SALIVARY TOTAL ANTIOXIDANT CAPACITY AND PERIODONTAL STATUS AMONG DIABETICS AND NON DIABETICS ATTENDING THIKA LEVEL 5 HOSPITAL

ANNETTE WAMBUI MURIITHI

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2019
DECLARATION

I declare that this research is my original work and has not been presented for the award of a degree in any other university.

Signed..................................................Date.................................................................

ANNETTE WAMBUI MURIITHI
APPROVAL

I, ANNETTE WAMBUI MURIITHI, hereby submit this thesis with the approval of my supervisors:

DR NELSON K. MATU

BDS (UoN), MSc.Dent (UWC, SOUTH AFRICA)

Department of Periodontology/Community and Preventive Dentistry

School of Dental Sciences, University of Nairobi.

Signed.................................................... Date.............................................

DR. ANDREW WETENDE

BDS (UoN), MDS (UoN)

Department of Periodontology/Community and Preventive Dentistry

School of Dental Sciences, University of Nairobi.

Signed.................................................... Date.............................................

DR. BENARD N.MUA

BDS, MPH, PgDip (STIs & HIV/AIDS), MBA (Strategic Management)

Department of Periodontology/Community and Preventive Dentistry

School of Dental Sciences, University of Nairobi.

Signed.................................................... Date.............................................
DEDICATION

This thesis is dedicated to my husband Kenneth and sons Nathaniel and Joshua whose unyielding love, support and encouragement throughout the entire process gave me strength to pursue and complete the research.
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LIST OF ABBREVIATIONS

CDC/AAP  Centre for Disease Control/American Academy of Periodontology
ABTS  2, 2’-azino-bis (3-ethylbenzothiazolidine-6-sulphonic acid)
BDS  Bachelor of Dental Surgery
BOP  Bleeding on Probing
CAL  Clinical Attachment Loss
CRP  C- Reactive Protein
ELISA  Enzyme Linked Immune-Sorbent Assay
FRAP  Ferric Reduced Antioxidant Potential
GCF  Gingival Crevicular Fluid
GI  Gingival Index
ICF  Implant Crevicular Fluid
KAVI  Kenya Aids Vaccine Initiative
KNH  Kenyatta National Hospital
MDS  Master of Dental Surgery
MPH  Masters in Public Health
MSc.Dent  Master of Science in Dentistry
ORAC  Oxygen Radical Antioxidant Capacity
PHD  Doctor of Philosophy
PPD  Periodontal Probing Depth
PS  Plaque Score
SPSS  Statistical Package for Social Sciences
TAC  Total Antioxidant Capacity
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<td>TBARS</td>
<td>Thiobarbituric acid reactive substances</td>
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<td>TEAC</td>
<td>Trolox Equivalent Antioxidant Capacity</td>
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<td>TRAP</td>
<td>Total Radical Trapping Antioxidant Parameter</td>
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<td>UoN</td>
<td>University of Nairobi</td>
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<td>WHO</td>
<td>World Health Organisation</td>
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DEFINITIONS OF TERMS

The following definitions are provided to ensure uniformity and understanding of the terms used in the present study.

**Biofilms**

Microbial communities which adhere to the tooth surface or other hard tissues within the oral cavity.

**Gingivitis**

This refers to inflammation of the gingival tissues. It results in signs and symptoms that are localised within the gingiva without any loss of periodontal attachment clinically. These signs and symptoms include bleeding on probing, bluish redness and swelling of the free gingival margin with loss of the knife edged shape.

**Clinical attachment loss**

This refers to the vertical distance from the cemento-enamel junction to the base of the periodontal pocket as measured by a periodontal probe. It is the migration of the junctional epithelium apically due to disease. Periodontal probing depths give an indication of the amount of clinical loss of attachment from the root surface.

**Periodontitis**

This is a multifactorial induced inflammatory disease affecting the supporting structures of the tooth. It is usually a progressive destructive process leading to pocket formation, loss of clinical attachment as well as periodontal ligament and alveolar bone destruction.

**Passive saliva**

Saliva obtained by pooling at the floor of the mouth without stimulation and thereafter the sample collected by drooling into a collection vessel.

**Whole saliva**

A mixture of secretions from all the salivary glands in the mouth and also contains serum exudates from the gingiva, food and cellular debris.
ABSTRACT

Background: Periodontitis and diabetes are common, complex, chronic diseases with an established bidirectional relationship. Both disease conditions present with reduced salivary antioxidant capacity whose key role is to protect gingival tissues from oxidative stress. Oxidative stress refers to an imbalance between the production of free radicals in the body that serve to eliminate a microbial challenge and antioxidant defences that protect body tissues from damage by excessive production of the free radicals. Reduced salivary total antioxidant capacity in diabetic patients may be one of the mechanisms by which poor glycemic control leads to increased severity of periodontal disease.

Objective: To investigate salivary total antioxidant capacity and periodontal status among diabetics and non diabetics attending Thika Level 5 Hospital.

Study design: Comparative cross sectional study.

Study area: Thika Level 5 Hospital diabetic outpatient clinic.

Study population: All diabetics and non diabetics who attended the Thika Level 5 Hospital diabetic clinic during the period of the study.

Materials and methods: A sample of 63 diabetics and 59 non diabetics were recruited into the study. A semi-structured questionnaire was used to obtain data on their socio-demographic characteristics, oral hygiene practices and oral health seeking behaviour. Saliva was collected from each of the participants. The principal investigator carried out a clinical examination to determine the participant’s oral hygiene, gingival index, and periodontal status using probing depths and clinical attachment loss.

Saliva collection: About 5ml of saliva was collected from each participant (unstimulated whole saliva). Collection was done between 9 and 11am through passive drool technique and transported in a cooler box to the laboratory for immediate centrifugation. The supernatant was collected and aliquoted into clean microvials prior to refrigeration at -80°C until processing.

Total Antioxidant Capacity analysis: Concentration of salivary TAC was determined using CS0790 Total Antioxidant Assay kit from Sigma- Aldrich Company (USA) by use of ELISA technique. The results are expressed in millimoles/l (mM/l). The laboratory stage of the study
was performed at the Kenya AIDS Vaccine Initiative (KAVI) - Institute of Clinical Research, College of Health Sciences, University of Nairobi.

**Data analysis and presentation:** The collected data was entered into Microsoft Excel program. The data was cleaned and exported into SPSS version 20 software. The results were then presented in form of text, figures and tables. Chi square test, Independent t-test and Spearman’s correlation test were used to analyse data.

**Results:** A total of 122 participants were included in the study with 63 (51.6%) being diabetics and 59 (48.4%) being non diabetics. The age ranged from 25-80 years. Salivary TAC ranged from undetectable levels to 0.252mM/l. There was a statistically significant difference within salivary TAC among diabetics and non diabetics ($X^2_{(1)} =11.718$ $p=0.001$) with diabetics having lower levels compared to the non diabetics. A statistically significant association between salivary TAC and gingivitis was found ($r=-0.289$ $p=0.001$) with lower levels of TAC detected among participants with severe forms of gingivitis. A negative correlation between CAL and TAC ($r=-0.158$ $p=0.084$) was also detected which was however not statistically significant.

**Conclusion:** Within the limitations of this study, the following were the conclusions. Diabetics had more severe forms of periodontal disease compared to non diabetics. Reduced amounts of salivary TAC was also mostly found among diabetics compared to non diabetics. There was an association found between salivary TAC and gingivitis, however, there was no association found between salivary TAC and periodontitis.

**Recommendation:** There is need to create more awareness among diabetics as well as their care givers on the relationship between diabetic status and periodontal health so that appropriate measures are taken to monitor for early signs of periodontal disease. There is also need to conduct further studies on salivary TAC using the various kits available in order to obtain a gold standard that would be used to obtain more conclusive results.
CHAPTER ONE: INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Periodontal diseases are common chronic conditions that cause destruction of the connective tissue and bone around the root area of the tooth [1]. These diseases are multifactorial inflammatory diseases, caused by interaction of microbial agents present in plaque bio film associated with host susceptibility and environmental factors[2]. It has been observed that invading P. gingivalis bacteria trigger the release of cytokines such as interleukin 8 and tumour necrosis factor α, leading to elevated numbers and activity of polymorphonucleocytes (PMN). As a result of stimulation by bacterial antigens, PMN produce the reactive oxygen species (ROS) superoxide via the respiratory burst as part of the host response to infection. Patients with periodontal disease display increased PMN number and activity. It has been suggested that this proliferation results in a high degree of ROS release, culminating in heightened oxidative damage to the gingival tissues, periodontal ligament and alveolar bone [1].

Neutrophils also appear to be crucial for the maintenance of periodontal health, as disease severity is increased in neutropenia, agranulocytosis and where cellular function is impaired, such as diabetes mellitus. Diabetes mellitus is a group of metabolic diseases characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycaemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Periodontitis is now considered the 6th complication of diabetes mellitus [3].

Periodontitis and diabetes are common, complex, chronic diseases with an established bidirectional relationship. That is, diabetes (particularly if glycemic control is poor) is associated with an increased prevalence and severity of periodontitis, and, severe periodontitis is associated with compromised glycemic control. Periodontal treatment (conventional non-surgical periodontal therapy) has been associated with improvements in glycemic control in diabetic patients, with reductions in HbA1c of approximately 0.4% following periodontal therapy [3]. For these reasons, management of periodontitis in people with diabetes is particularly important. The dental team therefore has an important role to play in the management of people with diabetes. An emerging role for dental professionals is envisaged, in which diabetes screening tools could be used to identify patients at high
risk of diabetes, to enable them seek further investigation and assessment from medical healthcare providers[3].

Diabetic patients have been shown to have reduced levels of salivary antioxidants thus lowering their capacity to withstand tissue damage caused by free radicals and oxidative stress [1]. Reduced levels of salivary antioxidants could serve as an additional mechanism through which diabetic patients present with more severe forms of periodontitis.

Antioxidants are defined as substances that are able to delay or prevent the oxidation of a substrate and exist in bodily tissues and fluids. Their function is to protect bodily tissues against free radicals. Free radicals are species capable of independent existence that contain one or more unpaired electrons. They are beneficial to the host if secreted in appropriate amounts and may act as powerful antibacterial agents or help in regulation of vascular tone in the endothelium. In healthy individuals there is an appropriate balance between free radicals and their scavengers. This balance is usually shifted to an unhealthy pro-oxidant state when production of free radicals is increased leading to cell damage and progressive inflammatory disease of the periodontium and periapical tissues. These free radicals may be important biomarkers of disease in the absence of clinical symptoms. Nitric oxide, a free radical gas is an inflammatory biomarker[4].

Oxidative stress is a condition which arises when there is an imbalance between levels of free radicals and its antioxidant defences. It is a major cause of inflammation resulting from increased production of free radicals and or a decrease in the antioxidant status. There is significant evidence linking chronic periodontitis and oxidative stress[5].

Saliva is a complex fluid in the oral cavity composed of a mixture of secreatory products from the major and minor salivary glands. It has multifunctional roles in the oral cavity and is important in maintaining oral health. About 99% of saliva is made of water while the remaining 1% is a complex of organic and inorganic molecules such as electrolytes, mucins, antiseptic substances, immunoglobulin, proteins and various enzymes. Saliva has various defence mechanisms such as immunological and enzymatic defence systems against microbes and promotes healing. One of the most important defence mechanisms is the antioxidant system[6]. Saliva is rich in antioxidants, mainly uric acid with lesser contributions from albumin, ascorbate and glutathione. It has been reported that uric acid is
the major antioxidant in saliva accounting for more than 85% of the total antioxidant capacity of resting and stimulated saliva from both healthy and periodontally compromised subjects[7].

