., & Menon, R. (2012). Amniotic fluid and maternal race influence responsiveness of fetal membranes to bacteria. *Journal of Reproductive Immunology, 96*(1), 68-78.

Perdichizzi, A., Nicoletti, F., La Vignera, S., Barone, N., D'Agata, R., Vicari, E., & Calogero, A. E. (2007). Effects of tumour necrosis factor-α on human sperm motility and apoptosis. *Journal of Clinical Immunology, 27*(2), 152-162.

Ponchietti, A., Erre, G., & Fenu, P. (2009). Effect of TNF-alpha blockers on sperm count and motility in patients with rheumatic diseases: a cross-sectional study. *Annals of Rheumatic Diseases, 68*, 229.

Pihlstrom BL, Tabak L. The National Institute of Dental and Craniofacial Research: research for the practicing dentist. . (2005).*Journal of the American Dental Association*, 136(6):728-37

Prabha, V., Sandhu, R., Kaur, S., Kaur, K., Sarwal, A., Mavuduru, R. S., & Singh, S. K. (2010). Mechanism of sperm immobilization by Escherichia coli. *Advances in Urology, 2010*.

Rahnama, M., Czupkałło, Ł., Kozicka-Czupkałło, M., & Łobacz, M. (2014). Gingival crevicular fluid– composition and clinical importance in gingivitis and periodontitis. *Polish of Journal of Public Health, 124*(2), 96-98.

Reynolds, J. J., & Meikle, M. C. (1997). Mechanisms of connective tissue matrix destruction in periodontitis. *Periodontology 2000, 14*(1), 144-157.

Rudolph-Owen, L. A., Cannon, P., & Matrisian, L. M. (1998). Overexpression of the matrix metalloproteinase matrilysin results in premature mammary gland differentiation and male infertility. *Molecular Biology of the Cell, 9*(2), 421-435.

Rydén, L., Buhlin, K., Ekstrand, E., de Faire, U., Gustafsson, A., Holmer, J., Nygren, Å. (2016). Periodontitis Increases the Risk of a First Myocardial Infarction: A Report From the PAROKRANK Study. *Circulation*, 115.020324.

Schenkein, H., Bradley, J., & Purkall, D. (2013). Anticardiolipin in Porphyromonas gingivalis antisera causes fetal loss in mice. *Journal of Dental Research, 92*(9), 814-818.

Sharma, A., Pradeep, A., & Raju P, A. (2011). Association between chronic periodontitis and vasculogenic erectile dysfunction. *Journal of Periodontology, 82*(12), 1665-1669.

Shibahara, H., Suzuki, T., Kikuchi, K., Hirano, Y., & Suzuki, M. (2005). Serum Matrix Metalloproteinase and Tissue Inhibitor of Metalloproteinase Concentrations in

Infertile Women Achieved Pregnancy Following IVF-ET. *American Journal of Reproductive Immunology,* 54(4), 186-192.

Slade, G., Offenbacher, S., Beck, J., Heiss, G., & Pankow, J. (2000). Acute-phase inflammatory response to periodontal disease in the US population. *Journal of Dental Research, 79*(1), 49-57.

Slauk, A. (1940). Plaque disease and dentist. *Publisher Gehlen, Leipzig.(Article in German).*

Slots, J. (1979). Subgingival microflora in periodontal disease. *Journal of Clinical Periodontology,* 6, 351-82.

Socransky, S. S., & Haffajee, A. D. (2005). *Periodontal microbial ecology. Periodontology 2000, 38*(1), 135-187.

Sorsa, T., Hernández, M., Leppilahti, J., Munjal, S., Netuschil, L., & Mäntylä, P. (2010). Detection of gingival crevicular fluid MMP-8 levels with different laboratory and chair-side methods. *Oral Diseases,* 16(1), 39-45.

Sorsa, T., Mäntylä, P., Rönkä, H., Kallio, P., Kallis, G. B., Lundqvist, C., Teronen, O. (1999). Scientific basis of a matrix metalloproteinase-8 specific chair-side test for monitoring periodontal and peri-implant health and disease. *Annals of the New York Academy of Sciences, 878*(1), 130-140.

Sorsa, T., Tjäderhane, L., Konttinen, Y. T., Lauhio, A., Salo, T., Lee, H. M., Mäntylä, P. (2006). Matrix metalloproteinases: contribution to pathogenesis, diagnosis and treatment of periodontal inflammation. *Annals of Medicine, 38*(5), 306-321.

Sorsa, T., Tjäderhane, L., & Salo, T. (2004). Matrix metalloproteinases (MMPs) in oral diseases. *Oral Diseases, 10*(6), 311-318.

Speroff, L., & Fritz, M. A. (2005). *Clinical Gynecologic Endocrinology and Infertility:* Lippincott Williams & Wilkins.

Theilade, E. (1986). The non-specific theory in microbial etiology of inflammatory periodontal diseases. *Journal of Clinical Periodontology.*

Thompson, R. J. (2003). DHEA supplement for increasing women's libido: US Patent 20,030,064,123.

Tsao, C. W., Liu, C. Y., Cha, T. L., Wu, S. T., Chen, S. C., & Hsu, C. Y. (2015). Exploration of the association between chronic periodontal disease and erectile dysfunction from a population-based view point. *Andrologia, 47*(5), 513-518.

Uitto, V. J., Suomalainen, K., & Sorsa, T. (1990). Salivary collagenase. Origin, characteristics and relationship to periodontal health. *Journal of Periodontal Research, 25*(3), 135-142.

Umeizudike, K., Ayanbadejo, P., Savage, K., Nwhator, S., Akanmu, A., & Ogunleye, O. (2016). Comparative periodontal status of human immunodeficiency virus-positive patients and controls in a dedicated human immunodeficiency virus clinic in Nigeria. *Nigerian Journal of Clinical Practice, 19*(1), 35.

Uppoor, A. S., Lohi, H. S., & Nayak, D. (2013). Periodontitis and Alzheimer's disease: oral systemic link still on the rise? *Gerodontology, 30*(3), 239-242.

Usin, M. M., Menso, J., Rodríguez, V. I., González, A., Tabares, S., Parodi, R., & Sembaj, A. (2016). Association between maternal periodontitis and preterm and/or low birth weight infants in normal pregnancies. *The, 29*(1), 115 *Journal of Maternal-Fetal & Neonatal Medicine* -119.

Vasconcelos, R. C., Costa, A. d. L. L., Freitas, R. d. A., Bezerra, B. A. d. A., Santos, B. R. M. d., Pinto, L. P., & Gurgel, B. C. d. V. (2016). Immunoexpression of HIF-1α and VEGF in Periodontal Disease and Healthy Gingival Tissues. *Brazilian Dental Journal, 27*(2), 117-122.

Wang, M., Krauss, J. L., Domon, H., Hosur, K. B., Liang, S., Magotti, P., Hajishengallis, G. (2010). Microbial hijacking of complement–Toll-like receptor crosstalk. *Science Signaling, 3*(109), ra11.

Weiss, G., Goldsmith, L. T., Taylor, R. N., Bellet, D., & Taylor, H. S. (2009). Inflammation in reproductive disorders. *Reproductive Sciences, 16*(2), 216-229.

White, D. J. (1997). Dental calculus: recent insights into occurrence, formation, prevention, removal and oral health effects of supragingival and subgingival deposits. *European Journal of Oral Sciences, 105*(5), 508-522.

Williams, R. (1973). Benefit and mischief from commensal bacteria. *Journal of Clinical Pathology, 26*(11), 811.

1

Clinical Correlates of a Lateral-Flow Immunoassay Oral Risk Indicator

S.O. Nwhator,* P.O. Ayanbadejo,† K.A. Umeizudike,† O.I. Opeodu,‡ G.A. Agbelusi,†
J.A. Olamijulo,§ M.O. Arowojolu,‡ T. Sorsa,‖ B.S. Babajide,¶ and D.O. Opedun#

Background: The aim of the present study is to investigate the clinical correlates of a novel lateral-flow immunoassay with bleeding on probing (BOP), oral hygiene, and periodontal probing depth. This report offers a simple, rapid, and highly sensitive tool that addresses two issues important to periodontists: 1) detecting active periodontitis, and 2) predicting chronic periodontitis.

Methods: Seventy-six of 86 males requiring seminal fluid analysis as part of a separate study were serially recruited into the study. After basic dental and periodontal examination under natural light and with the use of the community periodontal index of treatment needs (CPITN) probe, debris and calculus indices were recorded per participant. Participants were subsequently grouped into "good," "fair," and "poor" oral hygiene categories based on a simplified oral hygiene index. BOP was assessed with the ball-ended tip of the probe, and periodontitis was assessed with pocket probing as well as a lateral flow of neutrophil collagenase-2 immunoassay, which measures levels of matrix metalloproteinase-8.

Results: Neutrophil collagenase-2 immunoassay was 96% sensitive for poor oral hygiene, 95% sensitive for chronic periodontitis (defined as at least two sites with periodontal pockets), and 82.6% sensitive for at least two sites with BOP.

Conclusion: Neutrophil collagenase-2 immunoassay had a high sensitivity for at least two sites with BOP and two sites with periodontal pockets but a lower relationship for single-site pockets and BOP. *J Periodontol 2014;85:188-194.*

KEY WORDS

Immunoassay; matrix metalloproteinases; oral hygiene; periodontitis; risk.

* Department of Preventive Dentistry, University of Abuja Teaching Hospital, Federal Capital Territory, Abuja, Nigeria.
† Department of Preventive Dentistry, Faculty of Dental Sciences, College of Medicine, University of Lagos, Lagos, Nigeria.
‡ Department of Periodontology and Community Dentistry, University of Ibadan, Ibadan, Nigeria.
§ Department of Obstetrics and Gynecology, College of Medicine, University of Lagos.
‖ Department of Oral and Maxillofacial Diseases, Helsinki University Central Hospital, Helsinki, Finland.
¶ Research Laboratory, Department of Obstetrics and Gynecology, College of Medicine, University of Lagos.
# Department of Medical Microbiology and Parasitology, Lagos University Teaching Hospital, Lagos, Nigeria.

Current trends in periodontal medicine are changing long-held views about the mouth being a mere mirror of the body. A growing body of evidence suggests a two-way relationship,[1-3] yet to be fully understood. With the help of modern diagnostic and investigative procedures, it is now possible to predict the risk of chronic periodontitis (CP) in apparently healthy mouths. These scientific innovations and advances are hastening a change of opinion on traditional prevalence figures for periodontitis. It is now widely believed that CP is much more prevalent than was previously perceived and reported in traditional dental texts.[4-6]

With increasing evidence of a possible link between oral inflammatory burden and general health, the need to rapidly screen a population in an objective manner has never been greater. Developed in Germany and well researched in other parts of the world,[7,8] neutrophil collagenase-2 immunoassay has been used in periodontitis screening with claims about its ability to detect periodontal inflammation ahead of overt clinical signs and symptoms.

Neutrophil collagenase-2 immunoassay could potentially help in rapid screening of a target population with minimal equipment and expertise. If this tool closely correlates clinical parameters and shows enough sensitivity, it could be easily used in such rapid screening without the fear of missing

active periodontal lesions. Neutrophil collagenase-2 immunoassay promises this and even more: neutrophil collagenase-2 immunoassay has not only the ability to detect active periodontitis but, in fact, detects subclinical periodontal inflammation.[5] Neutrophil collagenase-2 (matrix metalloproteinase-8 [MMP-8]) concentration has been reported to rise in severe CP, especially in non-smokers,[7,8] and affects chronic inflammation in other parts of the body.[9]

One brand of neutrophil collagenase-2 immunoassay** uses lateral-flow immunoassay to detect and measure levels of active MMP-8, a collagenolytic enzyme whose levels rise ahead of overt signs of CP. Therefore, it estimates past and present experience of CP, as well as the risk of yet-to-be-observed CP.

If this tool does what it claims, it would be useful for dentists as an alternative to more cumbersome traditional measures involving estimations of clinical attachment loss (AL). However, it would serve another purpose: estimation of oral inflammatory burden when there is no dentist! That means that any target group could be easily screened by their health care provider. Only positive cases would then need additional preventive or curative attention of the oral/dental health care practitioner.

It was in light of these potential benefits that the present authors proceeded to study the clinical oral/periodontal correlates of this novel neutrophil collagenase-2 immunoassay.

MATERIALS AND METHODS

Two teaching hospitals in southwest Nigeria were involved: 1) University College Hospital, Ibadan, Nigeria; and 2) Lagos University Teaching Hospital, Lagos, Nigeria. The study was performed within a 4-month period, from August to November 2012, in accordance with the World Medical Association Declaration of Helsinki. This study was approved by ethical committees of participating hospitals as part of a wider study.

