THE RELATIONSHIP BETWEEN SALIVARY INTERLEUKIN-1 LEVELS AND PERIODONTAL HEALTH STATUS

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V60/87625/2016

A RESEARCH THESIS SUBMITTED IN PARTIAL FULFILLMENT FOR THE AWARD OF MASTER OF DENTAL SURGERY IN PERIODONTOLOGY

2019

DECLARATION

I declare that this thesis is my original work and has not been presented for the award of a degree in any other university. Reference to work done by others has been clearly indicated.

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DEDICATION

This work is dedicated to my father Stephen Muthima Mathenge.

ACKNOWLEDGEMENTS

I depended to a considerable extent on the support, dedication and assistance of others. It is for this reason that I hereby wish to express my sincere gratitude and appreciation to the following individuals and institutions whose remarkable concerted efforts made the completion of this study possible. I express my heartfelt gratitude to my supervisors Dr. Mulli and Prof. Evelyn Wagaiyu for their time and continuous guidance to ensure that a good and insightful thesis came out of the work.

Special recognition goes to all the patients who graciously participated in the study. Mr. Desmond Owino for his assistance in data analysis. KAVI labs in particular Mr. Mwaura for his valuable assistance during laboratory analysis. Baringo County for granting me study leave to pursue my post graduate degree.

My immediate family for their nourishing and understanding love, patience and wisdom during my prolonged absence from home. Lastly I thank the Almighty God for the strength, will, good health of body and mind that He gave me throughout the study period.

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LIST OF ABBREVIATIONS

AHEA	-	Associate of Higher Education Academy
BDS	-	Bachelor of Dental Surgery
BOP	-	Bleeding on Probing
BPE	-	Basic Periodontal Examination
CAL	-	Clinical Attachment Loss
CDE	-	Certificate in Dental Education
ELISA	-	Enzyme-linked immunosorbent assay
FADI	-	Fellow of Academy of Dentists International
FICD	-	Fellow of International College of Dentists
GI	-	Gingival Index
IL-1	-	Interleukin 1-beta
KAVI	-	Kenya Aids Vaccine Institute
KNH	-	Kenyatta National Hospital
MClin Dent	-	Master in Clinical Dentistry
Min	-	Minute
MPH	-	Masters in Public Health
MSc Dent	-	Masters of Science in Dentistry
ng/mL	-	nano grams per milliliter (nano gram=10^{-9}g)
PFA	-	Pierre Fauchard Academy
PGE_2	-	Prostaglandin E_2
PhD	-	Doctor of Philosophy
PPD	-	Periodontal probing depth
PS	-	Plaque Score
SPSS	-	Statistical Package for Social Sciences
UNDH	-	University of Nairobi Dental Hospital
WHO	-	World Health Organization
UON	-	University of Nairobi

ABSTRACT

Introduction: The periodontium consists of the gingiva, periodontal ligaments, cementum and alveolar bone. Together the four components function as a unit and are the supportive structures of the teeth, maintaining their function and stability.[1] Periodontal diseases are inflammatory conditions that affect these supportive structures.

Background: Inflammatory Periodontal diseases are the most common oral diseases in human kind and as such it's a serious public health issue and the second major cause of tooth loss.[2] Presently the diagnosis of inflammatory periodontal diseases relies on the analysis of a range of clinical parameters. These traditional methods of diagnosis only manage to describe past events such as attachment loss and thus are not able to detect current disease activity. Consequently, there is interest in developing new diagnostic markers which provide additional clinical information over and above those obtained by clinical examination. The use of saliva as a tool for diagnosis due to the fact that majority of biomarkers found in blood or in urine are also found in saliva samples which is easier and more safe to collect. The identification of molecular biomarkers of inflammatory periodontal disease found in saliva can objectively provide a rapid and accurate tool that can diagnose and monitor disease activity and would go a long way in the management of periodontal conditions.[3]

Objectives: The aim of the study was to investigate salivary IL-1 biomarker and how it relates to the periodontal health status of the participants.

Materials and Methods: This was a descriptive cross-sectional study. The study population was patients attending the University of Nairobi Dental Hospital (UONDH) for treatment of various oral facial conditions. They were interviewed using a structured questionnaire on Bio-data, socio-demographic characteristics, lifestyle habits and oral health status. The clinical examination comprised of periodontal evaluation based on the basic periodontal examination (BPE). Plaque and gingivitis were determined using Turesky modification of Quigley Hein, 1970 Index and Loe and Silness 1963 index respectively. Collection of whole saliva for analysis of salivary IL1- levels was done through the spit method.[4]Whole saliva samples were used and the levels of interleukin1 were quantified using ELISA.

Data analysis and presentation: Data collected was coded and analyzed using SPSS software, descriptive statistics, Independent T tests and ANOVA, Pearson's correlation and linear

regression were used to investigate the relationship between salivary-interleukin 1 and periodontal health status.

Results: 71 participants were recruited into the study. The age range of the participants was between 18-81 years. Out of the 71, 48 (67%) were females and 23(32.4%) were males. 39 (54.9%) of participants had gingivitis, 20(28.2%) had mild to moderate periodontitis and 12 (16.9%) had severe periodontitis.

The levels of IL-1βof participants with gingivitis was 461.67pg/mg those with mild to moderate periodontitis had a mean of 807.81pg/ml and those with severe had 1210.12pg/ml. The IL-1βranged from 41.50pg/ml -2808.21pg/ml with a mean of 680.47pg/ml ± 677.85SD. A statistical significant positive association was found between increased levels of IL- 1β and basic periodontal examination scores (f=6.510,p=0.013). A non-statistical significant association was found between IL-1β(pg/ml) scores and plaque scores (f=0.134, p= 0.716).

Conclusion: There was an increase in salivary IL-1β levels with disease severity suggesting a close association between salivary IL-1β and periodontitis.

Recommendation: In the future longitudinal studies with larger sample sizes are needed to validate salivary IL-1β as a marker of periodontal diseases.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.0 Introduction

Salivary biomarkers are under investigation as useful diagnostic tools that can be used to evaluate periodontal diseases, which are inflammatory conditions that affect the tissues supporting the teeth. The inflammatory forms of periodontal diseases may be divided into damaging and non-damaging forms.[5] Gingivitis is a non-damaging infectious disease and is characterized by soft tissue inflammation surrounding the teeth. Clinically it appears as a change in tissue colour and consistency and can be associated with swelling and tendency to bleed upon gentle probing with a periodontal probe.[6] Chronic periodontitis is an infectious disease that is damaging, there is inflammation of the periodontal tissues including bone, resulting in bone resorption leading to alveolar bone loss and apical migration of the epithelial attachment.[7] Sites with chronic periodontitis are characterized by bleeding on probing, increased probing pocket depths and may also show recession of the gingiva.

Chronic Periodontitis may occur at any age after permanent teeth erupt. However, the disease is mostly presents in adults and the aged. Certain distinctive forms of periodontal diseases occur in children and young adults and are of the aggressive type characterized by a high rate of periodontal tissue loss and other characteristic features.[8, 9,]

The inflammatory types of periodontal diseases are infectious in nature, and bacteria have been shown in literature to play a major role in their initiation and progression.[11] Over 80% of the population in Kenya suffers from one form or another of periodontal disease with chronic periodontitis prevalence reported to be at 10-85% while gingivitis is 0.2-90%.[12] The 2015 Kenya National Oral Health Survey found the overall prevalence of gingival bleeding to be 98.1% in adults.[13]

Periodontal diagnosis is based on clinical examination and radiographic assessments. The components of clinical examination of chronic periodontitis include the presence of dental plaque, bleeding on probing, probing pocket depth, furcation involvement, clinical attachment loss, tooth mobility, presence of calculus as well as radiographic examination to assess the

alveolar bone level. These clinical diagnostic parameters have been used for over 50years.[14] The limitations of these traditional diagnostic methods are that they determine disease severity rather than disease activity which may lead to under diagnosis. This inadequacy in diagnosis leads to incomplete diagnosis, poor periodontal therapy and eventually might lead to failure of periodontal treatment. For this reason, the use of biomarkers to measure health status, diseases onset, treatment response and outcome has become an added advantage to supplement these traditional methods.[15]

Many of the inflammatory biomarkers associated with oral diseases are found in saliva, serum and gingival crevicular fluid and include but are not limited to IL-1 , Il-6, Tumor necrosis factors, matrix metalloproteinases. These Inflammatory biomarkers are released by different cells during inflammation and hence can be important in clinical use for: diagnosis, prognosis and monitoring response to treatment. Interleukin -1beta is a biomarker that has been identified to be of use as a diagnostic or as a prognostic marker of periodontal destruction. This is because it is now known that IL-1beta is able to act on a large number of cells like fibroblasts, osteocytes, chondrocytes, osteoblasts, neutrophils and lymphocytes. This action on many different cell lines, suggests that periodontal destruction and repair in the progression of chronic periodontitis may partly be linked with IL-1beta.[16] This study therefore aims at investigating the relationship between salivary IL -1 biomarker and periodontal health status.

1.1 Literature Review

1.1.1 Pathogenesis of Periodontal Diseases

Pathogenesis is defined as the origin and progression of diseases. It is a step by step process that leads to the development of diseases, resulting in a series of changes in the structure and function. It's the process by which the etiologic factor causes the disease.[17] Page and Schroeder classified the histological changes that occur during the course of periodontal disease.[11] According to their study, the disease appears as mild inflammation referred to as the Initial lesion. Thereafter the gingiva becomes red, swollen and bleeds readily and this is referred to as the early lesion. If the condition is not treated, it becomes an established lesion. With disease progression, the alveolar bone and periodontal ligament are involved leading to the advanced lesion, where the transition to periodontitis begins. However, not all cases will progress to

periodontitis. Socransky and colleagues in 1982 in their study looked at the attachment level at two sites of each tooth in 22 subjects who had not received any periodontal treatment and had existing periodontal pockets and measured those sites every month for one year. [18] Majority of the sites, 82.8% of them did not show significant change throughout the year. Some sites 5.7% became deeper and 11.5% of the sites became shallower, during the period of the study. Amongst the sites where pocket depth increased, approximately half of them exhibited a cyclic deepening followed by spontaneous recovery to their original depth. The results of this study show that there is a dynamic process taking place with disease exacerbation followed by periods of remission and this may be the characteristic of periodontal diseases.

The current understanding is that the pathogenesis of inflammatory periodontal disease occurs as a result of interactions of periodontopathic micro-organisms and the host tissues. This process is modified by the immune status of the host, systemic and genetic factors and environmental exposures.[19] Lately, it has been proposed that also certain viruses may play a role in the progression of aggressive and chronic periodontitis.[20] In summary, inflammatory periodontal diseases are diseases of the periodontium initiated by micro biota and maintained by the host inflammatory response. There is infiltration of the periodontal supportive tissues with inflammatory cells and subsequent production of inflammatory mediators. The cellular infiltration as well as the inflammatory mediators that are produced lead to the destruction of the periodontal tissues. Several etiological factors lead to the progression of periodontal disease. These etiological factors have been divided into local, Occlusal, systemic and other risk factors.[19]

1.1.2 Local Factors of Inflammatory Periodontal Diseases

Plaque is known to be the main etiological factor of periodontal diseases. Plaque can be described as soft amorphous granular deposit which accumulates on the surface of the teeth, dental restorations and dental calculus.[19] The accumulation of plaque or bio film (well organized community of bacteria) around the teeth was designated as the primary cause of gingivitis. The removal of which leads to the disappearance of clinical manifestation. It has also been suggested that unresolved gingivitis and the continued accumulation of plaque will eventually lead to periodontitis.[19] This notion has however been challenged by findings from another classical study by Loe et al in 1986 where only 20% of a rural population deprived of regular dental care

suffered from severe periodontal breakdown.[21] It was also shown that a subset of patients under good periodontal maintenance and plaque control might still suffer from disease progression. Hence it was concluded that, other than plaque, other factors like certain environmental factors and the host systemic conditions play a major role in periodontal diseases progression.[19]

1.1.3 Occlusal Factors that Influence Inflammatory Periodontal Diseases

These are factors that alter the environment of the periodontium and might influence the spread of periodontal diseases. One of the known conditions that aggravate chronic periodontitis is trauma from occlusion (TFO). TFO per se does not cause the disease it requires the presence of plaque. Occlusal factors that can aggravate the spread of periodontitis include such factors as over function, which produces excessive stress on the teeth. Abnormal habits which include unilateral mastication, nail, lip, pencil, and cheek biting can cause traumatic injuries which affect both the periodontium and the tooth.[22]

1.1.4 Systemic Factors Affecting Inflammatory Periodontal Diseases

A number of systemic conditions in particular diabetes mellitus have been associated with periodontal diseases. Other systemic conditions associated with periodontitis are Papillion Le Fevre syndrome, Down syndrome and agranulocytosis. None of these diseases have been found to cause periodontal diseases, they are predisposing factors.

1.1.5 Other Risk Factors of Inflammatory Periodontal Diseases

Other factors associated or found to confound the prevalence of inflammatory periodontal diseases are: - smoking, age, diet, and economic status.[23] There is a strong co-relation between smoking and deterioration of periodontal health. Over a 20-year period, smokers had a significantly larger amount of plaque, more gingival inflammation and more progression rates of periodontal attachment loss compared to nonsmokers.[24]

1.1.6 AAP Classification of Periodontal Diseases and Conditions (1999)

The recommended classification of periodontal diseases is the 1999 classification. This classification system is accepted by the American Academy of Periodontology as well as the European Federation of Periodontology.[25] However, there is a new classification system of 2018

but it is still in the process of interrogation hence the reason for the continued use of the 1999 classification.

Table 1: Classification of Periodontal Diseases and Conditions (Armitage 1999)[25]

CLINICAL PARAMETER	OTHER DESCRIPTIVE CHARACTERICS
Gingival Diseases	a.) Dental-Plaque Induced gingival diseases b.) Non-Plaque induced gingival lesions
Chronic periodontitis	a.) Localized b.) Generalized (>30% of Active Sites are involved) • Slight: 1-2mm CAL • Moderate: 3-4mm CAL • Severe >5mm CAL
Aggressive Periodontitis	a.) Localized b.) Generalized (>30% of sites are involved) • Slight: 1-2mm CAL • Moderate: 3-4mm CAL • Severe: >5mm CAL
Periodontitis as a manifestation of systemic diseases	a.) Association with hematological disorders b.) Association with genetic disorders. c.) Not otherwise specified
Necrotizing Periodontal Diseases	a.) Necrotizing ulcerative gingivitis b.) Necrotizing ulcerative periodontitis
Abscesses of Periodontium	a.) Gingival Abscesses b.) Periodontal Abscesses c.) Pericoronal Abscesses
Periodontitis associated with Endodontic Lesions	a.) Combined periodontics- endodontic lesions
Developmental or Acquired Deformities or Conditions	a.) Localized tooth related factors that modify or predispose to plaque-induced gingival diseases periodontitis b.) Mucogingival deformities and conditions around teeth c.) Mucogingival deformities and conditions on edentulous ridges Occlusal trauma

1.1.7 Epidemiology of Periodontal Diseases

Gingivitis is the commonest type of periodontal diseases, and its occurrence is very common. On the other hand, periodontitis affects mainly the adult population and a small proportion of adolescents, but its prevalence and extent increases significantly with increasing age.[26] According to the WHO report of 1978 on periodontal diseases in mankind, 80% of young children in the world had gingivitis.[27] In Kenya, the prevalence of these diseases has been determined through several studies. One of the reviews on work done in Kenya by Ng'ang'a P.M in 2002 showed a prevalence of 60-70% among 45-60yrs old male and female subjects[28].

In another study conducted by Manji et al in Nairobi. Ninety percent (90%) of the examined 7536 primary school children of 5-14 years had gingivitis[29]. Most recently, the Kenya oral health survey of 2015[13] showed that the presence of bleeding gums occurred in three out of four children (75.6%) who were less than 15 years. Acute necrotizing gingivitis was reported at 0.8%. The adult population prevalence of gingival bleeding stood at 98.1%. The report concluded that the burden of oral diseases and conditions varied from low to high. Both children and adults had unmet dental caries and gum related treatment needs.

A study to analyze the periodontal condition and oral hygiene practices among Tanzanians, reported a prevalence of plaque at 65-100%, gingival bleeding on probing was found in 79-100% of the participants.[30]

A recent study estimating the occurrence, severity and extent of periodontitis in adults in the United States applying the CDC/AAP criteria established that over 50% of the population had periodontitis. In terms of severity 8.7% had mild, 30.0% had moderate and 8.5% severe periodontitis. Chronic periodontitis was also highest in males without a high school education.[31]

Most if not all of these studies confirm the need to address the widely prevalent periodontal diseases. These inflammatory diseases exhibit multi factorial etiological factors. One of the factors considered to contribute to the progression of inflammatory periodontal disease is the host systemic factors. Amongst the systemic diseases that impact on the progression of periodontal diseases is xerostomia.[32] A condition that leads to decreased saliva quantity due to

under production by the salivary glands. In cases where an individual's saliva production is minimal the oral cleansing and buffering functions of saliva are affected leading to dental caries and chronic periodontitis. Saliva is known to keep the oral tissues moist thus protecting them from physical injury and also since it contains antimicrobial factors it acts as the first line of defense against invading microorganisms. Thus, confirming the vital role saliva plays in arresting the progression of inflammatory periodontal diseases.

