TOTAL SALIVARY PROTEIN AND ITS RELATIONSHIP TO PERIODONTAL HEALTH IN AN ADULT KENYAN POPULATION

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DECLARATION

This thesis is my original work and has not been presented for the award of a degree in any other university

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DEDICATION

This thesis is dedicated to my Husband Lukwiya whose unyielding love, support and encouragement enriched my soul and inspired me to pursue and complete the research.

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LIST OF ABBREVIATIONS

AHEA	Associate of Higher Education Academy
BDS	Bachelor of Dental Surgery
BPE	Basic periodontal examination
CDC	Center for Disease Control
CDE	Certificate in Dental Education
FADI	Fellow of Academy of Dentists Iinternational
FICD	Fellow of International College of Dentistry
GCF	Gingival Crevicular Fluid
ITI	International Team for Implantology
MClinDent	Master in Clinical Dentistry
MUK.	Makerere University Kampala
PFA	Pierre Fauchard Academy
PhD	Doctor of Philosophy
PI	Principal investigator
UNDH	University of Nairobi Dental Hospital
UON	University of Nairobi

ABSTRACT

Introduction: Periodontal disease is highly prevalentand is one of the highest contributors to global oral health burden.Diagnosis of active periodontal disease provides a challenge for clinicians because the traditional periodontal diagnostic parameters are not very easyto use and they mostly only measure disease history. Advances in oral and periodontal disease diagnostic research is moving towards more objective measures such as biomarkers which can identify and quantify the presence of disease.

Saliva is a mirror of oral and systemic health and a valuable source for biomarkerswhich are specific for the unique and physiological aspects of periodontal diseases. Changes in quality and quantity of salivary proteins occur in different physiologic and pathologic states therefore measuring these may act as biomarkers for the periodontal phenotype.

The aim of this study is to analyze total salivary protein as a potential diagnostic biomarker for detecting inflammation of the periodontal tissues using simple biochemical methods.

Study objective: To determine the total protein content of saliva in a Kenyan adult population and investigate its relationship with periodontal health status.

Study population and sample size: one hundred and sixty-one study participants were selected from a pool of people attending University of Nairobi Dental Hospital.

Study area: The study was carried out at the University of Nairobi Dental Hospital (UNDH).The subjects were recruited from the OralDiagnosis and Periodontology clinics.

Study design: This was a hospital based descriptive cross-sectional study.

Materials and Methodology:one hundred and sixty-one participants were selected from a pool of individuals attending the University of Nairobi Dental Hospital during the period of study via systematic random sampling. Saliva was collected from each participant using the spit method followed by a periodontal examination. Total salivary protein was quantified using bicinchoninic acid assay.

Results: A total of 161 participants were recruited. The male to female ration was 0.85. The age of the participants ranged between 18 – 80 with a mean of 38.34 years (± 13.44 SD) and a median of 37.00.

The total salivary protein ranged between 0.11mg/ml to 12.17mg/ml (mean = 2.03mg/ml ± 1.97 SD and a median =1.38). Males had a statistically higher salivary protein levels (*mean* = 2.39±2.16*SD*) than females (*mean* = 1.72±1.75*SD*), *t* (140.220) = 2.156, *p* = 0. 033.Generally, the mean levels were higher in patients with periodontitis with statistically significant association between salivary protein levels and mild periodontitis levels (*r* = 0.594*, *p* = 0.020). However, onlya moderate, positive and non-statistically significant association was found between salivary protein levels and severe periodontitis levels (*r* = 0.359, *p* = 0.278).

Conclusion: The findings of this study suggests that total salivary proteinlevels could serve as biomarkers of inflammation in the periodontium.

Recommendation Total salivary protein should be considered as a potential adjunctive diagnostic tool for evaluating inflammatory periodontal diseases. However, there is need for more salivary proteomic studies with larger sample sizes and evaluation of individual proteins and their specific role in periodontal diseases and randomized controlled trials in Kenyans to fully exploit the potential of these biomarker

CHAPTER ONE
INTRODUCTION

1.1 Periodontal disease

Periodontitis is defined as an inflammatory disease of the supporting tissues of the teeth caused by specific microorganisms or groupsof specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone resulting in increased probing depth formation, recession, or both.[1]The clinical feature that distinguishes periodontitis from gingivitis is the presence of clinically detectable attachment loss. Thisloss often is accompanied by periodontal pocket formation and changes in the density and height of subjacent alveolar bone.[1]

Periodontal disease is the commonest oral health problem with over 90% of the population suffering from at least one form of this disease. In Kenya, Ng'ang'a, 2002 reported, in a review paper, a prevalence of chronic periodontitis (1-10%) and gingivitis (0.2 – 90%)[2]. Internationally, in the United States of America, chronic periodontitis and gingivitis have been reported at 47.2% and (50-100%) respectively in adults by the CDC[3]. It is a worldwide disease and according to the American Academy of Periodontitis, there are 3 subtypes of periodontitisnamely, chronicperiodontitis, aggressiveperiodontitisand periodontitis as a manifestation of systemic disease[4]

Chronic periodontitis is the most prevalent form of periodontal conditionsin adults, it is adestructive condition frequently found in the presence of local factorsand sub gingivalplaque. Diagnosis of active disease in chronic periodontitis presents a challenge for clinicians. Clinical and radiographic investigations remain the basis for patient evaluation and they are measurements of the history of the disease. The obtained values are also subject to considerable measurement error for example challenges of probing depth due to different probing pressure used by clinicians and areoften poorly tolerated by patients. The search for a clear, objective, easy to use measure of active disease identification is still ongoing.

The etiology of periodontal conditions is multifactorial based on an imbalance between bacterial products and host responses. Bacteria and their products have been shown to initiate the disease process and it requires the accumulation of a biofilm of bacteria at the gingival margin for the disease process to begin. This accumulation of bacteria biofilm requires the right conditions for it to happen. Bacteria have to contend with several mechanisms that are geared towards preventing their accumulation. One of these mechanisms is saliva.Saliva plays different roles including but not limited to; antimicrobial effect, clearance of microbes by the constant flow and swallowing by the individual, and prevention of biofilm adhesion through some of its components.[5]

1.1.1 Saliva

Saliva is abiological fluid that contains proteins and genetic molecules. Itis predominantly composed of 99% water but includes electrolytes(potassium,sodium,calcium ,chloride) andproteins(enzymes,immunoglobulins, antimicrobial factors,mucosal glycoprotein and albumin)[6].

Saliva is a mirror of oral and systemic health and a valuable source for clinically relevant information as it contains biomarkers specific to the unique physiological aspects of periodontal diseases. In addition, it has long been recognized as a potential diagnostic tool.

Saliva provides an easily available, noninvasive diagnostic medium that can be used to detect a wide range of diseases and clinical situations. Hence, ithas been discussed lately as an important biological material that could be used for developing new diagnostic tests [7].

Thereis evidence available to support changes in protein composition of whole saliva in the presence of chronic periodontitis. Some of the reported changes are: increased amounts of blood proteins(serum albumin and hemoglobin),immunoglobulins and salivary amylase[8]. In relation to protein quantification, scarce literature exists and none in the African and Kenyan settingin particular.

1.1.2 Diagnostic Parameters

Traditional periodontal diagnostic parameters used include probing depths, bleeding on probing, clinical attachment levels, plaque index, and radiographs assessing alveolar bone level.The strengths of the above tools are their ease of use, their cost effectiveness and they are relatively noninvasive,but also very inherently limited in that mostly only disease history can be assessed.Advances in oral and periodontal disease diagnostic research is moving towards moreobjective measures such as biomarkers which can identify and quantify the presence of disease.

Gingivitis and periodontitis are oral diseases that are characterized by chronic inflammation. The inflammatory process brings about degradation of the connective tissue components of the periodontal tissues with resultant plasma protein leakage into the gingival crevicularfluid and saliva[9]. Salivary protein and albumin concentrations can therefore be used as markers to detect inflammation of the periodontal tissues. Hence, the aim of this study was to analyze total salivary protein and correlate it with periodontal health status as a way of developing it as a potential diagnostic biomarker for inflammation of the periodontal tissues. Simple biochemical methods were used to assess the proteins in saliva of adult Kenyans.

CHAPTER TWO
LITERATURE REVIEW

2.1 Biomarkers

The American National Institute Of Health defined a biomarker as a "characteristic" that objectively measures and evaluates normal biological processes, pathogenic processes or pharmacological responses to therapeutic intervention[10]. Biomarkers of disease play an important role in life sciences and have begun to assume a greater role in diagnosis and monitoring oftherapeutic outcomes.Biomarkers allow earlier detection of disease, evolution of the disease process and have also been used to monitor response to treatment measures[10].If biomarkers are to assume their rightful role in routine practice, it is essential that their relationship to the mechanism of disease progression and their role in monitoring therapeutic interventions be more fully understood[11].

In oral diagnostics and more especially in the periodontology field, no known investigation based on biomarkers is currently being used in the routine diagnosis of periodontal disease.Most of the investigations available measure the disease history and rely heavily on the clinician's abilities to use the tools correctly, leaving room for error. Salivashould be developed as a diagnostic tool because of its non-invasive property, easy to use and is easily available, as well as the fact that collection of saliva does not require trained medical personnel[12].

2.2 Salivary components

Salivary fluid is an exocrine secretion consisting of approximately 99% water, containing a variety of electrolytes (sodium, potassium, calcium, chloride, magnesium, bicarbonate, phosphate) and proteins, represented by enzymes, immunoglobulins and other antimicrobial factors, mucosal glycoproteins, traces of albumin and some polypeptides of importance to oral health[13]. There are also glucose and nitrogenous products, such as urea and ammonia. These components interact and are responsible for the various functions attributed to saliva.

Salivary proteins have been shown to increase in many chronic diseases like cardiovascular disease and autoimmune diseases[14][15]. Plasma proteins (hemoglobin and albumin) and immunoglobulins[16] have also been shown to increase in periodontal disease.

Advances are being made towards developing diagnostic markers in saliva due tothe ease of availability of saliva[12],ease of collection, no specialized equipment required, lessinvasive, cost effectiveness of the testsand the possibility of being employed for mass screening as it facilitates repeated sampling even at short intervals[13].

2.3 Biomarkers in saliva

Numerous markers in saliva have been proposed as diagnostic tests for periodontal disease. These include intracellular enzymes such as Creatine Kinase, Lactate Dehydrogenase, Aspartate, Alanine Aminotransferase, Gama Glutamyl Transferase and Alkaline Phosphatase[17]. In periodontal disease, the tissuesbecome damaged, due to edema and destruction of cellular membranes during the inflammatory process.The intracellular enzymes are increasingly released into the gingival crevicular fluid (GCF) and saliva where their activity can be measured. Thus, the presence of these enzymes in saliva and GCF can be used as biochemical markers for determining the condition of the periodontal apparatus [17]. Although the activity of the mentioned enzymes can be quantified in saliva, their use as diagnostic markers is limited because these enzymes are also active in healthy persons.

Of interest is a particular group of enzymes released from damaged periodontal tissues as a result of host response. Enzymes such as Aspartate Aminotransferase, AlanineAminotransferase, GammaGlutamyl Transferase are enzymes engaged in metabolic processes of cells and are mostly present in soft tissue.These enzymes are indicators of cellular damage and their increased activity is a direct consequence of their increased release from soft tissues of the periodontium[18].

Alkaline Phosphatase and Acid Phosphatase are glycoproteins present in most hard tissues especially in bone,their increased activity in saliva is a direct consequence of

increased destruction of alveolar bone[19]. The potential value of alkaline phosphate was identified by Ishikawa and Cimasoni in1970[20]. Recently, a longitudinal studydemonstrated a 20 fold increase of alkalinephosphataseactivity at sites of 2mm or more of attachment loss[21].

Acid phosphatase is also associated with bone metabolism, it is present in neutrophils, desquamated epithelial cells, macrophages and several bacterial species. Actinobacillus,Capnoctophaga and Veillonella species are known toproduce acid phosphatase and it has been shown to be elevated in periodontal disease by several studies[18]. The mentioned bacterial species are known periodontal pathogens.

Majority of the biomarkers are protein in nature and measuring the total protein in saliva in patients with periodontitis and correlating it with health and severity of diseasecould revealthe possible use of total salivary protein as a potential biomarker. Saliva's main advantage is that apatient is able to collect samples at home or in other places when necessary. It is also easy to use unlike clinicalexamination and radiographic assessment.Tests based on saliva have already made strides in medicine for detection of certain antibodies and drugs[22],however none that arespecific and reproducible for periodontal disease are available as yet.

2.4 Salivary proteome analysis

Periodontal proteomic markers range from salivary protein markers like Immunoglobulin G to bone remodeling protein markers[23] . Proteomic markers are divided into specific and non-specific. Specific markers are immunoglobulins which characterize the presence of chronic or aggressive periodontitis. Nonspecific markers include enzymes, proteins, mucins, histatin, lactoferrin and lysosomal peroxidase.In addition,GCF,blood, serum products electrolytes, microorganisms, epithelial and immune cells, bacterial degradation products, and lipopolysaccharides, can be used for proteome analysis[24] . Any change in the composition of biomarkers specific for periodontitis could be used as diagnostic markers. Comprehensive analysis and identification of proteomic contents in saliva, is an essential first step towards the identification of protein markers for periodontal disease.

The National Institute of Dental and craniofacial Research Bethesda cataloged proteins in human saliva, the results of salivary and serum proteins revealed that the oral cavity has 3397 non redundant proteins of which 605are altered in pathological states,51 are only found in disease,3115from saliva,990 from oral mucosa and 1929 from plasma[25][26].

Protein secretion in salivary glands is an active process by acinar and ductal cells, Blood plasma proteins enter ductal saliva by several mechanisms including passive intracellular diffusion, active transport for example secretory Ig A, ultra-filtration and leakage via leaky patches at site of tissue damage. Regardless of the mechanism for secretion of these plasma proteins, it appears that saliva is an easily accessible source for monitoring many proteins that are present in the oral cavity[25].

2.5 Periodontal diseases and proteomics

Periodontal diseases usually refer to common inflammatory disorders known as gingivitis and periodontitis, which are caused by a pathogenic microbiota in sub gingival biofilm. Gaining an understanding of the human salivary proteome gives insight into the physiological and pathological processes relevant to periodontal health, and is crucial for the identification of meaningful biomarkers for periodontal diseases.

Periodontopathic bacteria usually produce virulence factors that cause degradation of host tissue. This can be either directdestruction orthroughactivating host response mechanisms which release biological mediators from host cells. These mediators in the presence of exaggerated response as happens in periodontal disease leads to host tissue destruction[27]. Host and bacteria products like enzymes, proteins and other inflammatory mediators can be potential salivary diagnostic biomarkers for periodontal diseases.

Bacterial products like lipopolysaccharide and bacterial DNA trigger the innate host defense resulting in recruitment of neutrophils, monocytes and activated macrophages to the site. These host cells in turn release numerous cytokines such as prostaglandins (PGE_2), tumor necrosis factor (TNF), and interleukins IL-1 and IL-6 which direct the

inflammatory process further. Consequently, collagenases like matrix metalloproteinases (MMP's) are produced by alveolar bone and polymorphonuclear leukocytes. In addition there are proteins emanating from serum,albumin and hemoglobin or other cells at inflamed sites, these products are released into the gingival crevicular fluid and periodontal pocket and can serve as proteomic biomarkers for periodontal diseases[28][29].

2.6 Salivary diagnostic markers and periodontal diseases

Current clinical diagnosis of periodontal disease is based on an oral examination, consisting of inspection of the gingival tissue on the buccal and lingual aspect of every tooth, conducting a periodontal screening and recording pocket depths for each tooth, checking attachment level, measuring plaque index, testing bleeding on probing, testing tooth mobility, checking for temperature changes using temperature probes and taking radiographs to assess bone loss. Rapid chairside tests are also available to test for specific periodontal pathogens as well as interleukin 1 alleles. However, some of these tests are not reliable. Studies to find a more specific, reproducible and rapid test are necessary to try eliminate the short falls of the current used practices. Severalstudies geared towards researching for diagnostic parameters that are rapid, easierand more sensitive have been conducted. These studiesinclude; bacterial studies and proteomic profile studies.

Bacterial studies; for example, Holt BJ et. al,2005, in an effort to isolate oral bacteria which cause periodontitis using classical invitro methods,isolated some bacterial species but only the cultivable species such as Porphyromonas gingivalis, Treponema denticola and Tannerella forsythia which were present as a complex biofilm in destructive periodontitis[30], However, it is known that majority of the oral bacterial species are uncultivable.

Salivary proteomic studies; Haigh BJ et. al,2010 studied comparative proteomic profiles in patients with periodontitis and healthy subjects, showed distinctive profiles with alterations of individual salivary proteins in the presence of periodontal inflammation[31].

In 2009, Ramseier CAand co-workers demonstrated that with the use of PCR and sensitive immunoassays identification of host and bacterially derived biomarkers is

possible especially in correlating them with periodontal diseases. This approach offered a significant potential for discovery of biomarkers signatures useful in development of a rapid chairside diagnostic test for oral diseases. Some enzymes such as MMP-8 and 9 have been used for chairside diagnostic tests[32]. However, a cheaper easier to use biomarker is still lacking.

The clinical value of salivary proteomic biomarkers in periodontal disease diagnosis is under experimental development and is based on detecting changes in the profile of molecules involved in inflammation, collagen degradation and bone loss[33].A study by Bostanci N et.al,2010reported an increase in bacterial, viral and yeast proteins in disease when compared with the healthy sites in gingival crevicular fluid[34].