Antioxidants have many health benefits that have made their evaluation in disease process very popular. Saliva is easily accessible and provides a non invasive method of evaluating the total antioxidant capacity it provides[6].

1.2 Literature Review

1.2.1 Periodontal disease
Periodontal disease is a host-microbial interaction in which both host and bacterial factors determine the outcome, such that changes in the balance between host and bacterial factors can result in a change from health to disease. The balance may be changed, for example, by a reduction in the host resistance, an increase in the microbial plaque bio film or an increase in bacterial virulence. The clinical manifestation of periodontal disease is further modified by local and/or systemic factors and/or environmental factors.

The dental bio-film is an organised bacterial community which forms when a solid structure is placed in an aqueous environment. In the oral cavity, the solid surfaces are either teeth or restorative materials – the metal, ceramics or acrylic in appliances. Dental bio-films differ from bio films on mucosal surfaces as they form on non-shedding surfaces; stable communities can therefore become established.

The primary colonisation consists of aerobic and facultative anaerobes such as Gram-positive cocci (e.g. streptococci). Gram-positive rods appear, increase in number and eventually outnumber the cocci. Gram-positive filaments, such as Actinomyces spp., may later predominate. There are specific surface receptors on the Gram-positive cocci and rods that allow the adherence of Gram-negative bacteria, which otherwise lack the ability to attach directly to pellicle. As time progresses there is a shift in the micro flora from Gram-positive to Gram-negative organisms, and an increase in heterogeneity of the microbial species.
1.2.1.1 Role of bacteria in periodontitis

a) Non-specific plaque hypothesis
This implies that no one specific bacterial species is more significant than another in its ability to cause periodontal disease. The implication from the non-specific plaque hypothesis is that all patients must maintain a high standard of oral hygiene to prevent periodontal Disease, as all bacteria are perceived as playing a role. Although the amount of plaque present may correlate well with disease severity in cross-sectional studies, it correlates poorly in longitudinal studies. This hypothesis does not consider variations in the dental bio-film that may affect its pathogenicity or, most importantly, host determinants.

b) Specific plaque hypothesis
This may help to explain why there are many patients who have considerable plaque deposits but only a minority suffer from severe destructive periodontitis. The implications from the specific pathogen hypothesis are that one need only worry about the bacterial pathogen responsible for periodontal disease, and therefore need only employ procedures that lead to the elimination of this species and not all other bacterial species. Treatment could specifically target the identified pathogen as one would do for any other mono specific infection such as tuberculosis or syphilis.

c) Multiple pathogen hypothesis
One major difficulty lies in identifying the possible combinations of pathogens that are important. It should be appreciated that this current list of periodontopathogens may be superseded once the results from bacterial culture and isolation using molecular techniques are combined and reinterpreted. One could, nevertheless, arbitrarily determine antibiotic sensitivities of the top ten periodontopathogens and then employ these antibiotics to eliminate the organisms[8]. *Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia, Treponema denticola, Fusobacterium nucleatum* and *Campylobacter spp.* are present in diseased sites and have been implicated in disease progression[9].

1.2.1.2 Host response

a. Innate immunity
Innate immune mechanisms include a number of relatively non-specific mechanisms, including the barrier effect of an intact epithelium. Oral mucosa is bathed in saliva, which
contains a number of protective factors. Bacteria can be recognised by non-clonal receptors, otherwise known as pattern recognition receptors. These receptors recognise substances such as lipopolysaccharide (LPS) from gram-negative bacteria and peptidoglycan from gram-positive bacteria. Innate responses are relatively non-specific and therefore there is an increased potential for bystander damage to tissues[10].

Neutrophils appear to be crucial for the maintenance of periodontal health, as disease severity is increased in neutropenia, agranulocytosis and where cellular function is impaired, such as leukocyte adhesion deficiency, lazy leukocyte disease and Papillon-Lefèvre, Chediak-Higashi and Down’s syndromes, as well as diabetes mellitus[11].

b. Adaptive immunity

The adaptive immune response is characterised by specificity, memory and the capacity to distinguish self from non-self. Once recognition of microbial antigens has taken place by the appropriate receptor on macrophages or dendritic cells, then cytokines are released which activate T and B cells, thereby engaging cell-mediated and humoral immune responses. The two arms of immunity therefore function together; the earlier responses being predominantly innate, subsequently helping to focus adaptive immune responses. In humoral or cell-mediated immunity, specificity of the responses is thought to limit bystander damage by focusing the adaptive or specific immune system.

Periodontal disease thus represents a complex interaction between host and microbes. In future one could employ strategies which improve the host's ability to prevent bacterial colonisation or eliminate the key pathogenic microbial species. One could also target systemic conditions where cellular function is impaired leading to a reduction in the host’s defence mechanisms, such as diabetes mellitus.

1.2.2 Diabetes mellitus

Diabetes mellitus is a heterogeneous chronic metabolic disorder principally characterised by persistent hyperglycaemia resulting from defects in insulin action and or defects in insulin secretion. It affects more than 171 million individuals worldwide and has reached epidemic status[12].
Several pathogenic processes are involved in the development of diabetes. These range from autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. The basis of the abnormalities in carbohydrate, fat, and protein metabolism in diabetes is deficient action of insulin on target tissues. Deficient insulin action results from inadequate insulin secretion and/or diminished tissue responses to insulin at one or more points in the complex pathways of hormone action. Impairment of insulin secretion and defects in insulin action frequently coexist in the same patient, and it is often unclear which abnormality, if either alone, is the primary cause of the hyperglycemia.[13]

Symptoms of marked hyperglycemia include polyuria, polydipsia, weight loss, sometimes with polyphagia, and blurred vision. Impairment of growth and susceptibility to certain infections may also accompany chronic hyperglycaemia. Acute, life-threatening consequences of uncontrolled diabetes are hyperglycemias with ketoacidosis or the non ketotic hyperosmolar syndrome.

The vast majority of cases of diabetes fall into two broad pathogenic categories. In one category, type 1 diabetes, the cause is an absolute deficiency of insulin secretion. Individuals who are at an increased risk of developing this type of diabetes can often be identified by serological evidence of an autoimmune pathologic process occurring in the pancreatic islets and by genetic markers. In type 2 diabetes which is more prevalent, the cause is a combination of resistance to insulin action and an inadequate compensatory insulin secretory response. In the latter category, a degree of hyperglycemia sufficient to cause pathologic and functional changes in various target tissues, but without clinical symptoms, may be present for a long period of time before diabetes is detected. During this asymptomatic period, it is possible to demonstrate an abnormality in carbohydrate metabolism by measurement of plasma glucose in the fasting state or after a challenge with an oral glucose load[13].

1.2.2.1 Effect of Diabetes mellitus on periodontal disease

Diabetes mellitus has been unequivocally confirmed as a major risk factor for periodontitis. The risk of periodontitis is increased by approximately threefold in diabetic individuals compared with non-diabetic individuals[14]. The level of glycemic control is of key importance in determining increased risk. For example, in the US National Health and
Nutrition Examination Survey (NHANES) III, adults with an HbA1c level of >9% had a significantly higher prevalence of severe periodontitis than those without diabetes after controlling for age, ethnicity, education, sex and smoking[14]. The importance of diabetes as a major risk factor for periodontitis became apparent in the 1990s in a number of cross-sectional and longitudinal studies investigating the Pima Indian population. The prevalence and incidence of periodontitis were greater in Pima Indians who had type 2 diabetes mellitus compared with those who did not, with an approximately threefold increased risk for periodontitis[15]. The majority of research has focused on type 2 diabetes mellitus as a risk factor for periodontitis, probably because both diseases have historically tended to develop in patients in their 40s and 50s. However, type 1 diabetes mellitus also increases the risk of periodontitis, and all patients with diabetes (including children and young adults) should be considered to be at increased risk of periodontitis[16].

1.2.2.2 Effect of periodontitis on diabetes mellitus

There has been much emphasis on the ‘two-way’ relationship between diabetes and periodontitis[15]. That is, not only is diabetes a risk factor for periodontitis, but periodontitis could have a negative effect on glycemic control. The first clear evidence to support this hypothesis came from investigations of individuals in the Gila River Indian community. Severe periodontitis at baseline was associated with an increased risk of poor glycemic control (HbA1c > 9.0%) at follow-up (minimum 2 years), suggesting that severe periodontitis was a risk factor for compromised diabetes management[15]. In addition, various studies have reported that the prevalence and severity of non-oral diabetes-related complications, including retinopathy, diabetic neuropathy, proteinuria and cardiovascular complications are correlated with the severity of periodontitis[13].

Both type 1 and type 2 diabetes mellitus are associated with elevated levels of systemic markers of inflammation[17]. The elevated inflammatory state in diabetes contributes to both microvascular and macrovascular complications, and it is clear that hyperglycaemia can result in the activation of pathways that increase inflammation, oxidative stress and apoptosis[18]. Elevated serum levels of IL-6 and TNF-α have been demonstrated in diabetes and obesity[17], and serum levels of IL-6 and C-reactive protein (CRP) have been shown to predict future occurrence of type 2 diabetes mellitus[19]. Elevated levels of CRP are also associated with insulin resistance, type 2 diabetes mellitus and cardiovascular disease[20]. TNF-α and IL-6 are the main inducers of acute-phase proteins, including CRP,
and both have been shown to impair intracellular insulin signalling, potentially contributing to insulin resistance[21]. Serum levels of IL-6 and CRP are also raised in patients with periodontitis, with IL-6 levels correlating with the extent of disease[22]. The systemic inflammation that is associated with periodontal disease may therefore enhance the diabetic state. Adipokines may also contribute to susceptibility to both periodontitis and diabetes, and the proinflammatory properties of leptin may be particularly important in up-regulating periodontal inflammation in people who are obese and/or have type 2 diabetes mellitus[13]. Diabetes mellitus and periodontal diseases are thus common chronic diseases observed worldwide[12]. Several mechanisms have been investigated on the effect of raised blood glucose levels on periodontitis and reduced levels of salivary antioxidants could serve as an additional mechanism through which diabetic patients present with more severe forms of periodontitis.

1.2.3 Antioxidants and disease process

A functional classification of antioxidants is in the way they act as classified by Lobo et al[23]:

i. Preventive antioxidants: - suppress the formation of free radicals e.g. superoxide dismutase, catalase, glutathione peroxidase & s-transferase, carotenoids, transferring, albumin, haptoglobin and caeruloplasmin.

ii. Radical scavenging antioxidants: - scavenge radicals to inhibit chain initiation and break chain propagation e.g. albumin, bilirubin, carotenoids, ubiquinol, uric acid, vitamin A, vitamin C and vitamin E.

iii. Repair and de novo enzymes: - they repair the damage and reconstitute membranes i.e. DNA repair enzymes, lipase, protease, transferase.

A study done by Tothova et al looked at salivary markers of oxidative stress in oral diseases. This clinical study showed an association between oral pathologies and established salivary markers of oxidative stress. This oxidative stress seems to be of local origin but currently, it’s unclear whether it is caused by an overproduction of reactive oxygen species due to inflammation or by lack of antioxidants. Intervventional studies, both in experimental animals as well as humans indicate that antioxidant treatment could prevent or slow down the progress of periodontitis[24].
A study done to evaluate and compare salivary concentration of reduced & oxidised glutathione, uric acid and ascorbic acid and total antioxidant capacity in subjects with diabetes and systematically healthy subjects with inflammatory periodontal disease concluded that a decrease in salivary reduced glutathione levels in patients with type I diabetes may have a role to play in periodontal tissue destruction by predisposing tissues to oxidative stress. In the role of oxidative stress in both diabetes and periodontitis, the compensatory mechanism of the body is partially collapsed because of excessive production of free radicals during periodontitis. The body is not able to cope with increased free radical generation attributable to diabetes thereby worsening the situation [1].