1.1.8 Saliva

Saliva is a viscous clear watery fluid secreted from the major and minor salivary glands. Saliva production averages 500-1000ml per day. The submandibular gland produces the most about 70% of the overall quantity, the parotid gland 25% and the sublingual glands about 5%. The minor salivary glands share the remaining insignificant quantity.[33]Saliva is composed of 98% water. Other important compounds, such as electrolytes, mucus, antimicrobial agents and various enzymes comprise the remaining 2%. Saliva pH falls between 6 and 7.4.[34] Whole saliva consists of secretions from all the salivary glands, serum and blood products such as neutrophils and their products. Loo and colleagues in 2010 found over 1000 proteins unique to whole saliva and not found in glandular saliva or in plasma[35]. These proteins in saliva may be derived from several sources including host defense systems, oral epithelium, the oral micro flora and diet. The resulting fluid is a complex mixture that reflects the metabolic status of the tissues involved in its production and its immediate environment. It is for this reason in the past decade, there is a growing interest in the discovery of salivary based diagnostics in the detection of many disease conditions through analysis of various products present in saliva. Saliva has been used to diagnose HIV-1 and 2, oral cancer and viral hepatitis as well as predicting the presence of inflammatory periodontal diseases. Saliva provides an easily available non-invasive diagnostic media for testing a range of diseases and clinical situations, due to the abundance of proteins and genetic molecules in whole saliva.[36]The study of Saliva and its components has proved to be useful in early detection of certain diseases, monitoring the course of diseases and detection of addictive drugs[36]. Saliva also possesses many important biological functions that are important for maintenance of homeostasis.[37]

1.1.8.1 Functions of Saliva

The many functions of saliva include antimicrobial activity, protection of soft tissue against desiccation, mechanical cleansing action, maintaining of pH, aids in post eruptive maturation of enamel [37]The presence of antimicrobial peptides have a role in protecting the oral cavity from infectious organisms.[38] Other important functions of saliva include digestion. Saliva plays a role in taste sensation, repair of soft tissue, maintaining the balance of oral micro flora, ensuring the stability of the oral environment and enamel remineralization. A lack of saliva also referred to as xerostomia can be due to insufficient saliva production (hypo salivation) or can be brought about by prescription medicines and autoimmune syndromes like sjogren's syndrome. Xerostomia can lead to microbial balance alteration allowing the growth of certain pathogens such as Candida species and Streptococcus. This has a negative impact on oral health and leads to tooth decay. In addition, Xerostomia can lead to acute irritation of the oral mucosa, difficulties in airflow, speaking, swallowing, food clearance and taste and this may lead to effects on the general health.

Human saliva has many biological functions which are essential for the maintenance of oral health. Local and systemic biomarkers are also present in saliva and these include electrolytes, blood products, enzymes, inflammatory markers as well as proteins associated with different diseases. Saliva therefore has molecular biomarkers of diagnostic potential for detecting oral and systemic diseases in large populations.[39]

1.1.9 Biomarkers in Saliva

Several definitions of biomarkers exist in literature. The national institute of health definition of a biomarker is a substance which has characteristics which can be objectively measured as well as evaluated to indicate normal biological process, pathogenic process or pharmacologic response to a therapeutic intervention.[40]According to WHO definition it can be a substance, or a process which is measurable in the body or its products. These measures can then be used to predict the incidence or outcome of a diseases.[41]

A biomarker thus can be used as an indicator of a biological state. In diagnosis of oral conditions, it has been difficult to identify biomarkers for screening, prognosis, evaluating the diseases activity and the efficacy of treatment. This is because no single biomarker demonstrates

the ability to be specific to a particular disease. An ideal oral diagnostic tool in general should provide useful information about the diagnosis, localization, and severity of disease.

There is need therefore for the continued search for diagnostic tests especially those based on multiple biomarkers. It is hoped that multiple biomarkers would improve the specificity and sensitivity of the test. Several potential biomarkers that have been studied to detect the presence and characteristics of periodontal diseases and have been classified as follows: markers that indicate presence of periodontal pathogens (bacteria, viruses and Candida), markers identifying presence of periodontal or gingival inflammation (Interleukin and TNF), markers that detect host immune response to certain pathogens and markers that detect products of tissue destruction (IgG & PMNs) (Table 2).

In regard to periodontal diseases the potential sources of these biomarkers are in oral fluids, which include gingival crevicular fluid (GCF) and saliva. Saliva and GCF are fluids which are easily accessible and contain both local and systemic derived markers of periodontal disease. They thus offer the basis for efficient, specific biomarker assessment for periodontal and other systemic diseases evaluation.[42]

Table 2: Salivary Biomarkers of Oral Diseases Adapted from Zhang 2009 and Priyanka 2012 [43,44]

SALIVA/ORAL FLUID BIOMAKERS	POSSIBILITIES FOR
DNA	Standard genotyping Bacterial infection Diagnosing carcinomas of the head and neck forensics
RNA	Viral/ bacterial identification Carcinomas of head and neck
Proteins	Diagnosing periodontitis Diagnosing carcinomas of the head and the neck Detecting dental caries
Mucin/glycoproteins	Detecting dental caries
Immunoglobulin	Diagnosing viruses (HIV, Hepatitis B and C)
Metabolites	Diagnosing periodontitis
Drugs and their Metabolites	Monitoring drug abuse Detecting the presence of drugs in the body
Viruses	Epstein Barr Virus reactivation(mononucleosis)
Cellular Material	Diagnosing carcinomas of the head and neck

Several studies have been done before to try and ascertain the constituents of whole saliva to find the different components that can be used as potential biomarkers of any biological process in the oral cavity. In the study by Mandel et al, the conclusion was that whole saliva comprises of secretions from the salivary glands, serum and blood products, such as neutrophils and their byproducts, GCF and other products from the oral mucosa including epithelial cells, electrolytes, microorganisms and their byproducts. The presence of these products in whole saliva makes it an ideal medium for use in salivary based diagnostic tests. These diagnostic tests which use saliva are able to detect diseases such as HIV 1 and 2, oral cancer and viral hepatitis A, B&C. They are also very useful in monitoring the presence of drugs such as cocaine and alcohol[45].

In a study to assess the presence of inflammatory mediators in saliva, Miller's found interleukin 1 , Matrix Metalloproteinase-8 (MMP-8), and Osteoprotegerin (OPG) in whole saliva, which are byproducts of inflammation and are directly related to inflammation, collagen degradation and bone turnover. Whole saliva thus contains multiple biomarkers that are useful in diagnosis, prognosis and monitoring of treatment. For this reason, this study used whole saliva to analyze the levels of the cytokine (salivary IL- 1) in those patients recruited in the study[46].

Studies that have investigated the pathogenesis of periodontal disease usually examine whether biochemical and immunological markers in saliva or GCF (Biomarkers) can indicate the severity of periodontal destruction and help predict future disease progression.[47] Most of the studies have shown that no single marker will fulfill all the criteria necessary for assessment of the clinical state of the periodontium and thus future research should be directed at the production of a 'marker package' which incorporates several markers. Multiple biomarkers would improve the specificity and sensitivity of a test and therefore make it possible to accurately predict the disease activity.[48] Salminen and Hernandez-Rios in 2014 developed a commercially available biomarker in Europe. The biomarker incorporates MMP-8, IL-1 , and P. gingivalis. The combination of the three biomarkers mentioned demonstrated a strong association with periodontitis. MMP-8 has also been developed as a diagnostic tool and is commercially available in European countries[49].

1.1.10 Biomarkers in Identification of Periodontal Diseases

Biomarkers are important mediators of inflammation which are associated with pathogenesis of many inflammatory diseases. There are some biomarkers in clinical use. One example being a commercially available chair-side mouth rinse which has an active MMP-8, with enough sensitivity and specificity to detect clinical signs of periodontitis.[51] Biomarkers are thus non-invasive diagnostic tools which can be used for determination of disease, prognosis and to monitor treatment response. Biomarkers help augment these traditional clinical periodontal diagnostic parameters. The traditional methods are easy to use, cost effective and are relatively non-invasive; however, they only tell the history of the disease and not the current status. [52] It is also challenging for clinical practitioners or investigators to determine the active phase of disease or identify those patients at risk of developing active disease. It is thus the reason why sensitive and disease specific salivary biomarkers come in handy to complement regular clinical examination. In addition, the use of diseases-specific biomarkers increases the specificity and sensitivity of obtaining accurate diagnosis and prognostic information. Finally, advances in oral and periodontal disease diagnostic research are moving towards methods whereby periodontal risk can be identified by objective measures of molecular indicators such as biomarkers which use the protein chip technology. This technology provides an opportunity to simultaneously and comprehensively study large numbers of proteins and compare them in health and disease.[53]

1.1.11 Current Trends in Biomarkers Research

Lately, there is continuous medical research on oral fluids to aid in diagnosis of both local and systemic diseases as well as in the analysis of drugs.[50] Oral biomarkers are being developed to help determine dosage and drug metabolism. Studies are investigating the possible uses of GCF and saliva in the screening of persons suspected of having been exposed to biological and chemical agents and also for screening for metabolites of drugs in persons abusing drugs.[50]

1.1.12 Limitations of Biomarkers

The criteria of an ideal diagnostic biomarker was proposed by Chapple and colleagues in 1993 and 1997. The studies proposed that an ideal diagnostic marker should be highly sensitive, use quantitative measures and be capable of analyzing a single periodontal site in health and disease. It should also be highly specific, reproducible and simple to perform. It should be rapid, non-

invasive and versatile in terms of storage and handling as well as transportation. It should be amenable to chair side use, economical and dependent on simple and robust instrumentation. Most of the available biomarkers are not able to meet all these requirements. Many lack the ability to definitively and accurately identify specific diseases especially in the early stages. They lack an easy and inexpensive way of sampling the diagnostic medium without discomfort to the individual. Saliva has been under study for biomarkers because of its availability and ease of collection without discomfort to the individual[54, 55].

1.1.13 Saliva Collection

The ultimate results when analyzing the salivary biomarkers depends on proper collection, transportation and storage of the saliva. Saliva collection is mainly done in two (2) ways: unstimulated saliva obtained from the floor of the mouth contains secretions of all salivary glands. Stimulated saliva composed mainly from parotid gland and produced upon stimulation with agents such as citric acid or chewing on gum.[56] It is prudent that during collection of saliva for biomarker assessment to consider the collection time as most analytes display a diurnal rhythm of collection and to also consider the location of saliva in the mouth as whole saliva which is commonly used for biomarker analysis, is known to collect under the tongue.[56]

Unstimulated whole saliva also called resting saliva is composed mainly of submandibular glands saliva together with saliva from sublingual gland and minor salivary glands. The characteristics of unstimulated saliva are that it is more viscous and is mucin–rich. Its secretion is usually greater than 0.25ml/min. In hypo salivation cases less than 0.1ml/min. Unstimulated saliva is usually collected by allowing saliva to pool in the floor of the mouth then spitting it into a specimen bottle.[57]

Stimulated whole saliva is produced upon stimulation and is mainly composed of parotid gland saliva and to some extent saliva from the submandibular gland. Characteristics of stimulated saliva reveal a thin, watery and amylase-rich fluid. Normally stimulated saliva secretion is usually greater than 1ml/min but in hypo salivation is less than 0.7ml/min. Saliva flow can be stimulated with a chewing gum, with or without citric acid.[57]

1.1.14 Comparison of Stimulated Saliva versus the Unstimulated Saliva

The collection of saliva samples for analysis can be done easily with the use of stimulating agents or with no stimulation. The unstimulated saliva is most frequently used in diagnostic tests since the stimulated one has a reduced concentration of biomarkers because of stimulating the glands. This dilution of biomarkers in stimulated saliva hinders the detection of the biomarkers of interest in diseases diagnosis.

Stimulated saliva, due to the method used for stimulation, may lead to contamination of the specimen which will compromise the biomarkers. Additionally, the stimulant may also interfere with the salivary potential hydrogen (pH).

The unstimulated whole saliva on the other hand is a mixture of secretions from all the salivary glands including substances derived from the gingival crevicular fluid, epithelial cells, micro-organisms and food debris.

Unstimulated saliva is commonly contaminated with food products and may at times be too viscous presenting problems during analysis. Its viscosity is determined by the quality of particles and the presence of bubbles.[58, 59] In this study to mitigate this problem, participants were requested to thoroughly rinse their mouths with water and spit into the spittoon as remove food debris, then after about five minutes the saliva that had pulled at the floor of the mouth was expectorated through a passive drool into the saliva collection tubes provided to them.

When saliva is collected, it should be frozen immediately at four degrees centigrade (snap freezing) for best concentration of biomarkers. However, it’s not always possible to immediately freeze the samples at temperatures of 4 degrees centigrade. There is therefore, a need to sometimes use saliva stabilizers because oral fluids contain enzyme derivatives, bacteria proteases and ribonucleases which degrade the biomarkers of interest. Saliva stabilizers help to preserve biomarkers at room temperature. An example of a stabilizer is sodiumazide.[60]

1.1.15 Collection of Saliva for Biomarkers Assessment

Standardizing saliva collection is useful in the comparative analysis of the biomarkers. The standardized schedules of collection could avoid possible biases. Saliva production follows a circadian rhythm and undergoes changes over 24 hours in both its quality and its quantity. Dawes showed a difference in concentration of sodium chloride and a circadian rhythm in flow rate in unstimulated saliva.[61] On the contrary stimulated saliva showed a cyclic rhythm in the concentration of urea, proteins and ions. Thus, having an effect on the time of day when saliva should be collected. It has been found that almost no changes occur in saliva composition from around 8am and just before noon. Therefore, in view of the study by Dawe et al and another by Navesh et al, the saliva samples best suited for this study were those samples collected before midday.[61,62] This helped to eliminate the confounding effect time may have on saliva biomarkers. Saliva storage also may interfere with the analytes hence the need for early storage of samples. Some analytes are unstable and can change at room temperature thus the need to freeze immediately after collection for best results. There is also the effect of freezing saliva for long periods which can greatly affect the measurement of most biological markers. It is recommended that it is always best to freeze samples at or below minus 20 degrees immediately after collection to preserve the samples for possible use in future studies. In the event that freezing immediately is not possible, then, to minimize degradation of unstable analytes and to prevent bacteria growth, refrigeration can be done at 4 degrees immediately after collection and maintained for less than 2 hours before freezing at minus 20 degrees.[60]

1.1.16 Advantages of Saliva Samples

The main reason in using saliva as a tool for diagnosis is the fact that the majority of biomarkers found in blood or in urine are also found in saliva samples. Infectious diseases causative agents such as some viruses, fungi and bacteria are also identified in saliva. Due to the ease and quick collection in a non-invasive way and easy storage of samples, there has been great interest in investigating the biomarkers present in saliva. Methods of saliva collection are easy and allow repeated measurements without the problems associated with drawing blood.[63]

A study by Mohammad and Javaid et al in 2016 on saliva use as a diagnostic fluid concluded that saliva popularity is increasing as compared to blood this is due to its non–invasive nature during

collection as compared to collecting blood. Making it possible for it to be collected by individuals who are not highly trained[64]. Its simple nature of collection makes it cost effective and thus ideal when screening large populations. Whole saliva contains serum constituents and is mostly preferred in the diagnosis of systemic diseases; on the other hand gland saliva is mostly preferred in the detection of pathologies associated with major salivary glands. Saliva analysis is currently being used for the detection of various conditions which include; infectious diseases, malignant diseases, hereditary, auto immune diseases, and endocrine disorders as well as the assessment of therapeutic drug levels. Moreover, saliva is available in adequate volumes making it an ideal bio-fluid for testing vulnerable populations where saliva specimens may be preferred to blood. Other advantages include minimal risk to the investigator of contracting blood borne pathogens like human immune deficiency virus and hepatitis. Saliva samples are easily collected, stored and preserved. Collection of samples is also possible at participant's immediate location without much inconvenience thus making saliva an ideal fluid to study compared to blood or urine.[64]

1.1.17 Disadvantages of Using Saliva as a Biomarker

Saliva is a heterogeneous fluid that shows varied concentration of constituents depending on the time of the day. Changes in the quantity and quality of saliva may therefore interfere with the composition of analytes.

Concentrations of some specific biomarkers in saliva are often much less compared to the same biomarker in plasma.[65] This drawback has been overcome by the availability of sensitive technologies such as enzyme-linked immunosorbent assay, mass spectrometry, next generation sequencing that are able to detect small quantities of the biomarker. Saliva can also be influenced by confounders specific to the oral cavity like recent eating can influence flow rate, excessive drinking of water can influence some biomarkers.[66]

1.1.18 Problems Associated with Saliva Collection

Food as well as drink particles and residues are often found in saliva samples. To address this, saliva collection protocols require participants to rinse their mouth with water to clean out the food debris before collection. Then the subjects are expected to wait approximately 5-10 minute

before collecting the sample. This ensures that the sample collected has not been diluted by recent hydration.[67] In this study to help mitigate this problem, each participant was requested to relax, avoid chewing and sit comfortably on the dental chair for a few minutes, then rinse the mouth with water before saliva collection.

In those participants who find saliva collection difficult research done has shown that ingestion of acid substances enhances flow rate. Unfortunately, this may subsequently change the pH of the sample and thus influence the concentration of specific analytes in saliva and render some biomarkers contaminated. Even the thought of food may affect the salivary flow rate diluting the concentration of the biomarker of interest.

Saliva is not a sterile bio-fluid. Viral as well as bacteria contaminate saliva samples. Saliva production is influenced by characteristics of an individual; some individuals may have a condition referred to as hypo salivation whereby an individual is produces less than adequate saliva while in hyper salivation saliva production is increased. Both of these two situations may present challenges during saliva collection and analysis. Exercise and stress have been known to slow salivary flow rate.[68] The aim during saliva collection is to ensure individuals are relaxed and not under pressure to produce saliva. The collection should also be done in the morning hours. This will enable collection of a reasonable sample that avoids many of these short comings.

1.1.19 Inflammatory Biomarkers of Periodontal Diseases

Traditionally, periodontal health has been defined by clinical parameters such as absence of gingival inflammation, lack of bleeding during probing, no clinical attachment loss and no alveolar bone loss as well as no pathological probing pocket depth.[69] However, other than these clinical measures, histological studies have also identified some local tissues inflammatory response even in clinically healthy sites.[70] These histological findings of the periodontium are consistent with levels of a group of host response molecules in healthy tissue that are generally considered to increase in magnitude and expand in response to gingivitis and periodontitis.[71]These molecules are referred to as inflammatory biomarkers of periodontal diseases. Many of these molecules have been analyzed in saliva and include cytokines such as

the IL-group, proteolytic enzymes, chemokines and, arachidonic acid metabolites these collectively contribute to tissue destruction including bone resorption. They are thus important in clinical use for disease prognosis and monitoring of treatment response.

1.1.20 Recent Studies on Biomarkers

Recent studies on biomarkers have revealed several inflammatory by products that can be used to identify disease presence. Host responses play a big role in the progression of the inflammatory lesions. At the cellular level, exposure to bacterial products and lipopolysaccharides lead to activation of monocytes/macrophages that elicits production of cytokines and inflammatory mediators examples include; interleukin -1 interleukin-6,TNF alfa (TNF) among others. This results in the release of matrix metalloproteinases (MMPs) which is thought to contribute to the destruction of the integrity of the periodontal tissues.[71]

Many of these inflammatory molecules are found in oral fluids which has enabled saliva usage as an important and easily accessible fluid for diagnosis. Saliva provides important diagnostic information of oral health and diseases.[73]Studies on different biomarkers have been done extensively, with each biomarker having its own advantage and drawbacks in Kenya.

