A COMPARATIVE STUDY TO ASSESS INTERLEUKIN - 1 POLYMORPHISMS AND THEIR RELATIONSHIP WITH CHRONIC PERIODONTITIS IN TWO KENYAN COASTAL COMMUNITIES.

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

This work is dedicated to my loving husband Dr Chris K. Wagaiyu and my two loving sons, Wagaiyu Wagaiyu and Kimani Wagaiyu for their inspiration and encouragement throughout the studies. They were part of the team throughout the time of my studies.

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TABLE OF CONTENTS

ABBREVIATIONS

A	Adenine
B	Buccal
Bis	A prefix used in chemical nomenclature
C	Cytosine
BOP	Bleeding on probing
CAL	Clinical attachment loss
CD4	Cluster definition 4
CDC/AAP	Centre for Disease Control/American Academy of Periodontology
CEJ	Cemento-enamel junction
CI	Confidence interval
COHO	Community Oral Health Officer
CP	Chronic periodontitis
D	Distal
DB	Disto-buccal
ddH_2O	Double distilled water
DL	Disto-lingual
dNTPs	Deoxynucleotide triphosphates
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
Exp	Expected
F	Facial
G	Guanine

GCF	Gingival crevicular fluid
HWP	Hardy Weinberg principle
IFNγ	Interferon gamma
IL-1	Interleukin-1
IL-1β	Interleukin -1 beta cytokine
IL-1α	Interleukin – 1 alpha cytokine
L	Lingual
LA	Loss of attachment
LAP	Localized aggressive periodontitis
LJP	Localized juvenile periodontitis
LPS	Lipopolysaccharides
M	Mesial
MB	Mesio-buccal
$MgCl_2$	Magnesium chloride
ML	Mesio-lingual
MMPS	Matrix metalloproteinases
MP	Mesio-palatal
MWM	Molecular weight marker
NIC	National Influenza Center
Obs	Observed
OR	Odds ratio
Ortho	Orthodontic appliance
PAG	Periodontitis associated genotype

PBS	Potassium buffer solution
PCR	Polymerase chain reaction
PD	Partial denture
PGE_2	Prostaglandin E_2
PHO	Public health officer
PI	Principal Investigator
PL	Plaque levels
PMNs	Polymorphonuclear cells
PPD	Probing pocket depth
QA/QC	Quality assurance /Quality control
RFLP	Restriction fragment length polymorphisim
RNA	Ribonucleic acid
RR	Relative risk
SOPs	Standard operating procedures
SNP	Single nucleotide polymorphism
T	Thyimidine
TBE	Tris/Borate EDTA
TEMED	Tetramethylethylenediamine
Th	T-helper
TNF-α	Tumour necrosis factor alpha
UV	Ultra violet

DEFINITION OF WORDS/ TERMS

Allele An allele is an alternative form of a gene (one member of a pair) that is located at a specific position on a specific chromosome. Two alleles for each gene are inherited, one from each parent. Paired alleles (one on each of two paired chromosomes) that are the same are called homozygous (1-1 and 2-2 in this study), and those that are different are called heterozygous (1-2).

Bisacrylamide One of the compounds of the polyacrylamide gel used in creating cross-links between polyacrylamide chains thus forming a network.

Buffer AE A solution that elutes the DNA from the membrane and allows stable storage of DNA for years in the refridgerator or freezer.

Buffer AL Lysis buffer used during DNA isolation. It causes cell lysis to expose DNA.

Buffer AW1 Contains Guanidinium chloride. This is used to denature proteins in the sample. These proteins will then flow through the column and will be discarded with the wash. This aids in purifying DNA.

Buffer AW2 70% ethanol. It is used to remove salts from the column and aid in purifying DNA

Cases Individuals found to be having chronic periodontitis as defined

	by loss of attachment of ≥ 3mm in two non-adjacent teeth.
Chronic periodontitis	An infectious disease resulting in inflammation of the supporting structures of the teeth, leading to loss of attachment and bone. If left long enough without treatment, it may lead to tooth loss.
Clinical attachment loss(CAL)	Apical migration of the junctional epithelium from the cemento-enamel junction.
Composite genotype	A positive composite genotype was considered when allele 2 of IL-1A -889 and allele 2 of IL-1B +3954 were present.
Controls	Individuals without chronic periodontitis, which means no/minimal bleeding on probing and no pockets of more than 3mm and no CAL.
Dental calculus	Calcium phosphate and carbonate, with organic matter, deposited on tooth surfaces. It is also defined as, “mineralized plaque”.
Electrophoresis	A process for separating protein molecules of varying sizes in a mixture by moving them through a block of gel (agarose or polyacrylamide) by means of an electric field.
Genotype	This is the specific genetic makeup of an individual with reference to a single set of traits or an entire complex of traits.
Haplotype	These are combinations of alleles that are adjacent to each other on the chromosome and are transmitted together.

Interleukins	Proteins signaling molecules that are secreted by a wide variety of cells. They are potent mediators/messengers between cells.
Interleukin-1 polymorphisms	Interleukin – 1 is a pro-inflammatory mediator (cytokine) which modulates the disease process in chronic periodontitis. It is controlled by a cluster of genes on the human chromosome 2q13. Polymorphism is the difference in DNA sequence that gives rise to different forms of the cytokine.
Microbial biofilm	The relatively undefined microbial community associated with a tooth surface or any non-shedding material.
Periodontal pocket depth	Is the distance between the gingival margin and the bottom of the periodontal pocket to the nearest whole millimeter. The periodontal pocket being the space between the periodontal tissues and the tooth created by the pathological migration of the attachment apparatus.
Phosphate Buffer Saline (PBS)	A salty solution of sodium chloride, sodium phosphate and potassium phosphate used to keep tissue cells and proteins intact during DNA extraction.
Proteinase K	Broad spectrum serine protease. Used in DNA extraction to remove contaminating proteins.
Risk factor	This applies to an exposure that is statistically related in some way to the outcome. For example, smoking is a risk factor for

chronic periodontitis.

TBE A buffer solution containing a mixture of tris base, boric acid and EDTA.

TEMED Is used with ammonium persulphate to catalyze the polymerization of Acrylamide when making polyacrylamide gel.

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LIST OF FIGURES

LIST OF PLATES

LIST OF TABLES

ABSTRACT

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Introduction: Chronic periodontitis (CP) is an infectious disease resulting in the inflammation of the supportive structures of teeth, which leads to loss of attachment and bone, and eventually tooth loss if left untreated. Genetic polymorphisms in the proinflammatory cytokine, interleukin-1 (IL-1A and IL-1B) isoforms have been associated with CP in Caucasians, Asians and Arabs but little is known about their role in Africans. Therefore, the aim of this study was to resolve the association between genetic polymorphisms in IL-1A and IL-1B isoforms and chronic periodontitis in two Kenyan coastal communities, the Taita and the Swahili.

Objectives: 1) To determine the association between socio-demographic characteristics, clinical features and CP amongst cases and controls in both ethnic groups. 2) To determine the severity of CP in both ethnic groups. 3) To determine the distribution of interleukin-1 genotype polymorphisms of IL-1B and IL-1A in both ethnic groups. 4) To evaluate the association between IL-1B and IL-1A with CP in both ethnic groups. 5) To compare the carriage rate of IL-1 polymorphisms amongst the Taita and Swahili participants.

Methodology: This was a population based case-control study. The test was, whether the marker genotypes distributed differently between the cases and controls. After informed consent, potential participants were interviewed using a modified version of the World Health Organization oral health survey questionnaire for age group 35-44years. A clinical examination was then conducted to assess the oral health status and data recorded in a data collection form. The presence of

dental plaque (PL) using a disclosing agent (produits dentaire vevey, Switzerland) and bleeding on probing (BOP) were recorded dichotomously. Presence of calculus, missing teeth and carious teeth were recorded. The examination also included the measurement of periodontal pocket depths and recession on six sites per tooth. Buccal swab samples were then obtained as per the manufacturer's instructions using the isohelix buccal swabs (BocaScientific, Isohelix, Kent, England). Deoxyribonucleic acid (DNA) was isolated from the swabs using QIAamp DNA purification protocol followed by polymerase chain reaction (PCR) amplification using specific primers to IL-1A (loci -889 & +4845) and IL-1B (loci -511 & +3954). The amplicons were digested using Nco1, Fnu4H1, Ava1 and Taq1 respectively. Restriction fragment length polymorphisms (RFLP) were recorded after running the digested amplicons on a 30 % PAGE, followed by visualization under ultra violet (UV) light after staining with ethidium bromide. Association analyses of the RFLP, demographic and clinical data were carried out using Pearson's Chi-squared (χ^2) test and risk was assessed by odds ratios (OR) with 95% confidence interval (CI). χ^2 and Mantel Hanzel tests were used to determine confounders and effect modifiers. Multivariate analyses were performed using binary logistic regression. The genotype frequencies were tested for Hardy-Weinberg equilibrium using the χ^2 test with one degree of freedom.

Results: Screening was done on approximately 500 individuals at each of the sites. The number recruited in Taita was 198; 99 cases and 99 controls. 200 were included in the study from old town Mombasa; 100 cases and 100 controls. The total number

of subjects 398 subjects; four loci (-511, -889, +3953 and +4845) per subject thus 1,592 samples were tested.

The distribution of socio-demographic features amongst the Taita revealed that the only feature associated with the risk of having chronic periodontitis was being married with an OR= 2.88(95% CI of 1.12- 7.58) and p=0.014. The mean number of teeth per individual having plaque in cases was 25(SD3.6) and for controls 14(SD 9.9).The difference demonstrated was, OR = 21, with 95% CI = 7.8-56.4, p<0.001. The mean number of teeth per individual found to be bleeding was 17(SD 10.2). There was more calculus amongst those with chronic periodontitis OR = 33.9, 95%CI = 13.3-86.3, p<0.001.The mean pocket depth was 1.58(SD1.01). 20.7% of the Taita participants were found to have ≥4mm CAL. The severity of CP according to the CDC/AAP classification was as follow: - 10(10.6%) had mild CP, 43(45.7%) had moderate CP and 41(43.6%) had the severe form.

No deviation from the Hardy Weinberg equilibrium was observed in any of the groups. Carriage of allele 2 at IL-1B +3954 (i.e. combination of '2-2' or'1-2' at locus +3954) was associated with CP in the Taita participants (OR = 1.94, 95%CI=1.01-3.70, p=0.045). There were no confounders or effect modifiers in the Taita participants and no association with severity of CP was observed in this population. Amongst the male Taita participants, none of them were found to have the homozygous allele 1 of IL-1A+4845 polymorphism. Amongst the female Taita participants, the controls had a higher frequency of homozygous allele 1 of IL-

1A+4845 with OR=0.137, 95%CI=0.016-1.14, p=0.034. None of the composite genotypes were associated with CP in the Taita participants.

Amongst the Swahili participants, none of the socio-demographic features were significant when cases were compared to controls. Plaque was present on a mean of 26(SD4.3) teeth per individual in cases and 18(SD5.6) teeth per individual in controls with OR = 9.2 (95%CI = 3.7-23.1), p<0.001. The mean number of teeth that had bleeding on probing of the gingival tissues was 25(SD 5.8). Periodontal probing depths of ≥4mm were found on 8(5.0%) teeth per individual. The mean pocket depth was 1.93(SD1.07). Mean clinical attachment loss of ≥4mm was present in 37(SD 30.6) of the sites per individual. None of the Swahili participants were found to have all teeth covered with calculus. When cases were compared to controls, the OR = 114.6, 95%CI = 33.1-397.2, p<0.001 showing a difference between cases and controls. Mild CP was present in 9(9%) participants, moderate CP in 35(35%) and the severe form of CP in 56(56%).

Carriage of allele 1 at IL-1A-889 (i.e. combination of '1-1' or '1-2' at locus -889) amongst the Swahili participants was associated with CP (OR = 3.16, 95%CI=1.644-6.083, p<0.001). The frequency of homozygous allele 2 ('2-2') at locus -889 was more prevalent in controls (90.8%) than cases (77.2%) with OR= 0.316, 95%CI=0.164-0.608, p=0.001 amongst the Swahili participants. Amongst the Swahili participants, allele 1 at locus IL-1A-889 was associated with mild, (OR=5.2, 95%CI=1.445-18.71, p=0.005), moderate (OR=4.51, 95%CI = 2.08-9.79, p<0.001)

and severe disease (OR=2.19, 95%CI=1.013-4.738, p=0.042). Futhermore, amongst the Swahili participants, plaque level was an effect modifyer in the association between IL-1B-511 polymorphism and CP. None of the composite genotypes was associated with CP in the Swahili participants. Amongst the male Swahili participants, allele 2 at IL-1A-889, was more frequent in the controls OR=0.229, 95% CI=0.069-0.748, p=0.009. Whereas amongst the female Swahili participants, the frequency of allele 1 at IL-1A-889 was higher in cases, OR=5.567, 95%CI=1.148-27.88, p=0.019.

When the carriage rate of the various alleles were compared amongst the Taita and Swahili participants, the Taita participants, who do not generally marry outside their ethnic group, had a lower frequency of alleles at three loci IL-1B-511 (heterozygotes, p=0.016), IL-1B+3954 (heterozygotes and homozygotes for allele 2, p= 0.004 and p<0.001 respectively) and IL-1B+4845 (homozygotes for allele 2, p=0.002). The only allele where the frequency was observed to be higher in the Taita participants was allele 1 at IL-1A-889, p<0.001.

Haplotype 3 (i.e. allele 1 at all the four loci) was significantly associated with CP amongst both the Taita (OR=2.4, 95%CI=1.1-5.14, p=0.022) and Swahili (OR=4.2, 95%CI=1.35-13.3, p=0.008) participants. Whereas, in the multivariate model, plaque (p<0.001), was the factor that remained related to CP in the Taita and also in the Swahili participants (p<0.001). Brushing of teeth in the evenings amongst the Taita participants was significant, OR=0.38, 95%CI=0.18-0.82, p=0.007. Whereas

amongst the Swahili participants, brushing in the mornings was significant, OR=0.04 95%CI=0.01-0.17, p<0.001. Oral health seeking behavior was poor especially amongst the Taita participants with only 19(9.6%) having visited a dental clinic.

Conclusions: The significant association of allele 2 at IL-1B +3954 with CP in the Taita participants, confirmed the importance of this genotype in disease pathogenesis since monocytes and polymorphonuclear leukocytes in those homozygous for this allele have been shown to produce 4-fold more IL-1β, a proinflammatory cytokine that promotes CP. Increased susceptibility to CP in the Swahili participants with allele 1 at IL-1A-889 was also not surprising. This genotype IL-1A-889 is associated with significantly lower transcriptional activity of the IL-1A gene and lower levels of IL-1α. Variations in IL-1α may affect production of IL-1β. This may lead to increased destruction of the periodontium in carriers of this genotype. The association of haplotype 3 and CP in the two African tribes suggests that the haplotype is an important risk factor to CP in Africans of Bantu origin.

The two ethnic groups demonstrated differences in the carriage rates of the various alleles studied. The Taita participants, who do not generally marry outside their ethnic group, had significantly lower frequencies at most of the loci studied as compared to the Swahili participants. When comparisons were made across gender in the two ethnic groups, it was only amongst the Swahili participants, where allele 2 at IL-1A-889 appeared to confer protection to the males and allele 1 at IL-1A-889 was associated with a 6 fold chance of getting CP in the females.

The relationship between plaque and CP in both African tribes suggests that it initiates progression of CP against a background of genetically susceptible individuals. Plaque and calculus were associated with clinical attachment loss in both ethnic groups. Brushing of teeth in the evenings protected the Taita participants from developing CP and brushing in the mornings for the Swahili participants. Oral health seeking behavior was poor in both ethnic groups. Plaque control as a well tested mode of prevention of CP has been proven in this study.

Recommendations: These findings advocate for inclusion of oral health disease prevention into public health programs to offer susceptible individuals a chance of retaining their teeth for longer. Furthermore, a similar study with a larger sample size of other Bantu groups, cushites and nilotes resident in Kenya is recommended to assess whether the same genetic polymorphisms are associated with chronic periodontitis.

CHAPTER 1

INTRODUCTION

This thesis is the report of a case control study to investigate genetic risk factors of chronic periodontitis in two different coastal communities; the Taita and the Swahili. The Taita are a rural community living in the Taita Hills and have limited access to dental services. The Swahili on the other hand are an urban community living in Mombasa old town with easy access to dental services. The chapter gives an overview of chronic periodontitis, aetiology of chronic periodontitis and risk factors associated with chronic periodontitis, including the genetic ones.

1.1 PERIODONTAL DISEASES

Periodontal diseases are conditions associated with dental plaque formation[1]. These diseases and conditions affect the tissues of the periodontium, leading to the destruction of the connective tissue attachment of the teeth. The connective tissue attachment includes the gingiva, the periodontal ligament, the root cementum and the alveolar bone[2]. Periodontal diseases are broadly categorized into eight groups[3] (reference is made to appendix 1). Gingivitis is the first group and is described as inflammation confined to the gingival tissues. Chronic periodontitis on the other hand is the second group and occurs when there is destruction of the periodontal ligament and the alveolar bone leading to the apical migration of the junctional epithelium. Chronic periodontitis is always preceded by gingivitis. However, gingivitis does not always progress to periodontitis[2]. This study focused on chronic periodontitis

because it has previously been shown that interleukin-1 gene polymorphisms are not associated with the development of gingivitis[4].

1.2 GLOBAL OVERVIEW OF CHRONIC PERIODONTITIS

Chronic periodontitis is described as an infectious disease initiated by bacterial organisms and propagated by the host response. It is a multifactorial disease with complex risk factors. Studies of populations across the world have shown that there are three different ways in which this disease progresses in any group of people[1]. The first group, the individuals develop a treatable form of the disease and will retain their teeth for life. The second group of individuals, approximately 11%, appears to be resistant to the disease and will not develop the disease thus retaining their teeth for life. The third group approximately 15% of the population is the rapidly progressive group. In this third group, the affected individuals will develop destructive chronic periodontitis and will eventually lose their teeth[1]. The three groups of people mentioned are otherwise healthy individuals with no underlying systemic illnesses like diabetes or neutrophil deficiencies. This implies that there are other factors that may be responsible for the variation in the progression of periodontitis. Genetic variation is considered as one of the risk factors which bring about differences in disease progression[5]. Studies carried out on reared – together and reared – apart twins on the development of gingivitis, probing depth and clinical attachment loss, have shown that between 38% and 82% of the variation seen in the progression of chronic periodontitis, could be attributed to genetic variation[5, 6, 7]. The prevalence of chronic periodontitis in Kenya will be reviewed.

1.3 OVERVIEW OF CHRONIC PERIODONTITIS IN KENYA

A national oral health survey has not been carried out in Kenya to date. Therefore, the exact prevalence of periodontal diseases is unknown. A few epidemiological studies on gingivitis and periodontitis in different groups of people have been carried out [8, 9,10,11,12,13,14,15] and will form the basis for this summary.

The prevalence for chronic periodontitis in Kenya has been reported to be between 1-10% and that of gingivitis varies from 0.2% - 90%[8]. One of the earliest studies carried out in Kenya was done in 1966 using the Russell periodontal index method and the report showed a high prevalence of periodontal diseases ranging from gingivitis to chronic destructive periodontitis[9]. However, this high prevalence could have been due to the fact that the Russell periodontal index includes gingivitis as part of periodontitis. This lack of separation between gingivitis and periodontitis will reflect as more disease being present because gingivitis is usually highly prevalent in any given population. The high prevalence has been questioned by Baelum and co-workers in 2002, where they compared several studies and showed that the prevalence of chronic periodontitis in Kenya is similar to that seen in other populations[10]. Another early study in 1978, reported on periodontal diseases in two northwestern tribes of Kenya[11]. The mode of examination for periodontal disease was by observation of the tissues with the naked eye rather than the use of an index. This method of examination was biased and cannot therefore be used to assess objectively the periodontal condition of the two Northwestern Kenyan tribes examined. The work done by Butt in 1986, looked at the periodontal condition of a

rural population and also reported a high prevalence[12]. However, this was again a biased study since the population examined was of patients already attending a dental clinic in Nyeri which is a town situated in Central Kenya. The method used for the examination of the diseases was also biased in that no recognized index was used and bone levels were recorded without indicating how this was assessed. It was not stated whether radiographs were taken to asses bone loss. Periodontal destruction was also recorded without probing the periodontal pockets. A study done on a group of 352 trainees aged between 18 and 26 years showed that gingivitis was widespread with a prevalence of 66% in males and 40% in females[13]. Destructive periodontal disease defined as recession of >3mm was recorded in 12 % of the subjects and pocket depths of > 3mm was recorded in 2% of individuals[13]. A subsequent more comprehensive study by Baelum and co-workers in 1988 examined a rural population for periodontal diseases and reported heavy plaque and dental calculus presence but only a fraction of the individuals experienced periodontal destruction[14]. In 1996, this group of scientists, recalculated data from six studies on attachment loss levels in different populations so as to compare periodontal destruction in a Kenyan and a Chinese adult population with other populations[15]. They reported that the attachment loss levels in the Kenyans and Chinese were quite similar to those in a Japanese population, in a Norwegian population, in a New Mexican group of adults and a population of young US adults aged between 35 and 60 years. [15] Thus they showed that the traditionally held view, that Africans suffer from more severe forms or more prevalent diseases of the periodontium do not hold.

It is difficult to determine the trend in chronic periodontitis experience in Kenya since most of the studies have been on gingivitis and of a descriptive nature rather than analytical or longitudinal. There is no study in Kenya that has looked at chronic periodontitis over time. Moreover, information from studies on the same population over time or on two or more separate occasions is not available. Additionally, in the absence of consecutive national oral health surveys, the accurate determination of the trend of chronic periodontitis experience is not possible.

1.4 AETIOLOGY OF CHRONIC PERIODONTITIS

The primary etiological factor in the initiation of chronic periodontitis is plaque with host susceptibility being reported as an important modifying factor in the progression of the disease[2]. Many patients with periodontitis do not have the classical risk factors, whereas others with comparable risk factors do not show the same level of periodontal destruction or progression[2]. This seems to indicate that other additional risk factors and aetiological factors may explain the differences seen[6]. Recently, several gene polymorphisms have been investigated as possible contributors to the increased host susceptibility. Studies on genetic susceptibility come from twins, [5,6] linkage studies, segregation analysis in families with aggressive forms of periodontitis [16, 17] and association studies[18, 19].

Besides genetic influences, environmental factors also affect the clinical expression of chronic periodontitis. These include cigarette smoking, age, gender, socio-economic factors, systemic illnesses like diabetes and stress[20, 21, 22, 23]. Patients who smoke have more teeth which are affected and greater attachment and bone loss

mean levels. They also do not respond well to periodontal therapies when compared to non-smokers[20]. Stress has also been associated with periodontal disease progression and this has been shown in cross sectional studies that revealed increased progression of periodontal breakdown in patients affected by psychological stress[23]. Therefore, it has been suggested that stress is a predictive factor for future clinical attachment loss[24].

1.5 GENES AND CHRONIC PERIODONTITIS

Genes alone do not determine whether an individual will develop the disease. It is the interplay between genetics and environmental factors which will eventually lead to the development of the disease. The different forms of genes or the variation in nucleotide sequence at a locus are known as alleles or allelic variants. Genetic polymorphisms occur when different alleles of a given gene coexist in the human population[25]. Polymorphisms arise as a result of gene mutations. All organisms undergo spontaneous mutations as a result of regular cellular functions[25]. Genetic polymorphisms together with environmental factors have been reported to influence the progression of chronic periodontitis in a complex way[18]. For example, in one of the early experimental studies on beagle dogs, approximately 25% of the dogs failed to develop periodontitis in spite of a uniformly sustained bacterial challenge showing that other factors possibly genetic contribute to the disease progression[26]. In humans, this finding was also observed in tea plantation workers in Sri-Lanka where despite not brushing their teeth and receiving no professional care, 11% of them did not develop periodontitis, yet they all had large amounts of plaque and dental

calculus as well as plaque-associated chronic gingivitis[1]. Some authors have attributed this to host susceptibility. This implies that individuals who inherit susceptibility alleles will develop the disease when exposed to risk factors of periodontal disease. A strong association has been observed between the severity of periodontitis and a specific genotype of the interleukin-1 (IL-1) gene cluster[27]. Patients carrying this periodontitis-associated genotype (PAG) were shown to demonstrate phenotypic differences, as indicated by elevated levels of the cytokine IL-1β in gingival crevicular fluid[27].

Interleukin-1 polymorphisms have not been investigated in the Kenyan population. It is in this regard that two local communities were identified for this study. The Taita are Bantus and occupy the southeastern part of Kenya, known as the Taita Hills, while the Swahili are found along the Kenyan coast and are of mixed heritage, with a Bantu and Arabic/European mixture. Both these groups though diverse in culture are found in the coastal area of Kenya. The reason for selecting these two groups was due to the fact that they were both of Bantu origin before the Swahili intermarried with outsiders from the Middle East and Europe, thus allowing the study of two ethnic groups of similar origins, but with diverse genetic and cultural make-up, yet occupying the same regional area.

CHAPTER 2

LITERATURE REVIEW

2.1 THE PERIODONTIUM AND PERIODONTAL DISEASES

The periodontium is unique in structure. It forms the point at which hard tissues breach the epithelial surface through the junctional epithelium. This junction of tooth and gingiva provides a potentially weak barrier through which bacteria and their products could enter the underlying connective tissues and subsequently the systemic circulation[28]. This junction between the tooth and the gingival tissues is known as the dentogingival junction and is formed by the junctional epithelium (JE) and gingival fibers. The attachment of the JE to the tooth is through the dental lamina. The underlying structures are composed of the gingival connective tissue, the periodontal ligament, the cementum, the alveolar bone and the vascular and nerve supplies which are all interdependent. In health, the biologic adaptation and renewal processes of these tissues maintain a harmonious relationship [29].

Various disease conditions affecting the periodontium have been defined and classified mainly based on clinical manifestations, including location, degree of tissue loss and rate of destruction. The most widely used and accepted classification of periodontal diseases is the one developed and discussed at the 1999 international workshop for the classification of periodontal diseases organized by the American Academy of Periodontology[3]. This classification distinguishes between eight groups of periodontal diseases and conditions (Appendix 1). In the present study, only chronic periodontitis, one of the categories of the various diseases, was studied.