The sample included a non-random, serial sampling of patients for whom a seminal fluid analysis had been ordered for various reasons. A total of 86 participants were recruited from two teaching hospitals, of which 76 were eventually included in the analysis, with 10 being excluded because of missing data. The only reason these patients were selected is because this study was part of a bigger study investigating the link between CP and subfertility. All study participants were non-smokers, except for five, constituting ≈5.8%. No other special characteristics or confounders were identified among the study population. Informed verbal consent was obtained for the original study,

which involved assessment of selected oral and periodontal parameters among males undergoing seminal fluid analysis.

Interviewer (examiner)-administered questionnaires were used as the research instrument, whereas neutrophil collagenase-2 immunoassay was used for rapid assessment of periodontitis. Demographic details were obtained from participants.

Intraoral Dental and Periodontal Assessment

Intraoral dental and periodontal assessment included the following: 1) debris index and calculus index scores; 2) bleeding on probing (BOP); and 3) basic periodontal examination using the community periodontal index of treatment needs (CPITN) probe. AL was not assessed. Oral hygiene scores were obtained by the sum of debris and calculus index scores.

All examinations were performed by standardized periodontists (SON, POA, KAU, OIO, and MOA).

Participants were grouped into "good," "fair," and "poor" oral hygiene categories using recommended cutoff points. BOP was assessed with the ball-ended tip of the CPITN probe.[10] Oral hygiene was calculated from the sum of debris and calculus index score and expressed as good (0 to 1.2), fair (1.3 to 3.0), and poor (3.1 to 6.0).[11] Participants were grouped into "good," "fair," and "poor" oral hygiene categories using recommended cut off points as detailed above. CPITN code 3 formed the basis of periodontal pocket assessment. The active MMP-8 neutrophil collagenase-2 immunoassay was used as a simple mouthrinse, and results were read as a color change.

Immunoassay Procedure

The immunoassay test was administered to participants according to the instructions of the manufacturer, which included a 30-second prerinse with tap water, followed by a 30-second wait, after which participants rinsed with the test liquid for 30 seconds. Patients poured the mouthrinse into a little collection cup that accompanied the test kit. Three milliliters of the rinse were drawn up into a syringe, and then a filter was placed on the syringe through which a maximum of four drops was placed in a lateral-flow immunoassay system which showed one single blue line for a negative result (no risk) and two blue lines for a positive result (increased risk). The result was read as a color change within 5 minutes.

The examiners practiced and were standardized with sample kits to get conversant with the color change, and because this was a single color (blue), bias was eliminated to the barest minimum.

Correlates of immunoassay results and clinical parameters were evaluated as follows. Results of

** PerioMarker, Dentognistics, Jena, Germany.

Table 1.

Correlates of Immunoassay With Oral Hygiene

Category	Immunoassay Negative	Immunoassay Positive	Total
Good	8	5 (38.5%)	13
Fair	15	23 (60.5%)	38
Poor	1	24 (96%)	25
Total	24	52	76

There was a direct relationship between oral hygiene and immunoassay result, with 96% of those with poor oral hygiene being immunoassay positive. Pearson χ^2 =15.29, df = 2, P = 0.000; likelihood ratio $\times2$ = 18.09, df = 2, P = 0.000.

periodontitis, defined as at least two sites per participant with periodontal pockets, were tabulated against immunoassay results and compared with patients with only one site with a periodontal pocket. A similar procedure and comparison was repeated with BOP sites.

Statistical Analyses

Data were analyzed using statistical software.[††] Sensitivity was expressed as the proportion of positive cases detected among the total number of cases. Specificity was not assessed because of the claims that the marker could detect even cases that were not clinically overt. Because there were 2 × 2 tables, Fisher exact statistic was used to estimate significance and 95% confidence level. Therefore, values $P \leq 0.05$ were accepted as statistically significant.

RESULTS

Correlates With Oral Hygiene

The sensitivity of neutrophil collagenase-2 immunoassay kit for poor oral hygiene was 24 of 25 or 96%, which was highly statistically significant (P = 000) (Table 1). The relationship between the neutrophil collagenase-2 immunoassay results and oral hygiene status was directly proportional.

Correlates With Periodontitis

The sensitivity of the neutrophil collagenase-2 immunoassay kit for periodontitis when measured as the presence of at least two periodontal pockets was 18 of 19 or 95%, which was highly statistically significant (P = 0.003) (Table 2).

Correlates With BOP

The sensitivity of the neutrophil collagenase-2 immunoassay kit for BOP when measured as the presence of at least two sites with BOP was 19 of 23 or 82.6%, which was highly statistically significant (P = 000) (Table 3).

Correlates With Apparently Normal Sites

Six of 43 apparently healthy sites showed increased risk with the neutrophil collagenase-2 immunoassay.

DISCUSSION

Recent advances in salivary diagnostics[12,13] have increased the knowledge of enzymatic interactions and how they correlate with the clinical picture in periodontitis. MMP-8 exists in active and latent forms that correlate clinically with periodontitis and gingivitis, respectively.[14-16]

The development of a lateral-flow-type assay that selectively detects levels of active MMP-8, which in turn confers the ability to selectively detect periodontitis, is an innovative technology.[‡‡] The present authors' interest in the use of neutrophil collagenase-2 immunoassay stems from the possibility of screening for periodontitis without the initial complex measurements of AL.

Three things are very important to the periodontist: 1) the presence of periodontitis, 2) the severity of periodontitis, and 3) the progression of periodontitis.[17] The sensitivity of active MMP-8 to address these three concerns appears to be in a decreasing order, meaning that although the marker is sensitive enough to detect periodontitis, it appears to be too sensitive for clinical classification of its severity. Its ability to detect very minute levels of active MMP-8, also called neutrophil collagenase-2, is both an advantage and a disadvantage.

Its advantage lies in its predictive ability because it is able to predict the likelihood of periodontitis among participants.[15] However, the threshold for the detection of likely cases against present (active) periodontitis appears too close for meaningful clinical patient classification and treatment planning. The reasons for this are not far-fetched: although likelihood/risk for periodontitis is detected as a color change, active cases with periodontal pockets also appear as a similar color change. Although it is true that a faint line might be a distinguishing factor, this did not always correlate with

Table 2.

Correlates of Immunoassay With Periodontitis

Number of Pockets	Immunoassay Positive	Immunoassay Negative	Total
≥2	1	18	19
<2	23	34	57
Total	24	52	76

There was a strong association between the number of pockets and immunoassay result, especially in the presence of two periodontal pockets. Fisher exact, $P = 0.003$.

Table 3.

Correlates of Immunoassay With BOP

BOP Sites	Immunoassay Positive	Immunoassay Negative	Total
>2	4	19	23
<2	15	6	21
Total	19	25	44

There was a strong association between the number of sites with BOP and immunoassay result, especially in the presence of two sites with BOP. Fisher exact, $P = 0.000$.

the clinical condition of the patients' periodontal status.

It is interesting to note that although 23 of 57 patients (40.4%) with one periodontal pocket and one of 19 patients (5%) with two periodontal pockets had a negative result with immunoassay, six of 43 patients (14.0%) with clinically healthy periodontal tissues had a positive test indicating a higher risk.

With that noted, it is important to recognize the very positive sides of neutrophil collagenase-2 immunoassay. This biomarker is very sensitive in detecting established periodontitis. A very strong correlation was found between having at least two sites with periodontal pockets and having a positive result for the active MMP-8 biomarker. This makes active MMP-8 a very useful tool in screening for periodontitis, especially when periodontitis is considered to be the presence of at least two sites with periodontal pockets.[18] This assertion is clearly reflected in the results of 18 of 19 sites with at least two periodontal pockets (95%) which showed as positive with neutrophil collagenase-2 immunoassay. This is in sharp contrast with its sensitivity for one periodontal pocket, which yielded just 34 of 57 (59.6%) sites.

The same phenomenon was repeated with bleeding on gentle probing. Although the sensitivity for sites with only one site with BOP was six of 21 (28.6%), a striking difference is seen for sensitivity of the marker for at least two sites with BOP, which

sharply increases to 19 of 23, or 82.6%. Based on this result, it appears that the immunoassay is more sensitive for moderate-to-severe periodontitis in which there is at least two teeth with interdental bone loss; this might look like a flaw. Conversely, this might actually reflect a previously overlooked limitation in the current estimation of periodontitis. A paradigm shift is suggested to consider periodontitis in terms of inflammatory mediators that immunoassay measures rather than a mere measurement of random periodontal pockets.

Probably the most interesting finding of this study was the correlates of neutrophil collagenase-2 immunoassay with oral hygiene. A directly proportional relationship was found between oral hygiene index score and neutrophil collagenase-2 immunoassay results. Although 38.5% of those with good oral hygiene tested positive for the marker, the proportion rose to 60.5% among participants with fair oral hygiene and climaxed at 96% among participants with poor oral hygiene.

Therefore, based on the experience of the present authors, neutrophil collagenase-2 immunoassay has a highly statistically significant ability to differentiate among different levels of oral hygiene ($P = 0.000$), periodontal pockets ($P = 0.003$), and BOP sites ($P = 0.000$). It also possesses high sensitivity to detect the presence of poor oral hygiene (96%), periodontitis (expressed as at least two sites with periodontal pockets, 95%), and BOP in at least two sites (82.6%).Despite all this, the ability of the immunoassay to detect lower levels of disease process and oral hygiene is very low. However, the confusing aspect of the present study is that despite the low sensitivity for sites with less than two pockets and BOP sites, six of 43 apparently healthy sites show increased risk, indicating the predictive ability of neutrophil collagenase-2 immunoassay. This predictive ability lies in the ability of the immunoassay to detect periodontitis even ahead of obvious clinical signs of periodontal breakdown, such as BOP and AL. It is unclear how the biomarker was able to detect six apparently healthy sites but miss some sites with at least one

periodontal pocket. It is possible that these sites account for the reported sensitivity figures in the present study because the tool does not lay claim to 100% sensitivity *ab initio.*

Another plausible explanation lies in the currently accepted concepts in periodontal disease progression. It is now generally agreed that contrary to the traditional view of inflammatory periodontitis being a continuous process of activity (linear theory),[19] periodontitis actually occurs in bouts of disease activity with intervening periods of quiescence (random burst theory)[20] and is even proposed to be occurring in a multilevel pattern, which seems to unite both theories.[21] Again, because different stages of disease activity may be evident at different sites in the same mouth, the possibility that the supposed false negatives are actual sites of quiescent (inactive/passive) periodontitis needs to be investigated further.

Because bleeding on gentle probing correlates positively with disease activity,[22] it was necessary to compare immunoassay results with BOP. Unfortunately, correlates between BOP and immunoassay are also not very sensitive in the present study. The 82.6% sensitivity, although impressive, only applies to the presence of at least two bleeding sites and bears a striking resemblance to a previous sensitivity report.[23]

Again, the natural concern of periodontists is how to differentiate BOP observed from that caused by gingivitis alone. Although gingivitis also causes BOP, the BOP elicited in the current study cannot be explained away as gingivitis alone. The reason for this is simple: gingivitis corresponds with code 1 of the CPITN used in this study. To conclusively differentiate periodontitis from gingivitis, patients with CPITN code 1 must be separately analyzed to determine the sensitivity of the immunoassay tool for gingivitis. Unfortunately, only one patient in the current study was found to have a maximum CPITN code 1 (CPITNMAX 1). It would be interesting to discover how the immunoassay fares in patients with a CPITNMAX 1 in the future.

At first look, the strong association between the immunoassay and BOP appear disturbing because immunoassay claims to measure active periodontitis and not gingivitis. Even more confusing is the strong association with poor oral hygiene. However, evidence in literature removes the confusion. First, BOP is not exclusive to gingival inflammation. It is, in fact, a recognized clinical risk indicator for AL and, therefore, an effective tool in the diagnosis and monitoring of active periodontal diseases.[24,25]

Eliciting BOP in a pocket on consecutive appointments is a strong predictor of activity,[26] with inflammation being the likely common denominator

because significantly more inflammation has been associated with sites that show BOP than BOP-free sites.[27]

Inflammation can also at least partially explain the strong correlation between immunoassay and poor oral hygiene. Worsening oral hygiene is a direct measure of plaque buildup, and an increasing prevalence of Gram-negative *Actinomyces* species naturally follows this overgrowth of indigenous oral microflora, as does the accumulation of other Gram-negative microflora.[28]

It has been estimated that ≈25% of plaque microflora will become Gram negative.[29] It is well known that >90% of the outer membrane of Gram-negative bacteria contains lipopolysaccharide (LPS),[30] which promotes a strong immune response. Poor oral hygiene directly raises levels of hydrolytic enzymes originating from plaque bacteria and polymorphous leukocytes.[31] MMP-8 levels are also directly proportional to LPS levels, with a 50-fold increase in MMP-8 levels experimentally induced through the injection of LPS,[32] thus easily explaining the strong association between poor oral hygiene and immunoassay in the present study.