In Kenya, a study by Mwai et al investigated the relationship between periodontal health status and levels of MMP-8 in 120 adults attending the University of Nairobi Dental Hospital. The study reported that in adults with no periodontitis MMP–8 was 22.68ng/ml, in those with mild periodontitis it was 44.55ng/ml, in those with moderate periodontitis, 46.34ng/ml and in those with severe periodontitis 156.62ng/ml. Within the limits of the study a positive association was drawn between salivary mmp-8 levels and periodontal health status. The study concluded that salivary mmp-8 should be considered as a probable diagnostic and adjunctive evaluation tool for assessing periodontal disease. However, they reported a further need to study this putative biomarker in better controlled and randomized longitudinal studies in the Kenyan population. Other studies elsewhere have been done on MMP-8[74].

A study to check the reliability of MMP-8 in the diagnosis of periodontitis when a systemic condition like diabetes is present was carried out by Costa. The results of this study questioned

the reliability of mmp-8 as a diagnostic tool in diabetic patients. The conclusion of the study was that for a biomarker to be useful, it should ideally be able to differentiate between gingivitis and periodontitis as well as be reliable in detecting periodontitis in the presence of co-morbid conditions such as diabetes and osteoarthritis. MMP-8 was not able to do this[75]. It is known that diabetes, liver, kidney, salivary gland diseases and those who have undergone organ transplants have been found to have increased levels of IL I beta in serum and subsequently in saliva. Chronic inflammation related to systemic diseases can increase levels of IL I beta in serum and consequently in saliva. Thus the reason in this study patients with systemic diseases were excluded to minimize false positive responses in periodontal healthy patients.

In the reported studies on oral-fluid based biomarkers, currently matrix metalloproteinase 8(MMP-8) and interleukin 1beta seem to be the most promising potential biomarkers for both diagnosing and possibly determining prognosis of chronic periodontitis. Levels of mmp8 and IL-1 have been shown to correlate with clinical and radiographic parameters of periodontitis.[76]

Salivary proteomic studies are also gaining a lot of popularity as most of the body's processes which include energy generation and production of cellular components as well as degradation of waste products involve protein functions.

In his thesis, Mulli and colleagues investigated proteomic salivary biomarkers based on antimicrobial peptides in periodontal diseases. This study reported that the levels of human neutrophil peptide-3 (HNP 1-3) and cathelicidin LL-37 were significantly elevated in aggressive periodontitis as compared with chronic periodontitis and gingivitis. Periodontal therapy was also found to reduce levels of HNP 1-3. The conclusion of the study was that salivary HNP 1-3 and LL-37 are potential novel biomarkers capable of differentiating periodontal diseases from a healthy periodontium with high specificity and sensitivity, and can be useful for point of care treatment, diagnosis, and for monitoring periodontal health status[38].

Among the several inflammatory and immune mediators identified in GCF and saliva, cytokines have attracted particular attention and are suspected to be involved in both inflammatory related alteration and repair in periodontal tissues. Certain cytokines have been hypothesized as

potentially useful diagnostic or prognostic markers of periodontal destruction. Interleukin 1 beta is a multifunctional inflammatory mediator able to mediate bone resorption by activating osteoclast and by stimulating prostaglandin E2 synthesis.[77] Evidence suggests IL-1 is produced by connective tissue cells such as fibroblasts and keratinocytes this happens as a result of stimulation by bacteria products.[78]The fact that IL-1 is now known to act on cells like fibroblast, chondrocytes, osteoblasts, osteocytes, osteoclast, neutrophils and lymphocytes pointing to the fact that destruction and repair in periodontitis is partly associated with interleukin1 .[79] In 1992, Kinane et al demonstrated that inflamed sites of patients with gingivitis had increased levels of IL-1 in the GCF and saliva[80]. Other studies that followed have shown a co-relation between increasing levels of IL-1 in the GCF and saliva with increasing gingival inflammation and increasing probing depth. Site specific increases were also observed in participants with untreated periodontitis and in an experimental gingivitis model. [81] Treatment of periodontitis was shown to result in drastic local decrease of IL-1beta suggesting that this cytokine is pertinent in periodontal destruction.

Currently, there is interest in the study of genetic association with periodontal diseases in particular that of the IL-1genotype. This genetic marker includes two (2) polymorphisms of the IL-1 gene cluster on chromosome 2, Kornman and co-workers demonstrated an association between a specific composite genotype of the IL-1gene cluster and periodontal disease severity.[82]

In a study by Wagaiyu et al to check the association of interlukin-1 polymorphisms with chronic periodontitis in the African population of Bantu origin found two (2) polymorphisms associated with chronic periodontitis, IL-1 +3954 in Taitas and IL-1 -889 in Swahilis and halotype 3 were associated with chronic periodontitis in both ethnic groups[83,84,85].

Engebretson et al associated the presence of the carriage of a specific IL-1 gene cluster composite polymorphism with increased IL-1beta expression in vivo. Among patients of similar diseases severity, those with the periodontitis associated genotype (GAP) demonstrated elevated levels of IL-1beta in GCF and gingival tissue in those who carry the GAP[86].

Based on the studies above and several others it is now clearer that biomarkers in saliva and GCF reveal a promising outlook as a key diagnostic medium for periodontal diseases. However, the challenge has been how to get all the diagnostic information from these oral fluids. As in many other diseases, periodontitis is heterogeneous in its etiology and thus the use of a single biomarker to offer insights into diagnosis is arguably not adequate. Among all the aforementioned salivary biomarkers interleukin1 has shown great potential as an indicator of periodontal diseases.

1.1.21 Interleukin-1 and Periodontal Disease

Inflammatory periodontal disease is known to be initiated by bacteria bio-film and propagated by the host response. It has been shown to have periods of activity and remission. Monocytes activated by the bacteria lipopolysaccharides secrete inflammatory mediators such as prostaglandin E2 (PGE2), TNF and interleukin-1 .[87]

Interleukin-1 is a pro-inflammatory cytokine. Cytokines are soluble proteins, which act as messenger molecules transmitting signals to other cells. During inflammation, they are secreted by immune cells in response to a pathogen. The secretion then leads to activation of other immune cells in order to respond to the pathogens. This induces release of metalloproteinases that contribute to the activation of osteoclasts and consequently, to bone resorption.[88] Interleukin-1 is synthesized by macrophages in it inactive form, it is then processed to its active form by caspace 1 (CASP1/ICE). It is involved not only in inflammation, but also in cellular activities which include cell proliferation, differentiation, and apoptosis.[89]Of the two isoforms of IL-1 that is,IL-1 and IL-1 , The latter (IL-1) is more potent in stimulating bone resorption and is the most common in chronic periodontitis.[90] It is produced during periodontal inflammation and tissue destruction.[91] In the periodontium, IL-1 is synthesized and secreted by connective tissue cells among which are the fibroblasts ,endothelial cells and the infiltrating leukocytes. Clinical studies, have shown increased levels of IL-1 detected in GCF and saliva associated with increased gingival inflammation and periodontal disease severity. The levels of IL-1 co-relate with several periodontal parameters such as bleeding on probing, deep periodontal pockets, as well as clinical attachment loss.[92]

Hence salivary interleukin 1 is a promising biomolecule that can be used for non-invasive diagnosis and possibly more importantly, assessing the severity, activity and progression of periodontal diseases since it is possible to detect and quantify it in saliva using molecular techniques such as ELISA. This study investigated the relationship between salivary interleukin 1 and clinical parameters of periodontal disease in an effort to find out if it is a valuable adjunct or substitute to traditional periodontal diagnostic methods.

1.1.22 Studies on Interleukin 1

Genetic polymorphisms together with environmental factors have been reported to influence the progression of chronic periodontitis in a complex way. The study by Kornman and colleagues reported a specific genotype of the polymorphic IL-1 gene cluster that was associated with diseases severity in periodontitis in non-smokers and distinguished severe periodontitis from those with mild diseases. In smokers, severe disease was not co-related with genotype. The cases of severe periodontitis patients (86%) were accounted for by either smoking or presence of IL-1 genotype. This study demonstrated that there are specific genetic markers that have been associated with increased IL-1 production and are viable indicators of susceptibility to severe periodontitis in adults[82].

Host susceptibly and its lack of in some individuals was reported in a study on tea plantation workers in Sri Lanka, where despite not brushing their teeth and receiving no professional care, 11% of them did not develop periodontitis yet they all had large plaque deposits and dental calculus as well as plaque associated chronic gingivitis.[21] This attribute of host susceptibility possibly implies the presence of genotype that exposes the individual to more disease. The implication being that individuals who inherit susceptible alleles will develop the disease when exposed to risk factors of periodontal diseases whereas those without the susceptible alleles will not develop the disease despite being exposed to the risk factors.

In 1999 Engebretson et al carried out a study to check whether gingival crevicular fluid (GCF) levels of interleukin 1 and Tumor Necrosis Factor alfa and gingival tissue levels of IL 1 , IL-1 and TNF alfa, co relate with the periodontitis associated genotype (PAG). They also examined the effect of conservative periodontal therapy on these levels. The study comprised of 22 adults

with moderate to advanced periodontitis. The results of the study identified 7 as PAG positive (+) and 15 as PAG negative (-). Comparing the two (2) groups, in shallow sites (<4mm) total interleukin 1 in GCF was 2.5 times higher for PAG (+) patients as compared to the PAG (-) prior to treatment, and 2.2 times higher than the PAG (-) after treatment. These differences were less apparent in deeper sites following treatment. A reduction in IL-1 concentration in GCF was seen for PAG (-) but not for PAG (+) patients. This could be due to the fact that the carriage of the PAG is associated with increased expression of IL-1 in vivo. The study concluded that patients carrying this periodontitis associated genotype (PAG) demonstrate phenotypic differences in that there is increased expression of the amounts of the cytokine as indicated by the elevated levels of the cytokine (IL-1) in gingival crevicular fluid[86].

Variations of the interleukin –I gene cluster have been proposed as genetic modifiers in inflammatory periodontal diseases. In a study by Wagaiyu and colleagues to investigate the association between IL-1beta and IL-1alfa isoforms and chronic periodontitis in two Kenyan ethnic groups, Taitas and Swahilis where 290 participants were recruited, 4 loci (-511, -889, +3953 and +4845) per subject equivalent to 1560 analysis were done. The study concluded that in the African population of Bantu origin the two-polymorphisms associated with chronic periodontitis are IL-1 +3954 in Taitas and IL-1 -889 in Swahilis. Interleukin 1 -511 allele was also associated with chronic periodontitis in the Swahilis when plaques levels were low[85]. As a follow up to the study by Wagaiyu, it is envisaged that a study on the correlation between IL1 levels in saliva and chronic periodontitis in Kenyans would reveal whether this cytokine is significant in detecting disease presence since the genotype has already been shown to be associated with chronic periodontitis. Hence the reason why this study investigated this correlation.

In a cross-section study by Craig et al on salivary biomarkers of existing periodontal diseases. Craig checked the relationship of periodontal disease and compared with the levels of interleukin 1- (IL-1), matrix metalloproteinase (MMP) and osteoprotegerin (OPG) in whole saliva of 57 adults. This was a case control study where 28 case subjects had moderate to severe periodontal disease and 29 subjects were healthy controls. The results showed significantly higher levels IL-1 and MMP-8 in the case subjects than in controls. There was a relationship between the

analytes and periodontal indexes whereas after adjustment for confounders, OPG did not. Increased salivary levels of MMP -8 or IL-1 greately increased the risk of periodontal disease. Combined elevated salivary level of MMP-8 and IL-1 increased the risk of periodontal disease by 45-fold and increased levels of all three biomarkers correlated with individual clinical parameters indicative of periodontal disease. The conclusion made was that salivary levels of MMP-8 and IL-1 are able to serve as oral biomarkers of chronic periodontitis. The clinical implications being that qualitative changes in the composition of salivary biomarkers is of great importance during the diagnosis and treatment of periodontal disease[46].

A study by Kaushik et al on salivary interleukin 1 levels in patients with chronic periodontitis before and after periodontal phase 1 therapy and healthy controls indicated that interleukin 1 levels are raised in the saliva of patients with chronic periodontitis which are reduced after phase 1 therapy suggesting a close association between salivary interleukin 1 and periodontitis. The study measured interleukin 1 beta levels in patients with chronic periodontitis before and after phase 1 therapy and compared with healthy controls. 28 patients with mild to severe generalized chronic periodontitis and 24 controls matched for age, race and ethnicity participated. Saliva samples were obtained from all patients. IL-1beta levels were measured using enzyme linked immune absorbent assay. Mean interleukin 1 beta levels in patients with periodontitis at baseline were (1,312.75pg/ml) and in controls (161.51pg/ml). Although treatment of patients with periodontitis resulted in significant reduction in IL-1 levels (mean 674.34pg/ml), this level remained significantly higher by four-fold when they were compared to the control subjects. There were significant correlations between IL-1 levels and all clinical parameters expect percentage sites with clinical attachment loss CAL 2mm.The data indicated that IL-1 levels are raised in the saliva of patients with chronic periodontitis which are reduced after phase 1 therapy suggesting a relationship between salivary IL-1 and periodontitis. The author recommended more longitudinal studies to validate salivary interleukin 1 as a marker of periodontal disease[93].

Most of the studies done on interleukin 1 have either been done in combination with other salivary biomarkers or have involved a look at the genes that code for interleukin 1 . Few clinical trial studies have been done to examine IL 1 alone and its relationship with periodontal health status. No study to the author's best knowledge has been done in Kenya to investigate IL

1 in saliva and its association to periodontal health status. Thus, the aim of this study was to investigate salivary interleukin 1 levels and correlate these levels to the periodontal health status of recruited individuals.

CHAPTER TWO

STATEMENT OF RESEARCH PROBLEM AND JUSTIFICATION

2.1 Problem Statement

Chronic inflammatory conditions affecting the supporting structure of the dentition referred to as periodontal diseases are considered a health issue globally. They lead to loss of the dentition and cause oral dysfunction and increase susceptibility to other diseases. Because of the high prevalence and potential systemic health problems, periodontal diseases have to be detected early and treated properly in order to preserve and improve as well as maintain the remaining teeth this will minimize the systemic sequelae that may arise.

Customary clinical parameters of periodontal diseases are used in dental practice because of their simplicity, reliability, and are less invasive yet several disadvantages exist with the current method in addressing the needs of the public. First, is the need for highly trained clinicians and an assistant to record the findings, secondly is the use of expensive diagnostic equipment such as radiographic machines to collect diagnostic information thus making the procedure very intensive in regard to time and labor. It also imposes significant financial costs to the consumer and it is subject to variations in the clinical readings of the many parameters even among experts and even on a day to day basis in the same examiner. Also important is that these clinical parameters do not determine the current status of the disease rather it is the history of the disease that is recorded. Additionally, some amount of damage has to occur before these diagnostic parameters are able to detect the presence of disease. Early disease detection plays an important role in successful therapy. Early diagnosis as well as early intervention reduces the severity and possible complication of the disease progression. To overcome these challenges the use of other complimentary methods such as molecular disease biomarkers has made all the difference in the diagnosis process. These biomarkers are able to reveal a hidden destructive threat before the disease becomes established or complicated and should therefore become essential diagnostic tools that can augment the traditional diagnostic methods.[94, 95] Continued research is thus needed to develop diagnostic biomarkers that are more accurate, efficient, less invasive, less tedious to do clinically and can also be used to monitor response to periodontal therapy.

2.2 Study Justification

This study investigated interleukin 1   and its relationship with inflammatory periodontal disease in an effort to come up with an ideal salivary biomarker that can be used to detect disease in its early stages when it is easy to treat.

There is no data in the Kenyan population that shows the range of the IL-1   in the saliva of periodontally healthy individuals. This baseline information is important because increased levels of this cytokine could be used as an indication of the presence of disease. This has been demonstrated by Kornman and co-workers in 1997 when they were able to associate a specific composite genotype of the IL-1 gene cluster and periodontal disease severity. Engebretson in 1999 also demonstrated that carriage of the specific IL-1gene cluster composite polymorphism is also related to increased IL-1beta expression in vivo. They demonstrated that among patients of similar disease severity, those with the periodontitis associated genotype (PAG) had increased levels of IL-1beta in GCF and in the gingival tissues.

Salivary interleukin-1beta represents a family of cytokines that are expressed in pathological conditions. It has been established that environmental, racial, ethnic, socioeconomic as well as genetic polymorphism and the carriage of the periodontitis associated genotype (PAG), alter the expression of the host response in periodontal diseases. This may lead to the expression of cytokines in the tissues which in effect alter the disease severity. Some genetic variations are associated with increased levels of cytokines in GCF and in saliva. This has been shown in individuals who carry the periodontitis associated genotype. Hence the need to carry out a study to assess the normal range of this cytokine, (IL1 ,) in saliva and then assess its correlation with inflammatory periodontal diseases since it has already been shown by Wagaiyu that inflammatory periodontal disease is associated with IL 1   polymorphisms in Kenyans.

In Kenya data exists among the Swahilis and the Taitas that show association of chronic periodontitis with genetic polymorphism of IL-1 at different specific loci for each ethnic group. Based on this mentioned study it is possible that IL-1beta phenotype may also show variations in other ethnic groups of the Kenyan population. This study assessed the salivary levels of cytokine IL1   coded by the IL 1   gene in a mixed ethnic group. The current study was based in Nairobi,

the cosmopolitan capital of Kenya, where it is known that the population is diverse and comprises many ethnic groups present in the country thus giving an ethnic mix probably more representative of the diverse Kenyan population. More importantly this study forms the baseline for mounting larger population-based studies on the subjects.

Based on the aforementioned it would be important to evaluate the expression of this salivary cytokine as it relates to health and disease in an attempt to identify an ideal salivary biomarker that will overcome the challenges experienced with the traditional diagnostic methods. This will also aid in the development of a chair side /point of care diagnostic tool that can identify patients at risk of periodontal diseases progression and assess treatment outcome. This may help ease the challenges of inaccurate diagnosis of periodontal diseases which may lead to inappropriate treatment.

2.3 Objectives

2.3.1 Main Objective

To investigate the relationship between salivary 1L-1 levels and periodontal health status in an adult Kenyan population.

2.3.2 Specific Objective

1. To measure the salivary IL-1 levels among adult patients recruited from those attending the University of Nairobi (UoN) dental hospital
2. To assess the periodontal health status of the recruited adult patients.
3. To evaluate the correlation between levels of salivary IL-1 and periodontal health status among the recruited adult patients.

2.4 Hypothesis

2.4.1 Null Hypothesis

There is no association between salivary IL-1 levels and periodontal health status among adult patients attending the UoN dental hospital.

2.4.2 Alternate Hypothesis

There is an association between salivary IL-1 levels and periodontal health status among adult patients attending the UoN dental hospital.

