SALIVARY FLOW RATE AND PERIODONTAL STATUS IN AN ADULT KENYAN POPULATION

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DECLARATION

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

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DEDICATION

This work is dedicated to my family, Zubedah, Nawal and Abubakar for their patience and support

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CONTENTS

LIST OF ACRONYMS

AHEA	Associate of Higher Education Academy
ANOVA	Analysis of variance
BDS	Bachelor of Dental Surgery
BPE	Basic periodontal examination
CDE	Certificate in Dental Education
FADI	Fellow of Academy of Dentists International
FICD	Fellow of International College of Dentistry
g	grams
GCAP	Graduate Certificate of Academic Practice.
GI	Gingival index
MClinDent	Master in Clinical Dentistry
Min	Minute
MSc	Master of Science
PFA	Pierre Fauchard Academy
PhD	Doctor of Philosophy
SD	Standard deviation
SFR	Salivary flow rate
SPSS	Statistical package of social scientists
UNDH	University of Nairobi Dental Hospital.
UoN	University of Nairobi
UWSFR	Unstimulated whole saliva flow rate
Yrs	Years

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ABSTRACT

Periodontal diseases are the commonest oral health problems with over 90% of the population suffering from at least one form of this disease. Ng'ang'a's literature review on oral health status in Kenya reported a prevalence of chronic periodontitis (1-10%) and gingivitis (0.2 – 90%). A recent Kenya National Oral Health Survey (KNOHS 2015) found the overall prevalence of gingival bleeding to be 98.1 % in adults. Several studies have linked periodontitis to alteration in saliva composition and flow rate.

Saliva is an important oral fluid with numerous functions that relate to the normal functioning of the body and maintaining the integrity of the oral tissues including the periodontium. Salivary flow rate is the amount of saliva one produces in a minute. This rate varies from population to population depending on age, sex, diet, geographical location and genetics. It may also be altered by chronic systemic diseases, medication and radiation therapy. The aim of this study was to establish the normal salivary flow rate (SFR) in an adult Kenyan population and relate it to periodontal status.

Study objective: To establish the salivary flow rate in a Kenyan adult population and investigate its relationship with periodontal status**.**

Setting: Oral diagnosis and Periodontology clinics at the University of Nairobi Dental Hospital.

Study design: A cross sectional study based at the University of Nairobi Dental Hospital. A total of 333 participants were recruited using systematic random sampling. Saliva was collected using spit method and periodontal evaluation was based on the basic periodontal examination (BPE). Plaque and gingivitis were determined using the Quigley Hein Index - (Modified by Turesky et al, 1970) and Silness and Loe 1964 indices respectively. Bio-data and social demographic information was obtained through a questionnaire.

Data Analysis: The data was coded, entered and analyzed using SPSS 20.0 software. Descriptive statistics, independent T-test and ANOVA, Pearson

correlation and linear regression were used to investigate the relationship between the study variables.

Results: A total of 333 participants were recruited in the study with a male to female ratio of 1:1.3. Age range was 18 to 45 years, with a mean of 32.2 years. The saliva flow rate (g/min) ranged between 0.14 – 1.98g/min in males and 0.08 – 1.68g/min in females. The mean SFR was 0.66 ±0.31g/min SD with a mode of 0.30g/min. Two hundred fifty-three participants were normal secretors within the range of 0.3 and 1.0g/min, forty-three were secretors with over 1.0 g/min while thirty-two were low secretors with a range of 0.1 and 0.29g/min.

Using independent sample t test, the periodontitis group had a statistically significant higher mean salivary flow rate (0.7g/min ± 0.3) than the gingivitis group (0.6g/min± 0.3) p = 0.04.

Conclusion: The average unstimulated salivary flow rate of the study population was 0.66g/min, which falls within the reported normal range. Within the limitations of this study, the data suggests that there may be a possible positive relationship between SFR and inflammatory periodontal destruction.

CHAPTER ONE

Introduction

Periodontal diseases are the commonest oral health problems. Globally, gingival bleeding is the most prevalent sign of this class of diseases, and the presence of deep periodontal pockets of greater than 6 mm have been reported to range from 10% to 15% in adult populations[1]. These variations reported in the prevalence of periodontal disease, have been associated with socio-environmental conditions, behavioral risk factors, general systemic health status of people for example diabetes, hypertension and HIV status and oral health policies of the different countries[1].

In Kenya, over 90% of the population suffers from at least one form of the disease with a reported prevalence of chronic periodontitis at 1-10% while for gingivitis is 0.2 – 90%[2]. A 2015 Kenya National Oral Health Survey found the overall prevalence of gingival bleeding to be 98.1 % in adults[3].

Saliva is an important oral fluid with numerous functions that relate to the normal functioning of the body and especially the oral structures. For instance, saliva keeps the oral tissues moistened protecting them from physical injury. The salivary proteins including the antimicrobial peptides also play an important role as a first line of defense against invading microorganisms. Changes in the quality or quantity of saliva may therefore have deleterious effects on the oral tissues. Hypo salivation describes a situation where an individual is unable to produce enough saliva, while hyper salivation is the opposite. Both hyper salivation and hypo salivation may present with challenges to oral health.

The quantitative state of saliva is determined using the salivary flow rate. Salivary flow rate is the amount of saliva produced by salivary glands in a given period of time, usually expressed in milliliters per minute or grams per minute (ml/min or g/min). Several studies have shown varying values reported as normal salivary flow rates. The variations could be explained partially by geographical, age, sex, race and genetic differences among the different groups studied[4-9]. There is minimal data describing the normal salivary flow

rate among Africans. Knowing that genetic and environmental factors may affect salivary flow rates, it is imperative that such values are established for a native African population. This would help to set up values for determining the diagnosis of salivary flow abnormalities in this population.

The purpose of this study was to establish the normal salivary flow rate in an adult Kenyan population and relate it to periodontal status. This will act as a benchmark for future salivary flow rate references for Kenyan adult population.

Literature review

Saliva is a secretory product of salivary glands, which in turn are under systemic control through the sympathetic and parasympathetic nervous system. As such, saliva can be viewed as a mirror of oral and systemic health status of an individual. It is therefore a valuable source for determining clinically relevant information. Other than its volumetric importance, it contains biomarkers for unique pathological aspects of chronic periodontitis and peri-implant disease. Therefore both quantitative and qualitative changes in saliva could be of diagnostic value in periodontology [10-11].

Low levels of saliva also known as hypo salivation has been shown to lower the oral clearing and buffering functions. As a result, it has been linked to rampant dental caries and chronic periodontitis. Such conditions are evident in patients who are undergoing head and neck radiotherapy. The radiation causes the destruction of the salivary gland's acini and therefore its secretory capacity[12-13].

Knowing the values of normal salivary flow rate (SFR) is very important when treating dental patients. Early diagnosis and treatment of hypo salivation would preserve the health of oral structures and lower the incidence of chronic periodontitis among others [14]. However, this can only be determined objectively if the normal range of SFR is known in the population.

Of the many methods that may be used to evaluate salivary function, collecting unstimulated whole saliva (UWS) is the method most frequently employed due to the ease with which it is conducted[4].

The normal range for SFR in different populations has been reported by different authors (Table 1). However, it is difficult to compare SFR reported by different studies due to variations in study design, differences in patient's gender, age, race, salivary gland size, diet, or bite force[4-8].

The studies in table 1 show a wide variation in the mean salivary flow rate further suggesting the possible contribution of race, age, geographic and other environmental factors in determining the flow rate.

Table 1: Unstimulated saliva flow rate (USF) from values of selected previous studies.