Although neutrophil collagenase-2 immunoassay demonstrated 96% sensitivity for CP, its cost might be an issue for resource-limited developing countries with poor and at times non-existent research funding. For such centers, there is at least the consolation that traditional measures of oral hygiene and BOP and periodontal pockets strongly correlate with the immunoassay. Such centers will unfortunately be stuck with these traditional measures for the foreseeable future.

However, affluent nations can be assured that at last a tool has been developed that can be used in the rapid screening of a population for CP. The fact that such screenings can be done by nurses, doctors, and trained members of the public makes the prospect even more promising.

The use of the CPITN probe imposes some limitation on this, being a measure of index of treatment needs rather than of periodontal status. A more useful tool would have been measurement of AL, which is a better index of periodontal status, an omission partially compensated for by the assessment of BOP. However, this omission was deliberate because the authors attempted to justify the rapid yet dependable nature of this novel neutrophil collagenase-2 immunoassay.

In the authors' opinion, the second limitation of not considering AL did not significantly affect the results. Although AL was not measured, probing depths (PDs) were measured, and in instances in which there was no recession, PD would be equal to AL. Therefore, the sensitivity of the test was

determined by its relationship with the number of pockets present, which is a measure of periodontitis. All pocket measurements were true pockets, in line with the criteria for CPITN. The examiners were competent to differentiate between true and false pockets. However, it is recommended that this study should be replicated in a full clinical setting with consideration for AL to further validate these findings.

CONCLUSION

Although neutrophil collagenase-2 immunoassay had a high sensitivity for at least two sites with BOP and two sites with periodontal pockets but low sensitivity for single-site pocket and BOP, the results must be interpreted knowing that AL was not assessed, which thus necessitates additional studies with full assessment of AL.

ACKNOWLEDGMENTS

REFERENCES

1. Preshaw PM, Alba AL, Herrera D, et al. Periodontitis and diabetes: A two-way relationship. *Diabetologia* 2012;55:21-31.
2. Kim J, Amar S. Periodontal disease and systemic conditions: A bidirectional relationship. *Odontology* 2006;94:10-21.
3. Offenbacher S, Barros SP, Altarawneh S, Beck JD, Loewy ZG. Impact of tooth loss on oral and systemic health. *Gen Dent* 2012;60:494-500, quiz 501-502.
4. Ligtenberg AJ, de Soet JJ, Veerman EC, Amerongen AV. Oral diseases: From detection to diagnostics. *Ann N Y Acad Sci* 2007;1098:200-203.
5. Taba M Jr., Kinney J, Kim AS, Giannobile WV. Diagnostic biomarkers for oral and periodontal diseases. *Dent Clin North Am* 2005;49:551-571, vi.
6. Eke PI, Dye BA, Wei L, et al. Prevalence of periodontitis in adults in the United States: 2009 and 2010. *J Dent Res* 2012;91:914-920.
7. Sorsa T, Tervahartiala T, Leppilahti J, et al. Collagenase-2 (MMP-8) as a point-of-care biomarker in periodontitis and cardiovascular diseases. Therapeutic response to non-antimicrobial properties of tetracyclines. *Pharmacol Res* 2011;63:108-113.
8. Sorsa T, Tjäderhane L, Salo T. Matrix metalloproteinases (MMPs) in oral diseases. *Oral Dis* 2004;10: 311-318.
9. Cox JH, Starr AE, Kappelhoff R, Yan R, Roberts CR, Overall CM. Matrix metalloproteinase 8 deficiency in mice exacerbates inflammatory arthritis through delayed neutrophil apoptosis and reduced caspase 11 expression. *Arthritis Rheum* 2010;62:3645-3655.
10. Ainamo J, Barnes D, Beagrie G, Cutress T, Martin J, Sardo-Infini J. Development of the World Health Organisation (WHO) community periodontal index

11. of treatment needs (CPITN). *Int Dent J* 1982;32: 281-301.
11. Greene JC, Vermillion JR. Oral hygiene index: A method for classifying oral hygiene status. *J Am Dent Assoc* 1960;61:172-179.
12. Giannobile WV. Salivary diagnostics for periodontal diseases. *J Am Dent Assoc* 2012;143(Suppl. 10):6S-11S.
13. Wei F, Wong DT. Point-of-care platforms for salivary diagnostics. *Chin J Dent Res* 2012;15:7-15.
14. Romanelli R, Mancini S, Laschinger C, Overall CM, Sodek J, McCulloch CA. Activation of neutrophil collagenase in periodontitis. *Infect Immun* 1999;67: 2319-2326.
15. Sorsa T, Hernández M, Leppilahti J, Munjal S, Netuschil L, Mäntylä P. Detection of gingival crevicular fluid MMP-8 levels with different laboratory and chair-side methods. *Oral Dis* 2010;16:39-45.
16. Teles R, Sakellari D, Teles F, et al. Relationships among gingival crevicular fluid biomarkers, clinical parameters of periodontal disease, and the subgingival microbiota. *J Periodontol* 2010;81:89-98.
17. Brown LJ, Löe H. Prevalence, extent, severity and progression of periodontal disease. *Periodontol 2000* 1993;2:57-71.
18. Klinger A, Hain B, Yaffe H, Schonberger O. Periodontal status of males attending an in vitro fertilization clinic. *J Clin Periodontol* 2011;38:542-546.
19. Löe H, Anerud A, Boysen H, Smith M. The natural history of periodontal disease in man. The rate of periodontal destruction before 40 years of age. *J Periodontol* 1978;49:607-620.
20. Socransky SS, Haffajee AD, Goodson JM, Lindhe J. New concepts of destructive periodontal disease. *J Clin Periodontol* 1984;11:21-32.
21. Gilthorpe MS, Zamzuri AT, Griffiths GS, Maddick IH, Eaton KA, Johnson NW. Unification of the "burst" and "linear" theories of periodontal disease progression: A multilevel manifestation of the same phenomenon. *J Dent Res* 2003;82:200-205.
22. Palcanis KG, Larjava IK, Wells BR, et al. Elastase as an indicator of periodontal disease progression. *J Periodontol* 1992;63:237-242.
23. Mäntylä P, Stenman M, Kinane DF, et al. Gingival crevicular fluid collagenase-2 (MMP-8) test stick for chair-side monitoring of periodontitis. *J Periodontal Res* 2003;38:436-439.
24. Claffey N, Egelberg J. Clinical indicators of probing attachment loss following initial periodontal treatment in advanced periodontitis patients. *J Clin Periodontol* 1995;22:690-696.
25. Leppilahti JM, Ahonen MM, Hernández M, et al. Oral rinse MMP-8 point-of-care immuno test identifies patients with strong periodontal inflammatory burden. *Oral Dis* 2011;17:115-122.
26. Biyikoğlu B, Buduneli N, Kardeşler L, Aksu K, Pitkala M, Sorsa T. Gingival crevicular fluid MMP-8 and -13 and TIMP-1 levels in patients with rheumatoid arthritis and inflammatory periodontal disease. *J Periodontol* 2009;80:1307-1314.
27. Lang NP, Adler R, Joss A, Nyman S. Absence of bleeding on probing. An indicator of periodontal stability. *J Clin Periodontol* 1990;17:714-721.
28. Aldredge WA. Bleeding on probing defined. *Dimens Dent Hyg* 2012;10:23-26.
29. Slots J. Subgingival microflora and periodontal disease. *J Clin Periodontol* 1979;6:351-382.

30. Papo N, Shai Y. A molecular mechanism for lipopolysac-charide protection of Gram-negative bacteria from anti-microbial peptides. *J Biol Chem* 2005;280:10378-10387.
31. Nakamura M, Slots J. Salivary enzymes. Origin and relationship to periodontal disease. *J Periodontal Res* 1983;18:559-569.
32. Vandenbroucke RE, Dejonckheere E, Van Lint P, et al. Matrix metalloprotease 8-dependent extracellular matrix cleavage at the blood-CSF barrier contributes to lethality during systemic inflammatory diseases. *J Neurosci* 2012;32:9805-9816.

Correspondence: Dr. S.O. Nwhator, University of Abuja Teaching Hospital, Federal Capital Territory, Abuja, Nigeria. E-mail: periodontologist2010@gmail.com.

2

Pilot Study on Oral Health Status as Assessed by an Active Matrix Metalloproteinase-8 Chairside Mouthrinse Test in Adolescents

Anna Maria Heikkinen,* Solomon O. Nwhator,[†] Nilminie Rathnayake,[‡] Päivi Mäntylä,* Päivi Vatanen,[§] and Timo Sorsa*[†]

Background: Matrix metalloproteinase (MMP)-8 is a major destructive collagenase involved in periodontitis and can be regarded as a periodontitis biomarker. A neutrophil collagenase 2 (active MMP-8 [aMMP-8]) oral fluid immunoassay has recently been demonstrated to be a periodontitis risk indicator among adults. The aim of this study is to investigate whether a point-of-care mouthrinse test based on an aMMP-8 immunoassay could identify patients with oral inflammatory burden (periodontitis and caries) among adolescents with early pathologic findings.

Methods: This cross-sectional study was carried out at the Kotka Health Center, Finland. First, the aMMP-8 chairside mouthrinse test was performed on enrolled individuals (adolescents aged 15 to 17 years, n = 47), and the results were read based on a color change within 5 minutes. Then, full-mouth clinical parameters of oral health were assessed, including periodontal, oral mucosal, and caries status.

Results: The sensitivity and specificity of the test for bleeding on probing were 71.8% and 77.5%, respectively ($P = 0.05$); for ≥1 site with probing depth (PD) ≥4 mm, 48.3% and 100% ($P <0.001$); for ≥2 sites with PD ≥4 mm, 63.6% and 100% ($P <0.001$); and for >2 sites with PD ≥4 mm, 76.5% and 96.7% ($P <0.01$). Regarding periodontitis (≥1 site with PD ≥4 mm), hardly any false-positive results were identified. The sensitivity of the immunoassay for ≥1 caries lesions was 76.5%, and the specificity was 96.7% ($P <0.01$).

Conclusions: In 5 minutes, the aMMP-8 chairside test showed promising results, recognizing oral inflammatory burden in adolescents with early initial signs of periodontitis. Caries lesions could also be detected, but less efficiently. *J Periodontol 2016;87:36-40.*

KEY WORDS

Adolescent; dental caries; immunoassay; matrix metalloproteinase 8; periodontitis.

Recent studies reveal that oral fluid biomarkers can be used for detection of both oral and systemic diseases, including periodontal disease.[1-7] Mouthrinse could offer an inexpensive and easy means of collecting oral fluid, in particular for an adjunctive point-of-care (POC) periodontitis diagnostic.[8] The authors have recently published a study based on salivary matrix metalloproteinase (MMP)-8 and -9 in relation to coronary heart disease and periodontal disease, wherein salivary levels of MMP-8 and -9 were associated with periodontal status.[4] MMP-8 is a major destructive collagenase in periodontitis,[7] and MMP-8 in oral fluids could have a predictive value.[7,9-11] Indeed, the progression of periodontitis has been repeatedly associated with pathologically excessive elevation of MMP-8 in oral fluids.[6,7,12,13] Immunoassays for MMP-8 detection, which target polymorphonuclear leukocyte (PMN)–type and fibroblast-type MMP-8 isoenzyme species in oral fluids, have been found to be beneficial to differentiate sites with periodontitis and gingivitis from healthy sites.[6,12] In addition, Hedenbjörk-Lager et al.[14] reported that elevated salivary MMP-8 levels seemed to be associated with caries lesions.

A number of studies have measured MMP-8 in gingival crevicular fluid and saliva,[1-4,7,13,15] but there is a gap in knowledge based on the utility of a POC

* Department of Oral and Maxillofacial Diseases, University of Helsinki and Helsinki University Hospital, Helsinki, Finland.
† Department of Preventive and Community Dentistry, Faculty of Dentistry, College of Health Sciences, Obafemi Awolowo University, Ife, Nigeria.
‡ Division of Periodontology, Department of Dental Medicine, Karolinska Institutet, Huddinge, Sweden.
§ Kotka Health Center, Kotka, Finland.

oral fluid MMP-8 test for chairside diagnostics of periodontal disease.[7] According to studies by Heikkinen and colleagues,[16,17] 10% to 15% of 15- to 16-year-olds were observed to suffer from initial chronic periodontitis (CP). In this context, a chairside mouthrinse test based on an active MMP-8 (aMMP-8) immunoassay for measuring oral inflammatory burden among adolescents is of high interest. The aim of this study is to assess the sensitivity and specificity of a POC collagenase 2 (aMMP-8)–based immunoassay‖ in detecting oral inflammatory burden, periodontitis, and caries among Finnish adolescents.