As demonstrated in a study by Haigh et al, 2010and many other studies,variationsin the quantity of the different proteins in oral fluidsoccurs depending on the periodontal health status.Quantifying the total protein components in the oral fluids could help investigate whethertotal salivary protein could be used as a diagnostic test. Development of a diagnostic test utilizing total salivary proteins in saliva will go a long way in identifying presence and progression of periodontal disease.

2.7 Statement of the problem and justification

2.7.1 Problem statement

Clinical diagnosis of active periodontal disease is a great challenge to clinicians worldwide[33]. Traditional diagnostic parameters mostly measure disease history, are invasive, timeconsuming and subjective as they depend on the clinician's experience, leaving margins for error. The search for biomarkers in saliva to be used in diagnosis of periodontal disease would provide a simple, non-invasive diagnostic tool that enables reliable evaluation of periodontal disease.

Saliva is essential in the prevention or progression of chronic periodontitis through bacterial clearing and antimicrobial properties. Different salivary enzymes and proteins like immunoglobulin, interleukins and collagenases have been demonstrated asbiomarkers for disease, as they are produced by the host in response to microorganism

invasion. The current literature on total protein in saliva shows a positive correlation between individual proteins(like interleukins and cytokines) and periodontal disease[8]. In the available literature, there is minimal data to no data on total saliva protein in the African and especially in adult Kenyans.

Periodontal diseases including chronic periodontitis, gingivitis and aggressive periodontitis are a major health burden that have contributed immensely towards decreasing the overall quality of life for a sizeable proportion of the population[35]. Identifying the biomarkers that might assist in diagnosis and hence early treatment of periodontal diseases would help improve periodontal health. It is with this in mind that this study was done to establish the total protein contentof saliva in a Kenyan population and investigate its relationship with periodontal health status.

2.7.2 Justification

There is hardly any data on total protein quantity in saliva in the African or Kenyan population, and especially how it relates to periodontal disease.Thus, this study determined the total protein quantity in saliva of an adult Kenyan population and correlatedit to the periodontal condition with the aim of finding a rapid test which can confirm the presence of disease. Having a chairside test that can confirm disease presence will enable dental practitioners to correctly diagnose and treat patients.

2.8 Objectives

2.8.1 Main objective

To determine the total protein content of saliva in a Kenyan adult population and investigate its relationship with periodontal health status.

2.8.2 Specific objectives

1. To measure the total protein content in saliva in study participants.
2. To assess the periodontal health status of the study participants.
3. To evaluate the correlation between total protein content in saliva and a healthy periodontium in study participants.

4. To evaluate the correlation between total protein content in saliva and gingivitis of study participants.
5. To evaluate the correlation between total protein content in saliva and chronic periodontitis in studyparticipants.

Variables

Variables	Measurement
Socio demographics	
Age	Number of years
Gender	Phenotypic appearance of the respondent, male or female
Occupation	Type of work the respondent engages in
Frequency of brushing	Number of times one brushes every day
outcome variable	
Total protein in saliva	mg/ml
Independent variables	
Oral hygiene status	Plaque score- (Silness and Loe Index-1964)
Gingival health status	Gingival index (Loe and Silness1963)
Periodontal status	Basic Periodontal Exam (British society of Periodontology 2011)

CHAPTER THREE
MATERIALS AND METHODS

3.1 Study design

This was ahospital based descriptive cross-sectional study based at the Universityof Nairobi Dental Hospital.

3.2 Study Area

The study was carried out at University of Nairobi Dental Hospital (UNDH), a dental teaching hospital that receives about 3500 patients annually. It runs specialized clinics in Oral Diagnosis, Pediatric dentistry, Prosthodontics, Oral and Maxillofacial and Periodontology. The hospital acts as a referral center for patients from all parts of Kenya. Thestudy participantswere recruited from both the Periodontology clinic and Oral Diagnosis clinic.

3.3 Study Population

This consisted of all consenting adult patients attending the Periodontology and Oral Diagnosis clinics at the University of Nairobi Dental Hospital during the period of the study.

3.4 Inclusion criteria

All consenting adult patients above age of 18 years attending the periodontology and oral diagnosis clinics at the University of Nairobi Dental Hospital during the time of the study. Individuals had to haveat least 16 teeth in the mouth for adequate representation.

3.5 Exclusion criteria

Medically compromised patients, especially those suffering from diseases known to alter normal composition of body fluids. Several diseases have been implicated including; diabetes, hormonal imbalance, autoimmune disorders such as sjogren’s syndrome, rheumatoid arthritis, salivary gland disease andsmokers. Screening of all possible

participantswas done through interview and using the form described in Appendix 1(appendix I). Those who did not meet the inclusion criteria were eliminated.

3.6 Sample size determination

The prevalence of periodontal disease has been reported to be between 60-80% in Kenyan adult population(36)using 70% as prevalence, the sample sizewas based on the formula by Kish and Leslie for cross sectional studies(37).

$$N = \frac{Z^2 P(1-P)}{C^2}$$

Where N=Sample size desired

P=assumed true prevalence of periodontal disease among adult Kenyans70%(36)

Z= standard normal deviate at 95%confidence interval corresponding to 1.96

C=margin of error at 5%(standard value, 0.05)

$$N=\frac{1.96x1.96x0.7x0.3}{0.05^2}$$

=322.69=323

However, using modified Kish Leslie formula for available sample size as patients visiting the dental hospital are less than 10,000 in one month

$n= no/(1 + (no - 1)/N)$

n=desired sample size for population less than 10000

no=desired sample size for population greater than 10,000

N=population estimate 300

N=156, 5 more people were included to allow dummy laboratory process to test equipment and reagents.

Total sample size is 161.

3.7 Sampling design and procedure

Systematic random samplingwas used during sample selection. All consenting patientsattending the Periodontology clinic and the Oral Diagnosis clinic on the day of data collection and who fit the inclusion criteria were given an equal chance to participate in the study. The systematic random sampling was based on selection of every secondsubject available in any of the two clinics on the data collection days. The selected patient (every alternate patient in the queue)was then subjected to the screening process, after obtaining consent, before being included in the study.

3.8 Data collection

Data collection tools,clinical examination and laboratory procedureincluded bio-data collection, periodontal examination, oral hygiene assessment and saliva collection. Data collection was done over a period of 3months from May 2018 to July 2018

The process begun with explaining the study and its purpose to the patient. A screening form was used to identify suitable study participants.Those who were found to meet the inclusion criteria and gave written informed consentwere recruited.

The principal investigator interviewed the participants and duly completed the questionnaire on biodata.Saliva was collected and finally clinical examination was carried out.

3.9 Data collection tools

A screening form (appendix I) was used to identifystudy participants who fit in the inclusion criteria. Translation was done for those who didn't speak English as the form was designed in English.

Bio data and social demographic information was obtained through a short questionnaire (Appendix II)by the principal investigator.

3.10 Saliva Collection

Saliva was collected using spit method.

3.11 Periodontal Examination

The periodontal examination was carried out as follows: Plaque was measured using the Silness and Loe 1964[38] index shown in Appendix III. The gingival health was measured using the Loe and Silness 1963[39] index shown in Appendix IV. The periodontal status was determined by using the basic periodontal examination(BPE)[40]shown in Appendix V.

3.12 Total protein levels

This was measured by **bicinchoninic acid assay(**Pierce**™ BCA Protein Assay Kit)**(a biochemical assay for determining the total concentration of protein in a solution.The Thermo Scientific™ Pierce™ BCA Protein Assay is a detergent-compatible formulation based on bicinchoninic acid (BCA) for the colorimetric detection and quantitation of total protein. This method combines the well-known reduction of Cu+2 to Cu+1 by protein in an alkaline medium (the biuret reaction) with the highly sensitive and selective colorimetric detection of the cuprous cation (Cu+1) using a unique reagent containing bicinchoninic acid. The purple-colored reaction product of this assay is formed by the chelation of two molecules of BCA with one cuprous ion. This water-soluble complex exhibits a strong absorbance at 562nm that is nearly linear with increasing protein concentrations over a broad working range (20-2000µg/mL). The BCA method is not a true end-point method; that is, the final color continues to develop. However, following incubation, the rate of continued color development is sufficiently slow to allow large numbers of samples to be assayed together. The macromolecular structure of protein, the number of peptide bonds and the presence of four particular amino acids (cysteine, cystine, tryptophan and tyrosine) are reported to be responsible for color formation with BCA.2 Studies with di-, tri- and tetrapeptides suggest that the extent of color formation is caused by more than the mere sum of individual colorproducing functional groups. Accordingly, protein concentrations generally are determined and reported with reference to standards of a common protein such as bovine serum albumin (BSA).

3.13 Data collection instruments and technique

3.13.1 Saliva collection

The sequence of participant assessment was as follows; Saliva was collected first before the clinical examination to prevent stimulation of the major and minor salivary glands as a result of introduction of examination equipment in the mouth

Saliva was collected using spit method as described by Navesh[41]. Because of itsconvenience for the study participants and the protocol beingeasily reproducible. The participants were instructed not to eat or brush one hour prior to saliva collection. Saliva was collected between 9am and 12 pm to avoid diurnal variations, study participantswere seated on a dental chair and allowed to relax. The Principalinvestigator requested each individual to gently lean forward and without swallowing or talking allow saliva to accumulate in the floor of the mouth for two minutes then spit the collected saliva into a sterileplasticcentrifuge tube(10ml tubes Sarstedt, Germany)
Unstimulated whole saliva of about 5ml was collected from each study participant and the collected saliva was immediately placed in a cool box with ice packs temperature 4 degreesCelsius for transportation to the laboratory within 2 hours.

3.13.2 Biodata

An interviewer- administered questionnaire was used to collect data on social demographics and medical history. This included age, sex, gender, employment, brushing habits, smoking status, marital status and drug history (Appendix II).

3.13.3 Clinical evaluation

Following saliva collection. Oral hygiene assessment and periodontal examination were carried out under illumination from the dental chair light. using disposable gloves, facial mask, a WHO periodontal probe,Dental mirrors and sterile Gauze.

3.13.4 Clinical Examination

This was donesequentially and recorded in a clinical form. A partial-mouth periodontal examinationwas done. Clinical assessmentwas done on a representative set of teeth and probing sites described by Kingman and Abandar 2002[42].Ramfjord teeth which are maxillary right and mandibular left first molars,maxillary left and mandibular right

firstpremolars and maxillary right and mandibularleft lateral incisors were used. Fleiss et. al, in 1987 found that Ramfjord teeth are anadequate representation of the rest of the dentition[44]. It has been shown that there are no significant differences between the full mouth examination and use of Ramfjord teeth [43][44]. The main advantage of Ramfjord teeth is that ittakes a much shorter time than a full mouth periodontal examination.

3.13.5 Oral hygiene assessment

Oral hygiene was assessed using the Plaque score index by (Silness and Loe 1964). (Appendix III) Plaque score was done first to avoid disrupting it while doing the gingival examination. Running the probe along the gingival margin as well as probing the pocket depths disrupts plaque accumulation

3.14 Gingival health

The Gingival Index (Loe and Silness, 1963) was used for the assessment of the gingival condition and it records the qualitative changes in the gingiva. It scores the marginal and interproximal tissues separately on the basis of 0 to 3.

The bleeding was assessed by running the probe gently along the wall of soft tissues of the gingival sulcusand waiting 30 seconds before visual inspection of the gingiva for areas of bleeding.

The scores on four areas of the tooth were summed up and divided by four to give the GI for the tooth. The GI for the individual was obtained by adding the values of each tooth and dividing by the number of teeth examined. A score from 0.1-1.0 implies mild inflammation; 1.1-2.0 is moderate inflammation and 2.1-3.0 signifies severe inflammation (Appendix IV).

3.15 Periodontal health assessment

Finally, data on periodontal status was collected by basic periodontal examination (BPE). All the present teeth wereexamined excluding the 3rd molars

1. The dentition was divided into 6 sextants upper right (17-14),upper anterior (13-23)upper left (24-27),lower right (47-44) lower anterior (43-33)lower left (34-37).
2. All teeth in each sextant were examined with exception of third molar unless 1st or 2nd molars are missing
3. For a sextant to qualify for recording, ithad to have had at least two teeth
4. A WHO probe was used. This has a ball end 0.5 mm in diameter and a black band from 3.5mm to 5.5mm, light probing force was used.
5. The probe was passed around all teeth in each sextant. All sites were examined to ensure that the highest score in the sextant was recorded before moving on to the next sextant.

This index integrates gingival inflammation, presence of calculus and overhanging margins and pocket depth to determine a particular score for a given sextant see (AppendixV)[40]

3.16 Infection control

Precautions were taken to protect the participants, the principle investigator and other users of the clinic from the risk of cross infection.

Disinfection of the dental chair before sitting the participant was done. The principle investigator thoroughly washed her hands and wore a clean whitecoat.Gloves and facemasks were also used.

Each study participant was draped with a disposable bib and given a disposable plastic tumbler for mouth rinsing.

Sterile instruments in a sterile dental instrument tray were used for the clinical evaluation

Prepacked sterile centrifugation tubes were used to avoid spillage and packed into a clean cool box at 4degrees Celsius fortransportation to the laboratory

Saliva handling was done under supervisionby alaboratory technologist in compliance with biosafety protocols

Waste disposal was according to hospital guidelines and the used instruments and trays were taken to the central sterilization unit for cleaning and then packaged, sterilized for the next clinical session

3.17 Data collection- laboratory stage

The laboratory stage was carried out at the Kenya AIDS Vaccine Initiative (KAVI), Institute of Clinical Research, College of Health Sciences University of Nairobi. It involved several stages.

3.18 Centrifugation and storage of saliva samples

Each saliva sample received in the laboratory was assigned a serial number (001-161) and recorded. The samples wereimmediately centrifuged at 1800rpm for 10 minutes at 20 degrees Celsius (Eppendorf® 5804 Centrifuge) to remove impurities and cellular debris and minimize turbidity of saliva which can negatively impact the accuracy of the results[45]The supernatant was collected and aliquoted in 500µL using micropipettes into clean microcap tubes (Micro tube 2ml,PP – Sarstedt, Germany) and appropriately labelled. Two aliquots were made from each saliva sample and kept in ultra- low temperature freezer at -80°C until processing (U360 Innova® freezer, New Brunswick Scientific, last serviced by Biologic Solutions Limited in May,2018)

Assay procedure

The total protein content for the samples was assayed using a commercial kit Bicinchoninic Acid Protein (**Pierce™ BCA) Assay**Kit according to the manufacturer's instructions.

Preparation of standards and working reagents

Preparation of diluted albumin (BSA) standards

As per manufactures instructions, the contents of one Albumin Standard (BSA) ampule were diluted into several clean vials. Thiswas done using the same diluentsterile -filtered, bioreagent (@sigma-Aldrich, Inc) as the sample. Each 1mL ampule of 2mg/mL Albumin Standard prepared a set of 9

of diluted standards for either working range. As shown in diagram

Table 1: Preparation of Diluted Albumin standards

Preparation of Diluted Albumin (BSA) Standards

Dilution Scheme for Standard Test Tube Protocol and Microplate Procedure (Working Range = 20-2,000 g/mL)

Vial	Volume of Diluent (L)	Volume and Source of BSA (L)	Final BSA Concentration (g/mL)
A	0	300 of Stock	2000
B	125	375 of Stock	1500
C	325	325 of Stock	1000
D	175	175 of vial B dilution	750
E	325	325 of vial C dilution	500
F	325	325 of vial E dilution	250
G	325	325 of vial F dilution	125
H	400	100 of vial G dilution	25
I	400	0	0 = Blank

3.19 **Preparation of BCAWorking Reagent**

As per manufactures instructions, the total volume of Working Reagent required was calculated.

(# standards + # unknowns) (# replicates) (volumeof WR per sample) = total volume WR required

The Working Reagent was prepared by mixing 50 parts ofBCAreagentA with 1 part of BCA reagent B (50;1)(reagent A:B). when reagent B was first added to reagentA, turbidity was observed that quickly disappeared upon mixing to yield a clear green Working reagent as shown in figure 1

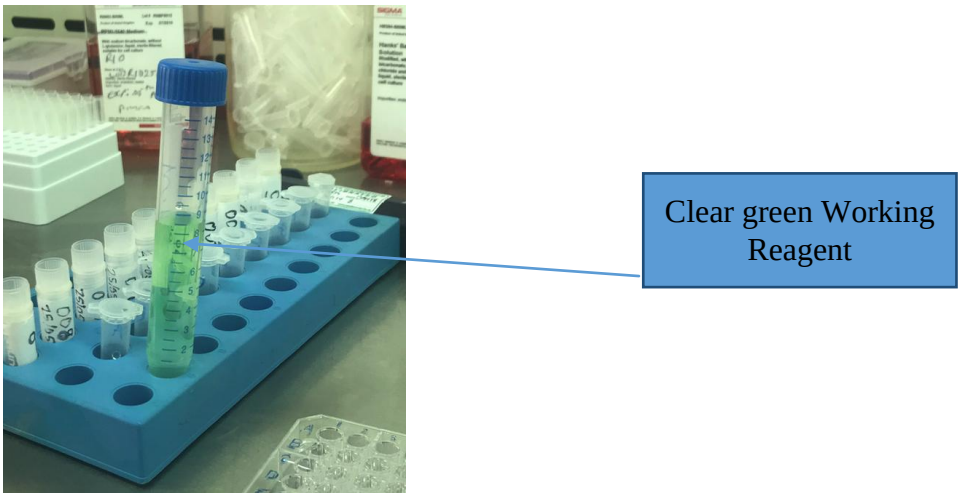

Figure 1: Showing Working Reagent

NB: Samples were diluted to *8 and 25 microliterof diluted sample (*8) was used

Plate preparation

25µL of each standard and sample were pipetted into a microplate well (Greiner -bio-one 96-Well Plates, Product No. 655001). Each microplate containing 12rows and 8columnsclearly indicating the positions of the standards and the samples. As shown in figure 2.