Uric acid could also have a role in the inflammatory pathology of periodontitis[25]. It is suggested that it may be useful in prognosis and diagnosis of chronic periodontitis. However, the mechanistic association of these parameters with inflammatory pathology of patients with periodontitis needs to be further elucidated. Studies have shown that non surgical periodontal treatment affected salivary total antioxidant capacity including uric acid content. Moreover, these biochemical parameters convincingly reflected periodontal status and tissue response on treatment [26].

Antioxidant constituents in plasma have been known to display sensitivity to dietary antioxidant intakes. The concentration of antioxidants in saliva does not appear to mirror those of plasma. The extent of dietary influence upon salivary antioxidant status is unclear. Urate is the predominant salivary antioxidant, with albumin and ascorbate providing minor contributions. Previous research has found reduced salivary antioxidant activity in patients suffering from periodontal disease. An improved understanding of the role antioxidants play in periodontitis, and the influence of nutrition on antioxidant status, may lead to a possible nutritional strategy for the treatment of periodontal disease [27].

1.2.4 Saliva as a diagnostic tool
Saliva can be collected by passive drool technique or by using oral swabs. In healthy individuals, depending on age and gender, unstimulated salivary flow rate is between 0.1-2ml/min. Additional factors influencing unstimulated saliva flow and composition include individual hydration, body posture, lighting, smoking, circadian and circannual rhythms and medication. Whole saliva represents a mixture of secretions of major salivary glands (submandibular, sublingual & parotid) and minor salivary glands plus gingival fluid. These
vary considerably and are affected by different forms of stimulation, time of day, diet, age, gender, disease states and pharmacologic agents. 60% of saliva is produced under resting conditions [6].

Gowrie et al stated that stimulation of saliva may increase the flow of gingival crevicular fluid which may result in a false increase in the concentration of antioxidants in saliva [28]. In a study done by Ahmadi et al, total antioxidant capacity of saliva and dental caries was evaluated. In his saliva sampling technique, unstimulated whole saliva specimens were collected in the morning and participants were asked to brush their teeth and not use any oral stimulation e.g. eating and drinking for 90 minutes prior to collection. Participants were sitting and anterior head position used. Whole saliva samples were obtained by expectorating into polypropylene tubes within 5 minutes. The saliva samples were first weighed then immediately put in ice and stored at 4 degrees Celsius and transferred to the laboratory up to 20 minutes. They were kept at -80 degrees Celsius until the analysis [6].

1.2.5 Total antioxidant capacity (TAC) in saliva

Total antioxidant capacity, defined as the moles of oxidants neutralised by one litre of solution, is a biomarker measuring the antioxidant potential of body fluids [29]. The antioxidant status in saliva is related to both oral hygiene and periodontal status. Dental hygiene procedures and scaling and root planning increased salivary TAC in 50% of the studies conducted on patients with periodontal disease [30]. It was also noted in some studies that salivary TAC increased during aging [31] while on the contrary, others reported that salivary TAC was higher in younger subjects [32]. Women had significantly lower salivary TAC than men. In this context, Kawamoto et al reported decreased levels of salivary TAC during the ovulatory phase compared to follicular phase in women with periodontal disease, but not in healthy women [33].

Results on smoking habit were conflicting as higher, lower or non significant different levels of salivary TAC have been found in smokers compared to non smokers. On the contrary, alcohol dependent subjects showed significantly lower TAC in blood and saliva as compared to those in the controls and alcohol withdrawal caused an increase in the TAC to near control values [29]. Type 2 diabetes has been associated with a decreased total antioxidant capacity. Battino et al conducted a study on antioxidant status in saliva and found that salivary TAC content was significantly lower in diabetes group compared to
healthy controls. When the salivary TAC in type 2 diabetic patients with and without periodontal disease was measured, it was found to be significantly lower in diabetic patients with periodontitis compared to healthy individuals with periodontitis [28].

Despite differences in assays, concordant results were found in the majority of cases. The different assays used to measure salivary TAC include:

i. Trolox Equivalent Antioxidant capacity (TEAC)
ii. Ferric Reducing Antioxidant Potential (FRAP)
iii. Oxygen Radical Antioxidant Capacity (ORAC)
iv. Total Radical Trapping Antioxidant Parameter (TRAP)
v. Enzyme Linked Immunosorbent Assay (ELISA)

The most common total antioxidant capacity tests were summarized by Rice-Evans in 2000 [34]. In 1996, the research group led by Benzie described the “ferric reducing–antioxidant power” (FRAP) method, which is very widely used today. The FRAP assay is based on the reduction of ferric ions to ferrous ions by the effect of the reducing power of the plasma (or a sample) constituents measured using spectrophotometry at 593nm [35]. Another important TAC method is the ORAC (oxygen radical absorbance capacity). ORAC assay was developed by Cao’s research team. The ORAC method is based on the ability of plasma constituents to trap peroxyl radicals formed from thermal decomposition of azo initiators (ABAP-2,2’-azobis[2-amidino propane]) and measurement of fluorescence decay of β-phycoerythrin (β- PE) (excitation wavelength of 540nm and emission wavelength of 565nm)[36]. Miller et al in 1993 described the “Trolox equivalent antioxidant capacity” (TEAC) method which was transformed into a commercial kit by Randox Laboratories Ltd. (UK). The method is based on formation of the ABTS•+ cation [2, 2’- azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] and its scavenging by antioxidant sample constituents (e. g., serum or food) measured by spectrophotometry (decay of green/blue chromatophore absorbance is inversely associated with antioxidant sample content and the control antioxidant is Trolox, a hydrophilic vitamin E analogue). For its relative simplicity and high analytical quality, TEAC kit (Randox) is widely used [37].
The authors indicated that method choice does not seem to be a critical point for salivary TAC measure. No single method is considered gold standard for TAC determination suggesting that many comparable methods can be used [28]. On the contrary, major concerns come from saliva collection. Saliva sampling has been improved by cotton and polypropylene salivette collection systems. It has been reported that samples collected using the cotton salivette TAC were comparable, but higher thiobarbituric acid reacting substance(TBARS) concentration were determined compared with unstimulated saliva. When TAC was examined with respect to flow rate, a significantly lower rate of antioxidant production was noted in patients with periodontal disease compared with controls for unstimulated saliva but not for stimulated saliva. Therefore, unstimulated saliva with and without flow rate normalisation could be the better approaches to measure salivary TAC [29].

In a study on antioxidant status of saliva by Battino et al, spectrophotometric assays quantifying levels of thiobarbituric acid reacting substances (TBARS) are used to evaluate salivary antioxidant status [7]. This is by use of an antioxidant assay kit (cat #709001; Cayman chemical). The reaction is based on ability of aqueous and lipid antioxidants to inhibit the oxidation of 3 ethylbenzthiazoline sulphonate- ABTS to ABTS+. The capacity of the antioxidants to prevent ABTS oxidation is compared to that of standard Trolox, a water soluble tocopherol analogue. Absorbance is measured at 405nm according to the kit manufacturer’s instructions using Tecan sunrise micro plate reader( Tecan Australia) [6].

In another study by Reshma et al, salivary TAC levels were estimated by using a spectrophotometer. A standardised solution of Fe-EDTA complex reacts with hydrogen peroxide by a Fenton type reaction leading to formation of hydroxyl radicals. These reactive oxygen species degrade benzoate, resulting in release of thiobarbituric acid reactive substances (TBARS). Antioxidants from the added saliva cause suppression of production of TBARS. This reaction can be measured spectrophotometrically and inhibition of colour development as total antioxidant capacity [38].

Salivary TAC measurements as done by Zhang et al were based on the measurements of the reduction of 2, 2’ azinobis (3-ethylbenzothiazoline-6-sulphoic acid; ABTS) radical. 225 micro litres of assay Reagent 1(acetate buffer, ph 5.8) was mixed with 5 micro litres of saliva and the absorbance measured at 420nm after 30 sec incubation. Afterwards, 20 micro
litres of Reagent 2 (ABTS, 30mm in acetate buffer, pH 3.6) were added into each sample and the absorbance measured after 5min incubation. TAC was calculated based on the differences in absorbance at 420nm before and after adding the Reagent 2. The assay was calibrated with trolox and the results expressed in terms of mm trolox equivalent per litre (mmoltroloxEquiv/L). TAC was evaluated because any antioxidants work together. Studies have shown that TAC evaluation was statistically significant than individual antioxidants and the measurement of any individual antioxidant may be difficult and expensive. TAC shows the action of all non enzymatic antioxidants [6].

Periodontal disease is thus a host-microbial interaction in which both host and bacterial factors determine the outcome, such that changes in the balance between host and bacterial factors can result in a change from health to disease. The balance may be changed, for example, by a reduction in the host resistance, an increase in the microbial plaque bio film or an increase in bacterial virulence. Systemic conditions where cellular function is impaired such as diabetes mellitus lead to a reduction in the host’s defence mechanisms, and consequently predisposing the patient to more severe forms of periodontal disease. Diabetes has been associated with a decreased total antioxidant capacity. Reduced salivary total antioxidant capacity in diabetic patients may be one of the mechanisms by which poor glycemic control leads to increased prevalence and severity of periodontitis. This is due to a reduction in the protective mechanisms offered by salivary antioxidants against the reactive oxygen species.

The current study aims at measuring the TAC in saliva of diabetic and non diabetic patients. The study aims to collect whole unstimulated saliva as TAC has been shown to be higher in unstimulated saliva and though studies have shown that parotid saliva is the major source of antioxidants[6] whole saliva is chosen for ease of collection. The results would be a guide for future research work in antioxidant activity in periodontitis.
CHAPTER TWO: STATEMENT OF THE RESEARCH PROBLEM AND JUSTIFICATION

2.1 Problem Statement
Periodontal disease has a high prevalence in Kenya, estimated to affect about 80% of the adult population [39]. Periodontal disease is also prevalent worldwide and a leading cause of tooth loss in adults. Diabetes on the other hand is also common worldwide and Kenya is no exception. The Ministry of Health estimates the prevalence of diabetes to be around 10% (3.5 million people). Screening studies have identified the prevalence of undiagnosed diabetes to be 14% [40]. Diabetes places a considerable economic burden on individuals, families and healthcare systems and is thus the cause of much human suffering. Several studies demonstrate an association between diabetes mellitus and periodontal disease. On one hand, diabetes mellitus is a risk factor for developing gingivitis and/or periodontitis in both type 1 and 2 diabetics. On the other hand, periodontal disease may impair or even hinder the control of blood sugar levels in these patients.

There is significant evidence linking reduced salivary total antioxidant capacity and periodontal disease. This is further compounded by the fact that diabetes further reduces salivary total antioxidant capacity levels which would normally protect the periodontal tissues against oxidative stress. If left unchecked, uncontrolled diabetic patients would suffer from severe forms of periodontal disease whose ultimate end point is tooth loss. This would impair proper mastication and speech, affect the normal function of the temporal mandibular joint as well as lead to aesthetic concerns that may lead to psychological alterations.