MATERIALS AND METHODS

This cross-sectional study was carried out in 2014 to 2015 in Kotka Health Center, Kotka, Finland. The study protocol was approved by the Ethics Committee of Helsinki University Central Hospital, Helsinki, Finland. Forty-seven adolescents (30 males and 17 females, aged 15 to 17 years) provided written informed consent and were enrolled to participate.

A positive aMMP-8 test is based on a cutoff of 25 ng aMMP-8 per milliliter of filtrate derived from 5 mL mouthrinse.[7] After comparing values from 130 patients with CP at six different cutoffs (20, 25, 30, 35, 40, and 50 ng/mL) by ELISA,[8,12] the cutoff of 25 ng/mL was chosen based on κ values.[7,8] One line on the test device indicates that the test has successfully analyzed the drop of mouthrinse and the result is negative. The result is positive if two lines are observed, indicating elevated risk for periodontitis or caries,[18] as presented in Figure 1. Low-risk (light line) and high-risk (dark line) groups were combined as all those with risk.

First, the aMMP-8 chairside test was performed on participants to identify individuals with elevated MMP-8. Second, full-mouth clinical parameters (visible plaque index [VPI], bleeding on probing index [BOP], sites with probing depth [PD] ≥4 mm, and sites with caries lesions) were evaluated with periodontal, oral mucosal, and caries assessments. The same person (AMH) performed the clinical measurements and the collection of saliva samples. The oral clinical examination was carried out using World Health Organization criteria.[19,20] Results were recorded in the dental health care system electronic records, as were smoking history and oral hygiene regimen.[16]

Clinical parameters among participants who were positive and negative were compared. Sensitivity (the

Figure 1.
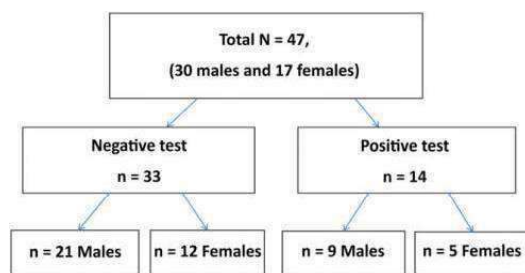*Two lines indicate elevated periodontitis risk among adults.*

Figure 2.
Study profile of aMMP-8 mouthrinse chairside test.

true-positive rate) and specificity (the true-negative rate) were measured. A test with 100% sensitivity will recognize all patients with the disease; a test with 100% specificity will rule out disease in all healthy patients. Results were analyzed with software¶ by cross tabulation and Fisher exact test.

RESULTS

Of the 47 participants (63.8% males and 36.2% females), 14 tested positive (Fig. 2). Recording of oral hygiene regimens revealed that females brushed their teeth more than males (83.3% compared to 16.7% at least once a day; $P = 0.03$). Only five of 47 adolescents were current smokers, two positive and three test negative. Smoking did not interfere with the test significantly.

Median (interquartile range) values for positive individuals according to VPI, BOP, and PD (sites with PD ≥4 mm) were 19.1% (9.01% to 48.0%), 12.5% (8.63% to 33.05%), and 11.0% (5.75% to 15.25%) ($P < 0.001$), respectively. Respective values for negative individuals were 8.9% (5.65% to 15.10%), 7.1% (3.10% to 13.10%), and 0.0% (0.0% to 1.50%). Figure 3 shows BOP and number of patients with sites with PD ≥4 mm among negative and positive adolescents.

The sensitivity of the immunoassay for BOP (cutoff point of 20% of positive sites) was 71.8%, and the specificity was 77.5% ($P = 0.05$). For ≥1 site with PD ≥4 mm, the values were 48.3% and 100% ($P < 0.001$); for ≥2 sites with PD ≥4 mm, 63.6% and 100% ($P < 0.001$); and for >2 sites with PD ≥4 mm, 76.5% and 96.7% ($P < 0.01$) (Table 1). The sensitivity of the immunoassay for ≥1 caries lesions was 76.5%, and the specificity was 96.8% ($P < 0.01$). Figure 4 shows participants identified as having periodontitis with the aMMP-8 test in 5 minutes chairside.

DISCUSSION

It is important to consider the sensitivity and specificity of a chairside oral fluid test for MMP-8 to detect
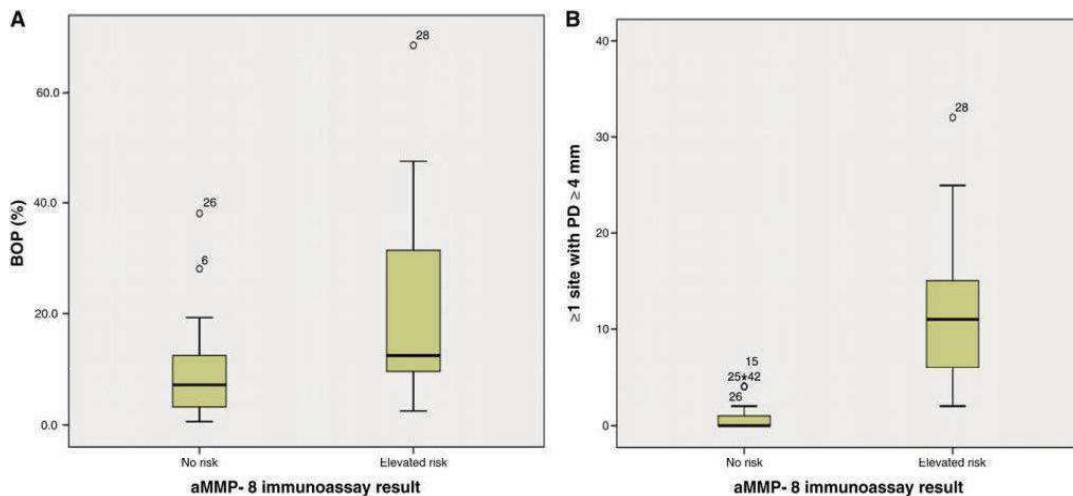
Figure 3.
*Box plot of BOP sites (cut point 20% positive) **(A)** and ≥1 site with PD ≥4 mm **(B)** among adolescents with no risk and with elevated risk for initial periodontitis.*

periodontitis. In a Nigerian study among adults, it was concluded that the neutrophil collagenase 2 (aMMP-8) immunoassay is a good periodontal risk indicator, especially for high PD (≥4 mm).[18] The POC mouthrinse assay uses the same MMP-8 antibodies as the immunofluorometric assay used in studies by Leppilahti and colleagues[9-11] that describe the predictive ability of gingival crevicular fluid MMP-8 analysis.[7] However, these effects are yet to be reported among adolescents. Hedenbjörk-Lager et al.[14] reported that elevated salivary MMP-8 levels seemed to be associated with caries lesions. Periodontitis is a major health problem among adults in Finland,[21,22] and according to a Finnish study, 10% to 15% of 15- to 16-year-olds were observed to suffer from initial CP.[16,17]

A chairside periodontal diagnostic assay based on aMMP-8 has been shown to 1) identify and screen susceptible sites and patients;[7,9,12] 2) differentiate active and inactive sites and patients;[9,11,12,15,19] 3) predict future disease progression;[7,9,19] and 4) monitor the effects of periodontal treatment.[7,11,15,23] Furthermore, in regard to potential clinical relevance and practical advantages, the chairside oral fluid MMP-8 test is inexpensive, quick, and easy for adjunctive POC screening of adolescents with elevated periodontitis risk, compared with conventional clinical methods using a probe. aMMP-8 POC mouthrinse testing can be used as an adjunctive diagnostic tool to detect initial periodontitis and caries lesions in adolescents.

Table 1.

Sensitivity and Specificity of the aMMP-8 Test According to BOP and ≥1, ≥2, or >2 Sites with PD ≥4 mm in Finnish Adolescents

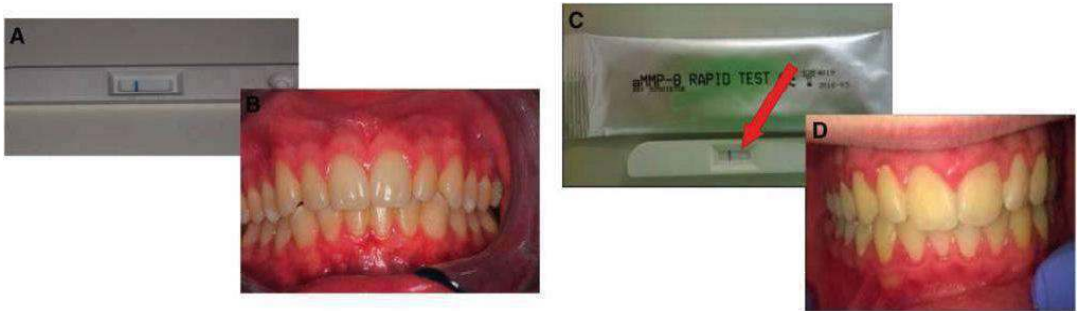| Clinical Parameter	Sensitivity (%)	Specificity (%)	P	False Positive
BOP (cut point 20% positive)[16,17]	71.8	77.5	0.05	9
≥1 site with PD ≥4 mm	48.3	100	<0.001	0
≥2 sites with PD ≥4 mm	63.6	100	<0.001	0
>2 sites with PD ≥4 mm	76.5	96.7	<0.01	1
≥1 caries lesion	76.5	96.7	<0.01	5

Figure 4.
One line indicates no periodontitis risk **(A** *and* **B)** *and two lines indicate elevated initial periodontitis risk* **(C** *and* **D)** *in male patients aged 16 years.*

CONCLUSIONS

In this pilot study among adolescents with early signs of periodontitis, the aMMP-8 chairside mouthrinse test showed promising results in recognizing oral inflammatory burden within 5 minutes, as previously reported.[18] The presence of caries lesions could also be detected using this aMMP-8 test, but less efficiently than periodontitis.

ACKNOWLEDGMENTS

REFERENCES

1. Mäntylä P, Stenman M, Kinane DF, et al. Gingival crevicular fluid collagenase-2 (MMP-8) test stick for chair-side monitoring of periodontitis. *J Periodontal Res* 2003;38:436-439.
2. Mäntylä P, Stenman M, Kinane D, et al. Monitoring periodontal disease status in smokers and nonsmokers using a gingival crevicular fluid matrix metalloproteinase-8-specific chair-side test. *J Periodontal Res* 2006;41:503-512.
3. Rathnayake N, Akerman S, Klinge B, et al. Salivary biomarkers of oral health: A cross-sectional study. *J Clin Periodontol* 2013;40:140-147.
4. Rathnayake N, Akerman S, Klinge B, et al. Salivary biomarkers for detection of systemic diseases. *PLoS One* 2013;24;8:e61356.
5. Herr AE, Hatch AV, Giannobile WV, et al. Integrated microfluidic platform for oral diagnostics. *Ann N Y Acad Sci* 2007;1098:362-374.
6. Sorsa T, Mäntylä P, Tervahartiala T, Pussinen PJ, Gamonal J, Hernandez M. MMP activation in diagnostics of periodontitis and systemic inflammation. *J Clin Periodontol* 2011;38:817-819.
7. Sorsa T, Gürsoy U, Nhwator S, et al. Analysis of matrix metalloproteinases in gingival crevicular fluid (GCF), mouthrinse and saliva for monitoring periodontal diseases. *Periodontol 2000* 2015; in press.
8. Leppilahti JM, Ahonen MM, Hernández M, et al. Oral rinse MMP-8 point-of-care immuno test identifies patients with strong periodontal inflammatory burden. *Oral Dis* 2011;17:115-122.
9. Leppilahti JM, Kallio MA, Tervahartiala T, Sorsa T, Mäntylä P. Gingival crevicular fluid matrix metalloproteinase-8 levels predict treatment outcome among smokers with chronic periodontitis. *J Periodontol* 2014;85:250-260.
10. Leppilahti JM, Hernández-Ríos PA, Gamonal JA, et al. Matrix metalloproteinases and myeloperoxidase in gingival crevicular fluid provide site-specific diagnostic value for chronic periodontitis. *J Clin Periodontol* 2014; 41:348-356.
11. Leppilahti JM, Sorsa T, Kallio MA, et al. The utility of gingival crevicular fluid matrix metalloproteinase-8 response patterns in prediction of site-level clinical treatment outcome. *J Periodontol* 2015;86:777-787.
12. Sorsa T, Hernández M, Leppilahti J, Munjal S, Netuschil L, Mäntylä P. Detection of gingival crevicular fluid MMP-8 levels with different laboratory and chairside methods. *Oral Dis* 2010;16:39-45.
13. Hernández M, Gamonal J, Tervahartiala T, et al. Associations between matrix metalloproteinase-8 and -14 and myeloperoxidase in gingival crevicular fluid from subjects with progressive chronic periodontitis: A longitudinal study. *J Periodontol* 2010;81:1644-1652.
14. Hedenbjörk-Lager A, Bjørndal L, Gustafsson A, et al. Caries correlates strongly to salivary levels of matrix metalloproteinase-8. *Caries Res* 2015;49:1-8.
15. Reinhardt RA, Stoner JA, Golub LM, et al. Association of gingival crevicular fluid biomarkers during periodontal maintenance with subsequent progressive periodontitis. *J Periodontol* 2010;81:251-259.
16. Heikkinen AM, Pajukanta R, Pitkäniemi J, et al. The effect of smoking on periodontal health of 15- to 16-year-old adolescents. *J Periodontol* 2008;79:2042-2047.
17. Heikkinen AM. (2011). Oral health, smoking and adolescence. University of Helsinki, Faculty of Medicine, Institute of Dentistry. Available at: http://urn.fi/URN:ISBN:978-952-10-7250-5. Accessed October 21, 2011.