200µL of the Working Reagent wasthen added to each well and the microplate mixedthoroughly on a plate shaker for 30 seconds as shown in figure 3. The Platewas thencovered and incubated at 37°C for 30 minutes, and cooled to Room temperature.

The absorbance measured at 562nm on a Tecan platereader (INFINITY M200) last serviced may 7th 2018 by biologic solutions Ltd Nairobi Kenya (appendix XII).

The results were read out from the Tecan plate reader software, copiedto Microsoft excel 2007 and exported for analysis.

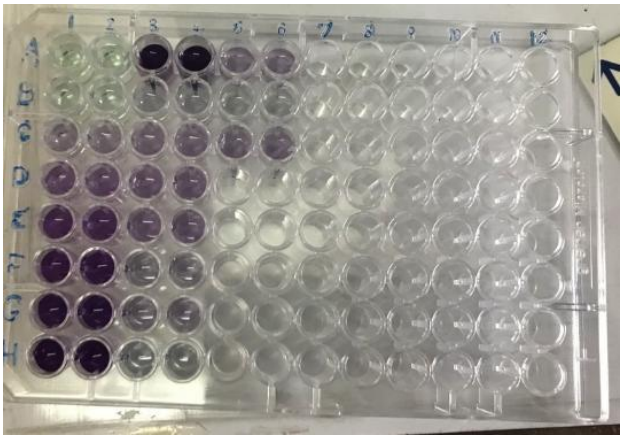

Figure 2: showing microplate well

Figure 3 Showing plate shaker

3.20 Calculation of total protein

A standard curve was prepared usingMicrosoft excel2007 software by plotting the average Blank-corrected 562nm absorbance on the x -axis against the corresponding concentration corresponding concentration on the y- axis A best fit line was generated by third orderpolynomial equation and used to calculate concentration of proteinin each saliva sample in µg/ml.The values were then converted mg/ml

Minimizing laboratory errors

Procedures were done following manufacturer's instructions regarding reconstitution, working concentrations, storage conditions, incubation periods and assay procedures. The procedures were performed using clean gloves to avoid contamination. The aspiration and washing process for each plate using the auto washer machine was thorough and consistent. At each step, a fresh reagent reservoir and pipette tips were used to avoid

cross contamination. Pipetting was done with great care and repeated at intervals to ensure accuracy. Reagents were reconstituted and used immediately to eliminate repeated thawing freezing cycles. The assays were performed in duplicates.

3.21 Reliability and validity, calibration

A number of measures were put in place to ensure that assessment tools produced stable, consistent and credible results. A pilot phase was carried out to ascertain the validity and reliability of questionnaires, clinical examination forms and instruments. Saliva collection protocol was also assessed in the pilot phase.

All the clinical measurements were carried out by the principal investigator. Intra examiner reliability was determined through double evaluation of every10^{th}patient by the principal investigator. For inter- examiner reliability, the principal investigator wascalibrated by one of thesupervisors(EW) who is a consultant periodontist. Cohen's kappa score was used to calculate both inter- examiner and intra-examiner reliability. A score of 80% was accepted for inter-examiner reliability.Cohen's κ was run to determine intra-rater reliability on whether the participants exhibited normal (>2mg/ml) or abnormal (<2mg/ml) total salivary protein levels. There was strong agreement between the researcher's grading, $\kappa = .893$ (95% CI, .349 to .895), $p < 0.001$.

Transportation, processing and storage of saliva samples were done in consultation with a senior laboratory technologist to ensure safety and viability. All the equipment and machines used in the study were calibrated and passed quality assurance and quality control checks

Dummy samples were used for a test run before the actual assay to confirm that the analytical procedures employed were suitable for their intended use. The samples were assayed against standard reagents and in duplicates for reliability and trueness. Repeat tests were carried out at given intervals to assess reproducibility and validity.

All the standards and reagents were sourced from the same supplier for precision and reproducibility. The principal investigator was trained on BCA and assisted by only one laboratory technician who was blinded to the clinical findings of the participants (the

clinical data were not available to the laboratory staff). Only equipment that had been calibrated according to set international standards wereused.

Data processing included cleaning and validation with elimination of entries that were obviously erroneous. Extreme outliers were excluded from tests of association through systematic statistical tests.

3.22 Data entry, analysis and presentation

The collected data was entered, cleaned and validated. Coding and analysis was done by Statistical Packages for Social Sciences (SPSS) 25.0 for windows (SPSS Inc. Chicago, Illinois, USA) and Microsoft Excel 2013.

Descriptive statistics were used in the analysis of categorical data like gender, frequency of brushing, smoking.Theseincluded frequencies and percentages. Continuous data like age, total salivary protein was described using mean, range and standard deviation

Comparison of means and proportions were done using independent samples t test. Analysis of variance (ANOVA) and Spearman's rank correlation were also used where appropriate. Independence of the association of totalsalivary proteinlevels with the disease status was done through hierarchical multiple linear regression analysis, whilst adjusting for confounders such as age strata and alcohol consumption

Confidence level was set at 95% (α level 0.05). Presentation of findings was done using tables andgraphs.

3.23 Ethical considerations

Permission to carry out this study was given by Kenyatta National Hospital – University of Nairobi Ethics andResearch Committeenumber P53/02/2018

Written consent was individually obtained for every subject. No study participant was subjected to any study procedure without a signed consent form. Signature or thumbprint was accepted as proof of voluntary consent.

Permission to carry out research at the University of Nairobi Dental Hospital wasgiven by the Dean, School of Dental Sciences and the Chairman of Department of Periodontology. Patients who required emergency treatment weretreated in the dental

school clinics, and those with periodontal disease referred to the periodontology clinic for further management.

3.24Consenting process

The process begun with an explanation to the participant of the aim of the study, followed by an explanation about the procedure for saliva sample collection and finally the process of the intra oral examination by the Principle Investigator. Any questionsbyparticipants were addressed. The consent was obtained by the participant signing the consent form or thumb print for those unable to sign (Appendix VII).

CHAPTER FOUR

RESULTS

4.1 Socio-demographic characteristics

A total of 161 participants were included in the study. Of the 161, 87 (54.0%) being female and74 (46.0%) were male. The age of the participants ranged between 18 – 80 with a mean of 38.34 years (± 13.44 SD) and a median of 37.00. The male participants were slightly older with a mean age of 38.78 (± 14.41 SD) compared to the female participants with a mean age of 37.95 (± 12.64 SD). The difference however, was not statistically significant (t (159) = 0.389, p = 0.698, two-tailed).

4.2 Total Salivary protein characteristics

The salivary protein of the participants ranged between 0.11mg/ml – 12.17mg/ml with a mean of 2.03mg/ml ± 1.97 SD and a median of 1.38.(figure 4)There was a positive statistically significant difference in the variance of salivary protein levels between gender where males (M = 2.39±2.16SD) had higher salivary protein levels than females (M = 1.72±1.75SD), t (140.220) = 2.156, p = 0.033, two-tailed (figure 5)(table 2)

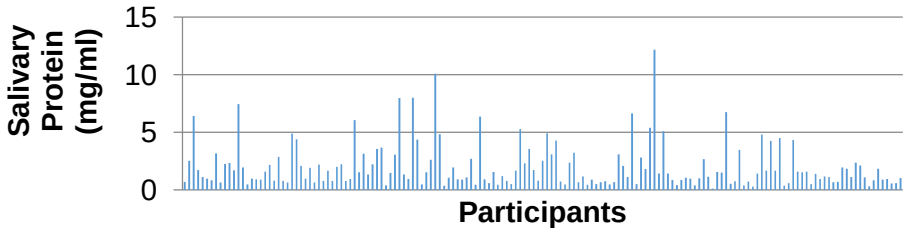

Figure 4: Distribution of salivary protein (mg/ml) by participants

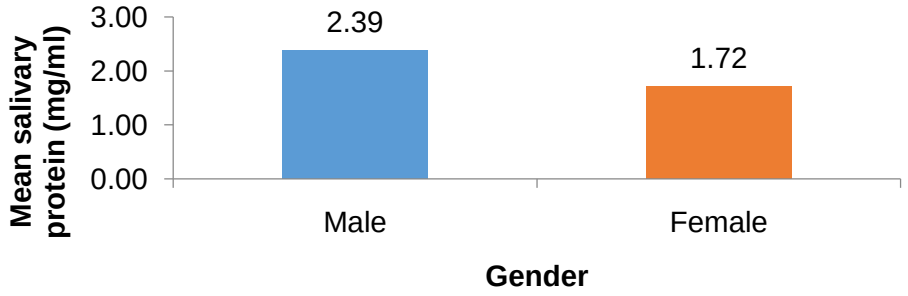

Figure 5: Comparison of mean salivary protein by gender

Table 2: Salivary protein characteristics of participants (n = 161)

Salivary protein (mg/ml)									
					95% Confidence Interval for Mean				
Characteristics		*n*	*M*	*SD*	Lower Bound	Upper Bound	*df*	Statistical Test	*p*
Gender	Male	74	2.39	2.16	0.06	1.29	140.220	$t = 2.156$	0.033*
	Female	87	1.72	1.75					
Education	None	2	1.95	1.60	-12.46	16.36	3, 157	$F = 0.142$	0.935
	Primary	8	1.62	2.03	-0.07	3.32			
	Secondary	81	2.09	2.07	1.63	2.55			
	Tertiary	70	2.00	1.89	1.55	2.45			
Marital status	Married	92	2.21	2.31	1.73	2.69	3, 157	$F = 0.633$	0.595
	Single	63	1.79	1.42	1.43	2.15			
	Divorced	3	1.52	0.84	-0.56	3.59			
	Widowed	3	1.88	1.23	-1.18	4.95			
Occupation	Non-skilled	14	1.20	0.85	0.71	1.69	3, 157	$F = 1.335$	0.265
	Skilled	55	2.34	2.18	1.75	2.93			
	Professional	77	1.95	1.96	1.50	2.39			
	Others	15	2.09	1.84	1.07	3.11			
Brushing	Once daily	66	1.93	1.98	1.44	2.42	2, 158	$F = 0.398$	0.672
	Twice daily	92	2.07	1.97	1.66	2.48			
	>Twice daily	3	2.91	2.05	-2.18	8.01			
Brushing aid	Conventional	160	2.04	1.97	-2.37	5.45	159	$t = 0.779$	0.437
(Tooth brush)	Electric	1	0.50	.					
Dentifrice	Conventional	160	2.03	1.98	-3.40	4.43	159	$t = 0.259$	0.796
(Tooth paste)	Herbal	1	1.52	.					
Alcohol consumption	Teetotaler	108	1.82	1.74	1.49	2.15	2, 158	$F = 1.868$	0.158
	Social drinker	49	2.47	2.37	1.79	3.15			
	Regular drinker	4	2.24	2.07	-1.06	5.54			

Independent-Samples t test was used for gender, brushing aid and dentifrice.

Analysis of Variance (ANOVA) was used for education, marital status, occupation, brushing and alcohol consumption.

* $p < 0.05$.

4.3 Oral Hygiene and Periodontal Health Status

4.3.1 Oral hygiene practices

Tooth brushing was reported by all participants. Majority 92 (57.1%) brushed their teeth twice daily with 66 (41.0%) brushing once daily and 3 (1.9%) brushing more than twice daily.

4.3.2 Oral hygiene status

Oral hygiene status of the participants was assessed using plaque scores. The plaque scores of the participants ranged between 0.17 – 3.00 with a mean of 1.25 $\pm$ 0.66 SD and a median of 1.08 showing that every participant had some degree of plaque deposits on their teeth surfaces. Majority, 113 (70.2%) had mild plaque while 40 (24.85) had moderate plaque and 8 (5.0%) had severe plaque.

An independent samples t test was performed comparing the plaque scores between gender elicited a positive statistically significant difference in the variances where male participants(M = 1.37$\pm$0.67SD) had higher plaque scores than female ones (M = 1.15$\pm$0.64SD), t (159) = 2.152, p = 0.033, two-tailed (table 3).

An analysis of variance showed that the difference in plaque scores among education levels of participants was statistically significant, with low education category having higher plaque scores$F$ (3, 157) = 4.383, p = 0.005, two-tailed as shown in table 2. Tukey's post hoc test revealed a statistically significant critical difference (M = 0.31, p = 0.005) in plaque scores between secondary (M = 1.38 $\pm$ 0.65 SD) and tertiary (M = 1.06 $\pm$ 0.60 SD) levels of education (table 3).

The plaque scores were put into three categories of mild, moderate and severe and tested against various variables for associations (table 4). There was statistical significance in the association between plaque scores and education (*Fisher's* = 6.604, p = 0.036, two-tailed)

Table 3: Comparison of socio – demographic characteristics and plaque index (n =161)

Plaque scores									
					95% Confidence Interval for Mean				
Characteristics		*n*	*M*	*SD*	Lower Bound	Upper Bound	*df*	Statistical Test	*p*
Gender	Male	74	1.37	0.67	0.02	0.43	159	$t = 2.152$	0.033*
	Female	87	1.15	0.64					
Education	None	2	0.83	0.24	-1.28	2.95	3, 157	$F = 4.383$	0.005**
	Primary	8	1.66	0.95	0.87	2.45			
	Secondary	81	1.38	0.65	1.24	1.52			
	Tertiary	70	1.06	0.60	0.92	1.21			
Marital status	Married	92	1.25	0.65	1.12	1.39	3, 157	$F = 0.207$	0.891
	Single	63	1.26	0.68	1.09	1.43			
	Divorced	3	1.14	0.97	-1.26	3.54			
	Widowed	3	0.97	0.65	-0.64	2.58			
Occupation	Non-skilled	14	1.48	0.60	1.13	1.82	3, 157	$F = 1.483$	0.221
	Skilled	55	1.34	0.70	1.15	1.53			
	Professional	77	1.16	0.66	1.01	1.31			
	Others	15	1.14	0.54	0.84	1.44			
Brushing	Once daily	66	1.14	0.66	0.98	1.31	2, 158	$F = 1.450$	0.238
	Twice daily	92	1.33	0.66	1.19	1.46			
	>Twice daily	3	1.22	0.82	-0.82	3.26			
Brushing aid	Conventional	160	1.24	0.66	-2.48	0.13	159	$t = 1.789$	0.077
(Tooth brush)	Electric	1	2.42	.					
Dentifrice	Conventional	160	1.25	0.66	-1.15	1.48	159	$t = 0.250$	0.803
(Tooth paste)	Herbal	1	1.08	.					
Alcohol consumption	Teetotaler	108	1.26	0.68	1.13	1.39	2, 158	$F = 0.078$	0.925
	Social drinker	49	1.22	0.64	1.03	1.40			
	Regular drinker	4	1.25	0.59	0.32	2.18			

Independent-Samples t test was used for gender, brushing aid and dentifrice.

Analysis of Variance (ANOVA) was used for education, marital status, occupation, frequency of brushing and alcohol consumption.

** $p < 0.01$.

* $p < 0.05$.

Table 4: Association of socio – demographic characteristics and plaque index (n = 161)

Characteristics		Plaque score					
		Mild	Moderate	Severe	Statistical Test		*p*
		n (%)	*n* (%)	*n* (%)			
Gender	Male	49 (43.4)	19 (47.5)	6 (75.0)	*Fisher's* 2.954	=	0.240
	Female	64 (56.6)	21 (52.5)	2 (25.0)			
Education	<= Secondary	57 (50.4)	27 (67.5)	7 (87.5)	*Fisher's* 6.604	=	0.036 *
	Tertiary	56 (49.6)	13 (32.5)	1 (12.5)			
Marital status	Married	65 (57.5)	21 (52.5)	6 (75.0)	*Fisher's* 1.316	=	0.558
	Single/widowed	48 (42.5)	19 (47.5)	2 (25.0)			
Occupation	Skilled/professional	96 (85.0)	28 (70.0)	8 (100.0)	*Fisher's* 5.497	=	0.055
	Non-skilled/Others	17 (15.0)	12 (30.0)	0			
Brushing	Once daily	46 (40.7)	18 (45.0)	2 (25.0)	*Fisher's* 1.037	=	0.575
	>= Twice daily	67 (59.3)	22 (55.0)	6 (75.0)			
Alcohol consumption	Teetotaler	75 (66.4)	27 (67.5)	6 (75.0)	*Fisher's* 0.214	=	1.000
	Social/regular drinker	38 (33.6)	13 (32.5)	2 (25.0)			

Fisher's Exact test was used for gender, education, marital status, occupation, brushing and alcohol consumption.

* $p < 0.05$.

