Levels of secretory immunoglobulin A in saliva and the periodontal health status of adult patients attending the University of Nairobi Dental Hospital

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Declaration

I, **Dr. Asif Jabir Mohamedali,** hereby declare that this thesis is my original work and has not been presented for the award of a degree or any other purposes in any other institution.

Signed..... **Date**..04/11/2021.

Supervisors' Approval

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Dedication

This thesis is dedicated to my wonderful family which has given me the best social, psychological, and logistical support. Without them, this thesis would have been impossible, and I am eternally grateful for their sacrifices in my pursuit of academic and clinical excellence.

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This thesis represents working relationships between numerous people and has come to fruition through the efforts of these relations.

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List of acronyms

AAP / CDC	American Academy of Periodontology / Centres for Disease control
BDS	Bachelor of Dental Surgery
BOP	Bleeding on probing
CAL	Clinical attachment loss
EIA	Enzyme immunoassay
ELISA	Enzyme Linked Immunosorbent Assay
ERC	Ethics and Research Committee
Fc region	Fragment Crystallizable region
GBD	Global Burden of Disease
GI	Gingival Index
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IPA	Isopropyl alcohol
J-chain	Joining chain
KEBS	Kenya Bureau of Standards
KNH	Kenyatta National Hospital
LSD	Least significant difference
MAHE	Manipal Academy of Higher Education
MPH	Master’s in Public Health
NSB	Non-Specific Binding wells

PhD	Doctor of philosophy
PIgR	Polyimmunoglobulin receptor
PS	Plaque score
RPM	Revolutions per Minute
SC	Secretory Component
SIgA	Secretory Immunoglobulin A
SPSS	Statistical Packages for Social Sciences
UON	University of Nairobi
WHO	World health organization

Definitions

These definitions will provide uniformity in the understanding of terms used throughout this study.

Gingivitis / Gingival inflammation

Describes inflammation of the gingival tissues without clinical attachment loss and presents with findings such as redness, oedema and bleeding on probing involving the free gingival margin.

Periodontitis

A chronic inflammatory disease that affects the supporting structures of the teeth (Periodontium), it is multifactorial and progressive in nature causing destruction of both hard and soft tissues leading to clinical attachment loss, periodontal pocket formation and alveolar bone destruction with the eventual outcome of tooth loss.

Probing depth (PD)

Is measured using a periodontal probe and is defined as the distance from the free gingival margin to the base of the gingival sulcus or gingival pocket.

Clinical attachment loss (CAL)

An increased CAL is found in periodontitis and is defined as the distance from the cementoenamel junction to the base of the sulcus or periodontal pocket. It is a derived value and represents distance from a fixed point the Cemento-enamel junction (CEJ).

Whole saliva

Describes the mix of saliva contributed from the various salivary glands both major and minor, the gingival crevicular fluid and mucosal transudate.

Unstimulated saliva

It represents the basal flow of saliva produced in the absence of any external or pharmacologic stimuli and is collected by allowing the saliva that has pooled to the floor of the mouth to be gently guided by drooling into an appropriate collection device.

Biological marker (Biomarker)

“A characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes or pharmacological response to therapeutic intervention

Vortex

Utilizing manually applied circular movements to generate centrifugal force on a fluid in a laboratory capped tube. This method is commonly used to gently mix fluids that have been pipetted together manually.

Abstract

Background: Periodontal diseases are a group of common chronic diseases affecting supporting structures of teeth. In Kenya, periodontitis affects 60% to 80% of the population. Secretory Immunoglobulin A (SIgA) is a key component of the host defence, its levels in saliva have been shown to possibly be affected by periodontitis. Salivary SIgA quantification may lead to a non-invasive method of detecting presence of and status of periodontitis.

Study objective: To determine the relationship between Secretory immunoglobulin A levels in saliva and periodontal health status

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

Study area: The study was carried out at the University of Nairobi Dental Hospital oral diagnosis and periodontology clinics.

Study population: Adult patients aged 18 years and above.

Materials and Methods: The sample size was 77 sampled conveniently. The saliva flow rate was determined in mL/min. The gingival index, plaque scores and clinical attachment loss measurements were recorded. Salivary SIgA quantification (µg/mL) was done. Extreme outliers were determined after establishing a laboratory range using a 4 Parameter non-linear curve fit. Values that fell beyond the assay range were re-run and those that were obviously erroneous were excluded.

Since SIgA is actively secreted into saliva, the saliva flow rate affects the final concentration of SIgA in saliva. To account for the differing saliva flow rates, we calculated the secretion rate of SIgA in µg/min.

The resulting data was analysed using descriptive statistics for means and standard deviations. An analysis of variance (ANOVA) model was employed, tests of homogeneity and post hoc comparison performed. Statistical significance was set at α value of $p < 0.05$.

Results: Periodontitis had a prevalence of 32(44.4%), of which 14(19.4%) had mild, 11(15.3%) had moderate and 7(9.7%) had severe periodontitis.

Levels of SIgA were 153.11µg/mL (±90.8 SD) for no periodontitis, 162.12µg/mL (±87.58 SD) for mild, 185µg/mL (±87.42 SD) for moderate and 327.33µg/mL (±204.84 SD) for severe periodontitis.

After saliva flow rate was accounted, secretion rate of SIgA (µg/min) was determined, 104.11µg/min (±74.30 SD) for no periodontitis, 130.85µg/min (±105.54 SD) for mild, 126.60µg/min (±89.58 SD) for moderate and 148.79µg/min (±40 SD) for severe periodontitis, p = 0.186.

No significant difference was seen between varying severities of periodontitis and SIgA levels in saliva.

Conclusion

These results indicate that the greatest overall effect on the final levels of SIgA was likely caused by the differing saliva flow rates among the participants, in this study.

CHAPTER ONE: INTRODUCTION

Periodontal diseases comprise of a group of inflammatory diseases that affect the supporting structures of teeth and are broadly grouped into gingivitis and periodontitis. Gingivitis is the inflammation of the gingival tissues, whilst periodontitis, is the inflammatory process that results in destruction of the periodontium and effects both soft and hard tissues[1].

Worldwide, periodontal diseases pose a global issue, affecting all races, genders, and socioeconomic divides. Dental caries and chronic periodontitis are the leading causes of tooth loss globally. As periodontitis progresses the number of teeth involved and severity of the attachment loss increases, leading to complications such as tooth migration, drifting and subsequently tooth loss. Loss of teeth results in impaired masticatory function which can compromise nutrition, and create aesthetic challenges that have effects on self-esteem and social interaction[2]. Chronic periodontitis has also been strongly associated to a number of systemic conditions such as, coronary heart disease[3], rheumatic diseases[4], metabolic disorders such as diabetes mellitus[5], among others.

In periodontal diseases the host response plays a crucial role in both the host defence process as well as the progression of disease[6]. It has been shown that whilst oral micro-organisms are required to initiate and propagate the disease process, they are unable to, on their own, cause periodontitis. It is only in combination with the hosts' response to the micro-organisms that results in the disease progression[7].

Secretory immunoglobulin A (SIgA) is a component of the host defence, found in the oral cavity as well as in secretory fluids in the gastrointestinal tract. In the oral cavity it is found in saliva, on mucosa as a transudate and in the gingival crevicular fluid (GCF). It plays a major role in the host defence and its' function generally is to prevent the initiation and progression of periodontal diseases. During the progression of periodontal disease changes in the local oral microbial environment and the host response occur. These changes in the host response may be reflected in the concentrations of salivary SIgA; hence, quantification of SIgA may be a useful biomarker for determining disease state and further our understanding on etiopathogenesis of periodontitis.

CHAPTER TWO: LITERATURE REVIEW

2.1 Periodontal disease

2.1.1 The periodontium and periodontal diseases

The periodontium represents the supporting structures of teeth that make up a single functional unit consisting of the gingiva, periodontal ligament, cementum, alveolar bone, and the associated neurovascular supply. This group of components creates a synergistic unit that maintains form and function of the teeth in the oral cavity. Periodontal disease is characterized by initial marked inflammation of the gingiva resulting in gingivitis, followed by progressive destruction of the supporting structures of teeth involving both soft and hard tissue destruction.

The unchallenged disease may cause significant alveolar bone loss, periodontal pocket formation and clinical attachment loss; broadly termed as chronic periodontitis. The likely outcome of progressing periodontal disease without intervention is tooth loss.

2.1.2 Epidemiology of periodontal disease

Periodontal disease has numerous effects on the worldwide population, ranging from systemic, lifestyle, aesthetic, and economic effects. The global burden of periodontal disease as a chronic disease has been investigated and surveys conducted by the WHO, utilizing the community periodontal index of treatment needs, found that a large percentage of the global population suffers from gingival bleeding and calculus deposits. Calculus is a local predisposing factor to periodontal disease. Utilizing the WHO global oral health data bank, it was found that a globally estimated 10 to 15% of the total population suffers from severe periodontitis with pocket formation ≥6mm[2]. The global burden of disease survey (GBD 1990 to 2010), showed that severe periodontitis had global prevalence of 11.2% showing an increase from 1990 to 2010, ranking severe periodontitis as the 6[th] most prevalent chronic disease worldwide [8].

The Kenya national oral health survey report (2015), found an overall prevalence of gingival bleeding in children was 75.7%, while an overall prevalence of 98.1% was found in adults, with the highest values of those recorded found in those aged 60 years and above, with a prevalence of 99.3%[9]. Another study done in Kenya observing

several age groups, the youngest between 15 to 24 years and the oldest of 55 to 65 years, found that the bleeding on gentle probing was present in 40% of all surfaces measured in the 15 to 24 years group, whilst 60% to 70% of surfaces measured in the older age groups were affected. Clinical loss of attachment was found to affect 5% to 10% of surfaces in the younger age groups, with a range of clinical attachment loss between 1 mm to 3 mm. In older age groups, 75% to 85% of measured surfaces had notable loss of attachment, of which 50% of all affected surfaces had a clinical attachment loss of >4mm. This study showed that the prevalence and severity of periodontal disease was also associated with an increase in age[10].

Overall, there is a clear understanding that periodontal disease of which specifically chronic periodontitis is a common chronic condition that imposes significant socioeconomic and health burden on the global and Kenyan population.

2.1.3 Classification of periodontal disease

The currently most used and accepted clinical classification of periodontal diseases, is the American Academy of Periodontology (AAP) classification, formulated by the 1999 international workshop for the classification of periodontal diseases and conditions[1]. In the case of population-based studies a challenge arose, where no universally agreed upon case definition was available for periodontitis. In February 2003 the Centre for disease control and prevention, division of oral health, along with the American Academy of periodontology (AAP), created a working group with a requirement to develop a standardized clinical case definition model[11]. This classification describes moderate and severe periodontitis providing more clear thresholds in measurement and therefore a clearer definition of a diseased site. In 2012 an update to the case definitions added mild periodontitis to the classification in order to obtain a more clear depiction of the total prevalence of periodontal disease[12]. In 2017 a workgroup was formed by the AAP and in 2018 published a new classification scheme for periodontal and peri-implant diseases and conditions[13]. Considering the litany of research utilizing the previous classification it was decided that using this scheme would be premature.

2.1.4 Diagnosis of periodontal diseases

Classically, the diagnosis of periodontal diseases is clinically based. Traditional diagnostic parameters include bleeding on probing, plaque indexing, probing depths and clinical attachment loss determination. These methods are generally invasive and time consuming in nature. Adjunctively, radiographs are used to determine alveolar bone levels and pattern of destruction. Whilst these mentioned methods are standardised among clinicians globally as well as being cost effective, they may show inconsistency between clinicians and are dependent on clinical skill and experience. It has also been shown that there is heterogeneity in the methods of measurement of disease such as use of different case definitions or classifications of disease or the use of different instruments between clinicians and researchers, further imparting inconsistency in measurement[14]. Furthermore, these methods are limited to measuring the disease history and not the status of the disease.

The use of saliva as a diagnostic tool shows immense potential, within which locating a reliable biomarker for disease could aid in early detection and treatment of periodontal disease, as well the potential in screening larger populations. Measuring such a component would potentially also provide information on the current disease status.

2.2 Pathogenesis of periodontal disease

2.2.1 Initiation and propagation

The understanding of periodontal disease has shifted over the years, from an early, primarily clinical, or classical paradigm of understanding to an infection and host response paradigm. The current understanding of periodontal diseases is that they are multifactorial. Gingivitis and periodontitis are both chronic inflammatory diseases.

The initiation of disease occurs with accumulation of bacteria on the surfaces of teeth, primarily along the free gingival margin and within the gingival sulcus at the tooth tissue interface. These initial bacterial cells invade the acquired salivary pellicle and colonize it eventually forming an organised and complex bacterial biofilm (dental plaque). This process is generally disrupted by normal salivary flow and oral hygiene practices. Once the initial bacterial biofilm is established the process of bacterial succession

occurs[15]. Other bacterial species begin to colonize the biofilm. It is notable that initial bacterial biofilms have a low diversity pool of microorganisms whilst mature biofilms possess higher diversity of microorganisms. Colony formation begins to take place by co-aggregation and adhesion of bacterial types that are synergistic. There is a general shift in the types of microorganisms from an initial gram positive to a more obligatory anaerobic gram negative population, which is likely caused by depletion of oxygen in the salivary pellicle due to the bacterial metabolic function[15]. Anaerobic gram-negative organisms have an increased virulence and produce more potent virulence factors such as lipopolysaccharides (endotoxins), which potentiate a greater immune inflammatory host response.

Therefore in summary, the exposure of gingival tissues to the bacterial biofilm and subsequent maturation of the biofilm results in the inflammatory response[16].
For periodontal disease to occur several factors are required to be present simultaneously. The presence of a virulent periodontal pathogen, a favourable local environment for disease and host susceptibility[17]. The resulting inflammatory response that occurs after bacterial exposure was alluded to in the study done prior by Page and Schroeder in 1976, in which a description of the progression of periodontal disease was made based on histological findings. They were grossly divided into, the initial lesion, early lesion, established lesion and the advanced lesion. As the lesion develops, in its initial stage there is a classic exudative vasculitis and as it progresses there is loss of perivascular collagen. The early lesion is noted to have increased infiltration of lymphocytes and mononuclear cells, due to the chemotactic and antigenic agents released by bacteria. The established lesion is seen at two to three weeks after continued exposure and shows an increase in plasma cell infiltration. It is important to note that up to this stage, significant involvement of alveolar bone and periodontal ligament has not occurred. At the stage of the established lesion, bleeding on probing is a common clinical finding and the disease is generally termed gingivitis. Extension of the inflammation and subsequently also the bacterial biofilm apically results in progression of the lesion, where the migration of the junctional epithelium occurs apically along with destruction of alveolar bone, periodontal ligament and disruption of tissue architecture resulting in fibrosis.

The histology of a dense plasma cell infiltration is the typical finding of the advanced lesion. This is clinically diagnosed as chronic periodontitis[18]. This histological finding of plasma cell infiltrate is of importance in relation to this study as the primary function of plasma cells in the cell mediated immunity is the production of immunoglobulins such as IgA.

2.2.2 Bacterial adherence

For bacteria to get established in the oral cavity and initiate the host response, they must resist the flow of saliva to be able to adhere to the dental hard and soft tissues. The adherence occurs between bacteria and host receptor sites found on the cell surfaces of oral tissues and within saliva. This type of bacterial adhesion is known generally as specific or stereochemical interaction and is akin to an antigen-antibody type of interaction[19]. This process of adherence is mediated by bacterial adhesins such as polysaccharides, carbohydrate binding proteins (lectins), lipoteichoic acids, glucosyltransferases, and glucans. These bacterial adhesins are generally components of the bacterial cell wall, or they may be present on associated structures of the bacterial cell such as fimbriae and fibrils. The receptor sites to which these bacterial adhesins can bind to in the oral cavity are found in, saliva (mucins, glycoproteins, IgA, IgG, amylase), as well as on other bacteria that are already bound to oral surfaces.

Not all bacteria are able to directly colonize the acquired salivary pellicle but do so indirectly, by adhering to primary bacterial colonizers. This process is called coaggregation and is critical in the formation of dental plaque. Coaggregation has been well demonstrated in vitro, while in vivo it has been observed microscopically in dental plaque, examples include, gram positive filaments covered by gram positive cocci (corn cobs), as well as large filaments surrounded by short filaments or gram negative rods (bristle brushes)[20].

Another mechanism of bacterial adherence is nonspecific in nature and involves ionic interactions such as calcium bridges between the negatively charged bacterial cell surfaces and the host surface, though this interaction does not entirely account for the specificity that certain organisms show to adhering to certain tissues in the oral cavity[19].

2.3 Saliva and biological markers

2.3.1 Saliva

Saliva is an oral biologic fluid that is exocrine in nature, composed of 99 % water, with the remainder consisting of electrolytes (calcium, phosphate, bicarbonate, chloride etc.), proteins (Immunoglobulin's, enzymes, and antimicrobial factors), mucosal glycoproteins, glucose, and nitrogenous compounds (urea / ammonia) among others. These components in combination confer to saliva its properties that aid in its numerous functions[21]. The major functions of saliva include taste, digestion, lubrication, remineralisation, and host defence among others. Whole or mixed saliva is a term used to describe the complex mix of secretions from both major and minor salivary glands, as well as from gingival crevicular fluid and the mucosal transudate. It also includes components from the local oral environment such as desquamated epithelial cells, food debris, and non-adherent oral bacteria. At rest the basal flow of unstimulated saliva varies due to variances in genetic, racial, environmental, dietary and drug related factors. Unstimulated saliva is the secretion that continuously lubricates the oral tissues and forms the salivary pellicle whereas stimulated saliva is produced as a result of gustatory, masticatory or pharmacological response and can be up to 7 ml/min in extremes, contributing 80% to 90% of the daily saliva production[21]. In the adult Kenyan population it was shown that the mean salivary flow rate was 0.66 g/min[22] a volumetric equivalent to 0.67 mL/min. Stimulated saliva shows greater variation in its constituents, shown in studies that compare unstimulated and stimulated saliva[23]. Therefore it is generally preferred to collect unstimulated saliva in order to achieve more reliable and consistent results[24].

It has been shown that due to the active nature of secretion of SIgA into saliva, its concentration is inversely proportional to salivary flow rates. Therefore, as saliva flow rate decreases there is more available time for active transport of SIgA into saliva hence a higher concentration and vice versa. This variation should be accounted for by firstly obtaining the flow rate of saliva (mL/min) and secondly calculating the secretion rate of SIgA (µg/min), which will account for the different flow rates of saliva in the study sample and provide more accurate results.

$$\underline{\textbf{Saliva flow rate (mL/min)}} = \mathbf{x} = \frac{\textbf{Volume of Saliva collcted (mL)}}{\textbf{Time (min)}}$$

Secretion rate of SIgA (µg/min) = Flow rate (mL/min) **x** SIgA concentration (µg/mL)

The use of saliva as a diagnostic tool is desirable as it is easily obtainable, its collection process is non-invasive and convenient, and therefore its use has greater patient compliance potential. Adequate saliva quantity and quality is critical in maintaining the oral health and preventing periodontal diseases. Therefore, investigating its components can lead to a greater understanding of the disease process and the role of saliva in that process.

2.3.2 Biological marker (Biomarker)

The biomarker definitions working group defined 'biomarkers' as follows.

"A characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes or pharmacological response to therapeutic intervention" [25]

Biomarkers can act as surrogates that can be validated for a specific clinical endpoint. A biomarker that accurately and consistently predicts a clinical outcome, beneficial or harmful, is considered a surrogate endpoint. While a specific biomarker can be validated for a specific clinical endpoint it is not necessarily indicative that the biomarker in question plays a role in the pathophysiology leading to that specific clinical outcome[26]. In the field of periodontology, diagnosis as described earlier, is primarily clinically driven and there is currently no routine diagnostic investigation being used for periodontal diseases that utilizes a biomarker despite the ease of obtaining saliva. The primary reason for this lies with the lack of adequate biomarkers as surrogate endpoints to predict clinical outcomes.

The need of an accurate, easy to use portable platform for detection of relevant biomarkers at a cost-effective margin is required for viability as a diagnostic tool.