Authors	Country	Number of participants, (male/female)	Age (yrs)	UWSFR ml/min (mean ± SD)	Notes
Yamamoto et al(4)	Japan	200, (100/100)	22 – 29	0.053 ± 0.032	Study done in healthy un medicated participants
Fenoll-Palomares et al(5)	Spain	159, (52/107)	44 ± 14	Range: 0.10-2.0, median: 0.48	Study done in healthy un medicated participants
Flink et al(6)	Swede n	1420, (663/757)	≥ 20	0.29 ± 0.24	Patients had complaints of dry mouth
Percival et al (7)	UK	116, (55/61)	≥ 20	0.50 ± 0.04 (male) 0.33 ± 0.03 (female)	Study done in healthy un medicated participants
Shern et al(8)	USA	51, (25/26)	54 ± 19	Mean: 0.61	Study done in healthy un medicated participants

Saliva

Saliva is the viscous, clear watery fluid secreted from the parotid, submandibular, sublingual and smaller mucous glands of the mucosa. It contains two major types of protein secretions: a serous secretion containing the digestive enzyme ptyalin and a mucous secretion containing the lubricating aid mucin. The pH of saliva falls between 6 and 7.4. Saliva also contains large amounts of potassium and bicarbonate ions, and to a lesser extent sodium and chloride ions. In addition, saliva contains several antimicrobial constituents, including thiocyanate, lysozyme, immunoglobulins, lactoferrin, transferrin and antimicrobial peptides such as alpha defensins.

Saliva possesses many important functions including antimicrobial activity, mechanical cleansing action, control of pH, removal of food debris from the oral cavity, lubrication of the oral cavity, remineralization of enamel and maintenance of the integrity of the oral mucosa[15-17]. The buffering effect of saliva helps to neutralize acids consumed thereby protecting the person from dental caries. Remineralization also takes place through the presence of calcium ions (ca++) and phosphates in saliva providing a pool of remineralizing ions. The presence of antimicrobial peptides, neutrophils, thiocynates and other antimicrobial molecules in whole saliva play an important role in protecting the oral cavity from infectious microorganisms. Recent research has demonstrated that salivary antimicrobial peptides especially human neutrophil peptide 1-3 and LL-37 can predict the presence and severity of chronic periodontitis [18].

Several pharmacological agents have been found to have a direct or indirect effect on daily salivary flow rates. These drugs may include but are not restricted to blood pressure medication, antidepressants, diuretics, quinolones and non-steroidal anti-inflammatory drugs[19]. All the above drugs depress salivary production. Other drugs like Ketamine used in sedation and anesthesia, greatly increases salivary secretion.

Systemic diseases including diabetes, hormonal imbalance, autoimmune disorders such as Sjogren’s syndrome, rheumatoid arthritis and systemic lupus erythromatosus also depress salivary flow rate.

Salivary flow rate has been shown to vary both with age and sex. According to a Nigerian study by Adenji et al 1996, there was a demonstrable increase in salivary flow rate from age 20 to 23 years thereafter followed by a gradual decrease[20]. In general, severe reduction in salivary flow rate has been shown to set in at 45 years of age. This phenomenon may partly be due to the advent of other underlying conditions that tend to set in around the same time such as diabetes, hypertension and menopause.

The same study by Adenji et al also reported that men are higher secretors of saliva than females. This was also supported by Percival et al 1994 [7]. In his study, Adenji reported mean unstimulated values of 0.43ml/min for males and 0.42 ml/min for females while Percival reported 0.5 and 0.33 respectively. This later study attributed the difference to the male subjects being more active physically compared to the females.

Saliva follows a circadian rhythm where both quantity and quality of saliva undergoes changes over 24 hours. In a study by Dawes 1972, Unstimulated whole saliva showed significant circadian rhythm in flow rate and in the concentration of sodium chloride while the stimulated saliva showed significant circadian rhythm in the concentration of protein, potassium, calcium, phosphate and urea[21]. This has a bearing on the time of day in which saliva should be collected. It has been found that no significant changes occur in saliva quantity between 8 am and just before midday. Therefore, the saliva samples for this study were collected within that time frame to eliminate the confounding effect time may have on saliva flow rate.

Methods of collecting saliva

Several saliva collection methods have been reported, including drooling, spitting, suction and use of cotton wool [22]. Spitting method is preferred because of its convenience to the patient. Methods like suction and use of cotton rolls cause a certain degree of stimulation in the oral cavity[23], which could interfere with un stimulated saliva measurement. In drooling, saliva is allowed to collect in the mouth over a given period of time and then directed by a straw into a collecting container. This method is very accurate in collecting unstimulated whole saliva but it is uncomfortable for the patient as

the patient has to keep the mouth open to allow the saliva to drool into the collecting container[24]. In the spit method, saliva is allowed to collect in the mouth and then spat in the collecting container over a specified time interval. This is a convenient method for the patient. However, spitting may lead to foaming of saliva, which may present with difficulty in taking accurate volumetric measurements. Weighing the saliva rather than measuring the volume overcomes this problem. The short collection time (5 min) makes the procedure more practical for the clinician and more acceptable for the patient.

Salivary flow measurement by means of weighing is the most commonly used method. This is because of its reported accuracy and simplicity in a clinical setting [22-23]. Variables that could influence salivary flow were minimized in this study by collecting saliva at the same time of the day, excluding participants who were on medication or had any systemic disease that could potentially influence the results.

Periodontal diseases

Periodontal diseases are a group of inflammatory conditions affecting the supporting structures of the teeth. Plaque has been shown to have an effect on the etiology of periodontal diseases. However, it is the body's inflammatory response to the plaque microbes that are responsible for the destruction seen in periodontal diseases [8].

The commonest form of periodontal disease is a chronic non-reversible inflammation of the supporting structures of teeth known as chronic periodontitis. The disease usually has a slow on-set which gradually progresses to loss of collagen fibres and detachment of the epithelial attachment to root surfaces. This is then followed by apical migration of the pocket epithelium and subsequent pocket formation. Left unattended, the disease progresses to alveolar bone destruction, recession of soft tissues and mobility of the teeth and possibly, tooth mortality[25].

Over 500 different species of bacteria have been found in the human oral plaque. Virulence factors exhibited by these bacteria such as gingipain proteinases, leukotoxin, and fimbrae allow penetration of the epithelial barrier and avoidance of host defense factors. The adhesins of bacteria, ionic and

hydrophobic interactions allow for the colonization of the tooth and gingival surfaces, causing periodontal tissue destruction and triggering host inflammatory response.

Saliva contains antimicrobial buffers and bacterial load clearing capacity that helps in keeping the periodontium and other tissues healthy. Consequently, factors that may affect salivary flow rate and composition may have a direct effect on the periodontal status of an individual. This is because potentially, saliva has a protective effect on the periodontium and this may be compromised if the flow rate is altered.

CHAPTER TWO

Statement of the problem and justification

Problem

Saliva composition and flow rate are important in the prevention or progression of chronic periodontitis through their bacterial clearing and antimicrobial properties [(26)]. The current literature on salivary flow rates showed that there are low, moderate or high secretors among individuals but these studies were based in Europe and USA and as such may not be representative of the native African population[(27)]. A study by Björnstad and Crossner showed obvious differences in salivary flow rate and buffering effect between school children from Greenland and Sweden[(28)]. This further illustrates the importance of being cautious when exchanging reference data between different cultures/ethnic groups. As such, there is need to have data for each different population.

To the best of the authors' knowledge, there is minimal data on salivary flow rate in the African setting and especially in Kenya. A Nigerian study on adolescents demonstrated differences between different sexes and age [(20)]. However, there was no attempt to determine the effect of salivary flow rate on the oral health status and especially periodontal health. Saliva plays an important role in protecting the oral cavity including the periodontal tissues from injurious processes by reducing the microbial load from the oral cavity or neutralizing their injurious effects. Variations in the salivary flow rate and hence its protective capacity may partly explain the wide variation in prevalence of chronic periodontitis (1-10%) and gingivitis (0.2 – 90%) in Kenya[(2)].

Periodontal diseases including chronic periodontitis, gingivitis and aggressive periodontitis are a major health burden that has contributed immensely towards decreasing the overall quality of life for a sizeable proportion of the population. Identifying the predisposing or aggravating factors would help the oral health care providers to mitigate against the effects of these factors on oral health and therefore improve periodontal health. It is with this in mind that

this study was set to establish the salivary flow rate in the study Kenyan population and investigate its relationship with periodontal health status.

Justification

Several studies report age, sex, diet, life style, geographical location, season of the year and genetics as major players in variation or differences in salivary flow rates [27-29]. As these factors widely vary among the different races and geographical location, it follows that there may be observed variations in the normal salivary flow rates among the different races. There are hardly any studies on salivary flow rate in Kenya and none to the best of the author's knowledge relating SFR with periodontal status.