Chronic periodontitis, identified in the above mentioned classification, is defined as an infectious condition of the supporting tissues of the teeth caused by specific microorganisms or groups of specific microorganisms, resulting in progressive destruction of the periodontal ligament, alveolar bone with pocket formation, recession or both[30]. The destructive changes seen in periodontal diseases are brought about by the interplay between the microbial biofilm at the gingival margin and the host response[31,32]. The extent to which progression of the disease will take place depends on the sex, age, race, socioeconomic status, education, psychological symptoms, genetic factors, nutritional factors, and systemic conditions[2]. All these risk factors and risk indicators have been studied extensively over the years [2]. In his review Albander in 2000, showed that global risk factors and indicators include geographical region, oral hygiene level, smoking, diabetes mellitus, age, gender, race-ethnicity, genetic factors, bacterial specificity, host response factors, viruses, socioeconomic status, osteopenia, osteoporosis, psychological factors and local factors[2]. These factors, he concluded had important modifying roles in the pathogenesis of chronic periodontitis. In Kenya, the absence of a national oral health survey prevents precise identification of risk factors of chronic periodontitis in the population. Nevertheless age, male gender, plaque, dental calculus, and rural residency have been identified as risk factors of periodontal diseases in Kenya[8, 14, 15].

The relationship showing that dental plaque leads to the development of gingival inflammation has been well documented starting with the experimental gingivitis

studies of Loe and co-workers, 1965[33]. It has been shown that after 8 hours, without oral hygiene, bacteria plaque is usually found at concentrations of 10^3 to $10^4/mm^2$ of tooth surface and increases by a factor of 100 to 1000[34]. Visible plaque is clinically evident after 36 hours and gingivitis develops within 7-21 days of plaque accumulation. The microbiota associated with plaque induced gingivitis is composed of approximately 56% gram positive species and 44% of gram negative species, of which 59% are facultative and 41% are anaerobic[35]. This association between bacterial deposits and gingivitis has further been demonstrated in epidemiological studies in many parts of the world where the amount of plaque deposits measured by the oral hygiene indices/plaque indices has been correlated to the severity of gingival inflammation[14,36,37,38,39,40,41].

Chronic periodontitis is the most frequently occurring form of periodontitis in mankind[3]. This form of periodontal disease can be considered an immunological disease since the localized chronic inflammation associated with the disease process exhibits several unique immunological features, which include elevated cellular and humoral immune responses[42]. The inflammatory response seen in chronic periodontitis is initiated by bacteria and their products, mainly lipopolysacharides (LPS), but the progression is determined by the host. The host response provides tissue resistance to bacterial invasion but also in the process initiates mechanisms that lead to tissue damage. It has become clear that the host derived enzymes known as matrix metalloproteinases (MMPs) as well as changes in osteoclast activity driven by cytokines and prostanoids cause most of the tissue

destruction in the periodontium[43]. IL1β cytokine has been shown to increase production of MMPs [43]. Periodontal disease is characterized by high concentrations of MMPs, cytokines and prostanoids in the periodontal tissues, whereas in the absence of disease, the exact opposite presentation is observed[44].

Extensive studies have been carried out over the years to examine the nature of the inflammatory infiltrate in chronic periodontitis so as to understand the disease process. This infiltrate is characterized by an influx of leukocytes. There are a number of factors that promote the recruitment of the leukocytes including bacterial products, cytokines, chemokines, lipid mediators, interactions between the innate and adaptive immune responses and the complement system[32]. This review will focus on cytokines, since the study investigated genetic factors that influence the production of cytokines. Cytokines are small peptides that are produced by many different cell types like the polymorphonuclear cells (PMNs), T cells, macrophages and dendritic cells among others. They are 'cell to cell' messengers and when they attach to a cell receptor, switch on particular intracellular messenger systems within the cell and this leads to a particular function that is associated with the activated system, such as the recruitment of inflammatory cells. Evidence that cytokines play a role in destruction of the periodontium has been shown on non-human primate models. Inhibition of interleukin-1 using human soluble IL-1 receptor type 1(sIL-IRI) significantly ($p<0.05$) reduced the progression of periodontal bone loss and loss of attachment in animals with induced periodontal disease compared to control animals which did not receive the inhibitor[45,46]. Additionally, to demonstrate that IL-1 has a

potentiating effect on periodontitis, an experiment was done on a rat model where an application of recombinant human IL-1β cytokine on a ligature was applied to the teeth over a two week period and the results showed accelerated alveolar bone destruction and inflammation[47]. Taken together, these studies show that IL-1 cytokine has a role to play in the destruction of the periodontium. IL-1 proteins (IL-1α and IL-1β) are encoded by the IL-1A and IL-1B genes respectively and variations in these genes may be of importance in the development of CP.

2.2 SUSCEPTIBILITY TO PERIODONTAL DISEASES

It is well known that not all gingivitis cases will progress to periodontitis and that bacteria in plaque are necessary but not sufficient for the development of periodontitis, a susceptible host is needed[2, 32]. Furthermore, gingivitis has been shown to be a poor predictor of the development of periodontitis in persons under the age of thirty[48]. Thus genetic variance and exposure to environmental risk factors have been suggested as the key determinants in individual disease susceptibility and progression.

The role of genes in influencing susceptibility to any disease process is well known[6]. There are monogenic disorders like Huntington's disease where almost everyone with the mutation will develop the condition and multifactorial diseases like periodontitis, where multiple genes are referred to as susceptibility genes or alleles [49]. This is because the multiple genes are only associated with the disease process. For these later diseases, individuals who inherit susceptibility alleles will not develop the condition unless they are exposed to environmental risk factors such as

pathogenic microorganisms, poor oral hygiene and cigarette smoking among others[50]. Trombelli et al in 2004 showed that exposure to the same amount of plaque results in various responses by the gingival tissues[51, 52]. They identified two groups of responders. These two groups were the high responders who quickly develop inflammation in the presence of plaque and the low responders who take a longer time[51, 52]. Thus the phenotype is determined by the interactions between the genes and the environment.

2.3 RELATIONSHIP BETWEEN PLAQUE, GINGIVITIS, AND LOSS OF ATTACHMENT (CHRONIC PERIODONTITIS)

The early studies by Loe and co-workers (1965), demonstrated the causal relationship between plaque and gingivitis[33]. However, the factors involved in the progression from gingivitis to periodontitis remain unclear. A study by Muller et al in 2000 on 127 young adults of 17-30 years, confirmed the relationship between plaque, bleeding and recession[52]. They reported that subjects with a strong and positive association between plaque and bleeding had more gingival recession than those without a positive association. Moreover, they also found that the risk of bleeding was increased by 67.4% or 2 fold in the presence of supra-gingival plaque. However, there was a large variation according to tooth type with odds ratios ranging from 1.4 in molars to 2.6 at lower premolars. The relationship between plaque and bleeding on probing was stronger on the buccal surfaces, with odds ratio of 2.6, 95% CI of 2.0-3.3. Grossi et al in 1994, while studying 1,426 subjects aged 25-74 years found that age was the factor most strongly associated with attachment loss; odds ratio = 1.7, 95% CI = 1.2-2.5 in 35-44year olds[53]. This association remained

significant even after controlling for gender, socio-economic status, income, education and oral hygiene. Another study of men only also found an association between attachment loss and bleeding in <65 year olds with odds ratio = 2.1, 95% CI = 1.5-3.1[54]. Plaque was also strongly associated with bleeding on the buccal surfaces in a different study by Muller and Heinecker 2002, where the odds ratio was found to be 1.80, 95% CI of 1.2-2.7[55]. When they investigated the palatal surfaces, plaque was associated with periodontal probing depth (odds ratio = 1.9, 95% CI = 1.2-2.8), thus showing that plaque is associated with bleeding on probing and attachment loss.

2.4 LOCAL FACTORS WHICH MODIFY PERIODONTAL DISEASES

There are factors in the literature that have already been established or implicated as modifiers of gingivitis and eventually periodontitis. These factors which modulate periodontal disease expression can be grouped into local factors and systemic factors. Local factors which have already been implicated in the deterioration of periodontal health include: developmental or anatomical tooth variations-palato-gingival groove, [56] enamel pearls, [57] crowding, [58] pathological tooth conditions-fractures, [59] caries, [60] gingival anatomical condition-recession defects, [61] frenum position, [62] iatrogenic factors such as subgingival restorative margins, [63] overhangs, [64] partial dentures, [65] orthodontic appliances, [66] maxillofacial anatomic variants such as inadequate upper lip coverage, [67] epipharyngeal adenoids, deviated nasal septum leading to mouthbreathing which changes plaque accumulation and gingivitis expression leading to increased gingivitis in the maxillary anterior

segment[67, 68]. The prevalence of these risk factors listed above or their role in periodontal disease has not been determined in the Kenyan population.

2.5 SYSTEMIC FACTORS WHICH MODIFY PERIODONTAL DISEASES

The systemic modifying factors which have been shown to modulate expression of periodontal disease include fluctuations in the sex hormones during puberty [69] and during pregnancy[70]. In addition, diabetics whether insulin dependent [71] or non-insulin dependent, [72] have significantly higher gingival inflammation compared with non-diabetics with similar plaque levels.

The issue of prevalence of chronic periodontitis in HIV infected persons is still unresolved with some studies showing increased prevalence [73, 74, 75] and others showing no difference with the normal population. Ndiaye et al 1997 [74] studied the extent of periodontal disease in an HIV infected population in Senegal that was not receiving antiviral or antimicrobial therapy and found that the number of individuals with sites of ≥6mm attachment loss was significantly higher in the HIV cohort compared to an HIV non-infected group. A longitudinal study where patients were followed for 20 months, reported that immunosuppression in combination with increasing age may be a risk factor for attachment loss[73]. The HIV positive subjects also developed significantly higher mean GI scores overtime compared with HIV negative individuals. This study however was on homo/bisexual individuals[73]. Other studies report similar levels of disease in HIV infected and uninfected individuals[75, 76, 77, 78, 79]. Thus an association between periodontal disease and immune status of HIV-infected individuals remains unclear. For example, polymorphisms at IL-1A–889

(rs1800587) and IL-1B+3953 (rs1143634) (subsequently renamed +3954) have been reported not to significantly affect periodontal parameters in HIV infected individuals in a study done by Price et al, 1999[80].

2.6 GENETIC RISK FACTORS IN PERIODONTAL DISEASES

Studies have shown that gingival tissue response to plaque varies significantly between individuals with similar plaque levels both quantitatively and qualitatively and that genes are the contributory factors to this variation[81, 82]. In experimental gingivitis trials, Down's syndrome individuals manifest more extensive and severe gingivitis than age and sex matched genetically healthy controls[83, 84]. Thus, demonstrating a genetic basis for the differences observed in the severity of gingivitis. Goodson et al. 2000 [85] compared seven (7) periodontally healthy IL-1 genotype-positive subjects (individuals having a combination of allele 2 of IL-1A [rs1800587] -889 plus allele 2 of IL-1B [rs1143634] +3954) with 13 periodontally healthy IL-1 genotype negative subjects and found significant differences in bleeding on probing between the two groups after ten days of plaque accumulation. They reported an increased susceptibility to the development of gingivitis in those who were genotype positive.

Kornman et al, 1997 [18] have demonstrated an association between specific composite genotype of the IL-1 gene cluster and periodontal disease severity. The genotype that was associated with chronic periodontitis was composed of a polymorphism in the gene for production of cytokine IL-1α (IL-1A [rs1800587] -889 or IL-1A [rs17561] +4845) and in the gene for production of cytokine IL-1β (IL-1B

[rs1143634] +3954). The degree of increased risk of severe periodontitis for genotype-positive patients was estimated to be about 6.8 times greater as compared to genotype-negative individuals. It is estimated that approximately 30 percent of the Caucasian population may be positive for this genetic marker[86]. This relationship was observed in non-smokers only. The effect of smoking on periodontitis is pronounced and thus masks this relationship and only on removal of smokers during data analysis were the effects of genetic predisposition observed[18].

Allele 2 (change of proteins from C to T designated as C/T) of the IL-1B+3953(renamed +3954) polymorphism has been linked to an increase in IL-1β cytokine production in vitro[87]. Engebretson and co-workers in 1999 [88] on the other hand showed that carriage of the IL-1 periodontitis associated genotype (IL-1A +4845 and IL-1B+3954) was also related to increase IL-1β cytokine expression in vivo. In their study, they showed that amongst patients with similar disease severity, those with periodontitis associated genotype (PAG) demonstrated elevated levels of cytokine IL-1β in GCF and in the gingival tissues. IL-1β has been shown to be a pro-inflammatory cytokine which is involved in periodontal tissue destruction[88]. A study by Lopez et al in 2005 [89] also showed that individuals carrying the positive genotype for IL-1 polymorphism (IL-1A-889 and IL-1B +3954) have a significantly greater risk for developing periodontitis.

Studies that have examined identical twins reared together and identical twins reared apart, determined that more than 30% of the clinical signs of disease severity were the result of genetic factors rather than environmental factors[5, 6, 7].

Furthermore, these studies on twins have indicated that a considerable amount of individual variability to periodontitis may be attributed to genetic rather than environmental factors[6]. These observations and the study on Sri-Lankan tea labourers by Loe and co-workers, [1] strongly suggest that genetics may have a role in chronic periodontitis.

One study investigated the effect of sibling relationship on the periodontal condition in a group of Indonesians deprived of professional dental care and reported that the results obtained supported the hypothesis that there is a genetic basis for chronic periodontitis and that chronic periodontitis aggregates in families[90]. They compared data from 23 families with three or more siblings in each family and reported a mean interproximal loss of attachment (LA) in this population of 0.29mm and a range for individual mean LA of 0 to 1.27mm. A longitudinal study on this same population of 23 families but on married couples showed that after 10 years of cohabitation, the periodontal condition of a spouse did not influence the partner's condition. This finding suggests that there is a genetic basis for chronic periodontitis since there were similarities in attachment loss between siblings and none between the spouses even after living together for 10 years under the same environmental and possibly the same behavioral exposure[91].

2.7 EVIDENCE FOR THE ROLE OF GENETIC POLYMORPHISMS IN CHRONIC PERIODONTITIS IN CAUCASIANS

One of the earliest studies in Caucasians on genetic polymorphisms and chronic periodontitis was by Kornman et al 1997, [18] where they reported a 7 fold increase in

severe periodontitis in those with PAG (IL-1A -889 combined with IL-1B +3954). Several studies done on Caucasians followed and they showed that there were variations in the carriage of the IL-1 alleles. IL-1A-889 (change of proteins from C to T designated C/T) varies from 43% to 90% in patients and from 35% to 79% in controls, as reported by a meta-analysis study by Laine et al in 2010[92].

A study by Gore et al 1998 on 64 sex and age matched Caucasians, 32 cases and 32 controls, showed that the frequency of genotype IL-1B +3953 (renamed +3954) allele 2 was significantly increased in patients with advanced periodontitis, when compared with those of the early and moderate categories collectively ($p=0.043$; $\chi^2 =4.10$, $df=1$) and also when compared with healthy controls although this did not reach significant levels ($p=0.084$; $\chi^2=2.99$. $df=1$). However, no significant differences in the distribution of IL-1B -511 [rs16944] and IL-1A -889 [rs1800587] allele 2 were found between patients of the three disease severities and controls[93].

In the year 2000, research that was carried out on twins provided further evidence that a person's genes play a major role in the onset and severity of periodontal disease. This study by Michalowicz et al in 2000 concluded that approximately half of the variance in periodontal disease in the population could be attributed to genetic differences[6]. The study examined periodontal health in 64 pairs of identical and 53 pairs of fraternal twins. The study found that between 48 and 59 percent of the differences in measures of periodontal disease such as attachment loss and probing depth, could be attributed to genetics. This heritability remained the same even when adjustments for behavioral variables including smoking were made[6]. Pociot et

al in 1992 reported that the IL-1B +3954 allele 2 polymorphism associated with severe periodontitis in their study was correlated with a two- to four-fold increase in IL-1β cytokine production in response to lipopolysaccharides (LPS) stimulation of monocytes[87].

A study by Lang et al in 2000, investigated the relationship between composite genotype and inflammation and reported that genotype-negative subjects had significantly lower percentages of bleeding on probing (BOP). They concluded that the increased BOP prevalence and incidence observed in IL-1 genotype-positive subjects indicated that some individuals had a genetically determined hyper-inflammatory response that was expressed in the clinical responses of the periodontal tissues[94].

There are also some studies that do not support the association between the composite genotype and chronic periodontitis. Meisel et al 2002 [95] found that non-smokers, even genotype positive individuals, were not at an increased risk to developing periodontitis. Furthermore, they also found that the composite genotype had a strong interaction with smoking. This might imply that smoking has a masking effect in the relationship between genes and chronic periodontitis.

Papapanou et al in 2001 [96] while reporting on 132 periodontitis patients who were age and sex matched with controls, showed that there was no association between composite genotype and periodontitis. Among the 205 subjects (cases and controls), 42.9% were genotype positive; 26.8% of the cases and 16.1% of the controls (χ^2

=0.24, df=1, p=0.624). However, IL-1 polymorphism had a statistical significant impact on severity of attachment loss (% sites with AL ≥ 6mm, p=0.008), indicating that genotype positive subjects had more severe attachment loss than genotype negative subjects. Struch et al in 2008 [97] also reported no significance (p=0.07) when studying IL-1B +3954 polymorphism in a Caucasian population of 893 chronic periodontitis patients and 493 controls. This study examined only one locus for the gene polymorphism. Addition of other loci or combinations of other polymorphisms may have produced a different effect.

A meta-analysis investigation by Nikolopoulos et al 2008 [98] to check whether IL-1A (-889) (C/T) and IL-1B (+3954) (C/T) were associated with CP reported that there was an association between chronic periodontitis and IL-1A (-889) (C/T) and IL-1B (+3954) (C/T) in Caucasians.

2.8 EVIDENCE FORTHE ROLE OF GENETIC POLYMORPHISM IN CHRONIC PERIODONTITIS IN ASIANS

The prevalence of IL-1 composite genotype is low among Asian populations. The prevalence reported in Chinese subjects is 2.3%, [99] 2% in Thai subjects, [100] 0.2% in Japanese persons, [101] and 14% in Indians[102]. Other Indian studies reported no association of IL-1B (+3954) and chronic periodontitis[103, 104]. Thus, the use of the composite genotype IL-1A (+4845) allele 2 and IL-1B (+3954) allele2 for determining susceptibility in Asian patients is questionable[111]. Other genotypes such as the IL-1B (-511) or IL-1A (-889) have not been reported in relation to CP in Indian subjects.

In one recent study on a South Indian population by Prakash and Victor 2010, [105] IL-1B (+3954) was reported as being present in 28% of the chronic periodontitis group and 8.7% of the control group, suggesting that this interleukin may have a role as a risk factor in this particular population. Another study on Indians also reported a positive association between the composite genotype (allele 2 of IL-1A (+4845) combined with allele2 of IL-1B (+3954)) and chronic periodontitis[106]. This implies that ethnicity has a role to play since other Indian populations did not report similar findings.

2.9 EVIDENCE FOR THE ROLE OF GENETIC POLYMORPHISMS IN CHRONIC PERIODONTITIS IN ARABS

In the Arab population, a recent study reported 52% of the non-smoking healthy young adults with gingivitis to be positive for the IL-1 composite genotype polymorphisms[107]. This is the highest percentage reported so far. The relationship between supra-gingival plaque and bleeding on probing was marginally significant (odds ratio of 1.674, 95% CI of 1.497-1.872), indicating that susceptibility possibly contributed by the presence of the IL-1 composite genotype may be attributed to the inflammatory changes observed.

2.10 EVIDENCE FOR THE ROLE OF GENETIC POLYMORPHISMS IN CHRONIC PERIODONTITIS IN AFRICANS

A study done on African Americans by Walker et al 2000,[108] has reported on the prevalence of IL-1 polymorphisms but in relation to aggressive periodontitis and not chronic periondontitis. They reported a high prevalence of IL-1 polymorphism in the African American population studied and concluded that this polymorphism would

provide little diagnostic or predictive information for localized aggressive periodontitis.

IL-1 polymorphisms in African populations are limited. The prevalence of IL-1A (+4845) allele 2 was 46.9 % in cases and 22 % in controls and IL-1B (+3954) 15.8 % in cases and 14.3 % in controls in a study on Xhosa in South Africa.This study showed that IL-1 composite polymorphism was not associated with severity of periodontitis in this South African population[109].

2.11 SOCIO-DEMOGRAPHIC RISK FACTORS OF PERIODONTAL DISEASES

Age, gender, socio-economic status, educational level, psychological stress, nutrition, smoking and even oral health seeking behaviour have been identified as sociodemographic risk factors that modify periodontal disease progression[110,111,112,113]. Smoking has been well documented to influence clinical expression of gingival inflammation[114]. Smokers express less gingivitis during experimental gingivitis trials despite there being no qualitative difference in plaque accumulation when compared with non-smokers [115]. The explanation may be that smoking byproducts exert local vasoconstriction which reduces blood flow, edema and clinical signs of inflammation[116]. Smoking may be an important risk factor in periodontitis and the effect of nicotine will be present even in those not genetically susceptible to disease. Thus smoking related susceptibility may obscure the genetic related susceptibility. It has been recognized that smoking exerts a stronger effect on chronic periodontitis than genetic polymorphism.

In several studies, by Borrell and co-workers, [117,118,119] it was found that African-Americans had a higher prevalence of chronic periodontitis than Caucasians (29.8% vs 17.7%) and this pattern remained after adjustment for age, gender, availability of insurance, time since last dental visit, history of diabetes, smoking and income. The association between race and periodontitis was significant. This effect of race may be modified by dental checkup visit frequency. African Americans with at least once a year checkup had a fourfold higher odds of established periodontitis than their Caucasian counterparts with dental checkups at least once a year; while African Americans with a dental checkup once every two years or less often were more than fourfold less likely to have established periodontitis than their Caucasian counterparts. This study showed that those who utilize dental services may be doing so because of an existing problem, thus implying that the African American group had more disease.

When they examined the effect of education, African Americans in the higher education group exhibited prevalences of periodontitis that were 2.3 and 4.9 times higher than those of their Caucasian and Mexican-American counterparts, respectively. This pattern was consistent for income categories as well. In general, the prevalence of periodontitis was highest among males, those without insurance, those without a dental visit within the last 6 months, those with a self-reported history of diabetes, and those who smoked. For marital status, the pattern for the prevalence of periodontitis was somewhat different for each racial/ethnic group, with married people exhibiting the lower prevalence regardless of their race/ethnicity.

African Americans exhibited a direct association between income and periodontitis, whereas their Caucasian counterparts exhibited the expected inverse association. The joint effect of income and neighborhood socio-economic status was significantly associated with higher odds of severe periodontitis among Caucasians of lower income, after adjustment for age, gender, recruitment center and education. Whereas for the African Americans, the odds ratio for the same association was OR=2.0; 95% CI = 1.2-3.3.

Utilization of oral health services among individuals in the developing world depends on the socio-economic status of the family, the size of the family, the availability of dental services, cultural beliefs and perceptions, educational status, gender and age. The distance travelled to the nearest health facility and availability of transport also have an impact on the utilization of any health service[120, 121, 122, 123, 124]. Reports from the few African studies available show low utilization of oral health services and the visits that are actually undertaken to the health facilities are done so for the relief of symptoms [74]. In the Taita Hills, individuals have to trek long distances in search of dental services whereas the Swahili do not have to travel such long distances as the services are easily accessible in Mombasa Town. The utilization of dental services by the two ethnic groups under study was investigated in this study.

The Taita are a Bantu speaking group of people who occupy the southeastern part of Kenya. They are thought to have migrated from central Africa around 100 BC and 700 AD along with other Bantu people and arrived in present day Kenya through Tanzania to settle in the Taita Hills. The Swahili, on the other hand, also having a

Bantu ancestry, trace their ancestry to the early Bantu communities of the first millennium AD and settled along the coastline[125]. Therefore, interactions between the two ethnic groups would have been limited thereafter due to their geographic locations[126]. These two groups of people although having a common origin subsequently evolved separately because of their geographical location and are now quite different with the Swahili being, Muslim and light-skinned. This is probably due to intermarrying with Arab and European merchants who came to the coastal belt looking for trade. They survive on a market economy. The Taita people remained dark-skinned, largely Christian and are subsistence farmers[126]. A study on genetic biodistance on the two ethinic groups showed two clear distinct groups with some overlap in similarity in the genetic loci studied[126]. This being an indication that the two groups are African in origin and have some similarities in their genetic makeup that comes from their common ancestry but are also clearly different in their genes, appearance, religion, culture and language.

CHAPTER 3

PROBLEM STATEMENT, JUSTIFICATION, OBJECTIVES, HYPOTHESES AND VARIABLES

3.1 PROBLEM STATEMENT

In Kenya, various studies have shown that only a fraction of the population seems to get destructive periodontitis[13, 14]. This observation is seen when different groups are examined even those born before 1957 when the diet and dental services were completely different from today[127]. It is therefore important to identify susceptible individuals early through the study of risk factors such as the interleukin-1 genotype, so as to provide early treatment and thus minimize the adverse effects of chronic periodontitis on the systemic health status of the individual. The interrelationships between periodontal health and systemic health have been confirmed by various studies[69,70,71,72].

Oral health is an integral part of general wellbeing in all individuals. In a report in 2000, [128] by the Surgeon General on oral health in America, it was stated that the oral cavity is a portal of entry as well as the site of disease for microbial infections that affect general health status. Thus the mouth can become the source of disease or pathological processes that affect other parts of the body. It has been reported that disease-causing oral bacteria can be inhaled, leading to respiratory conditions[129,130]. Chronic periodontitis is a risk factor for cardiovascular disease[131] and can make the control of diabetes more challenging[131]. Chronic periodontitis if left

untreated in susceptible individuals will eventually lead to tooth loss. Tooth loss leads to craniofacial imbalance and may subsequently affect the quality of life of the individual due to loss of self-esteem and poor nutrition. The effects on nutritional status is due to difficulties in chewing leading to selective eating of soft foods which are usually high in unrefined carbohydrates of poor nutritional value[132].