2.4 Secretory Immunoglobulin A (SIgA)

2.4.1 Synthesis and transport of secretory immunoglobulin A

Plasma cells produce a group of Y shaped proteins known as immunoglobulin's, which exist as several isotypes that have differing characteristics, and different mechanisms, but generally participate in the host defence as the primary component of the antibody

mediated immunity. The plasma cells located within salivary glands are found adjacent to the acini and ductal system, they are also found in the underlying connective tissue of the gingiva and oral mucosae. Plasma cells generally produce several immunoglobulins, but it has been shown that plasma cells of the oral cavity and those within the salivary glands are predominantly the IgA producing type. The process of synthesis and transport is depicted in figure 1 below.

The IgA produced is primarily J chain positive[27](joining chain). Polymeric IgA containing a J chain is secreted by the plasma cells, this molecule is then recognized by the polyimmunoglobulin receptor (PIgR), which is in the basolateral domain of the epithelial cells. IgA binds to this cell membrane receptor forming the IgA-PIgR complex. This complex is taken into the cell by endocytosis and transported from the basal end to the apical end of the epithelial cell by the process of transcytosis via a membrane bound vesicle, which eventually fuses with the cell membrane at the apical end of the cell. Once fusion occurs the PIgR in the IgA-PIgR complex undergoes proteolytic cleavage, leaving two remnants, one remnant portion initially links to IgA via weak bonds, this is the bound Secretory Component (bound SC), whilst the remaining unlinked remnant is thought to be released after cleavage as free SC[28]. The initially weakly linked, bound SC, will eventually covalently bind by disulphide bonds to IgA at the Fc region (Fragment crystallization region), during the process of exocytosis into the oral cavity, thereby creating a stronger bond which will stabilize the IgA-SC complex now termed secretory immunoglobulin A (SIgA)[29]. The result is a very stable salivary SIgA in the oral cavity resistant to proteolytic cleavage. The free SC is thought to possess similar properties to bound SC and is suggested to be capable of inhibiting bacterial adherence and neutralize bacterial toxins.

Due to the unique transport mechanism through the epithelium salivary SIgA is found on the mucosal surface as a transudate, in the GCF, as well as in the salivary secretions from both major and minor salivary glands.

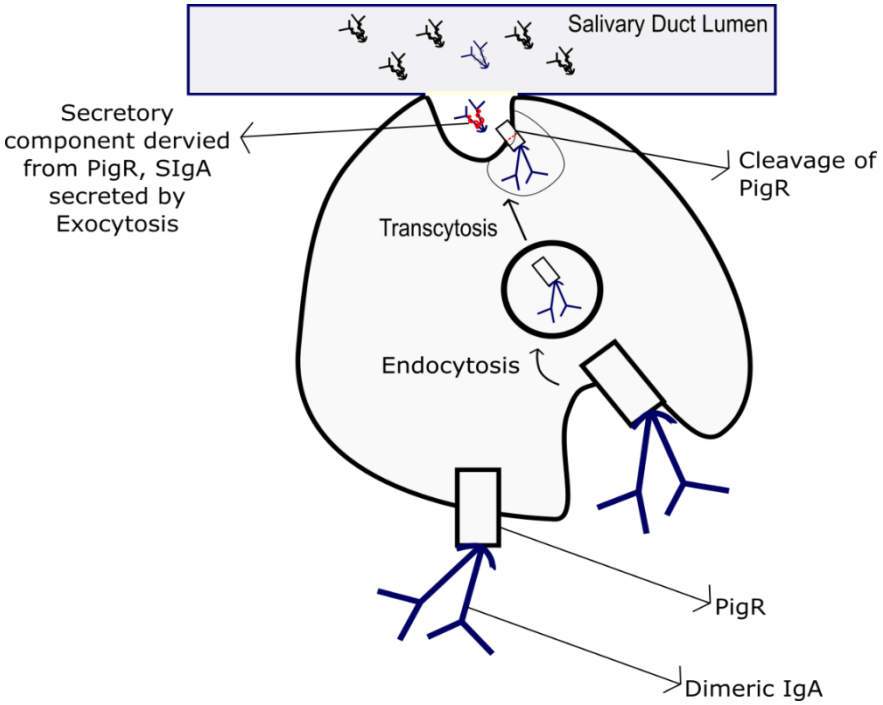

Figure 1: Illustration to depict synthesis and transport of SIgA after secretion of dimeric IgA from plasma cells. (Acini epithelial cell)

2.4.2 Structure of SIgA

It is a polymeric molecule, its components include; two or more IgA monomers which are linked by a J chain (Joining chain), and a secretory component (SC) (Figure 1)[20]. Each IgA monomer is composed of four polypeptide chains, two light chains and two heavy chains linked by covalent disulphide bonds. The J chain and SC are covalently bonded to the IgA molecule at the Fc region (Fragment crystallization region).

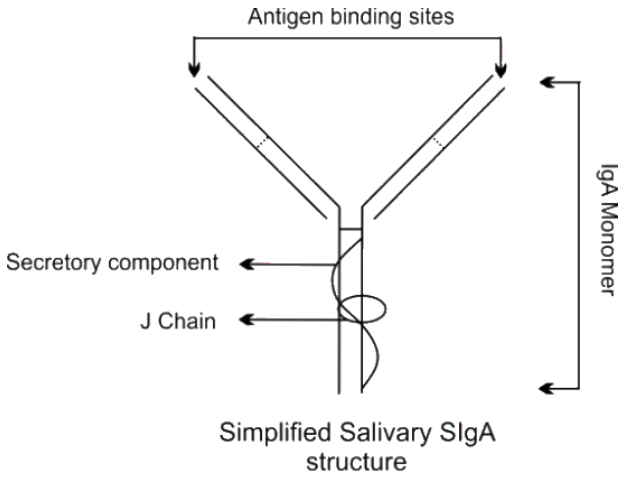

Figure 2; Adopted and redrawn from Marcotte and Lavoie

The secretory component as seen in figure 2 above is a heavily glycosylated protein produced during the transfer of IgA from its production site to the oral cavity through

the epithelium (described above). The SC is critical in stabilizing the polymeric IgA and protects it from proteolysis in secretions[30].

There are two IgA subclasses in saliva, IgA1 and IgA2 and they are present in saliva in roughly similar proportions[20]. There are minor structural differences between the two subclasses, which confer to them different characteristics. Antibodies that target bacterial proteins and carbohydrates are in the IgA1 subclass whilst antibodies against components of the bacterial cell wall such as lipoteichoic acid and lipopolysaccharide are found in the IgA2 subclass.

2.4.3 Role of salivary SIgA in host defence

It is the first line of defence against pathogens that invade, colonize, and adhere to oral tissue surfaces that are covered with the acquired salivary pellicle. Due to the secretory component bound to IgA, it is more resistant to proteolytic cleavage, thereby preserving its functions in the oral environment, and within saliva. Its numerous functions are described below.

Salivary SIgA and inhibition of bacterial adherence

Considerably the most important mechanism of salivary SIgA is its ability to prevent adherence of bacteria to oral tissue surfaces, thereby preventing bacterial invasion. It has been shown that SIgA prevents microbial attachment to buccal epithelial cells in vitro, such as oral streptococci[31] and candida albicans[32].

Furthermore, it has been shown that salivary SIgA has a high affinity for hydroxyapatite and prevents numerous bacterial species from adhering to the teeth surfaces. It has been suggested that bacterial cell wall components such as lipoteichoic acid confer a negative charge to the cell wall, and the resulting (non-specific) ionic interaction with positively charged ions of hydroxyapatite allow for these bacteria to adhere to teeth surfaces[33]. Salivary SIgA prevents adherence by binding to the bacterial adhesins that confer the negative charge and alters the surface charge of the bacterial cell wall preventing any ionic interaction.

The secretory component of SIgA also reduces hydrophobicity (reduces water repulsion) of cell structures, this then confers a hydrophilic property to the cell structures (reduces affinity for lipids)[34], and this property is suggested to prevent binding between bacterial adhesins and host receptor sites hence preventing adherence. SIgA is further shown to cause agglutination of bacteria, which increases

the ease of bacterial clearance by saliva, this activity has been shown to vary over time depending on the dominant antigen in the oral cavity[35].

Overall, these mechanisms result in a reduction in bacterial adherence to tooth and tissue surfaces.

Interaction with the complement system

SIgA has been shown to activate the complement system via an alternative pathway and this is believed to be caused by reduced opsonisation of bacteria bound to SIgA which results in reduced activation of the complement peptide C5a, that is responsible for intense chemotactic and inflammatory response[34].

The net result is a reduced inflammatory response which would be indicative of the potential protective role of salivary SIgA in periodontal disease.

Interaction with other components of saliva

Mucin is a component of saliva and has been shown to contain structures that inherently mimic the receptors of epithelial cells. These structures act as dummy binding sites for microorganisms which result in their entrapment and subsequent clearance. It has been suggested that SIgA facilitates this activity when incorporated into saliva, causing bacteria to become mucophillic facilitating their clearance by saliva[36]. Salivary SIgA has also been shown to enhance the activity of lactoperoxidase enzyme in saliva. This occurs by the binding of the enzyme to SIgA and thus stabilizing the enzyme and improving its enzymatic and antimicrobial properties significantly[37].

The net result is the increased clearance and enzymatic destruction of bacteria.

IgA proteases

It is interesting to note that several bacterial species can produce proteases that cleave IgA. Most of these proteases are directed towards the IgA1 subtype, whilst the IgA2 remains resistant to bacterial protease activity. Several microorganisms that colonize mucosal surfaces such as haemophilus influenzae and streptococcus pneumoniae among others can produce IgA proteases. Some resident oral microorganisms have also been shown to produce IgA specific proteases, such as streptococcus sanguis and streptococcus oralis. The role of the IgA proteases is to counteract the adherence

inhibiting ability of IgA. Whilst the production of these proteases to cleave IgA is limited to certain strains of microorganisms and the majority of the proteases produced are against the IgA-1 subtype, it has been shown in vitro that cleavage of SIgA can result in its inability to inhibit adherence of bacteria to tooth surfaces[38].

Therefore, as disease progresses the increased diversity of the organisms may impact the ability of SIgA to perform its functions adequately due to the production of IgA specific proteases. It has also been shown that the cleaved SIgA fragments are able to bind to antigens, but whether it prevents adhesion of bacteria is still a point of on-going research[38].

2.4.4 Detection of salivary SIgA

Several methods have been employed to detect salivary SIgA, these methods range from single or multiple radial diffusion techniques[40], particle enhanced nephelometric immunoassay, sandwich ELISA and competitive or indirect ELISA techniques. Competitive and Indirect ELISA / EIA test techniques are commonly employed for the quantification of SIgA in different body secretions including saliva[44]. These techniques are sensitive, can be calibrated to generate a custom laboratory range, and have a wide detection range. EIA assays have the potential of being automated and therefore using these detection methods are not only more accurate but can be potentially adapted for mass screening. Newer assays have also proven to be less cross-reactive with other components of saliva and therefore more specific.

Principles of Indirect competitive EIA

This method is an immunoassay technique that is the preferred choice for SIgA quantification and is inherently like ELISA. A constant amount of anti-human goat SIgA conjugated to horseradish peroxidase (Termed the antibody enzyme conjugate) is added to tubes that contain diluted saliva samples, standards, or controls. The constant amount of antibody enzyme conjugate binds to SIgA in the sample or standard. The remaining unbound free antibody enzyme conjugate is inversely proportional to the amount of SIgA in the sample or standard. After incubation, an equal amount of standard, sample or control is added to the plate wells in duplicate. The free antibody enzyme conjugate then binds to the SIgA coated wells. The already bound antibody enzyme conjugate in solution is removed via plate washing. This

bound SIgA enzyme conjugate is measured by adding the substrate tetramethylbenzidine (TMB), after incubation the reaction is stopped using a STOP solution of Methanesulfonic acid. The resulting colour change is read in a plate reader and optical densities determined at a wavelength of 450nM. (Figure 3 below)

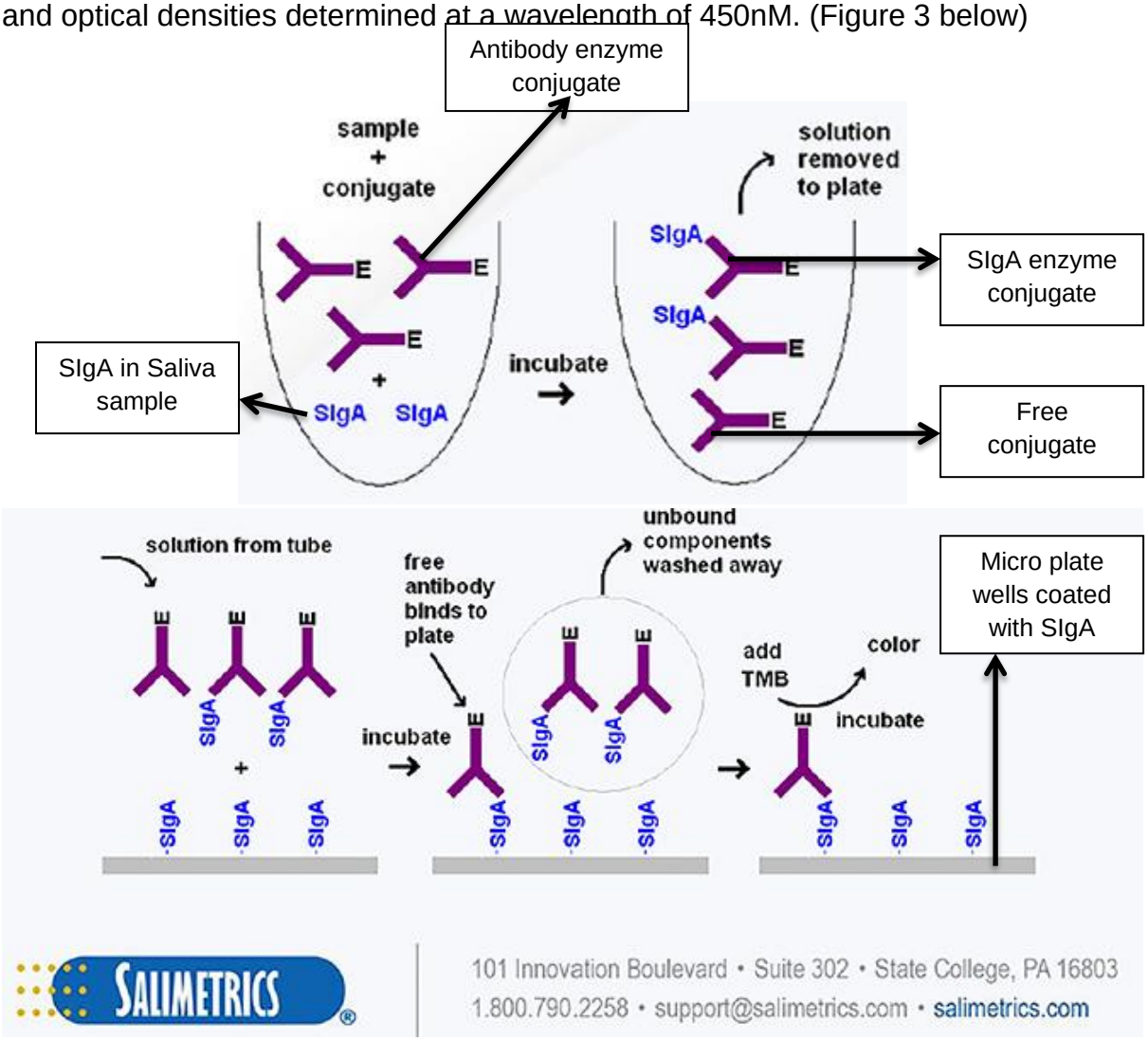

Figure 3: Depicts the test principle, the resulting bound conjugate to the plate wells undergoes a colour change when TMB and the STOP solutions are added. Optical densities are then read with a plate reader at 450nM wavelength.

2.5 Salivary SIgA and the periodontal health status

2.5.1 Salivary SIgA and oral hygiene status

Research has been undertaken to investigate the relation between dental plaque formation and salivary SIgA levels. It has been shown that slow plaque formers presented with significantly higher levels of salivary SIgA than fast plaque formers[39], suggesting that salivary SIgA has potentially a major role in the rate of dental plaque formation as well as its clearance. It is worth noting that very few attempts have been made to replicate these studies, and further examination is required.

2.5.2 Salivary SIgA and periodontitis

The relation between salivary SIgA and periodontal diseases has been investigated previously. It has been shown that there is a general increase in salivary concentration of SIgA in the presence of periodontal diseases, though this has not been frequently quantified in a meaningful way or compared to the severity of disease[40]. Studies have observed the changes in salivary SIgA before and after periodontal therapy, and found that there was an increase in salivary SIgA in relation to periodontitis and subsequently there was a reduction in salivary SIgA levels after periodontal therapy[41]. This study concluded that the magnitude of change after therapy was insufficient to use salivary SIgA as a determinant for clinical outcome[41]. However, it is worth noting this study did not take into consideration the varying lifecycles of plasma cells. This is especially important as it has been shown that short lived plasma cells undergo apoptosis within days whilst a subset population of long lived plasma cells may persist for longer periods of time[42], hence producing salivary SIgA for a greater time than what may be expected. Research carried out investigating the relationship between aggressive periodontitis (rapidly progressive periodontitis) and salivary SIgA levels, showed that there was a reduced concentration of SIgA in saliva of patients suffering from aggressive periodontitis[43] the authors considering its potential value in preventing the initiation of aggressive periodontitis.

Determining the levels of salivary SIgA in the healthy periodontium and in varying degrees of severity of chronic periodontitis will allow a more objective observation of the changes in salivary SIgA levels during the progression of disease.

CHAPTER THREE: PROBLEM STATEMENT AND JUSTIFICATION

3.1 Problem statement

Periodontal diseases are considered globally as of the most common chronic diseases in Africa and within Kenya in particular, a high prevalence of periodontal disease is evident. Studies in Kenya show that up to 80 % of the population had some degree of periodontal disease with large variations between age groups[10].

Timely diagnosis and prompt treatment of periodontal diseases greatly improves the clinical outcome; hence it is unfortunate that a large majority of patients present in advanced stages of periodontitis worsening the clinical outcomes and increasing the mortality of the teeth affected. The current diagnostic methods are primarily clinically driven, which measures only the disease history and do not provide the current disease status. Furthermore, the process is time consuming, subjective in nature and dependant on the clinician's skill and experience to improve consistency of results. Further complicating the matter, is the lack of universally agreed upon case definitions for diagnosing periodontitis, leading to the use of numerous reporting methods resulting in more uncertainties regarding disease state.

Periodontitis is an inflammatory disease initiated by bacteria and progresses due to the host mediated inflammatory response, where the unchallenged disease will progressively cause increased loss of attachment, destruction of the periodontium and eventual loss of teeth. The disease itself and the outcome of tooth loss bare a great socioeconomic burden upon society. The current body knowledge associated with the host response primarily deals with the mediators of inflammation, yet the function of the immune response, as an inhibitor to initiation and progression of periodontal diseases has not been well researched. Additionally, the current testing methods are more sensitive, possess less cross reactivity with other salivary proteins and are more reproducible, making quantification of salivary SIgA more accurate and specific.

The role of salivary secretory immunoglobulin A and its response in disease is important, to understand the progression of chronic periodontitis. The current study therefore explores to understand to a greater degree the role played by the

immunologic response in saliva and therefore whether it may be used to determine the status of disease as a biomarker.

3.2 Justification of the research

Periodontal diseases are a group of common inflammatory diseases with an evident global health burden[2]. The quantification of Salivary SIgA in health and disease will help in understanding its variation in the pathogenesis of periodontal. Furthermore, the results of this study may aid in determining the viability of salivary SIgA as a biomarker for periodontal diseases and may further aid in future molecular studies surrounding salivary SIgA. Its use as a biomarker may provide a method of measuring the current disease status reducing subjectivity of the clinically driven diagnostic method, whilst also possibly providing a simple, rapid, non-invasive screening method for periodontal diseases.