2.5 Variables

Table 3: Variables

VARIABLES	MEASUREMENT
Sociodemographic variables	
Age	number of years
Education level	level of education attained
Residence	Where participants currently reside
Gender	Male or female
Occupation	employed, self-employed, not employed
Independent Variables	
Gingivitis	gingival index (Loe & Sillness, 1963)
Periodontitis severity	(BPE)
Oral hygiene status (plaque)	(Turesky modification of Quigley&Hein1970)
Outcome Variables	
Salivary IL-1 levels	in ng/ml

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Location

The study location was the University of Nairobi (UoN) Dental Hospital in Nairobi, which offers undergraduate and post-graduate training in dentistry in Kenya. The hospital manages patients with different oral-facial conditions and diseases including periodontal diseases. It also caters for patients who have been referred from other public hospitals and private dental clinics in Nairobi and other parts of the country.

3.2 Study Population

Adult patients and accompanying individuals (those over 18 years) who presented at the Oral Diagnosis and Periodontology clinics of the University of Nairobi Dental Hospital and who gave written informed consent.

3.2.1 Inclusion Criteria

Adult patients and accompanying individuals who voluntarily consented to participate in the study and gave written consent or consent through the use of a thumb print.

3.2.2 Exclusion Criteria

All those patients who did not-consent. Patients below 18 Years of age are considered minors and are legally incompetent and therefore lack the capacity to decide. Patients who had a history of having received periodontal treatment within the last 6 months. Those with a history of anti-biotic or steroid use in past three months preceding the study. Antibiotics and periodontal treatment are known to interfere with the quality and quantity of the oral microbial ecology and this affects the inflammatory response that is responsible for the release of host molecules that lead to the clinical features of periodontitis. Pregnant and Lactating Mothers are also known to have an increased propensity to developing periodontal diseases due to the elevated levels of hormones in the blood circulation. Smoking has been shown to have a cumulative destructive effect on the periodontal tissue leading to immune impairment and up regulation of the cytokine. Smokers were also excluded for this reason. Lastly, those patients with diabetes or those who reported to have liver, kidney or salivary gland diseases and those who had undergone organ

transplant or cancer therapy were also excluded. IL 1 beta has been found in serum and subsequently in saliva of patients with chronic inflammation which is related to systemic diseases. The patients with inflammatory conditions were thus excluded to minimize false positive responses in periodontally healthy patients. Those patients who on oral examination showed excessive toothwear, reported history of bruxism or excessive tenderness of jaw muscles, obvious dental abnormalities such as broken tooth that would cause occlusal discrepancy were also excluded. This is because trauma from occlusion is known to aggravate progression of periodontal disease.

3.2.3 Preliminary Feasibility Assessment

A preliminary visit was made to the Kenya AIDS Vaccine Initiative (KAVI) laboratory at Kenyatta National Hospital (KNH), where saliva sample analysis was carried out.

The results of the feasibility visit concluded that KAVI laboratory has the necessary equipment and an up to date calibration schedule of the equipment that was used. The ELISA kit that was used to analyze IL 1 in saliva samples was purchased through the laboratory.

3.3 Study Design

This was a descriptive cross-sectional study using the convenient sampling method to recruit participants.

3.4 Study Period

The study was carried out between August 2018 and February 2019.

3.5 Sample Size Determination

The sample size was determined using the Kish, Leslie formula[96] where the prevalence of interleukin 1 that was reported by Kaushik [93] of 12% was used

Therefore, using Kish, Leslie formula for prevalence

$$n = \frac{z^2 pq}{d^2}$$

Where

n =desired size (for population greater than 10,000)

z = the standard normal deviation at the required confidence level (1.96 for 95% Confidence level)

p = proportion of the population estimated to have the characteristics being measured

$q = 1 - p$

d =level of statistical significance set.

$$N = \frac{(1.96 \times 1.96 \times 0.12 \times 0.88)}{(0.05 \times 0.05)}$$

$= \mathbf{162.26}$

The calculated sample size for a population > 10,000 was 162. However, the average number of new patients with periodontal diseases seen at the Periodontology and Oral Diagnosis clinic is about 4 per day giving120 patients per month. Using the formulae for a population of the less than 10,000

$$nf = \frac{n}{1 + n/N}$$

Where

nf =desired sample size (for population <10,000)

n =desired sample size (for population >10,000)

N = estimate of population size

$$nf = \frac{162}{1 + (162/120)}$$

$= \mathbf{69.9}$

The minimum sample size was determined to be **70** participants.

3.6 Sampling Procedure

Convenient sampling was used. All patients presenting at the oral diagnosis and periodontology clinics of the UON dental hospital and who met the inclusion criteria and consented to inclusion were recruited into the study.

3.7 Study Methodology

Participants were recruited from the Oral Diagnosis clinic and the Periodontology Clinic waiting rooms. The purpose of the study was explained to them and written consent obtained (Appendix

I). Data on the socio-demographic variables was then collected through a questionnaire. A screening form that is part of the questionnaire was filled by the principal investigator after consulting the participant (Appendix II). The participants were free to ask the Principle Investigator (EKM) for clarification of any question that were not clear to them. The questionnaire was administered by EKM. The participants were then invited for the saliva collection and periodontal examination which was done on a dental chair in the clinic.

Saliva collection

Saliva samples were collected before the periodontal examination so as to discourage the stimulation of the glands caused by introducing examination equipment into the oral cavity. Each participant was requested to relax, seat comfortably on the dental chair for a few minutes after thoroughly rinsing their mouth with water and spitting into the spittoon so as to remove food debris. They were asked to slightly lean forward and not to swallow or speak. After about 5 minutes, the saliva that had pooled at the floor of the mouth was expectorated through a passive drool into the saliva collection tubes provided to them. About 3ml of saliva was collected from each participant. Absorbent paper towels were provided for any spillage. Saliva collection was done between 8am-11pm before the periodontal examination. This is because minimal or no change occurs in saliva quantity and quality between 8am and just before midday. Saliva was collected during this time to eliminate any errors.[61]

Unstimulated saliva is preferred in biomarker analysis since stimulated saliva has a reduced concentration of them, which may hinder their detection.

Handling of saliva

The saliva bottles containing the samples were tightly capped to avoid any spillage of saliva content. They were wrapped in polythene bags with ice and placed inside a cooler box with ice packs. These were then transported to the KAVI laboratory at KNH for immediate processing. This was done where it was not possible, for various reasons to immediately transport the samples for processing at KAVI, within a maximum period of 2 hours to avoid bacterial contamination and degradation of analytes which occurs within a period of three to six hours

after saliva collection at room temperature. Therefore two hours was thought to be a safe period to avoid degradation of analytes and bacterial contamination.[60]

Disposable face masks and gloves were used during the saliva collection and clinical examination. A set of sterile graduated periodontal probes, mirrors and tweezers were used for each patient. The normal accepted disinfection of the dental unit was done between patients.

Laboratory techniques in the measurement of Salivary IL-1

The levels of interleukin 1 were measured in saliva samples with high sensitive enzyme linked immunosorbent assay (ELISA). The kit used was the Salimetrics Salivary IL-1 ELISA kit (101 Innovation Boulevard, Suite 302, State College, PA 16803). This is a sandwich immunoassay specifically designed and validated for the quantitative measure of salivary IL-1 . This assay kit is designed and optimized for salivary research use in human subjects. (Appendix VII).

Principle of the method of the salimetric salivary IL-1 kit

The laboratory procedure is described in Appendix VI

Periodontal examination

Periodontal health status was measured using Loe and Silness gingival Index of 1963 for assessing gingival inflammation, (Appendix III), plaque score was done using the Quigley- Hein Index Modified by Turesky 1970 (Appendix IV) and the periodontal status was assessed using the British Society of Periodontology, Basic Periodontal Examination (BPE) protocol.[97,98,99] (Appendix V).

For both gingival and plaque scores, the representative indexed teeth for partial mouth scoring were used. The nomenclature was according to the FDI numbering system and the following teeth were examined; upper right central incisor, upper left first premolar, lower left first molar, lower left second incisor, lower right first premolar and upper right first molar.

Procedure

To record inflammation of the gingiva each indexed tooth was examined by running a periodontal probe into the gingival sulcus on both lingual and buccal surfaces. After a span of 30 (thirty) seconds the scores were calculated as follows. A score of 0.1-1 is interpreted as mild inflammation; 1.1-2.0 moderate inflammation and 2.1-3 signifies severe inflammation.

Plaque scores were assessed using disclosing tablets and the assessment done using the Quigley Hein index-modified by Turesky 1970 (Appendix IV) disclosing tablets were used for detection and visual measurement of the plaque on the surface of the tooth both lingually and buccally of the indexed teeth. The readings were recorded in the examination form. In case a tooth was found to be absent, substitution of missing teeth was done by using the tooth immediately next to the space of the missing tooth. When a participant had no tooth in a given sextant, such a sextant was omitted.

British Society of Periodontology, Basic Periodontal Examination (BPE) protocol was used in assessing the periodontal status. This index integrates inflammation, calculus present, overhanging margins and pocket depth to score a given sextant. The teeth present in a particular sextant apart from the third molars are probed with a graduated periodontal probe. The presence or absence of bleeding, calculus, overhanging margins, pocket depth and furcation involvement determine a particular score of a given sextant. A score of zero to four was available for each sextant. All this was done by a single trained investigator (EKM) who was calibrated by a senior periodontologist (TK). Recordings were done by an assistant.

BPE is used for screening. The dentition is divided into 6 sextants and the highest sextant for each score is recorded. These sextans are: upper right (17-14), upper anterior (13-23) upper left (24-27) lower right (47-44) lower anterior (43-33) lower left (34-37). All teeth are examined with the exception of third molars unless the 2nd and 1st molars are missing. For a sextant to qualify for recording it must contain at least 2 teeth. A WHO probe was used, this probe has a ball end 0.5mm in diameter and a black band from 3.5mm to 5. 5mm.The probe was walked around each tooth in each sextant. All sites were examined to ensure that the highest score in each sextant is recorded before moving on to the next sextant. The scoring codes and an example

of a BPE score grid are illustrated in (Appendix V). Partial mouth scoring allows for more accurate reproducibility as well as easy collection of representative data from the patient.

3.8 Research Ethics

The participants of the study were adults aged above 18yrs. A full explanation in English and Kiswahili of the study was given to them before obtaining written consent. All patient information was treated with strict confidentiality. All patients paper records were kept in locked cabinets and electronic records within the data base was password protected. Only data entry personnel, clinicians overseeing the database and researchers involved in this project were allowed access hence confidentiality was maintained. Patient names were not used and instead numbered identifiers were used. Approval for the study was given by KNH/UON Ethics Committee board No KNH/ERC/A/417. The study commenced after the approval was given.

3.9 Benefits

The study has provided new knowledge on levels of salivary interleukin-1 in adult Kenyan individuals without periodontal disease as well as those with varying degrees of periodontal inflammation.

The information may be used to aid in the development of a chair side point of care 1nterleukin-1 biomarker diagnostic kit for assessing presence of inflammatory periodontal disease.

The information obtained in this study will form a basis for further salivary proteomic studies on interleukins.

The participants received a free dental checkup and those with periodontal problems were treated for their various periodontal diseases and conditions and appropriate referrals done for other oral facial conditions requiring management by other specialists.

The results of the study on interleukino1 will be published in the hope that it will provide new information on the levels of this cytokine in the Kenyan population and its association with severity of periodontal diseases.

This study has also contributed towards the award of the principal investigator's (EKM) Masters degree

3.10 Data Analysis and Presentation

The data collected was coded and entered into a computer using Microsoft Excel. Data cleaning was done by checking frequencies. Analysis was done using Microsoft Excel R software and statistical packages for social sciences (SPSS) version 20.0 for Windows. Pearson's correlation was used to analyze the relationships. Data has been presented in form of tables, bar charts and photographs.

3.11 Limitations of the Study

The study relied on the participant's knowledge on their general health condition and this may have missed out on undiagnosed systemic conditions. The study population was also a hospital based sample which tends to be a biased population.

Time limitations as a result of other academic and clinical schedules led to the study taking longer than the required time. In this study to calculate the gingival scores, plaque scores indexed teeth 16, 11, 24, 36, 32, 44 were used. As much as they have been shown to have epidemiological validity in the African population they do not give the complete picture of the entire periodontium.

Though BPE is quick and easy to carry out, it summarizes the periodontium in a readily communicable form and might miss out on isolated areas of the periodontium with severe periodontal disease as it does not record all the six sites per tooth.

3.12 Laboratory Work Challenges

Microbial contamination of reagents as well as poor handling of samples and reagents are among the most common laboratory challenges. This was avoided by using new disposable pipettes for each transfer of reagent to avoid contamination. Proper training and studying of the reference manual before commencement of laboratory work was done to avoid improper handling of the reagents.

3.13 Validity and Reliability

Kappa values for inter examiner difference were calculated and determined during calibration. For intra examiner variability repeat examinations of every 10th participant was done and kappa scores obtained.

The principal investigator (EKM) carried out all the clinical examinations and measurements. Data processing included data cleaning and validation.

A test run was carried out to streamline sample collection, transport, sample storage as well as to optimize the ELISA assay technique. The transport, handling and storage of samples were done in consultation with a trained laboratory technologist who has experience in ELISA analysis techniques. The principal investigator was trained on lab protocols. All samples were analyzed within 3 months of collection and care taken to prevent repeated thawing and freezing of samples. In the ELISA technique, IL-1 standards and samples were run in duplicate to evaluate reproducibility

The Salmetrics Salivary IL 1 Kit, specific for saliva is tested for criteria such as sensitivity, specificity, precision and lot-to-lot consistency. This ensures that the results are accurate and consistent. Finally, the technologist was blinded on the participants' clinical findings and periodontal diagnosis as the clinical examination form was not provided.

Sample collection and handling including transportation to the lab was done according to the recommendations provided by the manufacturer of the Kit.

3.14 Quality Assurance

The equipment that was used is calibrated regularly and the schedules are maintained with the last calibration schedule being May 2018. The equipment are checked on a weekly basis for any malfunction. The use of quality control samples in the lab was done to ensure that the ELISA readings are valid and the results are reliable.

CHAPTER FOUR

RESULTS

Inter-examiner and intra-examiner variability

Cohen's ( ) kappa was run to determine if there was agreement between the student and the supervisor's scores for periodontitis. There was statistically significant perfect agreement between the student and supervisor's scores, = 1.000 (95% CI, 0.431 to 0.786), $p = 0.046$.

Cohen's (k) kappa was also run for intra-examiner variability.

Table 4: Inter-examiner and intra-examiner kappa value scores for calculation of reliability

PT		GI score mean	P.S. score	BPE Score
1.	Student	2.33	3.5	3.16
	Supervisor	2	3.58	3
2.	Student	0.83	1.75	2.5
	Supervisor	1.75	1.45	3.6
3.	Student	1.08	2.5	2
	Supervisor	1.58	2.5	3.5
4.	Student	0.85	1.42	1.17
	Supervisor	1	1.33	1.5
5.	Student	0.75	1.08	2
	Supervisor	0.67	0.92	2
6.	Student	0.67	1.00	2
	Supervisor	1.00	1.33	2
7.	Student	1.45	1.75	2.5
	Supervisor	1.08	2.17	2.33

4.1 Socio-demographic Characteristics

A total of 71 participants were included in the study. Of the 71, 48 (67.6%) were females while 23 (32.4%) were males. The age of the participants ranged between 18 – 81 years with a mean of 42.01 (±16.73 SD). Males were slightly older with a mean of 42.22 (±18.64 SD) compared to females with a mean of 41.92 (±15.95 SD). An independent samples t test showed a non-statistically significant difference in means between the male and female groups (t (69) = 0.070, p = 0.944).

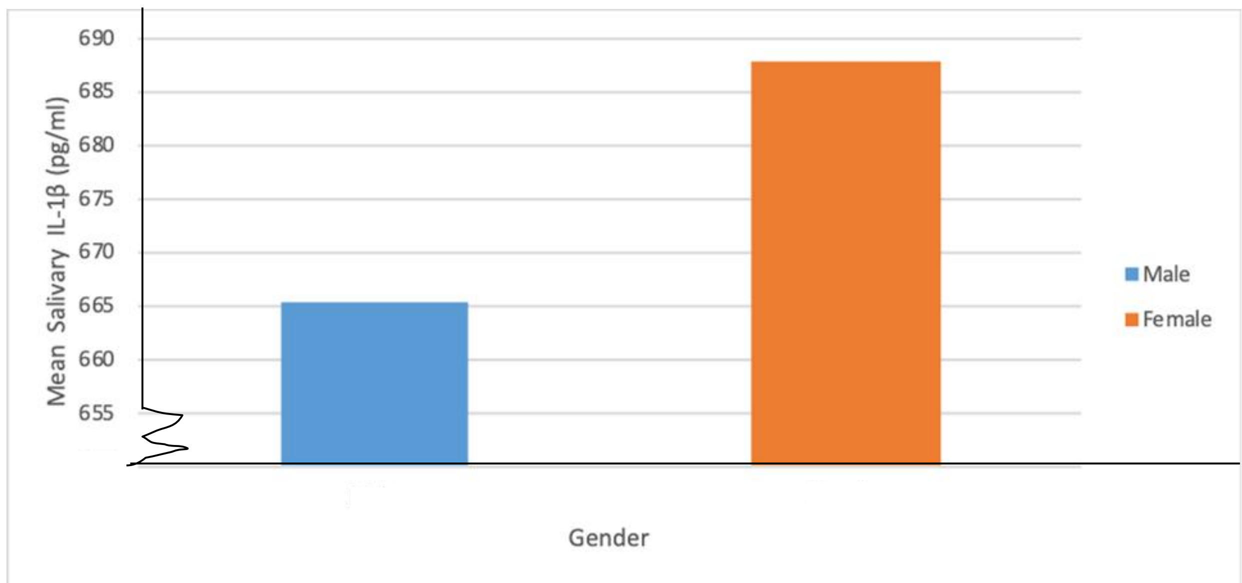

Figure 1: Comparison of mean salivary (IL-1 /ml) by gender

The IL-1 (pg/ml) of the participants ranged between 41.50 pg/ml – 2808.21pg/ml with a mean of 680.47 pg/ml ±677.85 SD. There was a non-statistically significant difference in the variance of IL-1 (pg/ml) levels between gender where females (*M* = 687.76±735.74*SD*) had higher IL-I levels (pg/ml) levels than males (*M* = 665.27±552.41*SD*), *t* (69) = 0.130, *p* = 0.897, two-tailed (Figure 2). The 71 samples for all participants were analysed. Salivary IL-1 was present in all the samples. Participants in the higher age group had slightly higher mean levels of IL-1 . There was a statistically significant association between IL-1 scores and level of education. Correlations between salivary IL-1 levels and other social demographics are summarized in table 5.