It was thus important to investigate this in a Kenyan adult population and correlate the findings with the periodontal status.

Objectives

Main Objective

To establish the normal salivary flow rate in a Kenyan adult population and investigate its relationship with periodontal status.

Specific objectives:

1. To establish the unstimulated salivary flow rate in a Kenyan adult population
2. To investigate the correlation between salivary flow rate and chronic periodontitis
3. To investigate the correlation between salivary flow rate and gingivitis

Variables

Table 2. Variables

Variables	Measurement
Socio demographics	
Age	Number of years
Sex	Phenotypic appearance of the participant, male or female
Occupation	Type of work the participant engages in
Smoking status	Describes the current smoking status of the participant. Active smoker, nonsmoker and previous smoker
Frequency of brushing	Number of times one brushes every day
Independent variables	
Salivary flow rate	Milliliters (ml) per minute (min)
Dependant variables	
Oral hygiene status	Plaque score- (Quigley Hein index- modified by Turesky et al 1970)
Gingival health status	Gingival index (Loe and Silness1963)
Periodontal status	Basic Periodontal Examination (BPE)

CHAPTER THREE

Materials and methods

Study design

The current study was a hospital based descriptive cross sectional study.

Study area

The study was carried out at University of Nairobi Dental Hospital (UNDH), a dental teaching hospital that receives about 3500 patients annually. It runs specialized clinics in oral diagnosis, pediatric dentistry, prosthodontics, oral and maxillofacial surgery and periodontology. The participants were recruited from both the periodontology clinic and oral diagnosis clinic. The periodontology clinic receives about 20 patients a day, whereas the oral diagnosis clinic receives approximately 40 patients and both clinics run from Monday to Friday. All new patients who come to the hospital go through the oral diagnosis clinic first before being referred to the other clinics.

Study population

The study population consisted of all patients who visited the periodontology and oral diagnosis clinics at the University of Nairobi Dental Hospital. In addition, those accompanying the patients and dental school staff were also included.

Inclusion criteria

All adult patients and accompanying persons between the age of 18 and 45 years attending the periodontology and oral diagnosis clinics at the University of Nairobi Dental Hospital during the time of the study (March 2015 to June 2014) were included in the study. The lower age limit of 18 years was because the study was targeting adult Kenyans while the upper age limit of 45 years was set to minimize the effect of confounding factors like diabetes, hypertension and some drugs, which may alter the salivary flow rate. The age of 45 years and above is a time when many individuals begin to experience the effect of lifestyle diseases such as hypertension and diabetes.

Exclusion criteria

Medically compromised patients, especially those suffering from diseases known to alter normal salivary flow rate were excluded. The screening process excluded mainly the following conditions; diabetes, hormonal imbalance, autoimmune disorders such as Sjogren's syndrome, rheumatoid arthritis and systemic lupus erythromatosus (Appendix I).

Also patients who had been on medication known to alter normal salivary flow rate for the last three months including but not limited to blood pressure medication, antidepressants, diuretics, quinolones and nonsteroidal anti-inflammatory drugs [19] were excluded.

Sample size determination

The prevalence of xerostomia in United States ranges from 10% to 40 % as is in most other countries in the northern hemisphere [30]. A prevalence of 40% was used in the calculation of the sample size using the Kish and Leslie formula for cross sectional studies. There are no local studies on xerostomia, hence the use of data from United States.

$$n_0 = \frac{Z^2 pq}{e^2}$$

Where:

Z= Z- score 95% confidence interval (1.96)

P= Reported prevalence of xerostomia

Q= 100-P

e = Precision of the study=5%

n_o= Sample size

Therefore;

$n_o = 1.96^2 \times 0.4 \times 0.6$

0.05×0.05

n_o = 368 participants. Since the total population was less than 10000, using the correction formula,

$$n = \frac{n_0}{1 + \frac{(n_0 - 1)}{N}}$$

Where:

N = the population size

n = sample size

The final sample size was **332.**

Participant recruitment

On every Data collection day, about 30 to 35 patients were available for participation in the study.

After thorough explanation and obtaining consent, the participants were screened on the basis of the inclusion and exclusion criteria. Successful participants were then subjected to systematic random sampling whereby every 3rd participant was entered into the study.

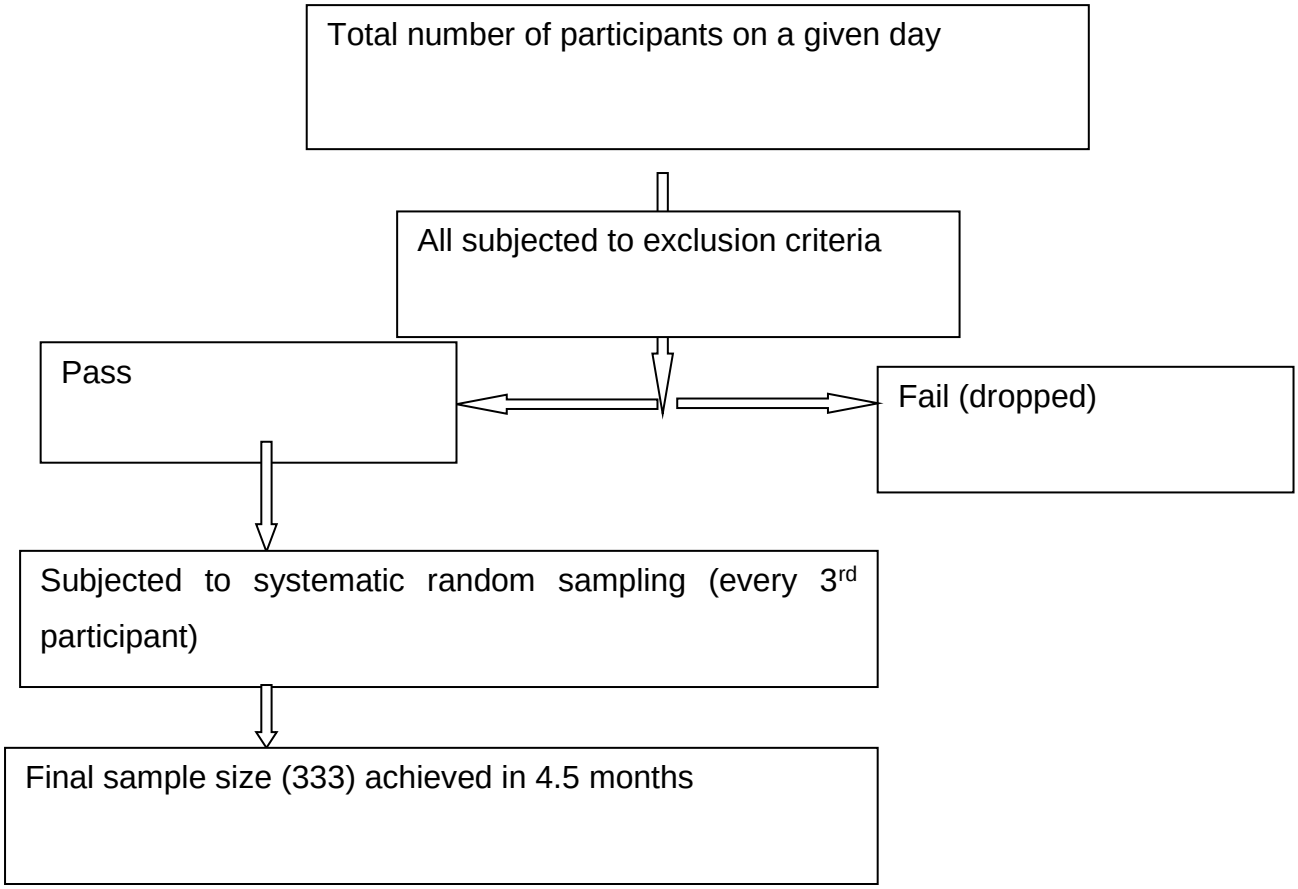

Figure1. Participant recruitment schematic

Data collection instruments and technique

Calibration

Data collection was carried out by the principal investigator who was calibrated by one of the supervisors (EW). Kappa values for inter examiner difference were calculated for BPE (0.8) which was considered good enough for the study. For Intra examiner variability, repeated examination of every 20th participant to adjust for intra- examiner errors was done.