2.2 Justification of the Study
Diabetes with poor glycemic control is associated with an increased prevalence and severity of periodontitis. Several mechanisms have been implicated in this association. Reduced salivary total antioxidant capacity in diabetic patients may be one of the mechanisms by which poor glycemic control leads to increased prevalence and severity of periodontitis. This is due to a reduction in the protective mechanisms offered by salivary antioxidants against the reactive oxygen species.
Substantial data is available in literature on the role of oxygen species and antioxidants in systemic pathology but little is available on oral diseases and specifically periodontitis. There is thus need to investigate salivary total antioxidant capacity and determine if there is a relationship between salivary total antioxidant capacity and severity of periodontal disease.

In the last decade, several methods have been developed for assaying the antioxidant activity of saliva, indicating an increasing interest of researchers and clinicians. However, no particular method has been considered gold standard [28]. This study will use an assay kit by Sigma Aldrich Company that has never been used before to investigate salivary TAC. This will widen the scope of application of kits that are available in the market. Saliva offers a promising diagnostic alternative, compared to blood sampling, for screening for inflammatory risk factors particularly among paediatric and geriatric populations where blood sampling may be difficult. Additional research is needed to validate salivary biomarkers and establish reference ranges and characterize the influence of diet, physical activity, and drug treatment [5].

This study will improve the quality of care given to diabetic patients during routine clinical follow up and may even indicate a need for antioxidant supplementation of such patients.

2.3 Objectives

2.3.1 Main objective
To investigate salivary total antioxidant capacity and periodontal status of diabetics and non diabetics at Thika Level 5 Hospital.

2.3.2 Specific objectives
i. To determine the severity of periodontitis in diabetics and non diabetics in the study population.
ii. To determine the salivary Total Antioxidant Capacity among diabetics and non diabetics in the study population.
iii. To compare the salivary Total Antioxidant Capacity in diabetics and non diabetics.
iv. To determine if there is any relationship between salivary total antioxidant capacity and the severity of periodontal disease.
2.4 Hypothesis

2.4.1 Null hypothesis
There is no association between salivary total antioxidant capacity and severity of periodontal disease.

2.4.2 Alternate hypothesis
There is an association between salivary total antioxidant capacity and severity of periodontal disease.

2.5 Study variables

Table 1: Study variables

<table>
<thead>
<tr>
<th>VARIABLES</th>
<th>MEASUREMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Socio-demographic variables</strong></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>Years</td>
</tr>
<tr>
<td>Gender</td>
<td>Male or female</td>
</tr>
<tr>
<td>Education</td>
<td>Primary, Secondary, Tertiary</td>
</tr>
<tr>
<td><strong>Independent (exposure) variables</strong></td>
<td></td>
</tr>
<tr>
<td>Gingival inflammatory status</td>
<td>Gingival index- Loe and Silness 1963[41]</td>
</tr>
<tr>
<td>Salivary total antioxidant capacity</td>
<td>Milli mol/L</td>
</tr>
<tr>
<td>Diabetic status</td>
<td>YES/NO</td>
</tr>
<tr>
<td><strong>Dependent (outcome) variables</strong></td>
<td></td>
</tr>
<tr>
<td>Severity of periodontitis</td>
<td>No, mild, moderate and severe periodontitis- CDC/AAP case definitions[42]</td>
</tr>
<tr>
<td><strong>Confounder variables</strong></td>
<td></td>
</tr>
<tr>
<td>Oral hygiene status</td>
<td>Plaque Index- Silness and Loe 1964[41]</td>
</tr>
</tbody>
</table>
CHAPTER THREE: MATERIALS AND METHODS

3.1 Study area
The study was carried out at Thika Level 5 Hospital diabetic outpatient clinic. Thika Level 5 Hospital is a public hospital located in Kiambu County, Thika town. The hospital provides both inpatient and outpatient services to an average of 20,000 and 350,000 patients respectively on an annual basis. It has a catchment population of 3-5 million people on average from Kiambu County as well as the neighbouring Nairobi, Murang’a, Kirinyaga and Machakos counties. The hospital has an outpatient diabetes clinic with a daily turnover of approximately 20-30 patients.

3.2 Study population
The study population consisted of all adult patients attending Thika Level 5 Hospital diabetic clinic between April and May 2018. It was assumed that they were diabetics based on their clinical records and that they were already on follow up by their physicians for diabetic management.

The comparative group consisted of adults within the diabetic clinic who were not attending the clinic but were accompanying the diabetic patients. A prior visit to the diabetic out-patient clinic had revealed that most of the patients were accompanied by persons who were not attending the diabetic clinic. They were requested to take a random blood sugar test and those with glucose levels between 3.2 and 7.8mmol/l were recruited.

3.3 Study design
This study was conducted at one point in time and was thus a comparative cross sectional study with both descriptive and analytic components.

3.4 Sample size determination
To estimate the difference in means between two independent samples, the formula for determining the sample sizes required in each comparison group was [43];

\[ n_1 = 2 \left( \frac{Z \sigma}{E} \right)^2 \]

Where \( n_1 \) = sample size required in each group

\[ Z = \text{the } z \text{ value corresponding to } 0.05 \text{ which is } 1.96 \]
σ = the standard deviation of the outcome variable taken as 4.68 gotten from a similar study [5]

E = the desired margin of error taken as 3.

\[ n_1 = 2 \left( \frac{1.96 \times 4.68}{3} \right)^2 \]

\[ n_1 = 36 \]

The sample size was thus 36 participants from each group giving a total sample size of 72 participants.

3.5 Sampling

3.5.1 Sampling for diabetic group
Systematic sampling method was used to select the study participants whereby the number of patients on the booking list formed the sampling frame. The diabetic out-patient clinic had a booking of around 30 patients per day. Out of the 30 booked patients, every 5th patient was recruited to get a total of 6 patients per day. A confirmation was sought from their file that they were type II diabetics. A screening form was used to select the participants and the clients who met the inclusion criteria were assigned numbers until the desired number of participants was reached.

3.5.2 Sampling for non diabetic group
Systematic sampling method was also used whereby the persons accompanying the booked diabetic patients formed the sampling frame. Every 5th client was recruited. A screening form was used to select the participants, who were then requested to undertake a random blood sugar test. The participants who met the inclusion criteria were assigned numbers that were also used to label their saliva samples in order to tally the clinical characteristics with the laboratory results.

3.6 Inclusion/exclusion criteria

3.6.1 Inclusion criteria for diabetic group
1. Persons who were 18 years and above and gave voluntary informed consent to participate in the study.
2. Persons with type II diabetes who attended the diabetic outpatient clinic at Thika Level 5 Hospital.
3.6.2 Exclusion criteria for the diabetic group
1. Persons who had less than 20 teeth present.
2. Persons who had a previous history of periodontal treatment within the past 6 months.
3. Females who were pregnant or lactating.
4. Persons with concurrent systemic illness such as rheumatoid arthritis.

3.6.3 Inclusion criteria for non diabetic group
1. Persons who were 18 years and above and gave voluntary informed consent to participate in the study.
2. Persons who accompanied the diabetic patients within the diabetic clinic.

3.6.4 Exclusion criteria for non diabetic group
1. Persons with random blood sugar levels >7.8mmol/l
2. Persons who had a previous history of periodontal treatment within the past 6 months
3. Females who were pregnant or lactating
4. Persons who had less than 20 teeth
5. Persons with concurrent systemic illness such as rheumatoid arthritis.

3.7 Pretesting of data collection tools
A pilot study was conducted to pre-test the questionnaires, clinical examination protocol and saliva collection methodology. The questionnaire was found to be clear and well understood by the participants. All individuals involved responded to the questions appropriately. The clinical examination was comfortable in the set up and captured the data required adequately. Each individual was able to expectorate whole saliva sample into the polystyrene tube with ease.

A dummy ELISA assay was carried out in duplicate in the laboratory to test the instruments as well as the protocol that was to be used. The instruments were valid and in good condition as evidenced by positive and consistent results. The laboratory protocol was well understood by the laboratory technician as well as the principal investigator.
3.8 Data collection instruments and technique
Data was then collected using various tools, equipment and techniques as outlined below:

3.8.1 Measurement of variables
3.8.1.1 Socio-demographic data
Data concerning socio-demographic variables was collected from participants using interviewer administered questionnaires.

3.8.1.2 Clinical examination
Clinical examination was done in a well lit room using natural light and examining lamps. Intra-oral examination was done by the principal investigator. Infection control and prevention protocol was observed by use of sterile equipment and use of personal protective gear. Sterile dental mirrors and Marquis periodontal probes were used for clinical examination. Plaque score was established using Silness-Loe index (1964) and gingival index using the Loe and Silness index (1963) on Ramfjord’s index teeth that have been shown to be of representative and epidemiological significance including the East African region [44]. Full mouth periodontal examination was done and probing pocket depth as well as bleeding on probing was measured at six sites per tooth (mesiobuccal, mid-buccal, distobuccal, mesiolingual, midlingual and distolingual) using sterile periodontal probes on all teeth excluding third molars. Clinical attachment loss was measured as the distance from the cement-enamel junction to the base of the sulcus.

Recession was measured as the distance between the heights of the free gingival margin to the CEJ. Clinical findings were recorded by a nurse who had been trained for purposes of the study in a periodontal probing chart to the nearest millimetre. The nurse was recruited to ensure aseptic technique was followed during clinical examination as well as ensure accuracy of recorded findings by avoiding constant removal of gloves by the principal investigator. Periodontitis was determined using the CDC/ AAP consensus definitions[45] as indicated in Appendix 7.

3.8.1.3 Saliva sample collection
Prior to saliva sample collection, participants were allowed to seat comfortably for a few minutes and thoroughly rinse their mouth. They then leaned forwards slightly without swallowing or speaking. After about 5 minutes, the saliva had pooled in the anterior floor
of the mouth. The saliva was then collected by passively drooling into a 50ml pre weighed airtight and serialised polystyrene tubes. This was according to the protocol of Navazesh et al 2008[26]. Absorbent paper towels were provided for any untoward spillages. Saliva collection was done between 8 am and 11 am. Samples in tubes were placed in a sealed polythene bag and covered with ice cubes and gel ice packs inside a cooler box. The samples were then taken to the laboratory where they were centrifuged at 1800 revolutions per minute for 10 minutes at 21°C, and stored in small aliquots in a refrigerator at -80 degrees Celsius awaiting analysis.

3.8.1.4 Assessment of total antioxidant capacity.
The test samples were analysed using spectrophotometry at the Kenya Aids Vaccine Initiative- Institute of Clinical Research (KAVI-ICR) laboratories by the same laboratory technician. A Tecan Elisa micro plate reader was used to measure the endpoint absorbance at 405nm. The CS0790 Total Antioxidant Capacity Assay kit from Sigma- Aldrich Company (USA) was used. The principle of the assay is formation of a ferryl myoglobin radical from metmyoglobin and hydrogen peroxide, which oxidises the ABTS (2,2azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) to produce a radical cation, ABTS⁺, a soluble chromogen that is green in colour and can be determined spectrophotometrically at 405nm. HX-Fe³⁺ + H₂O₂. X-[Fe⁴=O] + H₂O
ABTS + X-[Fe⁴=O] ABTS⁺+HX-Fe³⁺

In this equation, HX-Fe³⁺ is metmyoglobin and X-[Fe⁴=O] is ferryl myoglobin.
Antioxidants suppress the production of the radical cation in a concentration dependent manner and the colour intensity decreases proportionally. Trolox, a water soluble vitamin E analogue, serves as a standard or control antioxidant.