Chronic periodontitis is a multifactorial disease with gram negative anaerobes initiating the tissue destruction through a series of inflammatory and immunological changes. This is further complicated by gene polymorphisms of IL-1, [18] which modify the inflammatory response. Sociodemographic features have been extensively studied and also found to be associated with chronic periodontitis[2].

Thus the study of factors that may be associated with chronic periodontitis including gene polymorphisms of IL-1 will enable clinicians to identify susceptible individuals early enough to institute preventive measures and therefore minimize the destructive effects of the disease which may ultimately affect the general health of the individual.

3.2 JUSTIFICATION

The association of genetic polymorphisms of the IL-1 gene and chronic periodontitis in Kenya has not been studied to date. This study was carried out to investigate whether there is any association between interleukin-1 gene polymorphisms and chronic periodontitis in two Kenyan coastal communities, the Taita and the Swahili.The Taita are known to be conservative and practice endogamy, which

means that they do not typically marry outside of their ethnic group[133]. The Swahili on the other hand marry outside of their ethnic group (exogamy) and this might produce a greater amount of genetic variability in this group. They have been known through history to intermarry with Arabs among others[126]. Recently, studies on an Arab population reported them as having interleukin-1 polymorphisms in 52% of the population[107]. The determination of the interleukin-1 polymorphisms in the two Kenyan communities and their association with chronic periodontitis will help to explain whether mixed heritage would affect the genetic variability of IL-1 gene and subsequently chronic periodontitis.

3.3 RESEARCH QUESTION

Do genetic polymorphisms of IL-1B and IL-1A exist in two Kenyan coastal communities of Bantu origin and if they do, what is their relationship with chronic periodontitis?

3.4 GENERAL OBJECTIVE

To determine whether there is an association between genetic polymorphisms of IL-1 and chronic periodontitis amongst adult Taita and Swahili participants.

3.5 SPECIFIC OBJECTIVES

1. To determine the association between sociodemographic characteristics and chronic periodontitis amongst cases and controls in Taita and Swahili participants.

2. To determine the severity of chronic periodontitis amongst the cases in Taita and Swahili participants.
3. To determine the distribution of interleukin-1 genotype polymorphisms of IL-1B at position -511 [rs169447] and +3954 [rs1143634] and IL-1A at position -889 [rs1800587] and +4845 [rs17561] amongst cases and controls in both ethnic groups.
4. To evaluate whether there is an association between IL-1B (-511, +3954) and IL-1A (-889, +4845) polymorphisms and chronic periodontitis in both ethnic groups.
5. To compare the carriage rate of interleukin-1 genotype polymorphisms at IL-1B (-511, +3954) and IL-1A (-889, +4845) amongst Taita and Swahili participants.

3.6 NULL HYPOTHESES

1. There is no association between socio-demographic characteristics and chronic periodontitis amongst Taita and Swahili participants.
2. There is no association between genetic polymorphisms of IL-1 [IL 1B (-511, +3954) and IL 1A (-889, +4845)] and chronic periodontitis amongst the Taita and Swahili participants.
3. The alleles of interleukin-1 polymorphisms that are associated with chronic periodontitis are the same amongst the Taita and Swahili participants.

3.7 VARIABLES

The study variables that were assessed were grouped into three categories. These are; the independent, the socio-demograhic and the dependent variables. The measures of these variables are shown in table 1.

Table 1: Study Variables

Variables	**Measurement**
Independent/Explanatory variables	
Genetic marker - IL-1 genotype	IL-1A (-889, +4845), IL-1B (-511, +3954)
Oral hygiene practices	Oral hygiene habits like tooth brushing
Oral health seeking behavior	Whether they have had dental treatment or not.
Oral hygiene status	Presence or absence of plaque
Sugar consumption	Number of times in a day sugary food or drink is comsumed
Socio-demographic variables	
Age	Number of years
Gender	Whether male or female
Ethnicity	Whether Taita or Swahili
Education	Highest level of education attained
Marital status	Whether married, single or divorced/separated
Residence	Village where they came from
Occupation	Type of work done
Income	Earnings per month (low, middle and high)
Employment	Employed, self employed or unemployed
Dependent/Response variables	
Chronic periodontitis	• Clinical attachment Loss (CAL) • Pocket depth • Bleeding on probing (gingivitis)

CHAPTER 4

MATERIALS AND METHODS

This chapter describes the population examined, the methodologies used to collect the data and the laboratory procedures used to determine the genotype levels. The study area, population sampled and the design is described in detail as well as the sample size determination and selection. The data collection and laboratory procedures are also explained systematically and in detail.

4.1 STUDY AREA

Kenya is one of the countries located in the East African region. It covers an area of 582,000 square kilometers and is divided into forty seven counties. The population of Kenya was estimated to be 38.6 million in 2010[134]. Approximately 80% of this population lives in the rural areas and are mainly peasant farmers. Kenya's economy is mainly agriculturally driven and is slow growing. About 46% of the population lives below the poverty line with no access to health services especially oral health care.

4.1.1 SPECIFIC AREAS

Taita Taveta County is one of the forty seven counties in Kenya. It is located in the coastal region of the country. It lies approximately 200km northwest of Mombasa and 360 km southeast of Nairobi City (figure1). The county covers an area of 16,975 km^2 of which a bulk 62% or 11,100 km^2 is within Tsavo East and Tsavo West National Parks. The remaining 5,876 km^2 is occupied by ranches, sisal estates, water bodies such as Lakes Chala and Jipe in Taveta and the hilltop forests, which occupy less than 100 km^2.The population consists of approximately 250,000

people[134]. The terrain is varied with the altitude rising from 500m to 2,300 m above sea level at Vuria peak, which is the highest in the area. Mwatate and Wundanyi Divisions form part of Taita Taveta County. The study was conducted in Mwatate and Wundanyi Divisions. Taita Taveta County is where the Taita people are found.

In **Mombasa County**, the study was conducted mainly in the old town where most of the Swahili people are found[134]. Mombasa County lies along the Kenyan coast as shown on figure 1. It is divided into five regions; the town and island of Mombasa; the south coast –stretching from Mombasa to the Tanzanian border 135 km away. The north coast- covers the beaches from Mombasa up to Kilifi, 60 km away: Malindi and Watamu about 130 km to the northeast of Mombasa and Lamu Island and archipelago, 225 km further up from Malindi. The city has a population of 939,370, as per the 2009 census[134].

Figure 1: A map of Kenya showingTaita Taveta County and Mombasa County. (Adapted from softkenya.com)

4.2 STUDY DESIGN

This was a population based case-control study. The test was, whether the marker genotypes distributed differently between the cases and controls.The study began with the selection of cases from the defined populations (Taita and Swahili), followed by matching by age and gender of the cases with unaffected controls. The study population was; cases who were individuals with chronic periodontitis selected on the basis of having at least 2 non-adjacent teeth with proximal attachment loss of ≥ 3mm [135] and controls, individuals with a clinically healthy gingiva that did not bleed on probing and had no probing depth of >3mm. The controls were individuals who would have been designated study cases if they had developed the disease (figure 2).

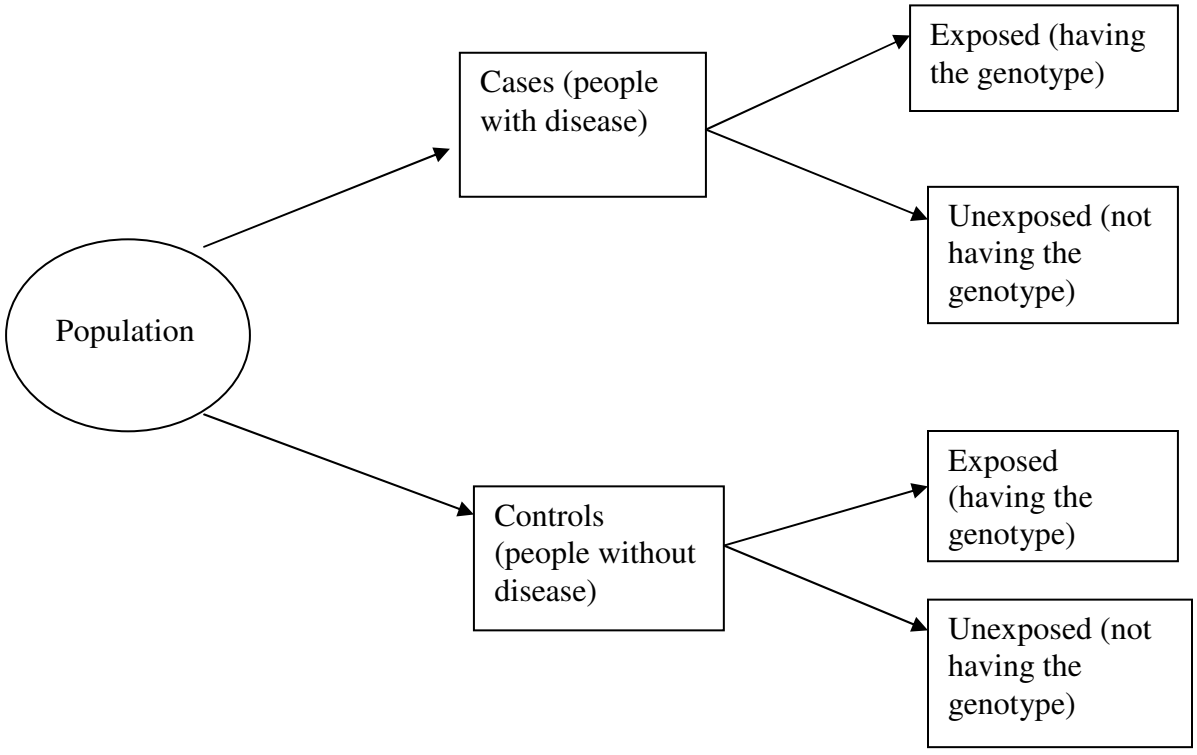


Figure 2: The design of the case control study

In the cases, proximal sites and non-adjacent teeth were selected, in order to minimize the likelihood of including lesions that are as a result of abrasion on the buccal surfaces, or habits in the interdental areas, rather than disease. The 3mm threshold was based upon studies which examined incremental attachment loss measurement, where the error of the recording method was calculated at 2.5 mm[136]. Recording >3mm attachment loss ensures that all cases actually have chronic periodontitis and of a moderate or severe form as recommended by Schafer et al 2011[137]. In this paper, Schafer et al 2011 recommend a higher level of severity in the case selection so as to improve the power of the study when numbers of less than 1000 are used. This high level of chronic periodontitis improves the statistical power of the study[138]. The severity of chronic periodontitis in this study was high as reported in chapter 5. There was also matching for age and sex so as to remove the need of statistical adjustments for these variables. Smokers and those with any systemic illness especially diabetes mellitus were excluded.

This being a population based case control study, the cases were selected from subjects who presented themselves to the various recreational centers and health centers and met the inclusion criteria. Some were recruited from door to door screening of subjects. The case control design allows for the selection of cases and controls without randomization as long as the inclusion criteria are adhered to[138]. The controls were recruited from the same area and were used to characterize the

distribution of the genotype since they had an equal chance of becoming cases if they had developed the disease.

4.3 STUDY POPULATION

The study participants were all adults of ages 35-44 years, (an age range recognized in the classification system and WHO as the age of occurrence for chronic periodontitis) residing in the Taita Hills and Mombasa old town. Taita participants were selected from a homogenous rural population with similar cultural, socio-economic status and level of education. In the same way, the Swahili participants were also selected from the same geographical area and similar cultural practices.

4.3.1 INCLUSION CRITERIA

a) Persons who were aged between 35-44 years. Chronic periodontitis is an adult onset condition commonly detected in the third and fourth decades of life[3].
b) Persons who had at least 18 teeth including 2 molars and 2 premolars in the same arch. This allowed representation of all tooth types including molars and premolars so as to capture the presentation of chronic periodontitis in single rooted teeth as well as multirooted teeth. Progression of CP may differ in the different tooth types[52].
c) Persons who consented to participate in the study

4.3.2 EXCLUSION CRITERIA

a) Persons with a history of periodontal treatment in the last six months. This would have interfered with disease definition by the inclusion of cases with a reduced but healthy periodontium as control subjects.

b) Persons who reported that they were using non-steroidal anti-inflammatory drugs, were pregnant, lactating mothers, persons found to be suffering from bleeding disorders and knew about it, persons on immunosuppressive chemotherapy, persons on substance abuse, smokers, any individual who was found to be on antibiotics or diabetic, were excluded since all these conditions are likely to modify the host response.

4.4 SAMPLE SIZE AND SAMPLING PROCEDURE

4.4.1 SAMPLE SIZE

The required minimum sample size for case control studies was calculated using a formula developed by Kirkwood and Sterne (2003)[139]. The exposure rate reported in the study by Kornman et al 1997 [18] was used since there are no known Kenyan African studies on genetic polymorphism and chronic periodontitis.

$$n = \frac{\{\beta\sqrt{[\pi_0(1-\pi_0) + \pi_1(1-\pi_1)]} + \alpha\sqrt{(2\pi'(1-\pi')}\}^2}{(\pi_0-\pi_1)^2}$$

Where α is the standard normal deviate corresponding to the two sided significance level, at 5% and β is the standard normal deviate corresponding to the one sided power of the test, at 80%;

π_0 is the estimated exposure in controls, at 0.23[18],

π_1 and π' were calculated from:-

$$\pi' = (\pi_0 + \pi_1)/2$$

$$\text{and } \pi_1 = \frac{\pi_0 OR}{1 + \pi_0 (OR - 1)}$$

OR = 2.5 which is the minimum OR considered clinically significant when comparing cases and controls in this study[136].

The specifications above yielded a sample size of 88.

Anticipating a 10% loss due to inadequate DNA collection, the sample size (per group) was 88/0.9 = 98.

A minimum appropriate sample ensures proper utilization of resources since sample size is often determined by logistic and financial considerations[137].

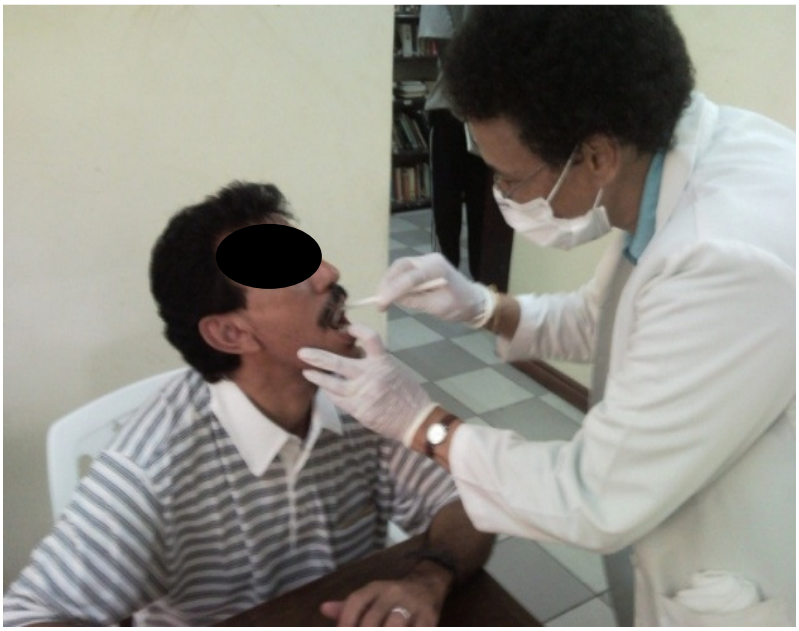
4.4.2 SAMPLING PROCEDURE

This being a case control study, a non-probability or convenient sampling method was used to accumulate the cases and controls according to the inclusion criteria[137]. Screening was done in Taita District followed by recruitment of those who met the

inclusion criteria. These individuals were selected from all those who presented themselves to Dembwa Health Center, Mpizinyi Health Center, Kidaya Ngerenyi Community Hall and Werugha Health Center. A chief's baraza was held prior to the dental examination to inform members of the public of the intended survey and explain the objectives of the study. At Kidaya Ngerenyi, most of the participants were drawn from members of the public attending a public health education and sensitization forum.

In Mombasa, screening took place in mosques where high concentrations of people of Swahili origin are usually found. The aim was to recruit participants from those attending prayers. The data collection took place at the Mosque grounds (picture 1) after the 1.00pm, 4.00pm and 6.00pm prayers. A different strategy was adopted when it became clear that participants of the female gender were few because in the Muslim culture, women stay indoors only venturing out when accompanied by their husbands or a male member of the family. Secondly, most of the women attend mosque prayers on Fridays only. A door to door strategy was adopted and 34 participants were recruited (picture 2). Finally, a camp was set up in a public area where area residents from Kaloleni usually meet socially and the final 99 participants were drawn from here. The areas covered were Kilifi, Kaloleni and Kuze all in old Town.

Picture 1: A photograph showing the mosque grounds where some of the clinical examinations took place



Picture 2: A photograph showing a typical clinical examination of a participant in his home

4.5 DATA COLLECTION TOOLS, CLINICAL EXAMINATION AND LABORATORY PROCEDURE

4.5.1 TOOLS FOR DATA COLLECTION

A modified WHO questionnaire on oral health seeking behavior, oral health practices and sugar consumption was used (Appendix 4). A Kiswahili version was used for those who did not understand English (Appendix 5). A clinical examination form was used to record data on recession on the following six sites, mesio-buccal, buccal, disto-buccal, mesio-lingual, lingual and disto-lingual areas. Probing depth measurements were also done on the same six sites per tooth. Bleeding on probing, calculus and plaque were recorded as present or absent. Dental caries was recorded on the mesial and distal surfaces on all teeth except third molars. (Appendix 6).

DNA collection was carried out using the isohelix buccal swabs (BocaScientific, Isohelix, Kent, England) (figure 3) by scrapping the cheek for a minimum of 30 seconds with the buccal swab, which was then returned to its plastic tube after air drying it for 30 seconds as shown in figure 4. This was then sealed with the attached top as shown in figure 4. This plastic tube was placed in a red biohazard resealable bag obtained from the laboratory and sealed ready for transportation to a -20^0C environment as per the manufacturer's instructions.

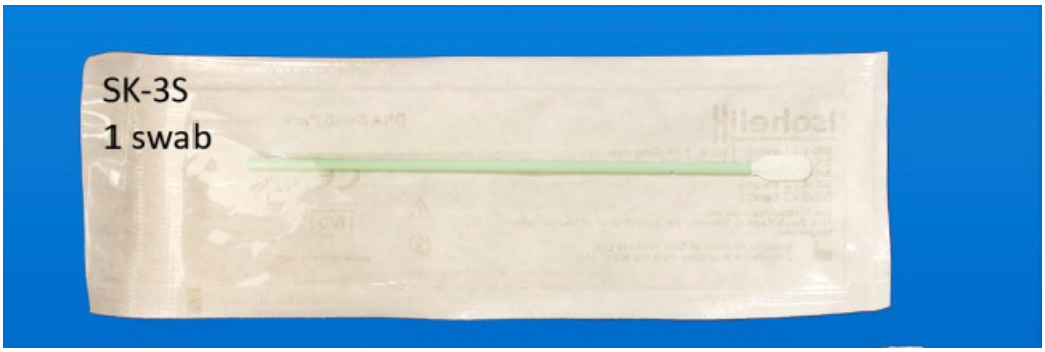
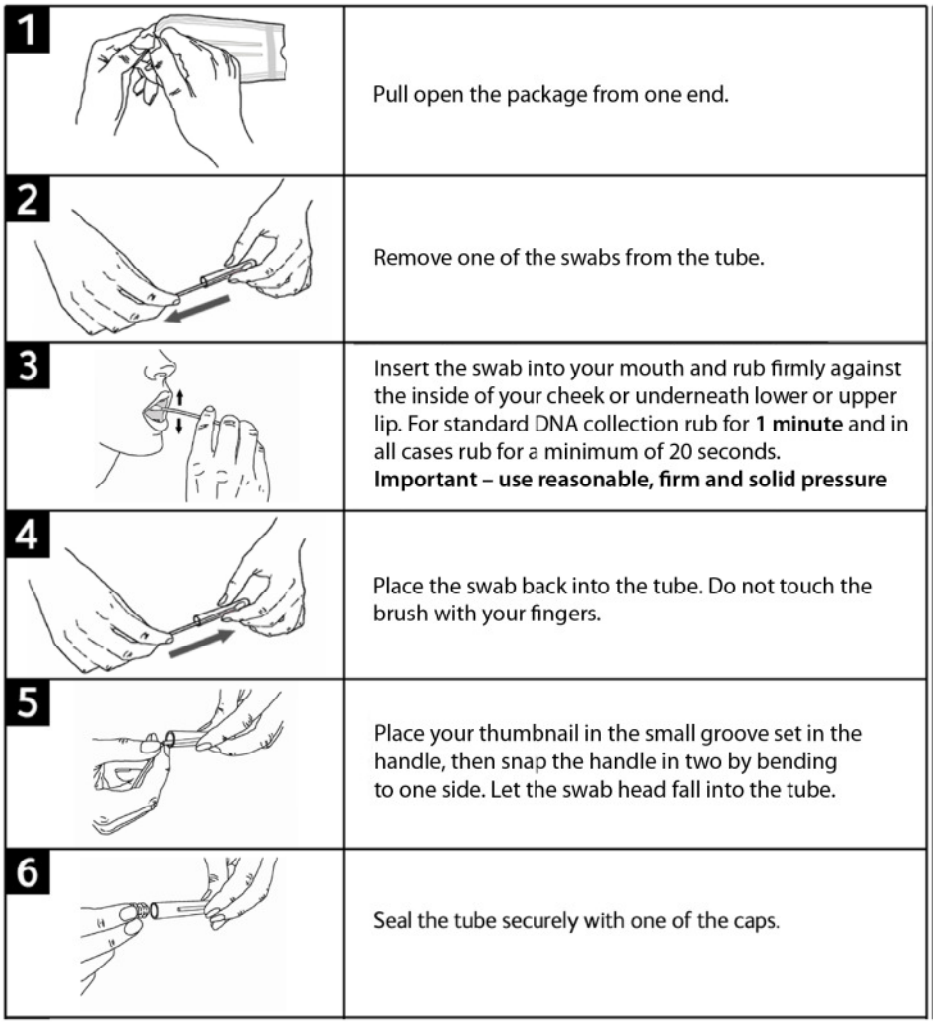


Figure 3: The isohelix buccal swabs used for DNA collection (BocaScientific).



www.isohelix.com

Figure 4. A self explanatory diagram showing how to collect DNA samples.

4.5.2 PERIODONTAL PARAMETERS

A full periodontal examination according to Papapanou et al 2001, [96] was carried out as follows:

4.5.2.1 PROBING POCKET DEPTHS (PPD)

This was the distance between the gingival margin and the bottom of the pocket to the nearest whole millimeter and was measured using a North Carolina (Hu-Friedy, Chicago III) periodontal probe.

4.5.2.2 CLINICAL ATTACHMENT MEASUREMENT

This was the distance between the cemento-enamel junction (CEJ) and the gingival margin. It was recorded using the Hu-Friedy periodontal probe to the nearest whole millimeter. This value was given a positive value in the presence of gingival recession and a negative value when the margin was found coronal to the CEJ. The sum of the two values (the probing pocket depth and the gingival margin/recession) was used to compute the clinical attachment loss (CAL).

4.5.2.3 DENTAL PLAQUE AND BLEEDING ON PROBING

The presence of dental plaque (PL) using a disclosing agent (produits dentaire vevey, Switzerland) and bleeding on probing (BOP) were recorded dichotomously. Bleeding on probing was deemed present if it occurred within 30s after running the probe back and forth along the gingival margin.

4.5.3 MISSING TEETH AND DENTAL CARIES

Missing teeth and dental caries were recorded.

4.5.4 DATA COLLECTION - PRELIMINARY PHASE

The fieldwork began in February 2010 when the initial visit to Taita District was made in order to familiarize with the area, address logistic issues and get to know the official requirements for obtaining permission to conduct the study. Discussions were held with the district medical officer of health and the public health officer (PHO). A tour of the area was conducted under the direction of the PHO. A pilot study was conducted to test the questionnaire and tolerance to the clinical examination.

4.5.5 ACTUAL DATA COLLECTION PHASE

The data collection phase for the whole study took eight months to complete starting from September 2010, after obtaining full registration and ethical approval (appendix 7 and 8). For the Taita participants, the actual data collection was done in four locations as previously mentioned in Mwatate and Taita Divisions. All the participants gave informed written consent before duly filling in the questionnaire under the guidance of a trained PHO.

The participants were first seen by the PHO, who read out the questions in English and explained in Kiswahili before duly entering the answers in the English questionnaires. Only English questionnaires were used in this area because the

PHO was more comfortable with the English version. The participants then proceeded to the Principal Investigator for the clinical/oral examination (figure 5).

4.5.6 CLINICAL EXAMINATION

The intra-oral examination in Taita District was carried out near the window under natural light and with the participant sitting on an ordinary chair as shown in the picture 3. The recording was done by a research assistant as shown in picture 3 as well.

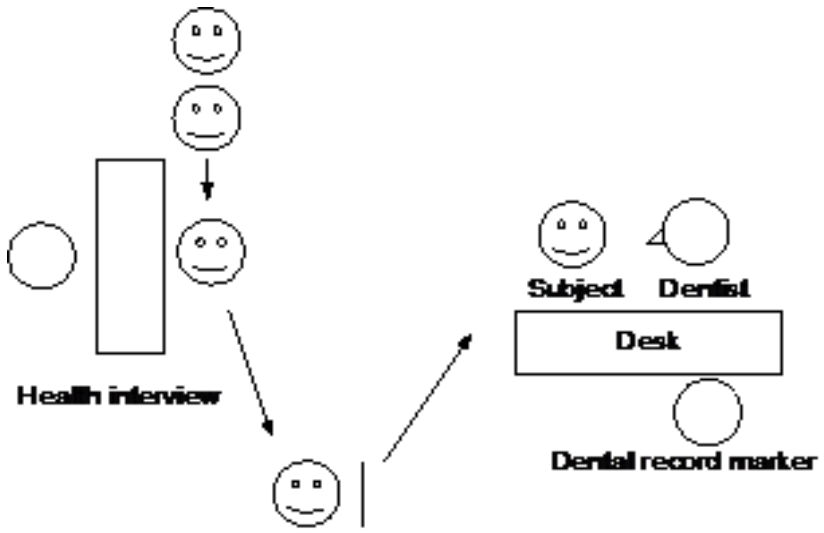


Figure 5: This is a flow chart showing the process followed during the clinical examination

Picture 3: The principal investigator carrying out a typical clinical examination in one of the sessions

The step-by-step procedure used in the intra-oral examination was to first collect the DNA sample using isohelix buccal swabs (BocaScientific, Kent, England). This was followed by the recording of missing teeth and roots. Gingival inflammation was then assessed by running the periodontal probe along the margin and waiting for 30 seconds before visual identification of areas of bleeding. Gingival recession was then recorded. This was followed by pocket depths measurements to the nearest millimeter using the Hu-Friedy periodontal probe. Recording of the presence or absence of calculus on the tooth surfaces followed and finally the recording of clinical dental caries was done. The participant was then given a disclosing tablet (produits dentaire vevey, Switzerland) to chew on so as to stain plaque. The presence or absence of plaque was visually identified and subsequently recorded.