4.4 Association between plaque and salivary protein.

The plaque scores were put into three categories of mild, moderate and severe and tested against mean salivary protein. Majority, 113 (70.2%) had mild plaque while 40 (24.85) had moderate plaque and 8 (5.0%) had severe plaque. The highest mean salivary protein was found in study participants with severe plaque deposits,the lowest mean salivary protein was observed in participants with mild plaque deposits (figure 6)

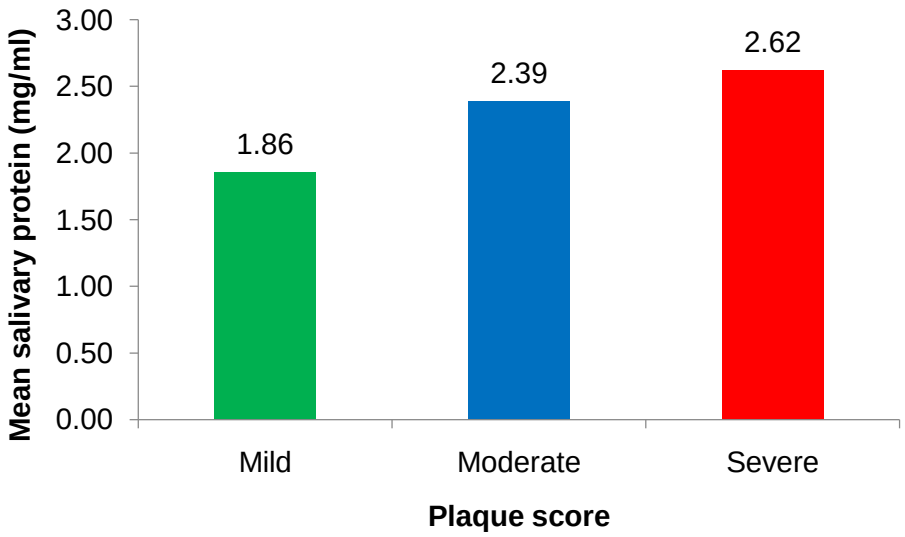

Figure 6: Comparison of salivary protein(mg/ml) by plaque severity

A linear regression curve estimation model revealed a non-statistically significant association between plaque and salivary protein as the predictor variable (β = 0.312, $F(1,159) = 1.768$, $R^2 = 0.011$, $p = 0.185$) (figure 7).

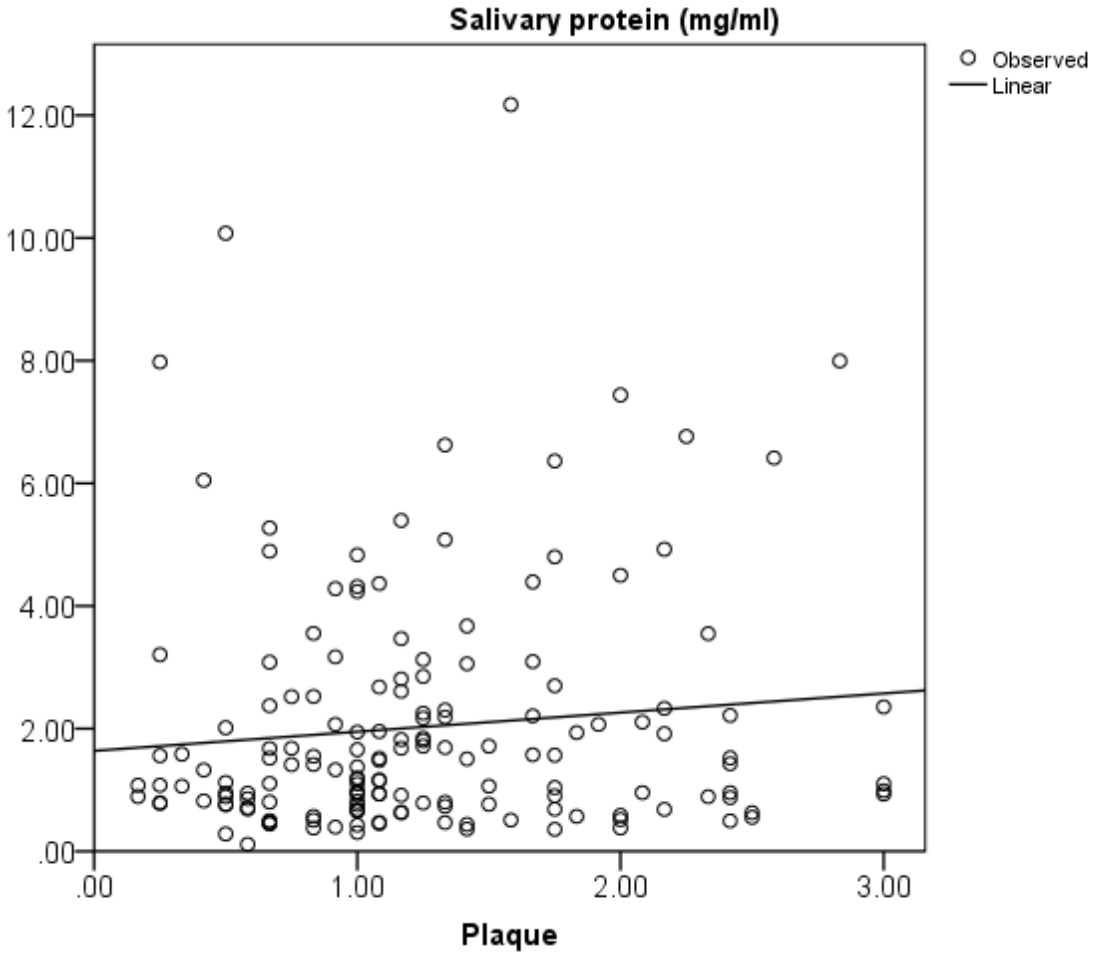

Figure 7: Regression plot model for plaque score and salivary protein

4.5 Association between plaque and periodontitis.

A linear regression curve estimation model elicited a positive statistically significant association between Basic Periodontal Examination (BPE) and plaque as the predictor variable (β = 0.873, $F(1,159)$ = 73.292, R^2 = 0.316, p< 0.001)(figure 8).

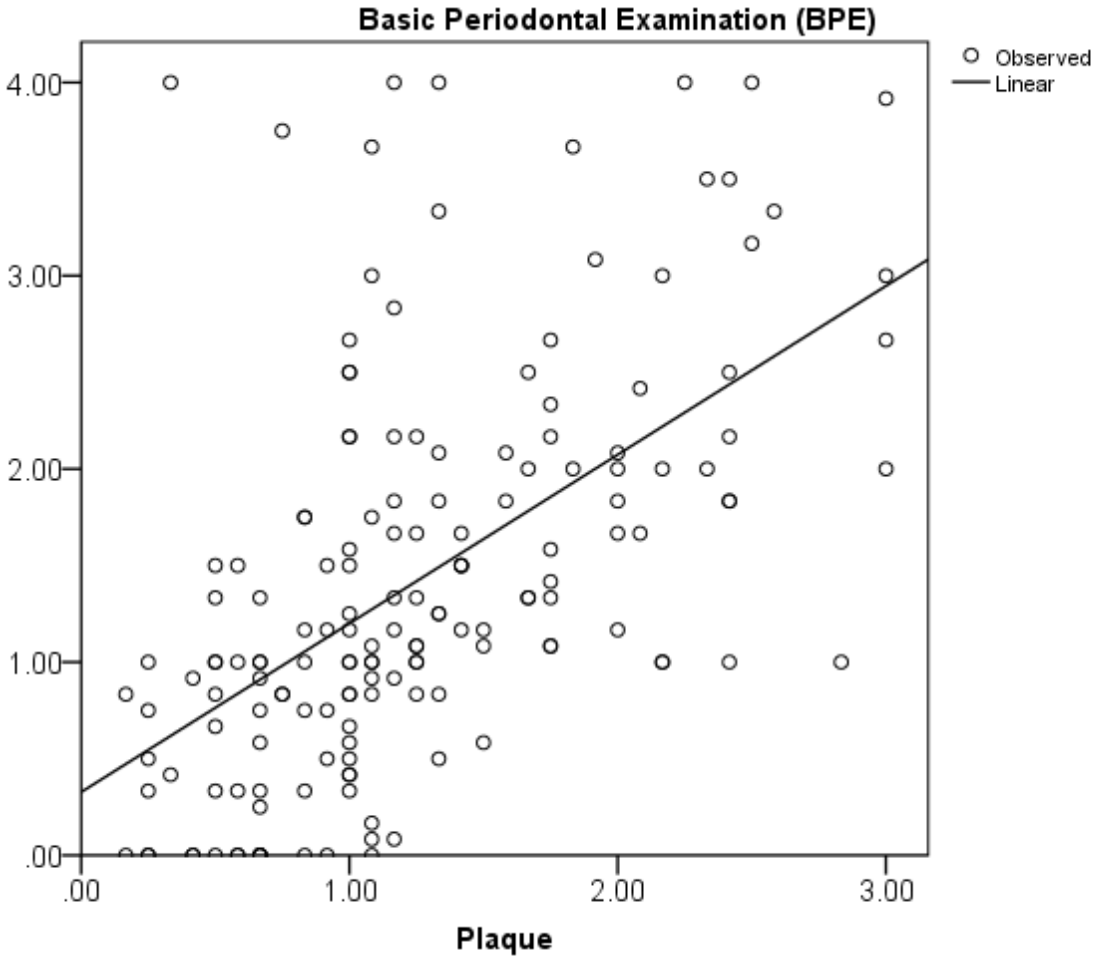

Figure 8: Regression plot model for plaque and Basic Periodontal Examination (BPE)

4.6 Association between plaque and gingivitis

A linear regression curve estimation model elicited a positive statistically significant association between gingival scores and plaque as the predictor variable (β = 0.740, $F(1,159) = 268.414$, $R^2 = 0.628$, $p < 0.001$) (figure 9).

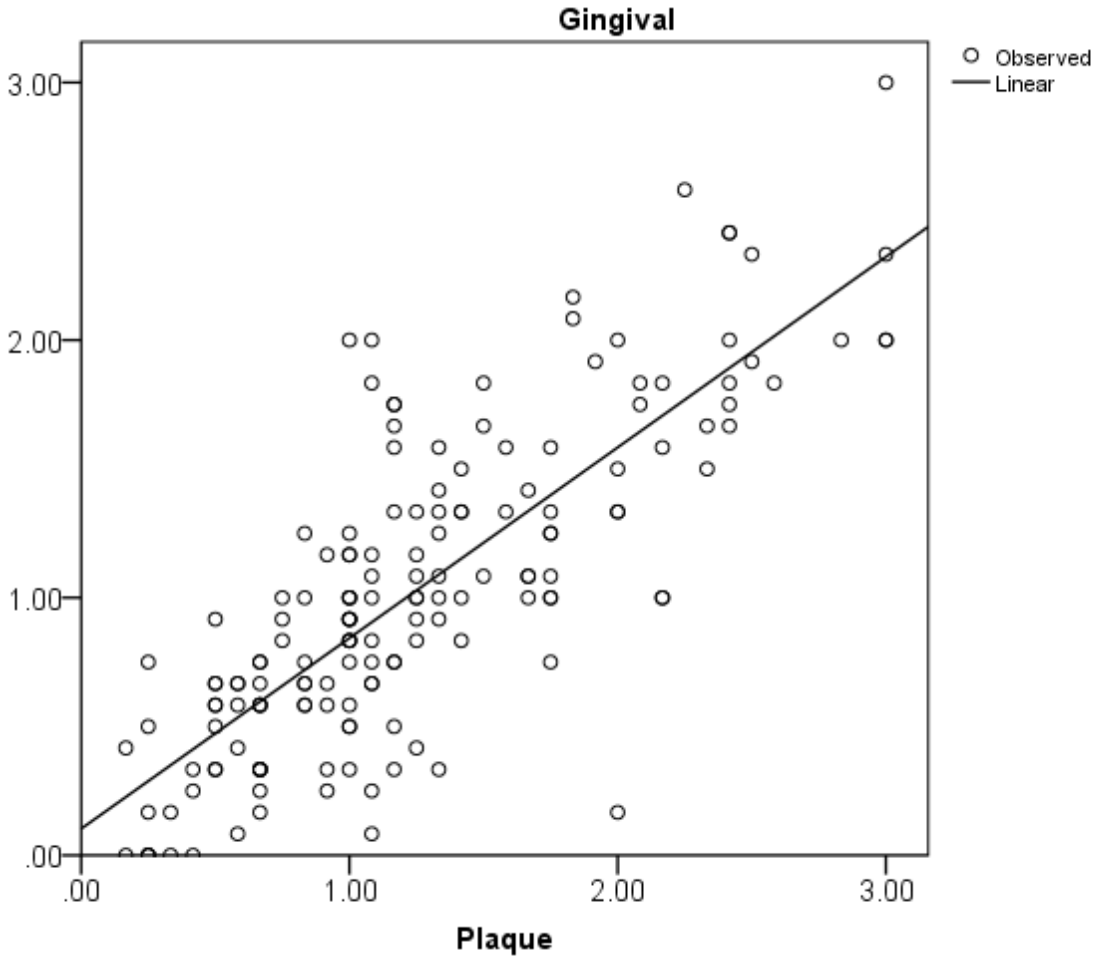

Figure 9: Regression plot model for plaque and gingival scores

4.7 Gingival inflammation (Gingivitis)

The degree of gingival inflammation was assessed using the gingival index Loe and Sillness 1963. The gingival scores of the participants ranged between 0.0 – 3.00 with a mean of 1.03 $\pm$ 0.62 SD and a median of 1.00 showing that every participant had some degree of gingivitis. Whereas 6 (3.7%) of the participants did not have gingival inflammation, majority 114 (70.8%) had mild gingival inflammation while 37 (23.0%) had moderate and 2 (1.2%) had severe gingival inflammation.

Social demographics and gingival scores

Anindependent samples t test was performed comparing the gingival scores between gender elicited a positive statistically significant difference in the variances where male participants (M = 1.13$\pm$0.65SD) had higher gingival scores than female ones (M = 0.94$\pm$0.58SD), t (159) = 1.992, p = 0.048, two-tailed (table 5).

An independent samples t test elicited a statistically significant difference in the variances of gingival scores between brushing aids used where participants who used electric tooth brushes (M = 2.42$\pm SD$) had higher gingival scores than participants who used conventional tooth brushes (M = 1.02$\pm$0.61 SD), t (159) = 2.283, p = 0.024, two-tailed (table 5).

There were non-statistically significant differences in the variances of gingival scores in relation to education levels, marital status, occupation, brushing frequency, dentifrice and alcohol consumption

Table 5: Relationship between gingival scores and demographic parameters (n = 161)

Gingival scores									
					95% Confidence Interval for Mean				
Characteristics		*n*	*M*	*SD*	Lower Bound	Upper Bound	*df*	Statistical Test	*p*
Gender	Male	74	1.13	0.65	0.00	0.38	159	$t = 1.992$	0.048*
	Female	87	0.93	0.985					
Education	None	2	0.46	0.18	-1.13	2.05	3, 157	$F = 2.331$	0.076
	Primary	8	1.27	0.60	0.77	1.77			
	Secondary	81	1.11	0.57	0.99	1.24			
	Tertiary	70	0.92	0.66	0.76	1.07			
Marital status	Married	92	1.05	0.58	0.93	1.17	3, 157	$F = 0.731$	0.535
	Single	63	0.97	0.65	0.80	1.13			
	Divorced	3	1.44	0.82	-0.60	3.49			
	Widowed	3	1.14	0.84	-0.96	3.23			
Occupation	Non-skilled	14	1.12	0.54	0.81	1.43	3, 157	$F = 0.759$	0.519
	Skilled	55	1.11	0.59	0.95	1.27			
	Professional	77	0.96	0.66	0.81	1.11			
	Others	15	0.98	0.59	0.65	1.31			
Brushing	Once daily	66	0.96	0.63	0.81	1.12	2, 158	$F = 1.339$	0.265
	Twice daily	92	1.09	0.61	0.96	1.21			
	>Twice daily	3	0.64	0.34	-0.20	1.48			
Brushing aid	Conventional	160	1.02	0.61	-2.61	-0.19	159	$t = 2.283$	0.024*
(Tooth brush)	Electric	1	2.42	.					
Dentifrice	Conventional	160	1.03	0.62	-0.27	2.17	159	$t = 1.538$	0.126
(Tooth paste)	Herbal	1	0.08	.					
Alcohol consumption	Teetotaler	108	1.05	0.59	0.94	1.16	2, 158	$F = 0.198$	0.821
	Social drinker	49	0.98	0.68	0.79	1.18			
	Regular drinker	4	1.02	0.77	-0.21	2.25			

Independent-Samples t test was used for gender, brushing aid and dentifrice.

Analysis of Variance (ANOVA) was used for education, marital status, occupation, brushing and alcohol consumption.

* $p < 0.05$.

A Fisher's exact test was performed to examine the relation between socio-demographic characteristics of participants and mean gingivalscores. The relation between education levels and gingival scores was statistically significant, Fisher's = 8.600, p = 0.019, two-tailed as shown in table6

There were non-statistically significant relations between gingival scores and gender, marital status, occupation, brushing frequency and alcohol consumption as shown in table 6.