18. Nwhator SO, Ayanbadejo PO, Umeizudike KA, et al. Clinical correlates of a lateral-flow immunoassay oral risk indicator. *J Periodontol* 2014;85:188-194.
19. Kramer IR, Pindborg JJ, Bezroukov V, Infirri JS; World Health Organization. Guide to epidemiology and diagnosis of oral mucosal diseases and conditions. *Community Dent Oral Epidemiol* 1980;8:1-26.
20. World Health Organization. Oral Health Surveys – Basic Methods, 3rd edition. Geneva, WHO; 1987.
21. Suominen-Taipale L, Nordblad A, Vehkalahti M, et al. Oral health in the Finnish adult population Health 2000 Survey. Helsinki, Finland: National Public Health Institute; 2004.

22. Heikkinen AM, Sorsa T, Pitkäniemi J, et al. Smoking affects diagnostic salivary periodontal disease biomarker levels in adolescents. *J Periodontol* 2010;81:1299-1307.
23. Buduneli N, Kinane DF. Host-derived diagnostic markers related to soft tissue destruction and bone degradation in periodontitis. *J Clin Periodontol* 2011;38(Suppl. 11):85-105.

Correspondence: Dr. Anna Maria Heikkinen, Department of Oral and Maxillofacial Diseases, University of Helsinki, PL 63 (Haartmaninkatu 8) 00014 Helsinki, Finland. Fax: +358-2941 58575; email: anna.m.heikkinen@helsinki.fi.

3
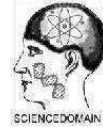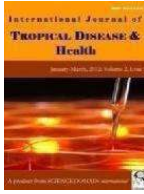
Black Women's Predisposition to Preterm Birth; Could We Be Near The Answer?

S. O. Nwhator[1*], K. A. Umeizudike[2], P. O. Ayanbadejo[2], G. A. Agbelusi[2], M. Arowojolu[3], T. Sorsa[4], O. I. Opeodu[3], J. A. Olamijulo[5], G. O. Akaba[6] and B. A. Ekele[6]

[1]*Lead Researcher, Periodontal Medicine Research Group, Senior Consultant Periodontologist, University of Abuja Teaching Hospital, FCT-Abuja, Nigeria.*
[2]*Department of Preventive Dentistry, Faculty of Dental Sciences, College of Medicine, University of Lagos, Nigeria.*
[3]*Department of Periodontology and Community Dentistry, University of Ibadan, Nigeria.*
[4]*Department of Oral and Maxillofacial Diseases, Helsinki University Central Hospital, Helsinki, Finland.*
[5]*Department of Obstetrics and Gynaecology College of Medicine, University of Lagos. Nigeria.*
[6]*Department of Obstetrics and Gynecology, University of Abuja Teaching Hospital, FCT-Abuja, Nigeria.*

Authors' contributions

All authors were involved in conceptualization, data collection and eventual approval of final draft for submission.

Original Research Article	*Received 30th August 2013* *Accepted 17th October 2013* *Published 20th November 2013*

ABSTRACT

Background: Pre-term Premature Rupture of Membranes (PPROM) is attributable to several causes including asymptomatic bacterial vaginosis among Caucasians and is commoner among black pregnant women. While malaria and high Body Mass Index (BMI) have been reported among Nigerians, the influence of metalloproteinases on PPROM has never been studied in Nigeria.
Methods: A qualitative estimation of active matrixmetalloproteinase-8 (a-MMP-8) to assess the effect of chronic periodontitis on time to conception led to an accidental discovery of widespread elevation of a-MMP-8 among pregnant participants. Values of a-MMP-8 were compared across demographics of participants as well as educational

Corresponding author: Email: periodontologist2010@gmail.com;

status, BMI and other parameters.

Results : One hundred and seventeen of 134 participants (117, 87.3%) had elevated a-MMP-8 based on a novel qualitative assessment using salivary diagnostics. Levels were increased across independent of age, Estimated Gestational Age(EGA), BMI, educational level and trimester.

Conclusion: This population of black pregnant women exhibited higher a-MMP-8 levels than reported among pregnant Caucasians independent of demographics, educational level and trimester of pregnancy. Reasons for the association need to be further investigated.

1. BACKGROUND

An association between periodontitis and increased time to conception has been reported. When increased time to conception was taken as conception occurring after 12 months, a study found greater prevalence of increased TTC among pregnant women suffering from periodontitis compared with periodontally-healthy controls (34.9% vs 25.7%)[1]. This observation was significantly pronounced among non-Caucasian women. (13.9% vs 6.2%, OR = 2.88). The workers therefore concluded that presence of periodontal disease may be a modifiable risk factor for increased time to conception particularly among non-Caucasian women.[1]

The current study —a cross-sectional estimation of periodontitis risk among black pregnant women at the University of Abuja Teaching Hospital- Nigeria, utilized a novel salivary bio-marker for periodontitis [2] to qualitatively assess levels of Neutrophil collagenase-2 (active matrix metalloproteinase-8) (aMMP-8). aMMP-8 selectively detects elevated levels of the metalloproteinase-8 and therefore qualifies as a selective risk-marker for chronic periodontitis [3,4]. The study however exposed a new finding— a shockingly large percentage of study participants had elevated aMMP-8 levels than has ever been reported. Authors suspected a racial connection because all participants were black women.

It is established that bacterial lipopolysaccaride upregulates metalloproteinases which in turn induce premature rupture of membranes and consequent preterm birth labor.[5,6] This mechanism however raises several questions. First, are there racial differences in the expression of aMMP-8 which might explain reported increased prevalence of preterm birth among black women? [7]

Secondly, since aMMP-8 responds to LPS stimulation [5,6], are there racial differences in the sensitivity to LPS? A racial difference in such sensitivity will translate to greater expression of aMMP-8 among blacks. If racial differences in sensitivity to LPS is indeed established from larger controlled studies, a major leap would have been made in addressing the question of greater prevalence of PPROM among black pregnant women. This would also make a case for aMMP-8 reduction interventions among this susceptible population.

2. METHODS

2.1 Consent and Confidentiality

The study was performed in accordance with the World Medical Association Declaration of Helsinki after ethical clearance from the University of Abuja Teaching Hospital. Written informed consent (appended signatures) was obtained from participants. Participation was completely voluntary personally identifiable information excluded.

2.2 Study Population, Setting and Instrument

Participants were pregnant women age range 20-45 years, mean age 30.6 years (standard deviation of 4.5), modal age of 30 years and Body Mass Index (BMI) of 17.8-46.9. Participants were systemically healthy non-smokers (except one smoker) with gravidity ranging from 1-8, parity of 0-5, estimated gestational age (EGA) of 4-36 weeks and last child birth duration (LCB) of 0-126 months. Duration of last child birth was computed as the time between last child birth and the time when the present study was conducted-measured in months.

2.3 Sampling

Purposive, non-random sampling method involving sequential recruitment of new willing patients registering at the antenatal clinic of the University of Abuja Teaching Hospital.

2.4 Calibration and Examination

One periodontologist and two dental therapists calibrated to recognize the color change performed all examinations. The percentage agreement was 100 percent due to the clarity of the blue color change (Fig. 1). aMMP-8 was qualitatively assessed using lateral flow immunoassay test kit --a point-of-care chair-side immunoassay kit which selectively detects elevated levels of aMMP-8. Participants mouth-rinsed with clean water for thirty seconds and waited for one minute.

A 30 second rinse with the distilled water followed and saliva expectorated into a small sterile dish that came with the kit. About 3 ml of the saliva was drawn up with the syringe with a filter fitted. Three to four drops of the saliva was placed in the immunoassay dish results read-off within 5 minutes and not beyond 10 minutes from starting time.

The result was read-off as a color change. One blue line indicated a negative test (normal aMMP-8) while two lines indicated a positive test (elevated aMMP-8) (Fig 1). Even a faint line indicated a positive test according to the manufacturer's instructions.

2.5 Statistical Analysis

This was performed with the PASW-18 (SPSS) statistical software. Uni-variate data such as frequencies, means and standard deviation were evaluated. Chi-square statistic was applied using cross-tabulations to examine the relationship between the outcome variable (aMMP-8 test result) and explanatory variables --age, estimated gestational age, BMI, last child birth etc. The confidence level of the test was set at 95% therefore p-values yielding results ≤ 0.05 were accepted as being statistically significant.

Fig. 1. aMMP-8 Test Kit showing blue control and test lines.

3. RESULTS

One hundred and thirty four participants participated in this study of which 117 (83.7%) had elevated aMMP-8 levels. aMMP-8 levels were generally elevated in participants across all age groups with no defined pattern. Participants aged 20-45 years showed no difference in aMMP-8 levels across all age groups. (Table 1).

Table 1. aMMP-8 levels by age group. aMMP-8 Levels were elevated across all age groups. X^2= 0.154, df= 2, p= 0.93

aMMP-8 Level by Age Group							
	aMMP-8 Level				Total		
	Normal		Elevated				
	n	%	n	%	n	%	
Age Group	20 to 25 Years	2	11.8	15	88.2	17	100
	26 to 31 Years	9	13.8	56	86.2	65	100
	> 31 Years	6	11.5	46	88.5	52	100
Total	17	12.7	87.3	117	134	100	

Based on BMI, 53 of 117(45.3%) were of normal weight, 46(36.8%) were overweight and 18 (15.4%) were obese. aMMP-8 levels were generally elevated across all BMI values with minor insignificant differences (p= 0.62) (Table 2).

Table 2. aMMP-8 levels by BMI. aMMP-8 Levels were elevated across all BMI values. X^2= 0.972, df= 2, p= 0.62

aMMP-8 Level by Basic Metabolic Index							
	aMMP-8 Level						
	Normal		Elevated		Total		
	n	%	n	%	n	%	
Basic Metabolic Index	Normalweight	8	15.1	45	84.9	53	100
	Overweight	4	8.7	42	91.3	46	100
	Obese	2	11.1	16	88.9	18	100
Total	14	12.0	103	88.0	117	100	

aMMP-8 levels were generally elevated in participants across all levels of education following a definite pattern: the percentage of participants with elevated aMMP-8 increased with level of educational qualification but the differences failed to attain statistical significance (p= 0.16) (Table 3).

Table 3. aMMP-8 levels by Qualification. aMMP-8 Levels were directly proportional to educational level. Differences did not attain statistical significance. X^2= 3.652, df= 2, p= 0.16.

aMMP-8 Level by Educational Qualification								
	aMMP-8 Level							
	Normal		**Elevated**		**Total**			
	n	**%**	**n**	**%**	**n**	**%**		
Qualification	Up to Secondary School	10	19.6	41	80.4	51	100	
	Diploma	4	9.5	38	90.5	42	100	
	Degree	3	7.3	38	92.7	41	100	
Total		17	12.7	117	87.3	134	100	

aMMP-8 levels were generally elevated in participants across all trimesters of pregnancy following a definite pattern but aMMP-8 levels which failed to attain statistical significance (p= 0.78) (Table 4).

Table 4. aMMP-8 levels by trimester. aMMP-8 Levels were directly proportional to trimester. Differences did not attain statistical significance. X^{2*}= 0.503, df= 2, p= 0.78

aMMP-8 Level by Pregnancy Trimester								
	aMMP-8 Level							
	Normal		**Elevated**		**Total**			
	n	**%**	**n**	**%**	**n**	**%**		
Trimester	1st Trimester	4	15.4	22	84.6	26	100	
	2nd Trimester	10	13.2	66	86.8	76	100	
	3rd Trimester	3	9.4	29	90.6	32	100	
Total		17	12.7	117	87.3	134	100	

*2 cells (33.3%) have expected count less than 5 hence table for description only, X^2 not valid.

aMMP-8 levels were also elevated in participants irrespective of duration of last child birth. The relationship between duration of last child birth and aMMP-8 levels followed no defined pattern (Table 5).