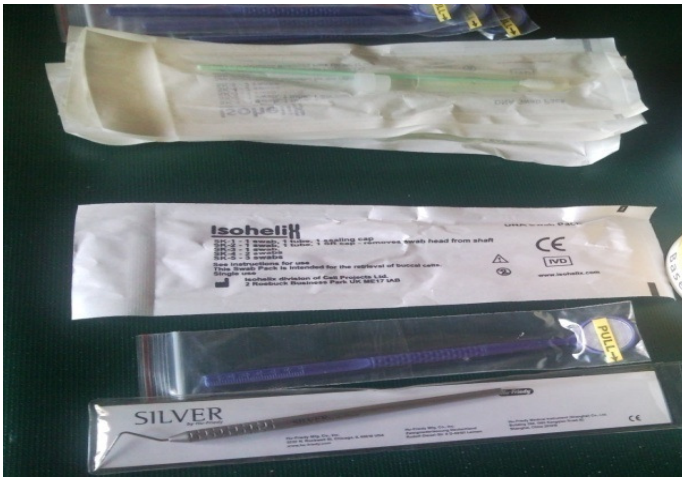
The procedure followed for data collection in Mombasa District was the same as that used in Taita District where the interview was carried out by a trained PHO and a Community Oral Health Officer (COHO) and the clinical examination by the Principal Investigator (figure 5). In Mombasa, the PHO was female to assist with the female participants and the COHO was male to help with the male subjects. This strategy was adopted because of the Muslim religious/cultural requirements that males who are not family members do not mix with females.

Most of the examinations in Mombasa took place outdoors in broad daylight except for the 34 who were recruited in the door to door strategy. In those examined in their homes, the examination took place next to a window under natural lighting. The questionnaires that were used in Mombasa were mainly the Kiswahili version. In the group of male participants, 86 Kiswahili questionnaires were duly completed and 22 English ones. In the female group, 5 English questionnaires and 87 Kiswahili ones were duly completed. The records were counter-checked at the end of each day by the PI to ensure that all the relevant information was captured and to match the cases with the controls.

Infection control

Only pre-packed sterile disposable dental mirrors and tweezers were used (picture 4). Periodontal probes were also sterilized and packed per patient. The DNA collection swabs were sterile and one was used for each individual. After DNA collection, the swabs were sealed in their individual containers as provided by the

manufacturer and labeled with the subject number. Disposable gloves and masks were used at all times.



Picture 4: The DNA collection kit, a disposable mirror and Hu-friedy periodontal probe

4.5.7 CALIBRATION

Calibration on the clinical parameters was done to the satisfaction of one of the supervisors who accompanied the PI to the field. Intra-examiner variability was assessed by re-examination of the clinical parameters of 38 Taita participants. Every fifth individual was examined and this should have given a total of 39 repeat examinations but one repeat examination was spoilt. Intra-examiner variability in Mombasa was assessed by re-examination of the clinical parameters of 27 participants.

4.6 LABORATORY PROCEDURES

The labeled swabs used to collect buccal cells from the cheeks of all the participants provided the DNA samples. Genomic DNA from cases and controls were analyzed

for polymorphisms within the IL-1A gene [-889 [140] and +4845 [141]] and the IL-1B gene [-511,[142] and +3954[143]]. Both analyses involved restriction fragment length polymorphism (RFLP) assays of PCR-amplified gene fragments before subjecting the digests to polyacrylamide gel electrophoresis.

4.6.1 DNA EXTRACTION

DNA was purified from buccal swabs using QIAmp DNA Minikit spin protocol (Qiagen, Turnberry Lane, Valencia, CA.)[144]. All centrifugation steps were carried out at room temperature (15-25^{0}C). Labeled buccal swabs were separated from the plastic stem and placed in a 2ml microcentrifuge tube (Sigma-Aldrich Inc, St Louis Missouri, USA). 400ml phosphate buffering solution (PBS) was added to the sample, followed by addition of 20µl of proteinase K (Qiagen, Turnberry Lane, Valencia, CA.) before 400µl of buffer AL was added to the sample. These were mixed immediately by vortexing for 15 s followed by incubation at 56^{0}C for 10min in a water bath. After the incubation, a brief centrifuging was carried out to remove drops from inside the lid. 400µl ethanol (96-100%) was added and mixed by vortexing and briefly centrifuged to remove drops from inside the lid. 600µl of the mixture was then placed in QIAamp Mini spin column (in a 2ml collection tube) without wetting the rim. The cap was closed and centrifugation performed at 6000xg for one minute. The QIAamp Mini spin column was removed and placed in a clean 2ml collection tube and the filtrate in the collection tube discarded. This process was repeated with the remaining 620ml mixture. The QIAamp Mini spin column was carefully opened and 500µl Buffer AW1 added without wetting the rim. The cap was closed and

centrifuging performed at 6000xg for one minute. QIAamp Mini spin column was placed in a clean 2ml collection tube and the filtrate in the collection tube discarded. QIAamp Mini spin column was carefully opened and 500µl Buffer AW2 added without wetting the rim. Centrifugation was performed at 16,100xg for 3min. The QIAamp Mini spin column was then placed in a new clean collection tube and the old collection tube with filtrate discarded. Centrifugation at 16,100xg for 1min was done. The QIAamp Mini spin column was placed in a clean 1.5ml microcentrifuge tube and 150 µl Buffer AE added and centrifugation done at 6000xg for 1min. The final DNA concentrate was measured with the NanoDrop 2000c (Thermo Scientific, Fermentas molecular biology tools, Waltham, Mass, USA).

4.6.2 GENOTYPING BY RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP)

The PCR reaction conditions were as previously described[18, 145]. Briefly, a reaction mix excluding Taq polymerase was prepared with template DNA added prior to heating at 95℃ for 15 min. Taq polymerase (Invitrogen Corporation, Grand Island NY, USA) was added and PCR started. The $MgCl_2$ and primer concentrations were varied in each type of reaction for the different loci ([rs1800587] -889, [rs17561] +4845, [rs16944] -511 and [rs1143634] +3954) and are detailed below.

The PCR reagents used were all from Invitrogen, Grand Island, NY, USA. All PCR products were stained with ethidium bromide (0.2 pg/ml) and visualized under ultraviolet light following electrophoresis. Reference is made to Appendix 9 for sample plates of the various loci.

4.6.2.1 IL-1A [rs1800587] -889

Gene-specific primers included the forward (IL-1A -889_F: 5'-AAG CTT GTT CTA CCA CCT GAA CTA GGC-3') and reverse (IL-1A -889_R: 5'- TTA CAT ATG AGC CTT CCA TG-3') primers both at 0.8 pm/µl. Amplification was carried out in a total volume of 50 µl reaction incorporating 1 µl Taq polymerase (5 U/µl), (Invitrogen Corporation, Grand Island NY, USA) 25 µl Invitrogen 2X reaction mix, 15µl nuclease free water, 2µl of the forward primer, 2µl of the reverse primer and 5 µl of DNA template (3-20ng/µl). The cycling conditions consisted of an initial denaturation step at 96℃ for 2 min, followed by 45 cycles of denaturation at 94℃ for 1 min, annealing at 50℃ for 1 min, and strand extension at 72℃ for 1 min followed by a final extension step at 72℃ for 5 minutes. The PCR amplicons were then digested at 37℃ for 10 min, with 6 units per 30 µl reaction of Nco1 (Thermo Scientific, Fermentas molecular biology tools, Waltham, Mass, USA) in a reaction mixture made up of 16 µl of nuclease-free water, 3 µl of 10x FastDigest buffer (Thermo Scientific, Fermentas molecular biology tools, Waltham Mass, USA), 10µl DNA (~0.2µg) and 1 µl FastDigest enzyme (Nco1) (Thermo Scientific, Fermentas molecular biology tools, Waltham, Mass, USA). After the restriction enzyme (RE) digestion, the enzyme was inactivated by heating the mixture for 5min at 65^0C. The restriction fragments were then separated by electrophoresis through a 30% polyacrylamide gel, and then stained by ethidium bromide (0.2 pg/ml) and visualized under UV light in the AlphaImager (Alpha Innotech Technologies ZA, California, USA). The results were documented by photography.

4.6.2.2 IL-1A [rs17561] +4845

The oligonucleotide primers IL-1A +4845 forward (5' - ATG GTT TTA GAA ATC ATC AAG CCT AGG GCA- 3') and IL-1A +4845 reverse (5'- AAT GAA AGG AGG GGA GGA TGA CAG AAA TGT- 3') [141] were used at 0.8 pm/µl. Amplification was carried out in a total volume of 50 µl reaction incorporating 1 µl Taq polymerase (5 U/µl) (Invitrogen Corporation, Grand Island NY, USA) under 25 µl Invitrogen 2X reaction mix, 15µl water, 2µl of the forward primer, 2µl of the reverse primer and 5 µl of DNA template (3-20ng/µl). Cycling conditions were as follows: initial denaturation at 95^{o}C for 1min, followed by 35 cycles at 94^{o}C for 1min, 56^{o}C for 1min, 72^{o}C for 2min and a final extension step at 72℃ for 5 min. Following amplification, the amplified DNA was digested with Fnu4H1 (SatI)-FD1644 restriction endonuclease (Thermo Scientific, Fermentas molecular biology tools, Waltham, Mass, USA) by incubation of a mixture made up of 16 µl of nuclease-free water, 3µl of 10x FastDigest buffer (Thermo Scientific, Fermentas molecular biology tools, Waltham, Mass, USA), 10 µl (~0.2µg) DNA and 1 µl Fnu4H1(SatI) (Thermo Scientific, Fermentas molecular biology tools, Waltham, Mass, USA) Fast Digest enzyme at 37^{0}C for 10min. The enzyme was inactivated by heating for 5min at 65^{0}C. The resultant fragments were separated by electrophoresis on a 30% polyacrylamide gel, and then stained by ethidium bromide (0.2 pg/ml). The fragments were then visualized under UV light in an AlphaImager (Alpha Innotech Technologies ZA, California, USA) and results documented by photography.

4.6.2.3 IL-IB [rs16944] -511

The primers used in this assay were IL-1B -511-Forward (5'-TGG CAT TGA TCT GGT TCA TC-3') and IL-1B -511-Reverse (5'- GTT TAG GAA TCT TCC CAC TT-3') at 1 µM concentration. Amplification was carried out in a total volume of 50 µl reaction incorporating 1 µl Taq polymerase (5 U/µl), (Invitrogen Corporation, Grand Island NY, USA), 25 µl Invitrogen 2X reaction mix, 15µl water, 2µl of the forward primer, 2µl of the reverse primer and 5 µl of DNA template (3-20ngµl). The cycling conditions included an initial denaturation at 96℃ for 1 min. followed by primer annealing at 50℃ for 1 min. and a strand extension at 72℃ for 1 min. This cycle was repeated twice followed by 35 cycles of 94℃ for 1 min, 50℃ for 1 min, 72℃ for 1 min and 3cycles of 94℃ for 1 min, 50℃ for 1 min and 72℃ for 1 min. Digestion of the PCR products was done using a mixture composed of 16 µl nuclease-free water, 3µl of 10xFastDigest buffer (Thermo Scientific,Fermentas molecular biology tools, Waltham, Mass, YSA) 10 µl (~0.2µg) DNA with 1 µl of 3 units of Ava1(Thermo Scientific, Fermentas molecular biology tools, Waltham, Mass, USA) per 30 µl reaction at 37℃ for 5min. The enzyme was inactivated by heating at 65^0C for 5min. The resultant fragments were separated by electrophoresis on a 30% polyacrylamide gel and then stained using ethidium bromide (0.2 pg/ml). The fragments were then visualized under UV light using and AlphaImager (Alpha Innotech Technologies ZA, California, USA) and the results documented by photography.

4.6.2.4 IL-IB [rs1143634] +3954

Here, the primers included IL-1B +3954-forward (5'- CTC AGG TGT CCT CGA AGA AAT CAA A-3') and IL-1B +3954-reverse (5'- GCT TTT TTC GTG TGA GTC CCG-3'). Both primers present at 2 pm/µl. Amplification was carried out in a total volume of 50 µl reaction incorporating 1 µl PCR Taq polymerase, (Invitrogen Corporation, Grand Island NY, USA), 25 µl Invitrogen 2X reaction mix, 15µl water, 2µl of the forward primer, 2µl of the reverse primer and 5 µl of DNA template (3-20ng/µl). Thermocycling was performed thus: 95℃ for 1 min followed by 94^0C for 1 min, 72℃ for 1 min cycled twice. These were followed by 35cycles of 95℃ for 1 min, 56℃ for 1 min, 72℃ for 1 min and 3 cycles of 95℃ for 1 min, 56℃ for 1min, 72℃for 5 min. The PCR fragments were digested at 37^0C for 5min and the reaction mixture was composed of 16µl nuclease-free water, 3µl of 10x FastDigest buffer, (Thermo Scientific, Fermentas molecular biology tools, Waltham, Mass, USA) 10µl (~0.2µg) DNA and 1µl of 10 units per 30 µl reaction of Taq 1 (Thermo Scientific, Fermentas molecular biology tools, Waltham, Mass, USA). The resultant fragments were separated by electrophoresis in a 30% polyacrylamide gel and then stained using ethidium bromide (0.2pg/ml). The products were then visualized under UV light using an AlphaImager (Alpha Innotech Technologies ZA, California, USA) and the results documented by photography.

4.6.3 DNA POLYACRYLAMIDE GEL ELECTROPHORESIS

4.6.3.1 GEL PREPARATION

The gel casting equipment (National Diagnostics, Atlanta, Georgia, USA) was used to prepare the polyacrylamide gel. Glass plates were cleaned with 70% alcohol on both sides, rinsed with ddH_2O and the gel cassette prepared according to lab SOPS. Adhering to the order in which the reagents are listed below, the following were mixed in a glass beaker:

- 12.5 ml 30% Acrylamide-Bis solution 29:1
- 3.75ml 10X TBE, pH 8.4
- 21.25 ml ddH_2O,
- 187.5µl 10% ammonium persulfate
- 75µl TEMED (always added last to prevent premature initiation of polymerization reaction)
- This gave a total volume = 37.5ml which was sufficient for 8 mini-gels.

The gel solution was injected in between the glass plates in the gel casting equipment using a 0.5mm thick gauge gel loading tips (Sigma-Aldrich, St. Louis, MO, USA). 15 well combs were fitted into place and the gel setting allowed for at least 45 minutes.

4.6.3.2 GEL ELECTROPHORESIS

The Molecular Weight Marker (MWM) and Molecular Weight Marker Buffer (MWMB) (Thermo Scientific, Fermentas molecular biology tools, Waltham, Mass, USA) were thawed on ice. The electrophoresis module was assembled. The casting dams were

filled with 0.5x TBE buffer and the combs removed. The wells were flushed with 0.5x TBE buffer by filling a syringe with the TBE buffer and then moving it slowly across the top of the gel as the buffer was expelled.

2µl of the blue orange 6x loading dye (Promega, Madison, USA) was added to each tube of PCR product and mixed. The tip was changed in between each addition. 20µl glycerol dye, 10 µl MW Marker V and 90µl MW Marker Buffer were mixed in a tube so as to prepare the Molecular Weight Marker (MWM) (Thermo Scientific, Fermentas molecular biology tools, Waltham, Mass, USA). This volume was sufficient for ten gels. 10µl of the PCR reactions and MWM were loaded onto the gel wells sequentially from left to right. The remaining volume of PCR product was stored at 4°C in the post-PCR fridge. Approximately 400ml of 0.5X TBE running buffer was poured into the minitank and connected to the electrodes, making sure that red terminal was connected to the anode and black to the cathode. This was left to run at 125V/20mA for 60 minutes.

4.6.3.3 GEL STAINING

While wearing double latex gloves, the running buffer was poured out into the sink and the gel plates removed from the assembly. A working solution of ethidium bromide solution was prepared in a shallow container by adding 30ul of a stock solution of ethidium bromide (1mg/ml) to 300ml distilled water. The glass plate was gently removed from the spacer plate and the gels cut out using a gel cutter. The gels were placed in the ethidium bromide solution for 15 minutes. The gels were then gently removed from the ethidium bromide solution and rinsed with ddH_2O for

2-5 minutes. A photograph was taken using the transilluminator Aplha Imager, (Alpha Innotech Technologies ZA, California, USA) according to manufacter's instruction.

4.6.4 VALIDITY OF THE PCR MACHINE

A calibrated GeneAmp® PCR System 9700 thermocycler (Applied Biosystems Inc; CA, USA) was used. This equipment was last calibrated by an external body in February 2011. The National Influenza Centre laboratory where the work was done has stringent and well validated Quality Assurance/Quality Control (QA/QC) program for equipments and procedures and the dictates of this program were strictly adhered to. External QA/QC was carried out at Center for Disease Control (CDC) in Kenya Medical Research Institute (KEMRI) which has similar equipments to the National Influenza Center (NIC) laboratory. In order to determine validity, 20 samples were repeated and the results were identical as demonstrated in the 12 samples displayed in figures 6 and 7.

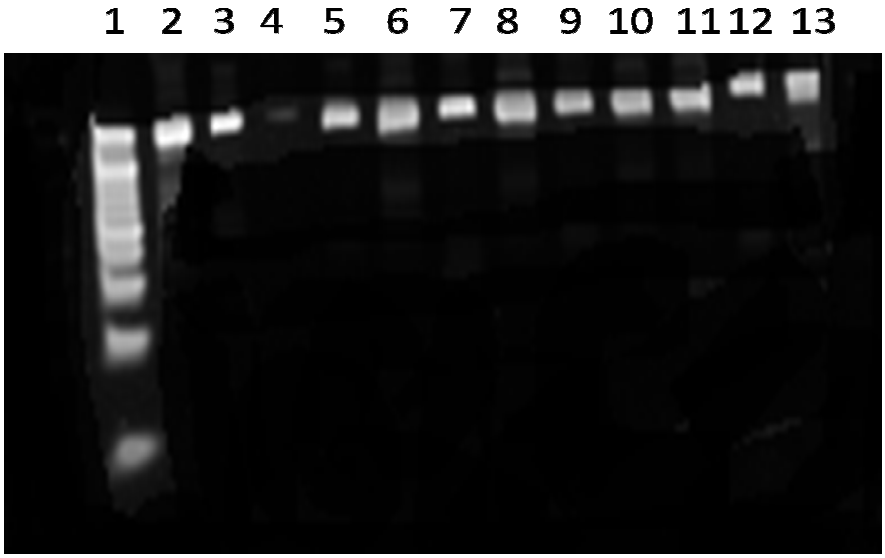


Figure 6: Lanes 1-13 beginning on the left shows the DNA ladder (20bp) then bands for samples 740-729 for locus -511. This work was done on 8th February 2012.

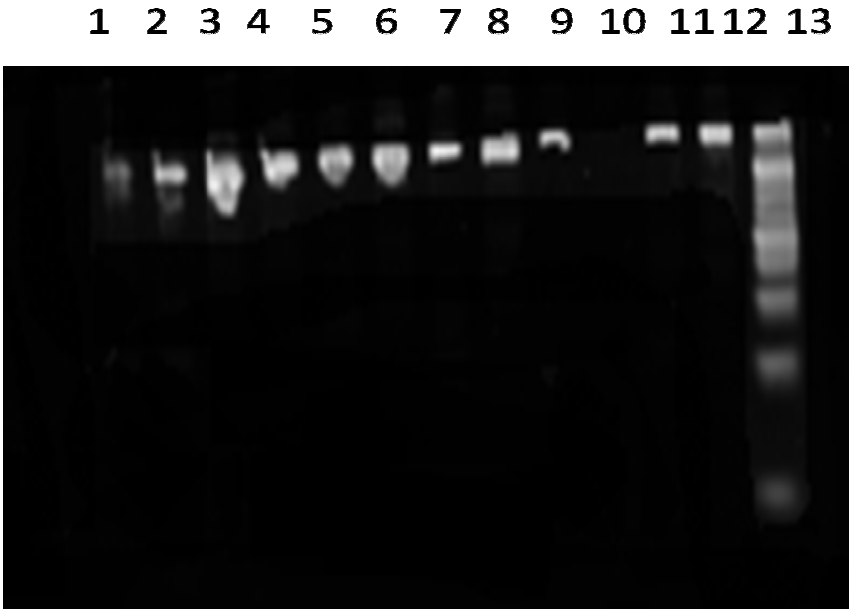


Figure 7: Lanes 1-13 beginning on the left shows bands for samples 729-740 and lane thirteen is the DNA ladder (20bp) for locus -511. This repeat work was done on 16th February 2012.

4.6.5 RELIABILITY OF LABORATORY PROCEDURE

Standard genotyping reagents and instrumentation from Applied Biosystems Fermentas Promega and Qiagen were used. All molecular screening methods which are PCR and RFLP based have been extensively validated[145,146].

4.7 METHODOLOGICAL CONSIDERATIONS

The field work was very challenging especially in the recruitment of matched healthy controls. This was overcome by the screening of as many participants as possible. The reconciliation of data at the end of each day by the PI also helped in keeping track of the cases and controls. In Taita Taveta County, a total of 502 participants were screened whereas in Mombasa County a total of 523. Having a PHO administering the questionnaire was extremely useful. They were familiar with the process and thus fairly accurate in gathering the information. The participants were also willing to give information and participate in the study because they felt comfortable dealing with somebody they knew.

As far as the laboratory procedures were concerned, optimization of the procedures took a long time. It took approximately four months to optimize all the reactions. The optimization process involved titrating template DNAs, trying out various magnesium ion concentrations, trying out various cycling temperatures and varying the dNTPs and enzyme concentrations. After optimization, while running the various reactions, the key to successful runs, was accurate labeling of all the samples.

Working with ethidium bromide was difficult since it is a known mutagen which can be easily absorbed through the skin. Therefore, the laid down guidelines for laboratory personnel working with ethidium bromide were used. The laboratory was well ventilated and equipped, with a designated area and a fume hood set aside specifically for working with this chemical. Eyes and skin protection were used at all times. UV protected eye wear with side shields were used. Disposable nitrile gloves were used and double gloving was practised at all times. Protective clothing and closed shoes were used to prevent skin contact in case of spillage. The entire laboratory SOPs were followed in storage and cleaning up after using this chemical. A decontamination solution was always used at the end of each session to clean the surfaces and this was made of 4.2 g of sodium nitrite, 20 ml of hypophosphorous acid (50%) and 300 ml of water.

4.8 MINIMIZING ERROR AND BIASES

The research assistants were trained public health officers working in the areas of study and were used to dealing with members of the public as well as to administer questionnaires in a language best understood by the subjects. Training was done on the questionnaires where each question was read out and explained to the PHO so as to introduce them to terms used in dentistry. Training on how to handle the skip questions was also done. All the clinical examinations were done by the PI. To collect the tissue samples required for DNA analysis, the Isohelix T-Swab made by Boca-Scientific, (Kent England) was used and the manufacturer's guidelines followed in handling the samples (figure 3 and 4). Only participants who met the

inclusion criteria were included in the study. All data collection tools were pre-tested and a total of 65(16%) repeat examinations were done. The equipments for the laboratory procedures were validated. Twenty repeat genotyping processes were done (Section 4.6.4).

The power calculations performed for this study showed that the sample size required in ascertaining the significance of association of periodontal disease and the studied genetic polymorphisms with an alpha value of 0.05 and 80% power were 98 for cases 98 for controls. This showed that the sample size was large enough to detect association with an acceptable level of confidence. The selection of extreme cases was also a factor that increased the power of the study and thus the chances of detecting the associations[137]. Case enrichment by using severe cases is good for reducing the phenotype heterogeneity and improving the power of the study. Matching for age and sex was done so as to remove the need for statistical adjustments of these variables. This also improves on the design of the study. Many studies have mis-matched cases and controls and this may affect the detection of association.

4.9 DATA ENTRY, PRESENTATION AND ANALYSIS

Data was double entered in Access by two different data entry clerks. It was cleaned in Epi-info as follows: - The entry of 50 records was entered, and then repeated by a different person in order to check for inconsistency. Only 2% of the repeat data was

found to be inconsistent (table 2). Data correction was then carried out to ensure correct data entry (table 3). Identification of the clinical data and the DNA samples of each individual were through the serial numbers given to the individuals during registration. Analysis was then done using the Statistical Package for Social Sciences version 17 (SPSS 17 Inc Chicago, Illinois, USA).

Table 2 (a): Frequency of matched repeat records and data fields from (a) clinical examination forms and (b) questionnaires before correction

(a) Clinical examination (n=number)

Records	Total n Compared	50	n with differences	1	% with differences	2%
Fields	Total n Compared	10100	n with differences	1	% with differences	0.0099%
Unmatched	In Table 2	0	In Table 3	0		

(b) Questionnaire

Records	Total n Compared	50	n with differences	1	% with differences	2%
Fields	Total n Compared	9850	n with differences	2	% with differences	0.0203%
Unmatched	In Table 2	0	In Table 3	0		

After correction of data

Table 3 (a): Frequency of matched repeat records and data fields from (a) clinical examination forms and (b) questionnaires after correction of data

a) Clinical examination (n=number)

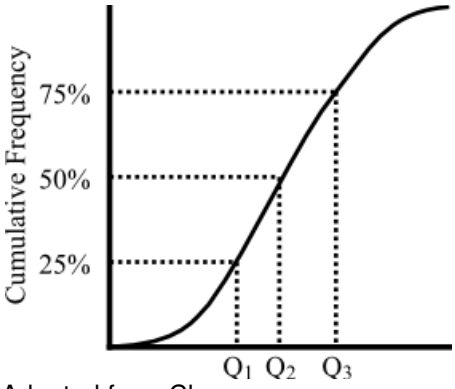
Records	Total n Compared	50	n with differences	0	% with differences	0%
Fields	Total n Compared	0	n with differences	0	% with differences	0%
Unmatched	In Table 2	0	In Table 3	0		

(b) Questionnaire

Records	Total n Compared	50	n with differences	0	% with differences	0%
Fields	Total n Compared	0	n with differences	0	% with differences	0%
Unmatched	In Table 2	0	In Table 3	0		

The clinical data was reported using cumulative frequency distribution graphs. The interpretation of these graphs is as reported below:-

For example, given a value of *x* you can estimate the number of values less than or greater than *x* by drawing a straight line that meets the cumulative frequency curve and then drawing a corresponding line to meet the other axis. The area above the frequency curve is interpreted as having values greater than x and that below the curve as having values less than x. In this study, the area below the frequency curve is interpreted as not having disease and that above the curve as having disease.