Table 5: Socio-demographic characteristics of participants by IL-1 (pg/ml) scores

					95% Confidence Interval of mean				
		n (%)	M	SD	Lower	Upper	Test statistic	Df	p
Age	18 – 30 Years	21 (29.6)	617.18	560.04	362.26	872.11	F = 2.122	2, 68	0.128
	31 – 45 Years	24 (33.8)	511.24	507.95	296.76	725.73			
	> 46 Years	26 (36.6)	887.81	850.11	544.44	1231.17			
Gender	Male	23 (32.4)	665.27	552.41	-367.86	322.88	t = 0.130	69	0.897
	Female	48 (67.6)	687.76	735.74					
Education	Primary	11 (15.5)	1183.54	974.85	528.62	1838.45	F = 3.890*	2, 68	0.025
	Secondary	24 (33.8)	573.85	573.91	331.51	816.20			
	Tertiary	36 (50.7)	597.84	580.19	401.53	794.15			
Brushing	Once daily	31 (43.7)	661.14	601.01	-360.14	291.49	t = 0.210	69	0.834
	Twice daily	40 (56.3)	695.46	739.13					
Dental floss	No	59 (83.1)	693.47	666.64	-354.03	507.81	t = 0.356	69	0.723
	Yes	12 (16.9)	616.58	758.51					
Inter dental brushes	No	69 (97.2)	683.60	685.25	-865.73	1087.46	t = 0.226	69	0.822
	Yes	2 (2.8)	572.74	457.15					
Dental visit	Never	16 (22.5)	529.11	461.65	283.12	775.11	F = 1.170	2, 68	0.317
	< 3 months ago	6 (8.5)	426.85	185.39	232.29	621.41			
	> 6 months ago	49 (69)	760.96	760.00	542.66	979.25			

Independent-Samples t test was used for gender, brushing frequency, dental floss and inter-dental brushing.
Analysis of Variance (ANOVA) was used for age, education and dental visit.
*. The mean difference is significant at the 0.05 level (less than 5% chance of being wrong).

4.2 Oral Hygiene Status

Oral hygiene status of the participants was assessed using plaque scores. The plaque scores of the participants ranged between 0.25 – 4.83 with a mean of 2.10 ± 0.86 SD showing that every participant had some degree of plaque deposits on their teeth surfaces. Majority, 38 (53.5%) had moderate plaque while 20 (28.2) had severe plaque and 13 (18.3%) had mild plaque. Out of the 71 samples analyzed salivary IL-1β was present in all participants 38(53.5%) with mild plaque accumulation had an average of 693.21pg/ml, moderate plaque was in 20 participants (28.2%) who had a mean salivary IL-1β of 640.97pg/ml and 13 (18.3%) with severe plaque deposits having a mean of 747.26pg/ml as shown in Figure 2.

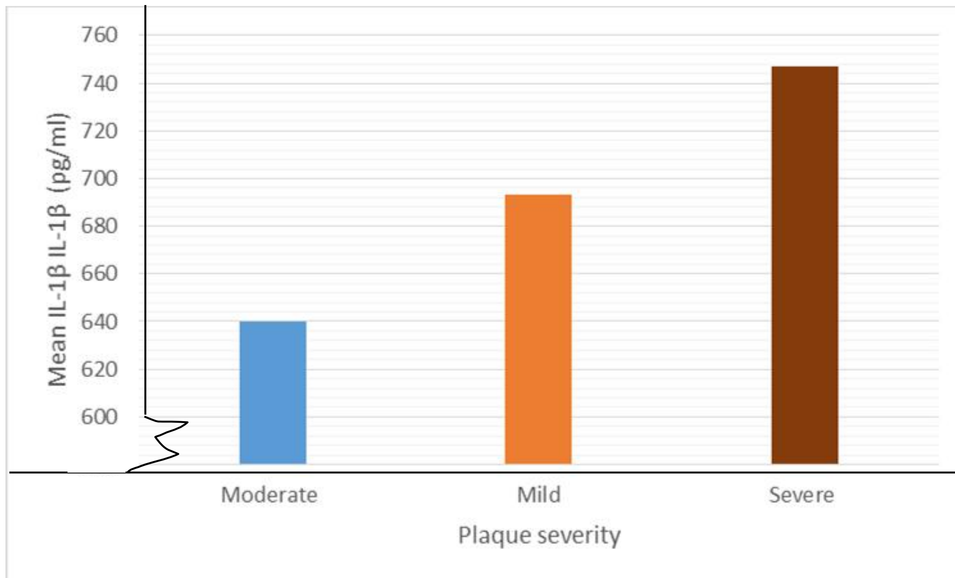

Figure 2: Comparison of mean IL-1 (pg/ml) by plaque severity

No statistically significant association was found between mean plaque scores and any socio demographic variable as summarized in table 6.

Table 6: Socio-demographic characteristics of participants by plaque scores

					95% Confidence Interval of mean				
		n (%)	M	SD	Lower	Upper	Test statistic	Df	P
Age	18 – 30 Years	21 (29.6)	1.87	0.64	1.58	2.16	F = 1.163	2, 68	0.319
	31 – 45 Years	24 (33.8)	2.13	0.78	1.80	2.45			
	> 46 Years	26 (36.6)	2.25	1.06	1.83	2.68			
Gender	Male	23 (32.4)	2.08	0.91	-0.46	0.42	t = 0.095	69	0.925
	Female	48 (67.6)	2.10	0.84					
Education	Primary	11 (15.5)	2.23	1.04	1.54	2.93	F = 1.583	2, 68	0.213
	Secondary	24 (33.8)	2.30	0.93	1.91	2.69			
	Tertiary	36 (50.7)	1.92	0.73	1.67	2.17			
Brushing	Once daily	31 (43.7)	1.95	0.84	-0.67	0.14	t = 1.289	69	0.202
	>= Twice daily	40 (56.3)	2.21	0.87					
Dental floss	No	59 (83.1)	2.08	0.78	-0.65	0.45	t = 0.366	69	0.716
	Yes	12 (16.9)	2.18	1.20					
Inter dental brushes	No	69 (97.2)	2.09	0.85	-1.52	0.95	t = 0.461	69	0.646
	Yes	2 (2.8)	2.38	1.47					
Dental visit	Never	16 (22.5)	1.81	0.74	1.42	2.21	F = 1.377	2, 68	0.259
	< 3 months ago	6 (8.5)	1.96	0.81	1.11	2.81			
	> 6 months ago	49 (69)	2.21	0.89	1.95	2.46			

Independent-Samples t test was used for gender, brushing frequency, dental floss and inter-dental brushing.
Analysis of Variance (ANOVA) was used for age, education and dental visit

All participants had plaque. The mean plaque score was 2.09 (±0.88SD). A greater number of participants had moderate plaque scores (53.5%). Gender, Education level and oral hygiene practices did not seem to influence the distribution of plaque. There was no statistically significant difference in severity of plaque distribution and other socio-demographic variables. Table 7 is a summary of the relationship of severity of plaque score with other socio-demographic variables.

Table 7: Sociodemographic characteristics by severity of Plaque score among participants

			Plaque score				
			<1.5	1.5 – 2.5	>2.5		
		n (%)	n (%)	n (%)	n (%)	Test statistic	*P*
Age	18 – 30 Years	21 (29.6)	5 (38.5)	12 (31.6)	4 (20.0)	Fisher's = 1.921	0.781
	31 – 45 Years	24 (33.8)	3 (23.1)	13 (34.2)	8 (40.0)		
	> 46 Years	26 (36.6)	5 (38.5)	13 (34.2)	8 (40.0)		
Gender	Male	23 (32.4)	3 (23.1)	15 (39.5)	5 (25.0)	Fisher's = 1.734	0.460
	Female	48 (67.6)	10 (76.9)	23 (60.5)	15 (75.0)		
Education	Primary	11 (15.5)	2 (15.4)	4 (10.5)	5 (25.0)	Fisher's = 5.333	0.246
	Secondary	24 (33.8)	2 (15.4)	14 (36.8)	8 (40.0)		
	Tertiary	36 (50.7)	9 (69.2)	20 (52.6)	7 (35.0)		
Brushing	Once daily	31 (43.7)	6 (46.2)	19 (50.0)	6 (30.0)	^{2} = 2.171	0.338
	Twice daily	40 (56.3)	7 (53.8)	19 (50.0)	14 (70.0)		
Dental floss	No	59 (83.1)	10 (76.9)	33 (86.8)	16 (80.0)	Fisher's = 1.140	0.585
	Yes	12 (16.9)	3 (23.1)	5 (13.2)	4 (20.0)		
Inter dental brushes	No	69 (97.2)	12 (92.3)	38 (100)	19 (95.0)	Fisher's = 3.100	0.212
	Yes	2 (2.8)	1 (7.7)	0	1 (5.0)		
Dental visit	Never	16 (22.5)	5 (38.5)	8 (21.1)	3 (15.0)	Fisher's = 2.800	0.598
	< 3 months ago	6 (8.5)	1 (7.7)	3 (7.9)	2 (10.0)		
	> 6 months ago	49 (69)	7 (53.8)	27 (71.1)	15 (75.0)		

Fisher's Exact test was used for age, gender, education, dental floss, inter-dental brushing and dental visit. Chi-Square test of association was used for brushing frequency.

A linear regression curve estimation model elicited a statistically significant association between basic periodontal examination scores and plaque scores as the predictor variable (= 0.569, $F(1,69) = 19.455$, $R^2 = 0.220$, $p < 0.001$).

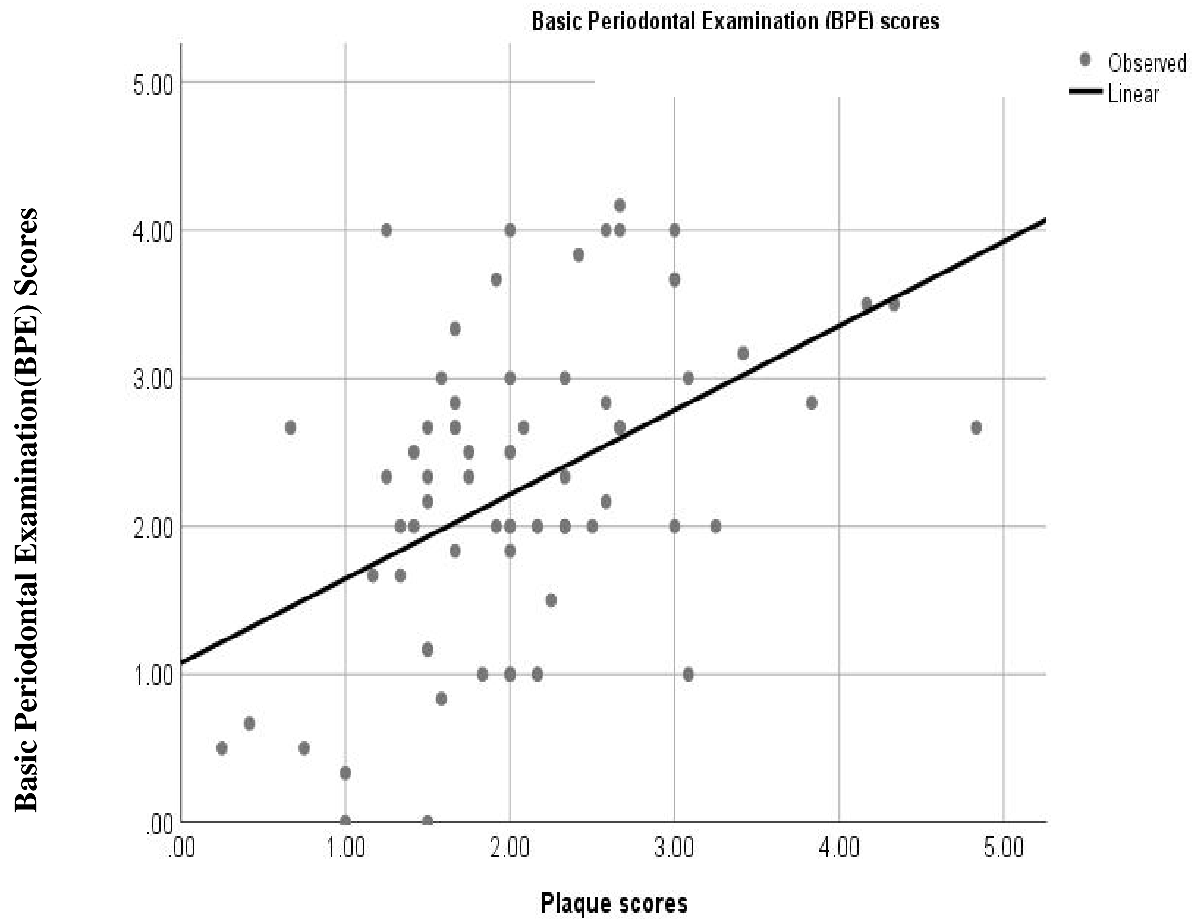

Figure 3: Linear Regression Curve 1 showing relation of BPE and Plaque score

A linear regression curve estimation model showed a non-statistically significant association between IL-1 (pg/ml) scores and plaque scores as the predictor variable (= 34.729, $F(1,69) = 0.134$, $R^2 = 0.002$, $p = 0.716$).

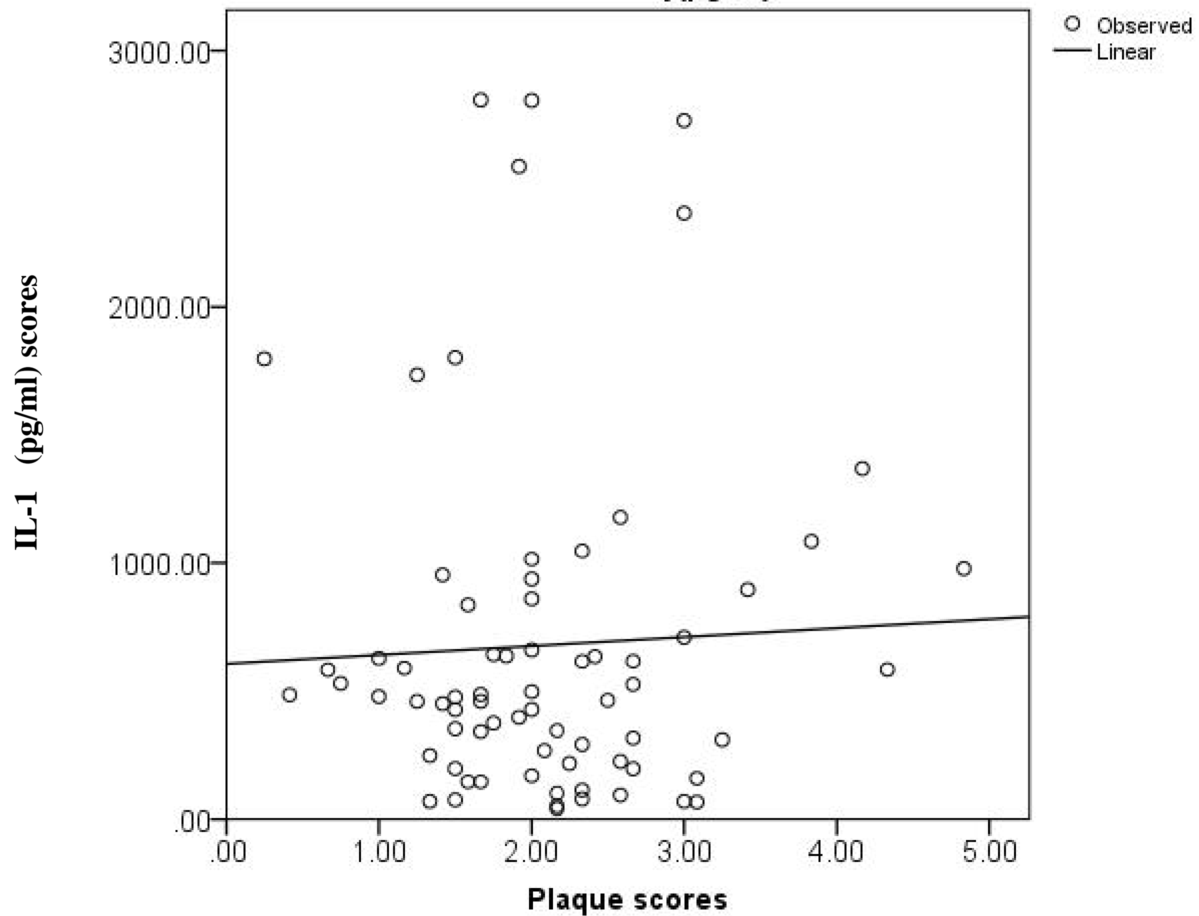

Figure 4: Linear Regression Curve 2 showing association between IL-1β and Plaque score

4.3 Gingival Inflammation (Gingivitis)

The degree of gingival inflammation was assessed using the gingival index. The gingival scores of the participants ranged between 0.17 – 2.50 with a mean of 1.29± 0.54 SD showing that every participant had some degree of gingivitis. Majority 46 (64.8%) had mild gingival inflammation while 24 (33.8%) had moderate and 1 (1.4%) had severe gingival inflammation. Among those with mild gingival inflammation they had a mean IL-1 level of 550.09 pg/ml those with moderate had 845.13pg/ml and the severe had 2726.29pg/ml as shown in figure 5 below.