No data exists on the levels of Salivary SIgA in the Kenyan population and minimal data in the African population overall, therefore measuring the salivary SIgA levels in the Kenyan population will be useful in determining the parameters found in this specific population.

3.3 Objectives and Hypothesis

3.3.1 Main Objective

To determine the relationship between levels of salivary secretory immunoglobulin A and the periodontal health status, in an adult Kenyan population.

3.3.2 Specific objectives

a) To assess the oral hygiene status among consenting adult participants attending the University of Nairobi Dental Hospital ,
b) To assess the gingival inflammation status among consenting adult participants attending the University of Nairobi Dental hospital,
c) To determine the clinical attachment loss among consenting adult participants attending the University of Nairobi Dental Hospital,

d) To determine Salivary SIgA levels in consenting adult participants attending the university of Nairobi Dental Hospital,
e) To investigate the relationship between Salivary SIgA levels and the periodontal health status among consenting adult patients attending the University of Nairobi dental hospital.

3.3.3 Null Hypothesis

There is no relationship between Salivary SIgA levels and periodontal health status in adult patients attending the University of Nairobi Dental Hospital.

3.3.4 Alternative Hypothesis

There is a relationship between Salivary SIgA levels and periodontal health status in adult patients attending the University of Nairobi Dental Hospital.

3.3.5 Expected direction of the hypothesis

Based on previous literature in this field of study it was expected there would be a direct relationship between periodontal disease and salivary SIgA levels.

3.3.6 Expected Variables

Table 1

Variable	**Measurement Unit**
Socio demographic characteristics	
Age	Number of years
Gender	Male or Female
Residence	Within or outside Nairobi County
Level of education	Highest academic qualification achieved
Dental visits	Frequency

Oral health practices	Tooth brushing, interdental cleaning, use of chemical plaque control frequency
Independent (exposure) variables	**Measurement Unit**
Oral hygiene status	Plaque score - Silness and Loe 1964
Gingival inflammation	Gingival index - Loe and Silness, 1963
Periodontitis severity	Clinical attachment loss – CDC/AAP 2012 case definitions criteria
Dependant (outcome) variables	**Measurement unit**
SIgA levels in unstimulated saliva	µg/ml
Secretion rate of SIgA (corrected levels of SIgA to account for differing saliva flow rates among study participants).	µg/min

CHAPTER FOUR: MATERIALS AND METHODS

4.1 Study Location

This study was carried out at the University of Nairobi Dental hospital, located off Argwings Kodhek road, opposite the Nairobi Hospital. The University Dental Hospital is a major dental referral hospital that serves a wide range of people from different locations of the country. The annual patient intake varies between 3000 to 3500 new patients per annum. The university trains both undergraduate and postgraduate students, and runs five clinics, namely, diagnostic, paediatric, prosthodontic, oral-maxillofacial and periodontology clinics. The location, patient inflow, and the wider socio demographic intake create a good location for research. The study participants were recruited from the oral diagnosis and periodontology clinics. Patients at the dental hospital may or may not present with periodontal disease to the dental hospital and this presented as an advantage as we collected data from both the periodontal healthy and patients with periodontal disease.

4.2 Study population

These were adult patients aged 18 years and over, who were attending oral diagnosis and periodontology clinics at the University of Nairobi dental hospital during the period of the study.

4.3 Study design

This was a descriptive cross-sectional study using a hospital-based population.

4.4 Sample size determination

The levels of salivary SIgA were evaluated in four groups, and the mean variance between them will be determined. The groups are as follows.

1) No periodontitis, μ_1
2) Mild periodontitis, μ_2
3) Moderate periodontitis, μ_3
4) Severe periodontitis, μ_4

It was set out to determine the variance between each group therefore this study employed an analysis of variance model and since the null hypothesis states that there is no relationship between levels of salivary SIgA and the periodontal health status, it implies no variation exists between groups, that is $\mu_1 = \mu_2 = \mu_3 = \mu_4$. The alternative hypothesis conversely states that a difference exists between groups' that is $\mu_1 \neq \mu_2 \neq \mu_3 \neq \mu_4$. According to Cohen 1988, to perform the statistical power analysis some overall factors were taken into consideration,

a) The significance level,
b) The effect size,
c) Desired power,
d) Estimated variance

The significance level is set at $\alpha = 0.05$, which represents the probability of wrongly rejecting the null hypothesis and committing a type 1 error. The effect size is the degree to which the null hypothesis is false (Cohen 1988), hence it describes the magnitude or amount of change from the null hypothesis. The effect size can be measured using raw values or standardised values and since we do not have previous data on levels of salivary SIgA in the Kenyan population, Cohen suggests the use of effect size values of, 0.1, 0.25 and 0.4, which represent small, medium, and large effect sizes respectively with the large effect size being more desirable. Statistical power represents the probability that a statistical test of significance will lead to a rejection of the null hypothesis at a specified value of the alternate hypothesis. Simply put it is the probability of correctly rejecting the null hypothesis. This is usually represented as 1-β, where β is the probability of wrongly accepting the null hypothesis, which is committing a type 2 error. The β is set as 0.2, therefore the power value is 1 - 0.2, giving us a power of 0.8 (80%).

The power function for one way ANOVA is.

$$f = \sqrt{\frac{\sum_{i-1}^{k} p_i \times (\mu_i - \mu)}{\alpha^2}}$$

Where.

$p_i = n_i \div N$

n_i = number of observations in group I

N = Total number of observations

μ_i = Mean of group I
μ = Grand mean
α^2 = error variance within groups

The values which were used in this study set for the powers analysis outlined by Cohen 1988:

f = Effect size: kept at 0.4 in this study (large effect size),
k = number of groups: 4 groups,
n = common sample size in each group: indicated as NULL (desired value),
sig. level (α) = significance level: set at 0.05,
Power = power of the test (1-β): 0.8,

Utilizing the power package developed by Stéphane Champely, current version 1.2-2, windows 10, on the R-script platform, which implements the powers analysis as outlined by Cohen in 1988, the sample size was calculated by inputting the data as described above and placing NULL in the n category, which generates the required sample size.

As per the method described above, n (sample size per group) was calculated at 18, giving a total sample size of 18×4 = 72. For this study a sample size of 77 was used with the additional 5 patients who were used to perform the preliminary phase of the study pretesting and piloting the research study.

4.5 Sampling

Convenience sampling was performed to select potential study participants, on patients attending the oral diagnosis and periodontology clinics at the University of Nairobi Dental hospital. The sampling period extended from September 2019 to January 2020, equating to five (5) months. The purpose of sampling in both clinics was to target the sample population of participants with and without periodontal disease, by increasing the available sample pool.

Patients were recruited into the study whilst in their respective waiting rooms or during history taking sessions. A verbal explanation of the study was given to them, screening was performed using the screening form. All potential participants who fit the inclusion criteria were provided a more detailed written and verbal explanation of the study individually and privately. Any questions raised by the participants were answered by the principal investigator. All patients who accepted to participate and provided voluntary informed consent were then subjected to the study process.

Participants that declined to participate were asked if they had any questions or in need of any advice and were provided the appropriate answers.

4.6 Inclusion and exclusion criteria

4.6.1 Inclusion criteria

1) Persons who were 18 years and older able to provide informed consent participated in the study.
2) Patients attending the oral diagnosis clinic or periodontology clinic, between 8 am and 12 noon. The time restriction was to account for the diurnal variation in unstimulated salivary flow.

4.6.2 Exclusion criteria

1) Individuals who were below the age of 18 years. This is because children and young adolescents have been shown to have lower levels of salivary SIgA compared to adults, this variation has been shown to be most significant below the age of 7 years[24].
2) Patients who declined to provide voluntary informed consent,
3) Patients who had undergone previous periodontal therapy over the past 12 months. Periodontal therapy has been shown to cause reduction in levels of salivary SIgA,
4) Females who were pregnant or lactating. These individuals have been shown to have an increased number of circulating plasma cells and increased levels of salivary SIgA during pregnancy[46],
5) Completely edentulous patients, or patients with less than 20 teeth. This would otherwise negatively affect the assessment of chronic periodontitis using clinical attachment loss measures effectively,

6) Medically compromised patients, in particular diseases and conditions which may affect the SIgA levels in secretory body fluids, examples include diabetes mellitus, autoimmune diseases, hormonal disorders, rheumatic diseases, and patients diagnosed with chronic viral infections such as HIV and HSV,
7) Diseases or conditions that may affect the normal functioning of the salivary glands and therefore affect salivary flow. Such conditions like sialolithiasis, sialadenitis, salivary gland neoplasms, autoimmune conditions affecting salivary glands such as Sjogren's syndrome, were excluded during screening. Salivary SIgA is actively secreted into saliva and therefore its levels are altered in salivary gland hypo function[24],
8) Patients consuming medications that may affect the antibody mediated immunity. Medications such as contraceptives, corticosteroids, chronic use of NSAIDS, antibiotic therapy, non-selective β blockers taken by hypertensive patients[47], and anticholinergic drugs,
9) Patients with chronic gastrointestinal diseases. Such as irritable bowel syndrome, ulcerative colitis were excluded, due to the evidence that suggests there is migration of plasma cells from mucosal associated lymphoid tissues (MALT) in the gut to the salivary glands which may affect the results[48]
10) Patients who are active smokers currently and previous smokers who have smoked more than 100 cigarettes. Smoking has been shown to affect the level of salivary SIgA possibly because of nicotine on vasculature and the general effects of smoking on salivary gland function and hence saliva flow rates are affected,

4.7 Minimizing errors and biases

Calibration of the principal investigator

To ensure reliability and validity of data collected there must be inter and intra examiner consistency. This was obtained through calibration of the principal investigator (A.J.M) by primary supervisor (H.A). The principal investigator performed data collection process in completion and the primary supervisor repeated this process for five participants during the preliminary phase. The data recorded by both examiners was compared and a Kappa score was established to determine inter-examiner reliability.

Kappa Score evaluation

For inter-observer reliability the Cohen's Kappa statistic was used. Five participants were recruited during the preliminary phase and were subjected to the study criteria. The formula used for Cohen's Kappa statistic is as follows.

$$\kappa = \frac{p_o - p_e}{1 - p_e} = 1 - \frac{1 - p_o}{1 - p_e},$$

Where.

P_O : Relative observed agreement among observers

P_e : The hypothetical probability of chance agreement

According to Cohen the K values corresponding to agreement are.

0 = agreement equivalent to chance.

0.1 – 0.20 = slight agreement.

0.21 – 0.40 = fair agreement.

0.41 – 0.60 = moderate agreement.

0.61 – 0.80 = substantial agreement.

0.81 – 0.99 = near perfect agreement

1 = perfect agreement.

Utilizing SPSS 25 which operates the inter-observer reliability test in accordance with Cohen's Kappa statistic, the kappa score was determined = 0.81 for n=5. This score suggests near perfect agreement, with the only limitation being the sample size used for the pilot (n=5) which was based on the number of available assays that could be safely run within the available kits without compromising total sample size. Once completing the preliminary phase and the calibration process was completed, the study moved to the data collection phase with the approval of all the supervisors.

Minimization of laboratory borne errors

The laboratory used had a temperature-controlled environment. All machinery used for the assay was in this environment, from the time of calibration of the machinery to the completion of the laboratory stage of the study. During the study only nitrile gloves were used to avoid risk of latex powder contamination of samples from latex gloves. All measuring devices were washed first with deionized water prior to use.

All graduated devices for measurements are manufacturer calibrated laboratory equipment which minimizes measurement errors. Pipettes used were the correct

ranges and lockable for the specific volume to be pipetted and these were colour coded for easy identification and had appropriate colour coded tips.

The laboratory staff (G.W) assigned to principal investigator (A.J.M) was blinded from the clinical data, and not privy to any confidential information regarding the participants. Furthermore, no data collection forms were submitted to the laboratory and all the laboratory data was collected separately on separate documentation.

4.8 Preliminary phase

Ethical approval was sought and obtained from KNH-UoN-ERC. Once approved the preliminary phase began, which comprised of calibration of the procedures for data collection and laboratory processes. CA Medlynks laboratory (Class F) was utilized; the laboratory equipment was evaluated and calibrated to ensure the laboratory process would go smoothly as per the manufacturers' recommendations.

A complete preliminary run was carried out; firstly, by obtaining approval from the respective chairmen of the departments of oral diagnosis and periodontology, five participants were used to perform the screening, consenting, bio data, saliva collection and clinical evaluation collection procedures with the primary supervisor (H.A.). This process ensured validity, reliability and consistency of the screening form, questionnaires, and interview procedure. This was then followed by a test run for the saliva collection procedure, to ensure familiarity and consistency in utilizing the saliva collection equipment and protocol, which would improve reliability of collection method.

The clinical examination process was then performed with the primary supervisor (H.A), to ensure that inter and intra examiner calibration was done to obtain consistent results following the outlined methodology. Finally, a laboratory test run was done utilizing the five additional samples collected and stored during the pilot run which allowed for testing and complete calibration of all equipment used. The test samples were then analysed using the © 2018 | Salimetrics, LLC - 5PK 1-1602-5 EIA assay for SIgA.

4.9 Screening and consenting process

Utilizing a screening form **(appendix 1)**, the potential participant was briefly questioned to determine if they fall within the study criteria. Once a determination of inclusion was made the consenting process was performed, and consisted of the following.

1) Verbal discussion with the potential participant regarding the study overall, its objectives and main outcome in an easy-to-understand manner,
2) The potential participant was free to ask questions during and after the discussion, which were answered accordingly and immediately,
3) An informed consent form **(appendix 2)** was utilized which described the study briefly, the forms were available in English and Swahili,
4) The potential participants questions regarding consent were answered, including any questions regarding data privacy and management,
5) Informed consent form was signed / thumb printed by the willing participant,
6) Participant was informed they may withdraw at any time from the study.

4.10 Data collection, clinical examination, and laboratory procedures

Data collection began in September 2019 and was completed in January 2020, during this period the following steps were carried out to collect the relevant data from participants:

4.10.1 Participant data collection

Interviewer administered questionnaire and bio data form was used to collect the socio demographic data and medical history from the patient **(appendix 3)**.

4.10.2 Saliva sample collection

Saliva sample collection was performed prior to clinical examination, to avoid stimulation of the oral cavity and potential alteration of the salivary flow rates by introduction of intraoral instruments. The “passive drool technique” was utilised to collect whole unstimulated saliva[49]. The time of day for saliva collection was also considered, to avoid the circadian rhythm variation that is seen in unstimulated salivary

flow rates after midday, hence sample collection was performed between 8 am to 12 noon.

The passive drool collection method[49] used involved instructing the participant to be seated on the dental chair upright, the participant was then instructed to allow saliva to pool in the oral cavity for a fixed time interval of 2 minutes, timed using a digital stopwatch. The participant was then instructed to place mouth over collection tube at the 2-minute interval and tilt their head forwards to gently guide saliva into the polypropylene collection tube (cryovial), until no saliva was seen flowing into the collection vial. This process was repeated utilizing the same fixed intervals of 2 minutes until minimum desired sample volume of 1 ml or more is achieved, and the total time and final volume was recorded. The collection tubes were labelled prior to collection of the sample with a sample code unique to each participant. The samples once collected were refrigerated immediately in a cooler box lined with sleeved frozen gel packs.

Using an infrared thermometer prior to sample collection, (ETEKCITY -50 ºC to 550ºC CFR 1040.10/11) the temperature of dummy samples filled with distilled water was measured after a period of 1 Hour in the gel lined cooler box, a range of temperatures of 1.5 C to 4.6 C was found and deemed optimal for temporary sample storage during collection period and transit. Within 2 to 4 hours of collection, the samples were transported to the laboratory where they were stored in a laboratory freezer with an active thermostat at -80ºC with an active thermostat. SIgA has been found to be stable in saliva at room temperature up to 6 hours, at 4ºC up to 48hrs and at -80ºC for 1.3 years[50]. Cotton based saliva collection methods were not utilized as it has been shown that these methods interfere with assay results and present with artificially low SIgA values[51]. Any samples collected that were visually contaminated with debris were either recollected or excluded. GCF was not collected, considering the setup up for this study, as it is more technique sensitive, and time consuming, hence has less reproducibility. The above instructions that have been outlined have also been recommended by the manufacturer of the assay kit, (© 2018 | Salimetrics, LLC.).

4.10.3 Clinical Examination

The oral hygiene status was recorded utilizing the plaque index described by Silness and Loe 1963[52] **(appendix 4)**. The gingival health status was recorded utilizing the gingival index described by Loe and Silness 1964[53] **(appendix 5)**. The probing depths and the clinical attachment loss were determined by recording a six-point chart examining all teeth in the mouth, excluding third molars. Utilizing the CDC/AAP 2012 case definitions criteria the severity of periodontal disease[12] was categorized **(appendix 6)**. In the case of the plaque and gingival index scores, the teeth selected were those as proposed by Ramfjord[54]. The teeth are the maxillary right first molar, maxillary left central incisor, maxillary left first premolar, mandibular left first molar, mandibular right central incisor, mandibular right first premolar. The replacement teeth in the event any of the first choice of teeth were missing are the maxillary right second molar, maxillary right central incisor, maxillary left second premolar, mandibular left second molar, mandibular left central incisor and mandibular right second premolar. The Ramfjord teeth have been popularly used in many studies including in an east African population.

It is worth noting that whilst the Ramfjord teeth act as excellent surrogates for representing gingivitis in the entire dentition as it is reliable, time saving and efficient, it has been shown to frequently underestimate chronic periodontitis hence not used for clinical attachment loss measurements[54] in this study.

Utilizing the clinical examination form **(appendix 7)**, the clinical examination was carried out in a sequential manner once an adequate saliva sample had been collected. Oral hygiene status assessment utilizing the plaque score index by Silness and Loe 1964 **(appendix 4)**, a standard explorer was used to measure the quantity of plaque on the selected teeth,

Gingival health status assessment was done utilizing the gingival index by Loe and Silness 1963, with a periodontal probe, which was gently inserted into the gingival sulcus until resistance is met and then run gently along the soft tissue wall of the gingival sulcus parallel to the tooth surface. The degree of gingival inflammation was assessed based on presence or absence of bleeding. The individual scores were then summated, and the mean obtained and categorized **(see appendix 5)**,

Assessment of the periodontal health status was done utilizing, a University of Michigan ‘O’ Probe with Williams markings at, 1, 2, 3, 5, 7, 8, 9mm. The procedure of measurement involves “walking” of the probe along the gingival sulcus in a fluid gentle motion, to aid in detection of pathologic pockets. Three readings of probing depths were taken at the buccal / facial and lingual / palatal aspects of teeth, specifically at the mesial, mid-buccal / mid-palatal and distal points of each tooth, for a total of 6 points per tooth. Gingival recession was measured by measuring the distance from the cementoenamel junction to the free gingival margin using the same periodontal probe. The probing depths and gingival recession values were simultaneously recorded on a six-point chart **(appendix 7)**, the clinical attachment loss was determined, recorded, and the severity of the disease categorized based on the CDC/AAP 2012 case definitions criteria **(appendix 6)**. Tooth mobility was measured utilizing miller’s classification of mobile teeth and recorded on the six-point chart **(appendix 7).**

4.10.4 Control of cross infection during data collection

Universal precautions were performed to prevent any form of cross infection and safeguard the patients, investigators, and others in the clinical workspace. These precautions were carried out prior to each participant being ushered into the clinic and after each participant left the clinic workspace.

These precautions included cleaning of surfaces such as the dental chair surfaces and attached surfaces with Isopropyl Alcohol IPA (ABV 70% KEBS validated). Surfaces used for placing documents, as well as stationary used were also wiped with alcohol laden cotton wipes. Secondary contact surfaces such as door handles and trash bin lids were sprayed with enzymatic disinfectant (Aniozyme) followed by a wipe down, which was provided by the clinic and represents standard aseptic protocol in both oral diagnosis and periodontology clinics. Other precautions taken were, all instruments and instrument cassettes used on participants were prior sterilized in the university validated and operated bulk steam autoclave and after each use they were cleaned and re-sterilised. Autoclaving tape was used to confirm validity of every sterilization cycle. Surgical face masks and nitrile gloves were used by principal investigator, whilst a two-sided bib with an inner hydrophobic side and outer absorptive side were used for the participants. These represented the disposable personal protective equipment

employed and were disposed of after each participant was examined. In addition, nitrile gloves used were disposed after saliva sample collection and handling, new gloves were donned and used for clinical examination.