A semi-structured questionnaire consisting of ten questions was used to collect socio-demographic data and oral hygiene practices. Participants who could read and write were allowed to fill the questionnaire while those who had difficulties were assisted. At any point, the participants were free to ask for clarification on any question that was not clear to them. Data on saliva flow

rate, periodontal status, plaque and gingival scores was collected using a clinical form. The periodontal status, plaque and gingival scores were done using the indices specified above in table 2 on variables. (Appendix II).

Unstimulated saliva was collected using the spit method over a period of five minutes. The flow rate was then calculated by using grams per minute (g/min). Collection was done before clinical examination to prevent stimulation of the major and minor salivary glands as a result of introducing examination equipment in the mouth. The participant was clearly informed on the protocol and a stop clock was used to time the period for saliva collection. A 40 ml plastic bottle, approximately 5cm in diameter with a tight fitting cover was used to collect saliva. The bottle was weighed before and after saliva collection using a calibrated digital balance (JY-09, Twins electronic Kitchen scale) to the nearest 0.1mg. The difference between the two weights was recorded as the saliva weight collected over the period of five minutes. This was then divided by the duration of collection (five minutes) to get the flow rate for each individual.

Plaque scores were obtained after using plaque disclosing tablets and the assessment was done based on Quigley Hein index- modified by Turesky et al 1970 (Appendix III). Disclosing tablets (produitis dentaires Vevey, Switzerland) were used to increase the sensitivity of detection and visual quantification of the plaque on the tooth surfaces. According to the FDI nomenclature, teeth 16 (upper right first molar), 11(upper right central incisor), 24 (upper left first premolar), 36 (lower left first molar), 31(lower left central incisor) and 44 (lower right first premolar) were used for detecting and recording plaque. The plaque scores on the bucal and lingual surfaces of the above teeth were dictated to the clerk who recorded them in their respective boxes. Substitution of missing teeth was done by using the tooth immediately mesial to the space for the missing tooth. When participants had no tooth or only one tooth in a given sextant, such a sextant was omitted. Partial mouth scoring was used because it allows for easy collection of representative data from the patient and allows for more accurate reproducibility.

Gingival scores were assessed using the Loe and Silness 1963 (Appendix IV). Partial mouth scoring was done using the same teeth as described above and same protocol for missing teeth adopted. Similar teeth were used in each individual for both plaque and gingival scores. In order to record gingival inflammation, each indexed tooth was examined by placing a periodontal probe into the gingival sulcus and gently running it around the lingual and buccal surfaces. A span of fifteen seconds was allowed before recording presence or absence of bleeding on probing. The average score was then calculated. A score from 0.1 to1.0 = mild inflammation; 1.1 to 2.0 = moderate inflammation and 2.1 to 3.0 signifies severe inflammation. [31]

Lastly, the periodontal status was assessed using British Society of Periodontology, Basic Periodontal Examination (BPE) protocol [32-33] (Appendix V). This index integrates gingival inflammation, presence of calculus and overhanging margins and pocket depth to determine a particular score of a given sextant.

All teeth present in a given sextant excluding the third molars were probed using a graduated periodontal probe with markings at every 3mm, and the deepest pocket noted. Factoring in presence or absence of bleeding, calculus and over hangs, a score of zero to four was recorded for each sextant (Appendix V)

Infection control

Disposable facemasks, tumblers and gloves were used during clinical examination. A set of sterile dental probe, mirror and tweezers were used for each patient. The dental chair was disinfected with hospital grade disinfectant each time before a new participant was examined.

Data analysis and presentation

The data collected was cleaned, coded, entered and analyzed by statistical package for social scientists (SPSS 20.0) for Microsoft windows®. Descriptive and inferential statistics were used. Levene's test of homogeneity of variance was used to determine the distribution of group variances within a given parameter.

Main outcome measures

The main outcome measures among the study participants during the study period were;

1. Plaque levels
2. Gingival scores
3. Salivary flow rate
4. BPE scores

Ethical considerations

Ethical approval was sought from and granted by the Kenyatta National Hospital and University of Nairobi Ethics, Research and Standards Committee (Appendix VI), approval number P660/11/2014.

Permission to carry out research at the university of Nairobi Dental Hospital was granted by the Dean, School of Dental Sciences and the Chairmen of Department of Periodontology and Oral and Maxillofacial surgery.

Voluntary written consent was obtained from each participant before obtaining any data. A name, signature or thumbprint was considered as sufficient proof of voluntary consent.

Study benefits

The participants received free dental checkups and information on their oral health status. Only five patients required emergency treatment due to acute pulpitis and this was provided.

Until the results of this study, there were no salivary flow rate values for adult Kenyans and Africans at large. The findings of this study can now be used as a bench mark for saliva flow rate among the adult Kenyan population. This data will be useful in many forms of saliva research in general in Kenya.

Limitations

During the four months' period of data collection, there was a change in the weather condition which could have affected saliva flow rate. Saliva flow rate has been reported to decrease with increasing temperature[34]

CHAPTER FOUR

Results

Socio-demographic Data

There were a total of 333 participants recruited with a male to female ratio of 1:1.3. The age of the participants ranged between 18-45 years with a mean of 32.2 years ± 8.1 SD.

There was no homogeneity of variance assessed by levene's test of equality of variances (F=4.917, p=0.027). An independent t-test was run on the data as well as 95%CI for the mean difference. It was found that there was no difference in the age of males (M=32.17±7.57SD) and females (M=32.26±8.44SD) included in the study t(331)=0.103,p=0.918.

The alternative non-parametric test (Mann-Whitney) for the T test showed a non-statistically significant difference in the means hence supporting the robustness of T-test despite the statistically significant levene's.

Salivary Flow Rate

The salivary flow rate (g/min) ranged between 0.14 – 1.98g/min in males and 0.08 – 1.68g/min in females. The mean SFR was 0.66 ±0.31g/min SD with a mode of 0.30 g/min. 256 participants were normal secretors within the range of 0.3 and 1.0g/min, 43 were high secretors with over 1.0 g/min while 32 were low secretors with a range of 0.1 and 0.29g/min as shown in table 3 below

Table 3: Secretors characteristics of participants

Variable		**Gender**		***X²***	***p-value***
		Male ***n(%)***	**Female** ***n(%)***		
Secretors				1.660	0.646
	Low	13(9.1)	19(10.1)		
	Normal	114(79.7)	142(75.1)		
	High	16(11.2)	27(14.3)		

There was no difference in the variances of secretors levels; very low (40±0), Low (32.2±9.09), Normal (32.1±7.85) and High (32.5±8.70) by age using Levene's test of homogeneity (F = 2.717, p = 0.068) as shown in table 4 below.

Table 4: Analysis of Variance of secretors level by age.

	Age (years)						
Secretors level	**n**	**M**	**SD**	**95% CI for Mean Difference**	**df**	**F**	**p**
Very low	1	40.0	.	.. .	3, 32	0.33	0.80
Low	32	32.2	9.09	28.98, 35.54			
Normal	256	32.1	7.85	31.19, 33.13			
High	43	32.5	8.70	29.83, 35.18			

There was no difference in the variances of salivary flow rate by gender; Males (M = 0.68±0.31 SD) and Females (M = 0.64±0.31 SD) using Levene's test of homogeneity (F = 1.158, p = 0.283). An Independent Sample t test was thus run on the data which showed a non-statistically significant difference in saliva flow rate (g/min) between Males (M = 0.68±0.31 SD) and Females (M = 0.64±0.31 SD), t (331) = 1.245, p = 0.214.

Oral Hygiene and Periodontal Health Status

Plaque scores

The average Plaque score was 1.94±0.72 SD for the study population with the males having a mean of 2.01±0.73 SD and females 1.89±0.71 SD.

An Independent Sample T test showed a non-statistically significant difference in average plaque scores between Males (M = 2.01±0.73 SD) and Females (M = 1.89±0.71 SD), t (331) = 1.494, p = 0.136.