Table 6: Association of socio-demographic characteristics, habits and gingival index (n = 161)

			Gingival index					
Characteristics		*n*	Absence	Mild	Moderate	Severe	Statistical Test	*P*
Gender	Male	74	2	48	21	1	Fisher's = 4.752	0.157
	Female	87	4	66	16	1		
Education	<= Secondary	91	1	66	23	1	Fisher's = 8.600	0.019*
	Tetiary	70	5	48	14	1		
Marital status	Married	92	3	69	18	1	Fisher's = 2.950	0.379
	Single/ Widowed	69	3	45	19	1		
Occupation	Skilled/ Professional	132	5	93	30	1	Fisher's = 1.011	0.813
	Non-Skilled/ Others	29	1	21	7	1		
Brushing	Once daily	66	4	46	16	1	Fisher's = 2.705	0.463
	Twice/ more daily	95	2	68	21	1		
Alcohol consumption	Teetotaler	108	3	81	23	1	Fisher's = 2.805	0.398
	Social/ Regular drinker	53	3	33	14	1		

Fisher's Exact test was used for gender, education, marital status, occupation, brushing and alcohol consumption.

* $p < 0.05$

4.8 Correlation between salivary total protein and gingivitis in study participants

The mean total salivary protein of study participants with Mild gingivitis was 1.87mg/ml,Moderate gingivitis was 2.45 mg/ml and Severe gingivitis 3.93mg/ml as shown in figure 10. The highest mean salivary protein was found in the severe gingivitis group.

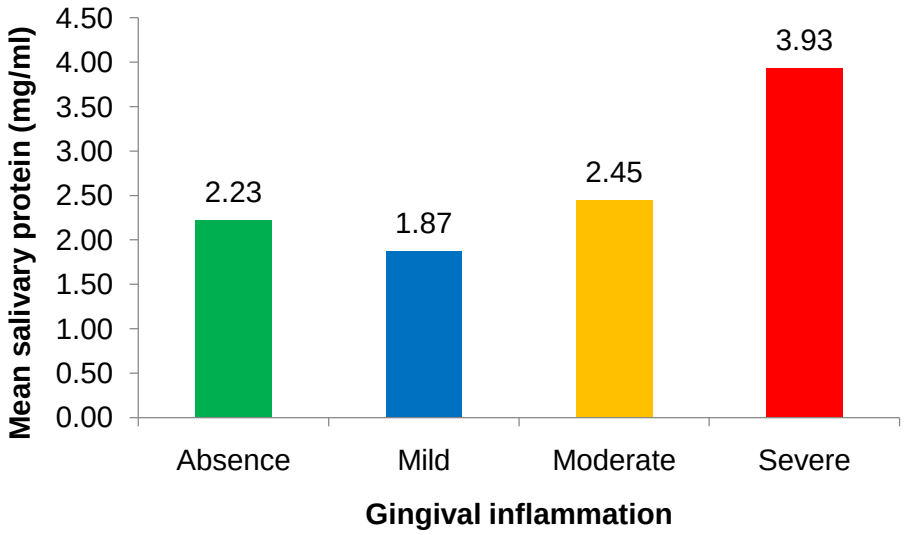

Figure 10: Comparison of salivary protein(mg/ml) by gingivitis

A linear regression curve estimation model (figure 11). showed a mild positive association between salivary protein and gingival inflammation as the predictor variable however it was not statistically significant (β = 0.196, $F(1,159)$ = 0.430, R^2 = 0.003, p = 0.513).

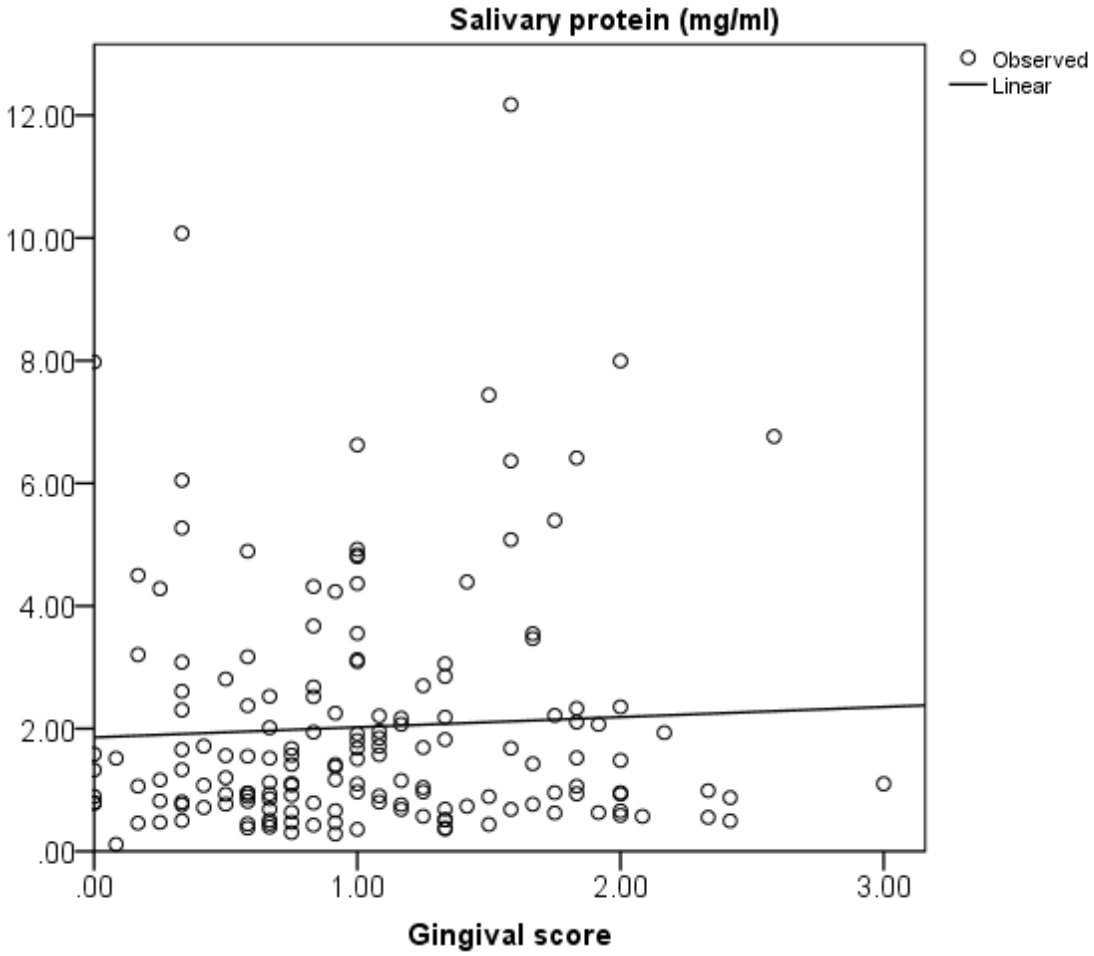

Figure 11: Regression plot model for salivary protein and gingival score

4.9 Correlation between gingivitis and periodontitis in study participants

A linear regression curve estimation model (figure 12) elicited a positive statistically significant association between Basic Periodontal Examination (BPE) scores and gingival scores as the predictor variable (β = 1.103, $F(1,159) = 124.447$, $R^2 = 0.439$, $p < 0.001$).

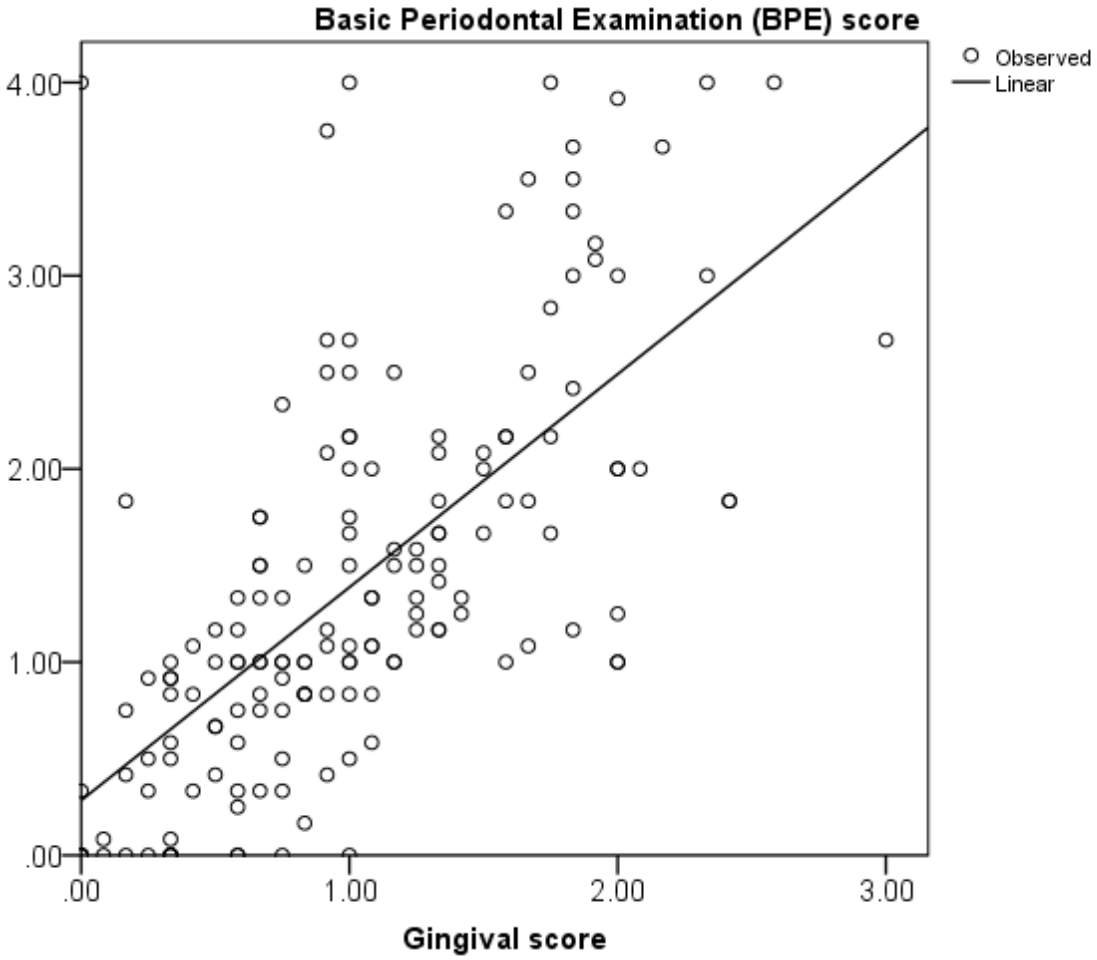

Figure 12: Regression plot model for Basic Periodontal Examination (BPE) and gingival scores

4.10 Periodontal health

The presence or absence of periodontitis and the severity thereof was assessed using Basic Periodontal Examination.

The Basic Periodontal Examination scores of the participants ranged between 0.0 – 4.0. Whereas 15 (9.3%) were healthy, majority 120 (74.6%) had gingivitis while 15 (9.3%) had mild and 11 (6.8%) had severe periodontitis.

An independent samples t test was performed comparing the mean Basic Periodontal Examination scores between gender elicited a positive statistically significant difference in the variances where male participants(M = 1.69$\pm$1.12SD) had higher scores than females (M = 1.19$\pm$0.89SD), t (138.64) = 3.074, p = 0.003, two-tailed as shown in table 7.There were non-statistically significant differences in the variances of Basic Periodontal Examination scores against education levels, marital status, occupation, brushing frequency, brushing aids, dentifrice and alcohol consumption as shown in table 7.

Table 7: Comparison of socio – demographic characteristics and means of Basic Periodontal Examination (n = 161)

Basic Periodontal Examination (BPE)									
					95% Confidence Interval for Mean				
Characteristics		*N*	*M*	*SD*	Lower Bound	Upper Bound	*df*	Statistical Test	*p*
Gender	Male	74	1.69	1.12	0.18	0.82	138.64	$t = 3.074$	0.003**
	Female	87	1.19	0.89					
Education	None	2	0.75	0.24	-1.37	2.87	3, 157	$F = 2.571$	0.056
	Primary	8	2.11	1.20	1.11	3.12			
	Secondary	81	1.52	0.93	1.32	1.73			
	Tertiary	70	1.24	1.09	0.98	1.50			
Marital status	Married	92	1.58	1.06	1.36	1.79	3, 157	$F = 2.025$	0.113
	Single	63	1.18	0.97	0.93	1.42			
	Divorced	3	1.75	1.09	-0.96	4.46			
	Widowed	3	1.33	0.74	-0.51	3.17			
Occupation	Non-skilled	14	1.74	0.93	1.21	2.28	3, 157	$F = 1.328$	0.267
	Skilled	55	1.55	0.98	1.29	1.82			
	Professional	77	1.30	1.11	1.05	1.55			
	Others	15	1.22	0.78	0.78	1.65			
Brushing	Once daily	66	1.29	0.99	1.04	1.53	2, 158	$F = 1.946$	0.146
	Twice daily	92	1.54	1.05	1.32	1.75			
	>Twice daily	3	0.67	0.58	-0.77	2.10			
Brushing aid (Tooth brush)	Conventional	160	1.42	1.03	-2.46	1.63	159	$t = 0.404$	0.687
	Electric	1	1.83	.					
Dentifrice (Tooth paste)	Conventional	160	1.43	1.03	-0.69	3.38	159	$t = 1.303$	0.194
	Herbal	1	0.08	.					
Alcohol consumption	Teetotaler	108	1.46	1.04	1.26	1.65	2, 158	$F = 0.241$	0.786
	Social drinker	49	1.35	1.03	1.05	1.64			
	Regular drinker	4	1.25	0.96	-0.27	2.77			

Independent-Samples t test was used for gender, brushing aid and dentifrice.

Analysis of Variance (ANOVA) was used for education, marital status, occupation brushing and alcohol consumption.

** $p < 0.01$.

A Fisher's exact test was performed to examine the relation between socio-demographic characteristics of participants and basic periodontal examination scores. The relation between gender and basic periodontal examination scores was statistically significant, with males having higher scores than female study participants Fisher's = 8.358, p = 0.036, two-tailed as shown in table 7.

A Fisher's exact test elicited a statistically significant relation between education levels and basic periodontal examination scores, with study participants with low education having higher scores. Fisher's = 14.126, p = 0.002, two-tailed as shown in table 8.

A Fisher's exact test elicited a statistically significant relation between marital status and basic periodontal examination scores, with the single category having lower scores than the other categories Fisher's = 12.445, p = 0.005, two-tailed as shown in table 8

Table 8: Association of socio-demographic characteristics and Basic Periodontal Examination (n = 161)

			Basic Periodontal Examination (BPE)					
Characteristics		*n*	Healthy	Gingivitis	Mild	Severe	Statistical Test	*p*
Gender	Male	74	4	50	10	8	Fisher's = 8.358	0.036*
	Female	87	11	68	5	3		
Education	<= Secondary	91	2	72	11	6	Fisher's = 14.126	0.002**
	Tertiary	70	13	46	4	5		
Marital status	Married	92	5	66	14	7	Fisher's = 12.445	0.005**
	Single/ Widowed	69	10	52	1	4		
Occupation	Skilled/ Professional	132	14	94	13	10	Fisher's = 1.866	0.600
	Non-Skilled/ Others	29	1	24	2	1		
Brushing	Once daily	66	6	50	5	4	Fisher's = 0.570	0.919
	Twice/ more daily	95	9	68	10	7		
Alcohol consumption	Teetotaler	108	9	79	11	9	Fisher's = 1.546	0.684
	Social/ Regular drinker	53	6	39	4	2		

Fisher's Exact test was used for gender, education, marital status, occupation, brushing and alcohol consumption.

** $p < 0.01$.

* $p < 0.05$.