Figure 1: Photograph showing different intensities of green colour in a plate
Protocol summary:

i. Sample preparation.

ii. Standard curve preparation.

iii. Prepare and add working solution.

iv. Incubate at room temperature for 2 minutes.

v. Add stop solution.

vi. Measure endpoint absorbance at 405nm.

Antioxidant concentration determination;
Salivary concentrations of TAC was obtained from a standard curve constructed as shown below.

![Figure 2: Salivary TAC standard curve](image)

Antioxidant concentration of the test samples were computer generated using the equation obtained from the linear regression of the standard curve:

\[ X \text{ (mM)} = y(A_{405}) - \text{Intercept} \times \text{dilution factor} \times \text{Slope} \]

Where; \(X\) (mM) – antioxidant concentration relative to the concentration of the Trolox standard.

\(y(A_{405})\) – average absorbance of test sample at 405nm

Intercept – intercept of the Y axis by the standard curve

Slope – slope of the standard curve, a negative value

Dilution factor – fold dilution of the original sample (only used if sample was diluted prior to adding to the well)
3.8.1.5 Quality assurance

The proper functionality of the equipment was ascertained by obtaining the maintenance schedules as well as records for equipment calibration and servicing. Each saliva sample was labelled using a serial number, stored and normal reference values established. The quality of data was ensured right from formulation of data collection tools. Proper coding and serialization of the forms was done. Records were clearly labelled and data entry done to precision. All collected data was entered in password protected systems. All questionnaires with data were kept in files in lockable cabinets in a restricted area.
3.8.1.6 Data analysis
Data was pre-coded and entered to the computer using Statistical Packages for Social Sciences (SPSS) 20.0 for Windows (SPSS inc. Chicago, Illinois, USA). Data cleaning was then done. Where necessary, Microsoft- Excel was used in conjunction with SPSS. Independent t-test was used to determine association between exposure and outcome variables. The Spearman’s correlation test was used to assess the relationship between diabetic status and the salivary total antioxidant capacity. The results of this study were then presented in form of text, figures, graphs, tables and charts among others.

3.9 Constraints
Ramfjord’s index teeth were used to calculate gingival and plaque score. As much as they have been shown to have epidemiological validity in the East African population, they do not give the true picture of the condition of the entire periodontium. Time constraints were also experienced due to compact post graduate academic and clinical schedules.

3.10 Confounders
Failure to disclose or inaccurate information provided by participants concerning exclusion criteria might have led to confounders in the study.

3.11 Ethical consideration
Approval to conduct the study was sought and obtained from the Kenyatta National Hospital- University of Nairobi Ethics and Research committee (P751/12/2017). Clinical examination was done following the standard operating procedures stipulated in the facility quality management standards manual. Participants who required emergency treatment were treated at the facility’s dental clinic.

Data confidentiality and anonymity of patients’ identity was ensured. All persons who met the inclusion criteria had an equal chance of being included in the study. Clinical examination and saliva sample collection was carried out under strict infection control measures. The participants were allowed to withdraw from the study at will without any dire consequences. There were no anticipated financial benefits either to the investigator or to the participants from this study.
3.12 Consent process
The principal investigator explained the purpose of the study to the participants in a language they understood best, any queries were answered and written informed voluntary consent was obtained from every participant before commencement of the study as shown in Appendix 1. In cases where the participant was illiterate, a witness confirmed the reading of the consent form to the potential participant after explanation of the purpose of the study and indicated in writing that the participant had freely given consent.

3.13 Benefits
This study provides new knowledge on the levels of salivary total antioxidant capacity (TAC) in diabetics and non diabetics in Kenya. Associations of salivary TAC with varying severity of periodontal disease as well as diabetic status, provides indicators for utility as a biomarker. The findings will expand dental practitioner’s current knowledge on assessment of periodontal disease activity among the diabetic patients in the Kenyan population. This will also form a vital platform for further salivary proteomic studies.
DISCLOSURE
The cost of the research was met by the principal investigator for academic purposes.
CHAPTER FOUR: RESULTS

This chapter represents the information obtained from the respondents using questionnaires, clinical examination as well as the laboratory findings. Key results include findings from the sociodemographic characteristics, clinical examination and salivary concentrations of total antioxidant capacity. The results are presented in both a descriptive as well as analytic manner.

4.1 Sociodemographic characteristics

A total of 122 participants ranging from 25-80 years were recruited into the study. Of the 122 examined, 63(51.6%) were diabetic and 59(48.4%) were non diabetic. Of the 63 diabetics, 37(58.7%) were females and 26(41.3%) were males. Among the non diabetics, 27(45.8%) were females while 32(54.2%) were males. Diabetics had a mean age of 53 years (±14.05SD) while non diabetics had a mean age of 41 years (±13.5SD). An independent sample t test showed a statistically significant difference in age between the two groups (t(120)=4.678 p=0.0001). Of the 122 participants, majority had attained secondary, college or university education at 70(57.3%). Among these, the non diabetics were the majority at 45(36.9%). Majority of the diabetics had attained either no education or primary level education at 38(31.1%). Non diabetics had a statistically significant higher level of education ($X^2(1)=16.678 p=0.0001$) compared to their diabetic counterparts. Table 2 below shows sociodemographic characteristics of participants.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Diabetes status</th>
<th>Test</th>
<th>p - value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diabetic n(%)</td>
<td>Non diabetic n(%)</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>26 (21.3)</td>
<td>31 (25.4)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>37 (30.3)</td>
<td>28 (23)</td>
</tr>
<tr>
<td>Education</td>
<td>None/ Primary</td>
<td>38 (31.1)</td>
<td>14 (11.5)</td>
</tr>
<tr>
<td></td>
<td>Secondary</td>
<td>25 (20.5)</td>
<td>45 (36.9)</td>
</tr>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>University</td>
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</table>
4.2 Oral hygiene practices
All participants brushed their teeth. Of the 63 diabetics, majority 33 (52.3%) brushed their teeth once a day while 30 (47.6%) brushed their teeth twice a day. In the non diabetic group, majority 31 (52.5%) brushed once a day while 28 (47.5%) brushed twice a day. There was no statistically significant difference between tooth brushing frequency and diabetic status \( (X^2_{(1)} = 0.0001 \ p = 0.986) \). Male participants generally tended to brush once daily while the female participants mostly brushed twice or thrice a day. The difference was statistically significant at \( (X^2_{(1)} = 6.653 \ p = 0.010) \). The oral hygiene practices of the participants are indicated in Table 3.

Table 3: Oral hygiene practices of participants

<table>
<thead>
<tr>
<th>Variable</th>
<th>Tooth brushing frequency</th>
<th>Test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>/once</td>
<td>twice/thrice</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>Age(years) 25-39</td>
<td>16 (13.1)</td>
<td>22 (24.4)</td>
<td>( X^2_{(3)}=11.335 ) 0.010</td>
</tr>
<tr>
<td>40-54</td>
<td>19 (15.6)</td>
<td>26 (21.3)</td>
<td></td>
</tr>
<tr>
<td>55-69</td>
<td>17 (14)</td>
<td>7 (5.7)</td>
<td></td>
</tr>
<tr>
<td>70-80</td>
<td>12 (10.0)</td>
<td>3 (2.5)</td>
<td></td>
</tr>
<tr>
<td>Gender male</td>
<td>37 (30.3)</td>
<td>20 (16.4)</td>
<td>( X^2_{(1)}=6.653 ) 0.010</td>
</tr>
<tr>
<td>female</td>
<td>27 (22.1)</td>
<td>38 (31.1)</td>
<td></td>
</tr>
<tr>
<td>Education none/primary</td>
<td>31 (25.4)</td>
<td>21 (17.2)</td>
<td>( X^2_{(1)}=1.861 ) 0.173</td>
</tr>
<tr>
<td>sec/coll/uni</td>
<td>33 (27.0)</td>
<td>37 (30.3)</td>
<td></td>
</tr>
<tr>
<td>Diabetes diabetic</td>
<td>33 (27)</td>
<td>30 (24.6)</td>
<td>( X^2_{(1)}=0.0001 ) 0.986</td>
</tr>
<tr>
<td>non diabetic</td>
<td>31 (25.4)</td>
<td>28 (23.0)</td>
<td></td>
</tr>
</tbody>
</table>

Key:
- sec secondary
- coll college
- uni university
4.3 Oral hygiene status

Oral hygiene status of participants was assessed using Silness and Loe plaque score index of 1964. The mean plaque score was 1.89 (±1.393SD) for the diabetics and 1.22 (±1.365SD) for the non diabetics. The scores were put into three categories and tested against various variables for association. There was a statistically significant difference between the plaque score and age of participants ($X^2_{(4)} = 34.431$, $p=0.0001$). The association between plaque score and smoking status was also statistically significant ($X^2_{(1)} =10.125$ $p=0.001$). A statistically significant difference was found between mean plaque scores and tooth brushing frequency ($X^2_{(1)} = 4.094$ $p=0.043$). Participants with higher levels of education had lower plaque scores compared to those who had lower levels of education ($X^2_{(1)}=11.570$ $p=0.001$).

Table 4: Oral hygiene status among the participants

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean plaque score</th>
<th>Test</th>
<th>p- value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-1 n (%)</td>
<td>2-3 n (%)</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>Diabetic</td>
<td>29 (23.8)</td>
<td>34 (27.9)</td>
</tr>
<tr>
<td></td>
<td>Non diabetic</td>
<td>35 (28.7)</td>
<td>24 (19.7)</td>
</tr>
<tr>
<td>Age(years)</td>
<td>25-39</td>
<td>32 (26.2)</td>
<td>6 (5.0)</td>
</tr>
<tr>
<td></td>
<td>40-54</td>
<td>25 (20.5)</td>
<td>20 (6.7)</td>
</tr>
<tr>
<td></td>
<td>55-69</td>
<td>5 (4.0)</td>
<td>19 (15.6)</td>
</tr>
<tr>
<td></td>
<td>70-80</td>
<td>2(1.6)</td>
<td>13(10.7)</td>
</tr>
<tr>
<td>Smoking</td>
<td>Never smoked</td>
<td>46 (51.1)</td>
<td>26 (28.9)</td>
</tr>
<tr>
<td></td>
<td>Former smoker</td>
<td>4 (4.44)</td>
<td>14 (15.6)</td>
</tr>
<tr>
<td>Brushing frequency</td>
<td>Once</td>
<td>28 (23.0)</td>
<td>36 (29.5)</td>
</tr>
<tr>
<td></td>
<td>Twice, thrice</td>
<td>36 (29.5)</td>
<td>22 (18.0)</td>
</tr>
<tr>
<td>Education</td>
<td>None, primary</td>
<td>18 (14.8)</td>
<td>34 (27.9)</td>
</tr>
<tr>
<td></td>
<td>Secondary, college, university</td>
<td>46 (37.7)</td>
<td>24 (19.7)</td>
</tr>
</tbody>
</table>
4.4 Gingivitis

The degree of gingival inflammation was assessed using the Loe and Silness gingival index of 1963. The mean gingival index for the diabetic group was 1.29 (±0.888SD) while that of the non-diabetic group was 0.80 (±0.783SD). Out of the 63 diabetics, 37 (30.3%) had either no or mild gingivitis clinically while 26 (21.3%) had either moderate or severe gingivitis. There was an association between diabetic status and mean gingival index \( t_{120} = 3.219 \) \( p=0.002 \) with most diabetics having higher mean gingival index scores compared to non-diabetics.