Adapted from Chegg.com

Descriptive and bivariate analyses were done. Associations between exposure variables and chronic periodontitis were done using Pearson Chi-squared and risk assessed by odds ratio (OR) with 95% confidence interval. Chi-squared and Mantel Hanzel were done to determine association between IL-1 and chronic periodontitis and possible confounders and effect modifiers identified. Multivariate analysis was carried out using the Binary Logistic Regression to test for significance of being a case in the presence of factors that were found to be significant at the bivariate stage. Hardy – Weinberg equilibrium for the four loci (-511, +3954, -889, +4845) in Taita and Swahili participants was tested for genotype frequency by chi square test, with 1 degree of freedom.

The three recognized haplotypes[147] as shown below were tested for association with chronic periodontitis:-

Haplotype 1: Allele 2 at IL-1A +4845 and allele 2 at IL-1B+3954

Haplotype 2: Allele 2 at IL-1B-511

Haplotype 3: Allele1 at the IL-1A and allele 1 at IL- 1B markers

The data was described by means and standard deviation (variance) for continuous variables. Mixed methods[148] were used to describe the oral health seeking behavior data because of the very few participants who had been to a dental clinic in both ethnic groups. The severity of chronic periodontitis according to the CDC/AAP definition [149] was used. The use of the CDC/AAP definition in this study was so as to avoid the under-estimation of disease by using both CAL and pocket depth.

4.10 ETHICAL CONSIDERATIONS

Ethical approval for the study was given by the Kenyatta National Hospital and University of Nairobi Ethics and Research Committee (Appendix 7). Permission to carry out the study in Taita District was obtained from the provincial medical officer, district commissioner and the district medical officer of health. In Mombasa, permission to carry out the study was obtained from the provincial director of medical services and medical officer of health, Mombasa Municipal Council. Permission was also obtained from the Imam of the Masjid Mihrab mosque in Kilifi area of Mombasa old town and Imam of the mosque in the Kisauni area. These were the only mosques in old town.

All the participants gave written informed consent after the purpose of the study, expected benefits and risks were explained to them by the PHO and COHO in a language they understood. Any queries were explained and the participants informed of their right to leave the study at any stage.

Emergency treatment was given where needed and referrals made. Facilitation to attend Voi District Hospital was given in a few cases where it was felt that the patient required follow up at the Hospital which was 50km away from the study site. In Mombasa, referrals were directed to the COHO accompanying the team and stationed at Coast General Hospital.

CHAPTER 5

RESULTS

This chapter presents the results of the data obtained from the field work as well as the laboratory procedures. The sequence followed in presenting the results involves the presentation of a description of the study population, the key variables and primary outcomes followed by bivariate analysis and finally the multivariate analysis.

5.1 RESULTS OF THE FIELD WORK, PILOT STUDY

A pilot study was conducted on five individuals to pre-test the questionnaire and clinical examination. The questionnaire was found to be clear and well understood. All individuals tested answered the questions adequately. The clinical examination was well tolerated including the buccal swap to collect DNA samples.

5.2 CALIBRATION AND INTRA-EXAMINER VARIABILITY

Calibration on the clinical parameters was done by re-examination of thirty eight Taita participants. The Cohen's kappa values obtained for calculus was 1.00, dental caries 1.00, plaque score 1.00, gingival bleeding 1.00, gingival recession 0.87 and probing pocket depth 0.86. All these values demonstrated satisfactory agreement.

5.3 SOCIO-DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF THE TAITA PARTICIPANTS

Socio-demographic characteristics

Of the 502 persons screened, only 198 were entered into the study according to the inclusion criteria. Of the 198 Taita participants examined, 99 were cases and 99 age

and sex matched healthy controls where there was no bleeding on probing. The age range for the participants was 35-44 years with a mean of 37.88 (SD 3.29). There were 64(32%) males and 134(67.7%) females OR = 0.97, 95% CI = 0.523-1.799. The difference was not significant (table 4). As far as education was concerned, more individuals with chronic periodontitis/cases 73(73.7%) had less than 8 years of schooling compared to controls 64(64.6%), but this was not significant. Married Taita participants with chronic periodontitis were 91(91.9%) and 79(79.8%) were controls, p=0.014 (table 4). Most of the Taitas were unemployed [74 (74.7%) cases and 62(62.6%) controls], p=0.266 and of middle income group (income ≥2000= but < 20,000= per month), p=0.641 but these values were not significant. Taita participants were mainly Christians 94.5%. In this study, there were only 11(5.5%) Muslim subjects. After the field study it was later realized that eight smokers were inadvertently included in the recruits. They were removed and excluded from subsequent analysis, leaving 94 cases and 94 controls.

Table 4: Distribution of socio-demographic features amongst the Taita participants

Variable	Cases n = 99	Controls n = 99	OR (CI)	P value
Gender				
Males	32 (32.3%)	32 (32.3%)	Reference	
Females	67 (67.7%)	67 (67.7%)	0.97 (0.523 –1.799)	0.923
Education				
< 8 years	73(73.7%)	64(64.6%)	1.54(0.80 – 2.95)	0.167
>8 years	26(26.3%)	35(35.6%)	Reference	
Marital status				
Married	91(91.9%)	79(79.8%)	2.88(1.12 – 7.58)	0.014*
Unmarried	8(8.1%)	20(20.0%)	Reference	
Employment				
Self employed	12(12.1%)	16(16.2%)	Reference	
Employed	13(13.1%)	21(21.2%)	0.83(0.26 – 2.58)	0.714
Unemployed	74(74.7%)	62(62.6%)	1.59(0.65 - 3.91)	0.266
Income levels				
Low	13(13.1%)	15(15.2%)	Reference	
Middle	86(86.6%)	82(82.8%)	1.21(0.51-2.90)	0.641
High	0(0.0%)	2(2.0%)	0.00(0.00-5.66)	0.491

P<0.05*

CLINICAL CHARACTERISTICS

Distibution of plaque present on tooth surfaces amongst the Taita participants

There was more plaque on the tooth surfaces of those with chronic periodontitis (cases) than in the control participants as shown in figure 8 below. The difference demonstrated between cases and controls was, OR = 21, with 95% CI = 7.8-56.4,

p<0.001. All those who had chronic periodontitis had plaque present on the tooth surfaces and the minimum number of teeth affected was eleven (11), whereas in the control subjects, the minimum number of teeth with plaque was one (1). 80% of the cases (y-axis) and 32% of controls (y-axis) had plaque on 23 tooth surfaces (x-axis) as can be seen on figure 8. Reference is made to the interpretation of the graph as explained on page 65. That is, the area above each line in figure 8 are those with disease and that below each line as those without disease.The median value on the y-axis (50% of individuals) showed plaque being present on 15 tooth surfaces in controls (x-axis) and 25 tooth surfaces in cases (x-axis). The mean number of tooth surfaces having plaque per individual in cases was 25(SD3.6) and for controls 14(SD 9.9).

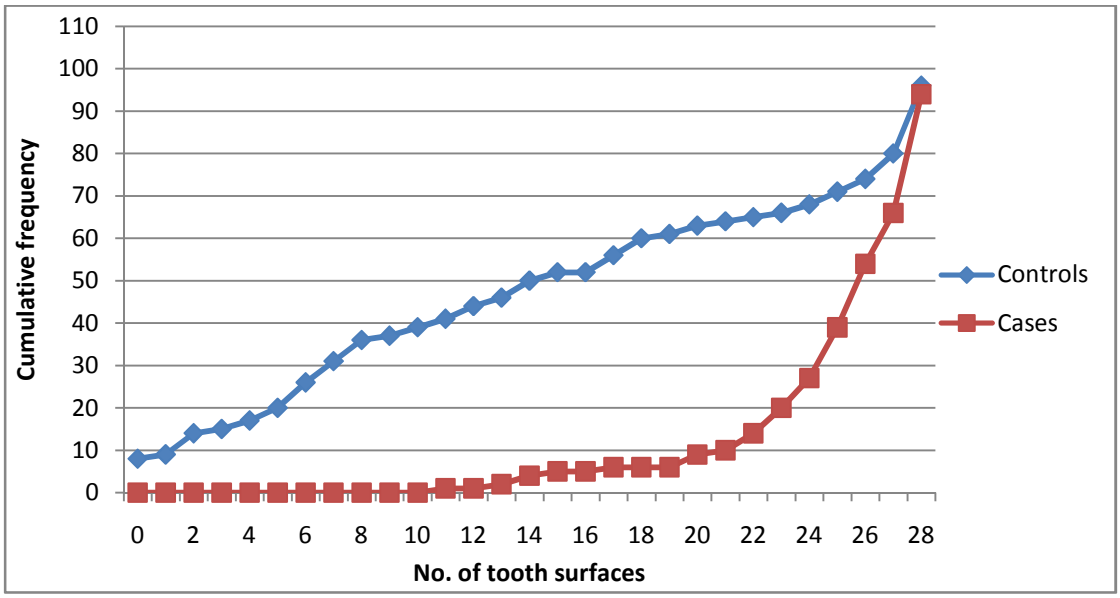


Figure 8: Cumulative frequency distribution of plaque on the tooth surfaces amongst the Taita participants

Figure 9 demonstrates that when the analysis of presence of plaque on the different tooth types (that is the incisors, canines, premolars and molars) was done, plaque was found on all the tooth types with more plaque being present on the molars. Approximately 90% of the individuals, had plaque on the first lower molars. Otherwise most individuals had plaque levels (~60-70%) that were almost similar in the upper and lower teeth as seen in figure 9 except for the upper right second premolar where only ~20% of individuals had plaque.

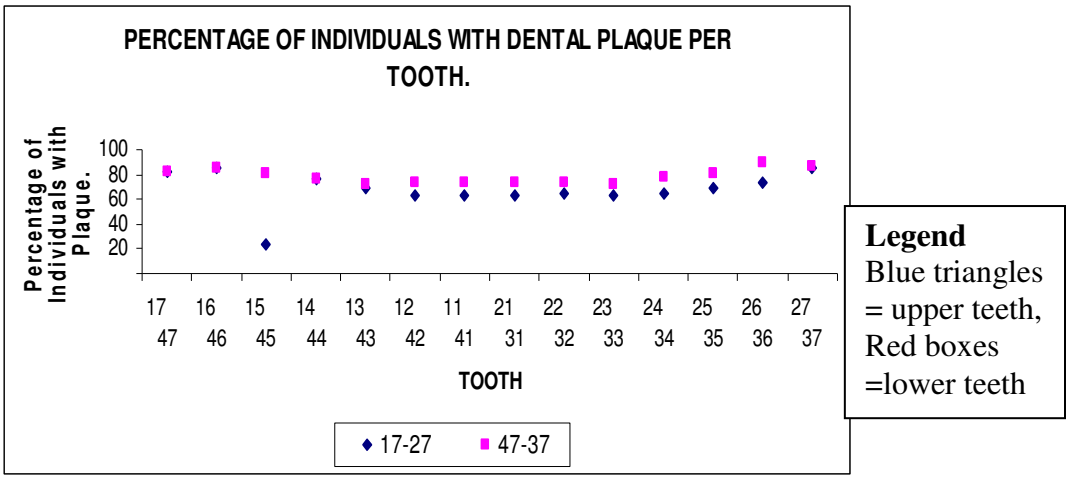


Figure 9: Percentage of individuals with dental plaque on each tooth type amongst the Taita participants in cases and controls.

Bleeding on probing of gingival tissues amongst the Taitas

Figure 10 below shows that most of the Taita participants with chronic periodontitis (cases) had bleeding on probing of the gingival tissues around the teeth with 88% having at least one tooth affected. The median value on the y-axis (50% individuals) showed that 24 tooth surfaces (x-axis) had gingival tissues that bled on probing in cases. When all 28 gingival sites (x-axis) were examined for bleeding, it was found that cumulatively, 6% of individuals had all gingival sites bleeding on probing. 30% had 27 gingival sites with bleeding out of a possible 28 gingival sites examined. The mean number of gingival sites per individual found to be bleeding was 17(SD 10.2). Only 6(0.2%) gingival sites were found to bleed on probing in the controls amongst the Taita participants.

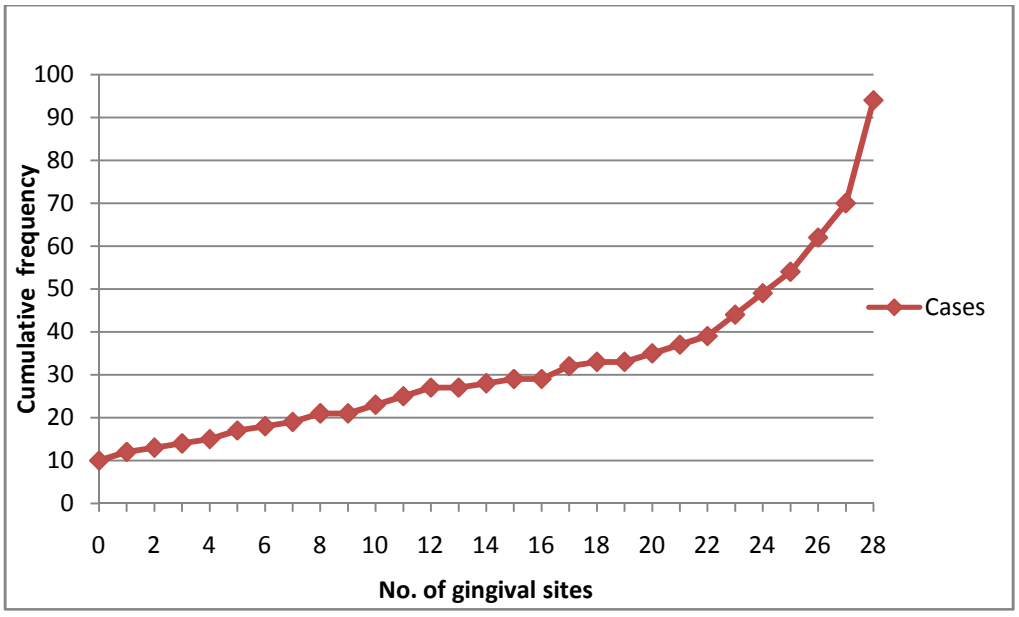


Figure 10: Cumulative frequency distribution of the number of teeth with bleeding on probing of the gingival tissues amongst the Taita participants

All the individuals had some bleeding on probing of the gingival tissues around the different tooth types examined (that is the incisors, canines, premolars and molars). Approximately 40% (y-axis) of the Taitas had bleeding on the gingival tissues in the upper right first molar (16) and ~38% in the lower right first molar (46), ~35% in the second right upper molar (17) and lower second right molar (47). On the left side, ~42% of the individuals had bleeding of the gingival tissues surrounding the lower canine (33), ~40% in the tissues around the first and second lower premolars (34,35) and ~32% on the tissues around the upper first and second molars (27,37) (figure 11).

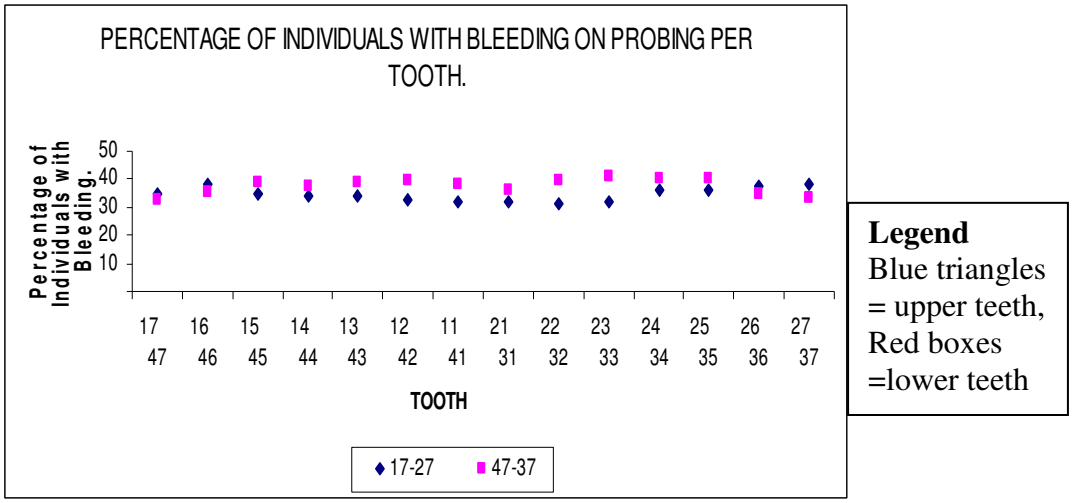


Figure 11: Percentage of Taita participants with bleeding on probing of gingival tissues surrounding the different tooth types in cases

Distribution of calculus on tooth surfaces amongst the Taita participants

Calculus was present mostly on the tooth surfaces of cases (figure 12). 90% of cases had at least one tooth surface with calculus while in the controls, it was 33%. Cumulatively, 45% of cases (y-axis) had calculus on 14 tooth surfaces (x-axis) and only 8% of controls (y-axis) had the similar number of teeth affected. The median value on the y-axis (50%) showed that 13 tooth surfaces (x-axis) had calculus in cases. The controls had less calculus with 22% of individuals, having calculus. Therefore there was more calculus on the tooth surfaces amongst those with chronic periodontitis OR = 33.9, 95%CI = 13.3-86.3, p<0.001.

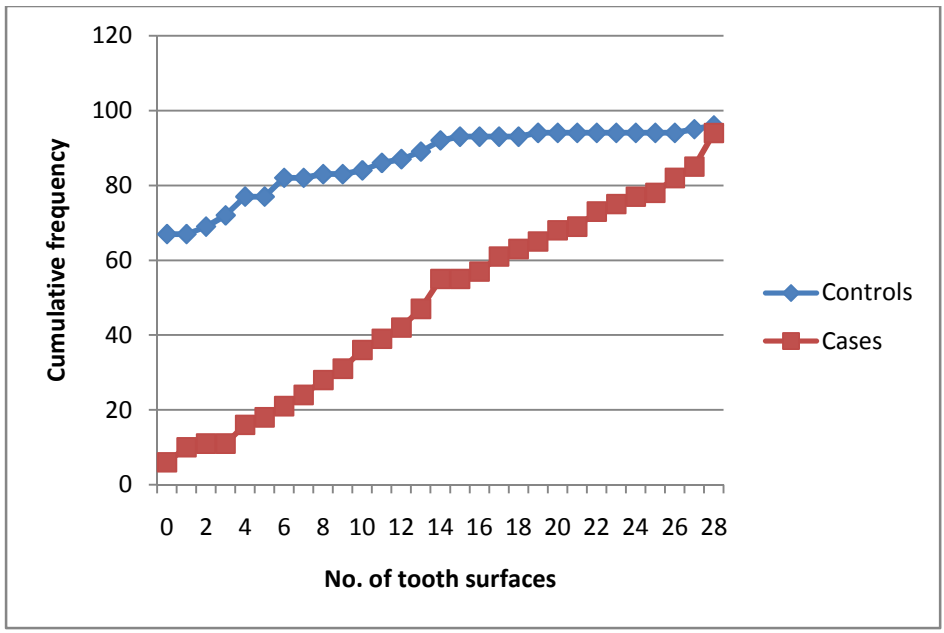


Figure 12: Cumulative frequency distribution of the number of tooth surfaces with calculus amongst the Taita participants

A majority of the individuals, approximately 68-70%, had calculus present in the lower anterior teeth (12,11,21,22) and 48-55% of individuals had calculus on the upper molars (17,16,27,26) as shown in figure 13.

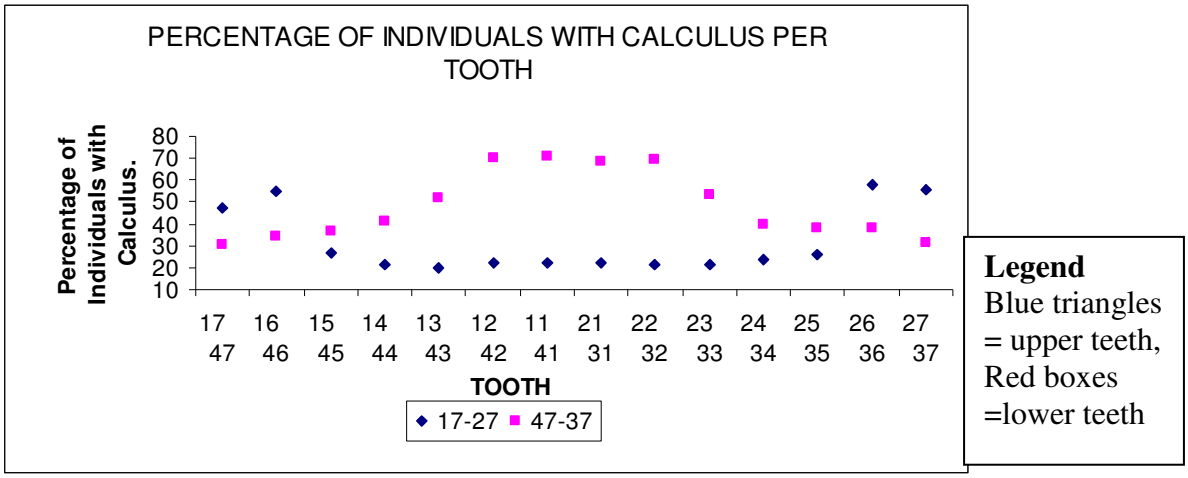


Figure 13: Cumulative frequency distribution of calculus on the surfaces of the different tooth types in cases and controls amongst theTaita participants

Distribution of periodontal pocket depths on different tooth types amongst the Taita participants

The presence of periodontal pockets of more than or equal to 4mm was low (3.8%) and the upper teeth were more affected than the lower teeth as seen in figure 14 below. Approximately 22% of the Taita participants had pockets of ≥4mm in upper

right first premolar (14) and ~ 20% of them had these pockets of ≥4mm in the upper second molars (17, 27) and upper left first premolar (24). The mean periodontal pocket depth was 1.58(SD1.01). 564 sites (3.8%) had pockets of ≥4mm.

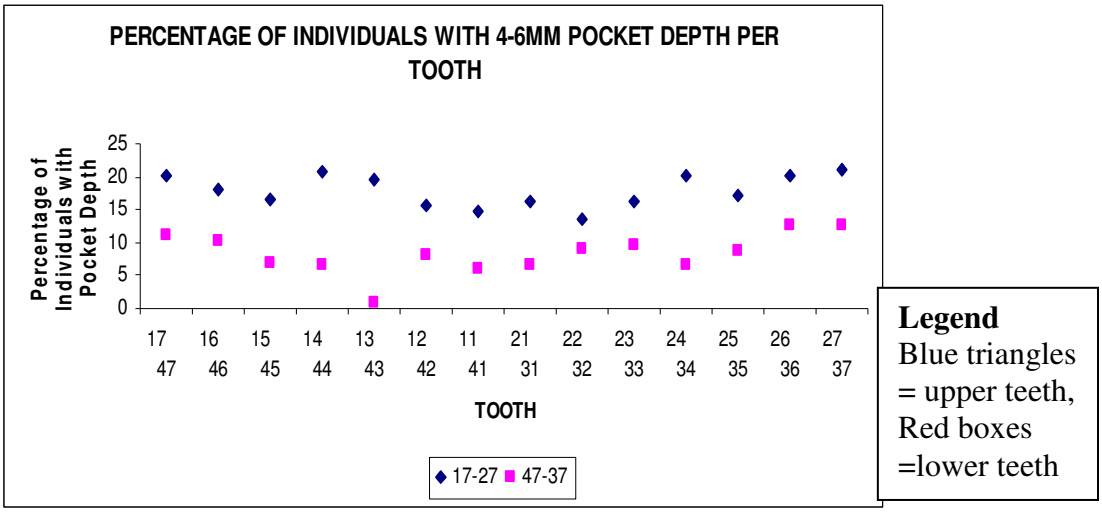


Figure 14: Percentage of different tooth types with periodontal pocket depths in cases amongst the Taita participants

Clinical attachment loss (CAL) amongst the Taita participants

The mean number of sites with ≥4mm CAL were 20 (SD 21.1), ≥5mm were 11 (SD17.9) and those with CAL of ≥6mm were 6(SD 11.2). The mean total CAL was 2.34(SD 1.09).

Dental caries

In the Taita participants very few individuals had dental caries. 14 (14.89%) individuals in the control group and 20 (21.28%) in the cases were found to have

dental caries. The individuals with dental caries had only one (1) affected tooth and no restorations.

Roots and missing teeth amongst the Taita participants

The presence of roots was evenly distributed between cases and control. 41(21%) individuals had roots and 157 (79%) did not. The Taita participants with retained roots, had mainly one root present, 23 (12%). The others were as follows; 9(5%) had 2 roots, 5(3%) had 3 roots, 2(1%) had 4 roots, 1(1%) had 5 roots and 1 (1%) 6 roots. 81(41%) of the individuals had missing teeth mainly due to extractions following the development of dental caries. The individuals with missing teeth had mainly one tooth missing 33(17%), 23 (12%) had two teeth missing, 13 (7%) had three missing teeth, 6 (3%) had four teeth missing, 3(2%) had five missing teeth and 3(2%) had six missing teeth. 117(59%) had all teeth present (figure 15).