Table 1. aMMP-8 levels by LCB duration. aMMP-8 Levels were elevated across all LCB durations. X^2=0.154,df= 2,p= 0.926

aMMP-8 by Last Child Birth Duration							
		aMMP-8 Level					
		Normal		**Elevated**		**Total**	
		n	**%**	**n**	**%**	**n**	**%**
LCB (months)	Up to 25 months	2	11.8	15	88.2	17	100
	Up to 31 months	9	13.8	56	86.2	65	100
	> 31 months	6	11.5	46	88.5	52	100
Total		17	12.7	117	87.3	134	100

4. DISCUSSION

Fetal membranes are susceptible to the effect of matrix metalloproteinases similar to other extracellular matrices anywhere in the body. Any mechanism leading to a premature degradation of these tissues are likely to lead to preterm birth. Matrix metalloproteinases have been implicated in the chain of events leading to preterm birth [8].

Reasons for higher prevalence of preterm birth among blacks have remained largely speculative. While asymptomatic bacterial vaginosis have been implicated [9], it is well-known that the final pathway in the pathogenesis of preterm birth is through the breakdown of these delicate membranes– an action performed by matrix metalloproteinases.[8] The question is; are blacks genetically predisposed to producing more matrix metalloproteinases than Caucasians?

Again, the role of tissue inhibitors of metalloproteinases (TIMPs) is considered as important as that of the matrix metalloproteinases themselves. [10] Now, did the (TIMPs) play any role in our findings and could their expression be racially influenced? Are these racial differences completely genetic, environmental or both?

While the current report lacks the strength to answer these questions, the authors believe that we have stumbled upon a probable explanation for the previously unexplained predisposition of black women to preterm birth albeit accidentally. These findings would hopefully provide a template for intense research into why black women are predisposed to preterm birth.

Contrary to recent findings among Caucasians [11], the present study found increased levels of mouthrinse aMMP-8 among pregnant women independent of their age, educational qualification, gestational age, duration of last child birth and BMI. While BMI had no impact in aMMP-8 assessment in the present study, it appeared to be a major risk factor for preterm birth in a Nigerian Teaching Hospital. [12]

The role of intrauterine infections in PPROM is well-reported in literature [13,14] but was not assessed in the current study. Equally established in literature is the mediating role of lipopolysaccaride (LPS) from the gram negative bacteria cell walls. LPS leads to the release of enzymes that remodel the extracellular matrix through activation of the innate immune system. [5,6] This position is easily explained by a report that LPS is recognized by the innate immune system.[15] The production of matrix metalloproteinases is a final pathway in the pathogenesis of preterm birth and PPROM. Authors therefore believe that aMMP-8 estimation is sufficient for the scope of the current study.

The fact that all participants in the present study were Nigerians raised the authors' suspicion of a racial twist in our observations. Only recently, a likely racial tendency in host responses to LPS was reported. [16] While African-Americans were twice more sensitive to E.coli, their Caucasian counterparts showed significantly greater sensitivity to LPS [16] The pathway through which this recognition occurs has been identified as a signal transduction pathway possessing the capacity to increase the production of pro-inflammatory cytokines and matrix metalloproteinases[13].

Furthermore, Ferdinand and colleagues [17] explored the genetic pathway in an attempt to unravel this unexplained predisposition of black women to PPROM. A possible explanation was mutations in the human gene CARD15 of the NOD1/APAF1 gene family and the

potentially protective TLR4 variant alleles but failed to find a difference between the expression of these mutant and alleles between black Americans and Caucasians. This means the pathogenesis of this differential prevalence is either not genetic or occurs through yet-to-be identified genetic pathways.

Perhaps the answer to the dilemma does not lie in complex genetics but in simple quantitative dynamics of matrix metalloproteinases. By this we propose that the simple reason why black women are selectively predisposed to preterm birth may be due to an increased production of matrix metalloproteinases. The genetic angle could then be considered in an attempt to explain why black women seem to produce more matrix metalloproteinases compared to their Caucasians counterparts.

Observations from recent studies are in favor of our position. Since LPS levels directly correlate with infection, it might possibly imply that black women simply suffer more intrauterine infections than their Caucasian counterparts. The finding of greater prevalence of amniotic fluid markers of inflammation by Guinn and colleagues and Fiscella corroborate this suggestion [18,19]. The infections angle is further supported by reports of elevated matrix metalloproteinases among women suffering from bacterial vaginosis compared with healthy controls.

In Nigeria, Adesiji and colleagues found no association between bacterial vaginosis and preterm birth [20]. The closest link was the association with malaria in a recent report [21]. A foundational statement by Fiscella —"Significantly higher rates of bacterial vaginosis among black women may account for nearly 30% of the racial gap in preterm births" [9] warrants further investigation among Nigerian women.

The likelihood that matrix metalloproteinases play a major role in determining the predisposition of black women to PPROM is further supported by a report which cited a polymorphism in MMP-9 promoter as being positively associated with an increased risk of preterm birth among African-Americans [7]. Their findings were further corroborated by the report by Fujimoto and colleagues which observed an increased risk of preterm birth attributable to a single nucleotide polymorphism in MMP-1 [22].

Moyer reported on the benefit of MMP-8 as a risk marker of intra-amniotic inflammation and a useful tool in identifying the presence of intra-amniotic inflammation in preterm PPROM patients. [8]

Apart from these possible mechanisms, at least two other positions have been proposed in literature. First, a hypothesis that defects in the caspase system could affect the programmed death of inflammatory cells leading to an overactive immune system which in turn potentially causes an increased release of inflammatory mediators. [23] It is well-recognized that African-Americans tend to have higher systemic inflammation than Caucasians [24] and more inflammatory variants of Pelvic inflammatory disease have been reported in Africans compared with Caucasians [25].

The second position is a microbiological explanation which posits that P. intermedia can up-regulate MMP production.[26] It is however not clear if there are any racial differences in P. intermedia and how this could have affected our findings.

5. CONCLUSIONS

There appears to be a yet-to-be explained racial difference in the differential production of aMMP-8 among black pregnant women compared with their Caucasian counterparts. This racial difference partly explains the differential preponderance of pre-term birth among black pregnant women.

CONSENT

The study was performed in accordance with the World Medical Association Declaration of Helsinki after ethical clearance from the University of Abuja Teaching Hospital. Written informed consent (appended signatures) was obtained from participants. Participation was completely voluntary personally identifiable information excluded.

ETHICAL APPROVAL

The study was performed in accordance with the World Medical Association Declaration of Helsinki after ethical clearance from the University of Abuja Teaching Hospital. Written informed consent (appended signatures) was obtained from participants. Participation was completely voluntary personally identifiable information excluded.

LIMITATIONS

The present study was a qualitative assessment of aMMP-8 levels which simply detected whether mouthrinse levels of aMMP-8 were normal or elevated among black pregnant women. Future studies should involve actual quantitative aMMP-8 measurements through well designed controlled studies using larger sample sizes in order to confirm or refute these findings.

COMPETING INTERESTS

Authors have declared that there are no competing interests.

REFERENCES

1. Hart R, Doherty D A et al. Periodontal disease ñ a further potentially modifiable risk factor limiting conception ñ a case for a pre-pregnancy dental check-up? Hum. Reprod. 26(suppl 1): i69-i71 doi:10.1093/humrep/26.s1.47.2011
2. Sorsa T, Tervahartiala T, Leppilahti J et al. Collagenase-2 (MMP-8) as a point-of-care bio-marker in periodontitis and cardiovascular diseases. Therapeutic response to non-antimicrobial properties of tetracyclines. Pharmacol Res. 2011;63(2):108-113.
3. Sorsa T, Tjäderhane L, Salo T. Matrix metalloproteinases (MMPs) in oral diseases. Oral Dis 2004;10:311–318.
4. Sorsa T, Hernández M, Leppilahti J, Munjal S, Netuschil L, Mäntylä P. Detection of gingival crevicular fluid MMP-8 levels with different laboratory and chair-side methods. Oral Dis. 2010; 16(1):39-45.
5. Romero R, Kadar N, Hobbins JC, Duff, GW. Infection and labor: The detection of endotoxin in amniotic fluid. Am J Obstet Gynecol. 1987;157:815-819.

6.   Romero R, Roslansky P, Oyarzun E et al. Labor and infection. II. Bacterial endotoxin in amniotic fluid and its relationship to the onset of preterm labor. Am J Obstet Gynecol. 1988;158(5):1044–1049.

7.   Ferrand PE, Parry S, Sammel M, Macones GA, Kuivaniemi H, Romero R, Strauss JF 3rd. A polymorphism in the matrix metalloproteinase-9 promoter is associated with increased risk of preterm premature rupture of membranes in African Americans. Mol Hum Reprod. 2002;8(5):494-501.

8.   Paula Moyeri. MMP-8 Test may help identify which preterm PPROM patients have 1ntra amniotic inflammation. medscape. 2007;13.

9.   Fiscella K. Racial disparities in preterm births. The role of urogenital infections. Public Health Rep. 1996r;111(2):104-113.

10.   Biyikoğlu B, Buduneli N, Kardeşler L, Aksu K, Pitkala M, Sorsa T. Gingival   crevicular fluid MMP-8 and -13 and TIMP-1 levels in patients with   rheumatoid   arthritis   and inflammatory periodontal disease. J Periodontol. 2009;80(8):1307-1314.

11.   Gürsoy M, Könönen E, Tervahartiala T et al. Longitudinal study of salivary proteinases duringpregnancy and postpartum. J Periodontal Res. 2010; 45(4):496-503.

12.   Mokuolu OA, Abdul IF, Adesiyun O. Maternal Factors Associated With Early Spontaneous Singleton Preterm Delivery in Nigeria. Trop J Obstet Gynaecol. 2002;19:32-35.

13.   Parry S, Strauss JF 3rd. Premature rupture of the fetal membranes. N Engl J Med. 1998 5;338(10):663-670.

14.   Torricelli M, Conti N, Galeazzi LR et al. Italian Pre-Term Network Study Group 3. Epidemiology of early pre-term delivery: Relationship with clinical and histopathological infective parameters. J Obstet Gynaecol. 2013;33(2):140-143.

15.   Medzhitov R, Preston-Hurlburt P, Janeway CA Jr. A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. Nature. 1997;388(6640):394-397

16.   Peltier MR, Drobek CO, Bhat G et al. Amniotic fluid and maternal race influence responsiveness of fetal membranes to bacteria. Reprod Immunol. 2012;96(1-2):68-78

17.   Ferrand PE, Fujimoto T, Chennathukuzhi V, et al. The CARD15 2936insC   mutation and TLR4 896 A>G polymorphism in African Americans and risk of preterm premature rupture of membranes (PPROM). Mol Hum Reprod. 2002;8:1031–1034

18.   Guinn DA, Goldenberg RL, Hauth JC, Andrews WW, Thom E, Romero R.Risk factors for the development of preterm premature rupture of the membranes after arrest of preterm labor. Am J Obstet Gynecol. 1995;173(4):1310-1315.

19.   Diaz-Cueto L, Cuica-Flores A, Ziga-Cordero F, et al. Vaginal Matrixmetalloproteinase levels in pregnant women with bacterial vaginosis. J Soc Gynecol Investig. 2006; 13(6):430-434

20.   Adesiji YO, Taiwo SS, Adekanle DA, Oboro VO, et al. Bacteria Vaginosis and Pregnancy Outcome in Osogbo, Nigeria. Research Journal of Medical Sciences. 2007;1:195-198.

21.   Omole-Ohonsi DA, Attah R A. Risk Factors of Preterm Deliveries at Aminu Kano Teaching Hospital, Kano, Nigeria. South Asian Journal of Medical Sciences. 2012; 1(1):3-10.

22.   Fujimoto T, Parry S, Urbanek M, et al. 3rd. A single nucleotide polymorphism in the matrixmetalloproteinase-1 (MMP-1) promoter influences amnion cell MMP-1 expression and risk for preterm premature rupture of the fetal membranes. J Biol Chem. 2002;277(8):6296-302.