The saliva collection tubes were pre sterilized and came in individual pouches which were opened and used at the time of sample collection. Any opened tubes that were unused for whatever reason were disposed of into biological waste bins using the dental hospitals' disposal protocol. Whilst spillage of saliva was rare during collection, when it occurred the saliva tubes were tightly shut and the tubes were cleaned with IPA alcohol wipes, due to the low vapour pressure of IPA it evaporates rapidly reducing risk of sample contamination in comparison to other disinfectants. Personal hygiene when working with saliva is critical and this was maintained using hand washing both by participants and principal investigator. Samples were carried in a medical grade cooler box which would facilitate movement from the hospital to laboratory environment; therefore, the cooler had a biohazard sign printed on all visible sides. The cooler box housed several freeze gel packs for cooling which had an outer polyethylene sleeve which was removed and disposed of at the laboratory after each transportation use. All surfaces of the cooler box and gel packs were cleaned with the IPA and the gel packs were covered with a new sleeve and re frozen.

On completion of laboratory assays, the samples, dilutions, and all materials that came in contact with saliva were considered contaminated and classified as biological waste and were disposed of using the laboratory disposal protocol. It is worth noting that the global COVID-19 pandemic caused by the SARS-COV-2 pathogen had not yet begun in Kenya and the protocols used in this study remain the gold standard for handling saliva related biologic material and waste.

This study did not require or receive approval for maintaining samples for future testing or study, hence they were disposed of appropriately.

4.10.5 Laboratory procedures

Utilizing the SIgA assay kit by © 2018 | Salimetrics, LLC, 5PK 1-1602-5 EIA, the saliva samples were prepared and analysed using the manufacturer's instructions and optimizations from the pilot study.

Assay details

Table 2: SIgA EIA Details and contents of kit

Catalogue number	Format	Assay time	Sample volume	Sensitivity	Assay range
1-1602	96 well plate	Approximately 4 hours	25µL	2.5µg/ml	2.5µg/ml - 600µg/ml

Microtiter plate	1 x 96 well plate
SIgA standard	1 vial ; 100µl
SIgA controls	2 vials ; 50µl each
SIgA antibody enzyme conjugate	1 vial ; 50µl
SIgA diluent concentrate (5X)	1 bottle ; 50ml
Wash buffer concentrate (10X)	1 bottle ; 100ml
TMB substrate solution	1 bottle ; 25ml
Stop solution(Methanesulfonic acid)	1 bottle ; 12.5ml
Adhesive plate covers	2 covers

Figure 4: Assay kit contents at time of opening (Plate removed and placed aside safely in foil pack.

Preparation of Diluent and SIgA standards

Prior to preparing standards or samples, the 1X SIgA diluent was prepared by diluting 50 mL of the SIgA diluent concentrate into 200 mL of deionized water. This is the diluent used throughout the procedure.

A volume of 30 µL of 1X SIgA diluent was pipette into capped tubes labelled Q1 – Q5. Serial dilution using 15 µL of the 600 µg/mL standard into tube Q1, the process was repeated serially moving down each tube from Q1 to Q5 as shown in figure 5 below. Gentle and thorough vortex of the tubes was done at each dilution step.

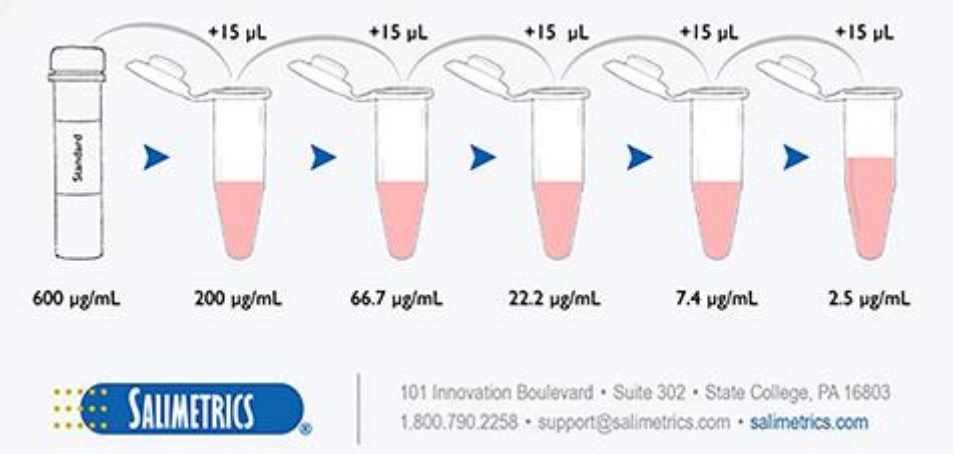

Figure 5: Serial dilutions of standards to prepare a total of 6 standards

Sample preparation

The samples once collected were stored in the polyethylene cryovials at -80ºC. The samples to be run on the day were removed from the freezer and allowed to thaw. Once thawed at room temperature they were gently vortex then spun in a centrifuge at a centrifugal force of 1500 g for 15 minutes. The clarified supernatant was extracted and placed in new cryovials and labelled to the corresponding sample.

Sample dilution: A volume of 100 µL of 1X SIgA diluent was transferred into empty laboratory capped tubes, for each sample to be diluted and labelled with sample codes. Into each of these tubes 25 µL of clarified supernatant saliva sample was pipette from the corresponding cryovial. Each tube was then vortex, to mix the diluent and sample thoroughly.

Final dilution for samples, standards, and controls: Using new empty 5 mL capped tubes labelled for each sample, standard and control, 4 mL of the 1X SIgA diluent was added to each of these empty tubes. To this, 10 µL of the previously prepared diluted samples and standards were pipette into corresponding tube and mixed. The controls

provided in assay kit were prepared by pipetting 10 µL of undiluted controls into the corresponding labelled tubes containing 4 mL of 1X SIgA diluent.

Incubation with antibody enzyme conjugate

The conjugate provided in the kit was diluted to a ratio of 1:120 by pipetting 25µL of the conjugate into a 5 mL capped tube containing 3 mL of 1X SIgA diluent. The now diluted antibody enzyme conjugate was mixed thoroughly. After mixing, 50 µL of the diluted conjugate was added to each labelled tube containing the final dilutions of the standards, samples, and controls. Each tube was mixed by inversion then incubated for 90 minutes at room temperature.

Transfer to assay microtiter plate

The tubes containing the incubated samples, standards and controls were then mixed by inversion and a volume of 50 µL from each tube was transferred to individual corresponding wells on the microtiter plate using a pipette. The location of each sample, standard and control was pre-determined according to the micro plate design, and a laboratory worksheet was created to keep track of the plate design in the lab **(appendix 9).** The plate was then covered with an adhesive plate cover and mixed on a plate rotator continuously for 90 minutes at 400 rpm at room temperature.

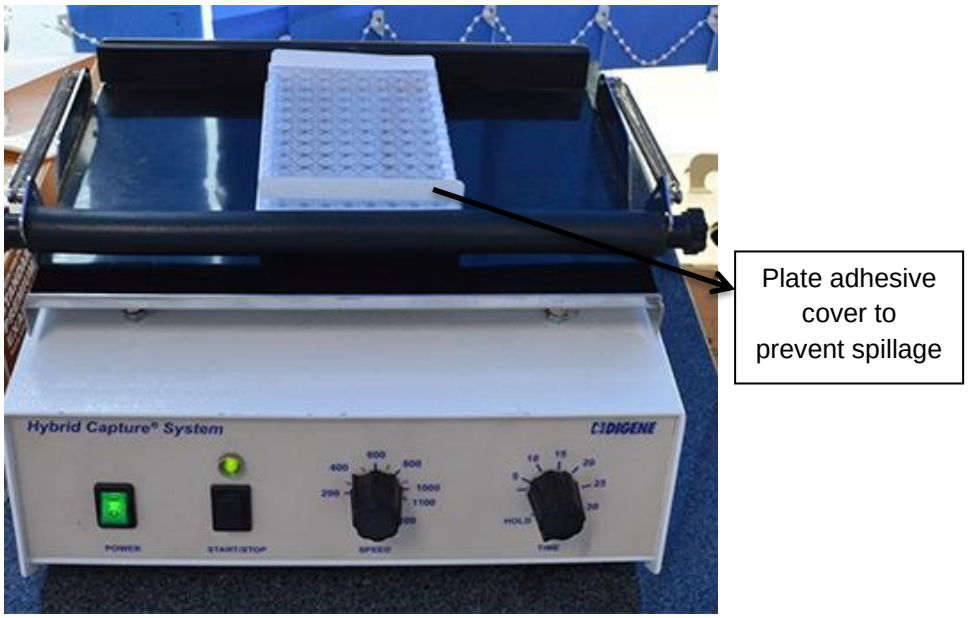

Figure 6: Plate rotator, securely holds plate, with variable RPM and timer

Plate preparation

The 1X wash buffer was prepared during the wait period, by diluting the wash buffer concentrate (10X) with deionized water in a volume of 100 mL: 900 mL respectively. The wash buffer was added to the pre calibrated plate washer reservoir. After plate mixing with conjugate was complete, the plate was transferred to the plate washer, where the plate was washed with the wash buffer 6 times, using a 300 µL cycle. The plate was blotted after the last wash to remove excess wash buffer.

Figure 7: Plate washer calibrated for six, 300µL cycles

Plate reaction

Once plate washing was complete 50 µL of tetramethylbenzidine (TMB) was added to all wells using a multichannel / repeater pipette excluding the non-specific binding wells (NSB). Following which the plate was covered with a plate adhesive cover and mixed on the plate rotator for 5 minutes at 500 rpm, after which the plate was placed in a dark environment for 40 minutes at room temperature.

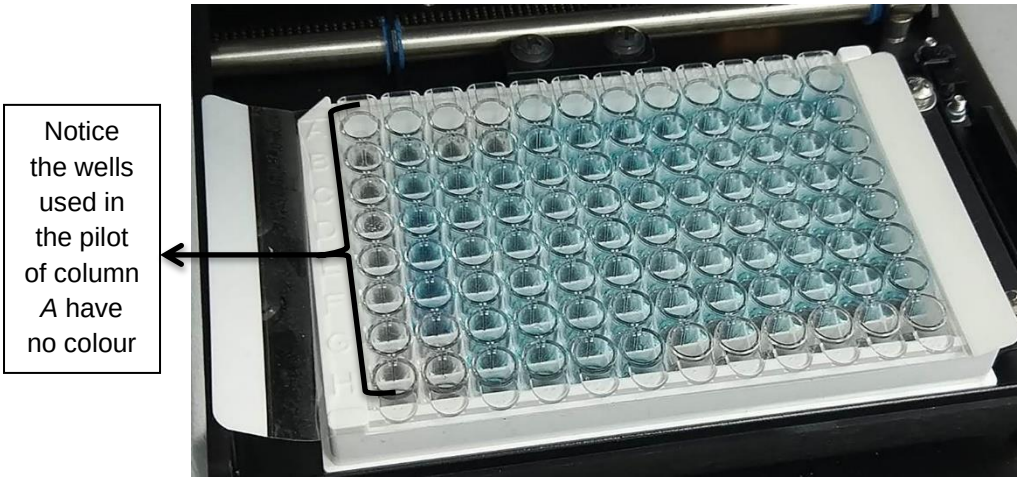

Figure 8: Plate being placed into a dark chamber for incubation

After the incubation the plate is uncovered, and the wells appear greenish blue as shown above, to these wells 50 µL of Stop solution (Methanesulfonic acid (CAS No. 75-75-2)) was added and mixed on plate rotator for 3 minutes. This results in the green-blue colour to change to yellow (See **appendix 9** for full plate images)**.** Once all wells have changed to yellow the plate cover is removed.

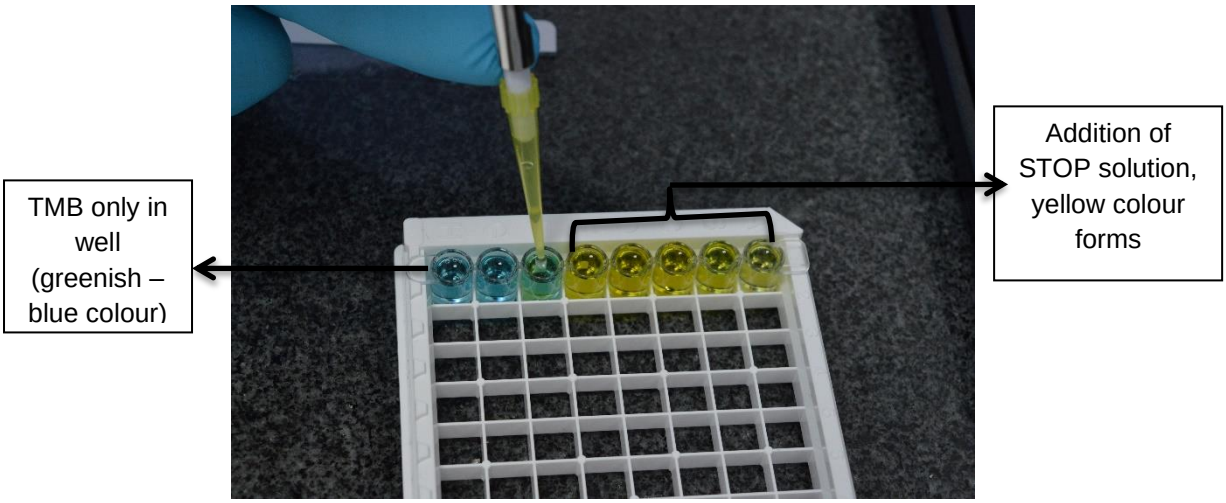

Figure 9: STOP solution being added to pilot wells. Notice the varying intensities of final colour changes. (This image was captured during the pilot run)

Reading the plate

The plate was placed into a micro plate reader (ELX-808 Bio Tek), the plate was coded, and read at 450 nm. It was read within 5 minutes of completing the plate reaction with the stop solution. The plate reader data was then extracted using the

GEN 5 software by Bio Tek, Version 2.0.9 – Windows 10, 64-bit architecture, to obtain the optical densities of each well.

Biologic waste management and laboratory precautions

During all procedures and especially those that handled saliva samples and potential toxic substances such as stop solutions / substrates (Methanesulfonic acid / TMB), nitrile rubber gloves and eyewear was worn for protection, along with a laboratory coat. All containers that housed samples, dilutions or other secondary products of the assay were also considered biologic waste along with the used assay kit. All materials were disposed of at the end of the laboratory stage using biologic waste disposal system employed by the laboratory that uses controlled air incineration.

4.11 Computation, calculation and data entry

4.11.1 Initial computation

The data is extracted from the ELX808 plate reader and using the GEN 5 software protocol the data was displayed as optical densities for each well. This data was transferred to Microsoft Excel 2010 on Windows 10.

4.11.2 Generating the standard curve and calculation of results

A 4-parameter non-linear regression curve fit data reduction software was used which had been specifically approved for Salimetrics SIgA quantification and was used to determine the best fit standard curve. Using the standard curve, the levels of SIgA were determined in **µg/mL**. Values that fell beyond the assay range were re-run and those that were obviously erroneous were excluded.

4.11.3 Data Entry and Analysis

Data entry was performed utilizing statistical packages for social sciences (SPSS) version 25, 64-bit protocol (SPSS, IBM Corporation 2017). Microsoft Excel 2010 on the windows 10 platform was also used. The power package on the R-script platform was also used.

During the preliminary phase the Cohen's Kappa statistic was determined for inter-observer variation (refer to preliminary phase). Statistical tests were carried out on

continuous variables such as age, plaque scores, gingival index scores and periodontal health status, utilizing measures for central tendency and dispersion. Independent samples t-test was used for comparison of mean values between two independent groups. The analysis of variance (ANOVA) was also utilized to determine variation between multiple groups, with tests of homogeneity, when variances were assumed unequal a multiple comparison test i.e., Tamhane's T2 post hoc test was performed and when equal variances were assumed the LSD (Least significant difference) post hoc test was performed for multiple comparisons. For correlations that had less than 5 values per cell, the Fisher's exact test was utilized as a more accurate method for comparisons. The findings are presented in various formats such as graphs, tables, and bar charts.

4.12 Ethical considerations

For the study to be carried out approval from the Kenyatta national hospital research, ethics, and standards committee (KNH-UoN ERC) was obtained **(Approval number: P144/02/2019)**. Each participant was explained to the study contents and what it entails. The participants then provided written informed consent and no potential participant/s were recruited to the study without a signed consent form (Appendix 2). Patients who required emergency treatment were treated at or referred to, the prerequisite dental hospital clinics and were not subjected to the study. All patients who did not wish to participate were allowed to decline with the express explanation that they would face no victimization of any kind, assuring that the principal investigator follows the core values of justice and beneficence of research. Furthermore, patients who declined to participate were still entitled to advice regarding their oral / dental health concerns from the principal investigator.

Approval for collection of data within the university of Nairobi dental hospital was obtained from the unit of oral diagnosis and chairman of unit of periodontology. Data protection measures were undertaken; data was backed up on an offline hard disk drive using an automated system, all files were encrypted, and the encryption key was available to the principal investigator and primary supervisor only. Note that no personal identification information of participants was collected and only data regarding the study itself was stored.

The biologic material i.e., saliva samples, were not to be kept for longer than the period of the study and hence were disposed of appropriately at the end of the laboratory phase along with all associated biologic waste materials. The laboratory was not given access to any participant information further protecting the participant's data and providing a blind.

4.13 Study outcomes and benefits

The intent of this study is to add to the foundational knowledge on severity of periodontal diseases and its relationship to Salivary SIgA in an adult Kenyan population. The study will also seek to provide the baseline values of salivary SIgA and potentially aid in determining the viability of SIgA as a biomarker for periodontal diseases. The results may further aid in future molecular studies in this field.

This thesis will be submitted in partial fulfilment of requirements for the award of, Master of Dental Surgery (MDS) degree in periodontology at the University of Nairobi School of dental sciences.

CHAPTER FIVE: RESULTS

5.1 Results from the preliminary phase

During the preliminary phase all the data collection methods and materials were tested. The screening forms were found to be efficient, and the questionnaires were easy to understand and use. The collection of saliva was also determined to be accepted well by all participants without any signs of aversion to the method further noting that minimal spillage occurred during the pilot.

A Cohen's Kappa score was determined as described previously; the kappa statistic was found to be 0.81 indicating near perfect agreement**.** The laboratory preliminary run was also performed, using this data a laboratory range was established, the results were validated, and the study moved from the preliminary phase to the actual data collection phase.

5.2 Sociodemographic characteristics

A total of 72 participants were recruited for the study. Of these, 35(48.6%) were males while 37(51.4%) were females. The sample age ranged from 19.0 – 70.0 years with a mean age of 35.6(±12.9 SD) years, a median of 32.0 years and a mode of 23.0 years.

An independent samples t-test showed no significant difference in mean age (years) between males (M = 35.74, ±13.61SD) and females (M = 35.43, ±12.36 SD), t = 0.101, df = 70, p = 0.920. Male and female participants appeared normally distributed within the study sample, whereas the age groups appeared well ranged within the study sample.

The socio-demographic variables were evaluated between the genders and depicted in Table 3 below using the Fishers exact test and Pearson Chi Squared. Most participants resided in Nairobi and there was equal representation of both males and females within all age groups. The majority had a higher education without much disparity between the genders p = 0.369.