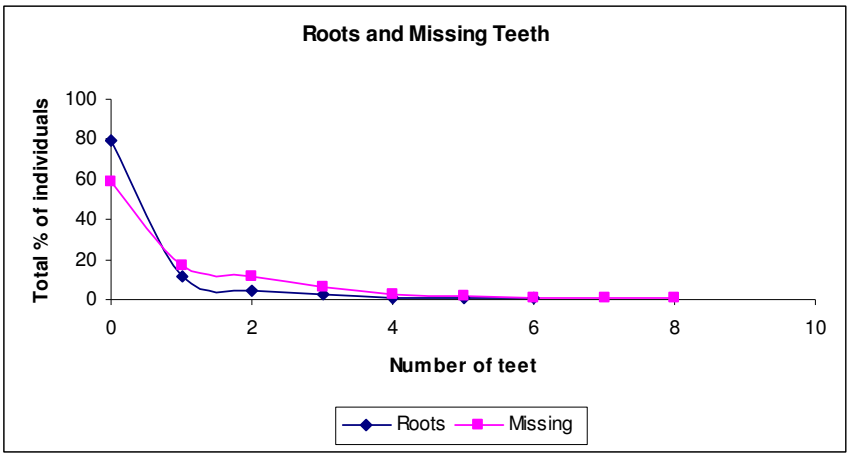


Figure 15: Distribution of roots and missing teeth amongst the Taita paticipants

5.3.1 SEVERITY OF CHRONIC PERIODONTITIS AMONGST THE TAITA PARTICIPANTS

The severity of chronic periodontitis was categorized into 3 groups according to the CDC/AAP consensus on case definitions of periodontal diseases [163] as shown in table 5.

Table 5: CDC/AAP consensus on case definition of periodontal diseases [163].

Disease category	**CAL**	**PPD**
Severe periodontitis	More than 2 interproximal sites with CAL of more or equal to 6mm (not on the same tooth)	**AND** More than 1 interproximal site with PD of more or equal to 5mm
Moderate Periodontitis	More than 2 interproximal sites with CAL of more or equal to 4mm (not on the same tooth)	**OR** 2 or more interproximal sites with PD of more or equal to 5mm (not on the same tooth)
Mild periodontitis	Neither moderate nor severe periodontitis.	

Third molars excluded.

The severity of CAL in the Taita participants according to the CDC/AAP definition [163] is as shown in table 6. Ten percent (10.6%) of the Taita participants had the mild form of the disease; 43(45.7%) had the moderate form and 41(43.6%) had the severe form of chronic periodontitis.

Table 6: Distribution of chronic periodontitis according to severity amongst Taita participants

Disease Severity according to CAL (CDC/AAP)	Number	Percent
Mild	10	10.6%
Moderate	43	45.7%
Severe	41	43.6%

Risk estimates of being a case amongst the Taita participants in the presence of plaque at the tooth level

The risk of being a case in the presence of plaque was 45 fold on the lower first left incisor and upper first left molar; this being the highest estimate. The range for risk estimate was from 6 to 45 fold on the lower left second molar. The upper molars and premolars also demonstrated relatively high risk estimates (40 fold for upper first molar and 44 fold for upper left premolar, for example). In the lower teeth, the premolars and the incisors also demonstrated a high risk estimate as shown in table7.

Table 7: Risk estimate of being a case when plaque is present on the tooth surface amongst theTaita participants

Tooth		95% Confidence Interval	
	Value	Lower	Upper
17	40.781	5.419	306.912
16	32.123	4.236	243.617
15	22.642	6.675	76.794
14	20.096	7.989	50.552
13	10.939	5.274	22.686
12	15.337	7.066	33.289
11	14.667	6.766	31.792
21	19.063	8.294	43.812
22	15.429	7.089	33.581
23	14.017	6.455	30.437
24	44.8	13.244	151.543
25	28.75	8.494	97.314
26	32.422	4.279	245.68
27	11.955	3.48	41.074

Tooth		95% Confidence Interval	
	Value	Lower	Upper
47	11.441	3.321	39.417
46	12.363	2.788	54.821
45	18.667	5.498	63.38
44	23.927	7.081	80.856
43	12.875	5.419	30.59
42	32.313	9.588	108.901
41	21.6	7.357	63.414
31	45.08	10.519	193.19
32	31.633	9.383	106.646
33	20	7.478	53.488
34	35.782	8.331	153.689
35	28.5	6.618	122.732
36	18.417	2.39	141.928
37	6.892	1.952	24.331

The risk of being a case in the presence of calculus ranged from 8 fold, on the upper central incisors to 23 fold on the lower left canine. This relationship was not as strong as that for plaque and chronic periodontitis as shown in table 8. All teeth were found to be at risk of having chronic periodontitis in the presence of calculus.

Table 8: The risk of being a case when calculus is present on the tooth surface amongst theTaita participants

Tooth		95% Confidence Interval	
	Value	Lower	Upper
17	16.68	6.939	40.099
16	20.513	8.957	46.978
15	10.18	3.76	27.561
14	13.676	4.008	46.665
13	15.354	4.513	52.235
12	15.354	4.513	52.235
11	8.699	3.215	23.532
21	8.294	3.061	22.475
22	14.179	4.156	48.38
23	21.269	4.914	92.051
24	15.124	4.447	51.437
25	17.756	5.221	60.387
26	14.022	6.558	29.978
27	20.517	9.075	46.387

Tooth		95% Confidence Interval	
	Value	Lower	Upper
47	9.333	4.142	21.03
46	9.024	4.162	19.564
45	14.488	6.347	33.074
44	14.171	6.685	30.043
43	13.154	6.59	26.255
42	20.192	9.602	42.462
41	21.326	9.969	45.625
31	18.215	8.682	38.217
32	19.952	9.484	41.972
33	23.154	10.921	49.087
34	10.842	5.241	22.428
35	11.559	5.472	24.419
36	10.677	5.039	22.624
37	12.331	5.065	30.018

5.4 FREQUENCY OF IL -1B AND 1A GENOTYPES AND ALLELES AMONGST THE TAITA PARTICIPANTS

5.4.1 INTERPRETATION OF RFLP PATTERNS

Interleukin-1B polymorphism was tested at loci -511 and +3954, whereas interleukin-1A was tested at -889 and +4845. The genotype frequency was tested for allele 1 and 2 at the four loci tested. Thus the presence of homozygous allele 1 (1-1), presence of heterozygous allele 1and 2 (1-2) and the presence of homozygous allele 2 (2-2) were tested.

The pattern of the RFLP fragments for IL-1B at locus -511 were 190bp + 114bp (allele 1) or 304bp (allele 2). The presence of the three bands indicated a heterozygous individual. The RFLP products for IL-1B at locus +3954 consisted of three fragments of 12bp+ 85bp + 97bp (allele 1), two fragments of 12bp + 182bp (allele 2) and all the five were present in heterozygous individuals.

The patterns of the RFLP fragments for IL-1A at Locus -889 were 83bp + 16bp (allele 1), 99bp (allele 2) and a mixture of the three bands for heterozygous individuals. Finally, the pattern for IL-1A at locus + 4845 were 124bp + 29bp in subjects homozygous for allele 1, and one fragment of 153bp in subjects homozygous for allele 2. When all the three fragments were present, the individuals were considered heterozygous.

Frequency of IL-1B -511 and +3954 genotypes and alleles amongst Taita participants

Table 9 shows the various frequency distributions of the four genotypes in cases and controls amongst Taita participants. A higher frequency of heterozygous IL-1B (-511) was found in cases 47(63.5%) than in controls 37 (57.8%), p=0.112. Allele 1 of the IL-1B -511 polymorphism was carried by 57 (38.5%) cases and 49(38.3%) controls, p=0.968. Of these, only 5 (6.8%) cases and 6(9.4%) controls were homozygous, p=0.784.

On the other hand, allele 2 of IL-1B polymorphism at -511 was carried by 91(61.5%) and 79(61.7%) cases and controls respectively, with homozygous distribution of allele 2 at 22(29.7%) and 21(32.8%) in cases and controls, with a p value of 0.801.

The homozygous distribution of allele 1 at locus +3954 was 34(54%) and 34(70.8%) in cases and controls respectively, p=0.914. Homozygous allele 2 was 8(12.7%) and 3(6.3 %) in cases and controls respectively, p=0.112. The heterozygous distribution of allele 1 and 2 (1-2) was 21(33.3%) in cases and 11(22.9%) in controls with a p-value of 0.045, which was significant.

The carriage rate for allele 1 polymorphism at position +3954 was 89(70.6%) in cases and 79(82.3%) in controls, OR=0.52 95%CI=0.27-0.99, p=0.045. Allele 2 was carried by 37(29.4%) and 17(17.7%) in cases and controls respectively, OR=1.936 95%CI=1.009-3.698, p=0.44.

Frequency of IL-1A -889 and +4845 genotypes and alleles amongst Taita participants

Homozygous frequency for allele 1 of -889 was 36(41.4%) and 28(34.1%) for cases and controls, p=0.183. Heterozygous frequency was 34(39.1%) and 36(43.9%) for cases and controls, p=0.849. Homozygous distribution for allele 2 was also not significant, with 17(19.5%) and 18(22%) for cases and controls respectively, p=0.906.

The carriage rate of allele 1 and allele 2 were 106(60.9%) and 92(56.1%) for allele 1 and 68(39.1%) and 72(43.9%) for allele 2 in cases and controls, p=0.368 (Table 9).

The frequency of the homozygous allele 1 for +4845 was found to be statistically significantly distributed between cases, 1(1.4%) and controls, 8(11.6%) although the numbers are low, p=0.018. Heterozygous for +4845 was 58(78.4%) and 50(72.5%)

for cases and controls respectively, p=0.181. Homozygous for allele 2 was 15(20.3%) and 11(15.9%) for cases and controls respectively, p=0.367.

The carriage rate for allele 1 polymorphism at position +4845 was 60(40.8%) and 66(47.8%) in cases and controls respectively. Whereas the carriers of allele 2 polymorphism at +4845 in cases and controls, were 88(59.5%) and 72(52.2%) respectively, p=0.215.

Table 9: Distribution of IL-1B and IL-1A genotype and allele frequencies amongst the Taita participants

		Cases (n=94)		Controls (n=96)				95% CI for OR	
Genotype		**n**	**%**	**n**	**%**	**P-Value**	**OR**	**Lower**	**Upper**
IL-1B-511	1--1	5	6.8	6	9.4	0.784			
	1--2	47	63.5	37	57.8	0.112			
	2--2	22	29.7	21	32.8	0.801			
Allele	1	57	38.5	49	38.3	0.968	1.01	0.62	1.64
	2	91	61.5	79	61.7				
IL-1B+3954	1--1	34	54	34	70.8	0.914			
	1--2	21	33.3	11	22.9	0.045*			
	2--2	8	12.7	3	6.3	0.112			
Allele	1	89	70.6	79	82.3				
	2	37	29.4	17	17.7	0.045*	1.94	1.01	3.70
IL-1A-889	1--1	36	41.4	28	34.1	0.183			
	1--2	34	39.1	36	43.9	0.849			
	2--2	17	19.5	18	22	0.906			
Allele	1	106	60.9	92	56.1	0.368	1.22	0.79	1.88
	2	68	39.1	72	43.9				
IL-1A+4845	1--1	1	1.4	8	11.6	0.018*			
	1--2	58	78.4	50	72.5	0.181			
	2--2	15	20.3	11	15.9	0.367			
Allele	1	60	40.5	66	47.8	0.215	0.74	0.46	1.19
	2	88	59.5	72	52.2				

Significance at *p<0.05, OR = odds ratio, CI = confidence interval

Hardy Weinberg Principle

Allele -511

$$p = \frac{2\times Obs(AA)+Obs(Aa)}{2\times(Obs(AA)+Obs(Aa)+Obs(aa)}$$

$$p = \frac{(2 \times 11)+84}{2 \times 138} = \frac{106}{276} = 0.38$$

$q = 1- p = 0.62$

HWP expectations

$Exp\ (AA) = p^2n = 0.38^2 \times 138 = 19.93$

$Exp\ (Aa) = 2pqn = 2x0.38 \times 0.62 \times 138 = 65.02$

$Exp\ (aa) = q^2n = 0.62^2 \times 138 = 53.05$

Pearson Chi-square test

$\chi^2 = \Sigma(O - E)^2 / E$

$= (11 – 19.93)^2 / 19.93 + (84 – 65.02)^2 / 65.02 + (43 -53.05)^2 /53.05 = 11.42$

Locus -511, $\chi^2 = 11.42$, $p<0.05$

Locus +3954, $\chi^2 = 6.48$, $p<0.05$

Locus -889, $\chi^2 = 3.84$, $p<0.05$

Locus +4845, $\chi^2= 40.5$, $p<0.05$

χ^2 distribution with 1df (3.84) shows that equilibrium exists for IL-1B -511, +3954 and IL-1A -889 and +4845 in the Taita participants .

5.4.2 FREQUENCY OF IL-1A AND IL-1B COMPOSITE GENOTYPE AMONGST THE TAITA PARTICIPANTS

The frequency distribution of homozygous allele1at IL-1A-889 and IL-1B +3954 was 5(8.1%) and 2(4.4%) in cases and controls respectively, p=0.236 (Table 10). That of heterozygous allele 1 and 2 (1-2) and homozygous allele 1 (1-1) at -889 together with homozygous allele 1 and heterozygous allele 1 and 2 (1-1/1-2) at +3954 were 28(45.2%) and 20(44.4%) respectively, p=0.156. The heterozygous frequency at -889 (1-2) and +3954 (1-2) was 12(19.4%) and 11(24.4%) in cases and controls respectively, p=0.782. The frequency distribution for heterozygous (1-2) together with homozygous allele 2 (2-2) at -889 and +3954 were 6(9.7%) and 9(20.0%) in cases and controls, p=0.444. The freqency found for homozygous allele 2 at -889 and homozygous allele 1 at +3954 together was 7(11.3%) and 2(4.4%) in cases and controls respectively, p=0.082. Homozygous allele 2 in both -889 and +3954 was 4(6.5%) and 1 (2.2%) in cases and controls respectively, p=0.166 (table 10).

The positive genotype which is allele 2 +889 and allele 2 +3954 was carried by 29(46.8%) of cases and 23(51.1%) controls, p=0.287.The difference between cases and controls was not significant (table 10).

Table 10: Distribution of the IL-1A-889 and IL-1B+3954 composite genotypes amongst the Taita participants

Composite genotype	Cases (n=100)		Controls (n=100)		P-value
	N	%	N	%	
1--1/1--1	5	8.1	2	4.4	0.236
1--2/1--1 0r 1--1/1--2	28	45.2	20	44.4	0.156
1--2/1--2	12	19.4	11	24.4	0.782
1--2/2--2 0r 2--2/1--2	6	9.7	9	20.0	0.444
2--2/1--1	7	11.3	2	4.4	0.082
2--2/2--2	4	6.5	1	2.2	0.166
Positive genotype	29	46.8	23	51.1	0.287

5.4.3 DISTRIBUTION OF IL-1 GENOTYPES AND ALLELES ACCORDING TO GENDER AMONGST THE TAITA PARTICIPANTS

Males

IL-1B polymorphism at position -511 heterozygous (1-2) had 16(66.7%) carriers in cases and 12(63.2%) in controls, p=0.293. Homozygous distribution for allele 1 at -511 was 3(12.5%) in cases, 1(5.3%) in controls, p=0.3 and allele 2 was 5(20.8%) in cases, 6(31.6%) in controls, p=0.738. Table 11 shows that at loci +3954, the highest frequencies were 8(44.4%) and 11(78.6%) in cases and controls respectively, p=0.401. None of the male controls were homozygous for allele 2 at +3954. The highest frequency distribution for loci IL-1A -889 was homozygous for allele 1, where 13(44.8%) and 10(41.7%) was the distribution among cases and controls, p=0.421. At loci +4845, the most frequent distribution was heterozygous (1-2), which were 19(82.6%) and 15(88.2%) for cases and controls respectively, p=0.286. None of the male Taita participants had allele 1 of loci +4845 (table 11).

Females

Amongst the female Taita participants, the highest frequency at loci -511 was heterozygous at 31(62%) and 25(55.6%) for cases and controls with a p = 0.228 (table 11). At loci +3954, the highest frequency was for homozygous for allele 1, which was 26(57.8%) and 23(67.6%) for cases and controls respectively, p=0.5. At loci -889, the frequency was 23(39.7%) and 18(31%), p=0.29, for homozygous allele 1 and 22(37.9%) and 28(48.3%), p=0.347, for heterozygous (1-2) in cases and controls respectively. For loci +4845, the highest frequency which was not significant was for heterozygous at 39(76.5%) and 35(67.3%), p=0.369 for cases and controls. The least frequent homozygous allele1 at locus +4845 was 1 (1.5%) in cases and 8(11.6%) in controls, p=0.034. The difference was significant (table 11) and OR=0.137, 95%CI=0.016-1.14.

Table 11: Distribution of genotypes and allele frequencies in males and females amongst theTaita participants

		Males (n=58)					Females(n=132)				
Genotype		**Cases (n=29)**		**Controls (n=29)**			**Cases (n=65)**		**Controls (n=67)**		
		n	**%**	**N**	**%**	**P-Value**	**N**	**%**	**n**	**%**	**P-Value**
-511	1 -1	3	12.5	1	5.3	0.3	2	4.0	5	11.1	0.261
	1--2	16	66.7	12	63.2	0.293	31	62.0	25	55.6	0.228
	2--2	5	20.8	6	31.6	0.738	17	34.0	15	33.3	0.614
+3954	1--1	8	44.4	11	78.6	0.401	26	57.8	23	67.6	0.5
	1--2	8	44.4	3	21.4	0.094	13	28.9	8	23.5	0.206
	2--2	2	11.1	0	0.0	0.15	6	13.3	3	8.8	0.279
-889	1--1	13	44.8	10	41.7	0.421	23	39.7	18	31.0	0.29
	1--2	12	41.4	8	33.3	0.269	22	37.9	28	48.3	0.347
	2--2	4	13.8	6	25.0	0.487	13	22.4	12	20.7	0.759
+4845	1--1	0		0			1	1.5	8	11.6	0.034*
	1--2	19	82.6	15	88.2	0.286	39	76.5	35	67.3	0.369
	2--2	4	17.4	2	11.8	0.389	11	21.6	9	17.3	0.576

Significance at*P< 0.05

5.4.4 **DISTRIBUTION OF IL-1B AND 1A IN THOSE WITH MILD, MODERATE AND SEVERE FORMS OF CHRONIC PERIODONTITIS AMONGST THE TAITA PARTICIPANTS.**

When the association between alleles and the severity of chronic periodontitis was tested, carriage rate of allele 1 polymorphism at position IL-B locus -511, was 4 (33.3%) for mild CP, 29 (41.4%) for moderate CP and 24 (38.7%) for severe CP. For total allele 2 at the same position was 8 (66.7%) for mild CP, 41 (58.6%) for moderate CP and 38 (61.3%) for severe CP, p=0.854.

For IL-1B at locus +3954 the results showed that for allele 1, the number of Taita participants having this allele were 8 (66.7%) in those with the mild form of CP, 42

(70.0%) in those with the moderate form and 39 (72.2%) in those with the severe form of CP. Allele 2 on the other hand was, 4 (33.3%) for mild CP, 18 (30.0%) for moderate and 15 (27.8%) for severe CP, p=0.919.

The results for IL-1A at locus -889 according to severity of CP were, for allele 1, the number of Taita participants having this allele were, 11 (61.1%) in those with mild CP, 51 (63.8%) in those with moderate CP and 44 (57.9%) in those with severe CP. Whereas, allele 2 at the same locus (-889), 7 (38.9%) individuals had mild CP, 29 (36.3%) moderate CP and 32 (42.1%) severe CP. There were no significant differences in the frequency of allele 1 and 2 and severity of CP at IL-1A locus -889, p=0.755.

IL-1A at locus +4845, the frequency distribution of allele 1 was 7 (38.9%) in the mild form of CP, 29 (41.4%) in the moderate form and 24 (40.0%) in the severe form of CP. Wheras, for allele 2, at the same locus (+4845), 11 (61.1%) of the Taita participants had mild CP, 41 (58.6%) had moderate CP and 36 (60.0%) had severe CP. There were no significant differences in severity of CP and allele 1 and 2 at locus +4845, p=0.975 (table 12).

Table 12: Distribution of IL-1B and IL-1A genotype and allele frequencies with mild, moderate and severe chronic periodontitis amongst the Taita participants

		Mild CP (n=10(10.6%))		Moderate CP (n=43(45.7%))		Severe CP (n=41(43.6%))		
Genotype		**n**	**%**	**n**	**%**	**N**	**%**	**P-Value**
IL-1B -511	1—1	0	0.0	3	8.6	2	6.5	0.666
	1—2	4	50.0	23	65.7	20	64.5	0.728
	2—2	4	50.0	9	25.7	9	29.0	0.421
Allele	1	4	33.3	29	41.4	24	38.7	0.854
	2	8	66.7	41	58.6	38	61.3	
IL-1B+3954	1—1	3	50.0	17	56.7	14	51.9	0.799
	1—2	2	33.3	8	26.7	11	40.7	0.653
	2—2	1	16.7	5	16.7	2	7.4	0.533
Allele	1	8	66.7	42	70.0	39	72.2	0.919
	2	4	33.3	18	30.0	15	27.8	
IL-1A-889	1--1	3	33.3	18	45.0	15	39.5	0.751
	1--2	5	55.6	15	37.5	14	36.8	0.628
	2--2	1	11.1	7	17.5	9	23.7	0.622
Allele	1	11	61.1	51	63.8	44	57.9	0.755
	2	7	38.9	29	36.3	32	42.1	
IL-1A +4845	1--1	0	0.0	1	2.9	0	0.0	0.549
	1--2	7	77.8	27	77.1	24	80.0	0.784
	2--2	2	22.2	7	20.0	6	20.0	0.915
Allele	1	7	38.9	29	41.4	24	40.0	0.975
	2	11	61.1	41	58.6	36	60.0	

Significance at*P<0.05

When the severity of chronic periodontitis was compared in cases and controls (table 13), the frequency values for mild chronic periodontitis at locus -511 allele 2,

were 8(66.7%) in cases and 79(61.7%) in controls, p= 0.735. At locus +3954 allele 2, 4(33.3%) Taita participants in cases and 17(17.7%) in controls, p=0.197, were found to have this allele. At position -889, 7(38.9%) in cases and 72(43.9%) in controls, p=0.683, were found to have allele 2. At position +4845, 11(61.1%) and 72(52.2%0 in cases and controls respectively, p=0.474 were found to have allele 2 (table 13 (a)).

When individuals with moderate chronic periodontitis were compared to controls, the frequency distribution for allele 2 position -511 were, 41(58.6%) in cases and 79(61.7%) in controls respectively, p=0.664. At position +3954, 18(30.0%) in cases and 17(17.7%) in controls, p=0.073, were found to have allele 2. At position -889, the individuals found to have allele 2 were 29(36.3%) in cases and 72(43.9%) in controls, p=0.255. At position +4845, 41(58.6%) and 72(52.2%) in cases and controls respectively, had allele 2, p=0.384 (table 13 (b)).

In severe chronic periodontitis, the frequency distribution for allele 2 at position -511 were, 38(61.3%) in cases and 79(61.7%) in controls, p=0.954. Whereas, at position +3954, 15(27.8%) in cases and 17(17.7%) in controls, p=0.148, had allele 2. At position -889, 32(42.1%) in cases and 72(43.9%) in controls, p=0.793 had allele 2. Finally the Taita participants having allele 2 at position +4845 were, 36(60.0%) and 72(52.2%) in cases and controls respectively, p=0.727 (table 13 (c)).

Table 13. Distribution of IL-1B and IL-1A genotype and allele frequencies with a) mild, b) moderate and c) severe chronic periodontitis amongst Taita participants in cases and controls

a) Mild CP		Cases (n=10)		Controls (n=96)				95% CI for OR	
Genotype		n	%	n	%	p value	OR	Lower	Upper
Allele (IL-1B -511)	1	4	33.3	49	38.3	0.735	0.806	0.23	2.819
	2	8	66.7	79	61.7				
Allele(IL-1B+3954)	1	8	66.7	79	82.3	0.197	0.43	0.116	1.594
	2	4	33.3	17	17.7				
Allele (IL-1A-889)	1	11	61.1	92	56.1	0.683	1.23	0.454	3.331
	2	7	38.9	72	43.9				
Allele(IL-1A +4845)	1	7	38.9	66	47.8	0.474	0.694	0.254	1.896
	2	11	61.1	72	52.2				

*p<0.05, OR = Odds Ratio, CI = Confidence Interval

b) Moderate CP		Cases (n=41)		Controls (n=96)				95% CI for OR	
Genotype		n	%	n	%	p-val	OR	Lower	Upper
Allele (IL-1B -511)	1	29	41.4	49	38.3	0.664	1.14	0.629	2.066
	2	41	58.6	79	61.7				
Allele(IL-1B+3954)	1	42	70	79	82.3	0.073	0.502	0.234	1.075
	2	18	30	17	17.7				
Allele (IL-1A-889)	1	51	63.8	92	56.1	0.255	1.376	0.793	2.386
	2	29	36.3	72	43.9				
Allele(IL-1A +4845)	1	29	41.4	66	47.8	0.384	0.771	0.431	1.38
	2	41	58.6	72	52.2				

*p<0.05, OR = Odds Ratio, CI = Confidence Interval

c) Severe CP		Cases (n=41)		Controls (n=96)				95% CI for OR	
Genotype		**n**	**%**	**n**	**%**	**p-val**	**OR**	**Lower**	**Upper**
Allele (IL-1B -511)	1	24	38.7	49	38.3	0.954	1.018	0.546	1.899
	2	38	61.3	79	61.7				
Allele(IL-1B+3954)	1	39	72.2	79	82.3	0.148	0.559	0.253	1.237
	2	15	27.8	17	17.7				
Allele (IL-1A-889)	1	44	57.9	92	56.1	0.793	1.076	0.62	1.865
	2	32	42.1	72	43.9				
Allele(IL-1A +4845)	1	24	40	66	47.8	0.311	0.727	0.393	1.345
	2	36	60	72	52.2				

*p<0.05, OR = Odds Ratio, CI = Confidence Interval

5.5 SOCIO-DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF THE SWAHILI PARTICIPANTS

Socio-demographic characteristics

523 subjects were screened in the survey in Mombasa but only 200 were recruited into the study according to the inclusion criteria. These 200 Swahili participants were examined and duly completed the questionnaires. There were 108 males and 92 females. Of these, 54 were male cases with 54 age and sex matched controls. On the other hand, 46 were female cases with 46 age and sex matched controls. There was no difference in the distribution of male and female participants OR = 1.00(95% CI = 0.57-1.74); p=1.000. The level of income was divided into three categories, low level (<Kshs 2000), middle level (>Kshs 2000 and <Kshs 20,000) and high level

(>Kshs 20,000). Most of the Swahili participants, 69(34.5%) were in the middle level income group p=0.160, but this was not statistically significant. However, 98 (49%) of them did not declare their income. In terms of education, 74 (74.0%) cases and 67 (67.0%) controls were found to have gone to school for eight (8) years or less. The majority of the Swahili participants 150(75%) were married, 193(96.5%), Muslims by faith and came from Mombasa. 28 (29.0%) cases and 31 (31.0%) controls were employed, 18 (18.0%) cases and 21 (21.0%) controls were self-employed and 54(54.0%) cases and 48 (48.0%) controls were unemployed (table 14). None of the socio-demographic variables were significant when cases were compared with the controls.