There was a statistically significant difference in plaque scores with age; 18-30 years (M= 1.81± 0.74), 31-40 years (2.0±0.66), above 40(2.14±0.73, P= 0.003) as shown in the table 6 below.

Table 5 showing how plaque score varied with age.

	Plaque score					
Age	**n**	**M**	**SD**	**95% CI for Mean Difference**	**df**	**F**	**p**
18-30 Years	156	1.81	0.74	1.68, 1.92	2, 33	6.08*	0.003
31-40 Years	106	2.00	0.66	1.87, 2.12			
Above41 Years	71	2.14	0.73	1.97, 2.31			

Fisher's least significant Difference post Hoc test found the above difference to exist between the age group of 18-30 (M= 0.19 p= 0.03) and the other two groups as shown in the table 7 below

Table 6: showing the variation of plaque score with age.

	Plaque score			
18 – 30 Years	**Mean Difference**	**SE**	**95% CI for Mean Difference**	**p**
31 – 40 Years	-0.19*	0.09	-0.36, -0.01	0.031
Above 41 Years	-0.34*	0.10	-0.53, -0.13	0.001
31 – 40 Years	**Mean Difference**	**SE**	**95% CI for Mean Difference**	**p**
18 – 30 Years	0.19*	0.09	-0.01,0 .36	0.031
Above 41 Years	-0.15	0.11	-0.35, 0.06	0.183
Above 41 Years	**Mean Difference**	**SE**	**95% CI for Mean Difference**	**p**
18 – 30 Years	0.34*	0.10	-0.13, 0.53	0.001
31 – 40 Years	0.15	0.11	-0.06,0 .35	0.183

Fisher's Least Significant Difference Post Hoc Test

Gingival health

The average Gingival Score (Loe and Silness 1963) ranged between 0 – 2.75 with a mean of 1.31±0.5 SD. There was a statistically significant difference in the variances of gingival scores by age using levene's test of homogeneity (F = 4.077, p = 0.018).

ANOVA test was thus run on the data and a statistically significant difference in means; 18-30 years (1.2±0.52), 31-40 years (1.33±0.45) and those above 40 years (1.52±0.41,P =0.001) was detected as shown in table 8 below.

Table 7: Analysis of Variance of gingival score by age.

	Gingival score						
Age	**n**	**M**	**SD**	**95% CI for Mean Difference**	**df**	**F**	**p**
18-30 Years	156	1.20	0.52	1.12, 1.28	2, 33	10.9*	0.001
31-40 Years	106	1.33	0.45	1.23, 1.41			
Above 41 Years	71	1.52	0.41	1.42, 1.62			

**where p<0.05*

Periodontal status

The Basic Periodontal Examination (BPE) scores ranged between 0 – 4.0. Forty eight participants (14.4%) presented with at least one sextant in the mouth with severe periodontitis (BPE score of 4), one hundred and nineteen (35.7%) with mild periodontitis (BPE of 3), one hundred and forty three (42.9%) with gingivitis (BPE of 2 and 1) and twenty three participants (6.9%) were healthy (BPE of 0) as shown in figure 2.

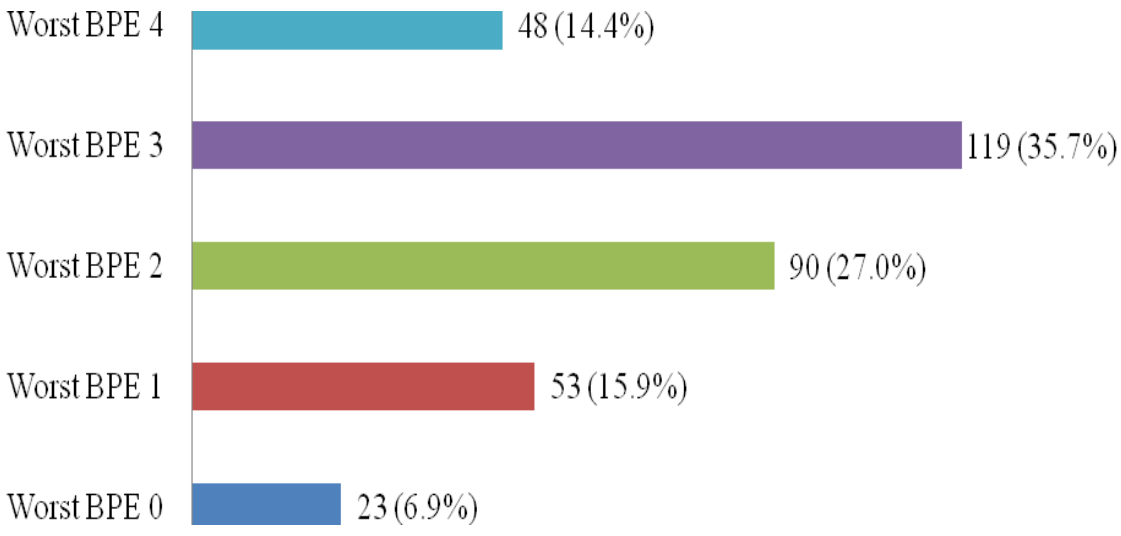

Figure 2: Distribution of BPE scores

A binomial logistic regression model revealed a non-statistically significant association between periodontitis and gender, F(1, 165) = 0.037, R^2 = 0.000, n = 167, p =0.847.

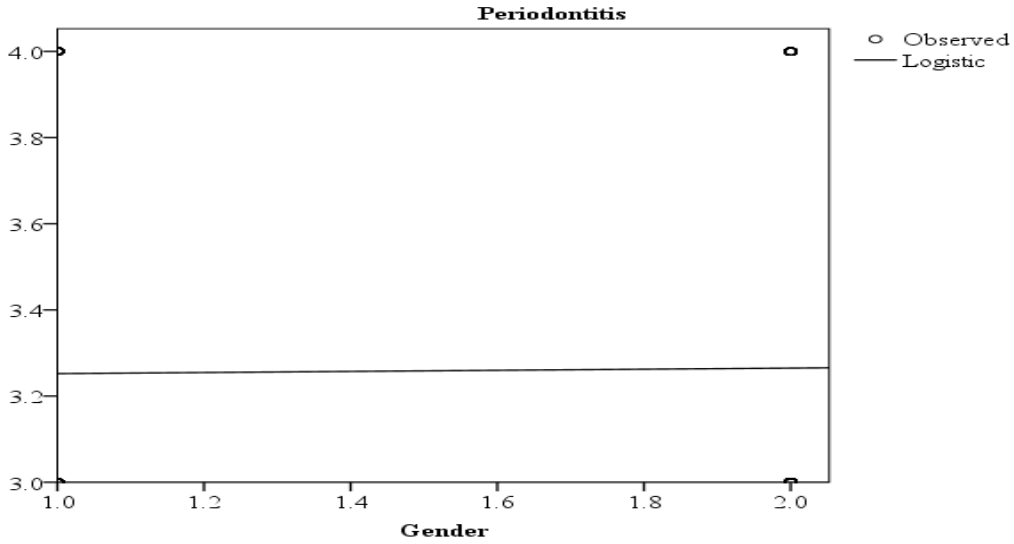

Figure 3: Plot model of periodontitis and gender.