Table 3: Associations of socio-demographic variables among the gender groups

Characteristics		n (%)	Gender		Statistical test
			Male	Female	
			n (%)	n (%)	
Age (grouped)	18 – 30 years	33 (45.9)	16 (22.2)	17 (23.6)	$X^2 = 0.018$,
	31 – 45 years	23 (31.9)	11 (15.3)	12 (16.7)	df = 2,
	>= 46 years	16 (22.2)	8 (11.1)	8 (11.1)	p = 0.991
Residence	Nairobi	56 (77.8)	31 (43.1)	25 (34.7)	Fisher's = 4.591*,
	Outside Nairobi	16 (22.2)	4 (5.6)	12 (16.7)	df = 1, p = 0.032
Occupation	Self employed	45 (62.5)	24 (33.3)	21 (29.2)	Fisher's = 3.806,
	Employed	17 (23.6)	9 (12.5)	8 (11.1)	df = 2,
	Unemployed	10 (13.9)	2 (2.8)	8 (11.1)	p = 0.149
Education	Primary	7 (9.7)	2 (2.8)	5 (6.9)	Fisher's = 1.992,
	Secondary	25 (34.7)	11 (15.3)	14 (19.4)	df = 2,
	Higher education	40 (55.6)	22 (30.6)	18 (25.0)	p = 0.369

Pearson Chi-Square (χ^2) test for association was used for age groups variable.
Fisher's exact test for association was used for residence, occupation, and education variables. df; Degrees of Freedom, *p<0.05

5.3 Oral Hygiene practices among participants

All participants performed tooth brushing, 34(47.2%) brushed once daily and 38(52.8%) brushed twice daily. Additionally, 60(83.3%) used a conventional dentifrice (paste) and 12(16.7%) used herbal dentifrices (paste). Interdental cleaning was performed by 13(18.1%), whilst 59(81.9%) performed no form of interdental cleaning.

5.4 Oral Hygiene status of participants

All participants had their plaque scores evaluated, with an overall mean of 0.94(±0.55). The mean plaque scores for males were 1.10(±0.50 SD) and females 0.79(±0.57 SD). An independent samples t-test was performed, and this elicited a significant difference in mean plaque scores between males and females (Table 4) indicating males had a higher mean plaque score than females.

An Analysis of Variance (ANOVA) was performed (Table 4). It showed that there was a significant difference seen for mean plaque scores among the different age groups, $F(2, 69) = 13.341$, $p<0.001$ indicating as age increased the mean plaque scores were also increased.

Table 4: Sociodemographic variables and plaque scores

		Plaque scores			95% Confidence Interval of the difference		
Characteristics		n (%)	M	SD	Lower	Upper	Statistical test
Gender	Male	35 (48.6)	1.10	0.50	0.06	0.56	t = 2.464*, df = 70, p = 0.016
	Female	37 (51.4)	0.79	0.57			
Age (grouped)	18 – 30 years	33 (45.9)	0.66	0.37	0.53	0.80	F = 13.341***, df = 2, 69, p < 0.001
	31 – 45 years	23 (31.9)	1.02	0.62	0.75	1.29	
	>= 46 years	16 (22.2)	1.40	0.43	1.17	1.63	
Residence	Nairobi	56 (77.8)	0.94	0.58	-0.33	0.30	t = 0.077, df = 70, p = 0.938
	Outside Nairobi	16 (22.2)	0.95	0.47			
Occupation	Self employed	45 (62.5)	1.00	0.56	0.83	1.17	F = 1.019, df = 2, 69, p = 0.366
	Employed	17 (23.6)	0.90	0.54	0.62	1.18	
	Unemployed	10 (13.9)	0.74	0.53	0.36	1.12	
Education	Primary	7 (9.7)	1.16	0.74	0.47	1.85	F = 1.495, df = 2, 69, p = 0.231
	Secondary	25 (34.7)	1.03	0.61	0.78	1.28	
	Higher education	40 (55.6)	0.85	0.47	0.70	1.00	

Independent Samples t-test (t) was used for gender and residence variables.
Analysis of Variance (ANOVA) (F) was used for age groups, occupation, and education variables.
M; Mean, SD; Standard Deviation. df; degrees of freedom, ******* p<0.001, ***** p<0.05.

On further examination of the age groups and plaque scores a Tamhane's T2 post hoc test elicited a significant difference in mean plaque scores between age group 18 – 30 years and age group ≥ 46 years ($p<0.001$) as shown in table 5 below. This indicated the age group ≥ 46 years had significantly higher plaque scores than other age groups.

Table 5: Comparison of mean plaque scores and participants age groups

Characteristics		Mean Difference	95% Confidence Interval		p
Age group	Age group		Lower	Upper	
18 – 30 years	31 – 45 years	-0.36	-0.72	-0.01	0.056
	>= 46 years	-0.74***	-1.06	-0.42	<0.001
31 – 45 years	18 – 30 years	0.36	0.01	0.72	0.056
	>= 46 years	-0.38	-0.80	-0.04	0.086
≥ 46 years	18 – 30 years	0.74***	0.42	1.06	<0.001
	31 – 45 years	0.38	-0.04	0.80	0.086

Tamhane's T2 test was used for post hoc comparison of means for all variables.
***p<0.001

5.5 Comparison between oral hygiene practices and the oral hygiene status of participants

To further evaluate the oral hygiene practices performed by participants and the effects on oral hygiene status, an independent samples t-test and analysis of variance (ANOVA) was performed (Table 6).

Participants who brushed twice daily 38(52.8%) and those that used interdental cleaning aids 13(18.1%) showed lower mean plaque scores of 0.87(±0.54 SD) and 0.80(±0.51 SD) respectively.

The number of dental visits appeared to have no effect on the mean plaque scores with most participants reporting their last dental visit was >6 months previously 46(63.9%) and 17(23.6%) having never attended a dental appointment.

The use of either conventional or herbal toothpaste also did not appear to have an overall effect on the plaque scores p = 0.52, which is somewhat expected as mechanical cleaning is known to be the most effective measure of plaque control.

Table 6: Oral health practices and plaque scores

Characteristics		Plaque scores			95% Confidence Interval of the difference		Statistical test
		n (%)	M	SD	Lower	Upper	
Brushing frequency	Once	34 (47.2)	1.02	0.57	-0.10	0.42	t = 1.204, df = 70, p = 0.233
	Twice	38 (52.8)	0.87	0.54			
Toothpaste	Conventional	60 (83.3)	0.96	0.53	-0.24	0.47	t = 0.647, df = 70, p = 0.520
	Herbal	12 (16.7)	0.85	0.70			
Interdental cleaning	None	59 (81.9)	0.97	0.56	-0.17	0.51	t = 1.004, df = 70, p = 0.319
	Dental floss/ Interdental brush	13 (18.1)	0.80	0.51			
Dental visit	None	17 (23.6)	0.94	0.63	0.62	1.27	F = 0.273, df = 2, 69, p = 0.762
	< 6 months	9 (12.5)	1.07	0.50	0.69	1.45	
	> 6 months	46 (63.9)	0.92	0.54	0.75	1.08	

Independent Samples T test (t) was used for brushing frequency, toothpaste, and interdental cleaning.
Analysis of Variance (ANOVA) (F) was used for dental visit variable.
M; Mean.
SD; Standard Deviation.

5.6 Saliva flow rate (mL/min)

The salvia flow rate ranged from 0.18 mL/min – 1.60 mL/min with a mean rate of secretion of 0.72(±0.38 SD) mL/min, a median of 0.69 and a mode of 0.50. An analysis of variance (ANOVA) elicited a significant difference in the mean rates of saliva secretion between the age groups, $F(2, 69) = 5.801$, $p<0.05$ (Table 7)

An LSD post hoc test revealed significant differences for the age group "≥46 years" which showed significantly lower rates of saliva secretion in comparison to the other age groups ($p<0.05$).

Table 7: Age and saliva flow rates

		Rate of saliva secretion (mL/min)			95% Confidence Interval of the difference		
Characteristics		n (%)	M	SD	Lower	Upper	Statistical test
Age	18-30	33(45.8)	0.84	0.37	0.71	0.97	F = 5.801*,
	31-45	23(31.9)	0.72	0.37	0.56	0.88	df = 2, 69,
	≥ 46	16(22.2)	0.47	0.30	0.31	0.63	p < 0.05

Analysis of Variance (ANOVA) (F) was used for all variables.
M; Mean. SD; Standard Deviation.
df; degrees of freedom. *p<0.05

A means plot was generated to demonstrate the differences between saliva flow rate and age as shown (Figure 10)

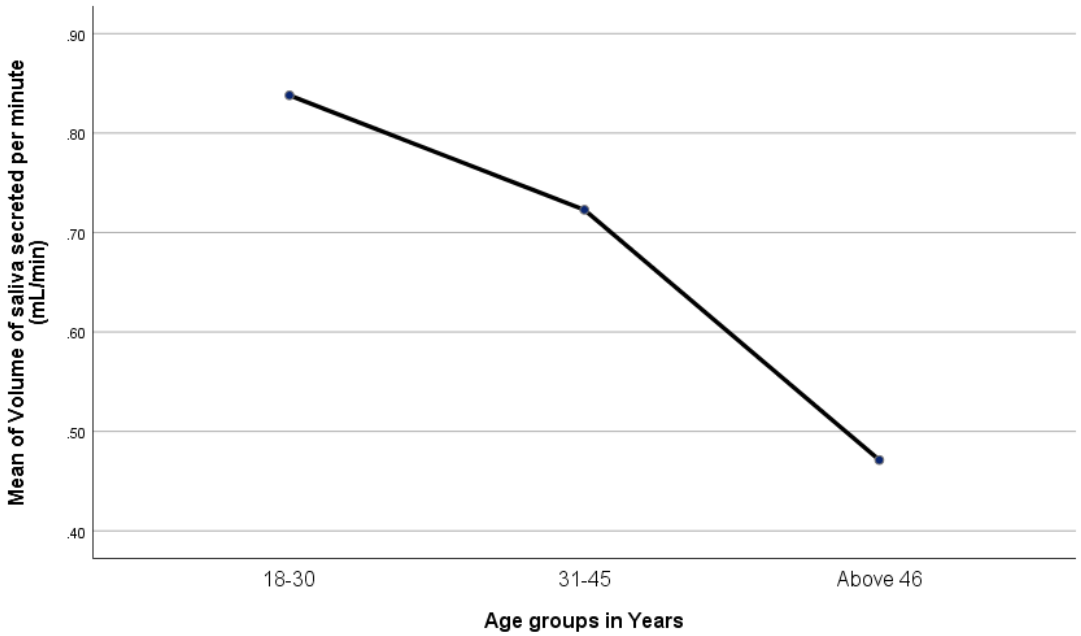

Figure 10: Means plot for Age and saliva flow rate

The means plot in Figure 10 shows that as age increases, there is a decrease in saliva secretion with a sharper decline being noticeable between groups “31-45 years” and “≥ 46 years”.

5.7 Gingival inflammation (gingivitis) among participants

The degree of gingival inflammation (gingivitis) was measured using the gingival index described by Loe and Silness 1963. The gingival index scores ranged from 0.1 –2.7 with a mean score of 1.0(±0.57 SD), a median of 1.0 and a mode of 1.1.

Therefore, all participants had some varying degree of gingival inflammation with most participants having mild gingival inflammation 38(52.8%), whilst 29(40.3%) had moderate inflammation and 5(6.9%) had severe gingival inflammation.

To evaluate the relationship between gingival inflammation and mean plaque scores, an analysis of variance (ANOVA) was performed (Table 8 below) and this significant difference was seen between the means of gingival inflammation and plaque scores, $F(2,69)=68.770, p<0.001$. In addition, a Pearson correlation coefficient (r) elicited a statistically significant, strong, positive association between mean plaque scores and gingival index scores ($r = 0.865$, $p < 0.001$). This indicated that as plaque scores increased gingivitis severity also increased.

Table 8: Gingival inflammation and plaque score of participants

Characteristics		Plaque scores			95% Confidence Interval of the difference		Statistical test
		n (%)	M	SD	Lower	Upper	
Gingivitis	Mild	38 (52.8)	0.52	0.28	0.43	0.61	F = 68.770***, df = 2, 69, p < 0.001
	Moderate	29 (40.3)	1.37	0.38	1.22	1.51	
	Severe	5 (6.9)	1.67	0.30	1.28	2.04	

Analysis of Variance (ANOVA) (F) was used for all variables.
M; Mean.
SD; Standard Deviation, df; degrees of freedom, ***p<0.001

An LSD post hoc test was done (Table 9) to identify the most significant groups and elicited a significant difference in mean plaque scores between mild gingivitis and moderate gingivitis (p<0.001), and between mild gingivitis and severe gingivitis (p<0.001), indicating as plaque score increased gingivitis severity increased.

Table 9: LSD post hoc comparison for mean plaque scores and gingivitis

Characteristics		Mean Difference	95% Confidence Interval		p
Gingivitis	Gingivitis		Lower	Upper	
Mild	Moderate	-0.85***	-1.01	-0.69	<0.001
	Severe	-1.14***	-1.45	-0.83	<0.001
Moderate	Mild	0.85***	0.69	1.01	<0.001
	Severe	-0.29	-0.61	0.02	0.068
Severe	Mild	1.14***	0.83	1.45	<0.001
	Moderate	0.29	-0.02	0.61	0.068

LSD test was used for post hoc comparison of means for all variables. ***p<0.001

A curve estimation linear regression model (Figure 11) showed significant difference between gingival index scores and mean plaque scores as the predictor variable, (R^2 = 0.749, β = 0.865, and F (1, 70) = 208.578, p < 0.001.)

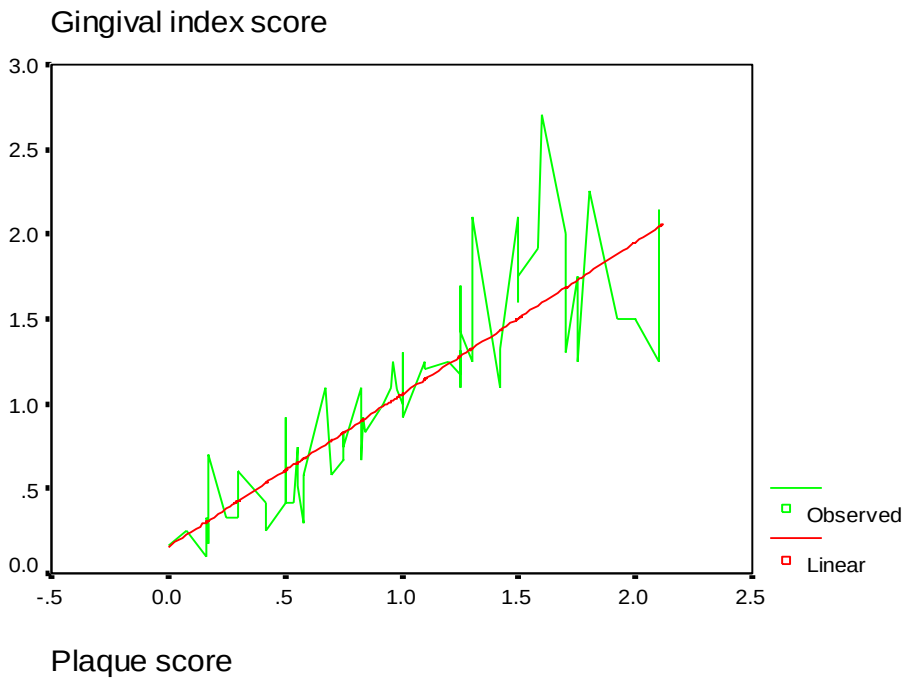

Figure 11: Linear regression model plot for gingival index score and plaque score

5.8 Periodontitis among participants

Out of a total of 72 participants 40(55.6%) had no periodontitis and 32(44.4%) had periodontitis. Of these participants 14(19.4%) had mild periodontitis, 11(15.3%) had moderate periodontitis and 7(9.7%) had severe periodontitis.

A comparison of socio-demographic variables and periodontitis was performed using Fisher's exact test, which elicited a significant relationship between age groups and periodontal disease severity (p<0.001) as shown in table 10 below. These results indicate that an increasing severity of periodontal disease was found with an increasing age among the study participants.

Table 10: Socio-demographic variables and periodontitis

Characteristics		n (%)	Chronic periodontitis None n (%)	Mild n (%)	Moderate n (%)	Severe n (%)	Statistical test
Gender	Male	35 (48.6)	17 (23.6)	8 (11.1)	6 (8.3)	4 (5.6)	Fisher's =1.365, df = 3, p = 0.714
	Female	37 (51.4)	23 (31.9)	6 (8.3)	5 (6.9)	3 (4.2)	
Age groups(Years)	18 – 30	33 (45.9)	26 (36.1)	6 (8.3)	1 (1.4)	0	Fisher's = 28.025***, df = 6, p < 0.001
	31 – 45	23 (31.9)	12 (16.7)	5 (6.9)	4 (5.6)	2 (2.8)	
	≥46	16 (22.2)	2 (2.8)	3 (4.2)	6 (8.3)	5 (6.9)	
Occupation	Self employed	45 (62.5)	25 (34.7)	9 (12.5)	6 (8.3)	5 (6.9)	Fisher's =0.793, df = 6, p = 0.992
	Employed	17 (23.6)	10 (13.9)	3 (4.2)	3 (4.2)	1 (1.4)	
Education	Primary	7 (9.7)	4 (5.6)	0	1 (1.4)	2 (2.8)	Fisher's = 5.876
	Secondary	25 (34.7)	12 (16.7)	5 (6.9)	5 (6.9)	3 (4.2)	df=6
	Higher education	40 (55.6)	24 (33.3)	9 (12.5)	5 (6.9)	2 (2.8)	p= 0.392

Fisher's exact test for association was used for all variables.
df; Degrees of Freedom.
***p<0.001

An evaluation of oral hygiene practices and periodontitis was performed using the Fisher's exact test (Table 11). There were no significant differences seen between the oral hygiene practices of participants and periodontitis severity in this study. Though not statistically significant it is worth noting that those who brushed twice daily and performed interdental cleaning had the lowest overall frequency of periodontal disease and with the lowest overall severity when periodontitis was present.

Table 11: Oral hygiene practices and periodontitis

Characteristics		n (%)	Chronic periodontitis				Statistical test
			None	Mild	Moderate	Severe	
			n (%)	n (%)	n (%)	n (%)	
Brushing (Daily)	Once	34 (47.2)	17 (23.6)	9 (12.5)	5 (6.9)	3 (4.2)	Fisher's = 2.061, df = 3, p = 0.560
	Twice	38 (52.8)	23 (31.9)	5 (6.9)	6 (8.3)	4 (5.6)	
Toothpaste	Conventional	60 (83.3)	34 (47.2)	12 (16.7)	10 (13.9)	4 (5.6)	Fisher's = 4.049, df = 3, p = 0.256
	Herbal	12 (16.7)	6 (8.3)	2 (2.8)	1 (1.4)	3 (4.2)	
Interdental cleaning	None	59 (81.9)	31 (43.1)	12 (16.7)	10 (13.9)	6 (8.3)	Fisher's = 1.333, df = 3, p = 0.721
	Dental floss/ Interdental brush	13 (18.1)	9 (12.5)	2 (2.8)	1 (1.4)	1 (1.4)	
Dental visit	None	17 (23.6)	10 (13.9)	3 (4.2)	1 (1.4)	3 (4.2)	Fisher's = 4.040, df = 6, p = 0.671
	< 6 months	9 (12.5)	6 (8.3)	1 (1.4)	1 (1.4)	1 (1.4)	
	>= 6 months	46 (63.9)	24 (33.3)	10 (13.9)	9 (12.5)	3 (4.2)	

Fisher's exact test for association was used for all variables.
df; Degrees of Freedom.

5.9 Levels of SIgA (µg/mL) and secretion rate of SIgA (µg/min) in saliva among participants

Of the 72 samples, 4 exceeded the parameters of the assay range and were re-run. The subsequent results fell within the assay range hence all 72 samples were included in the study and this process, recommended by the manufacturer of the assay, ensured that all samples remained within the valid laboratory range to avoid skewing of data unfavorably.

Levels of SIgA (µg/mL) in saliva

All samples had detectable levels of SIgA with the levels ranging from 12.5µg/mL to 526.7µg/mL, with a mean level of SIgA of 176.80(±105.01 SD), a median of 149.00µg/mL and a mode of 58.5µg/mL (Table 12)

An analysis of variance (ANOVA) showed that a significant difference was seen in the mean levels of SIgA between age groups F (2, 69) =5.252, p<0.05 (Table 12). An LSD post hoc test revealed a significant difference for mean levels of SIgA between groups 18-30 years and ≥46 years as well as between groups, 31-45 years and ≥46 years (p<0.05). This indicated that the group ≥46 years had significantly higher levels of SIgA than all other age groups.