Table 14: Distribution of clinical characteristics and socio-demographic data amongst the Swahili participants

Variable	Cases	Controls	OR (95% CI)	P Value
Gender				
Males	54(54%)	54(54%)	Reference	
Females	46(46%)	46(46%)	1.00(0.57-1.74)	1.000
Education (Years)				
<=8	74 (74.0%)	67 (67.0%)	1.53(0.80-2.92)	0.167
>8	26 (26.0%)	33 (33.3%)	Reference	
Marital Status				
Unmarried	17 (17.0%)	28 (28.0%)	Reference	
Married	80 (80.0%)	70 (70.0%)	1.88(0.90-3.94)	0.067
Level of income				
Low level	2(4.2%)	0(0.00%)	Reference	
Middle level	34(70.8%)	35(64.8%)	0.00(0.00-4.25)	0.160
Low level	12(25.0%)	19(35.2%)	0.00(0.00-3.06)	0,094
Employment				
Employed	28 (28.0%)	31 (31.0%)	1.03(0.67-1.58)	0.899
Self-employed	18 (18.0%)	21 (21.0%)	Reference	
Unemployed	54(54.0%)	48(48.0%)	1.31(0.59-2.94)	0.472

P<0.05*

Calibration

Intra-examiner variability was assessed by re-examination of the clinical parameters of 27 participants. The Cohen's kappa [164] values obtained for calculus was 1.00, dental caries 1.00, plaque score 0.91, gingival bleeding 1.00, gingival recession 0.83 and probing pocket depth 0.81. All these values demonstrated satisfactory agreement.

CLINICAL CHARACTERISTICS

Distribution of plaque present on tooth surfaces amongst the Swahili participants

The distribution of plaque in cases and controls of Swahili participants is shown in figure 16. In the cases, all individuals had plaque present on at least 14 tooth surfaces cumulatively whereas in the controls, all individuals had plaque present on at least 5 tooth surfaces. When comparing presence of plaque on 27 tooth surfaces (x-axis) out of a possible 28 examined tooth surfaces, figure 16 shows that 10% of controls (y-axis) had plaque present and 60% of cases (y-axis) cumulatively. The median on the y-axis (50%) showed that 17 tooth surfaces (x-axis) had plaque in ccontrols and 26 tooth surfaces (x-axis) in cases. The mean number of teeth with plaque was 26(SD4) tooth surfaces per individual in cases and 18(SD6) tooth surfaces per individual in controls with OR = 9.2 (95%CI = 3.7-23.1), $p<0.001$ when cases were compared to controls.

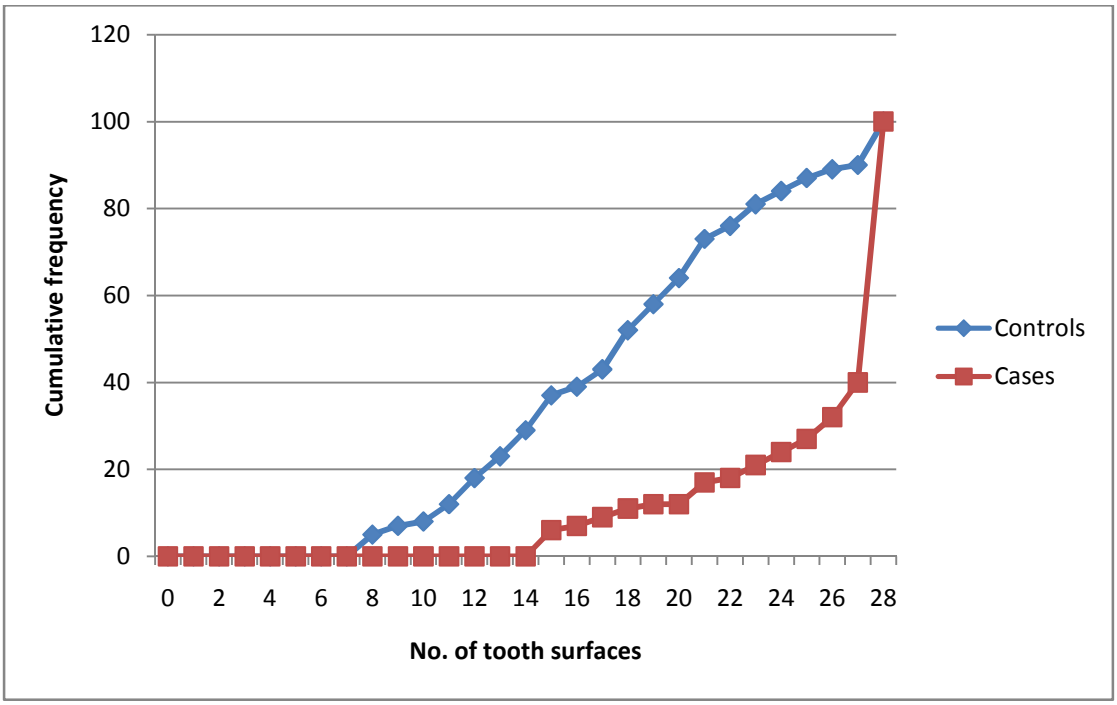


Figure 16: Cumulative frequency distribution of tooth surfaces with plaque amongst the Swahili participants by cases and controls

Plaque was present mainly on the lower tooth surfaces when the different tooth types (incisors, canines, premolars and molars) were assessed amongst the Swahili participants (figure 17 below). Approximately 53% of individuals (y-axis) had plaque in the upper anterior teeth (11,12,21,22), canines(13,23) and premolars (14,15,24,25) whereas the lower anterior teeth (41,42,31,32), canines (43,33) and premolars (44,45,34,35) had ~85% plaque present. More Swahili participants had

plaque ~ 95% in the right upper first and second molars (16,17) when compared to the lower first and second molars (46,47) (~90% and ~87% respectively). However on the left side, more of them had plaque ~88% on the lower first and second molars (36,37) as compared to ~ 82% in the upper first and second molars (26,27) (figure 17).

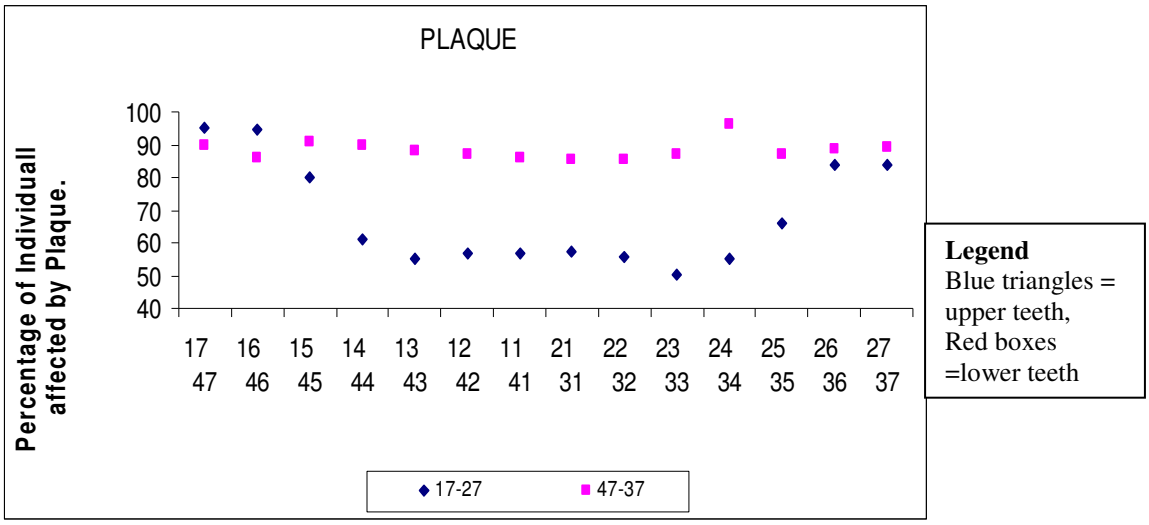


Figure 17: Percentage of individuals with plaque present on each different tooth type amongst the Swahili participants

Bleeding on probing of gingival tissues amongst the Swahili participants

The mean number of teeth that had bleeding on probing of the gingival tissues was 25(SD 6). There was minimal bleeding recorded in the control participants and it was

only on 29(1.07%) sites out of a total of 2800 sites examined. All the cases had at least one tooth where the gingival tissues bled on probing. When the gingival tissues surrounding 27 teeth out of a possible 28 were examined, it was found that approximately 64% (y-axis) of the teeth had tissues that bled on probing in the Swahili participants. The median on the y-axis (50% of individuals) showed bleeding of gingival tissues around 26 tooth surfaces (x-axis).

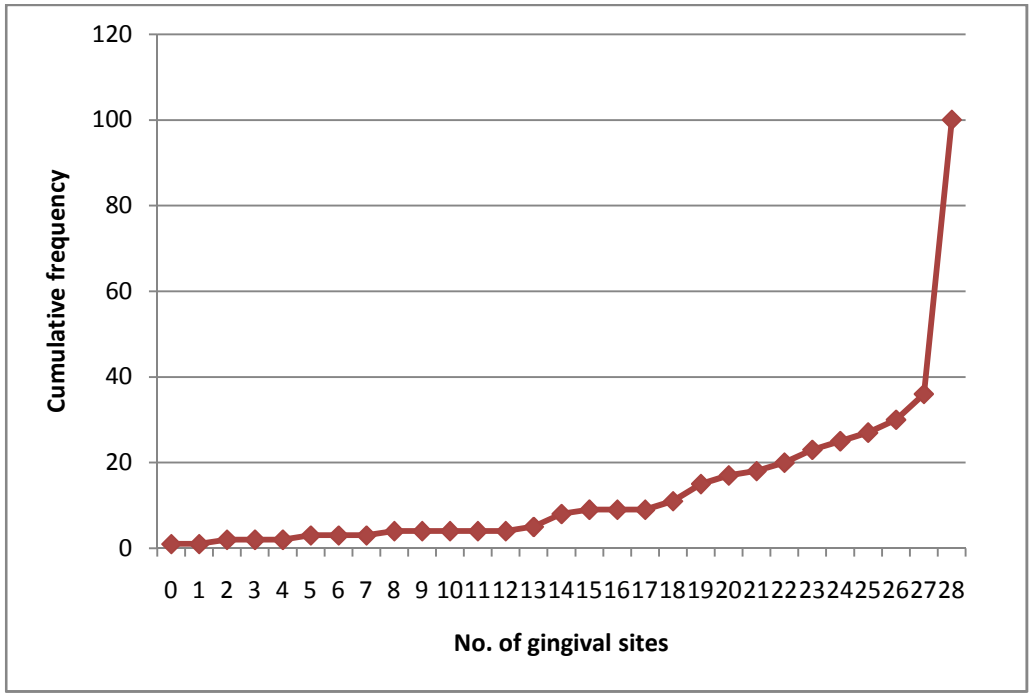


Figure 18: Cumulative frequency distribution of bleeding on probing of the gingival tissues around the teeth amongst the Swahili participants

When individual tooth types (incisors, canines, premolars and molars) were examined, more individuals had bleeding in the lower teeth than the upper anterior teeth as shown in figure 19 below. The upper right first molar (16) and second molar (17) had bleeding of approximately 47% (y-axis), whereas the lower first molars (46)

there were ~ 44% of the Swahili participants with bleeding (Figure 19). On the left side, the lower first molar (36) there were ~40% of individuals with bleeding on probing and ~45% of individuals with bleeding on the upper first molar (27). Approximately 47% of individuals had bleeding on the lower second molars (37) and ~45% on the upper second molar (27).

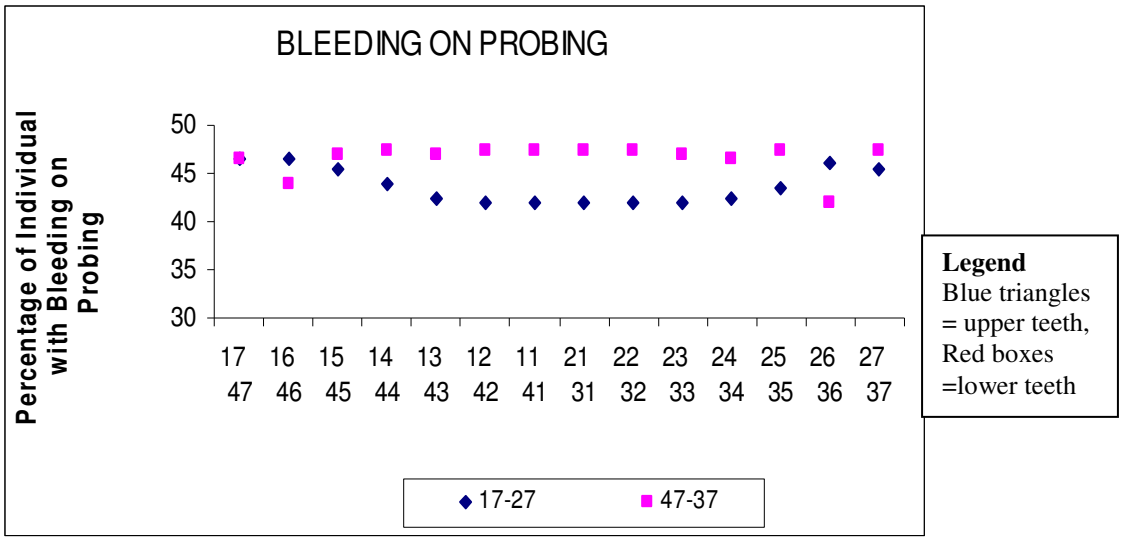


Figure 19: Percentage of individuals with bleeding on probing of the gingival tissues around each tooth amongst the Swahili participants

Presence of calculus amongst the Swahili participants

The teeth that had calculus were mainly in the cases. As shown in figure 20 below, 3% of cases (y-axis) and 78% (y-axis) of controls did not have any calculus on the tooth surfaces. In 14 tooth surfaces out of a possible 28 examined (x-axis), 61% of cases cumulatively and 5% of controls had calculus respectively. The median on the y-axis (50% of the individuals) showed calculus beng present on 19 tooth surfaces in

cases (x-axis). Less than 50% of the control individuals had calculus. None of the Swahili participants were found to have all teeth covered with calculus. When cases were compared to controls, the OR = 114.6, 95%CI = 33.1-397.2, p<0.001 showing a difference between cases and controls.

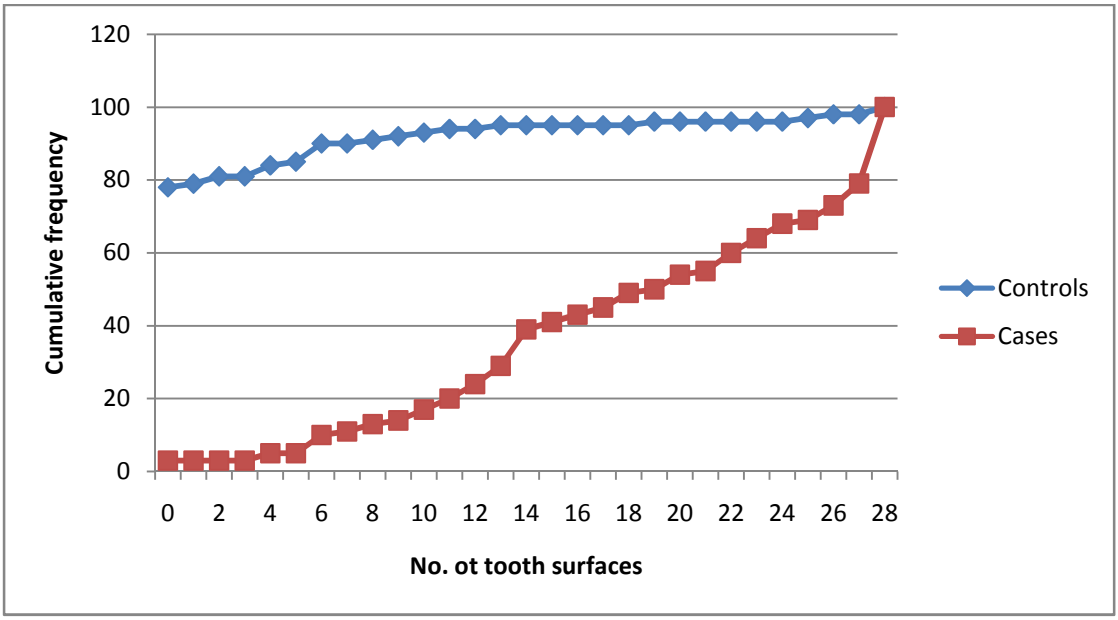


Figure 20: Cumulative frequency distribution of calculus on the tooth surfaces amongst the Swahili participants by cases and controls

There were appproximately 50% of Swahili participants with calculus on the lower anterior teeth (12,11,21,22) and upper right first molar (16). Approximately 45% had calculus on the upper right second molar (17) and left upper first molar (26). (figure 21 below).

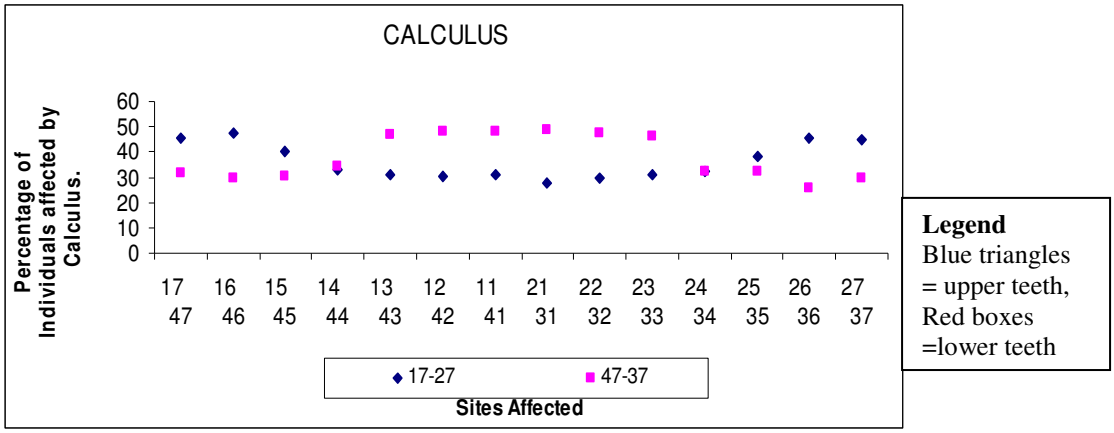


Figure 21: Percentage of individuals with calculus on the different tooth types amongst the Swahili participants

Distribution of periodontal pocket depths amongst the Swahili participants

On probing the periodontal pockets, it was found that depths of 1-3mm which are considered to be within normal range were present in both cases and controls. The cases had probing depths of more than 3mm but none of the controls had pockets of more than 3mm. Periodontal probing depths of more than or equal to 4mm were found on 8.33(5.0%) sites per individual. The mean pocket depth was 1.93(SD1.07).

When the different tooth types were examined in each individual, for the distribution of pocket depths, it was found that most Swahili partcipants had more or less an even distribution of pockets in all the lower and upper teeth as shown in figure 22 below. Approximately 23% of individuals had pockets depths of 3-5mm in the right lower second premolar (45). 20% had the same pocket depths in the upper second premolar (15). On the left, ~20% of individuals had pockets of 3-5mm on the lower first and second premolars (24,25).

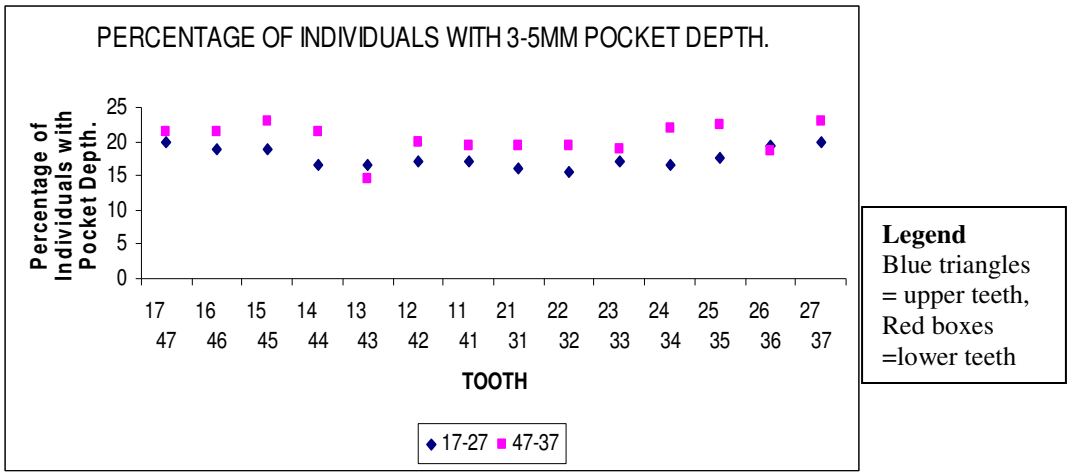


Figure 22: Cumulative frequency distribution of 3-5mm pocket depths on the different tooth types amongst the Swahili participants

Clinical attachment loss amongst the Swahili participants

Mean clinical attachment loss of more than or equal to 4mm was present in 37(SD 30.6) of the sites per individual that were examined. When CAL of more than or equal to 5mm and more than or equal to 6mm were considered, 25(SD 28.6) and 16(SD 24.8) sites per individual were affected respectively. The mean total CAL was 3.13(SD 1.07).

Dental caries

Swahili participants had very few teeth presenting with dental caries. In the controls, 2 individuals had one carious tooth, 8 individuals had two carious teeth and 3 individuals were found with 3 carious teeth. None of the controls had more than 4 teeth with caries. In the cases, 9 individuals had one carious tooth, 5 had two carious teeth and only one individual had 3 carious teeth. Additonally, one was found to have 4 carious teeth and another individual with 5 carious teeth. Hence in the Swahili participants, 87% of the controls and 83% of the cases were free from dental caries.

Roots and missing teeth

Only cases were found to have root remnants. Control subjects did not have root remnants. They had either carious teeth, sound teeth or very few missing teeth which had been extracted after developing dental caries.

5.5.1 SEVERITY OF CHRONIC PERIODONTITIS AMONGST THE SWAHILI PARTICIPANTS

Amongst the Swahili participants, the distribution of disease according to the CDC/AAP definitions[163] revealed a high prevalence of severe chronic periodontitis. Table 15 shows that mild periodontitis was present in only 9 (9%) individuals, moderate in 35(35%) and severe in 56(56%).

Table 15 : The Severity of chronic periodontitis amongst the Swahili participants

Disease severity according to CAL (CDC/AAP)	Number	Percent
	N	%
Mild	9	9
Moderate	35	35
Severe	56	56

Risk estimates of being a case amongst the Swahili participants in the presence of plaque on the tooth surfaces

The risk of having chronic periodontitis amongst the Swahili participants ranged from as low as 1.12 in the lower first left molar (36) to 14.94, in the upper right canine and second incisor. Thus the risk of being a case amongst the Swahili participants in the presence of plaque was 14 fold in the upper right anterior region and as low as one fold, in the lower left molar region. Plaque did not seem to confer a risk on the upper right first and second molar area as well as the lower right and left first molars (table 16). Therefore, plaque was positively associated with chronic periodontitis in most teeth.

Table 16: Risk estimates of being a case when plaque is present on the tooth surfaces amongst the Swahili participants

		95% Confidence Interval				95% Confidence Interval	
	Value	Lower	Upper		Value	Lower	Upper
17	2.41	0.605	9.608	47	3.912	1.24	12.343
16	2.842	0.731	11.046	46	1.894	0.757	4.74
15	10.123	3.765	27.215	45	3.31	1.029	10.647
14	10.921	5.439	21.925	44	4.171	1.483	11.728
13	14.942	7.444	29.993	43	6.38	2.103	19.357
12	14.942	7.444	29.993	42	7.169	2.378	21.608
11	9.681	4.999	18.747	41	7.579	2.522	22.779
21	10.375	5.309	20.274	31	7.579	2.522	22.779
22	9.947	5.153	19.201	32	7.169	2.378	21.608
23	10.601	5.513	20.386	33	13.821	3.153	60.577
24	9.333	4.87	17.886	34	3.586	1.127	11.412
25	10.725	5.112	22.502	35	5.774	1.616	20.633
26	5.505	2.153	14.07	36	1.12	0.527	2.378
27	5.58	2.182	14.268	37	6.951	1.977	24.444

The only tooth that showed that there was risk of clinical attachment loss (chronic periodontitis) in the presence of bleeding was the lower right central incisor (OR = 26.25, 95% CI = 10.04 – 68.635).

In the presence of both plaque and calculus, the risk of having chronic periodontitis was significant on the upper right central and lateral incisors (11,12), the lower right central incisor (41) and the lower canines (43,33) (figure 23).