A binomial logistic regression model revealed a statistically significant association between periodontitis and gingival scores, F (1, 165) = 8.327, R^2 = 0.048, n = 167, p =0.004 as shown in figure 4 below

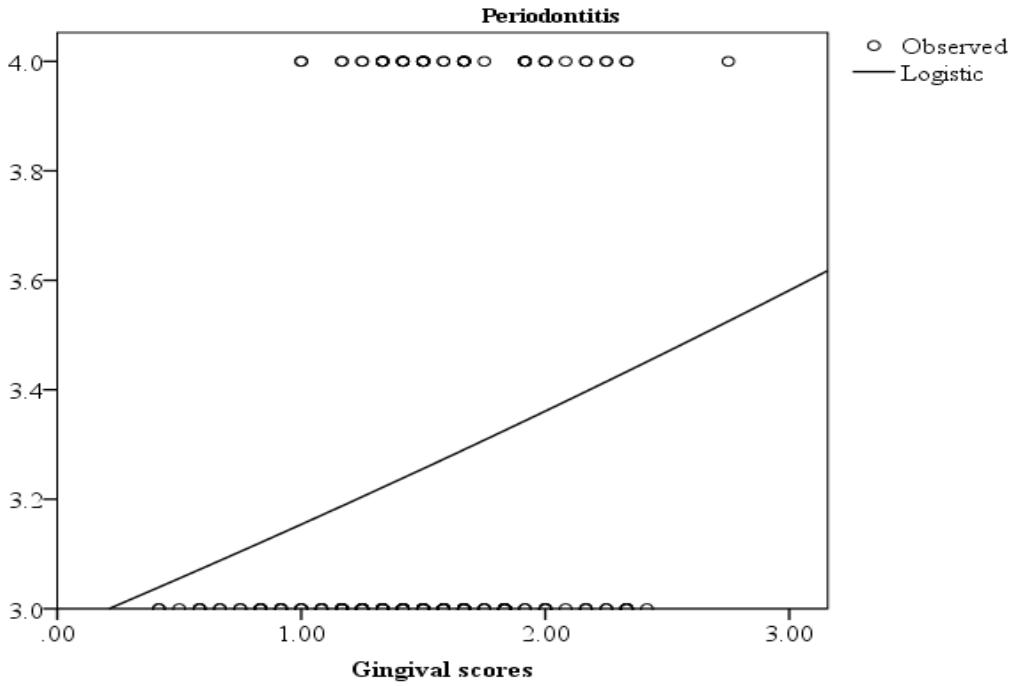

Figure 4: Plot model of periodontitis and gingival scores.

Relationship between salivary flow rate and periodontal status

There was a statistically significant difference between saliva flow rate of the participants who had periodontitis (M=0.68±0.33 SD) and those who had gingivitis (M=0.62±0.28 SD) with p=0.039 as shown by the distribution histogram in figure 5 below and independent sample t test in table 10.

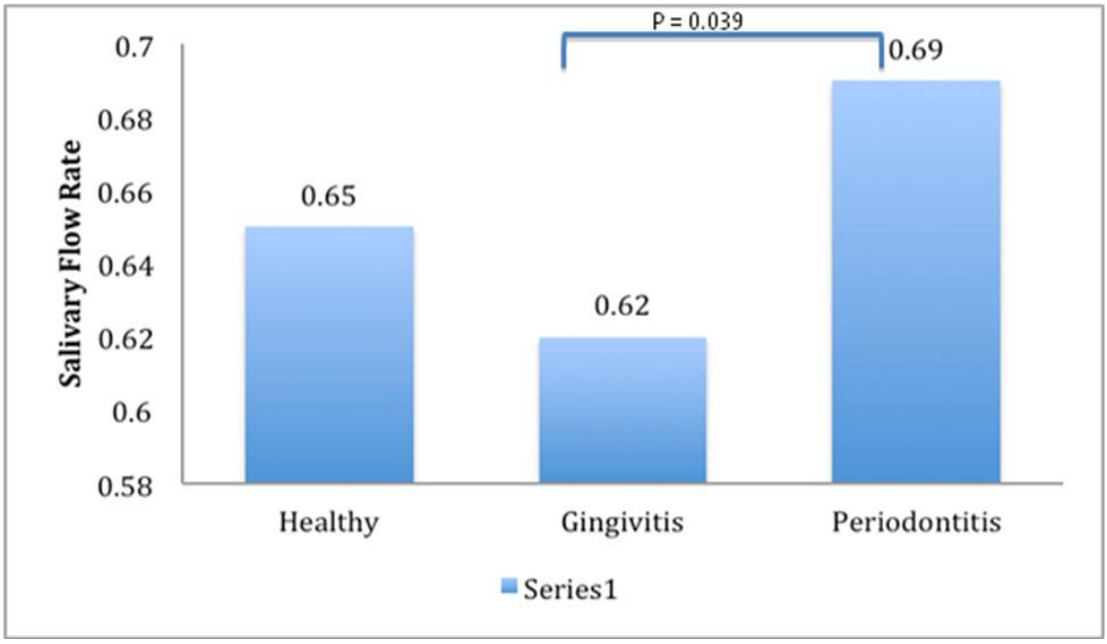

Figure 5: Distribution of mean salivary flow rate of participants with gingivitis and periodontitis.

Table 8: Relationship between salivary flow rate with periodontal health status.

	Saliva flow rate (g/min)						
BPE scores	**N**	**M**	**SD**	**95% CI for Mean Difference**	**df**	**T**	**p**
Healthy	23	0.65	0.29	-0.09, 0.16	164	0.55	0.58
Gingivitis	143	0.62	0.28				
BPE scores	**N**	**M**	**SD**	**95% CI for Mean Difference**	**df**	**T**	**p**
Healthy	23	0.65	0.29	-0.17, 0.10	188	-0.52	0.60
Periodontitis	167	0.69	0.33				

BPE scores	N	M	SD	95% CI for Mean Difference	df	T	p
Gingivitis	143	0.62	0.28	-0.14, -0.003	30	-2.07*	0.04
Periodontitis	167	0.68	0.33				

*p < 0.05.

A linear regression test elicited a statistically significant prediction of saliva flow rate (g/ min) from severity of sextant – BPE scores, F(1, 308) = 4.298, R^2 = .014, n = 310, p=.039 as shown in figure 10 below.

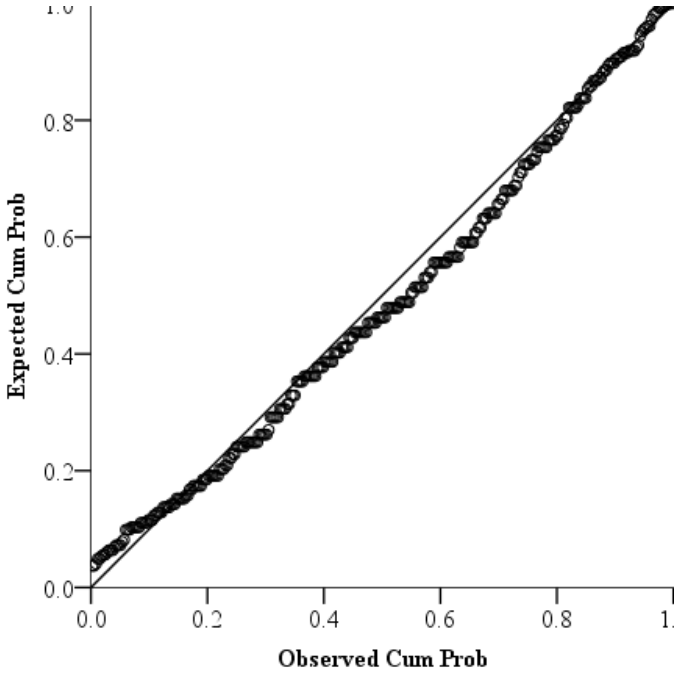

Figure 6 showing prediction of salivary flow rate (g/min) from severity of BPE scores.

Association between other variables

Pearson Product-Moment Correlation coefficient test for association elicited a statistically significant correlation between age and plaque (R^2 = 0.047, P= 0.001), age and gingival scores (R^2 = 0.072, p= 0.001), age and BPE scores (R^2 = 0.132, P= 0.001), plaque and gingival score (R^2 = 0.563, P= 0.001), plaque and BPE scores (R^2 = 0.299, P= 0.001) and Gingival and BPE (R^2 = 0.469, P= 0.001) scores as shown in table 9.

Table 9 showing association of variables.

Age	**Coefficient, r**	**R^2**	**P**
Plaque scores	0.218*	0.047	0.001
Gingival scores	0.269*	0.072	0.001
Sextant – BPE scores	0.363*	0.132	0.001
Plaque scores	**Coefficient, r**	**R^2**	**P**
Gingival score	0.750*	0.563	0.001
Sextant – BPE scores	0.547*	0.299	0.001
Gingival scores	**Coefficient, r**	**R^2**	**P**
Sextant – BPE scores	0.685*	0.469	0.001

*.The correlation is significant at 0.05 level

CHAPTER FIVE

Discussion

An alpha level of 0.05 was used for all statistical tests. Intra-examiner variability using Cohen's kappa was 0.85, which was considered good enough for the study.