A linear regression curve estimation model (figure 13)elicited a positivestatistically significant association between basic periodontal examination (BPE) and age as the predictor variable (β = 0.297, $F(1,159)$ = 18.529, R^2 = 0.104, p< 0.001).with the older study participants having higher BPE Scores

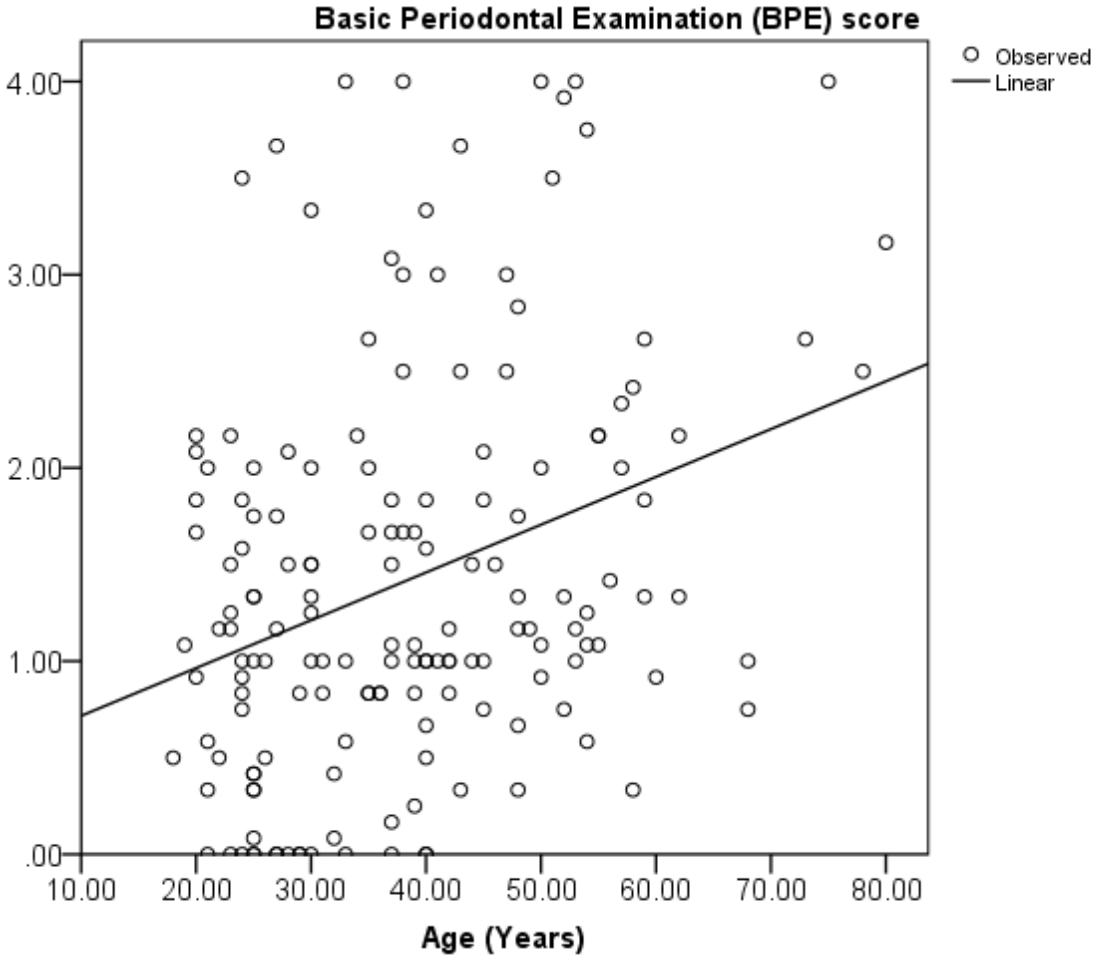

Figure 13: Regression plot model for basic periodontal examination (BPE) and age

4.11 The correlation between salivary total protein and periodontal status in study participants

The mean salivary protein for healthy study participants was 2.42mg/ml,for study participants with gingivitis 1.92mg/ml, mild periodontitis 1.87mg/ml and severe periodontitis 2.84mg/ml as shown in figure 14

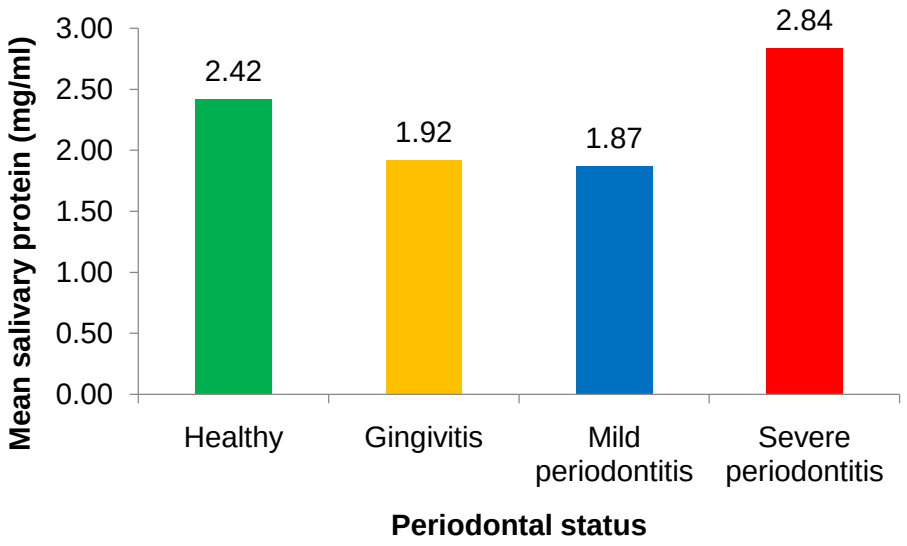

Figure 14Comparison of salivary protein(mg/ml) by Periodontal status

There was a non-statistically significant difference in the variance of salivary protein levels and periodontitis (table 9).

Table 9: Salivary protein characteristics of periodontitis (n = 161)

Salivary protein (mg/ml)									
					95% Confidence Interval for Mean				
Characteristics		*n*	*M*	*SD*	Lower Bound	Upper Bound	*df*	Statistical Test	*p*
BPE	Healthy	15	2.42	3.07	0.72	4.13	3, 157	$F = 0.964$	0.411
	Gingivitis	120	1.92	1.78	1.60	2.25			
	Mild periodontitis	15	1.87	1.76	0.90	2.85			
	Severe periodontitis	11	2.84	2.35	1.26	4.42			

Analysis of Variance (ANOVA) was used for periodontitis.

A Pearson product-moment correlation coefficient showed a mild, positive and non-statistically significant association between salivary protein levels and gingivitis levels ($r = 0.146$, $p = 0.113$).

A Pearson product-moment correlation coefficient showed a strong, positive and statistically significant association between salivary protein levels and mild periodontitis levels ($r = 0.594$*, $p = 0.020$).

A Pearson product-moment correlation coefficient showed a moderate, positive and non-statistically significant association between salivary protein levels and severe periodontitis levels ($r = 0.359$, $p = 0.278$).

A linear regression curve estimation model elicited a statistically significant association between mild periodontitis levels and salivary protein levels as the predictor variable ($\beta = 0.102$, $F(1,13) = 7.075$, $R^2 = 0.352$, $p = 0.020$).

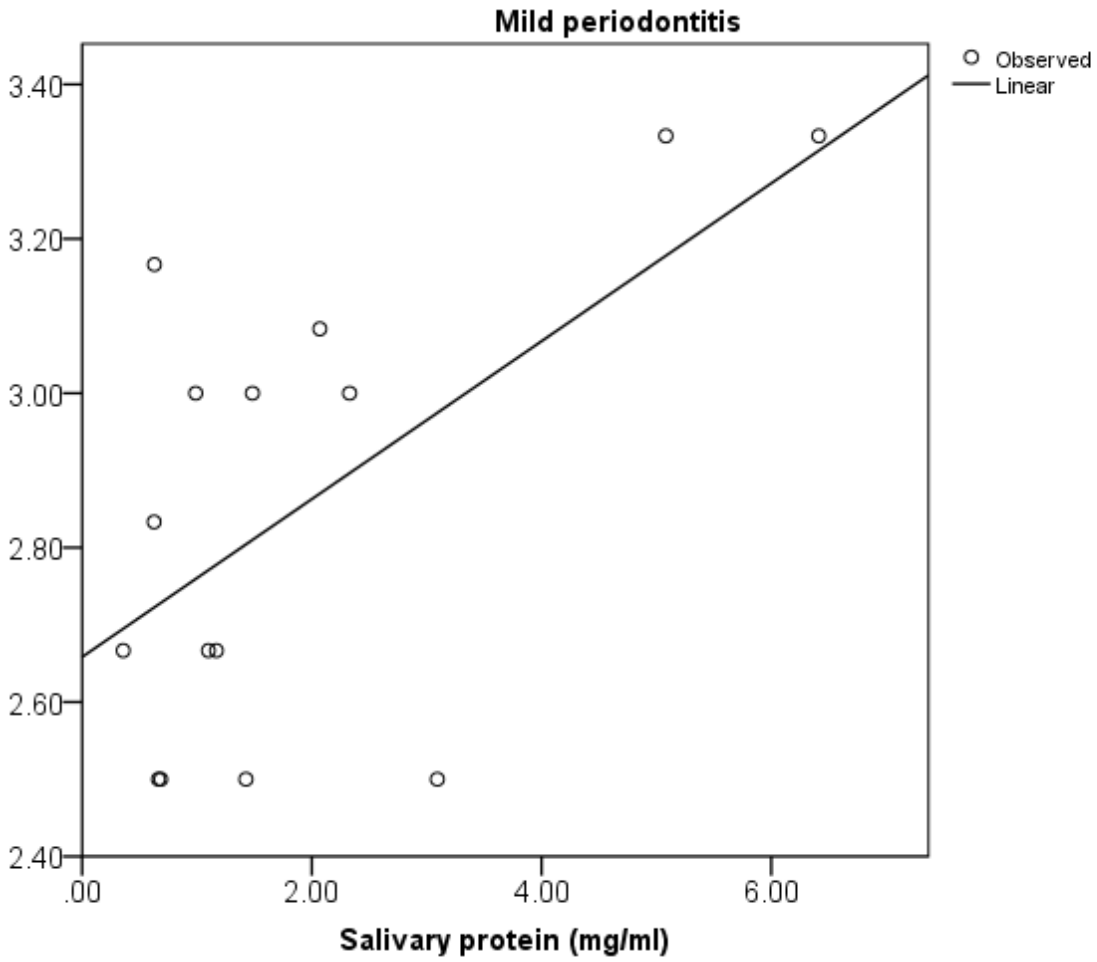

Figure 15: Regression plot model for salivary protein and mild periodontitis

A linear regression curve estimation model (figure16) revealed a mild positive association between total salivary protein and BPE scores however it wasn't -statistically significant. ($\beta = 0.696$, $F(1,159) = 1.982$, $R^2 = 0.012$, $p = 0.161$).

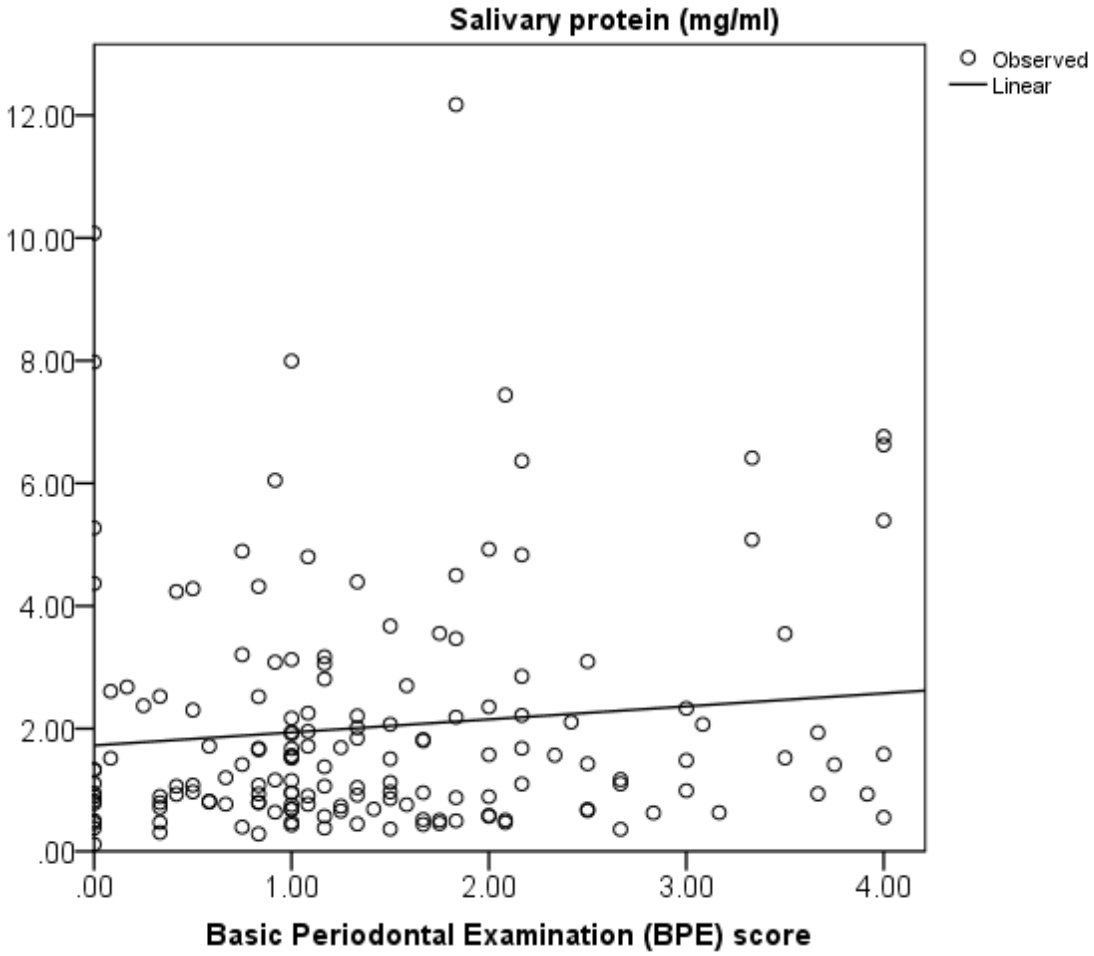

Figure 16: Regression plot model for Basic Periodontal Examination (BPE) and salivary protein

4.12 Association of other variables

A Pearson product-moment correlation coefficient showed a non-statistically significant association between age and salivary protein ($r = 0.025$, $p = 0.751$).

A Pearson product-moment correlation coefficient showed a non-statistically significant association between age and plaque ($r = 0.094$, $p = 0.235$).

A Pearson product-moment correlation coefficient elicited a mild a positive statistically significant association between age and gingival scores ($r = 0.158$, $p = 0.045$) with an increase in age leading to higher gingival scores.

A Pearson product-moment correlation coefficient elicited a statistically significant association between age and basic periodontal examination ($r = 0.323$, $p < 0.001$) showing older study participants having higher BPE Score.

CHAPTER FIVE
DISCUSION

5.1 Social demographics

The age of the participants ranged between 18 – 80years with a mean of 38.34 years ± 13.44 SD and a median of 37.00.Only consenting adults were included in the study. The age range shows variability and diversity in the age of patients seeking dental care at the university of Nairobi Dental School and general population by extension. There were more female participants than males in the study.Thisis in agreementwith Ashley et al 2015 who reported thatfemale subjectshave better health seeking behavior than males[46]

Majority of the participants had secondary education (50.6%) and tertiary education (43%). A study by Ahmed et al 2004 showed that education levels played a big role in patients' health seeking behavior [47]butalso, university of Nairobi dental school is located in the urban setting where the population is well educated.

5.2 Total salivary protein

The salivary protein of the participants ranged between 0.11mg/ml – 12.17mg/ml with a mean of 2.03mg/ml in agreement with the average normal total protein of 0.5-2mg/ml [48].The highest protein concentration was found in the periodontitis group this is similar to a study by Henskens et al 1993[9]

There was a statistically significant difference in the variance of total protein levels between gender where male participants had higher salivary protein levels than females. This finding differs from a study done by Pavitra et al 2013[49]where there was no significant difference between gender in respect to total protein however the study by Pavitra et al 2013 had a much smaller sample size of 39 study participants compared with the current study which had 161 participants.

The highest mean salivary protein was found in study participants with severe plaque deposits, the lowest mean salivary protein was observed in participants with mild plaque deposits however the difference was not statistically significant this in agreement with an earlier study byMirkovic et al 1998 [50]that showed that there is no effect of dental plaque on salivary protein composition

The highest mean salivary protein was found in the severe gingivitis group followed by moderate gingivitis category ,and the least total salivary protein was in the mild gingivitis group .This is in agreement with a study done byGonçalves et al 2011[51]that showed there in an increase in total salivary protein with severity of gingivitis however in our current study the difference was not statistically significant .

5.3 Periodontal health - Gingivitis/Periodontitis

The degree of gingival inflammation was assessed using the gingival index (Loe and Sillness 1963). The gingival scores of the participants ranged between 0.0 – 3.00 with a mean of 1.03 $\pm$ 0.62 SD and a median of 1.00 showing that every participant had some degree of gingivitis.

The positive statistically significant association between gingival index and plaque scores from this study confirmed known concepts of the role of dental plaque in the pathogenesis of gingival inflammation [52].

Positive correlations were observed between education levels and gingival score that were statistically significant. Individuals with higher level of education presented with lower degree of gingival inflammation. A study by Peeran et al 2015[53] demonstrated that education level plays a role in in oral hygiene practices. This reinforces the importance of oral hygiene education in reducing prevalence of periodontal disease practices.

Periodontitis was assessed using BPE definitions. There was significant positive association between periodontitis and increasing age. Plausible explanations for increased severity with increasing age in periodontal diseases is as a result of longer duration of exposure to risk factors over the years such as, periodontopathic bacteria, decreased manual dexterity hence compromised plaque control and undiagnosed concurrent systemic diseases [54].

A statistically significant association between severity of periodontal disease and plaque and gingivitis is further evidence to the existing known concepts that gingival inflammation precedes periodontal breakdown.

5.4 Association of total salivary protein and periodontal status

A positive relationship between periodontal health and total salivary protein was shown in this study. This study showed a strong, positive and statistically significant association between salivary protein levels and mild periodontitis levels (r = 0.594*, p = 0.020).These results are similar to a studyby Shaila et al 2013, who reported a significant rise in salivary total protein in gingivitis and periodontitis [(55)]. Henskens et al 1993 also reported a similar finding [(56)].

A mild positive association in protein concentrations in healthy group, gingivitis group and severe periodontitis existed however it was not statistically significant. The failurein finding significant correlations could be attributed to the small sample sizes in the different groups in the current study.

5.5 Oral hygiene practices

Tooth brushing is a form of mechanical plaque control and is the most relied upon oral hygiene practice worldwide[(57)]·All study participants brushed their teeth.Concerning the frequency of brushing, majority of the participants in this study 92(57%) brushed their teeth twice daily with 66(41%) brushing once daily and 3(0.02%) more than two times in a day. This is in agreement with a study done in a similar urban setting in Germany where majority brushed their teeth twice daily[(58)]. The findings are, however, in contrast to a local study done on a rural Kenyan population which found that majority brushed their teeth once daily[(59)]and also several other rural communities around the world(60) . The disparity is attributed to difference in the study population studied.In this study the population is an urban one while the other is rural. Although majority brushed their teeth twice daily, the relatively high percentage of those brushing once daily (41%) points to the need for more oral health education in the population.