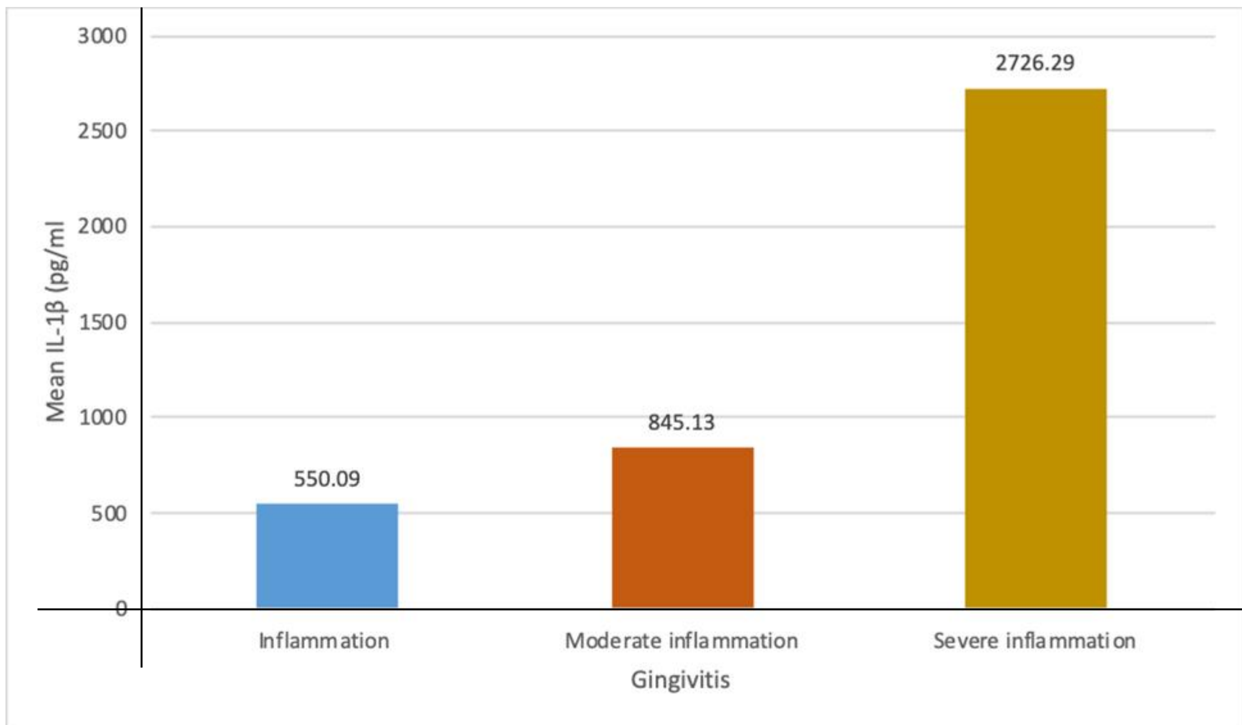

Figure 5: Comparison of mean salivary IL-1 (pg/ml) by gingivitis

All participants had gingival inflammation. The mean gingival score was 1.3(±0,52 SD) with a range of 0.17-2.5. Slightly higher level of gingival inflammation were noted in older age groups, level of education, brushing habits, the number of dental visits had an influence on the gingival scores but they were not statistically significant. Table 8 is a summary of the social demographics characteristics of the participants.

Table 8: Socio-demographic characteristics of participants by gingival inflammation

					95% Confidence Interval of mean				
		n (%)	M	SD	Lower	Upper	Test statistic	Df	P
Age	18 – 30 Years	21 (29.6)	1.12	0.40	0.94	1.30	F = 1.477	2, 68	0.236
	31 – 45 Years	24 (33.8)	1.35	0.50	1.14	1.56			
	> 46 Years	26 (36.6)	1.37	0.66	1.10	1.64			
Gender	Male	23 (32.4)	1.29	0.60	-0.27	0.28	t = 0.013	69	0.990
	Female	48 (67.6)	1.29	0.52					
Education	Primary	11 (15.5)	1.40	0.78	0.87	1.93	F = 0.482	2, 68	0.619
	Secondary	24 (33.8)	1.33	0.45	1.14	1.52			
	Tertiary	36 (50.7)	1.23	0.52	1.06	1.41			
Brushing	Once daily	31 (43.7)	1.26	0.57	-0.32	0.20	t = 0.465	69	0.644
	Twice daily	40 (56.3)	1.32	0.53					
Dental floss	No	59 (83.1)	1.30	0.54	-0.34	0.35	t = 0.004	69	0.997
	Yes	12 (16.9)	1.30	0.60					
Inter dental brushes	No	69 (97.2)	1.29	0.51	-0.74	0.83	t = 0.111	69	0.912
	Yes	2 (2.8)	1.25	1.53					
Dental visit	Never	16 (22.5)	1.14	0.60	0.82	1.46	F = 2.056	2, 68	0.136
	< 3 months ago	6 (8.5)	1.01	0.41	0.58	1.45			
	> 6 months ago	49 (69)	1.38	0.52	1.23	1.53			

Independent-Samples t test was used for gender, brushing frequency, dental floss and inter-dental brushing.
Analysis of Variance (ANOVA) was used for age, education and dental visit.

No statistically significant finding was found between gingival index and social demographic variables as summarized in table 9.

Table 9: Sociodemographic characteristics by Gingival index among participants

			Gingival index				
			1	2	3		
		n (%)	n (%)	n (%)	n (%)	Test statistic	*P*
Age	18 – 30 Years	21 (29.6)	4 (33.3)	17 (33.3)	0	Fisher's = 4.601	0.330
	31 – 45 Years	24 (33.8)	4 (33.3)	17 (33.3)	3 (37.5)		
	> 46 Years	26 (36.6)	4 (33.3)	17 (33.3)	5 (62.5)		
Gender	Male	23 (32.4)	5 (41.7)	15 (29.4)	3 (37.5)	Fisher's = 0.989	0.654
	Female	48 (67.6)	7 (58.3)	36 (70.6)	5 (62.5)		
Education	Primary	11 (15.5)	3 (25.0)	5 (9.8)	3 (37.5)	Fisher's = 6.169	0.157
	Secondary	24 (33.8)	2 (16.7)	20 (39.2)	2 (25.0)		
	Tertiary	36 (50.7)	7 (58.3)	26 (51.0)	3 (37.5)		
Brushing	Once daily	31 (43.7)	5 (41.7)	23 (45.1)	3 (37.5)	Fisher's = 0.247	0.999
	Twice daily	40 (56.3)	7 (58.3)	28 (54.9)	5 (62.5)		
Dental floss	No	59 (83.1)	10 (83.3)	43 (84.3)	6 (75.0)	Fisher's = 0.790	0.779
	Yes	12 (16.9)	2 (16.7)	8 (15.7)	2 (25.0)		
Inter dental brushes	No	69 (97.2)	11 (91.7)	51 (100)	7 (87.5)	Fisher's = 5.794	0.076
	Yes	2 (2.8)	1 (8.3)	0	1 (12.5)		
Dental visit	Never	16 (22.5)	5 (41.7)	10 (19.6)	1 (12.5)	Fisher's = 3.619	0.429
	< 3 months ago	6 (8.5)	1 (8.3)	5 (9.8)	0		
	> 6 months ago	49 (69)	6 (50.0)	36 (70.6)	7 (87.5)		

Fisher's exact test was used for age, gender, education, dental floss, inter-dental brushing, dental visit and brushing frequency.

A linear regression curve estimation model elicited a statistically significant association between gingival scores and plaque scores as the predictor variable (= 0.432, $F(1, 69) = 60.594$, $R^2 = 0.468$, $p < 0.001$).

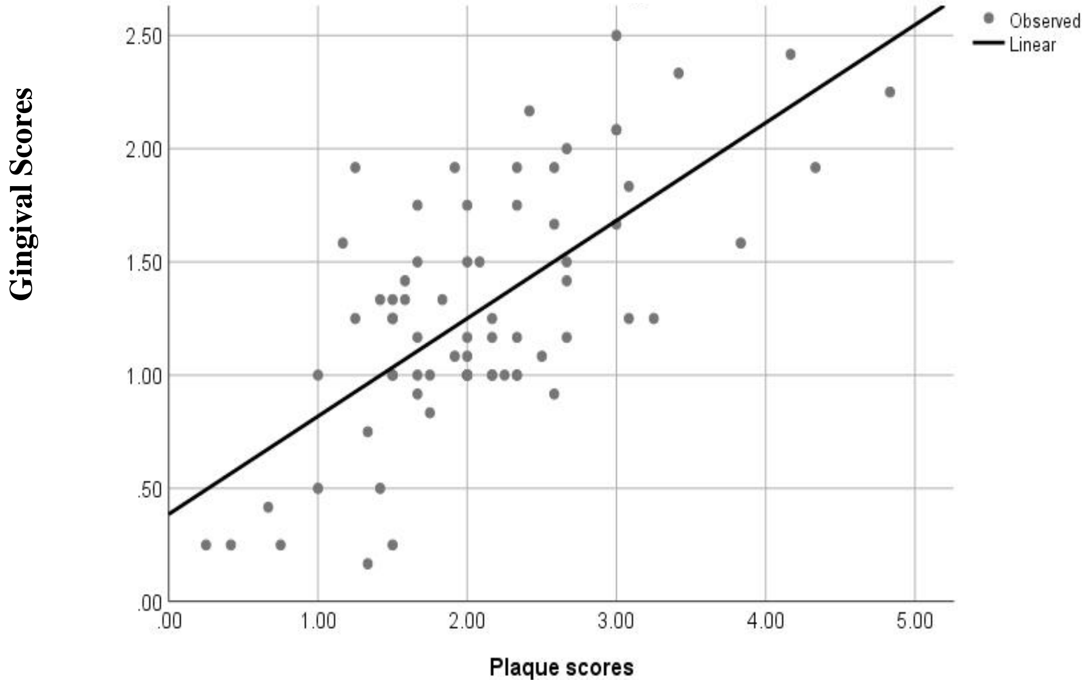

Figure 6: Linear Regression Curve 3 showing association between gingival score and plaque score

A linear regression curve estimation model elicited a statistically significant association between basic periodontal examination scores and gingival scores as the predictor variable (= 1.216, F(1,69) = 46.136, R^2 = 0.401, $p < 0.001$).

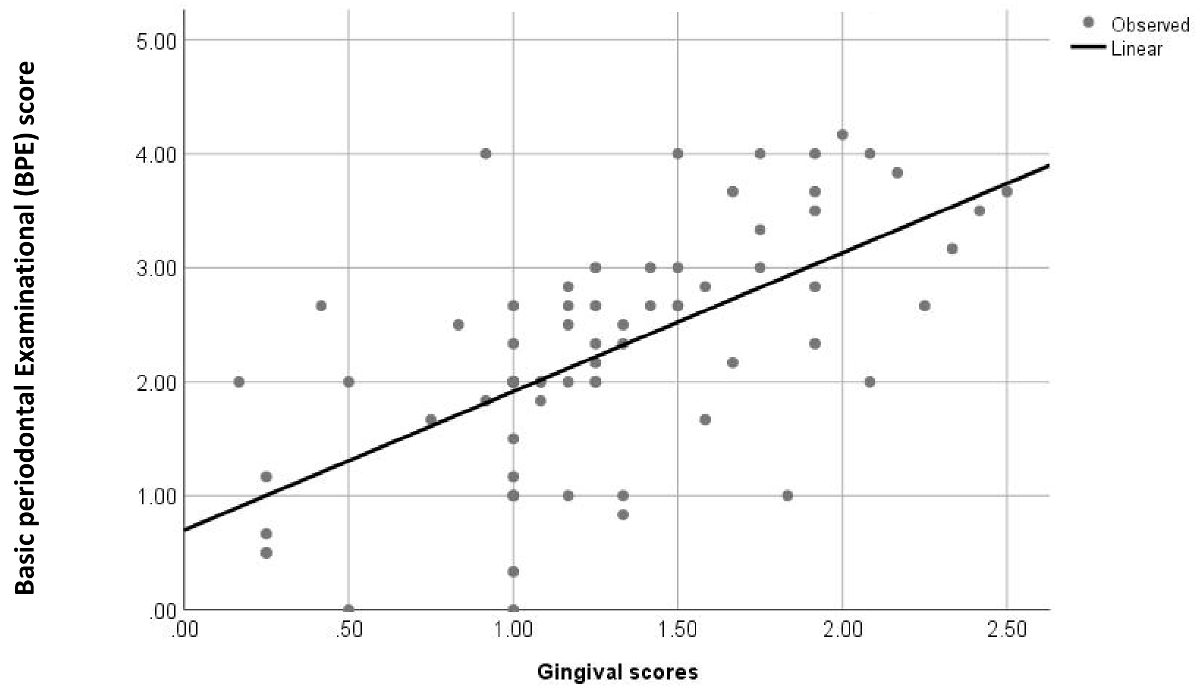

Figure 7: Linear Regression Curve 4 showing association between BPE) and gingival scores

A linear regression curve estimation model showed a non-statistically significant association between and gingival scores as the predictor variable (= 265.589, F(1,69) = 3.266, R^2 = 0.045, p = 0.075).

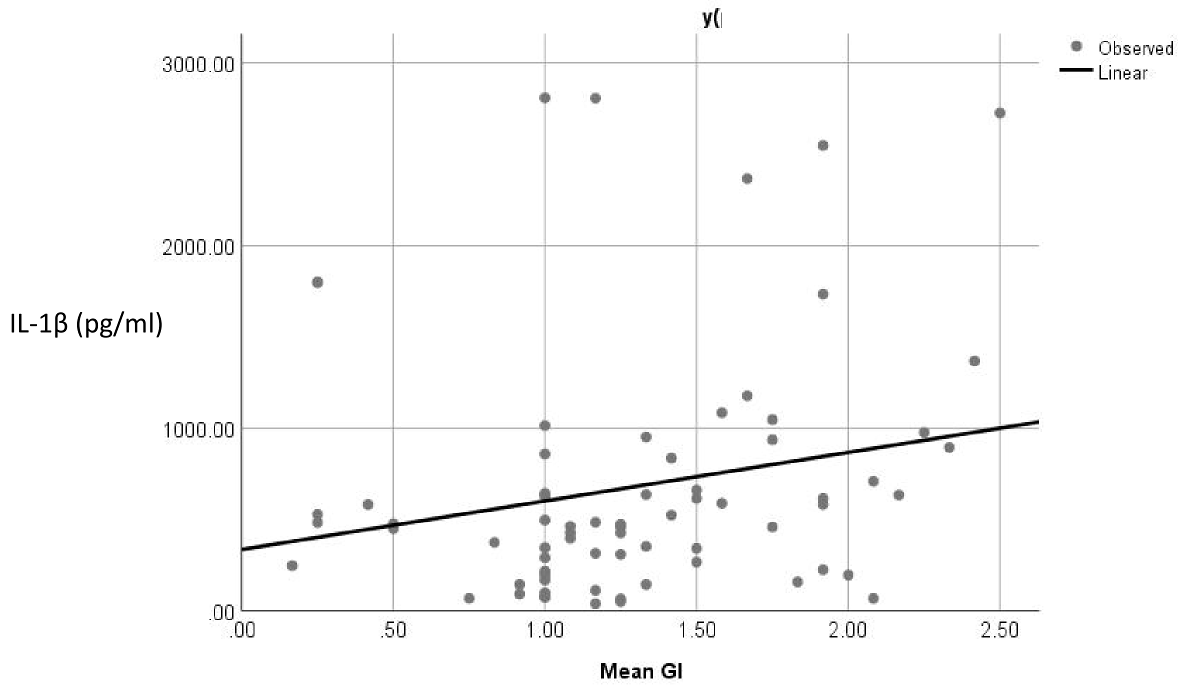

Figure 8: Linear Regression Curve 5 showing association between IL-1β and gingival scores

4.4 Periodontitis

The Basic Periodontal Examination scores of the participants ranged between 0 – 4. Whereas 2 (2.8%) were healthy, majority 37 (52.1%) had gingivitis while 20 (28.2%) had mild and 12 (16.9%) had severe periodontitis. Of the 71 samples collected 39(54.9%) had gingivitis with a mean IL-1 of 369.33pg/ml.20 participants (28.2%) with mild to moderate periodontitis had a mean IL-1 OF 807.81 pg/ml. Participants with severe periodontitis were 12 representing (16.9%) had the highest levels of IL-1 1,210.12pg/ml as shown in figure 9.

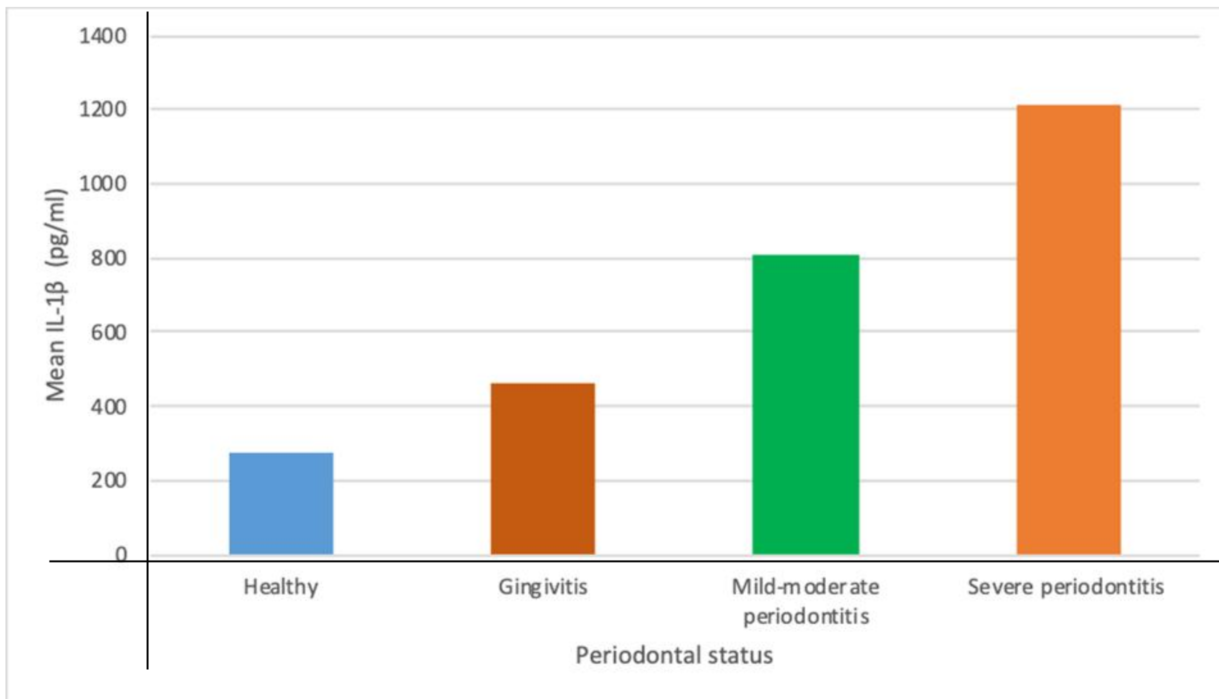

Figure 9: Distribution of mean salivary IL-1 (pg/ml) by periodontal status

The BPE scores levels ranged from 1-4. With a mean of 2.29(±1.05SD). Higher levels of BPE scores were noted in the older age group, those with a primary level of education. Oral hygiene practices also had an implication on the mean BPE scores. There was a statistically significant association between BPE scores and dental visits (f=3.291; p=0.043).There was no statistically significant association between mean BPE scores and other social demographic variables as summarized in table 10.