Global Literature (reference is made to table 1 in the literature review section) has shown a high variability in the value of unstimulated whole salivary flow rate. For instance, Yamamoto et al[4] reported 0.053 Fenoll- Palomares et al [5]0.48, Percival et al [7]0.33, Shern et al [8]0.61 and Foglio et al [9]0.643). The current study found the unstimulated salivary flow rate to be 0.66g/min. This variability in flow rate reported by various studies is thought to be due to gender, age, collection method, temperature and diurnal changes. These factors however, seem not to be sufficient in explaining the high variability of the flow rate. The assumption is that there are other variables like diet, geographical location and genetics that could contribute to the observed differences. The above studies have been conducted in different geographical locations and racial groups.

The unstimulated salivary flow rate (g/min) was found to be 0 .66±0.31. This was close to the values that were reported by Shern et al [8] of 0.61ml/min in a USA population and Foglio et al [9] of 0.64 in Italy. However the sample sizes were only 51 and 81 participants respectively in the two studies reported above. The current findings greatly varied from the values Yamamoto et al[4] reported for the Japanese population (0.053). This may be as a result of racial differences between the African population and Asian population.

Earlier studies [5, 7, 35] have also shown that women have a lower salivary flow rate than men. This was also the finding in this study although the difference was not statistically significant. This could be attributed to the small salivary glands reported in women[36] and a higher frequency of oral dryness[37].

Pearson Product-Moment Correlation coefficient test for association revealed a strong, positive correlation between plaque scores and gingival scores (β = 0.750, R^2 = 0.563 p < .001). Study respondents who had higher plaque scores had higher gingival scores. The accuracy of predicting gingival scores will improve by approximately 56.3% if the prediction is based on plaque scores, F (1, 331) = 425.752, R^2 = 0.563, n = 333, p < .001. This is in line with the current literature that gingival inflammation is primarily caused by pathogenic sub gingival plaque[38], and therefore the higher the plaque score, the more severe the gingival inflammation is likely to be.

In addition, table nine demonstrates the strong correlation between plaque scores and BPE scores. Gingival inflammation is the first step in periodontal tissue breakdown that finally culminates into clinical attachment loss (CAL). Therefore it is possible to predict BPE scores with a 29.9% accuracy based on plaque scores, F (1, 331) = 141.287, R^2 = 0.299, n = 333, p < .001. Thus the higher the plaque score, the worse the BPE score[39].

Correlation of saliva flow rate and periodontal disease

Within the limitations of the study, SFR was higher among the subjects with periodontitis compared to those who had gingivitis (M = .62, SD = .28), t(331) = 2.020, p = 0.04). It was not possible to demonstrate a statistically significant difference in SFR between those who were healthy compared to those with periodontitis or gingivitis. However the levels where highest among the periodontitis patients compared to the healthy and gingivitis

This was different from Mulki et al[40] findings which did not find a difference in saliva flow rate between the participants who had periodontitis and those who were considered normal. This could be as a result of the saliva collection protocol that was used. In Mulki and co-workers protocol, 5 mls of saliva were collected regardless of how long it took, then the flow rate was calculated from the time duration which differed for each patient. 5 mls of saliva were required in his study to allow for determination of qualitative composition of saliva. In addition, the criteria for periodontitis that was used was based on loss of attachment with pocket depth of ≥5 mm in at least eight sites. This could have led to elimination of localized periodontal disease. Also patients with gingivitis

on a reduced periodontium could have been mistaken for periodontitis. In Mulki and co-workers study, he reported a high concentration of saliva protein and albumen in patients with periodontitis. This could have altered the density of saliva that was detected by the g/min scale.

Periodontitis is an inflammatory condition of the periodontal tissue. The observed increase in saliva flow rate could be in part attributed to increase in inflammatory exudates (crevicular fluid) and in part to the body's defense mechanism by increasing saliva flow rate so as to deliver inflammatory mediators and immune cells to the site of infection. Saliva possesses many important functions most importantly its antimicrobial activity, mechanical cleansing action, control of pH [15-17] among others. The presence of antimicrobial peptides, neutrophils, thiocynates and other antimicrobial molecules in whole saliva play an important role in protecting the oral cavity from infectious microorganisms. Therefore, it would be expected that in response to an inflammatory attack to the body, increasing salivary flow rate would be a reasonable body's response.

Conclusion

The unstimulated salivary flow rate of the study population was 0.66g/min, which falls within the reported normal range. Within the limitations of this study, the data suggests that there may be a possible positive relationship between SFR and inflammatory periodontal destruction.

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Appendices

Appendix I: screening form

Kindly tick Yes or No as applicable to your health.

Condition	Yes	No
Diabetes		
Sjogren's Syndrome		
Rheumatoid Arthritis		
Systemic Lupus Erythromatosus		
Undergoing radiotherapy		
Hypertension		
Hormonal imbalance		
Chronic use of any medication (please specify)		

Appendix II: Data collection tool (Saliva flow rate and periodontal status in an adult Kenyan population)

A: Questionnaire. (Tick appropriately)

Code: …….. Date……..

1. Sex ☐ male ☐ female 2. Age…….. (in full years)

3. Level of education ☐ none ☐ primary ☐ secondary ☐ tertiary

4. Marital status ☐ married ☐ single ☐ divorced/separated widow/widower ☐

5. Occupation ☐ self employed ☐ employed ☐ un employed

6. Frequency of brushing. ☐ none ☐ once a day ☐ twice a day ☐ others

7. Type of brushing aid. ☐ Chewing stick ☐ hand toothbrush ☐ electric toothbrush

8. Type of dentifrice used. ☐ Conventional Tooth paste ☐ Herbal tooth pastes ☐ mouthwashes ☐ ash ☐ others

9. Smoking ☐ Current smoker ☐ nonsmoker ☐ previous smoker

10. Alcohol consumption. ☐ Teetotaler ☐ Social drinker ☐ Regular Drinker

B: Saliva

Time	Wt of bottle (g)	Wt of bottle and saliva (g)	Wt of Saliva (g)	Flow rate (g/5)
5 min				

C :**Plaque Score: (Quigley and Hein modified by Turesky 1970).**

Tooth	16		11		24		36		31		44	
Surface	F	L	F	L	F	L	F	L	F	L	F	L
Score												
Total score						Average score						

D: Gingival Score. (Loe and Silness 1963)

Tooth	16		11		24		36		33		44	
Surface	F	L	F	L	F	L	F	L	F	L	F	L
Score												
Total score						Average score						

E: Basic Periodontal Examination (BPE)

1 **2** **3**

6 **5** **4**

Appendix III. Plaque Index. QuigleyHein index- modified by Turesky et al 1970)