There was a significant positive association between plaque scores and gingival index category at \( X^2_{(1)} = 21.828 \) \( p=0.0001 \). Table 5 summarises the distribution of gingival inflammation among participants.
### Table 5: Gingival index among participants

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean gingival index</th>
<th>Test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-1</td>
<td>2-3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>X²(t)=</td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic</td>
<td>37</td>
<td>26</td>
<td>6.224</td>
</tr>
<tr>
<td>Non diabetic</td>
<td>(30.3)</td>
<td>(21.3)</td>
<td></td>
</tr>
<tr>
<td>Age(years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25-39</td>
<td>33</td>
<td>5</td>
<td>12.795</td>
</tr>
<tr>
<td></td>
<td>(4.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40-54</td>
<td>(27.0)</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>55-69</td>
<td>31</td>
<td>(11.5)</td>
<td></td>
</tr>
<tr>
<td>70-80</td>
<td>(25.4)</td>
<td>10</td>
<td>(7.4)</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>51</td>
<td>21</td>
<td>0.119</td>
</tr>
<tr>
<td>Former</td>
<td>(56.7)</td>
<td>(23.3)</td>
<td></td>
</tr>
<tr>
<td>Brushing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Once</td>
<td>41</td>
<td>23</td>
<td>1.440</td>
</tr>
<tr>
<td>Twice, thrice</td>
<td>(33.6)</td>
<td>(18.9)</td>
<td></td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None, primary</td>
<td>30</td>
<td>22</td>
<td>5.263</td>
</tr>
<tr>
<td>Secondary, college,</td>
<td>(24.6)</td>
<td>(18.0)</td>
<td></td>
</tr>
<tr>
<td>Plaque scores</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-1</td>
<td>30</td>
<td>28</td>
<td>21.828</td>
</tr>
<tr>
<td>2-3</td>
<td>(45.9)</td>
<td>30</td>
<td>(24.6)</td>
</tr>
<tr>
<td></td>
<td>(23.0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.5 Periodontitis

The presence or absence of periodontitis and the severity thereof was assessed using consensus CDC/AAP definitions. Out of the 63 diabetics, 11 (17.5%) had either no or mild periodontitis, while the majority, 52 (82.5%) had either moderate or severe periodontitis. Of the 59 non diabetics, 31 (52.5%) had either no or mild periodontitis while 28 (47.5%) had either moderate or severe periodontitis. The difference was statistically significant at (X$^2_{(1)}$ = 16.611 p=0.0001). The older age groups had more severe forms of periodontitis compared to the younger age groups at (X$^2_{(3)}$ = 38.048 p=0.0001). Male participants also had more severe forms of periodontitis compared to the females at (X$^2_{(1)}$ = 10.846 p=0.001). There was also a significant association between gingival index category and periodontitis. Mean clinical attachment loss was 1.643 (±0.776SD mm) for the diabetic group and 1.230 (±0.561SD mm) for the non diabetic group. Association between periodontitis and diabetic status is shown in Table 6.

Table 6: Periodontitis among the participants

<table>
<thead>
<tr>
<th>Variable</th>
<th>Periodontitis</th>
<th></th>
<th></th>
<th>Test</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-1 n(%)</td>
<td>2-3 n(%)</td>
<td>Test</td>
<td>p value</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>11 (9.0)</td>
<td>46 (37.7)</td>
<td>X$^2_{(1)}$=10.846</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>female</td>
<td>31 (25.4)</td>
<td>34 (27.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GI category</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-1</td>
<td>38 (31.1)</td>
<td>46 (37.7)</td>
<td>X$^2_{(1)}$=13.965</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>2-3</td>
<td>4 (3.3)</td>
<td>34 (27.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>diabetics</td>
<td>11 (9.0)</td>
<td>52 (42.6)</td>
<td>X$^2_{(1)}$=6.611</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>non diabetics</td>
<td>31 (25.4)</td>
<td>28 (23.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A linear regression curve estimation model elicited a statistically significant positive association between CAL and plaque as the predictor variable ($\beta=0.524$, F(1,88) = 33.349, $R^2=0.275$, p<0.001) as shown in Figure 4 below.
4.6 Salivary Total Antioxidant Capacity

In the 122 saliva samples analysed, the levels of TAC ranged from undetectable levels to 0.252mM/l. In individuals with diabetes, the TAC levels were either undetectable or significantly low. Non diabetic individuals however mostly had detectable levels of salivary TAC. A Pearson correlation coefficient showed a statistically significant difference between salivary TAC and diabetic status having controlled for age at r=0.277 p=0.002.

Figure 6: A box plot showing salivary TAC against gingivitis
A Pearson correlation coefficient also showed a non statistically significant negative association between age and salivary TAC ($r=-0.004$, $p=0.969$).

**4.7 Association between Salivary Total Antioxidant Capacity and gingivitis severity**

A Pearson correlation coefficient elicited a statistically significant negative association between gingivitis and TAC ($r=-0.289$ $p=0.001$).

A linear regression curve estimation model elicited a statistically significant negative association between TAC and gingival index as the predictor variable ($\beta=-0.294$, $F (1, 88) =8.307$, $R^2=0.294$, $p=0.005$) as shown in Figure 6 below.

![Regression plot model for TAC and gingival index](image)

**Figure 7: Regression plot model for TAC and gingival index**

**4.8 Association between Salivary Total Antioxidant Capacity and severity of periodontitis**

A Pearson correlation coefficient showed a negative association between CAL and TAC ($r=-0.158$ $p=0.084$) having controlled for age which was however not statistically significant. There was a non-statistically significant difference between salivary TAC and severity of periodontitis among diabetics ($X^2(1) =0.053$ $p=0.818$) as well as in non diabetics ($X^2(1) =0.336$ $p=0.562$). There was also a negative correlation between salivary TAC and CAL having controlled for age at $r=-0.158$ $p=0.084$. This was however not statistically significant.
4.9 Limitations of the study
This study was conducted in a hospital set up and extrapolation of the findings to the rest of the population may thus be a challenge. In addition, the setting did not allow appropriate randomisation as the investigator did not have control over the participants who visited the facility for treatment. Thus, a potential selection bias may have been introduced. This being a cross sectional study, the timing may not have been fully representative as the study only captured the population at one point in time. Age and gender differences between the two groups would also have been a confounder; however, this was eliminated by purposely matching the two groups with regard to these two variables.
CHAPTER FIVE: DISCUSSION, CONCLUSION & RECOMMENDATIONS
This chapter is dedicated to discussing the major findings of the study as highlighted in the results section in line with the study objectives and existing knowledge. The chapter interprets the findings and provides explanations that account for the findings linking them to the existing literature. The chapter also provides concluding remarks and gives recommendations.

5.1 Discussion
5.1.1: Sociodemographic characteristics
There was an age difference between the diabetic and non diabetic group. Age is an important confounder as periodontal disease severity tends to increase with advancing age. The effect of age was however minimised by controlling for age during statistical analysis. The age ranged from 25-80 years implying that only adults who could give informed consent participated in the study and indicated variability and diversity in ages of individuals visiting the diabetic clinic and the general population by extension. The gender disparity between the two groups was not statistically significant. However, there were more males than females seeking treatment at the facility’s diabetic clinic which may indicate a slightly higher prevalence of type 2 diabetes among males compared to females. This is in agreement with the findings by the International Diabetes Federation who reported that globally, more males are diagnosed with diabetes and in 2013 there were 14 million times more men affected with diabetes than women. Gender differences arise from sociocultural processes, such as different behaviours of women and men, exposition to specific influences of the environment, different forms of nutrition, life styles or stress, or attitudes towards treatments and prevention [46].

Concerning the level of education, majority of the participants had attained primary (37.4%) and secondary (35.8%) education. This could be due to the fact that the study was conducted in a peri-urban setting. There was a significant difference in the level of education between the two groups with the diabetics having attained lower levels of education. This could probably be attributed to the stigma attached to this disease condition as well as the health challenges encountered that may have hindered their progression academically.
5.1.2 Oral hygiene practices
All participants in this study brushed their teeth. Tooth brushing is a form of mechanical plaque control and is the most relied upon oral hygiene practice worldwide[47]. Concerning the frequency of brushing, majority of participants overall brushed their teeth once a day. This is in agreement with a study done on an Indian population[48] and could be attributed to low levels of awareness on oral health among the participants. This underscores the need for more oral health education in the population. The difference in tooth brushing frequency between diabetics and non diabetics was however not statistically significant. Most of the male participants brushed once a day while the female participants mostly brushed twice a day. This could probably be attributed to the fact that females tend to be more particular on general hygiene practices including oral hygiene. Similar findings were reported by Dafi S.T where more females than males reported brushing their teeth on a regular basis[49].

5.1.3 Oral hygiene status
Oral hygiene status of participants was assessed using Silness and Loe plaque score index of 1964. There was no significant difference in the plaque scores between the diabetics and non diabetics. This meant that any difference detected between the two groups in terms of periodontal disease would not be attributed to the oral hygiene status of the groups. Plaque scores of the participants increased with increasing age and this generally tends to be the norm. This could probably be attributed to a reduction in manual dexterity during tooth brushing with increasing age. Participants who brushed once also tended to have higher plaque scores compared to those who brushed twice or thrice. Again, this is expected as once daily brushing leaves plaque deposits to remain on tooth surfaces for a longer duration predisposing participants to higher plaque scores. This could also be attributed to the fact that tooth brushing is the most relied upon form of mechanical plaque control. Participants who had attained higher levels of education had lower plaque scores and vice versa as education tends to increase awareness on importance of oral health as well as oral hygiene practices [50].

5.1.4 Gingivitis/Periodontitis
Gingivitis was assessed using Loe and Silness gingival index of 1963. The mean gingival index was 1.29 (±0.888SD) for the diabetics and 0.80 (±0.783SD) for the non diabetics showing that all participants had some degree of gingivitis. The statistically significant
association between gingival index and plaque scores confirmed the role of dental plaque in the pathogenesis of gingival inflammation[47]. Individuals with higher level of education presented lower degree of gingival inflammation due to increased awareness in oral hygiene practices and lower plaque levels.

Periodontitis was assessed using consensus CDC/AAP definitions[42]. The significant positive association between periodontitis and age was as a result of longer duration of exposure to risk factors. Males also presented with more severe forms of periodontitis compared to females and this could be due to poor oral hygiene status. A statistically significant association was found between severity of periodontitis and diabetic status indicating the possible influence of type 2 diabetes on periodontal disease as reported by Mealey and colleagues[51]. This is also in agreement with a similar study done in South Africa where diabetics suffered more advanced periodontal disease compared to non diabetics[52].

5.1.5 Association of salivary TAC and periodontal clinical status
The periodontal clinical status in this study was described using plaque score, gingival index and CDC/AAP periodontal disease classification. While the plaque score gives the state of the oral hygiene, gingival index corresponds to the degree of inflammation (gingivitis). CDC/AAP periodontal disease classification describes the severity of the disease based on probing pocket depth and clinical attachment loss.

Statistically significant association was found between salivary TAC and diabetic status. Diabetics presented mostly with undetectable levels of salivary TAC as compared to the non diabetics who mostly had detectable levels. Similar findings were reported by Pendylala et al in 2013 [28]. This strong correlation is attributed to the many biochemical pathways associated with hyperglycemia that have been implicated in the increased free radical production in diabetic subjects thus depleting the antioxidant reserves [53].