5.6 Oral hygiene status

Oral hygiene status of the participants was assessed using Sillness and Loe plaque score 1964index. Every participant had some degree of plaque deposits on their teeth surfaces. Majority, 113 (70.2%) had mild plaque while 40 (24.85) had moderate plaque and 8 (5.0%) had severe plaque.

A positivestatistically significant difference in the variances where male participantshad higher plaque scores than females wasshown. This could be explained by the fact that in this study more females reported to brush twice daily compared to males although the difference was not statistically significant and these findings are similar to astudy done by Davidson et al 2007 which found higher frequency of brushing in females[61].This is also in agreement with studies done on relationship between gender and oral health status that suggest males tend to have poorer oral hygiene than females [62][63].

The study participants with low education had a statisticallysignificant higher plaque scores than the rest of the groups this could be attributed todecrease in awareness due to lowereducation. Studies have shown thatparticipants with higher level of education are more enlightened on oral hygiene practices.[64]. Syrjaelae et al 2010 reported that ina logistic regression model showed that gender and education were the most significant variables related to daily brushing and gingival health[62]

Predictably, plaque scores correlated positively with increasing severity of gingival disease. The microbial plaque biofilm has been implicated in the initiation and propagation of gingival inflammation in many studies.A non-statistically significant association between plaque and salivary protein was noted in this study

5.7 Limitations of the study

The study was carried out in a hospital set up. Extrapolation of the findings to the rest of the population may not be applicable. Moreover, the setting did not allow for adequate randomization due to the fact that the investigator did not have control over those who visited the facility for treatment. As such a potential selection bias may have been introduced. Being a cross sectional study, the snapshot timing may not have been fully representative as the study only captured the population at a single point in time. The study design also lacked the ability to make causal inference between the variables. Basic periodontal examination Lastly, the study was conducted parallel to other post graduate

academic activities with heavy cost implications. As such, there were both time and financial limitations.

5.8 Conclusion

Within the limitations of this study, it can be concluded that the findings of the present study suggest that salivary proteins may serve as markers of inflammation of the periodontium.Further investigations are needed to identify the specific proteins involved in total salivary protein in saliva of participants with varying degrees of periodontal disease.

5.9 Recommendation

Total salivary protein should be considered as a potential adjunctive diagnostic tool for evaluating periodontal disease. However, there is need for more salivary proteomic studies and evaluation of individual proteins and their specific role in periodontal diseases.Randomized controlled trials in Kenyans would be useful to fully exploit the potential of these biomarker

Conflict of interest

The study was carried out as a partial fulfillment for the award of Masters of Dental Surgery in Periodontology at the University of Nairobi as well as for scientific purposes. The cost of the study was solely met by the principal investigator. There was no conflict of interest.

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APPENDICES

Appendix I: Screening Form

Date: serial No:

	yes	no
Do you suffer from Diabetes?		
Do you have any type of Heart disease?		
Do you Smoking?		
Have you been diagnosed withSJOGERNS syndrome (a disease that causes dry mouth, dry eyes and difficulty swallowing)?		
Do you suffer from Rheumatoid arthritis?		
Have you been diagnosed with Salivary gland disease?		
Are you pregnant? (When was your last menstrual period?)		

Appendix II: Questionnaire

Total salivary protein and its relationship with the periodontal status

1. Sex male female
2. Age……. (in numbers)
3. Level of education none primary secondary tertiary
4. Marital status married single divorced/separated widow/widower
5. Occupation non-skilled professional job others
6. Frequency of brushing. none once a day twice a day More than twice a day
7. Type of brushing aid. Chewing stick conventional hand toothbrush electric toothbrush
8. Type of dentifrice used. Conventional Tooth paste herbal tooth pastes
9. Alcohol consumption. Teetotaler Social drinker Regular Drinker

Appendix III: Plaque Index. Silness-Loe 1964(0-3)

Score	Criteria
0	No plaque
1	A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may be seen in situ only after application of disclosing solution or by using a probe
2	Moderate accumulation of soft deposits within the gingival pocket or the tooth and gingival margin which can be seen with the naked eye
3	Abundance of soft matter within the gingival pocket or on the surface of tooth and gingival margin

Appendix IV: Gingival index (Loe and Silness 1963)

Score	Criteria
0	Normal gingiva/ absence of inflammation
1	Mild inflammation: slight change in color and slight edema. No bleeding on probing
2	Moderate inflammation: redness, edema, and Bleeding on probing
3	Severe inflammation: marked redness and edema, ulceration and tendency toward spontaneous bleeding

Appendix V: Periodontal examination

Basic Periodontal Examination (BPE)

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 >3.5 mm, but supra- or sub gingival calculus/overhangs (black band 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: Clinical form

Total salivary protein

Patient code……	Date of collection……...

Time	Patient code	Sample test volume	Total protein in μg/ml

Plaque Score: Silness-Loe Index-1964 (0-3)

Tooth	16		12		24		36		32		44	
Surface	F	L	F	L	F	L	F	L	F	L	F	L
Score												
Total score						Average score						

Gingival ScoreSilness –Loe index 1984(o-3)

Tooth	16		12		24		36		32		44	
Surface	F	L	F	L	F	L	F	L	F	L	F	L
Score												
Total score					Average score							

Basic Periodontal Examination (BPE)

Tooth	1st sextant		2nd sextant		3rd sextant		4th sextant		5th sextant		6th sextant	
Surface	F	L	F	L	F	L	F	L	F	L	F	L
Score												
Total score					Average score							

Appendix VII: Budget

CATEGORY	PARTICULARS	UNITS	UNIT COST	TOTAL (KSHS)
Proposal Development	Internet search for literature	N/A	6000	6000
	Printing and binding proposal copies	10	2000	20000
	Institutional review board fees	N/A	5000	5000
	Purchase of endnote (reference manager)	1	25000	25000
	Saliva collection kit	170	400	68000
	Stationary	Assorted	40000	4000
	Disposable examination kit	400	200	66,400
	Disclosing tablets	Tin	2	5000
	Disposable gloves	6	500	3000
	Face masks	1box	500	500
	Oral hygiene pack	400	100	33500
	Bca kits/lab fees	60000		60000
Data collection	Research assistants' lunch and transport allowance	2 for 30 days	1200	60,000
	Principal investigator lunch and transport	1 for 30 days	600	18,000
Data entry and analysis	Statistician	1	25,000	25,000
Report writing			10000	10,000
	Thesis copies	10	2000	20000
Grand total				429,640

Appendix VIII: Time frame

Time /activity	**march 17 / Jan 18**	**fe18**	**Jan118/ feb18**	**March18 / June18**	**Jul18 / Oct18**	**Nov18 / Mar 19**	**June 2019**
Proposal development							
Departmental and school approval							
Ethics approval							
Data collection							
Data Analysis							
Report writing							
Submission							

Appendix IX (a): Consent Form

UNIVERSITY OF NAIROBI (UoN)

COLLEGE OF HEALTH SCIENCES KNH-UoN ERC KENYATTA NATIONAL HOSPITAL (KNH)

P O BOX 19676 Code 00202 Email: uonknh_erc@uonbi.ac.ke **P O BOX 20723 Code 00202**

Telegrams: varsity Website: http://www.erc.uonbi.ac.ke **Tel: 726300-9**

(254-020) 2726300 Ext 44355 Facebook: ttps://www.facebook.com/uonknh.erc **Fax: 725272**

Twitter: @UONKNH_ERC ttps://twitter.com/UONKNH_ERC

Telegrams: MEDSUP, Nairobi

PARTICIPANT INFORMATION AND CONSENT FORM

SAMPLE ADULT CONSENT

FOR ENROLLMENT IN THE STUDY

Title of Study: Total salivary protein and its relationship to Periodontal Health in an adult Kenyan population

Principal Investigator\and institutional affiliation: Dr.Patience Nassimbwa University of Nairobi

Co-Investigators and institutional affiliation:N/A

Introduction:

I would like to tell you about a study being conducted by the above listed researchers. The purpose of this consent form is to give you the information you will need to help you decide whether or not to be a participant in the study. Feel free to ask any questions about the purpose of the research, what happens if you participate in the study, the possible risks and benefits, your rights as a volunteer, and anything else about the research or this form that is not clear. When we have answered all your questions to your satisfaction, you may decide to be in the study or not. This process is called 'informed consent'. Once you understand and agree to be in the study, I will request you to sign your name on this form. You should understand the general principles which apply to all participants in a medical research: i) Your decision to participate is entirely voluntary ii) You may withdraw from the study at any time without necessarily giving a reason for your withdrawal iii) Refusal to participate in the research will not affect the services you are entitled to in this health facility or other facilities. We will give you a copy of this form for your records.

May I continue? YES / NO

This study has approval by The Kenyatta National Hospital-University of Nairobi Ethics and Research Committee Protocol No. _____

What is this study about?

The study is aimed at establishing the total salivary protein of the Kenyan adult population and its relationship with periodontal status. The information I get is part of my research for a thesis as a partial fulfillment for the degree of master of dental surgery in Periodontology.

How do you participate?

I shall ask you some questions on the knowledge and practices of your oral health. I shall examine your mouth and record some observations. I will get a sample of your saliva for

five minutes. The examinations shall be carried out using clean (sterile) instruments and no invasive procedures shall be performed.

WHAT WILL HAPPEN IF YOU DECIDE TO BE IN THIS RESEARCH STUDY?

If you agree to participate in this study, the following things will happen:

You will be interviewed by a trained interviewer in a private area where you feel comfortable answering questions. The interview will last approximately 5 minutes. The interview will cover topics such as oral hygiene practices and knowledge.

After the interview has finished youwill be asked to collect saliva for about 5 minutes in the mouth and spit saliva in a sterile container**,**

We will ask for a telephone number where we can contact you if necessary. If you agree to provide your contact information, it will be used only by people working for this study and will never be shared with others. The reason why we may need to contact you is in the unlikely event some biodata is lost.

ARE THERE ANY RISKS, HARMS DISCOMFORTS ASSOCIATED WITH THIS STUDY?

Medical research has the potential to introduce psychological, social, emotional and physical risks. Effort should always be put in place to minimize the risks. One potential risk of being in the study is loss of privacy. We will keep everything you tell us as confidential as possible. We will use a code number to identify you in a password-protected computer database and will keep all of our paper records in a locked file cabinet. However, no system of protecting your confidentiality can be absolutely secure, so it is still possible that someone could find out you were in this study and could find out information about you.

Also, answering questions in the interview may be uncomfortable for you. If there are any questions you do not want to answer, you can skip them. You have the right to refuse the interview or any questions asked during the interview.

It may be embarrassing for you to have oral examination. We will do everything we can to ensure that this is done in private. Furthermore, all study staff and interviewers are professionals with special training in these examinations/interviews.

You may feel some discomfort when doing intra oral examination. In case of an injury, illness or complications related to this study, contact the study staff right away at the number provided at the end of this document. The study staff will treat you for minor conditions or refer you when necessary.

ARE THERE ANY BENEFITS BEING IN THIS STUDY?

You may benefit by receiving free periodontal examination. We will refer you to a hospital for care and support where necessary. Also, the information you provide will help us better understand of total protein in saliva and correlation with periodontal health. This information is a contribution to science with the aim of finding a rapid test which can confirm the presence of disease. Having a chairside test that can confirm disease presence will enable dental practitioners to correctly diagnose and treat patients.

WILL BEING IN THIS STUDY COST YOU ANYTHING?

N/A

WILL YOU GET REFUND FOR ANY MONEY SPENT AS PART OF THIS STUDY?

There will be no requirement that needs you to spend any money, but if by any slight chance there is liability caused by part of this study, a refund will be in order

WHAT IF YOU HAVE QUESTIONS IN FUTURE?

If you have further questions or concerns about participating in this study, please call or send a text message to the study staff at the number provided at the bottom of this page.

For more information about your rights as a research participant you may contact the Secretary/Chairperson, Kenyatta National Hospital-University of Nairobi Ethics and Research Committee Telephone No. 2726300 Ext. 44102 email uonknh_erc@uonbi.ac.ke.

The study staff will pay you back for your charges to these numbers if the call is for study-related communication.

WHAT ARE YOUR OTHER CHOICES?

Your decision to participate in research is voluntary. You are free to decline participation in the study and you can withdraw from the study at any time without injustice or loss of any benefits.

CONSENT FORM (STATEMENT OF CONSENT)

Participant's statement

I have read this consent form or had the information read to me. I have had the chance to discuss this research study with a study counselor. I have had my questions answered in a language that I understand. The risks and benefits have been explained to me. I understand that my participation in this study is voluntary and that I may choose to withdraw any time. I freely agree to participate in this research study.

I understand that all efforts will be made to keep information regarding my personal identity confidential.

By signing this consent form, I have not given up any of the legal rights that I have as a participant in a research study.

I agree to participate in this research study:	**Yes**	**No**
I agree to have saliva preserved for later study:	Yes	No
I agree to provide contact information for follow-up:	Yes	No

Participant printed name:

_____

Participant signature / Thumb stamp _____ Date _____

Researcher's statement

I, the undersigned, have fully explained the relevant details of this research study to the participant named above and believe that the participant has understood and has willingly and freely given his/her consent.

Researcher ‘s Name: Dr. Patience Nassimbwa **Date**: _____

Signature

_____

Role in the study: principal investigator

For more information contact

The Principal Investigator

Dr. Nassimbwa patience

School of Dental Sciences, University of Nairobi,

Tel: 0721365744.

Lead Supervisor

Prof. Evelyn Wagaiyu

Associate Professor

Department of Periodontology/Community and Preventive Dentistry, School of Dental Sciences, University of Nairobi

0722672567

The Secretary/Chairperson,

Kenyatta National Hospital-University of Nairobi Ethics and Research Committee

Telephone No. (254-020) 2726300-9

Email: uonknh_erc@uonbi.ac.ke.

Appendix X (b): Kiswahili version

FOMU YA RIDHAA

SAMPULI YA RIDHAA YA MTU MZIMA

YA USAJILI WA UTAFITI

Mada ya utafiti: Ujumla wa protini ya mate na uhusiano wake na afya ya ufizi meno miongoni mwa watu wazima nchini Kenya.

Mkuu wa uchunguzi na uhusiano wa taasisi: Daktari Patience Nassimbwa

Chuo kikuu cha Nairobi

Wachunguzi wenza na uhusiano wa taasisi: Haihusiki

Utangulizi:

Ningetaka kukueleza kuhusu utafiti unaofanywanawatafitiambaowametajwahapojuu. Lengo la fomuhiiyaridhaanikukuwezeshakufanyauamuziwaiwapoutashirikikatikautafiti au la. Kuwamwepesiwakuulizaswalilolotekuhusiananalengo la utafiti,ninihufanyikaiwapoutashirikiokwenyeutafiti, hatarinamanufaayautafiti, hakiyakokamamtualiyejitoleakwahiarinajambojinginelolotekuhusiananautafiti au fomuhiiambalohalijaeleweka. Baadayakuyajibumaswaliyakovilivyo, wawezakuamuakushirikikwenyeutafiti au kutoshiriki. Mchakatohuuunafahamikakama 'ridhaainayofahamika'. Pindituutakapoelewanakukubalikuwakwenyeutafiti, nitaombaulinakilijinalakonakutiasahihikwenyefomuhii. Yafaauelewe sharia zakawaidaambazohutumiwanawashirikiwotekatikautafitiwakimatibabu: i) Uamuziwakowakushirikiniwahiarikabisa ii) Wawezakujiondoakwenyeutafitiwakatiwowotebilakupatianasababuyakufanyahivyo. Iii) Kukataakushirikiokwenyeutafitihakutaathiriwajibuuanaopaswakutekelezakatikakituohiki cha afyaamavituovinginevyo. Tutakupanakalayafomuhiikwaajiliyarekodizako Nawezakuendelea? NDIO / LA

UtafitihuuumeidhinishwanahospitaliyaKitaifaya Kenyatta-Kamatiyamaadilinautafiti Chuo Kikuu Cha Nairobi, Nambariyaitifaki. _____

Utafitihuuunahusunini?

Utafitihuuunanuwiakupataujumlawaprotiniya mate miongoni mwa wa watu wazima nchini Kenya na uhusiano wake na afya ya ufizi. Habarinitakazopatanisehemuyautafitiwanguwatasnifuambayonisehemuyaukamilifuwa shahada yauzamilikatikaupasuajinaafyayaufizi.

Nitashirikivipi?

Nitakuulizamaswalikuhusiananaunayofahamukwenyeafyayakinywa.Nitakiangaliakinywa chakonaniyanakilinitakayoyaona. Nitachukuasampuliya mate yakokwadakikatano.Uchunguziutafanywakwakutumiavifaasafina hakuna shurutisholitakalofanywa.

NI NINI KITAKACHOFANYIKA IWAPO UTAAMUA KUWEKO KWENYE UTAFITI?