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

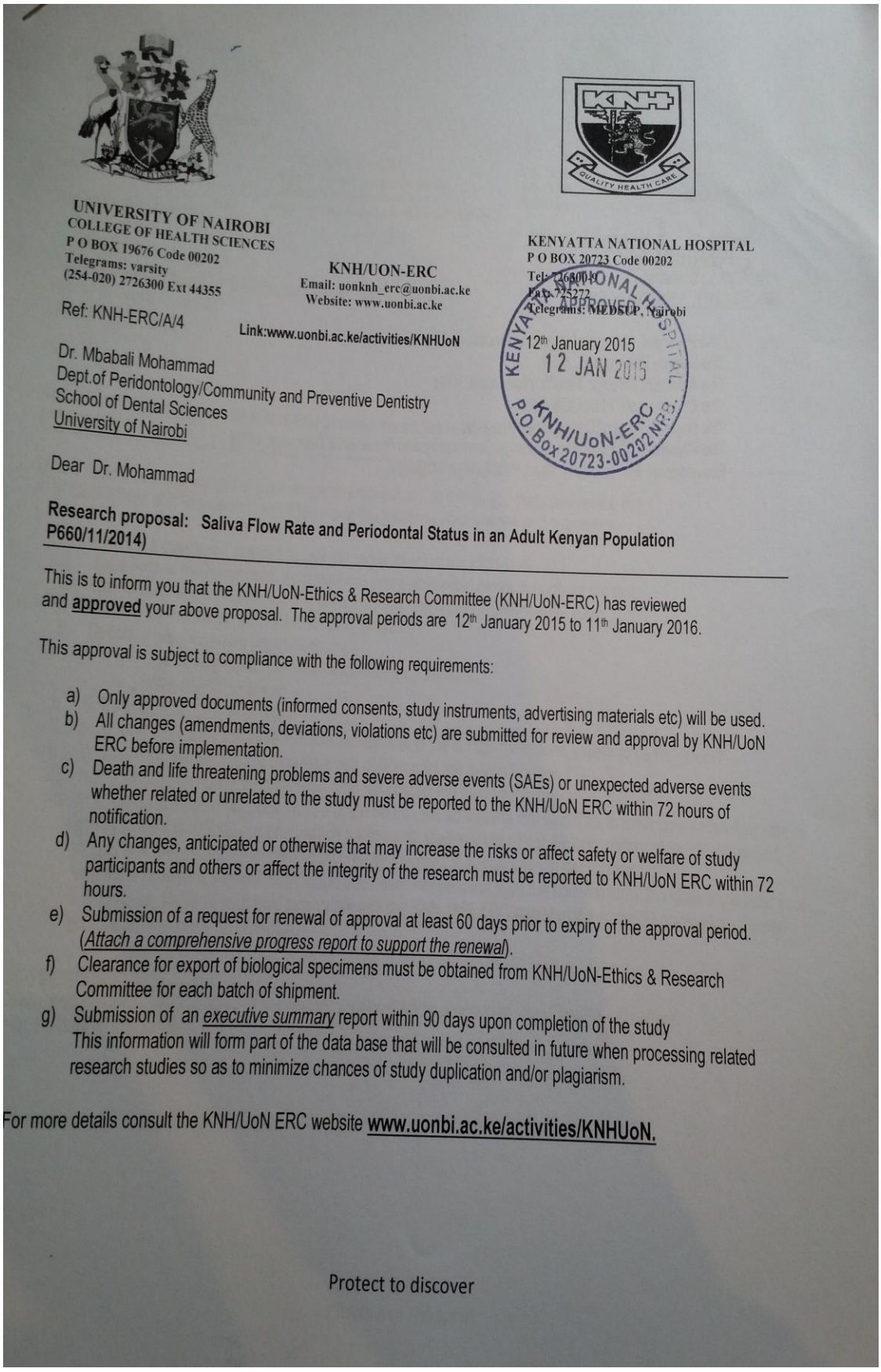
Appendix IV: Gingival index (Loe and Silness 1963)

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

Appendix V. Basic periodontal examination (BPE) (32-33)

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

Appendix VI: Ethics approval



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12th January 2015

KENYATTA NATIONAL HOSPITAL APPROVED 12 JAN 2015 KNH/UoN-ERC P.O. Box 20723-00202 NAIROBI

Dr. Mbabali Mohammad
Dept.of Peridontology/Community and Preventive Dentistry
School of Dental Sciences
University of Nairobi

Dear Dr. Mohammad

Research proposal: Saliva Flow Rate and Periodontal Status in an Adult Kenyan Population P660/11/2014)

This is to inform you that the KNH/UoN-Ethics & Research Committee (KNH/UoN-ERC) has reviewed and **approved** your above proposal. The approval periods are 12th January 2015 to 11th January 2016.

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 severe 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) 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*).
f) Clearance for export of biological specimens must be obtained from KNH/UoN-Ethics & Research Committee for each batch of shipment.
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.

For more details consult the KNH/UoN ERC website **www.uonbi.ac.ke/activities/KNHUoN.**

Protect to discover

Appendix VII: Consent Form

The purpose of the study

I, Dr. Mbabali Muhammad from the University of Nairobi would like to seek your consent for your participation in a study aimed at establishing the saliva flow rate of the Kenyan adult population and its relationship with periodontal status. The information I get is part of my research for a thesis as a partial fulfillment for the degree of master of dental surgery in Periodontology.

How do you participate?

I shall ask you some questions on the knowledge and practices of your oral health. I shall examine your mouth and record some observations. I will get a sample of your saliva for five minutes. The examinations shall be carried out using clean (sterile) instruments and no invasive procedures shall be performed.

Voluntary participation

Your participation in the study is voluntary. You can terminate your participation in the study at will without any consequences. Also understand that the participation in the study does not entail any financial benefit.

Anticipated risk

No risk is anticipated for participating in the study

Confidentiality

The information you provide me and my team will be kept in strict confidence and only used for the purpose of this research. No information, by which your identity can be revealed, will be released or published.

If you are satisfied with my explanation and you are willing to have your child participate, please sign the consent form.

Consent form

I_____of_____

Having understood the nature of study as explained to me by Dr. Mbabali Muhammad of The University of Nairobi, I am willing to participate in the study

Name _____ **signed** _____ **Date** _____

Patient

I confirm that I have explained the nature of the study to the patient.

Name _____**Signed** _____ **Date**

Principal Investigator:

For more information, please contact:

The Principal Investigator

Dr. Mbabali Muhammad,

School of Dental Sciences, University of Nairobi,

Tel: 0716 747919.

The Supervisors

Dr. Tonnie K. Mulli,

Lecturer,

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

0708414997

Prof. Evelyn Wagaiyu

Associate Professor

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

0722672567

KNH- UoN ERC secretariat

Kenyatta National Hospital

P.O. Box 70723

Nairobi

Tel. 726300-9

Kiambatisho Vb: Fomu Ya Idhini

Kiini cha Utafiti

Mimi,Dr. Mbabali Muhammad kutoka Chuo Kikuu cha Nairobi ningengependa kutafuta idhini yako kwa ushiriki wako katika utafiti wenye lengo la kuanzisha kiwango cha mtiririko wa mate wa watu wazima nchini Kenya na uhusiano wake na hali ya periodontal. Habari ntakayoipata itakuwa sehemu ya utafiti wangu kwa Thesis kama kutimiza mahitaji kwa ajili ya shahada ya upasuaji wa meno katika Periodontology .

Jinsi gani unaweza kushiriki?

Nami kuuliza baadhi ya maswali juu ya maarifa na mazoea ya afya yako ya mdomo. Nami kuchunguza mdomo wako na rekodi baadhi ya uchunguzi . Nami kupata sampuli ya mate yako kwa dakika tano . Mitihani watachukuliwa nje kwa kutumia safi (kuzaa) vyombo na hakuna taratibu vamizi litatekelezwa .

Ushiriki wa hiari

Ushiriki wako katika utafiti ni hiari. Unaweza kusitisha ushiriki wako katika utafiti katika mapenzi bila madhara yoyote. Pia kuelewa kwamba kushiriki katika utafiti haina leda faida yoyote ya kifedha.

Hatari kutarajia

Hakuna hatari ya kutarajia kwa ajili ya kushiriki katika utafiti huu.

Siri

Habari kutoa mimi na timu yangu yatawekwa katika imani kali na kutumika tu kwa madhumuni ya utafiti huu. Hakuna habari, ambayo utambulisho wako inaweza kuwa wazi , itakuwa iliyotolewa au kuchapishwa.

Kama wewe ni kuridhika na maelezo yangu na wewe ni tayari kuwa na mtoto wako kushiriki , tafadhali saini fomu ya ridhaa.

Fomu ya idhini

Mimi_____

Baada ya kufahamu hali ya utafiti vile nilivyoelezwa na Dk Mbabali Muhammad wa Chuo Kikuu cha Nairobi, nina nia ya kuwa na mtoto wangu kushiriki katika utafiti

Jina _____ saini _____ Tarehe _____

Mgonjwa

Nami nathibitisha kuwa nimemweleza jinsi ya utafiti kwa mgonjwa.

Jina _____ saini _____ Tarehe ____

Mpelelezi mkuu

Kwa habari zaidi, tafadhali wasiliana na:

Mpelelezi Mkuu

Dk Mbabali Muhammad,
Shule ya Sayansi ya meno, Chuo Kikuu cha Nairobi,
Tel: 0716747919.

Wasimamizi

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0708414997

Prof. Evelyn Wagaiyu

Associate Professor

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