Negative correlations were found between salivary TAC and gingival index. Increased amounts of salivary TAC were associated with lower gingival index scores and vice versa. Similar findings were reported by Pendylala et al in 2013[28] despite having used a different antioxidant kit for the assay. This correlation is attributed to the fact that decreased amounts of salivary antioxidants leads to increased oxidative damage within the
oral cavity that manifests as inflammation [54]. One possible source of the oxidative stress identified in type 2 diabetes patients with periodontitis may be hyperactive/reactive neutrophils as both periodontitis and diabetes are associated with neutrophil priming and enhanced ROS release, correlating with both severity of periodontal disease and glycemic control [55]. There was however no statistically significant association found between salivary TAC and CAL as well as periodontitis among diabetics and non diabetics. This is in contrast to a previous study where the salivary total antioxidant capacity was significantly lower in diabetics and non diabetics with periodontitis when compared to diabetics and non diabetics without periodontitis [28]. The findings of the current study are however similar to a study done in 1994 where saliva from patients with periodontitis did not have a lower TAC than that from healthy individuals. This was attributed to the fact that some of the extracellular antioxidants may be derived from gingival crevicular fluid (GCF) rather than from pure ductal saliva. The increased production of GCF associated with gingivitis and periodontitis may then balance the local decrease of antioxidants [34]. The inconsistent findings could be attributed to the different antioxidant assay kits used as well as differences in the laboratory protocol.

5.2 Conclusion
Based on the findings of this study, the following were the conclusions:-

1. Periodontitis severity was influenced by diabetic status whereby more diabetics suffered from severe periodontitis compared to non diabetics.
2. Levels of salivary TAC were influenced by diabetic status and subsequently had a relationship with periodontal clinical status.
3. Reduced amounts of salivary TAC mostly found in the diabetics corresponded to an increase in gingivitis disease severity.
4. There was no association found between salivary TAC and periodontitis severity.

5.3 Recommendations
Based on the findings of this study, the following were the recommendations:-

1. There is need to create more awareness among diabetics as well as their care givers on the relationship between diabetic status and periodontal health so that appropriate measures are taken to monitor for early signs of periodontal disease.
2. There is need to conduct further studies on salivary TAC using the various kits available in order to obtain a gold standard that would be used to obtain more conclusive results.

5.4 Conflict of Interest
The study was carried out as a partial fulfilment for the award of Masters of Dental Surgery degree in Periodontology at the University of Nairobi as well as for scientific purposes. The cost of the study was fully met by the principal investigator. There was thus no related conflict of interest.
REFERENCES


2. Lucarini, G., et al., Uncoupling of Vascular Endothelial Growth Factor (VEGF) and Inducible Nitric Oxide Synthase (iNOS) in Gingival Tissue of Type 2 Diabetic Patients. Inflammation 2015.


APPENDICES

APPENDIX 1: INFORMED CONSENT FORM (ICF) - ENGLISH VERSION

This informed consent form is for Thika Level 5 Hospital adult diabetics and non diabetics attending the diabetic clinic and who I am inviting to participate in the research titled “SALIVARY TOTAL ANTI-OXIDANT CAPACITY AND PERIODONTAL STATUS OF DIABETICS AND NON DIABETICS ATTENDING THE THIKA LEVEL 5 HOSPITAL”. I Annette Wambui Muriithi am the principle investigator and a postgraduate student at University of Nairobi Dental School Department of Periodontology, Community and Preventive Dentistry. I am the sole sponsor of the research project.

This informed consent form has two parts:-

- Information sheet (to share information about the study with you)
- Certificate of consent (for signatures if you chose to participate)

You will be given a copy of the full informed consent form.

Part 1: Information sheet.

Introduction
I am Dr. Annette Wambui Muriithi, a postgraduate student at University of Nairobi Dental School Department of Periodontology, Community and Preventive Dentistry. I am doing research on diabetes and periodontal status which is very common in this country and region. I am going to give you information and invite you to be part of this research. You do not have to decide today whether or not you will participate in this research. Before you decide, you can talk to anyone you feel comfortable with about the research.

This consent form may contain words that you do not understand. Please ask me to stop as we go through the information and I will take time to explain. If you have questions later, you can ask them of me or of another researcher.

Purpose of the research
Diabetes is affecting many people’s gums in our community. I want to find ways to stop this from happening. I believe that you can help me by letting me examine your gums and collect some of your saliva for testing. This may help me in finding out if your blood sugar
has a negative effect on your gums. This might help us to know how better to take care of your gums.

**Type of research intervention**
This research will involve your participation in responding to a questionnaire, having your gums examined for about 10 min and providing a small amount of saliva into a plastic container.

**Participant selection**
You are being invited to take part in this research because I feel that your blood sugar status can contribute much to our understanding and knowledge as dental health care providers.

**Voluntary participation**
Your participation in this study is entirely voluntary. It is your choice whether to participate or not. If you chose not to participate, all the services you receive at this centre will continue and nothing will change.

**Procedures**
We are asking you to help us know more about gum disease in diabetics. We are inviting you to take part in this research project. If you accept, you will be asked to:

Answer a few questions guided by myself. If you do not wish to answer any of the questions involved in the survey, you may skip them and move to the next question. The information collected is confidential, your name is not being included in the forms; only a number will identify you and no one else except myself will have access to your survey.

**Duration**
The research takes place over 2 months in total. During this time, you will only be required to participate in this study once and this will take about 30 minutes.

**Risks**
There is a risk that you may share some personal information by chance, or that you may feel uncomfortable talking about some of the topics. However, we do not wish for this to happen.
You do not have to answer any question or take part in the survey if you feel the questions or procedures are too personal or if talking about them makes you feel uncomfortable.

**Benefits**
There is no direct benefit to you but your participation is likely to help us find out more about how to treat and prevent gum disease among diabetic patients.

**Reimbursements**
You will not be provided any incentives to take part in the research.

**Confidentiality**
The research being done in the community may draw attention and if you participate you may be asked questions by other people in the community. I will not be sharing information about you to anyone outside of the research team. The information that I collect from this research project will be kept private. Any information about you will have a number on it instead of your name. Only the researcher will know what your number is and it will not be shared or given to anybody.

**Sharing the results**
Nothing that you tell us today will be shared with anybody outside the research team, and nothing will be attributed to you by name. The knowledge that we get from this research will be shared with you and your community before it is made widely available to the public.

**Right to refuse or withdraw**
You do not have to take part in this research if you do not wish to do so, and choosing to participate will not affect your treatment or treatment-related evaluations in any way. You may stop participating in the study at any time that you wish without your job being affected.
Who to contact

If you have any questions, you can ask them now or later. If you wish to ask questions later, you may contact any of the following:

Annette Wambui Muriithi.
P.O Box 70016, 00400,
Nairobi.
Tel. 0722396133
annette.muriithi@yahoo.com

This proposal has been reviewed and approved by the KNH-UoN Ethics and Research Committee, which is a committee whose task it is to make sure that research participants are protected from harm. If you wish to find about more about the KNH-UoN ERC, contact;

Email: uonknh_erc@uonbi.ac.ke
Telephone number +254-20 2726300 Ext 44355
Part II: certificate of consent

I have been invited to participate in research about gum disease and diabetes. I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions I have asked have been answered to my satisfaction. I consent voluntarily to be a participant in this study.

Print Name of Participant__________________

Signature of Participant__________________

Date______________________________

If illiterate

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of witness____________     Thumb print of participant __________

Signature of witness ______________

Date______________________________

Day/month/year

Statement by the researcher/person taking consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands that the following will be done:

1. Answering a few questions administered by the investigator

2. Undergoing a full periodontal examination

3. To provide a saliva sample in a plastic container

I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.
A copy of this ICF has been provided to the participant.

Print Name of Researcher/person taking the consent______________________________

Signature of Researcher/person taking the consent______________________________

Date ___________________________ Day/month/year.

**Who to contact**

If you have any questions, you can ask them now or later. If you wish to ask questions later, you may contact any of the following:

Annette Wambui Muriithi.

P.O Box 70016, 00400, Nairobi.

Tel. 0722396133
annette.muriithi@yahoo.com

Dr. Nelson K Matu

P.O. Box 19676, Nairobi

Tel. +254202713618

Email: nelson.matu@uonbi.ac.ke

KNH/UoN ERC

Email: uonknh_erc@uonbi.ac.ke

Telephone number +254-20 2726300 Ext 44355

Website: http://www.erc.uonbi.ac.ke
APPENDIX 2: CONSENT FORM SWAHILI VERSION

Fomu hii ya ridhaa ni kwa ajili ya wagonjwa wa kisukari na wasio na kisukari katika Thika Level 5 Hospital. Ni ya kwakaribisha kushiriki katika utafiti wenye jina "SALIVARY TOTAL ANTIOXIDANT CAPACITY AND PERIODONTAL STATUS OF DIABETICS AND NON DIABETICS ATTENDING THE THIKA LEVEL 5 HOSPITAL". Mimi Annette Wambui Muriithi, ni mwanafunzi katika Chuo Kikuu cha Nairobi Shule ya meno idara ya Periodontology. Mimi ni mdhamini mkuu wa utafiti wa utafiti.

Hii fomu ya ridhaa ina sehemu mbili:

• Karatasi ya taarifa (kushiriki ki habari kuhusu utafiti)
• Hati ya ridhaa (kwa saini kama aliamua kushiriki)

Utapewa nakala ya fomu hii ya ridhaa

Sehemu ya 1: Karatasi ya taarifa

Utangulizi


Fomu hii ya idhini huenda ina maneno ambayo huelewi. Tafadhali niulize mimi ili nichukue muda kueleza. Kama una maswali baadaye, unaweza pia kuniuliza mimi au mtafiti mwingine tutakayekuwa naye.

Madhumuni ya utafiti

Ugonjwa wa kisukari huathiri fizi za watu wengi katika jamii yetu. Nataka kupata njia za kuzuua hili kutokea. Naamini kuwa unaweza kunisaidia kwa kuruhusu mimi kuchunguza ufizi wako na kukusanya baadhi ya mate yako kwa ajili ya kupima. Hii inaweza kunisaidia mimi
katika kutafuta kama kiwango cha sukari kwenye damu yako ina athari katika ufizi wako. Hii inawezu kutusaidia kujua jinsi nzuri ya kutunza ufizi wako.

**Aina ya utafiti**
Utafiti huu utahusisha ushiriki wako katika kukabiliana na maswali, baadaye ufizi wako kuchunguza kwa karibu 10 na kutoa kiasi kidogo cha mate katika chombo plastiki.

**Uteuzi wa washirika**
Unaalikwa kushiriki katika utafiti huu kwa sababu kiwango cha sukari kwenye damu yako inawezu kuchangia sana kuongeza maarifa ya wanaotoa huduma ya afya ya meno.

**Ushiriki wa hiari**
Kushiriki kwako katika utafiti huu ni kwa hiari yako. Ni uchaguzi wako kama kushiriki au la. Kama atachagua kushiriki, utaendelea kupokea huduma zote katika kituo hili na hakutakuwa na mabadiliko yoyote.

**Utaratibu**
Sisi twakuomba wewe utusaidie kujua zaidi kuhusu ugonjwa wa ufizi katika wagonjwa wenyewe kisukari. Ukikubali kujiunga katika utafiti huu, utaulizwa: -
Kujibu maswali machache ukiongozwa na mimi. Habari zinazokusanywa ni za siri, jina lako halitakuwepo, bali utatambulishwa na nambari utakayopewa na hakuna mtu mwingine ila mimi atakayeweka kufikia utafiti wako.

**Muda**
Utafiti huu unafanyika katika kipindi cha miezi mbili kwa jumla. Kwa wakati huu, utatakiwa kushiriki katika utafiti huu mara moja tu, na hii itachukua muda wa dakika thelathini.