23.   Beutler B. Autoimmunity and apoptosis: the Crohn's connection. Immunity. 2001;(1):5-14.

24.   Brown MD, Feairheller DL, Thakkar S, Veerabhadrappa P, Park JY. Racial differences in tumor necrosis factor-α-induced endothelial microparticles and interleukin-6 production. Vasc Health Risk Manag. 2011;7:541-550.
25.   Taylor BD, Darville T, Ferrell RE, Ness RB, Haggerty CL. Racial Variation in Toll-like Receptor Variants Among Women With Pelvic Inflammatory Disease. J Infect Dis. 2013;207(6):940-946.
26.   Guan SM, Shu L, Fu SM et al. Prevotella intermedia upregulates MMP-1 and MMP-8 expression in human periodontal ligament cells. FEMS Microbiol Lett. 2009;299(2):214-222.

4

Volume 4 / Issue 5 / October 2014

amhsr

An Official Publication of Enugu state NMA

NMA

Annals of Medical & Health Sciences Research

Could Periodontitis Affect Time to Conception?

Nwhator SO, Opeodu OI[1], Ayanbadejo PO[2], Umeizudike KA[2], Olamijulo JA[3], Alade GO[2], Agbelusi GA[2], Arowojolu MO[1], Sorsa T[4]

Department of Preventive Dentistry, University of Abuja Teaching Hospital, Abuja, [1]Department of Periodontology and Community Dentistry, University of Ibadan, Oyo, [2]Departments of Preventive Dentistry, and [3]Obstetrics and Gynecology, University of Lagos, Lagos, Nigeria, [4]Department of Oral and Maxillofacial Diseases, University of Helsinki, Helsinki, Finland

Address for correspondence:
Dr. Solomon Olusegun Nwhator,
Department of Preventive Dentistry,
University of Abuja Teaching
Hospital, Abuja, Nigeria.
E-mail: periodontologist2010@gmail.com

Abstract

Background: Chronic periodontitis is gaining increasing prominence as a potential influnce on systemic health. Time to conception has been recently investigated in relation to chronic periodontitis among Caucasians. The authors set out to replicate the study among Nigerian pregnant women. **Aim:** The etiology of many medical conditions have been linked with the state of the oral health and one of such is the time to conception (TTC) among women. This study was aimed to assess the effect of periodontitis on TTC. **Subjects and Methods:** A cross-sectional study in a hospital setting involving 58 fertility clinic attendees and 70 pregnant controls using the simplified oral hygiene index, community periodontal index (CPI) and matrix metalloproteinase-8 immunoassay. Statistical analysis used included Spearman's rank order correlation statistic, Z-statistic and logistic regression. **Results:** Good oral hygiene correlated with shorter TTC (<1 year) than fair oral hygiene, but not statistically significant. The odds of increased conception were higher with CPI (odds ratio [OR]: 0.482, 95% confidence interval [CI]: 0.259-0.895, $P = 0.02$), periodontitis risk (OR 0.157, 95% CI 0.041-0.600, $P < 0.01$) and age (OR 0.842, 95% CI 0.756-0.938, $P < 0.01$). **Conclusion:** Chronic periodontitis was positively associated with increased TTC in the present study. The authors are recommending that women in child bearing age should be encouraged to have regular preventive dental check-ups in order to maintain good oral and periodontal health.

Keywords: Fertility, Oral hygiene, Periodontitis, Time to conception

Introduction

Almost two decades ago, Offenbacher *et al.* in their study have reported possible links between chronic periodontitis and adverse pregnancy outcomes.[1-3] Other potential links include cardiovascular events,[4] kidney disease,[5] endometriosis,[6] oral cancer,[7] erectile dysfunction,[8] low sperm count,[9] and increased time to conception (TTC)[10] have also been reported.

Explanations for the observed associations include a dose-response relationship between prostaglandin E-2, periodontal disease activity and decreasing birth weight.[2] Other

explanations include endotoxins released from Gram-negative bacteria[11] and direct vascular endothelial infection by periodontal microorganisms.[12] Systemic exposure to oral bacteria through periodontitis releases inflammatory mediators capable of initiating or supporting atherosclerosis.[13]

The evidence to support a link between periodontitis and increased TTC is still emerging. "Low-grade systemic inflammation associated with periodontal disease". may have a local effect within the endometrium."[10] This position is corroborated by several studies.[14-16]

An association between chronic periodontitis and increased TTC among black women is worrisome.

Nigerian researchers have reported a possible link between poor oral hygiene and low sperm count.[17] However, the association between chronic periodontitis and increased TTC has not been reported among Nigerians hence the need for the present study.

Subjects and Methods

Ethics

The study satisfied the Helsinki declaration and received the approval of the institutional review board. It formed part of a series of multi-center studies on the association between the chronic periodontitis and fertility among Nigerians. Verbal informed consent was obtained from participants prior to inclusion in the study. Consecutive fertility clinic attendees and pregnant antenatal clinic (ANC) controls participated in the study.

Sampling

This is the first study of its kind in Nigeria therefore, prevalence figures were not available. This made it only practicable to adopt a convenience sampling technique of consecutive new patients registering for ANC and fertility clinics of the University of Abuja Teaching Hospital.

Inclusions and exclusions

Inclusion criteria were being pregnant or attempting to get pregnant. Patients had to be clinically healthy to be included. Patients on fertility medications and those who got pregnant through fertility medications were included, but under fertility clinic attendees. Further exclusions were not considered. Fertility was based on gravidity rather than parity, which means that a woman who suffered an abortion would still have been considered fertile despite not having a baby.

In order to eliminate inadequate copulation as a confounder, a history of regular sexual intercourse was important before being classified as infertile or non-pregnant.

Using pre-tested, closed-ended examiner-administered questionnaires, data on age, past dental visit, oral hygiene practice and smoking status were obtained. Others included TTC among pregnant women and time since trying for pregnancy among yet-to-conceive women (tetrodotoxin [TTX]) were obtained.

Periodontal examination was performed under natural illumination and parameters including oral hygiene index score (OHIS), community periodontal index (CPI) and periodontitis risk score using matrix metalloproteinase-8 (MMP-8) (neutrophil collagenase-2). The kit works on the principle of lateral flow immunoassay. Lateral flow immunoassay which predictively reveals periodontal inflammation, existing or hidden ones and is associated with clinical signs of periodontal tissue destruction.[18] MMP-8 immunoassay also predicts successful treatment outcomes.[19-21]

A previous report[18] stated that this immunoassay kit is "96% sensitive for poor oral hygiene, 95% sensitive for chronic periodontitis and 82.6% sensitive for bleeding on probing."

The immunoassay test was performed using a simple mouthrinse previously approved by the institutional review board. Based the immunoassay results, patients were grouped either as a three-tier "high risk," "low risk" and "no risk" group or a two-tier "no risk" and "risk" group. The classification was based on the depth of color change. Obtained OHIS results were classified as either good, fair or poor "Good" (0-1.2), "Fair" (1.3-3.0) and "Poor" (3.1-6.0).[22] The result of the rapid periodontitis risk immunoassay was read-off as color change.

Statistical analysis

TTC was recoded into two groups - <1 year and >1 year. Though a continuous variable, TTC was so grouped in consonance with acceptable standards for infertility. A woman is not considered infertile until after 1-year of unprotected sexual intercourse with their male spouse. OHIS was recoded into good (0-1.2), fair (1.3-3.0) and poor (>3.1). Data were analyzed using software package used for statistical analysis (SPSS version 18 (PASW statistics, IBM). Preliminary analysis performed to assess associations between groups using the Chi-square statistic, Z statistic and risk analysis. An attempt to further explore the strength of associations between the oral hygiene and TTC was made using Pearson's correlation statistics. However, not all variables were normally distributed among pregnant ANC attendees as assessed by Shapiro-Wilk's test ($P < 0.05$). A Spearman's rank order correlation statistic was therefore performed as a non-parametric alternative after preliminary analysis showed the relationship to be monotonic. For the same reasons, a Spearman's rank order correlation test was performed as a non-parametric alternative to assess the relationship between OHIS and TTX. Age-matched comparisons were performed between the non-pregnant fertility clinic attendees and their pregnant controls using the Z statistic.

Since the relative contribution of the various independent variables to the occurrence of the dependent variable (TTC) was inconclusive in most instances, a logistic regression was performed. This incorporated six explanatory variables namely age, frequency of dental visit, oral hygiene index, oral hygiene practice, CPI score and periodontitis risk indicator (oral risk indicator). The logistic regression model to evaluate the likelihood that participants have increased TTC (>1 year) was statistically significant, χ^2 (12) =40.862, $P < 0.0015$, explained 38.3% (Nagelkerke R^2) of the variance in TTC and correctly classified 75.6% of cases. The sensitivity of the model was 66.7%, specificity was 81.3%, positive predictive value was 69.6% and negative predictive value was 79.2% respectively.

Results

A total of 128 women aged range from 23 to 48 years (mean age = 33.9 [5.04]) participated in this study. The number comprised of 70 pregnant ANC attendees (mean age = 32.8 [4.81]) and 58 non-pregnant fertility clinic attendees (mean age = 35.3 [5.00]).

TTC and oral hygiene

Overall, the association between oral hygiene and TTC was not significant ($P = 0.09$). Despite this, further analysis was conducted to determine if associations existed with smaller subgroups. The odds of conception within 1 year was greater in participants with good oral hygiene (odds ratio [OR]: 0.79, 95% confidence interval [CI]: 0.3210-1.3771) than fair oral hygiene (OR: 0.56, 95% CI: 0.3210-1.3771). The association translates approximately to 44% versus 36%, which failed to achieve statistical significance (Z statistic = 1.099, $P = 0.27$). There was also a weak positive correlation between oral hygiene and TTC among pregnant ANC attendees albeit insignificant (rs[69] =0.201, $P = 0.09$) [Table 1].

A Students' *t*-test and age-matched *Z*-statistic found no significant differences in oral hygiene scores between test and control groups (Z statistic = −0.6124) [Table 2]. However, a moderate, but significant positive correlation between oral hygiene and waiting time without pregnancy existed (TTX) (rs[39] =0. 327, $P = 0.04$) [Table 3].

TTC and periodontitis

Initial MMP-8 immunoassay results showed no significant differences between the two groups except in the 38-42 year old age group [Table 4]. Only CPI score 2 (calculus) differed significantly between both groups. Non-pregnant fertility clinic attendees had significantly more calculus deposits than their pregnant counterparts [Table 5].

Oral hygiene and periodontitis logistic regression

After logistic regression analysis, oral hygiene remained irrelevant to TTC ($P = 0.47$). The odds of increased TTC were higher with CPI (OR: 0.482, $P = 0.02$), periodontitis risk (OR: 0.157, $P < 0.01$) and age (OR: 0.842, $P < 0.01$) [Table 6].

Discussion

The study was aimed to explore the association between the chronic periodontitis and TTC. The main assessment tool for periodontitis has been described as "an effective tool in the diagnosis and monitoring of active periodontal diseases."[23,24] Considerations of periodontitis experience among pregnant fertility clinic attendees would have been sufficient for evaluation. Most of the analysis was therefore considered separately for ANC attendees and non-pregnant fertility clinic attendees.

Within the limits of a cross-sectional, case-control study compared with the pioneer study[10] in this area, the results are quite disturbing and call for further evaluation in a larger sample of Africans. Unlike a recent Nigerian study[17] which reported a statistically significant association between poor oral hygiene and low sperm count, the present study found no such associations. The closest observation included greater odds of conception within 1-year among women with good oral hygiene which failed to attain statistical significance (Z statistic = 1.099, $P = 0.27$). This observation, however, needs to be treated with caution due to the limited sample size.

Unfortunately, there is no basis for comparison with the pioneer work because it did not consider oral hygiene. Interestingly though, the popular media in quoting this work have alluded more to oral hygiene than chronic periodontitis actually evaluated in the study. Findings from oral hygiene evaluations should stimulate great concerns.