Table 12: Socio-demographic variables and Levels of SIgA (µg/mL)

		Levels of SIgA (µg/mL)			95% Confidence Interval of the difference		
Characteristics		n (%)	M	SD	Lower	Upper	Statistical test
Gender	Male	35 (48.6)	182.4	113.3	143.4	221.3	Fisher's=67.1, df = 70, p = 0.493
	Female	37 (51.4)	171.55	97.8	138.9	204.2	
Age (grouped)	18 – 30 years	33 (45.9)	157.85	100.8	122.2	193.6	F = 5.252* df = 2, 69, p = 0.008*
	31 – 45 years	23 (31.9)	154.7	76.3	121.7	187.7	
	≥ 46 years	16 (22.2)	247.7	122.8	182.2	313.1	
Occupation	Self employed	45 (62.5)	182.54	113.4	148.5	216.6	Fischer's=144.5, df =140, p = 0.454
	Employed	17 (23.6)	162	94.8	113.3	210.8	
	Unemployed	10 (13.9)	176.1	86.8	114	238.2	

Fischer's exact test was used for gender and occupation variables, Analysis of Variance (ANOVA) (F) was used for age group variables. ***p<0.001, *p<0.05.

Secretion rate of SIgA (µg/min) into saliva

SIgA is secreted actively into saliva; therefore, the flow rate of saliva will affect the final concentrations of SIgA in saliva in inverse correlation. Since all participants have different saliva flow rates, we must account for this to avoid inaccurate results, hence we calculate the more reliable secretion rate of SIgA in (µg/min) as follows:

Secretion rate of SIgA **(µg/min)** = Saliva flow rate (ml/min) X Level of SIgA (µg/ml)

The secretion rate of SIgA in µg/min ranged from 12.5 – 392.5µg/min with a mean rate of 117.09(+81.14 SD), a median of 93.56 and a mode 12.50µg/min (Table 13)
On evaluating secretion rate of SIgA and the socio-demographic variables (Table 13), no statistically significant differences were found between the rate of secretion of SIgA and any of the socio-demographic variables.

Table 13: Socio-demographic variables and secretion rate of SIgA (µg/min)

		Secretion rate of SIgA (µg/min)			95% Confidence Interval of the difference		
Characteristics		n (%)	M	SD	Lower	Upper	Statistical test
Gender	Male	35 (48.6)	133.36	89.86	102.4	164.2	Fisher's=67.3, df = 71, p = 1.00
	Female	37 (51.4)	101.70	69.68	78.46	124.9	
Age groups	18 – 30 years	33 (45.9)	128.27	98.93	93.19	163.3	F = 0.534 df = 2, 42.3, p = 0.590
	31 – 45 years	23 (31.9)	106.42	64.12	78.69	134.1	
	≥ 46 years	16 (22.2)	109.35	61.13	76.78	141.9	
Occupation	Self employed	45 (62.5)	112.19	82.62	87.37	137.0	Fischer's=146 df =142, p = 1.00
	Employed	17 (23.6)	125.30	87.68	80.22	170.3	
	Unemployed	10 (13.9)	125.17	67.63	76.78	173.5	

Fischer's exact test was used for gender and occupation variables
Analysis of Variance (ANOVA) (F) was used for age group variables.
M; Mean, SD; Standard Deviation, df; degrees of freedom.

5.10 Levels of SIgA and Secretion rate of SIgA in saliva, and their association to gingival inflammation

Levels of SIgA in saliva (µg/mL) and gingival inflammation

An analysis of variance (ANOVA) was performed and elicited a significant difference in the means for gingival index scores and salivary levels of SIgA (µg/mL) (Table 14 below), $F(2, 69) = 4.870^*$, $p<0.05$. This indicated that as gingival inflammation increased levels of uncorrected SIgA in saliva increased.

Table 14: Mean levels of SIgA and gingival inflammation

Characteristics		Level of SIgA in saliva (µg/mL)			95% Confidence Interval of the difference		Statistical test
		n (%)	M	SD	Lower	Upper	
Gingivitis	Mild	38 (52.8)	145.90	87.82	117.03	174.76	F = 4.870*, df = 2, 69, p = 0.011
	Moderate	29 (40.3)	201.29	115.06	157.52	245.06	
	Severe	5 (6.9)	269.70	86.51	162.28	377.12	

Analysis of Variance (ANOVA) (F) was used for gingival index score variable.
M; Mean, SD; Standard Deviation, df; degrees of freedom,
*p<0.05.

An LSD post hoc test elicited statistically significant differences for the levels of SIgA (µg/mL) in saliva between mild gingivitis and moderate gingivitis and between mild gingivitis and severe gingivitis. This shows that the levels of SIgA in saliva were significantly increased once gingival inflammation exceeded that of a mild severity (Table 15).

Table 15: Mean levels of SIgA and increasing severity of gingival inflammation

Characteristics		Mean	95% Confidence Interval		
Gingivitis	Gingivitis	Difference	Lower	Upper	P
Mild	Moderate	-55.39*	-104.44	-6.35	0.027
	Severe	-123.81*	-218.45	-29.18	0.011
Moderate	Mild	55.39*	6.35	104.44	0.027
	Severe	-68.41	-164.74	27.91	0.161
Severe	Mild	123.81*	29.18	218.44	0.011
	Moderate	68.41	-27.91	164.74	0.161

LSD test was used for post hoc comparison of means for all variables. *p<0.05

Secretion rate of SIgA into saliva (µg/min) and gingival inflammation

An Analysis of Variance (ANOVA) showed a no significant difference seen in mean rate of secretion of SIgA in saliva (µg/min) among the gingivitis groups (Table 16). F (2, 69) = 0.986, P = 0.378.

This contrasts with the observations for mean levels of SIgA (µg/mL) and gingival inflammation, as such these results indicate that the secretion rate of SIgA (µg/min) was less affected by the increasing severity of gingival inflammation.

Table 16: Mean secretion rate of SIgA and gingival inflammation

Characteristics		Secretion rate of SIgA (µg/min)			95% Confidence Interval of the difference		Statistical test
		n (%)	M	SD	Lower	Upper	
Gingivitis	Mild	38 (52.8)	112.43	84.68	84.59	140.26	F = 0.986, df = 2, 69, p = 0.378
	Moderate	29 (40.3)	114.76	75.43	86.07	143.45	
	Severe	5 (6.9)	166.10	86.32	58.91	273.27	

Analysis of Variance (ANOVA) (F) was used for all variables.
M; Mean.
SD; Standard Deviation. df; degrees of freedom.

5.11 Levels of SIgA and secretion rate of SIgA into saliva and their association with periodontitis

Levels of SIgA in saliva (µg/ml) and periodontitis

An analysis of variance (ANOVA) was performed for the mean levels of salivary SIgA and periodontitis. A significant difference was elicited for mean levels of SIgA among periodontitis groups F (3, 68) = 7.013, P<0.001 (Table 17 below) which indicates an increase in the levels of SIgA in saliva with an increasing severity of periodontal disease p<0.001.

Table 17: Levels of SIgA and periodontitis groups

Characteristics		Level of SIgA in saliva			95% Confidence Interval of the difference		Statistical test
		n (%)	M	SD	Lower	Upper	
Periodontitis	None	40 (55.6)	153.11	90.08	124.30	181.92	F = 7.013***,
	Mild	14 (19.4)	162.12	87.58	111.55	212.69	df = 3, 68,
	Moderate	11 (15.3)	185.88	87.42	127.15	244.60	p < 0.001
	Severe	7 (9.7)	327.33	132.44	204.84	449.81	

Analysis of Variance (ANOVA) (F) was used for all variables.
M; Mean, SD; Standard Deviation,
df; degrees of freedom,
***p<0.001

To evaluate the specific groups of periodontitis that showed the most significant increases in the levels of SIgA, an LSD post hoc test was performed (Table 18) and elicited statistically significant differences between the group severe periodontitis and the groups ‘none’, ‘mild’ and ‘moderate’ periodontitis severities. This indicates the most significant increase in uncorrected SIgA levels occurred in the group of severe periodontitis.

Table 18: Mean levels of SIgA and periodontitis

Characteristics		Mean Difference	95% Confidence Interval		
Periodontitis	Periodontitis		Lower	Upper	P
None	Mild	-9.01	-67.12	49.09	0.758
	Moderate	-32.77	-96.47	30.93	0.308
	Severe	-174.22***	-250.88	-97.56	<0.001
Mild	None	9.01	-49.09	67.12	0.758
	Moderate	-23.76	-99.19	51.63	0.532
	Severe	-165.21***	-251.82	-78.59	<0.001
Moderate	None	32.77	-30.93	96.47	0.308
	Mild	23.76	-51.63	99.15	0.532
	Severe	-141.45**	-231.91	-50.98	0.003
Severe	None	174.22***	97.56	250.88	0.001
	Mild	165.21***	78.59	251.82	0.001
	Moderate	141.45**	50.98	231.91	0.003

LSD test was used for post hoc comparison of means for all variables.***p<0.001, **p<0.01

Secretion rate of SIgA into saliva (µg/min) and periodontitis

An analysis of variance (ANOVA) test showed that there was no significant difference seen between the mean secretion rate of SIgA (µg/min) in saliva and periodontitis groups, F (3, 21.6) = 1.753, p = 0.186 (Table 19). This indicates that increasing severity of periodontitis did not cause a significant increase in the rate of secretion of SIgA into saliva and this contrasts with the results for the levels of SIgA in saliva (µg/mL). These results indicate that the saliva flow rate has a greater effect on the levels of SIgA (µg/mL) than was expected.

Table 19: Mean secretion rate of SIgA and periodontitis

		Secretion rate of SIgA(µg/min)			95% Confidence Interval of the difference		
Characteristics		n (%)	M	SD	Lower	Upper	Statistical test
Periodontitis	None	40 (55.6)	104.11	74.30	80.351	127.877	F = 1.753,
	Mild	14 (19.4)	130.85	105.54	69.911	191.790	df = 3, 21.6
	Moderate	11 (15.3)	126.60	89.59	66.410	186.793	p = 0.186
	Severe	7 (9.7)	148.79	40.00	111.803	185.793	

Welch's Analysis of Variance (ANOVA) (F) was used for periodontitis variables, M = Mean

5.12 Levels of SIgA compared to the Secretion rate of SIgA among participants

The levels of SIgA and secretion rates of SIgA show contrasting results when evaluated against the study variables. The primary difference between the two measurements is the consideration of saliva flow rate. To more clearly depict and explain these differences, bar graphs have been plotted below (Figure 12 and 13)

From figure 12 below, we can see the levels of SIgA are evidently increased as both gingival inflammation and periodontitis severity increase, these findings are significant, $p<0.05$ and $p<0.001$ respectively. This measurement is uncorrected does not account for the differing saliva flow rates in the study sample.

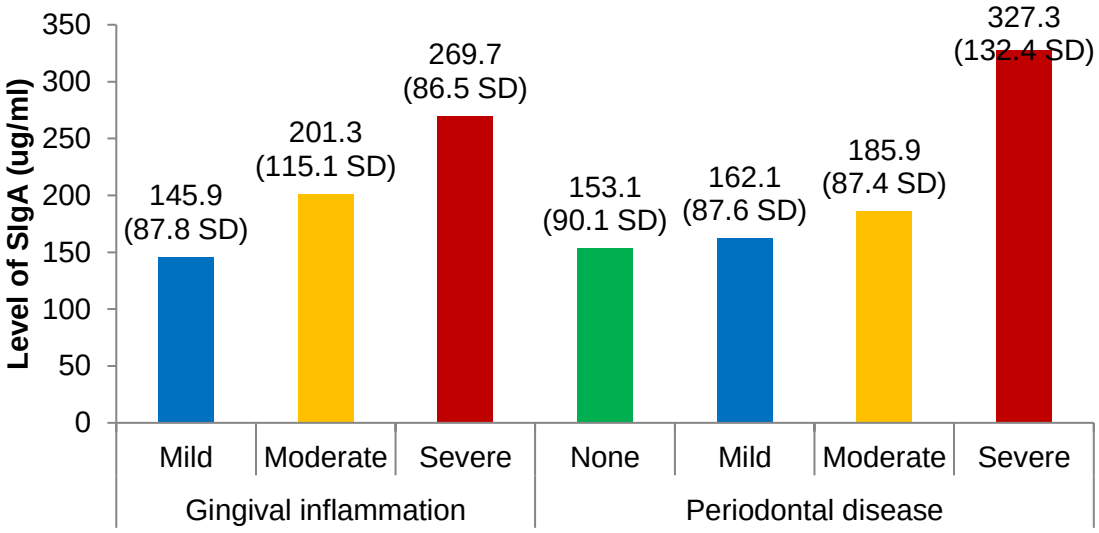

Figure 12: Levels of SIgA and gingival inflammation and periodontitis severity

In contrast the results found when evaluating the relationship between the secretion rate of SIgA and the severities of both gingival inflammation and periodontitis (Figure 13 below), indicate that there was no significant effect on the secretion rates of SIgA as gingival inflammation and periodontitis increased in severity. This result does account for the differing saliva flow rates in the study sample.

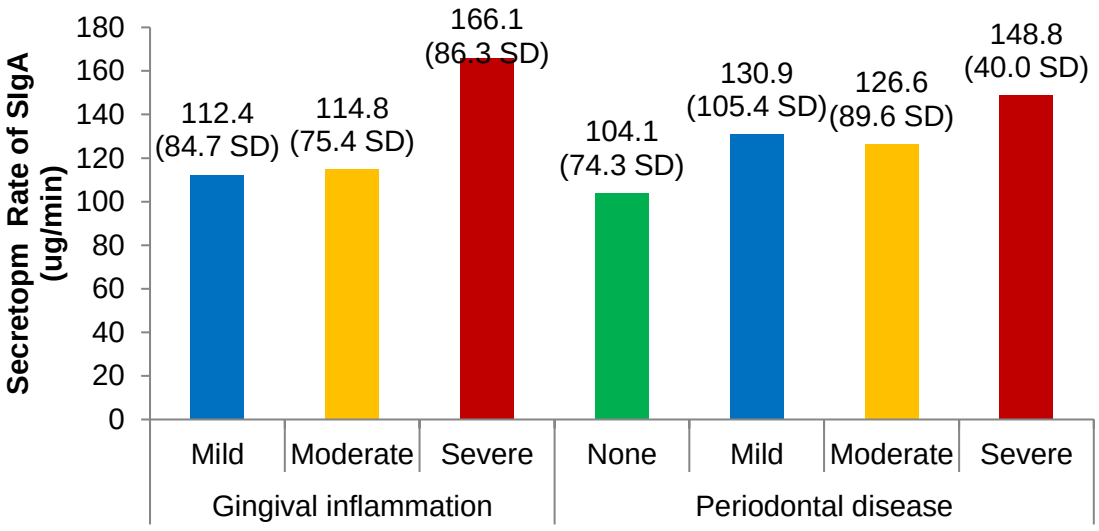

Figure 13: Secretion rate of SIgA and gingival inflammation and periodontitis severity

Therefore, these results indicate that the greatest effect on the final quantity of SIgA in saliva is not the increasing severity of gingival inflammation or periodontitis. From these results we can infer that in this study the greatest effect on salivary SIgA levels was caused by the differing saliva flow rate within the study sample.

CHAPTER SIX: DISCUSSION

This study was designed to assess the relationship between secretory immunoglobulin A in saliva and the periodontal health status. The null hypothesis stated that there would be no discernible relationship between SIgA levels in saliva and the periodontal health status with the alternative hypothesis stating a measurable difference would exist.

This study was a descriptive cross sectional study with hospital based study groups, utilizing convenience sampling to obtain a total of 77 screened and consenting participants, with 5 samples being used for a preliminary pilot phase and 72 for the study sample size determined using the Power's analysis outlined by Cohen in 1988[55].

The samples were evaluated for the levels of SIgA in µg/mL, using a saliva specific enzyme immunoassay (EIA) kit by Salimetrics LLC USA. It is known from previous studies that a key limitation in SIgA quantification is the fact that SIgA is actively secreted into saliva. Therefore, the saliva flow rate will affect the final levels of SIgA in saliva.

To account for this limitation in this study we determined the saliva flow rate (mL/min) and the levels of SIgA (µg/mL) from which we then calculated the Secretion rate of SIgA (µg/min). This allowed us to account for the differing saliva flow rates among the study sample participants. It has been frequently noted by the author that in previous studies[41 40 56 57], the differing saliva flow rates among participants are not being accounted for and failure to do so could potentially skew the results.

The use of a saliva specific EIA in this study provides greater accuracy, reliability and specificity which reduce cross reactions with other salivary analytes in comparison to other measurement methods.

6.1 Sociodemographic characteristics

Of the total participants 35(48.6%) were male and 37(51.4%) were female, with an age range of 19 to 70 years and a mean of 35.6(±12.9SD) years. This indicated that there was a relatively normal distribution between males and females seeking treatment at the study location, and the age range of seeking treatment was diverse. This is likely due to the nature of the study area at the university hospital being both a referral centre and located in an urban setting.

Out of the 72 participants, 45(62.5%) were self-employed, 17(23.6%) employed and 10(13.9%) were unemployed. It was also noted that 40(55.6%) had a higher education which constituted of some form of formal training such as college or university education. The high numbers of formal education are likely due to the metropolitan location of the university hospital and the proximity to university institutions.

The mean age was 35.58(±SD 12.9), the most populous age group was 18 – 30 years, and an overall range of 19 to 70 years, with a near equivalent distribution between males and females in all age groups indicating that health seeking behaviour between males and females in this study was similar. This may be possibly related to the overall higher number of participants in this study that had a formal education.

6.2 Oral hygiene practices and status among participants

All participants in this study performed tooth brushing, 47.2% once daily and 52.8% twice daily whilst interdental cleaning was performed by 18.1%. The results are in line with the findings from the Kenya national oral health survey 2015, which showed 36.9% brushed once daily, 43% twice daily and 9.3% brushing more than twice daily[9]. These results indicate that a large portion of the population require oral health education to express the benefits of more frequent mechanical plaque control.

Mechanical tooth brushing has been shown to be the most effective method at plaque control[58], it is notable that participants who brushed once daily had a plaque score of

1.02(±0.57 SD) while those who brushed twice daily scored a lower 0.87(±0.54 SD), though these results were not statistically significant in this study (p = 0.233)
All participants who performed interdental cleaning also brushed twice daily; these individuals had a mean plaque score of 0.80(±0.51 SD) in comparison to those who did not perform interdental cleaning who scored 0.97(±0.56).

From these findings in this study, we noted that mechanical tooth brushing twice daily reduced plaque scores by 14.7% and interdental cleaning further reduced these scores by another 8% with a cumulative decrease in plaque scores of 22.7%. These results reiterate the importance of mechanical plaque control and the importance of oral health education in the population[59 60].

There was also a notable decrease in plaque scores in accordance with increasing level of education, from 1.16(±0.74 SD) for those who achieved a primary school education and 0.85(±0.47 SD) for those with a higher education, this indicates a relationship between education and oral hygiene practices though not statistically significant in this study, p = 0.231. It has been shown that exposure to higher levels of education or to specific oral health education from younger ages has a benefit to the overall oral hygiene status of a population[61].
Also noted was the significant increase in plaque scores with an increase in age specifically with the age group ≥ 46 years having higher plaque scores overall (p< 0.001). Reasons for this may be due to reduced manual dexterity to perform adequate oral hygiene measures, as well as the lack of oral health education and access to oral health care which can significantly impact members of all age groups.

A study done in Poland showed that simple oral hygiene instructions and single dentist visits can mitigate these findings, regardless of age groups or level of education and these oral health instructions are capable of effectively reducing the quantity of dental plaque and the frequency of dental plaque related diseases in both adolescents and adults[61].

6.3 Saliva Flow rate

The saliva flow rate (rate of saliva secretion) was measured in this study as it has been shown that numerous analytes in saliva including SIgA are secreted actively into saliva. The rate at which saliva is secreted will have an impact on the concentration of the target analytes in saliva. Generally, the rule of thumb is that the slower the saliva flow rate, the greater the time available for active movement of SIgA into saliva hence the greater the final concentration (i.e., inverse correlation).