Table 10: Socio-demographic characteristics of participants by BPE scores

					95% Confidence Interval of mean				
		n (%)	M	SD	Lower	Upper	Test statistic	Df	p
Age	18 – 30 Years	21 (29.6)	1.94	0.94	1.51	2.37	F = 1.507	2, 68	0.229
	31 – 45 Years	24 (33.8)	2.37	0.98	1.95	2.78			
	> 46 Years	26 (36.6)	2.44	1.15	1.98	2.91			
Gender	Male	23 (32.4)	2.33	1.05	-0.44	0.62	t = 0.352	69	0.726
	Female	48 (67.6)	2.24	1.05					
Education	Primary	11 (15.5)	2.79	1.36	1.87	3.70	F = 2.050	2, 68	0.137
	Secondary	24 (33.8)	2.32	0.90	1.94	2.70			
	Tertiary	36 (50.7)	2.08	0.99	1.74	2.41			
Brushing	Once daily	31 (43.7)	2.05	1.04	-0.88	0.11	t = 1.554	69	0.125
	Twice daily	40 (56.3)	2.44	1.03					
Dental floss	No	59 (83.1)	2.34	1.03	-0.25	1.06	t = 1.242	69	0.218
	Yes	12 (16.9)	1.93	1.07					
Inter dental brushes	No	69 (97.2)	2.26	1.05	-1.82	1.18	t = 0.429	69	0.669
	Yes	2 (2.8)	2.58	0.82					
Dental visit	Never	16 (22.5)	1.94	1.15	1.33	2.55	F = 3.291*	2, 68	0.043
	< 3 months ago	6 (8.5)	1.56	0.78	0.74	2.37			
	> 6 months ago	49 (69)	2.47	0.98	2.18	2.75			

Independent-Samples t test was used for gender, brushing frequency, dental floss and inter-dental brushing.

Analysis of Variance (ANOVA) was used for age, education and dental visit.

*. The mean difference is significant at the 0.05 level (less than 5% chance of being wrong).

Of the 71 participants 32(45.1%) Had periodontitis those with severe periodontitis were 12(16.9%) and the ones with mild to moderate periodontitis were 20(28.2%). A statistically significant association was found between periodontitis and education level (f=11.14 p=0.05).Association between periodontitis and other social demographic variables are shown in table 11.

Table 11: Severity of periodontitis with demographic characteristics

			Periodontitis					
			1	2	3	4		
		n (%)	n (%)	n (%)	n (%)	n (%)	Test statistic	*p*
Age	18 – 30 Years	21 (29.6)	1 (50.0)	14 (37.8)	5 (25.0)	1 (8.3)	Fisher's = 6.644	0.308
	31 – 45 Years	24 (33.8)	0	13 (35.1)	7 (35.0)	4 (33.3)		
	> 46 Years	26 (36.6)	1 (50.0)	10 (27.0)	8 (40.0)	7 (58.3)		
Gender	Male	23 (32.4)	1 (50.0)	10 (27.0)	8 (40.0)	4 (33.3)	Fisher's = 1.742	0.616
	Female	48 (67.6)	1 (50.0)	27 (73.0)	12 (60.0)	8 (63.7)		
Education	Primary	11 (15.5)	0	4 (10.8)	1 (5.0)	6 (50.0)	Fisher's =11.141*	0.049
	Secondary	24 (33.8)	1 (50.0)	12 (32.4)	8 (40.0)	3 (25.0)		
	Tertiary	36 (50.7)	1 (50.0)	21 (56.8)	11 (55.0)	3 (25.0)		
Brushing	Once daily	31 (43.7)	2 (100)	18 (48.6)	8 (40.0)	3 (25.0)	Fisher's = 4.284	0.191
	>= Twice daily	40 (56.3)	0	19 (51.4)	12 (60.0)	9 (75.0)		
Dental floss	No	59 (83.1)	2 (100)	29 (78.4)	17 (85.0)	11 (91.7)	Fisher's = 1.229	0.789
	Yes	12 (16.9)	0	8 (21.6)	3 (15.0)	1 (8.3)		
Inter dental brushes	No	69 (97.2)	2 (100)	36 (97.3)	19 (95.0)	12 (100)	Fisher's = 2.181	0.999
	Yes	2 (2.8)	0	1 (2.7)	1 (5.0)	0		
Dental visit	Never	16 (22.5)	1 (50.0)	11 (29.7)	2 (10.0)	2 (16.7)	Fisher's = 7.332	0.245
	< 3 months ago	6 (8.5)	0	5 (13.5)	1 (5.0)	0		
	> 6 months ago	49 (69)	1 (50.0)	21 (56.8)	17 (85.0)	10 (83.3)		

Fisher's Exact test was used for age, gender, education, dental floss, inter-dental brushing, dental visit and brushing frequency.
* The mean difference is significant at the 0.05 level (less than 5% chance of being wrong).

A linear regression curve estimation model elicited a statistically significant association between IL-1 (pg/ml)scores and basic periodontal examination scores as the predictor variable (= 190.918, F(1,69) = 6.510, R^2 = 0.086, p = 0.013).

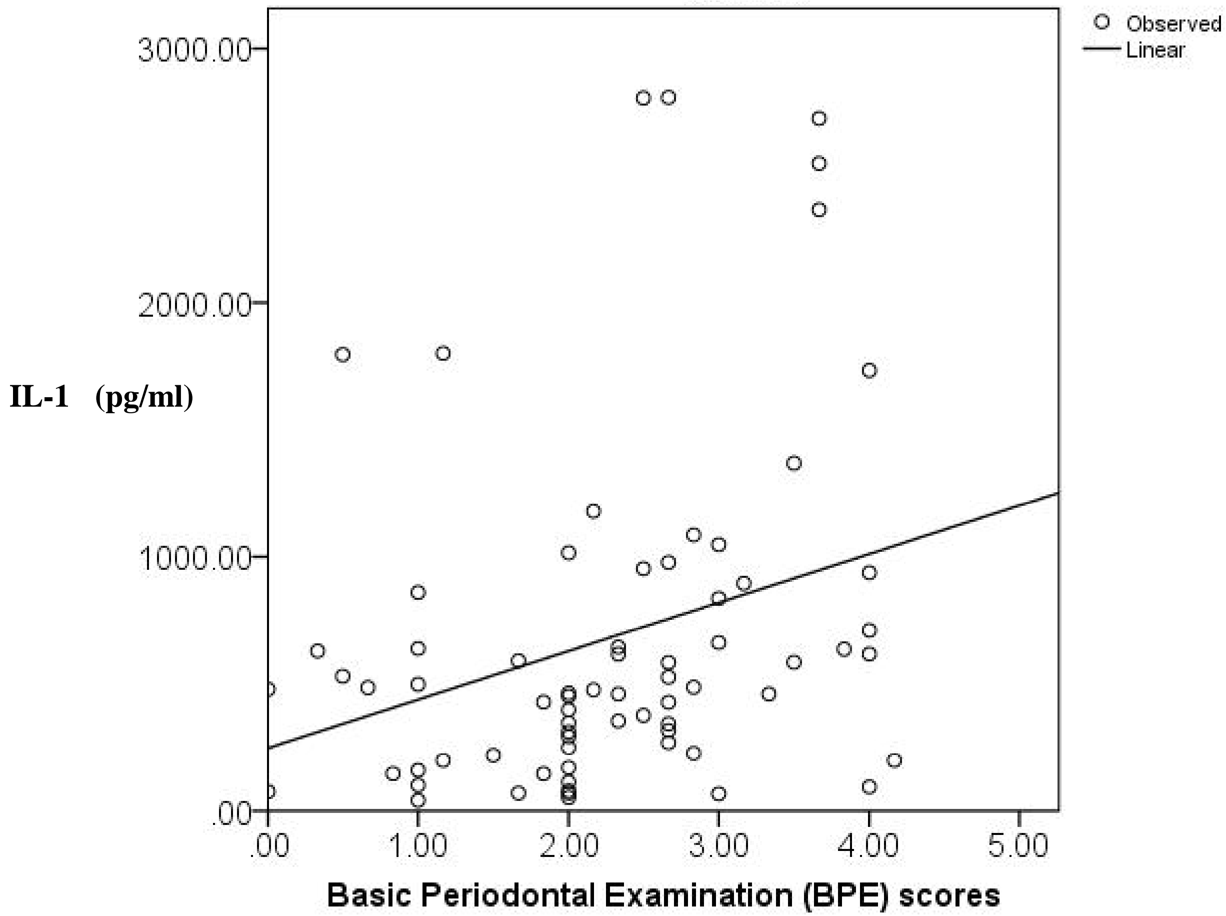

Figure 10: Linear Regression Curve 6 showing association between IL-1β and BPE

CHAPTER FIVE

DISCUSSION

An alpha level of 0.05 was used for all statistical tests inter-examiner variability using Cohens kappa was run to determine whether there was an agreement between the supervisor and the investigator on the scores for periodontitis. There was statistically significant agreement between the supervisor scores and the investigators k=1.000 (95% Cl, 0.431 to 0.786) p=0.046. The Cohens kappa score of 1.00 was considered good for the study. Intra- examiner variability of 7 (seven) repeat samples showed 1.00 level of agreement using Cohen (k) Kappa analysis which is considered good for the study.

5.1 Socio-demographic Characteristics

The participants were aged between18-81yrs this implies that different age groups of patients seek dental treatment at the University of Nairobi Dental hospital which was the study area. There was also a higher ratio of adult females to males seeking dental treatment indicating females have better heath seeking behavior than the males. This is in agreement with a study by Thompson 2016[100] which showed gender differences in health seeking behavior, with women reporting they visited primary care providers to a greater extent than men did. Thirty seven (50.7%) of participants had a tertiary education this could be explained by the fact that the study was carried out in an Urban setting where the population tends to be better educated. All participants reported brushing their teeth at least once a day, however few use dental floss 12(16.9%) and 2(2.8%) used interdental brushes. This points to emphasizing the need to educate patients on other adjunctive oral hygiene measures that help in improving plaque control.

Only 6 (8.5%) of participants had gone to seek the services of a dental health care provider in the previous 3 months before the study. This could best be explained by a study by Devaraj that looked at the reasons of use and non-use of dental service and concluded that majority of the people were “problem oriented visitors” rather than “prevention oriented visitors”. The main reason of visiting being pain[101].

5.2 Oral Hygiene Status

In current study, age, education level, oral hygiene habits and the number of dental visit seemed to have an influence on the plaque scores of the participants. Higher plaque scores were observed in those with advanced age (p=0.319, f=1.163), this could possibly be due to the reduced manual dexterity in controlling dental plaque with advancing age and also due to increased tooth surfaces as a result of age changes in the periodontium leading to gum recession. The higher the education level the lower the plaque sores this may be attributed to knowledge on good oral hygiene practices among the better educated.

The association between the mean gingival scores and mean plaque score was statistically significant (= 0.432, F (1,69) = 60.594, R^2 = 0.468, p < 0.001). Several studies have reported that plaque is a risk factor for gingivitis. This study is in agreement with studies by Kinane and Loe et al that show that there is an association between plaque and gingival inflammation[19, 21].

5.3 Oral Hygiene Practices

Routine tooth brushing is the principle method by which individuals remove plaque and control plaque related diseases. In this study, all participants brushed at least once daily with those brushing more than twice daily (56.7%) being associated with higher plaque scores. Other adjuncts to tooth brushing which include use of dental floss was reported in (16.8%) of participants and use of interdental brushes in (2%). In this study, it was observed that the frequency of brushing did not correlate with decrease in the plaque score as expected. Participants who brushed twice or more had higher mean plaque scores of 2.21(±0.87SD) as compared to those who brushed once with a mean of 1.95(±0.84SD). Studies including that by Harvey have shown that brushing more frequently has a greater effect in retarding plaque accumulation[102]. However this study found the opposite which could be attributed to participants not understanding the questions and possibly giving incorrect answers during data collection. Participants who used interdental brushes and dental floss also had higher plaque scores compared with those who did not. The findings in this study agrees with another that looked at the efficacy of inter dental mechanical plaque control that failed to demonstrate that flossing and interdental brushing was effective in plaque removal.[103]

5.4 Gingival Inflammation (Gingivitis)

The degree of gingival inflammation was assessed using the Loe & Silness gingival index. The gingival scores of the participants ranged between 0.17 – 2.50 with a mean of 1.29± 0.54 SD showing that every participant had some degree of gingivitis. Majority 46 (64.8%) had mild gingival inflammation while 24 (33.8%) had moderate and 1 (1.4%) had severe gingival inflammation. Those with mild gingivitis had a mean of 550.09pg/ml of IL-1 , followed by those with moderate with a mean of 845.13 pg/ml and lastly those with severe had a mean of 2726.69 pg/ml. There was a positive co-relation between increase in age and increase in gingival scores. (p=0.236) however the relationship was not statistically significant. Increasing age is a known risk factor of periodontal diseases and advance age could have led to decrease in manual dexterity leading to poor plaque control. Both male and female participants had a mean gingival score of 1.29.The level of education of the participants had a co-relation with the gingival scores, with increase in education level related to decrease in the gingival scores however the difference was not statistically significant (p=0.619). A study by Gomes showed that education level had a direct influence on patients' knowledge and behavior regarding the causes of oral diseases and hence need for continuous education on dental diseases preventive measures[104]. No statistically significant association was found between gingival scores and other social demographics. A statistically significant association was found between gingival scores and plaque scores as the predictor variable (= 0.432, F (1, 69) = 60.594, R^2 = 0.468, p < 0.001). This is in agreement with the classical study by Loe in 1986 that pointed to dental plaque as the main cause of gingivitis[21].

5.5 Periodontitis

The clinical parameters associated with periodontitis include increase in probing depth, clinical attachment loss. These parameters were evaluated through a full mouth examination and graded using the BPE index. Approximately 28.2% of participants had mild to moderate periodontitis with another 16.9% having severe periodontitis. This could be explained by the fact that the participants were drawn from a population of patients seeking treatment in a teaching dental hospital. There was a statistically significant association between BPE scores and the number of dental visits (f=3.291, p = 0.043). The highest mean BPE score (2.47±0.98SD) was found in the participants who had not visited the dentist for the past 6 months. This could be explained by the

fact that patients who don't receive routine periodontal therapy and are non-compliant tend to have poor periodontal health and increased tooth loss. A study by Thomas [looking] at tooth loss in maintenance patients found that the patient who presented for maintenance more often was less likely to loose a tooth[105]. Another study by Kumar and another by Poudyal et al showed that low utilization of dental services in selected populations lead to lack of dental education hence increased dental plaque, gingivitis and periodontitis and severity of diseases as depicted by the high BPE scores in those with poor utilization of dental services (>6 months)[106,107].

There was a positive correlation observed between increasing age and BPE scores. The possible explanation being prolonged exposure to risk factors and difficulty in controlling plaque with advancing age. Participants with a higher level of education were also found to have lower mean BPE scores 2.08±0.99SD. There was a statistically significant association between periodontitis and education level (f=11.141) this could be attributed to the participants being enlightened about proper oral hygiene practices. The findings of this study agree with those of Oliver and Brown et al that showed more periodontal diseases in persons with less education. Hence the importance of emphasizing health education in teaching institutions to help in reducing the prevalence of periodontal diseases[108].

In this study there was a statistically significant association between BPE scores and gingival scores confirming findings in the literature. The pro inflammatory cytokine IL-1 level is influenced by the degree of periodontal tissue inflammation and destruction as shown in this study. This is also true from several studies including a study by Gamonal et al that looked at the levels of IL-1 in crevicular fluid in adult periodontitis patients and found the cytokine increased as the diseases got worse[109]. Though this study used saliva samples. It is true to say that given that inflammatory mediators are continuously washed into saliva by gingival crevicular fluid, whole saliva is an easy alternative to GCF in determining analytes present.

5.6 Occurrence of Salivary IL-1

IL-1 is a cytokine secreted by many cells lines in response to inflammatory processes. Its levels are strongly influenced by the level of periodontal tissue inflammation and destruction. In relation to this study the mean IL1 levels among the participants was (676.51±644.07SD).

Increasing age was associated with increase in levels of IL-1 levels a possible explanation could be that the continued exposure to risk factors such as plaque due to advanced age leads to increase levels of the cytokine. However there was a statistically significant association between IL-1 and education levels (f=2.122, p=0.128). Participants with higher levels of education were found to have statistically significant lower IL-1 levels mean. This is likely due to participants being better enlightened on oral hygiene practices. Better oral hygiene practices is associated with lower plaque scores. Lower plaque scores co-relate positively with decrease with gingival inflammation. Gingivitis which is an inflammatory condition increases levels of salivary IL-1 . Increased salivary IL-1 is associated with severity of periodontal diseases. A study by Paulauder showed that education levels influence the oral conditions and should be considered when assessing risk and during treatment planning hence the importance of oral health education during preliminary phases of treatment to help reduce the prevalence of periodontal diseases[110]. No statistical association was found between IL-1 and the number of dental visits, tooth brushing habits and the other oral hygiene practices. Of note is an observation in this study where the quantity of plaque did not co- relate with the levels of interleukin 1 .Participants with mild plaque deposits having a mean IL-1β of 693.21pg/ml, which was higher than those with moderate plaque deposits who had a mean IL-1β levels of 640.97pg/ml. Those with severe plaque deposits had a mean of 747.26pg/ml a plausible explanation could be that IL-1 implicated in the pathogenesis of periodontal disease is influenced by the degree of periodontal tissue inflammation and destruction and not necessarily by the amount of plaque present

In addition to that is the fact that production of IL-Iβ in the absence of diseases has also been demonstrated in cells such as keratinocytes, mucosal epithelial cells, eccrine sweat glands and many other cells. Hence the reason why minimal amount of IL-1 was present in all the participants. It is also known that detection of cytokines and cytokine-containing cells in clinically healthy subjects is due to the small number of mononuclear cells and PMNs that are usually present in clinically healthy tissue.

In this study, higher amounts were detected in subjects with severe diseases and minimal amounts in patients with no disease. However, the mean levels of the IL-1 were slightly higher as compared to other similar studies by Tobon and another by Miller et al . This could be

explained by factors such as type of saliva collected, pretreatment procedures, storage conditions, use of preservatives, sensitivity and specificity of the immune assay used, as well as the study population[111,46].