In the present study, oral hygiene significantly positively correlated with increasing waiting time among non-pregnant fertility clinic attendees albeit weakly (rs[39] =0. 327, $P = 0.04$) and there were statistically more fertility clinic attendees with calculus than pregnant controls. Direct associations between oral hygiene and fertility issues have only been reported among Nigerian males.[17] It is not clear how calculus deposits affect TTC since calculus is traditionally believed to be incapable of inducing inflammation without plaque. This position is now contested as evident from the statement

Table 1: Association between OH and TTC (rs (69)=0.201, P>0.05)

Tables	Time conception		Total
	>1 year	**≤1 year**	
OH			
Fair	9	24	33
Good	11	26	37
Total	20	50	70

Odds of for good OH: fair OH for conception within 1 year=1.411 (95% CI=0.3210-1.3771). OH: Oral hygiene, TTC: Time to conception, CI: Confidence interval

Table 2: Age-matched evaluation between pregnant ANC attendees and non-pregnant FC attendees

Subjects and controls	23-27	28-32	33-37	38-42	43-48
ANC group	*n*=4	*n*=34	*n*=20	*n*=10	*n*=2
OHIS	0.93 (SD=0.71)	1.24 (SD=0.78)	1.22 (SD=0.65)	1.71 (SD=0.93)	1.50 (SD=0.47)
	Variance=0.22	Variance=0.61	Variance=0.42	Variance=0.88	Variance=0.22
FC group	*n*=2	*n*=18	*n*=21	*n*=13	*n*=4
OHIS	2	1.40 (SD=0.80)	1.26 (SD=0.65)	1.42 (SD=0.76)	1.77 (SD=0.98)
		Variance=0.64	Variance=0.42	Variance=0.58	Variance=0.96
Comment	N/A	Not significant	Not significant	Not significant	Not significant
		P>0.05	*P*>0.05	*P*>0.05	*P*>0.05

No significant associations in all instances (*P*>0.05). ANC: Antenatal clinic, SD: Standard deviation, OHIS: Oral hygiene index score, FC: Fertility clinic

"inability to clearly differentiate effects of calculus versus "plaque on calculus."[25] Full explanations of these effects might still elusive until we can confidently differentiate these effects.

Table 3: Association between OH and waiting time without pregnancy (TTX) (rs (39)=0.327, P=0.037)

Statistical tool	TTX	OHIS
Spearman's rho		
Correlation coefficient	1.000	0.327
Significant (2-tailed)		0.037*
N	41	41

*Correlation is significant at the 0.05 level (2-tailed). OH: Oral hygiene, TTX: Tetrodotoxin, OHIS: Oral hygiene index score

Table 4: Age-matched Z statistic for comparisons of periodontitis risk between pregnant (ANC) and non-pregnant FC groups

Subjects and controls	23-27	28-32	33-37	38-42	43-48
Pregnant ANC group	n=4	n=34	n=20	n=10	n=2
% ORI+VE high periodontitis risk	75	91.2	75.0	100	50
Non-pregnant FC group	n=2	n=18	n=21	n=13	n=4
% ORI+VE high periodontitis risk	50	77.8	76.2	69.2	75
Significance	Z=0.61	Z=1.35	Z= −0.09	Z=1.93	Z= −0.61
	P=0.54	P=0.18	P=0.93	P=0.05	P=0.54

Only age-group 38-42 showed significant a significant association. ANC: Antenatal clinic, FC: Fertility clinic, ORI+ve: Oral risk indicator-positive

Assessment of periodontitis in the present study went beyond dependence on CPI code 3 which could be very limited in its usefulness. The current study used a modern rapid chair-side MMP-8 immunoassay kit, which has proven sensitivity, specificity and good correlation with periodontal clinical parameters.[18] Initial analysis detected differences only in the 38-42 years age group. After regression analysis, periodontitis strongly associated with increased TTC ($P < 0.01$). This corroborates the pioneer study[10] only second to age as the most important predictor of TTC - again corroborating the pioneer study.[10]

A pertinent question to ask should be "how exactly does periodontitis affect fertility and TTC?" It is important to note that the pioneers of this observation never claimed or insinuated a causal relationship between periodontitis and infertility. They only, - as in the current study - reported an unexplained association. It has been suggested, however, that periodontitis affects TTC in a similar fashion to other inflammatory conditions such as endometriosis,[21] polycystic ovarian syndrome[16] and hydrosalpinges.[15] Periodontitis is believed to exert an "endometrial effect" as commonly reported

Table 5: Comparisons of CPI scores between pregnant (ANC) and non-pregnant FC groups showed significantly more calculus deposits in the non-pregnant FC group

CPI scores	ANC group (n=70) %	FC group (n=58) %	Z-score	P
0	22/70 31.4	15/58 25.9	0.69	0.49
1	23/70 32.9	12/58 20.7	1.54	0.12
2	22/70 31.4	28/58 48.3	1.94	0.05
3	3/70 4.3	3/58 5.2	−0.24	0.81

P=0.05. ANC: Antenatal clinic, FC: Fertility clinic, CPI: Community periodontal index

Table 6: Binary logistic regression analysis

Assessed parameters	B	SE	Wald	df	Significant	Exp (B)	95% CI for Exp (B)	
							Lower	Upper
Step 1*								
OHIS	0.467	0.639	0.535	1	0.465	1.596	0.456	5.588
CPI	−0.731	0.316	5.339	1	0.021	0.482	0.259	0.895
ORI			8.074	2	0.018			
ORI (1)	−1.164	0.786	2.192	1	0.139	0.312	0.067	1.458
ORI (2)	−1.851	0.683	7.333	1	0.007	0.157	0.041	0.600
OH			0.240	2	0.887			
OH (1)	0.415	0.847	0.240	1	0.624	1.515	0.288	7.974
OH (2)	−21.604	22170.638	0.000	1	0.999	0.000	0.000	
Cleaning			2.237	2	0.327			
Cleaning (1)	1.811	1.211	2.237	1	0.135	6.116	0.570	65.629
Cleaning (2)	−18.748	28053.533	0.000	1	0.999	0.000	0.000	
Age	−0.172	0.055	9.692	1	0.002	0.842	0.756	0.938
PDV			0.527	3	0.913			
PDV (1)	0.370	0.827	0.200	1	0.655	1.448	0.286	7.324
PDV (2)	−19.679	17114.582	0.000	1	0.999	0.000	0.000	
PDV (3)	0.340	0.536	0.402	1	0.526	1.405	0.492	4.012
Constant	4.014	2.327	2.976	1	0.084	55.366		

Dependent variable: TTC (recoded into <1 year and >1 year). Significant variables: CPI (OR=0.482, 95% CI 0.259-0.895, P=0.021), periodontitis risk (OR=0.157, 95% CI=0.041-0.600, P=0.007) and age (OR=0.842, 95% CI=0.756-0.938 P=0.002). CI: Confidence interval, SE: Standard error, OHIS: Oral hygiene index score, CPI: Community periodontal index, OH: Oral hygiene, PDV: Past dental visit ORI: Oral risk indicator, TTC: Time to conception, OR: Odds ratio

in the literature[14-16,21] similar to these systemic inflammatory conditions. This explains a possible link between periodontitis and increased TTC as observed in the current study.

Conclusion

There were significant associations between TTC and age ($P < 0.01$), periodontitis ($P < 0.01$) assessed with a MMP-8 chair-side oral rinse.

Recommendations

• The authors recommend that women in child bearing age should be encouraged to have regular preventive dental check-ups in order to maintain good oral and periodontal health
• This study lacks the power to establish a causal link between chronic periodontitis and prolonged TTC but warrants a need for periodontal consultation in women trying to conceive.

Limitations

Limitations to studies like the current one often involves the difficulty of ruling out all possible confounders. Such confounders include factors unrelated to the studied group such as low sperm count in the spouse. However, patients attending fertility clinic would usually have gone through a screening stage to rule out spouse-associated problems before commencement of fertility treatment.

Another apparent limitation of this study is the use of CPI code 3 and OHIS. It is noteworthy however, that OHIS measures oral hygiene (and not periodontal status) in this study. Also, CPI was only adjunctive to the use of the highly sensitive MMP-8 immunoassay which is "an effective tool in the diagnosis and monitoring of active periodontal diseases."[23,24]

Average number of sex attempts was eliminated as a confounder by considering those "actively trying for pregnancy."

The authors did not consider stress in this study. It is not clear how stress could have affected our findings.

Finally, previous abortions could have posed as confounders but confounder eliminated by basing TTC on gravidity rather than current pregnancy alone.

Definitions

rs in Spearman's rank order correlation = 1 for perfect positive and − 1 for perfect negative correlation.

References

1. Offenbacher S, Katz V, Fertik G, Collins J, Boyd D, Maynor G, *et al*. Periodontal infection as a possible risk factor for preterm low birth weight. J Periodontol 1996;67:1103-13.

2. Offenbacher S, Jared HL, O'Reilly PG, Wells SR, Salvi GE, Lawrence HP, *et al*. Potential pathogenic mechanisms of periodontitis associated pregnancy complications. Ann Periodontol 1998;3:233-50.

3. Offenbacher S, Lin D, Strauss R, McKaig R, Irving J, Barros SP, *et al*. Effects of periodontal therapy during pregnancy on periodontal status, biologic parameters, and pregnancy outcomes: A pilot study. J Periodontol 2006;77:2011-24.

4. Flores MF, Montenegro MM, Furtado MV, Polanczyk CA, Rösing CK, Haas AN. Periodontal Status Affects C-Reactive Protein and Lipids in Stable Heart Disease Patients From a Tertiary Care Cardiovascular Clinic. J Periodontol 2013;Jun 27. [Epub ahead of print].

5. Ismail G, Dumitriu HT, Dumitriu AS, Ismail FB. Periodontal disease: a covert source of inflammation in chronic kidney disease patients. Int J Nephrol 2013;2013:515796.

6. Kavoussi SK, West BT, Taylor GW, Lebovic DI. Periodontal disease and endometriosis: Analysis of the National Health and Nutrition Examination Survey. Fertil Steril 2009;91:335-42.

7. Feller L, Altini M, Lemmer J. Inflammation in the context of oral cancer. Oral Oncol 2013;49:887-92.

8. Grant WB, Sorenson M, Boucher BJ. Vitamin D deficiency may contribute to the explanation of the link between chronic periodontitis and erectile dysfunction. J Sex Med 2013;10:2353-4.

9. Klinger A, Hain B, Yaffe H, Schonberger O. Periodontal status of males attending an in vitro fertilization clinic. J Clin Periodontol 2011;38:542-6.

10. Hart R, Doherty DA, Pennell CE, Newnham IA, Newnham JP. Periodontal disease: A potential modifiable risk factor limiting conception. Hum Reprod 2012;27:1332-42.

11. Saini R, Saini S, Saini SR. Periodontitis: A risk for delivery of premature labor and low-birth-weight infants. J Nat Sci Biol Med 2010;1:40-2.

12. Fong IW. Infections and their role in atherosclerotic vascular disease. J Am Dent Assoc 2002;133 Suppl:7S-13.

13. Bhardwaj A, Bhardwaj SV. Periodontitis as a risk factor for cardiovascular disease with its treatment modalities: A review. J Mol Pathophysiol 2012;1:77-83.

14. Weiss G, Goldsmith LT, Taylor RN, Bellet D, Taylor HS. Inflammation in reproductive disorders. Reprod Sci 2009;16:216-29.

15. Johnson N, van Voorst S, Sowter MC, Strandell A, Mol BW. Surgical treatment for tubal disease in women due to undergo in vitro fertilisation. Cochrane Database Syst Rev 2010;CD002125.

16. Hart R, Norman R. Polycystic ovarian syndrome – Prognosis and outcomes. Best Pract Res Clin Obstet Gynaecol 2006;20:751-78.

17. Nwhator SO, Umeizudike KA, Ayanbadejo PO, Opeodu OI, Olamijulo JA, Sorsa T. Another reason for impeccable oral hygiene; oral hygiene-sperm count link. JCDP. [In press]

18. Nwhator SO, Ayanbadejo PO, Umeizudike KA, Opeodu OI, Agbelusi GA, Olamijulo JA, et al. Clinical correlates of a lateral-flow immunoassay oral risk indicator. J Periodontol 2014;85:188-94.

19. Mäntylä P, Stenman M, Kinane D, Salo T, Suomalainen K, Tikanoja S, *et al*. Monitoring periodontal disease status in smokers and nonsmokers using a gingival crevicular fluid matrix metalloproteinase-8-specific chair-side test. J Periodontal Res 2006;41:503-12.

20. Mäntylä P, Stenman M, Kinane DF, Tikanoja S, Luoto H,

Salo T, *et al*. Gingival crevicular fluid collagenase-2 (MMP-8) test stick for chair-side monitoring of periodontitis. J Periodontal Res 2003;38:436-9.

21. Barnhart K, Dunsmoor-Su R, Coutifaris C. Effect of endometriosis on in vitro fertilization. Fertil Steril 2002;77:1148-55.

22. Greene JC, Vermillion JR. Oral hygiene index: A method for classifying oral hygiene status. J Am Dent Assoc 1960;61:172-8.

23. Haffajee AD, Socransky SS, Lindhe J, Kent RL, Okamoto H, Yoneyama T. Clinical risk indicators for periodontal attachm ent loss. J Clin Periodontol 1991;18:117-25.

24. Lindhe J, Haffajee AD, Socransky SS. Progression of periodontal disease in adult subjects in the absence of periodontal therapy. J Clin Periodontol 1983;10:433-42.

25. White DJ. Dental calculus: Recent insights into occurrence, formation, prevention, removal and oral health effects of supragingival and subgingival deposits. Eur J Oral Sci 1997;105:508-22.