**Hatari**
Kuna hatari kwamba unawezu kueleza baadhi ya taarifa binafsi, au kwamba wanawezu kuhisi wasiwasi kuzungumza juu ya baadhi ya mada. Hata hivyo, si kwamba tunataka hili kutokea. Sio lazima ujibu swali lolote au kushiriki katika utafiti ikiwa unahisi maswali au taratibu ni binafsi.
**Faida**
Hakuna faida moja kwa moja kwako wewe lakini kushiriki kwako kuna uwezekano wa kutusaidia kujua zaidi kuhusu jinsi ya kutibu na kuzuia ugonjwa wa ufizi kati ya wagonjwa wa kisukari.

**Usiri**

**Kushiriki matokeo**
Hakuna chochote utakachotuambia leo kiajelezea mtu yeyote nje timu ya utafiti. Maarifa tutakayopata sisi kutoka utafiti huu utawezekana kwa pamoja na jamii yako kabla ya kuweza kupatikana kirahisi kwa umma.

**Haki ya kukataa au kuondoka**
Si lazima kushiriki katika utafiti huu na kukataa kwako hakutaathiri matibabu yako au matibabu yanayohusiana tathmini kwa njia yoyote. Unaweza kuacha kushiriki katika utafiti wakati wowote unaotaka bila kuathiri kwa namna yoyote ile. Kama una maswali yoyote, unaweza kuuliza sasa hii au baadaye. Ukitaka kuuliza maswali baadaye, unaweza kuwasiliana na namna za zifuatazo:
Annette Wambui Muriithi.
SLP 70016, 00400,
Nairobi.
Nambari ya simu: 0722396133.
anette.muriithi@yahoo.com

Washiriki wa utafiti wana kinga dhidi ya madhara. Ukitaka kupata ujumbe zaidi juu ya IRB, wasiliana na;
Barua pepe: uonknh ERC@uonbi.ac.ke

Namba ya simu +254-20 2726300 Ext 44355
Sehemu ya II: Hati ya idhini

Nimekuwa mmoja wa walioalikwa kushiriki katika utafiti kuhusu ugonjwa wa fizi na ugonjwa wa kisukari. Nimesoma maelezo yaliyotangulia. Nakubali kwa hiari kuwa mshiriki katika utafiti huu.

Jina la mshiriki ______________________
Sahihi ya mshiriki ______________________
Tarehe ______________________________

Kama hawajui kusoma na kuandika

Ninatoa ushahidi kwamba mshiriki mtarajiwa amesomewa kwa usahihi maandiko ya utafiti huu na amepewa nafasi ya kuuliza maswali. Ninathibitisha kwamba mtu huyu ametoa ridhaa kwa uhuru.

Jjina la shahidi ____________ kidole cha mshiriki
Sahihi ya shahidi ____________
Tarehe ____________________________

Siku / mwezi / mwaka

Taarifa iliyotolewa na mtafiti / mtu anayechukua kibali

Mimi kwa uwezo wangu wote, nimewezwa kumjulisha mshiriki kuhusu utafiti wangu na yanayohitajika kutoka kwake:-

1. Kujibu maswali kadhaa yatakayosimamiwa na mchunguzi.

2. Utafiti wa ufizi wake.


Ninathibitisha kwamba mshiriki alipewa nafasi ya kuuliza maswali kuhusu somo, na maswali yote aliyouliza yakajibiwa kwa usahihi na kwa kadri ya uwezo wangu. Ninathibitisha kwamba mtu huyu hajalazimishwa kutoa kibali, na ridhaa imetolewa kwa uhuru na hiari yake.
Nakala ya ICF hii imetolewa kwa mshiriki.

Jina la Mtafiti _______________________

Sahihi ya Mtafiti _______________________

Tarehe _______________________________

Siku / mwezi / mwaka.

Kama una maswali yoyote, unaweza kuuliza sasa hii au baadaye. Ukitaka kuuliza maswali baadaye, unaweza kuwasiliana nami kwa njia zifuatazo:

Annette Wambui Muriithi.

SLP 70016, 00400,

Nairobi.

Nambari ya simu: 0722391633.

anntette.muriithi@yahoo.com

Dr. Nelson K Matu

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Email: nelson.matu@uonbi.ac.ke

KNH/UoN ERC

Email: uonknh_erc@uonbi.ac.ke

Telephone number +254-20 2726300 Ext 44355

Website: http://www.erc.uonbi.ac.ke
APPENDIX 3: QUESTIONNAIRE/ BIODATA FORM.

SALIVARY TOTAL ANTIOXIDANT CAPACITY AND PERIODONTAL STATUS AMONG DIABETICS AND NON DIABETICS ATTENDING THIKA LEVEL 5 HOSPITAL.

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

Age (Years)........................................  File
number.................................................................

Gender: Male.........  Female........

Highest level of education:

Primary.........  Secondary.........  College.........  University........

Tooth brushing habits: Once daily.........  twice daily..........  Thrice daily........

Other..............................................................

Inter dental cleaning aids: Dental floss: No.........  Yes.........  If yes, state
frequency........

Interdental brushes: No.........  Yes.........  if yes, state frequency..............................

Last dental visit:

Never been to a dentist.........  Less than 3 months ago.........  3-6 months ago........

>6 months ago..........

Modifying factors:

Tobacco cigarette smoking: Never smoked.........  Former smoker >3 yrs................

Current smoker..............................
LABORATORY SAMPLE ANALYSIS DATA FORM:

Saliva sample serial No: ...................... Date and time of collection........................................

Date and time sample received, centrifuged and supernatant stored.................................

Spectrophotometry analysis:

Salivary Total Antioxidant Capacity level: ................................................................. Micro moles/L

Laboratory technologist:
Signed..........................................................................................................................
APPENDIX 4: QUESTIONNAIRE/ BIODATA FORM SCREENING SEGMENT.

Tick where applicable.

1. Persons below the age of 18 years

2. Periodontal procedure within the past 6 months

3. Concurrent systemic illness for example rheumatoid arthritis

4. Pregnancy or lactation

5. Current smoker or smoking history in the last 3 years

6. Edentulous patients and patients with less than 20 teeth.
APPENDIX 5: CLINICAL EXAMINATION FORM

PLAQUE SCORE: Silness and Loe Index 1964

<table>
<thead>
<tr>
<th>Tooth</th>
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</thead>
<tbody>
<tr>
<td>Surface</td>
<td>F</td>
<td>L</td>
<td>F</td>
<td>L</td>
<td>F</td>
<td>L</td>
<td>F</td>
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<td>Score</td>
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<td></td>
</tr>
</tbody>
</table>

Total: __________ Mean: __________

GINGIVAL INDEX: Loe- Silness index 1963

<table>
<thead>
<tr>
<th>Tooth</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Surface</td>
<td>F</td>
<td>L</td>
<td>F</td>
<td>L</td>
<td>F</td>
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<td>F</td>
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<td>Score</td>
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<td></td>
</tr>
</tbody>
</table>

Total: __________ Mean: __________
Gingival health status: ____________________________________________________________________

Plaque score: Silness and Loe 1964

<table>
<thead>
<tr>
<th>Scores</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No plaque</td>
</tr>
<tr>
<td>1</td>
<td>A film of plaque adhering to the tooth and the free gingival margin. The plaque may be seen in situ <strong>only</strong> after passing a probe on the tooth surface.</td>
</tr>
<tr>
<td>2</td>
<td>Moderate accumulation of soft deposits within the gingival sulcus, or the tooth surface and gingival margin which can be <strong>seen with the naked eye</strong>.</td>
</tr>
<tr>
<td>3</td>
<td><strong>Abundance</strong> of soft matter in the gingival sulcus and/or on the tooth and gingival margin.</td>
</tr>
</tbody>
</table>
**Gingival index;** Silness and Loe 1963

<table>
<thead>
<tr>
<th>Scores</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal, absence of oedema and no bleeding on probing.</td>
</tr>
<tr>
<td>1</td>
<td>Presence of oedema with absence of bleeding.</td>
</tr>
<tr>
<td>2</td>
<td>Oedema present, glazing and bleeding on probing.</td>
</tr>
<tr>
<td>3</td>
<td>Oedema, ulcerations with spontaneous bleeding.</td>
</tr>
</tbody>
</table>

**Gingivitis severity**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>No gingivitis</td>
</tr>
<tr>
<td>0.1-1</td>
<td>Mild gingivitis</td>
</tr>
<tr>
<td>1.1-2</td>
<td>Moderate gingivitis</td>
</tr>
<tr>
<td>2.1-3</td>
<td>Severe gingivitis</td>
</tr>
</tbody>
</table>
APPENDIX 6: PERIODONTAL PROBING CHART.

Maxillary arch

<table>
<thead>
<tr>
<th>Tooth</th>
<th>17</th>
<th>16</th>
<th>15</th>
<th>14</th>
<th>13</th>
<th>12</th>
<th>11</th>
<th>21</th>
<th>22</th>
<th>23</th>
<th>24</th>
<th>25</th>
<th>26</th>
<th>27</th>
</tr>
</thead>
<tbody>
<tr>
<td>palatal(mm)</td>
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<tr>
<td>recession(mm)</td>
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<tr>
<td>CAL(mm)</td>
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<tr>
<td>facial(mm)</td>
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<tr>
<td>recession(mm)</td>
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<tr>
<td>CAL(mm)</td>
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</tbody>
</table>

Mandibular arch

<table>
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<tr>
<th>Tooth</th>
<th>47</th>
<th>46</th>
<th>45</th>
<th>44</th>
<th>43</th>
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<th>32</th>
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<th>34</th>
<th>35</th>
<th>36</th>
<th>37</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lingual(mm)</td>
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<tr>
<td>recession(mm)</td>
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<td>CAL(mm)</td>
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<td>facial(mm)</td>
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<td>recession(mm)</td>
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<td>CAL(mm)</td>
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</table>

Number and measure of interproximal sites with CAL...........
PPD....................................................
APPENDIX 7: CDC/AAP CONSENSUS PERIODONITIS CLASSIFICATION.

<table>
<thead>
<tr>
<th>Disease category</th>
<th>Clinical attachment loss</th>
<th>Periodontal pocket depths</th>
</tr>
</thead>
<tbody>
<tr>
<td>severe periodontitis</td>
<td>More than 2 interproximal sites with CAL of more or equal to 6mm (not on the same tooth)</td>
<td>AND 2 or more interproximal sites with PPD of more than or equal to 5mm.</td>
</tr>
<tr>
<td>Moderate periodontitis</td>
<td>More than 2 interproximal sites with CAL of more or equal to 4mm (not on the same tooth)</td>
<td>OR 2 or more interproximal sites with PPD of more than or equal to 5mm.</td>
</tr>
<tr>
<td>Mild periodontitis</td>
<td>More than 2 interproximal sites with CAL of more or equal to 2mm (not on the same tooth)</td>
<td>OR 2 or more interproximal sites with PPD of more than or equal to 4mm.</td>
</tr>
<tr>
<td>No periodontitis</td>
<td>No evidence of mild, moderate or severe periodontitis.</td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX 8: REFERRAL LETTER

UNIVERSITY OF NAIROBI
SCHOOL OF DENTAL SCIENCES

Referral Consultation form

The opinion of professor/Dr./Mr./Ms ________________________________ In the __________________________ Consulting Clinic is requested regarding the management of the following patient:

Name ______________________________ Age __________ Gender

________________________________________________________

Clinic/Ward ____________________________

OP/ IP No. _____________________________ Date __________

Provisional/confirmed Diagnosis ____________________________

Clinic Summary ____________________________

________________________________________________________

Request by Dr ____________________________

Sign __________________________________

Date ________________________________