5.7 Association of IL-1 and Periodontitis

Salivary IL-1β increased with increase in the severity of chronic periodontitis. Varied levels of IL-1 were found within the various groups of periodontitis. The means were 277.1pg/ml,461.67pg/ml,807.81pg/ml and 1210.12 pg/ml in healthy, gingivitis, mild moderate periodontitis and those with severe periodontitis respectively. Other similar studies that were done before found slightly different values an example is the study by Tobon and colleagues that found the mean salivary IL-1 in chronic periodontitis patients equals 543.78pg/ml and Aggressive periodontitis equals 510.65pg/ml with healthy controls mean to be 295.75pg/ml[111]. The figures in this study show slightly higher amounts of the cytokine that could have been attributed to several factors, which include salivary flow, type of saliva collected in this case being the unstimulated saliva, pre-treatment procedures e.g. filtration and centrifugation as well as storage procedures might have affected the quantity of different analytes in saliva. Hence the IL-1 salivary concentration in this study might have been greater than that detected in other studies. Hence the reason why the overall salivary IL-1 found in this study were higher than those described by Miller and Tanaka[46,112]. Finally the genetic as well as racial differences in the Kenyan population may be responsible for the varied high mean concentrations of salivary IL-1 compared with other similar studies done before. This could be supported by experimental studies by Korman and colleagues & Engebretson et al that showed that although IL-1 can be an indicator of gingival inflammation and susceptibility of periodontal diseases other factors that can increase the expression of these cytokine include environmental, racial, ethnic, socioeconomic as well as genetic polymorphism and the carriage of the periodontitis associated genotype (PAG) [82,86].

The studies by Korman and Engebretson confirms that apart from gingival inflammation other factors contribute to the expression of the cytokine[82,86]. These studies could confirm the reason why, there was a non-statistically significant association between gingival scores and IL-1 levels.

5.8 Diagnostic Utility of IL-1

A linear regression curve estimation model (figure 6) elicited a statistically significant association between IL-1ß (pg/ml) scores and basic periodontal examination scores as the predictor variable. This highlighted the association of the levels of diseases severity and levels of IL-1 . This may imply that IL-1 in saliva could be used to measure disease severity.

The level of IL-1β increased as the diseases severity got worse hence reinforcing the concept that salivary IL-1 levels indicates severity of periodontal disease, This study was in agreement with that by Tobon and colleagues that concluded that elevated IL-1 concentrations may be one of the host response components associated with the clinical manifestations of periodontal diseases[111].

5.9 Conclusions

The data indicates that salivary IL-1 levels were raised in the saliva of patients with severe periodontal disease suggesting a close association between salivary IL-1 and chronic periodontitis. There was a positive correlation between salivary IL-1 levels and BPE scores.

5.10 Recommendations

IL-1 might be potentially useful in distinguishing health from disease. In the future longitudinal studies with larger sample sizes are needed to validate salivary IL-1 as a marker for periodontal diseases. There is need to explore a combination of other biomarkers in addition to IL-1 . Using a combination of biomarkers as a chairside diagnostic device may be more accurate in the diagnosis of periodontal disease.

5.11 Sources of Funding

The principal investigator solely met the cost of the study.

The study was meant for scientific and academic purposes.

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APPENDICES

APPENDIX I: CONSENT FORM

Date ………………….

Participant code……………

Project title-This research entitled the relationship between salivary interleukin one beta and periodontal status among adults attending the university of Nairobi dental hospital is being carried out by Dr, Eliab K. Muthima, a post-graduate student at the department of Periodontology, University of Nairobi, School of Dental Sciences towards his attainment of a masters of Dental Surgery

Objective of the study- The objective of the study is to investigate the relationship between salivary 1L-1 levels and periodontal health status in an adult Kenyan population.

Procedure to be followed- The research will entail collection of a small sample of saliva following rinsing the mouth with clean water. Clinical examination will be done using sterile dental instruments on the teeth and gums.

Risks-There is no health risk posed to participants during the examination.

Confidentiality-All the information gathered during this study will be treated with utmost confidentiality and will only be used for purposes of this research. Participation is voluntary you may opt out of the study at your own free will without any threats or dire consequences.

Benefits-No monetary compensation or reward of any form will be offered to participants. The findings of this research may be of great use in understanding, evaluation, treatment and follow up of periodontal diseases in the Kenyan setting using current emerging technology and globally accepted methodology.

Period of study-The study will be conducted in November and December 2018 and the participants will be interviewed and examined only once during the period of the study

Role of Ethics Research Committee-The role of the Kenyatta National Hospital /University of Nairobi Ethics and Research Review Committee (KNH/UON-ERRC is to review biomedical research in order to help safeguard the dignity, rights, safety and well being of all actual or potential research participants. A cardinal principle of research involving human participants is "respect for dignity of persons". The goals of research, while important, should never be permitted to override the health and well being and care of research participants. KNH/UON-ERRC shall takes into consideration the principles of justice. Justice requires that the benefits

and burdens of research be distributed fairly among all groups and classes in society, taking into account age, gender, socio-economic status, culture, and ethnic consideration.

Declaration of the participant Having read and understood the above information and with any concerns I may have had having been answered satisfactorily by the principal investigator Dr. Muthima I do hereby voluntarily consent to participate in this research.

Signed by participant ……………………… Date ………………………

.

Declaration by the principal investigator

I have explained to the participant the purpose and expected benefits of this study and have also answered his/her question and concerns regarding the research on the date on this consent form.

Signed by investigator………………………….. Date…………………………………

SWAHILI VERSION

FOMU YA KUTAFUTA IDHINI KUTOKA KWA WAHUSIKA KATIKA UTAFITI

Kiini cha utafiti-Utafiti huu unaitwa Uhusiano kati ya salivary IL-1 na afya ya ufizi kati ya watu wazima katika hospital ya matibabu ya meno katika chuo kikuu cha Nairobi. Unafanywa na daktari Muthima Eliab ambaye ni mwanafunzi katika chuo kikuu cha Nairobi

Kanuni za utafiti-Katika utafiti huu hali ya afya ya ufizi na uchafu katika meno za wahusika utachunguzwa kwa kutumia vifaa safi. Pia sampuli kidogo ya mate itachunguzwa baaada ya kuosha mdomo na maji safi.

Madhara na manufaa ya utafiti-Hakuna madhara ya aina yoyote kwa wahuska katika utafiti huu. Hakuna malipo yakifedha au aina nyingine ambayo washirika watapewa kwa kushiriki katika utafiti huu. Matokeo ya utafiti huu yataweza kusaidia madaktari na wanasayansi kuelewa magonjwa ya ufizi na jinsi ya kuyatibu vyema zaidi kwa kutumia mbinu za kisasa zinazokubaliwa kote duniani.

Hifadhi ya nakala ya habari utakazotoa-Habari zote zitakazotolewa na wahusika zitatumiwa kwa utafiti huu peke yake. Pia zitawekwa kwa njia ya kibinafsi na kisiri katika rekodi ambazo mchunguzi mkuu ataziweka vyema. Wahusika watashiriki kwa utafiti huu kwa hiari yao bila kushurutishwa au kulazimishwa na yeyote. Wahusika pia wanaweza kujiondoa kutoka kwa utafiti huu wakati wowote bila vitisho au madhara yoyote.

Baada ya kusoma na kuelewa maelezo haya, na baada ya maswali yote niliyokuwa nayo kuhusu utafiti huu kujibiwa na Daktari Muthima ninakubali kuhusishwa katika utafiti huu kwa kutiasahihi hapa chini.

Sahihi ya mshiriki………………………………Tarehe…………………………………

Maelezo ya mchunguzi mkuu

Nimemweleza mshiriki kuhusu maudhui na manufaa ya uchunguzi huu, nimejibu maswali aliyokuwa nayo.

Siku ambayo imetiwa sahihi hapa chini

Sahihi ya mchunguzi mkuu………………… **Tarehe**……………………

APPENDIX II: QUESTIONNAIRE/BIODATA FORM

Salivary IL-1 levels and periodontal health status among adults attending the University of Nairobi Dental Hospital

Date ………………………… Saliva sample serial/code number……………………….

Age (Years)……………………Hospital File no…………………………………

Gender: Male ☐ Female ☐

Residence……………………………………….. Occupation…………………………….

Highest level of education

Primary ☐ Secondary ☐ Tertiary ☐

Tooth brushing habits

Once daily twice daily ☐ thrice daily ☐

Other…………………………………………………………….

Interdental cleaning

Do you use dental floss: No ☐ Yes ☐

If yes, state frequency…………………………………………………….

Inter dental brushes No ☐ Yes ☐

If yes, state frequency…………………………………………………….

Last dental visit

Never been to a dentist ☐ less than 3 months ago ☐ 3-6 months ago ☐

>6 months ago ☐

Questionnaire (screening segment)

Screening (TICK)

		Yes	No
1.	Persons below the age of 18 years.		
2.	Periodontal procedure within the last 6 months.		
3.	Antibiotic therapy within the past 6 months.		
4.	Concurrent systemic illness for example rheumatoid arthritis. renal, kidney diseases, diabetes,		
5.	Pregnancy or lactation		
6.	Current smoker or history of smoking in the last 3 years		
7.	Patients who have less than 20 teeth.		
8.	Patients suspected to have symptoms or show signs of trauma occlusion.		

APPENDIX III: GINGIVAL SCORE (LOE-SILNESS INDEX 1963)

Tooth	16		11		24		36		32		44	
Surface	F	L	F	L	F	L	F	L	F	L	F	L
Score												

Total ……………… Mean ……………… Gingival Health Status …………………

Scoring criteria for Gingival Score (Loe-Silness Index 1963)

Score	**Criteria**
0	No gingivitis - Normal, absence of Oedema and no bleeding on probing
1	Mild Gingivitis - Presence of Oedema with absence of bleeding
2	Moderate Gingivitis - Oedema Present, glazing and bleeding on probing
3	Severe Gingivitis - Oedema, ulcerations with spontaneous bleeding

APPENDIX IV: PLAQUE SCORE: QUIGLEY AND HEIN MODIFIED BY TURESKY ET AL 1970

Tooth	16		11		24		36		32		44	
Surface	F	L	F	L	F	L	F	L	F	L	F	L
Score												

Total …………………. Mean……………………

Scoring criteria for Plaque Score: Quigley and Hein modified by Turesky et al 1970

Scores	**Criteria**
0	No Plaque
1	Separate flecks of plaque at the cervical margin of the tooth.
2	A thin continuous band of plaque at the cervical margin of the tooth.
3	A band of plaque wider than 1mm covering less than a 1/3 of the crown of the tooth.
4	Plaque covering at least one-third but less than two-thirds of the crown of the tooth.
5	Plaque covering two-thirds or more of the crown of the tooth.

APPENDIX V: BASIC PERIODONTAL EXAMINATION (BPE) SCORE GRID

4	**3**	**3***
-	**2**	**4***

Both the number and the *should be recorded if a furcation is detected E.g. if the score of as sextant is 3* It means the probing depth is 3.5-5.5mm plus a furcation involvement in the sextant

Score	**Criteria**
0	No pockets>3.5 mm, no calculus/overhangs, no bleeding after probing (black band completely visible)
1	No pockets>3.5 mm, no calculus/overhangs, but bleeding after probing (black band completely visible)
2	No pockets>3.5mm, but supra or sub-gingival calculus/overhangs (black bands completely visible)
3	Probing depth 3.5-5.5 mm (black band partially visible, indicating pocket of 4-5 mm)
4	Probing depth >5.5 mm (black band entirely within the pocket indicating pocket of 6 mm or more)
*	Furcation involvement.

APPENDIX VI: ELISA KIT MANUAL

Reagent Preparation

- Bring all reagents to room temperature and mix before use. A minimum of 1.5 hours is recommended for the 12 mL of IL-1β Assay Diluent used in Step 8 and 11 to come to room temperature.
- Bring microtitre plate to room temperature before use. ***It is important to keep the foil pouch with the plate strips closed until warmed to room temperature, as humidity may have an effect on the coated wells.***
- Prepare 1X wash buffer by diluting Wash Buffer Concentrate (10X) 10-fold with room-temperature deionized water (100 mL of Wash Buffer Concentrate (10X) to 900 mL of deionized water). ***Dilute only enough for current day's use and discard any leftover reagent.*** (If precipitate has formed in the concentrated wash buffer, it may be heated to 40ºC for 15 minutes. Cool to room temperature before use in assay.)
- Reconstitute each Control vial with 1.0 mL of deionized water. (We recommend sterile water if you plan to store at 2-8ºC.) Let sit 20 minutes at room temperature before using. Mix well immediately before use. ***Use reconstituted controls within 4 hours at room temperature or refrigerate at 2-8ºC for up to 48 hours.***

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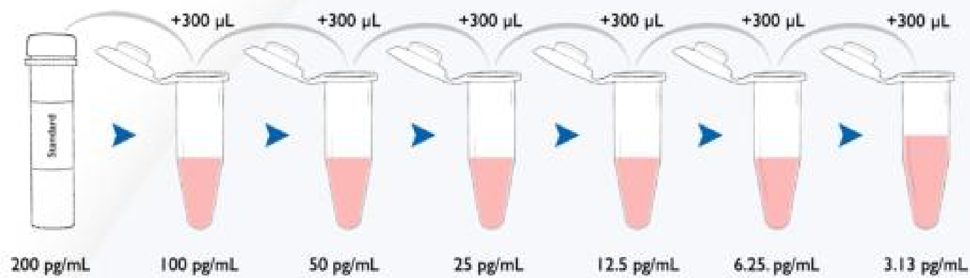
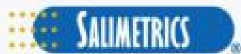
- Reconstitute IL-1β Standard with deionized water according to the volume on the standard vial label. (We recommend sterile water if you plan to store at 2-8°C.) Let sit 20 minutes at room temperature before using. Mix well immediately before use. ***Use reconstituted standard within 4 hours at room temperature or refrigerate at 2-8°C for up to 48 hours.***
- Prepare serial dilutions of the IL-1β Standard as follows:
  - Label six polypropylene microcentrifuge tubes or other small tubes 2 through 7.
  - Pipette 300 µL of IL-1β Assay Diluent into tubes 2 through 7. ***Do not use IL-1β Sample Diluent to dilute the standard curve.***
  - Serially dilute the standard 2X by adding 300 µL of the 200 pg/mL standard (tube 1) to tube 2. Mix well.
  - After changing pipette tips, remove 300 µL from tube 2 to tube 3. Mix well.
  - Continue for tubes 4, 5, 6 and 7.
  - The final concentrations of standards for tubes 1 through 7 are, respectively, 200 pg/mL, 100 pg/mL, 50 pg/mL, 25 pg/mL, 12.5 pg/mL, 6.25 pg/mL and 3.13 pg/mL. Standard concentrations in pmol/L are 11.8, 6.0, 3.0, 1.5, 0.7, 0.4 and 0.2 respectively.
  - IL-1β Assay Diluent is used for the Zero Standard.





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Procedure

Step 1: Read and prepare reagents according to the Reagent Preparation section before beginning assay. Determine your plate layout. Here is a suggested layout. (Standards, controls, and saliva samples should be assayed in duplicate.)

	1	2	3	4	5	6	7	8	9	10	11	12
A	200 Std	200 Std	Ctrl-H	Ctrl -H								
B	100 Std	100 Std	Ctrl-L	Ctrl -L								
C	50 Std	50 Std	SMP-1	SMP-1								
D	25 Std	25 Std	SMP-2	SMP-2								
E	12.5 Std	12.5 Std	SMP-3	SMP-3								
F	6.25 Std	6.25 Std	SMP-4	SMP-4								
G	3.13 Std	3.13 Std	SMP-5	SMP-5								
H	0 Std	0 Std	SMP-6	SMP-6								

Step 2: Keep the desired number of strips in the strip holder and place the remaining strips back in the foil pouch. Reseal the foil pouch with unused wells and desiccant. Store at 2-8°C.

Step 3: Pipette 12 mL of IL-1β Assay Diluent into each of two different disposable tubes. (Scale down proportionally if not using the entire plate.) Set aside for Step 8 and Step 11.

Step 4: Dilute saliva samples 15X in IL-1β Sample Diluent using 20 µL saliva to 280 µL IL-1β Sample Diluent. ***Do not dilute samples in IL-1β Assay Diluent.***

Step 5:

- Pipette 100 µL of standards, controls, and diluted saliva samples into appropriate wells.
- Pipette 100 µL of IL-1β Assay Diluent into 2 wells to serve as the Zero Standard.

Step 6: Place adhesive cover provided over plate. Mix plate on a plate rotator ***continuously*** at 500 rpm for 1 hour at room temperature.

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Step 7: Wash the plate 4 times with 1X wash buffer. A plate washer is recommended. However, washing may be done by gently squirting wash buffer into each well with a squirt bottle, or by pipetting 300 µL of wash buffer into each well and then discarding the liquid over a sink. After each wash, the plate should be thoroughly blotted on paper towels before turning upright. If using a plate washer, blotting is still recommended after the last wash.

Step 8: Dilute the antibody conjugate 1:500 by adding 24 µL of the antibody conjugate to the 12 mL of IL-1β Assay Diluent. (Scale down proportionally if not using the entire plate.) Antibody conjugate tube may be centrifuged for a few minutes to bring the liquid down to the tube bottom. Immediately mix the diluted antibody conjugate solution and add 100 µL to each well using a multichannel pipette.

Step 9: Place a new adhesive cover (provided) over plate. Mix plate on a plate rotator ***continuously*** at 500 rpm for 2 hours at room temperature.

Step 10: Repeat wash procedure from Step 7.

Step 11: Dilute the Streptavidin-HRP 1:200 by adding 60 µL of the Streptavidin-HRP to the 12 mL of IL-1β Assay Diluent. (Scale down proportionally if not using the entire plate.) Streptavidin-HRP tube may be centrifuged for a few minutes to bring the liquid down to the tube bottom. Immediately mix the diluted Streptavidin-HRP solution and add 100 µL to each well using a multichannel pipette.

Step 12: Mix plate on a plate rotator ***continuously*** at 500 rpm for 20 minutes at room temperature.

Step 13: Repeat wash procedure from Step 7.

Step 14: Add 100 µL of TMB Substrate Solution to each well with a multichannel pipette.

Step 15: Mix plate on a plate rotator ***continuously*** at 500 rpm in the dark (covered) for 20 minutes at room temperature.

Step 16: Add 50 µL of Stop Solution with a multichannel pipette.

Step 17:

- Mix on a plate rotator for 3 minutes at 500 rpm. If green color remains, continue mixing until green color turns to yellow. Be sure all wells have turned yellow.

  Caution: Spillage may occur if mixing speed exceeds 600 rpm.

- Wipe off bottom of plate with a water-moistened, lint-free cloth and wipe dry.
- Read in a plate reader at 450 nm. Read plate within 10 minutes of adding Stop Solution. (For best results, a secondary filter correction at 620 to 630 nm is recommended.)