To account for the differing saliva flow rates between the study participants that are caused by genetic, environmental, and age-related factors among others, we determined the saliva flow rate for each participant using the formula.

$$\underline{\textbf{Saliva flow rate}} = \mathbf{x} = \frac{\textbf{Volume (mL)}}{\textbf{Time (min)}}$$

The saliva flow rate ranged from 0.18 mL/min – 1.0 mL/min with a mean of 0.72(±0.38 SD). It was previously found in a study by Mbabali et al[22] in a Kenyan population that the mean mass of saliva secreted per minute was 0.66(±0.31 SD)g/min. When converted to volume via $\underline{p = m \div V}$ where p = density of saliva at 1.007g/mL and m = mass at 0.66g/mL, the volume is found to be 0.67(±0.312 SD) mL/min. The comparison between the two studies found that the mean values, minimums, and maximums were within comparable ranges with small differences likely attributed to age and diurnal variations of the study sample population.

This study also found that the age group ≥46 years showed lower saliva flow rates than other age groups, ($p < 0.05$), which is of importance as both gingival and periodontal disease tends to be more frequent in older age groups. These results are expected as it is known that saliva secretion tends to be reduced as age increases[62] and this decrease is multifactorial in nature.

6.4 Gingival inflammation (Gingivitis)

All participants showed some degree of gingival inflammation with a score ranging from 0.1 to 2.7 and a mean of 1.0($\underline{\pm}$0.57 SD). Whilst the majority had mild gingivitis 38(52.8%), the remainder, 29(40.3%) had moderate gingivitis and 5(6.9%) had severe gingivitis. The cumulative percentiles are in line with what is expected in the population

as was found in the national oral health survey 2015, where a 98.1% prevalence of gingival inflammation was found with no particular gender bias[9].

An increase in age also showed a significant increase in gingival inflammation, this can likely be attributed to the reduced dexterity and hence reduced plaque control capability, and an overall greater period of exposure to risk and etiological factors.

It was also found that an increase in the dental plaque scores showed a strong positive association with increasing severity of gingival inflammation and this association was statistically significant, ($p < 0.001$). This is expected as there is a known strong correlation between plaque scores and severity of gingival inflammation that was elucidated very well in the classical paper by Loe et al, 'Experimental gingivitis in man'[16].

6.5 Periodontitis

Of the 72 participants, 40(55.6%) had no periodontitis whilst the remainder had varying severities of periodontitis. A relatively high number of participants had mild and moderate periodontitis 14(19.4%) and 11(15.3%) respectively. This is likely since the participants for the study were enrolled at the university dental hospital and were likely seeking treatment at the hospital. Comparing to prevalence studies done previously in Kenya which showed an affected population range of 1%-10% for chronic periodontitis [63], this study had a higher frequency of occurrence of periodontitis which is likely due to the location the study was carried out at and improved health seeking behaviour in the metropolitan environment.

It was noted that gender, level of education or occupation did not show any statistically significant association with periodontitis in this study. On the other hand, there was strong positive association between increasing age and periodontitis ($p<0.001$). Similar associations have been found previously, in the 26 year long longitudinal study by Mayfield et al[64] where the participants showed increased loss of attachment and probing depths with an increase in age. The likely causes of this are the increased period of exposure to risk factors, increased frequency of Para functional conditions,

reduced salivary flow rates and reduction in dental plaque control due to reduced manual dexterity.

Oral hygiene practices performed did not show any significant associations with periodontitis severity in this study. It is worth noting that participants who had never visited the dentist or those whose last dental appointment was > 6 months prior had greater frequency of mild and moderate periodontitis than other participants.

6.6 Salivary levels of SIgA and the Secretion rate of SIgA

Salivary IgA constitutes a major immune response factor secreted by plasma cells in the vicinity of salivary gland acini, which undergoes modification through the epithelium as it is secreted to form salivary SIgA. It is actively secreted into saliva with no regard to diffusive or osmotic potential.

To account for the differing saliva flow rates among the study sample, once the levels of SIgA were determined the following formula was used to determine the secretion rate of SIgA:

Secretion rate of SIgA (µg/min) = Saliva flow rate (mL/min) X Levels of SIgA (µg/ml)

The levels of SIgA ranged from 12.5µg/mL to 526.7µg/mL with a mean level of SIgA of 176.80(±105.01 SD), whilst the secretion rate of SIgA ranged from 12.55µg/ml/min – 392.5µg/ml/min with a mean rate of 117.09(+81.14 SD).

It was found in this study, there was a significant association seen between age and the levels of SIgA, ($p < 0.05$), but once we accounted for the differing saliva flow rates among the study sample and determined the secretion rate of SIgA there was no significant association noted between the secretion rate of SIgA and age, ($p = 0.590$). These results indicate that the saliva flow rate had a large impact on the levels of SIgA in saliva and this effect outweighed that of the participants' age.

A similar finding was noted for gingival inflammation where a strong positive significant association between the levels of SIgA and gingivitis was observed ($p < 0.05$). Once

we accounted for the saliva flow rate, there was no significant association between secretion rate of SIgA and gingival inflammation ($p = 0.378$). These results indicated in this study; the saliva flow rate appeared to have a greater impact on the levels of SIgA than gingival inflammation severity.

In the case of periodontitis, it was found in this study that, an increase in periodontitis severity resulted in a significant increase in the levels of SIgA in saliva, ($p<0.001$). Here it was found that the most significant difference occurred in the group of severe periodontitis, where most likely, the long-standing chronic inflammation resulted in greater recruitment of plasma cells into salivary gland vicinity.
Nonetheless when the secretion rate of SIgA was calculated to account for saliva flow rate, there was no significant association seen ($p= 0.186$) between secretion rate of SIgA and periodontitis severity. Though the secretion rate of SIgA was increased for the group of severe periodontitis this was not significant.

Once again, these results indicate that the saliva flow rate had a major impact on the levels of SIgA and this effect was greater than what was observed for periodontitis and its increasing severities.

The results in this study are clear that the levels of SIgA are increased in saliva with increasing severities of gingival inflammation and periodontitis, but this significance only exists when the flow rate of saliva is not considered. When the saliva flow rate is considered, the secretion rate of SIgA into saliva shows no significant differences with increasing severity of gingival inflammation and periodontitis. Hence saliva flow rates have a greater impact on the final concentrations of SIgA than the other variables examined in this study.

We therefore accept the null hypothesis and reject alternative hypothesis and conclude from this study that increasing severity of periodontitis does not impact the final levels of SIgA in saliva.

Previous studies had found significant increases in SIgA with an increase in gingival inflammation and periodontal disease, though these studies used less specific and sensitive methods of quantification[40 41 56] and more importantly the majority did not

account for the differing salivary flow rates in their study sample which may potentially affect the results[20 41].

6.7 Diagnostic utility of SIgA

This study was designed to consider the diagnostic potential of SIgA as a biomarker for periodontal disease and to evaluate changes it undergoes during the etiopathogenesis of periodontitis. The assay utilized was designed for salivary use specifically. The assay kits used were of the EIA (Enzyme immunoassay kits) variety, and this is of significance as these systems can be automated which would be vital to reduction in cost of operation and utilization. These considerations were made to allow potential increase in future scope of the study.

In this study we found that SIgA may have some limitations as a diagnostic surrogate endpoint for periodontitis, due to the increased complexity of requiring the saliva flow rate to be measured and secretion rate of SIgA to be calculated. But due to the ease and non-invasive nature of collection, along with excellent specificity of the detection method for SIgA it should be evaluated further along with other salivary analytes to reach a robust consensus.

When evaluating previous literature on SIgA, some studies concluded that there was an increase in the levels of SIgA as gingival inflammation and periodontitis severity increased, many of these studies did not account for differing saliva flow rates among study participants [40 56 65].

6.8 Study Limitations

Since this study design was cross sectional in nature it is not possible to evaluate over time the true relationship between salivary SIgA and periodontitis severity. It was not possible within the scope of this study to also evaluate the quantities of plasma cells in the salivary gland vicinity or the quantities of polyimmunoglobulin receptors which play a role in the synthesis of SIgA as these require separate quantification methods to evaluate. Another study limitation involves the study sample population, since the study was carried out at the university dental hospital the participants enrolled into the

study were those seeking dental treatment at the hospital. This may present a challenge in making inferences to the general population at large.

The measurement methods used such as Ramjford's teeth are useful and efficient in screening and represent the dentition well, but they are not an infallible measurement and cannot give a complete picture of the periodontium on their own.

The study being biochemical in nature required numerous materials, some of which such as the assay kits, were not available locally and were required to be imported and be subjected to import process, costs, regulations, and licencing. As such this created a significant financial burden, which was solely burdened by the principal investigator. Another limitation was the overall time available to perform this study which was dictated by the constraints of the postgraduate curriculum and additionally made difficult due to the SARs Cov – 2 (Covid – 19) pandemic affecting the globe.

6.9 Conclusion

This study within its limitations was able to assess the periodontal health status of participants utilizing gingival inflammation measures and clinical attachment loss. We were also able to successfully quantify the levels of SIgA in saliva, determine the salivary flow rate and calculate the secretion rate of SIgA thereby successfully accounting for the varying rates of saliva production and secretion between different members of the study participants.

We found in this study that there was no significant association between secretion rate of SIgA into saliva and gingival inflammation or periodontitis severity. Therefore, this led us to conclude that in this study the saliva flow rate had a greater effect on the final levels of SIgA than gingival inflammation or periodontitis.

The use of salivary SIgA as a rapid diagnostic and screening utility should be carefully considered, as the salivary flow rate has a major impact on SIgA concentration in saliva, this likely increases the complexity of the screening and diagnostic process. Furthermore, since the salivary flow rate affects SIgA levels significantly, it may not be

plausible to consider SIgA as a true surrogate endpoint of disease and hence its reliability as a biomarker for periodontitis may be questioned.

6.10 Recommendations

In this study it was noted that the use of SIgA as a biomarker could be challenging due to the need to account for saliva flow rate. Further studies should be carried out to evaluate the precise relationship and effects of saliva flow rate on SIgA. Furthermore, a longitudinal type of study as well as a controlled studies should be carried out to further evaluate the relationship between SIgA and periodontitis.

6.11 Conflicts of interest

There were no conflicts of interest associated with this study. All costs of the study including the time utilized were burdened by the principal investigator for both scientific and academic purposes.

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APPENDICES

Levels of secretory immunoglobulin A in saliva and the periodontal health status in adult patients attending the university of Nairobi dental hospital

Appendix 1: Screening form

Screening check list (TICK where appropriate)

☐ Person below the age of 18

☐ Undergone periodontal therapy over the past 12 months

☐ Invasive dental procedure over the past 6 months for example extraction of teeth

☐ Any surgical procedure over the past 6 months

☐ Undergone an antibiotic therapy within the past 6 months

☐ Undergoing long term drug therapy such as contraceptives or corticosteroids

☐ Chronic gastrointestinal disorders for example irritable bowel syndrome

☐ Pregnant or lactating

☐ Auto immune disorder or metabolic disorders for example diabetes mellitus

☐ Any condition affecting the salivary glands for example neoplasms

☐ Edentulous or has less than 20 teeth

☐ Hypertensive or suffering from cardiovascular disease that requires long term management for example with β adrenergic antagonists

☐ Cigarette smoker

Levels of secretory immunoglobulin A in saliva and the periodontal health status in adult patients attending the university of Nairobi dental hospital

Appendix 2: Informed consent form`

CONSENT FORM (STATEMENT OF CONSENT - ENGLISH)

Participant's statement

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

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

I agree to participate in this research study: (Circle where valid)	**Yes**	**No**
I agree to have my saliva sample preserved for later study:	Yes	No
I agree to provide contact information for follow-up:	Yes	No

Participant printed name: _____

Participant signature / Thumb stamp_____ **Date**

_____

Researcher's statement:

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

Researcher 's Name:_____ **Date**:

_____

Signature _____

Role in the study:_____

For more information you may contact the following persons.

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uonknh_erc@uonbi.ac.ke

Levels of secretory immunoglobulin A in saliva and the periodontal health status in adult patients attending the university of Nairobi dental hospital

Appendix 2: Informed consent form

FOMU YA RIDHAA (SWAHILI)

Kauli ya mshiriki

Nimeisoma fomu hii ya ridhaa ama nimesomewa ujumbe. Nilipata fursa ya kujadiliana kuhusu utafiti huu na mtafiti. Maswali yangu yamejibiwa kwa lugha ambayo naielewa. Nimeelezewa manufaa na hatari ziliwepo.

Naelewa kuwa ushiriki wangu kwa utafiti huu ni wa hiari na naweza kujiondoawa wakati wowote.Nimekubali kwa hiari kushiriki katika utafiti huu.

Naelewa juhudi zitafanywa ili kuuhifadhi habari yangu wa kibinafsi.

Kwa kutia sahihi fomu hii ya ridhaa, sijaiacha haki zangu kisheria kama mshiriki katika utafiti.

Nimekubali kushiriki katika utafiti huu:	**Ndio**	**La**
(Jibu ambapo inahitajika)		
Nimekubaliana mate yangu inaweza kuhifadhiwa kwa ajili ya utafiti wa baadaye,	☐	☐
Nimekubali kupeana nambari za simu ili nifuatiliwe:	☐	☐

Jina la mshiriki lililochapishwa: _____

Sahihi ya mshiriki / alama ya kidole _____**Tarehe** _____

Kauli ya mtafiti

Mimi, ambaye ni mtafiti mkuu, wameeleza kikamilifu utafiti huu wa utafiti huu kwa mshiriki ambaye ametajwa hapo juuna naamini ya kwamba mshiriki ameelewa na akatoa ridhaa yake kwa hiari.

Jina la mtafiti: Dr Asif Jabir Mohamedali **Tarehe:** _____

Sahihi _____

Kazi yake katika utafiti: Mkuu wa uchunguzi

Kazi yake katika utafiti: Mkuu wa uchunguzi

Kwa habari zaidi zungumza na

Mkuu wa uchunguzi
Daktari Asif Jabir Mohamedali
Shule ya kisayansi ya meno, Chuo Kikuu Cha Nairobi,
Nambari ya simu ni ; 0788110521

Msimamizi mkuu
Daktari Hudson Alumera
University of Nairobi Department of Periodontology/Community and Preventive Dentistry, School of Dental Sciences

Katibu/ Mwenyekiti ,
Hospitali ya Kitaifa ya Kenyatta-Kamati ya maadili na utafiti Chuo Kikuu Cha Nairobi,
Nambari ya simu. (254-020) 2726300-9
Barua pepel: uonknh_erc@uonbi.ac.ke.

Levels of Secretory immunoglobulin A and the periodontal health status in adult patients attending the university of Nairobi dental hospital

Appendix 3: Questionnaire and bio data form

Date……. Saliva sample serial code……..

Age (Years) …….. File number……..

Gender: Male ☐ Female ☐

Residence: Within Nairobi ☐, Outside Nairobi ☐

Occupation: Self ☐, Employed ☐, Unemployed ☐

Level of education, Primary ☐, Secondary ☐, Higher education ☐

Tooth brushing: Zero daily ☐, Once daily ☐, Twice daily ☐, Thrice daily ☐, Use of a dentifrice Yes ☐, No ☐, if yes, Herbal ☐ Conventional ☐ Other……

Interdental cleaning: None ☐, Dental floss ☐, Interdental brush ☐,
Other……
If yes to interdental cleaning, frequency…….

Previous dental visit: None ☐ Less than 6 months ago ☐
More than 6 months ago ☐ If yes, what procedure was carried out…….

<u>Modifying factors</u>

Alcohol consumption: None ☐, Social drinker ☐, Regular drinker ☐,

Medically compromised status (such as allergies, asthma among others), if yes specify; ……

Appendix 4: Oral hygiene assessment

Plaque index by Silness and Loe 1964

Score	Criteria
0	Gingival area free of plaque
1	Plaque cannot be observed by the naked eye in situ but can be visualized on the point of a probe
2	Plaque is visible to the naked eye as a thin to moderately thick layer
3	Heavy accumulation of plaque and or soft matter at the gingival margin and tooth surface

Appendix 5: Gingival health assessment

Gingival index by Loe and Silness 1963

Score	Criteria
0	Absence of inflammation
1	Mild inflammation; mildly red margin and oedema but no bleeding on probing.
2	Moderate inflammation: reddened margin with oedema and bleeding on probing is present.
3	Severe inflammation: more intense redness with oedema and occasional ulcerations, the margins tend to bleed spontaneously with very little provocation.

Gingivitis severity based on gingival index: Loe and Silness 1963

0	No gingivitis
0.1 to 1	Mild gingivitis
1.1 to 2	Moderate gingivitis
2.1 to 3	Severe gingivitis

Appendix 6: CDC-AAP (2012) case definitions of periodontitis

Case	Definition
No periodontitis	No evidence of mild, moderate or severe periodontitis.
Mild periodontitis	≥2 interproximal sites with attachment loss ≥3mm and probing depths of ≥4mm (not on the same tooth) OR one site with probing depth of ≥5mm.
Moderate periodontitis	≥2 interproximal sites with clinical attachment loss of ≥4mm (not on the same tooth) OR ≥2 interproximal sites with probing depths of ≥5mm (not on the same tooth).
Severe periodontitis	≥2 interproximal sites with clinical attachment loss ≥6mm (not on the same tooth) and ≥1 interproximal site with probing depth ≥5mm.

Levels of Secretory immunoglobulin A and the periodontal health status in adult patients attending the university of Nairobi dental hospital

Appendix 7: Clinical examination form

Secretory immunoglobulin A

Participant saliva sample code	Date of collection

Time of collection (24hr)	Time taken to collect sample (min)	Volume of saliva collected (mL)	SIgA in µg/ml

Plaque score: Silness and Loe 1964

Tooth											
Surface	F	L	F	L	F	L	F	L	F	L	F	L
Score											

Total…….. Mean……..

Gingival index: Loe and Silness 1963

Tooth											
Surface	F	L	F	L	F	L	F	L	F	L	F	L
Score											

Total…….. Mean…….. Inference……

Levels of Secretory immunoglobulin A and the periodontal health status in

adult patients attending the university of Nairobi dental hospital

Periodontal probing chart

Maxillary arch

Tooth	**17**			**16**			**15**			**14**			**13**			**12**			**11**			**21**			**22**			**23**			**24**			**25**			**26**			**27**		
Palatal																																										
Recession (mm)																																										
CAL (mm)																																									
Facial																																									
Recession (mm)																																									
CAL (mm)																																									
Tooth Mobility																																									

CAL* Clinical attachment loss, *Third molars excluded

Mandibular arch

Tooth	**47**			**46**			**45**			**44**			**43**			**42**			**41**			**31**			**32**			**33**			**34**			**35**			**36**			**37**		
Lingual																																										
Recession (mm)																																										
CAL (mm)																																									
Facial																																									
Recession (mm)																																									
CAL (mm)																																									
Tooth Mobility																																									

CAL* Clinical attachment loss, *Third molars excluded

Severity based on CDC/APP case definitions

☐ No periodontitis, ☐ Mild periodontitis, ☐ Moderate periodontitis,

☐ Severe periodontitis.

Levels of Secretory immunoglobulin A and the periodontal health status in adult patients attending the university of Nairobi dental hospital

Appendix 8: Laboratory analysis form

Participant saliva sample code …….. Date of collection…….

Time of collection …….

Date and time sample received ……...

Time centrifuged and supernatant stored …….

Analysis by indirect ELISA.

Salivary Secretory immunoglobulin A level: ……**µg/ml**

Laboratory technologist: **Signed** ……

Appendix 9: Study images and paraphernalia used

	1	2	3	4	5	6	7	8	9	10	11	12
A	Boosted	Boosted	Ctrl – H	Ctrl- L	1	1	2	2	3	3	4	4
B	Q1	Q1	5	5	6	6	7	7	8	8	9	9
C	Q2	Q2	10	10	11	11	12	12	13	13	14	14
D	Q3	Q3	15	15	16	16	17	17	18	18	19	19
E	Q4	Q4	20	20	21	21	22	23	23	23	24	24
F	Q5	Q5	25	25	26	26	27	27	28	28	29	29
G	Zero	Zero	30	30	31	31	32	32	33	33	34	34
H	NSB	NSB	35	35	36	36						

Legends: 'Q': Substrates/Standards
'Zero': Zero wells
'NSB': Non-specific binding wells
'Ctrl- H': Control – High
'Ctrl-L': Control – Low
Numbers: Samples in duplicate.