Iwapoutakubalikushirikikwenyeutafiti, mambo yafuatayoyatafanyika:

Utahojiwanamtuambayeamepitiamafunzokatikamahalipasiriambapoutawezakuyajibumaswali. Mahojianohayoyatachukuwayapatamudawadakikatano. Mahojianohayoyatahusishamadakama vile usafikinywaninaufahamuwausafikinywaninajinsiyakufanyausafihuo. Mahojianoyalikamilikautaulizwaukusanye mate kwadakikatanokuokakinywaninakuyatiakatikachombosafi. Tutakuulizautupenambariyasimuambayotutatumiakuwasilianaiwapotutahitajikakufanyahivyo.Ukikubalikutupanambariyasimuitatumiwatunawatafitikatikautafitihuunakamwehaitapewamtumwingineyeyote.Sababuyetukuchukuanambariyakoyasimuniilituwezekuwasilianaweiwapo data itapotea.

JE, KUNA HATARI ZOZOTE AU MADHARA YANAYOHUSISHWA NA UTAFITI HUU?

Utafitiwakimatibabuunauwezowakusababishahatarizakisaikolojia, katikamahusiano, hisianakimwili.Yafaatujaributuwezavyokupunguzahatarihizo.Hatarimojaambayoyawezakutokeaniukosefuwasiri.Yoteutakayotuambiayatabakikuwasiri.Tutatumiakodifulanikukutambuakatikatarakilishiiliyona neon la siri. Data nanakalazetuzotetutazifungiakwakabati. Hatahivyo,hakunachombo cha kuhifadhisiriyakoambachonisalamakabisanahuendamtuakafumbuakwambaulishirikikatikautafitinaapatehabarikukuhusu.

Aidhaakujibumaswalikwenyemahojianohuendakukawakugumukwako.Iwapokunamaswalihutakikujibuwawezakuyaacha.Unahakiyakukataamahojiano au swalilolotelitakaloulizwakwenyemahojiano.

Inawezekanaliwenijambo la aibukwakokufanyiwauchunguzi.Tutahakikishayakwambayote hayoyatafanyiwamahalipasiri.Halikadhalikawatakaofanyamahojianoniwatuwenyeweledinaujuzi. Huendausihisivizuriwakatiwakukaguliwakinywani. Pakitokeayakwambaumejeruhiwa ,umekuwamgonjwa au shidanyingineinayohusiananautafitihuuimetokeapiganambariutakayoonamwishonimwanakalahiiharakaiwezekanavyo.Wahudumuwatakutibumagonjwamadogomadogo au wakutumekwinginekoiwapoitahitajikakufanyahivyo

KUNA MANUFAA YOYOTE KATIKA UTAFITI HUU?

Huendautafaidikakwakupatauchunguziwaufizibilamalipo.Tutakutumahospitaliniiwapotuttahitajikakufanyahivyo.Habariutakayotupaitasaidiakuelewavyemauhusianowaprotinikatika mate naafyayaufizi. Habarihiyoitachangiaufahamukatikasayansinaniayakupatanakudhibitishaugonjwakwanjiayaharaka.

Ugonjwaukishadhibitishwapapohaponadaktariwatawezakuwachunguzazaidinakuwatibuwagonjwa.

JE KUWEPO KATIKA UTAFITI HUU KUTAKUGHARIMU CHOCHOTE? : HAIHUSIKI

UTARUDISHWA PESA ZOZOTE UTAKAZOTUMIA KATIKA UTAFITI?

Hakuna jambololotelitakalokupelekeawewekutumiapesa, lakiniiwapopesazakozitumike,utaregeshewa .

IWAPO UKUMBANE NA MASWALI SIKU ZA USONI

Iwapoutakuwanamaswali Zaidi kuhusuutafitihuutafadhalipigasimu au utumearafakwanambariiliyokomwishonimwanakalahiiilikuwasilianawahudumuwetu. Kwa habari Zaidi kuhusuhakiyakokamamshirikiwautafitiwawezakuzungumzanakatibu/Mwenyekiti, HospitaliyaKitaifaya Kenyatta-Kamatiyamaadilinautafiti Chuo Kikuu cha Nairobi, Nambariyasimu 2726300 Ext. 44102 Baruapepe:uonknh_erc@uonbi.ac.ke.

Wahudumuwatakulipahelazakoukishatumianambarihiziiwapomawasilianoyatahusuutafiti

.

CHAGUO LAKO LINGINE NI LIPI?

Uamuziwakowakushirikikatikautafitihuuniwahiari.Unaruhusayakukataakushirikikatikautafitinawawezakujiondoakatikautafitibilahasarayoyotenabilakukiukwakwahakiyako.

FOMU YA RIDHAA

Kauliyamshiriki

Nimeisomafomuhiiyaridhaaamanimesomewaujumbe.

Nilipatafursayakujadilianakuhusuutafitihuunamtafiti.

Maswaliyanguyamejibiwakwalughaambayonaielewa.

Nimeelezewamanufaanahatariziliwepo.

Naelewakuwaushirikiwangukwautafitihuuniwahiarinanawezakujiondoawawakatiwowote.

Nimekubalikwahiarikushirikikatikautafitihuu.

Naelewajuhudizitafanywailikuuhifadhihabariyanguwakibinafsi.

Kwa kutiasahihifomuhiiyaridhaa, sijaiachahakizangukisheriakamamshirikikatikautafiti.

.

Nimekubalikushirikikatikautafitihuu:	**Ndio**	**La**
Nimekubali mate yahifadhiweyatumikebaadaye:	Ndio	La
Nimekubalikupeananambarizasimuilinifuatiliwe:	Ndio	La

Jina la mshirikililochapishwa: _____

Sahihiyamshiriki / alamayakidole_____Tarehe_____

Kauliyamtafiti

Mimi, ambayenimetiasahihi, nimetoamaelezokamilikuhusiananautafitihuukwamshirikiambayeametajwahapojuunanaa miniyakwambamshirikiameelewanaakatoaridhaayakekwahiari.

Jina la mtafiti: Dr. Patience Nassimbwa **Tarehe**: _____

Sahihi_____

Kaziyakekatikautafiti: Mkuu wa uchunguzi

Kwa habarizaidizungumzana

Mkuu wa Uchunguzi

Dr. Nassimbwa patience

Shuleyakisayansiya meno, Chuo Kikuu Cha Nairobi,

Nambariyasimu: 0721365744.

Msimamizimkuu

Prof. Evelyn Wagaiyu

Associate Professor

Department of Periodontology/Community and Preventive Dentistry, School of Dental Sciences, University of Nairobi

0722672567

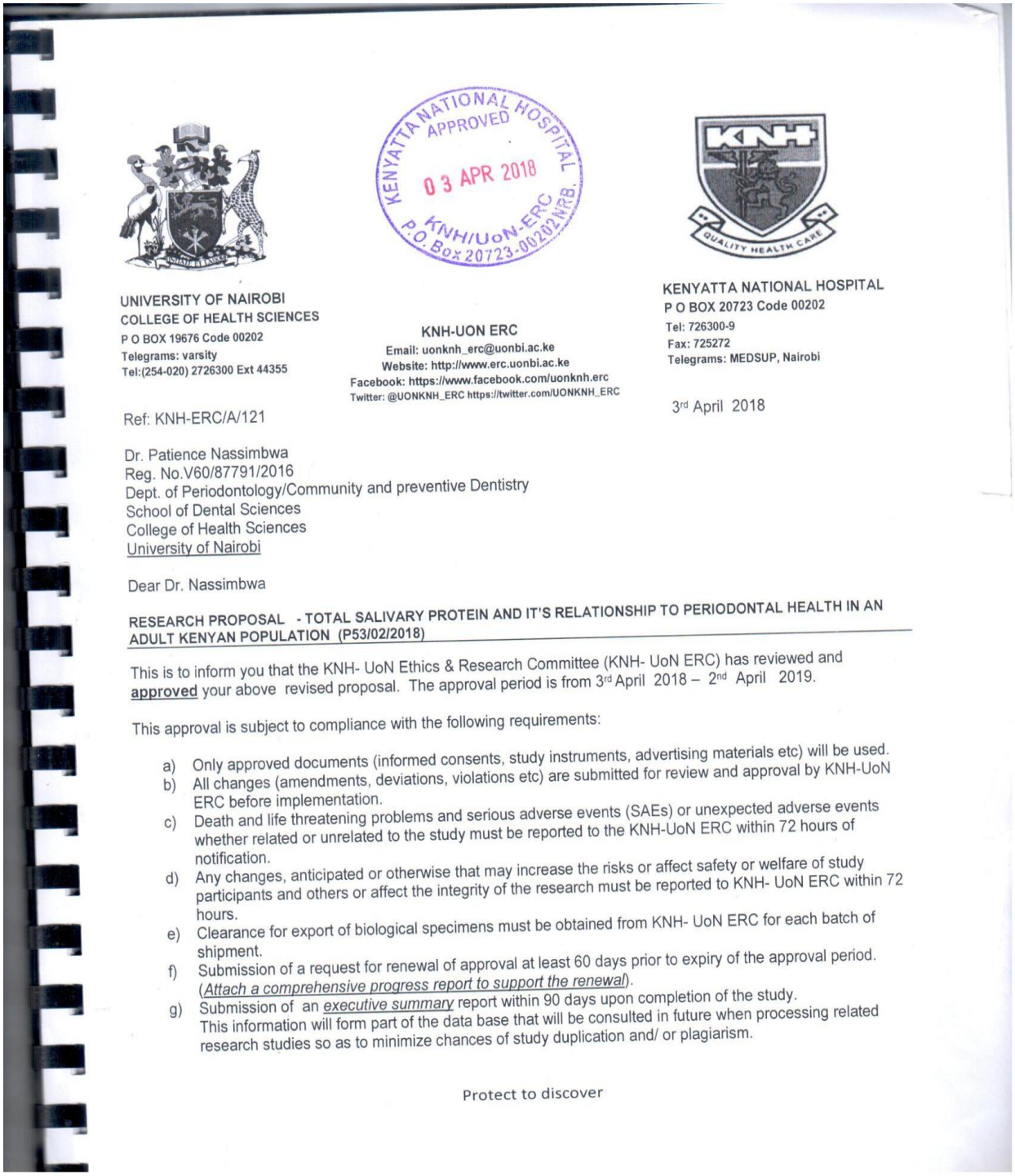
Katibu/ Mwenyekiti ,

HospitaliyaKitaifaya Kenyatta-Kamatiyamaadilina utafiti Chuo Kikuu Cha Nairobi,

Nambariyasimu. (254-020) 2726300-9

Baruapepel: uonknh_erc@uonbi.ac.ke.

Appendix -XI-Research approval letter



KENYATTA NATIONAL HOSPITAL APPROVED 03 APR 2018 KNH/UoN-ERC P.O. Box 20723-00202 NRB.

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Facebook: https://www.facebook.com/uonknh.erc
Twitter: @UONKNH_ERC https://twitter.com/UONKNH_ERC

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Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/A/121

3rd April 2018

Dr. Patience Nassimbwa
Reg. No.V60/87791/2016
Dept. of Periodontology/Community and preventive Dentistry
School of Dental Sciences
College of Health Sciences
University of Nairobi

Dear Dr. Nassimbwa

RESEARCH PROPOSAL - TOTAL SALIVARY PROTEIN AND IT'S RELATIONSHIP TO PERIODONTAL HEALTH IN AN ADULT KENYAN POPULATION (P53/02/2018)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH- UoN ERC) has reviewed and **approved** your above revised proposal. The approval period is from 3rd April 2018 – 2nd April 2019.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH-UoN ERC before implementation.
c) Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
e) Clearance for export of biological specimens must be obtained from KNH- UoN ERC for each batch of shipment.
f) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
g) Submission of an *executive summary* report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/ or plagiarism.

Protect to discover

For more details consult the KNH- UoN ERC website **http://www.erc.uonbi.ac.ke**

Yours sincerely,

PROF. M. L. CHINDIA
SECRETARY, KNH-UoN ERC

c.c. The Principal, College of Health Sciences, UoN
The Deputy Director, CS, KNH
The Chairperson, KNH-UON ERC
The Assistant Director, Health Information, KNH
The Dean, School of Dental Sciences, UoN
The Chair, Dept. of Periodontology/Community and Preventive Dentistry, UoN
Supervisors: Prof. Evelyn Wagaiyu, Dr.Tonnie Mulli, Dr. James Mutave

Appendix XII-Service record for the Plate reader

BIOLOGIC SOLUTIONS MEDICAL & LABORATORY SOLUTIONS PROVIDER

TECAN READER SERVICE RECORD

Date of Visit	07/05/ 2018
Make and Model number:	TECAN INFINITE M200
Instrument serial number:	908007098
Check all mechanical Parts:	PASS
Lens inspection and cleaning	PASS
Next Service	Nov 2018
Part Used	None
QC Plate Test	PASS
Comments:	IN GOOD WORKING CONDITION ATTACHED TEST REPORT. **Test Equipment:** **DVM: Multimeter** **S/N: 12380128** **Cal Date: MARCH 2018**
Sticker No:	BSL577E

Completed by: ROBINSON M. OUKO **Site** : PBMC LAB

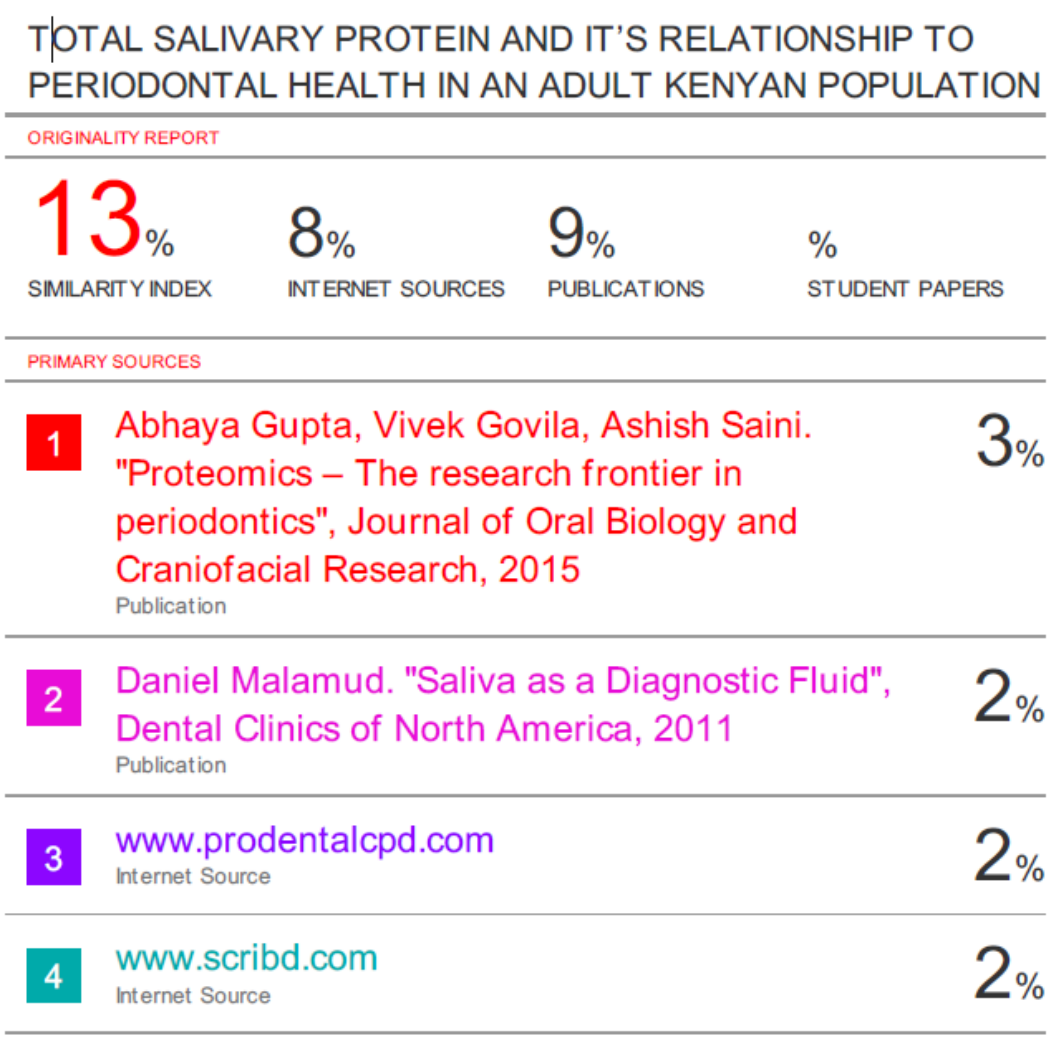
Signature:

Date : 07/05/2018

Renewed June 2018

BIOLOGIC SOLUTIONS
Biologic Solutions Ltd | Panari Sky Centre Mombasa Road | P.O. Box 15078 - 00100, Nairobi Kenya
Tel +254 731 076 764 | info@biologic.co.ke | www.biologic.co.ke

Appendix XIII-Plagiarism Report (Similarity Index)



TOTAL SALIVARY PROTEIN AND IT'S RELATIONSHIP TO PERIODONTAL HEALTH IN AN ADULT KENYAN POPULATION

ORIGINALITY REPORT

13%	8%	9%	%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Abhaya Gupta, Vivek Govila, Ashish Saini. "Proteomics – The research frontier in periodontics", Journal of Oral Biology and Craniofacial Research, 2015 Publication	3%
2	Daniel Malamud. "Saliva as a Diagnostic Fluid", Dental Clinics of North America, 2011 Publication	2%
3	www.prodentalcpd.com Internet Source	2%
4	www.scribd.com Internet Source	2%