Image 1: Plate worksheet used to maintain continuity in the laboratory with proposed plate design (Image of first plate sheet)

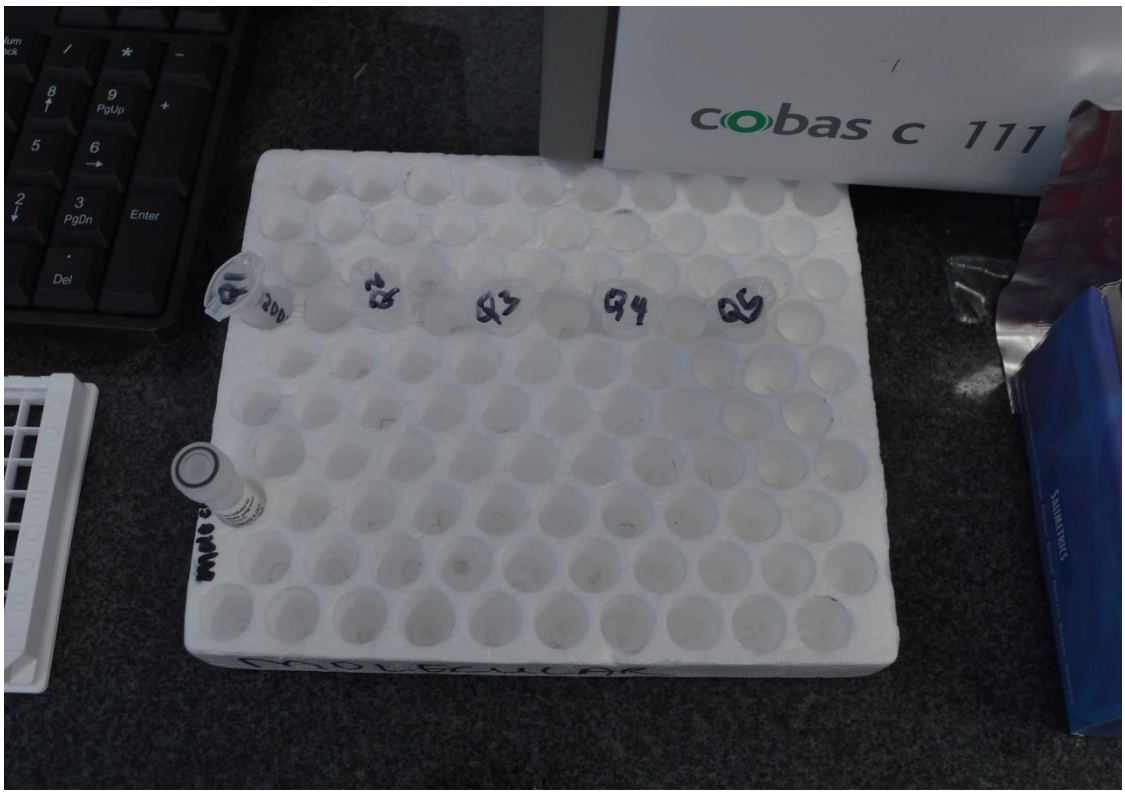
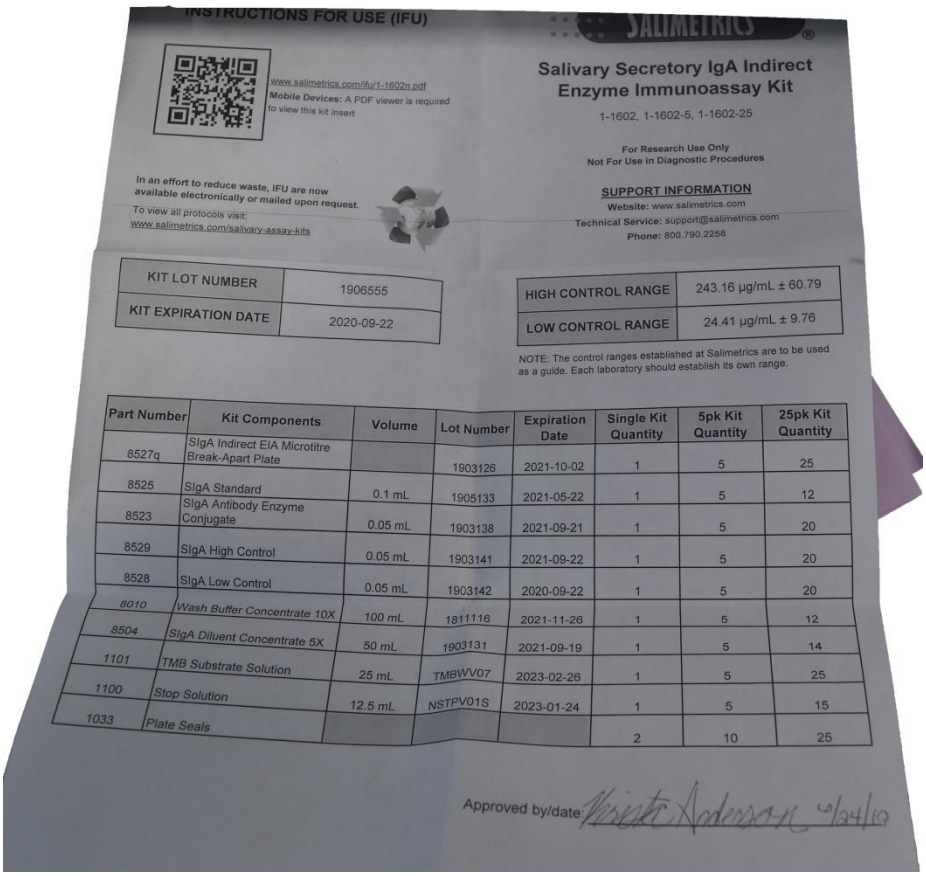


Image 2: Preparations of standards in laboratory capped tubes (initial dilution)

Image 3: Final dilutions of standard's during pilot and the pilot sample dilutions (1 to 5) number 5 is off screen



INSTRUCTIONS FOR USE (IFU)

SALIMETRICS

www.salimetrics.com/ifu/1-1602n.pdf
Mobile Devices: A PDF viewer is required to view this kit insert

Salivary Secretory IgA Indirect Enzyme Immunoassay Kit

1-1602, 1-1602-5, 1-1602-25

For Research Use Only
Not For Use in Diagnostic Procedures

In an effort to reduce waste, IFU are now available electronically or mailed upon request.
To view all protocols visit:
www.salimetrics.com/salivary-assay-kits

SUPPORT INFORMATION
Website: www.salimetrics.com
Technical Service: support@salimetrics.com
Phone: 800.790.2258

KIT LOT NUMBER	1906555
KIT EXPIRATION DATE	2020-09-22

HIGH CONTROL RANGE	243.16 µg/mL ± 60.79
LOW CONTROL RANGE	24.41 µg/mL ± 9.76

NOTE: The control ranges established at Salimetrics are to be used as a guide. Each laboratory should establish its own range.

Part Number	Kit Components	Volume	Lot Number	Expiration Date	Single Kit Quantity	5pk Kit Quantity	25pk Kit Quantity
8527q	SIgA Indirect EIA Microtitre Break-Apart Plate		1903126	2021-10-02	1	5	25
8525	SIgA Standard	0.1 mL	1905133	2021-05-22	1	5	12
8523	SIgA Antibody Enzyme Conjugate	0.05 mL	1903138	2021-09-21	1	5	20
8529	SIgA High Control	0.05 mL	1903141	2021-09-22	1	5	20
8528	SIgA Low Control	0.05 mL	1903142	2020-09-22	1	5	20
8010	Wash Buffer Concentrate 10X	100 mL	1811116	2021-11-26	1	5	12
8504	SIgA Diluent Concentrate 5X	50 mL	1903131	2021-09-19	1	5	14
1101	TMB Substrate Solution	25 mL	TMBWV07	2023-02-26	1	5	25
1100	Stop Solution	12.5 mL	NSTPV01S	2023-01-24	1	5	15
1033	Plate Seals				2	10	25

Approved by/date: Kristi Anderson 6/24/19

Image 4: Kit contents and laboratory control ranges, kit validation sheet

Whitney Lundskow 8 Jul 2020, 03:17

to me

Hello Asif,

Yes you may use this diagram. We just ask you please reference the source and it mentions Salimetrics.

Thank you!

Whitney

From: Asif Mohamedali <asif.dr521@gmail.com>
Sent: Tuesday, July 7, 2020 6:13 AM
To: Whitney Lundskow <wlundskow@salimetrics.com>; Asif Mohamedali <asif.dr521@gmail.com>
Subject: Kit insert use

Dear Whitney

I hope you are well and keeping safe.

I would like to request for fair use of information from the kit insert of the SigA kits I purchased from salimetrics.
I would like to use specifically the diagram that depicts the test principle (page 4) and wanted to confirm if I have the right to use this diagram in my study on SigA that utilised your kits.

Thanks for your assistance and I look forward to hearing from you

Have a good day and week ahead

Sincerely

Dr. Asif J Mohamedali

Image 5: Detailing permissions process for using test principal diagram

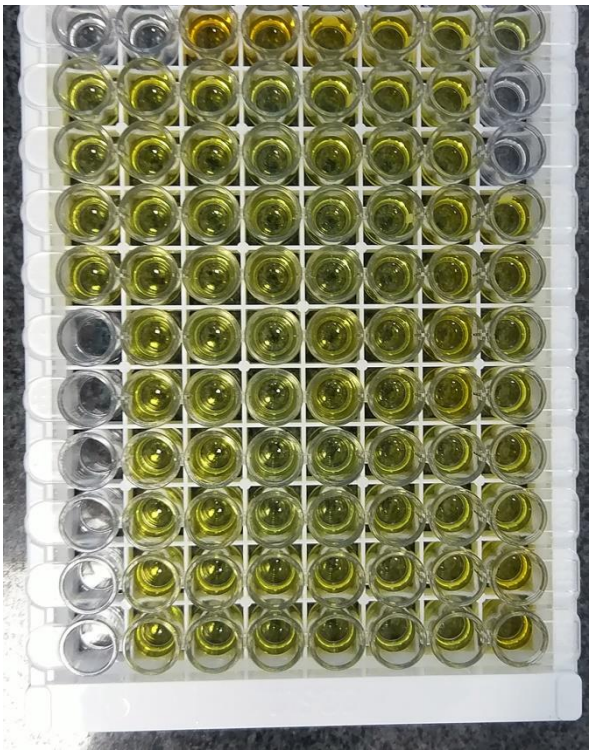
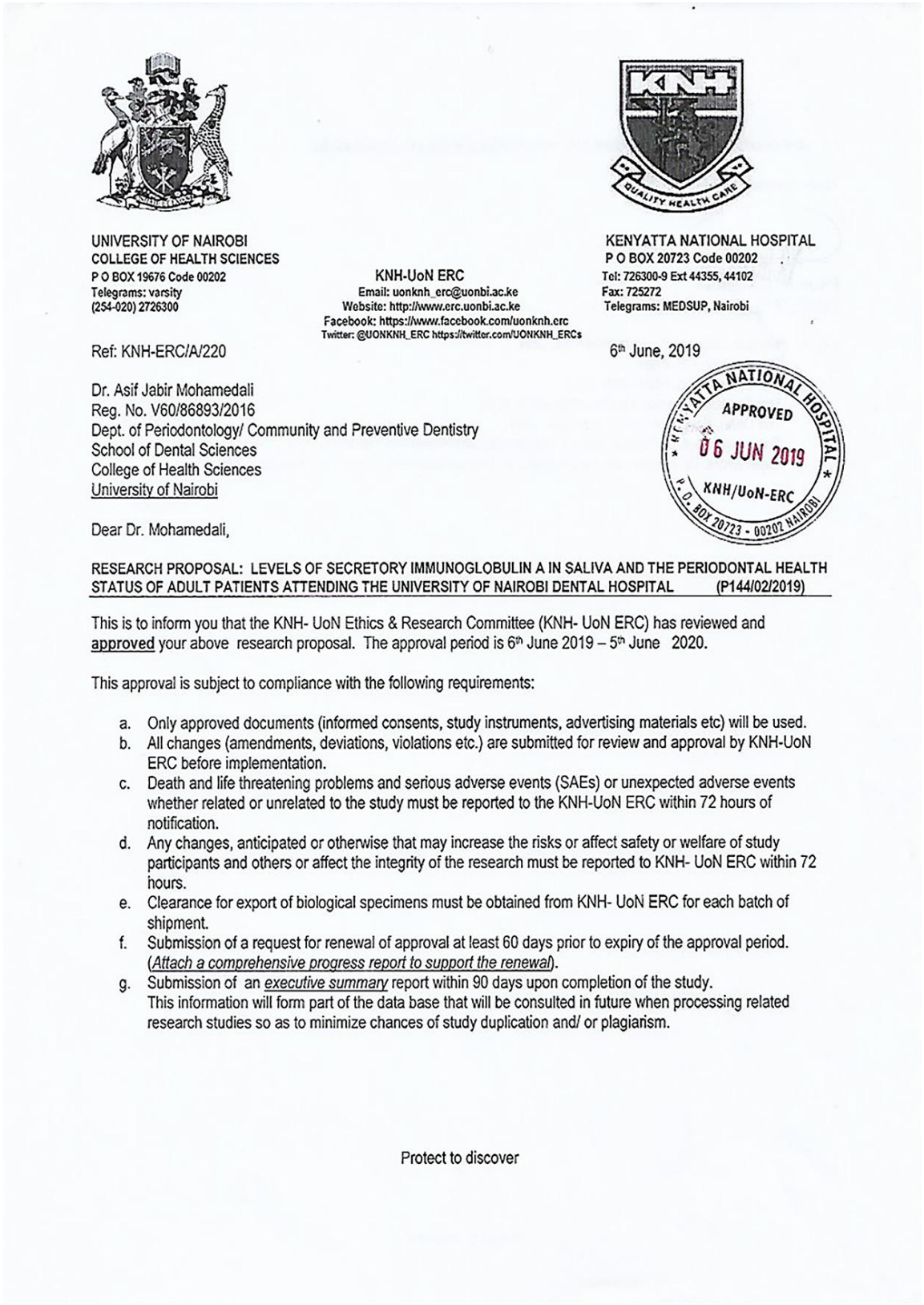


Image 6: Empty wells top left are Zero and NSB, remaining wells with no colour are blanks maintained for re-run flexibility, notice the variation in colour gradients of the Stopped plate, typical of a complete plate reaction. (First plate in series)

Image 7: Infrared thermometer designed for inanimate object temperature monitoring, used to determine temperature suitability of temporary storage box for samples during data collection period.

Appendix 10: Ethics approval document (KNH-UoN-ERC)



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Ref: KNH-ERC/A/220

6th June, 2019

KENYATTA NATIONAL HOSPITAL
APPROVED
06 JUN 2019
KNH/UoN-ERC
P. O. BOX 20723 - 00202 NAIROBI

Dr. Asif Jabir Mohamedali
Reg. No. V60/86893/2016
Dept. of Periodontology/ Community and Preventive Dentistry
School of Dental Sciences
College of Health Sciences
University of Nairobi

Dear Dr. Mohamedali,

RESEARCH PROPOSAL: LEVELS OF SECRETORY IMMUNOGLOBULIN A IN SALIVA AND THE PERIODONTAL HEALTH STATUS OF ADULT PATIENTS ATTENDING THE UNIVERSITY OF NAIROBI DENTAL HOSPITAL (P144/02/2019)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH- UoN ERC) has reviewed and approved your above research proposal. The approval period is 6th June 2019 – 5th June 2020.

This approval is subject to compliance with the following requirements:

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

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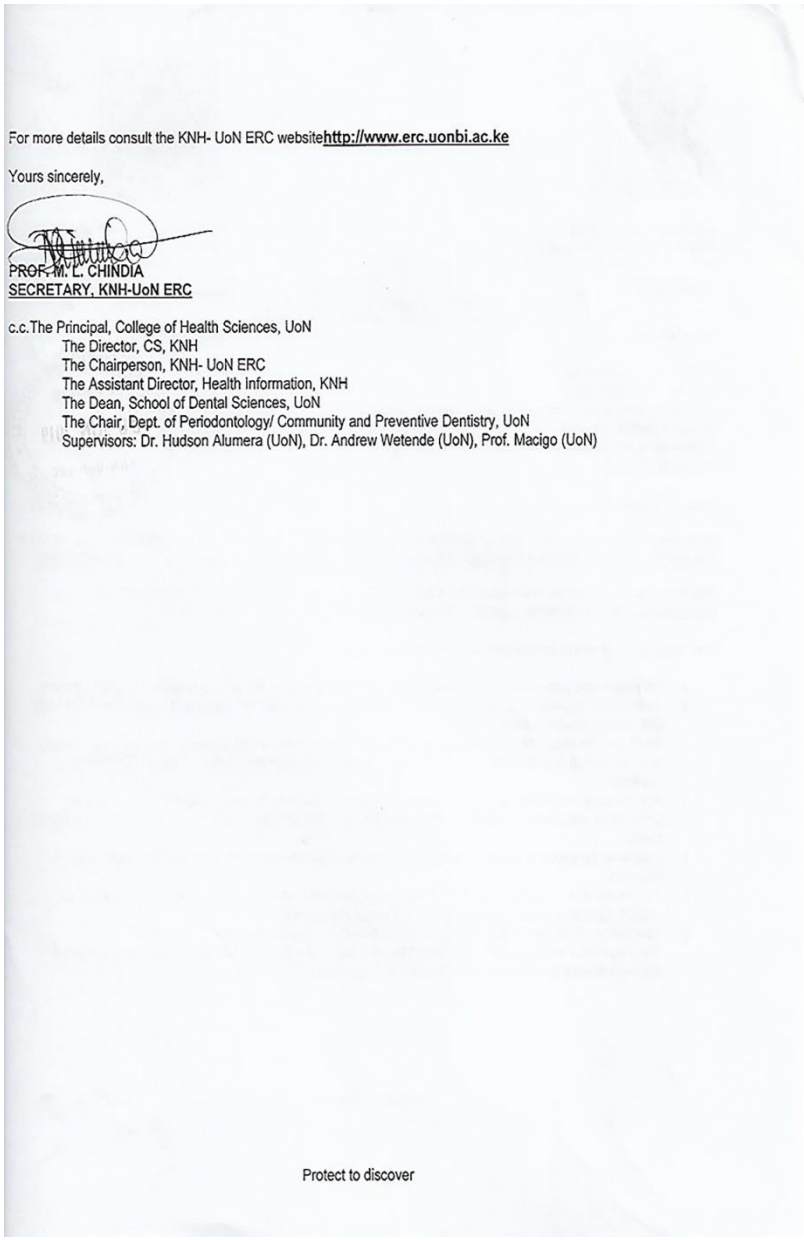
Scan 1: Ethical approval from KH-UoN-ERC for this study



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

Yours sincerely,

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

c.c. The Principal, College of Health Sciences, UoN
The Director, CS, KNH
The Chairperson, KNH- UoN ERC
The Assistant Director, Health Information, KNH
The Dean, School of Dental Sciences, UoN
The Chair, Dept. of Periodontology/ Community and Preventive Dentistry, UoN
Supervisors: Dr. Hudson Alumera (UoN), Dr. Andrew Wetende (UoN), Prof. Macigo (UoN)

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Scan 2: Ethical approval from KH-UoN-ERC for this study

Levels of secretory immunoglobulin A in saliva and the periodontal health status of adult patients attending the University of Nairobi Dental Hospital

by Asif J Mohamedali

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DEPARTMENT OF DENTAL SCIENCES
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Levels of secretory immunoglobulin A in saliva and the periodontal health status of adult patients attending the University of Nairobi Dental Hospital

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