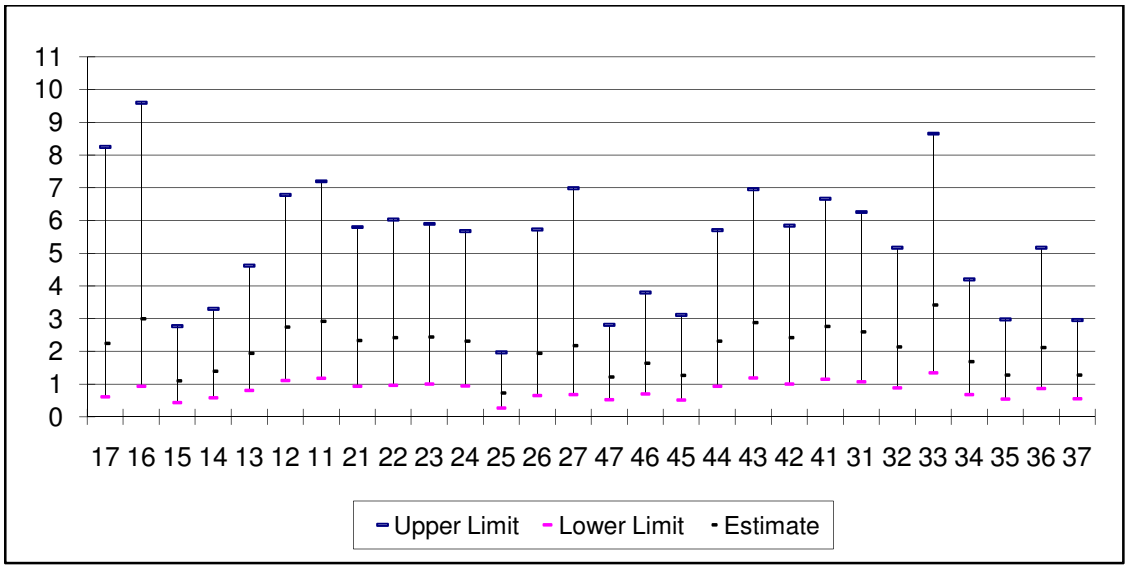


Figure 23 : Risk estimates (OR and CI) of having chronic periodontitis in the presence of plaque and calculus on the tooth surfaces amongst the Swahili participants

5.6 FREQUENCY OF INTERLEUKIN-1B AND 1A GENOTYPES AND ALLELES AMONGST THE SWAHILI PARTICIPANTS

Interleukin-1 polymorphisms were tested at locus -511, +3954, -889 and +4845. The genotype frequency was tested for allele 1 and 2 at the four loci. Thus the presence of allele 1 alone (1-1), presence of allele 1 and 2 (1-2) and the presence of allele 2 alone (2-2) were tested and the results are presented in table 17 below.

Frequency of IL-1B -511 and +3954 genotypes and alleles

Table 17 shows the various frequency distributions of the four genotypes in cases and controls. An insignificant higher frequency of heterozygous IL-1B (-511) was found in cases 67(69.8%) than amongst the controls 66(69.5%), p=0.881. The frequency of homozygous allele 1 was very low with only 4(4.2%) and 6(6.3%)

individuals in cases and controls respectively, p=0.516. Homozygous distribution of allele 2 was 25(26%) and 23(24.2%) in cases and controls, p=0.741, (table 17).

The frequency distribution of allele 2 at -511 was higher than allele 1. The frequencies that were found were, 117(60.9%) and 112(58.2%) in cases and controls respectively for allele 2 and 75(39.1%) and 78(41%) in cases and controls respectively for allele 1, p=0.983.

Interleukin-1B +3954 on the other hand showed an insignificant higher frequency distribution of homozygous allele 1 of 43(48.9%) and 35(41.4%) in cases and controls, p=0.246. Heterozygous distribution between cases and controls, was 29(33%) and 29(34.1%) respectively, p=1.000. The homozygous distribution of allele 2 (2-2) was the lowest, with the cases and controls having 16(18.2%) and 21(24.7%) respectively, p=0.363. The total frequency of allele 1 was insignificantly higher in cases 115(65.5%) than controls, 99(58.2%), with p value of 0.174.

Frequency of IL-A -889 and +4845 genotypes and alleles amongst Swahili participants

Homozygous frequency for allele 1 of -889 was significantly higher for cases than controls, 15(16.3%) and 5(5.1%) respectively, with a p value of 0.018. Heterozygous frequency was 6(6.5%) and 4(4.1%) for cases and controls respectively with a p value of 0.516. Homozygous distribution for allele 2 (2-2) was significant, p <0.001, with a distribution of 71(77.2%) and 89(90.8%) in cases and controls respectively (table 17 below).

The carriage rate of allele 2 polymorphism at -889 in cases was 148(80.4%) and controls 182 (92.9%), whereas the frequency of allele 1 was 36(19.6%) and 14(7.1%) in cases and controls, p< 0.001, OR = 3.16, 95%CI =1.644-6.083.

At loci +4845, the frequency of the homozygous allele 1 for +4845 was the lowest, with cases at 5(5.7%) and controls at 6(6.7%), p=0.756. Heterozygous for +4845 was 59(67%) and 56(62.2%), p=0.668 and homozygous for allele2 was 24(27.3%) and 28(31.1%) in cases and controls respectively p=0.519. The frequency for allele 2 carriage rate at loci +4845 was 107(60.8%) and 112(62.2%) for cases and controls respectively, p=0.782 (table 17 below).

Table 17 : Distribution of IL-1B and IL-1A genotype and allele frequencies amongst the Swahili participants

		Cases (n=94)		Controls (n=96)			95% CI for OR		
Genotype		**n**	**%**	**n**	**%**	**P-Value**	**OR**	**Lower**	**Upper**
IL-1B -511	1—1	4	4.2	6	6.3	0.516			
	1—2	67	69.8	66	69.5	0.881			
	2—2	25	26	23	24.2	0.741			
Allele	1	75	39.1	78	41.1	0.691	0.92	0.611	1.386
	2	117	60.9	112	58.9				
IL-1B+3954	1—1	43	48.9	35	41.2	0.246			
	1—2	29	33	29	34.1	1.000			
	2—2	16	18.2	21	24.7	0.363			
Allele	1	115	65.3	99	58.2	0.174	1.35	0.875	2.089
	2	61	34.7	71	41.8				
IL-1A-889	1—1	15	16.3	5	5.1	0.018*			
	1—2	6	6.5	4	4.1	0.516			
	2—2	71	77.2	89	90.8	0.001*			
Allele	1	36	19.6	14	7.1	<0.001*	3.16	1.644	6.083
	2	148	80.4	182	92.9				
IL-1A +4845	1--1	5	5.7	6	6.7	0.756			
	1--2	59	67	56	62.2	0.668			
	2--2	24	27.3	28	31.1	0.519			
Allele	1	69	39.2	68	37.8	0.782	1.062	0.693	1.628
	2	107	60.8	112	62.2				

*p<0.05, OR= Odds Ratio, CI = Confidence Interval

Hardy Weinberg Principle

Allele -511

$$p = \frac{2 \times Obs(AA) + Obs(Aa)}{2 \times (Obs(AA) + Obs(Aa) + Obs(aa))}$$

$$p = \frac{2 \times 10 + 133}{2 \times 191} = \frac{153}{382} = 0.4$$

$q = 1 - p = 0.6$

HWP expectations

$Exp(AA) = p^2 n = 0.4^2 \times 191 = 30.56$

$Exp(Aa) = 2pqn = 2 \times 0.4 \times 0.6 \times 191 = 91.68$

$Exp(aa) = q^2 n = 0.6^2 \times 191 = 68.76$

Pearson Chi-square test

$\chi^2 = \Sigma(O - E)^2 / E$

$= (10 - 30.56)^2 / 30.56 + (133 - 91.68)^2 / 91.68 + (48 - 68.76)^2 / 68.76 = 38.66$

Locus -511, $\chi^2 = 38.66$, $p<0.05$

Locus +3954, $\chi^2 = 14.66$, $p<0.05$

Locus -889, $\chi^2 = 85.22$, $p<0.05$

Locus +4845, $\chi^2 = 23.80$, $p<0.05$

χ^2 distribution with 1df (3.84) shows that equilibrium exists for IL-1B -511, +3954 and IL-1A -889 and +4845 in the Swahili participants.

5.6.1 FREQUENCY OF IL-1A AND IL-1B COMPOSITE GENOTYPE AMONGST THE SWAHILI PARTICIPANTS

The frequency distribution of the composite genotype is shown on table 18 below. Heterozygotes for the composite genotype, 1-2/1-2, were found to be 0% in controls. There was equal distribution of homozygous allele 2 at locus -889 [27(32.5%)] and homozygous distribution of allele 1 at locus +3954 [27(32.5%)] with a p value of 1. The positive genotype (allele 2 polymorphism at -889 and allele 2 polymorphism at +3954) distribution was recorded in 65(78.3%) cases and 76(91.6%) controls, with a p value of 0.088.

Table 18: Distribution of the IL-1A-889 and IL-1B+3954 composite genotypes amongst the Swahili participants

	Cases (n=100)		Controls (n=100)		
Composite genotype	**n**	**%**	**n**	**%**	**P-Value**
1--1/1—1	9	10.8	3	3.6	0.074
1--2/1--1 0r 1--1/1—2	9	10.8	4	4.8	0.152
1--2/1—2	3	3.6	0	0.0	0.081
1--2/2--2 0r 2--2/1—2	19	22.9	29	34.9	0.098
2--2/1—1	27	32.5	27	32.5	1
2--2/2—2	16	19.3	20	24.1	0.462
Positive genotype	65	78.3	76	91.6	0.088

5.6.2 DISTRIBUTION OF INTERLEUKIN-1 GENOTYPES AND ALLELES ACCORDING TO GENDER AMONGST THE SWAHILI PARTICIPANTS

Male Swahili participants

Homozygous frequency for allele 1 at -511 was 2(3.8%) for cases and 3 (5.8%) for controls, p=0.647. At locus +3954 the frequency was 24(52.2%) in cases and 19(42.2%) in controls, p=0.326. At locus -889, the frequency was 6(11.8%) in cases

and 3(5.7%) in controls, p=0.296 and at locus +4845, it was 2(41%) and 3(6.3%) in cases and controls respectively, p=0.647.

The homozygous distribution for allele 2 at the different loci were: - for locus -511 was 14(26.4%) in cases and 12(23.1%) in controls, p=0.653. For locus +3954, the carriage rate was 8(17.4%) in cases and 14(31.1%) in controls, p=0.152. Locus -889 the carriers were 40(78.4%) in cases, 50(94.3%) in controls, p=0.01 (OR=0.228, 95%CI= 0.07-0.75) and finally at locus +4845, 14(28.6%) cases and 15(31.3%) controls were carriers, p=0.828. Of note is that none of the male Swahili participants in the control group were heterozygous for locus -889. Therefore, heterozygotes at locus -889, had a distribution of 5(9.8%) in cases only.

Female Swahili participants

Amongst the female Swahili participants, homozygous distribution of allele 1 at the different loci were; 2(4.7%) in cases and 3(7.0%) in controls at locus -511, p=0.646. Locus +3954, the frequency was 19(45.2%) in cases and 16(40.0%) in controls, p=0.519. Locus -889, the distribution seen was 9(22.0%) and 2(4.4%) in cases and controls respectively, p=0.024 (OR=5.57, 95%CI=1.15-27.88) and at locus +4845, the carriage rate was 3(7.7%) in cases and 3(7.1%) in controls, p=1.000. Homozygous distribution for allele 2: at locus -511 was 11(25.6%) and 11(25.6%) in cases and controls, p=1.000. At locus +3954, the frequency was 8(19.0%) in cases and 7(17.5%) in controls, p=0.778. Locus -889, the frequency was 31(75.6%) and 39(86.7%) in cases and controls respectively, p=0.051; and at locus +4845, the frequency was 10(25.6%) in cases, and 13(31.0%) in controls, p=0.47 (table 19 below).

Table 19: Distribution of IL-1 genotypes and alleles according to gender amongst the Swahili participants

Genotype		Male (n=108)					Female(n=92)				
		Cases (n=54)		Controls (n=54)			Cases (n=46)		Controls (n=46)		
		n	%	N	%	P-Value	n	%	n	%	P-Value
-511	1--1	2	3.8	3	5.8	0.647	2	4.7	3	7.0	0.646
	1--2	37	69.8	37	71.2	1.000	30	69.8	29	67.4	0.828
	2--2	14	26.4	12	23.1	0.653	11	25.6	11	25.6	1.000
+3954	1--1	24	52.2	19	42.2	0.326	19	45.2	16	40.0	0.519
	1--2	14	30.4	12	26.7	0.653	15	35.7	17	42.5	0.662
	2--2	8	17.4	14	31.1	0.152	8	19.0	7	17.5	0.778
-889											
	1--1	6	11.8	3	5.7	0.296	9	22.0	2	4.4	0.024*
	1--2	5	9.8	0	0.0	0.022*	1	2.4	4	8.9	0.168
	2--2	40	78.4	50	94.3	0.01*	31	75.6	39	86.7	0.051
+4845	1--1	2	4.1	3	6.3	0.647	3	7.7	3	7.1	1.000
	1--2	33	67.3	30	62.5	0.558	26	66.7	26	61.9	1.000
	2--2	14	28.6	15	31.3	0.828	10	25.6	13	31.0	0.47

*P< 0.05

5.6.3 DISTRIBUTION OF INTERLEUKIN-1B AND 1A IN THOSE WITH MILD, MODERATE AND SEVERE FORMS OF CHRONIC PERIODONTITIS AMONGST THE SWAHILI PARTICIPANTS

Regarding interleukin-1B polymorphism at locus -511, the frequency for allele 1 was 7(43.75%) in mild CP, 26(39.39%) in moderate CP and 42(38.18%) in severe CP. As for allele 2 at the same locus (-511), the frequencies were 9(56.25%) in mild, 40(60.61%) in moderate and 68(61.82%) in severe chronic periodontitis p=0.91 (table 20 below). The allele 1 frequency at locus +3954 was found to be 9(56.25%) in mild CP, 40(68.97%) in moderate CP and 66(64.71%) in severe CP. As for allele 2 the frequency distribution was 7(43.75%), 18(31.03%) and 36(35.29%) in mild, moderate and severe CP respectively, p=0.63. The distribution of allele 1 at locus -889 was 4(28.57%), 17(25.76%) and 15(14.42%) for mild, moderate and severe forms of CP. Allele 2 at locus -889 the frequencies were 10(71.43%), 49(74.24%) and 89(85.58%) for the three categories of severity of CP respectively, p=0.13.At locus +4845, the frequency of allele 1 was 6(42.86%) for mild CP, 24(41.38%) for moderate CP and 39(37.50%) for severe CP. The frequency for allele 2 at locus +4845 was 8(57.14%), 34(58.62%) and 65(62.50%) for mild, moderate and severe CP, p=0.85 (table 20).

Table 20: Distribution of IL-1B and IL-1A genotype and allele frequencies amongst Swahili participants with mild, moderate and severe chronic periodontitis

		Mild CP (n=9(9%))		Moderate CP (n=35(35%))		Severe CP (n=56(56%))		
Genotype		**n**	**%**	**n**	**%**	**N**	**%**	**P-Value**
IL-1B -511	1--1	0	0.00	3	9.09	1	1.82	0.224
	1--2	7	87.50	20	60.61	40	72.73	0.285
	2--2	1	12.50	10	30.30	14	25.45	0.559
Allele	1	7	43.75	26	39.39	42	38.18	0.91
	2	9	56.25	40	60.61	68	61.82	
IL-1B+3954	1--1	2	25.00	15	51.72	26	50.98	0.396
	1--2	5	62.50	10	34.48	14	27.45	0.172
	2--2	1	12.50	4	13.79	11	21.57	0.533
Allele	1	9	56.25	40	68.97	66	64.71	0.63
	2	7	43.75	18	31.03	36	35.29	
IL-1A-889	1--1	2	28.57	7	21.21	6	11.54	0.394
	1--2	0	0.00	3	9.09	3	5.77	0.599
	2--2	5	71.43	23	69.70	43	82.69	0.297
Allele	1	4	28.57	17	25.76	15	14.42	0.13
	2	10	71.43	49	74.24	89	85.58	
IL-1A +4845	1--1	0	0.00	2	6.90	3	5.77	0.769
	1--2	6	85.71	20	68.97	33	63.46	0.874
	2--2	1	14.29	7	24.14	16	30.77	0.413
Allele	1	6	42.86	24	41.38	39	37.50	0.85
	2	8	57.14	34	58.62	65	62.50	

Significance at P<0.05

The severity of chronic periodontitis was subjected to further analysis by comparing the frequency of IL-1B and IL-1A genotypes in mild, moderate and severe CP with controls (table 21). The results show that allele 1 frequency at locus -889, was

4(28.57%) for mild chronic periodontitis and 14(7.1%) for the controls, p=0.005 and OR=5.2, 95%CI=1.445-18.71.

The frequency for allele 1 at locus -889 for moderate chronic periodontitis was 17(25.76%) and 14(7.1%) for controls, p=<0.001, OR=4.51, 95%CI=2.08-9.79.

The severe form of chronic periodontitis showed a distribution of 15(14.42%) in cases and 14(7.1%) in controls, p=0.042, OR=2.19, 95% CI = 1.013 – 4.738.

Table 21. Distribution of IL-1B and IL-1A genotype and allele frequencies in a) mild, b) moderate and c) severe chronic periodontitis amongst Swahili participants.

a) Mild chronic periodontitis

		Cases (n=16)		Controls (n=100)				95% CI for OR	
Genotype		**n**	**%**	**n**	**%**	**P-Value**	**OR**	**Lower**	**Upper**
IL-1B -511 Allele	1	7	43.75	78	41.1	0.83	1.11	0.399	3.125
	2	9	56.25	112	58.9				
IL-1B+3954 Allele	1	9	56.25	99	58.2	0.88	0.92	0.328	2.592
	2	7	43.75	71	41.8				
IL-1A-889 Allele	1	4	28.57	14	7.1	0.005*	5.2	1.445	18.71
	2	10	71.43	182	92.9				
IL-1A +4845 Allele	1	6	42.86	68	37.8	0.706	1.24	0.411	3.713
	2	8	57.14	112	62.2				

*p<0.05, OR = Odds Ratio, CI = Confidence Interval

b) Moderate chronic periodontitis

		Cases (n=16)		Controls (n=100)				95% CI for OR	
Genotype		**n**	**%**	**n**	**%**	**P-Value**	**OR**	**Lower**	**Upper**
IL-1B -511 Allele	1	26	39.39	78	41.1	0.813	0.93	0.527	1.654
	2	40	60.61	112	58.9				
IL-1B+3954 Allele	1	40	68.97	99	58.2	0.148	1.6	0.845	3.01
	2	18	31.03	71	41.8				
IL-1A-889 Allele	1	17	25.76	14	7.1	<0.001*	4.51	2.08	9.79
	2	49	74.24	182	92.9				
IL-1A +4845 Allele	1	24	41.38	68	37.8	0.624	1.16	0.64	2.13
	2	34	58.62	112	62.2				

c) Severe chronic periodontitis

		Case (n=16)		Controls (n=100)				95% CI for OR	
Genotype		**n**	**%**	**n**	**%**	**P-Value**	**OR**	**Lower**	**Upper**
IL-1B -511 Allele	1	42	38.18	78	41.1	0.625	0.887	0.548	1.435
	2	68	61.82	112	58.9				
IL-1B+3954 Allele	1	66	64.71	99	58.2	0.292	1.32	0.79	2.18
	2	36	35.29	71	41.8				
IL-1A-889 Allele	1	15	14.42	14	7.1	0.042*	2.19	1.013	4.738
	2	89	85.58	182	92.9				
IL-1A +4845 Allele	1	39	37.50	68	37.8	0.962	0.988	0.6	1.627
	2	65	62.50	112	62.2				

P=0.05*, OR = odds ratio, CI=confidence interval,

5.7 COMPARISON OF THE CARRIAGE RATE OF IL-1A AND IL-1B GENOTYPE AND ALLELE FREQUENCIES FOR ALL PARTICIPANTS IN BOTH ETHNIC GROUPS

When the genotype and allele frequencies were analyzed in all participants (cases and controls) in both ethnic groups, it was found that there were more Swahili participants who were heterozygous at loci -511 ($p<0.001$) and +3954 ($p=0.004$), than the Taita participants (table 22 below). The Swahili participants also had a higher frequency of allele 1and allele 2 at locus +3954 than the Taita participants with a p value =0.001. The frequency distribution of homozygous allele 2 at +3954 ($p<0.001$), -889 ($p<0.001$) and +4845 ($p=0.002$) was also higher amongst the Swahili participants (table 22 below).

The frequencies that were found to be higher amongst the Taita participants, when compared to the Swahili participants were at locus -889. Homozygous for allele 1 was higher ($p<0.001$), heterozygous for the same locus was also higher at ($p<0.001$) and the carriage rate of allele 1 was also higher ($p<0.001$).

The frequency of allele 1 at locus -511 was 38.4% for Taita participants and 40.1% for Swahili participants, $p=0.67$. The frequency of allele 1 at +3954 was 75.7% for Taita participants and 61.8% for Swahili participants, $p=0.001$. That of allele 2 was 24.3% for Taita participants and 38.2% for Swahili participants. The difference in the frequencies between the two ethnic groups was significant, $p=0.001$. The frequency of allele 1 at -889 was 58.6% in Taita participants and 13.2% in Swahili participants, $p=<0.001$. The frequency of allele 1 at +4845 was 44.1% amongst Taita participants and 38.5% amongst the Swahili participants. On the other hand, allele 2 was 55.9%

and 61.5% amongst Taita and Swahili participants respectively, p=0.154 (table 22 below).

Table 22. Comparison of the distribution of IL-1B and IL-1A genotype and allele frequencies for all subjects amongst the Taita and Swahili participants

		Taita (n=190)		Swahili (n=200)		
Genotype		**n**	**%**	**N**	**%**	**P-Value**
IL-1B -511	1--1	11	8.0	10	5.2	0.73
	1--2	84	60.9	133	69.6	<0.001*
	2--2	43	31.2	48	25.1	0.749
Allele	1	106	38.4	153	40.1	0.67
	2	170	61.6	229	59.9	
IL-1B+3954	1--1	68	61.3	78	45.1	0.513
	1—2	32	28.8	58	33.5	0.004*
	2—2	11	9.9	37	21.4	<0.001*
Allele	1	168	75.7	214	61.8	0.001*
	2	54	24.3	132	38.2	
IL-1A-889	1--1	64	37.9	20	10.5	<0.001*
	1--2	70	41.4	10	5.3	<0.001*
	2--2	35	20.7	160	84.2	<0.001*
Allele	1	198	58.6	50	13.2	<0.001*
	2	140	41.4	330	86.8	
IL-1A +4845	1--1	9	6.3	11	6.2	0.733
	1--2	108	75.5	115	64.6	0.896
	2--2	26	18.2	52	29.2	0.002*
Allele	1	126	44.1	137	38.5	0.154
	2	160	55.9	219	61.5	

*P< 0.05

5.8 COMPARISON OF IL-1B AND IL-1A BETWEEN THE TAITA AND SWAHILI PARTICIPANTS WITH CHRONIC PERIODONTITIS

When a comparison was made between the Taita and the Swahili participants cases only, it was found that in the heterozygous individuals at locus -511, the Swahili participants had more alleles at 47(63.5%) than the Taita participants at 67(69.8%), p=0.016.

The frequency distribution of homozygous allele 1 at -889 was significantly higher amongst the Taita participants at 36(41.1%) than the Swahili participants at 15(16.3%), p<0.001. Heterozygous frequency again was significantly higher in the Taita participants at 34(39.1) than the Swahili participants at 6(16.5) (p<0.001). However, homozygous distribution of allele 2 at -889 was significantly higher in the Swahili participants as compared with the Taita participants (p<0.001). The frequency distributions found at -889 homozygous allele 2 were 17(19.5%) and 71(77.2%) for Taita and Swahili participants respectively.

The carriage rate of allele 1 and allele 2 (locus -889) was significantly different between the two ethnic groups. There was a higher frequency distribution of allele 1 amongst the Taita participants with chronic periodontitis. Thus, 106(60.9%) was observed in Taita participants and 36(19.6%) in the Swahili participants p<0.001. On the other hand, a higher frequency distribution of allele 2 was observed amongst the Swahili participants, 148(80.4%) as compared to 68(39.1%) amongst the Taita participants, p<0.001 (table 23 below).

Table 23. Comparison of the distribution of IL-1B and IL-1A genotype and allele frequencies for participants with chronic periodontitis amongst both ethnic groups

		Taita (n=94)		Swahili (n=100)		
Genotype		**n**	**%**	**n**	**%**	**P-value P<0.05**
IL-1B -511	1—1	5	6.8	4	4.2	0.662
	1—2	47	63.5	67	69.8	0.016*
	2—2	22	29.7	25	26.0	0.795
Allele	1	57	38.5	75	39.1	0.918
	2	91	61.5	117	60.9	
IL-1B+3954	1—1	34	54.0	43	48.9	0.331
	1—2	21	33.3	29	33.0	0.289
	2—2	8	12.7	16	18.2	0.113
Allele	1	89	70.1	115	65.3	0.386
	2	38	29.9	61	34.7	
IL-1A-889	1—1	36	41.4	15	16.3	<0.001*
	1—2	34	39.1	6	6.5	<0.001*
	2—2	17	19.5	71	77.2	<0.001*
Allele	1	106	60.9	36	19.6	<0.001*
	2	68	39.1	148	80.4	
IL-1A +4845	1—1	1	1.4	5	5.7	0.114
	1—2	58	78.4	59	67.0	0.701
	2—2	15	20.3	24	27.3	0.162
Allele	1	60	40.5	69	39.2	0.807
	2	88	59.5	107	60.8	

*P<0.05

5.9 COMPARISON OF GINGIVITIS AND PLAQUE BETWEEN THE TAITA AND SWAHILI PARTICIPANTS

Comparison of gingivitis between the two ethnic groups showed that Swahili participants with chronic periodontitis had more sites with bleeding on probing than the Taita participants, with OR = 3.144(95% CI = 1.303 – 7.59) p=0.007. When plaque distribution was investigated, it was found again that the Swahili participants had more sites with plaque than the Taita participants, OR = 1.185(95% CI = 1.084-1.296) (tables 24 and 25 below).

Table 24: Comparison of bleeding on probing between the two ethnic groups

Risk factor	Cases		Controls		P-value
	N	%	N	%	
Taita	17.31	39.4	6	17.1	0.007*
Swahili	25.02	60.6	29	82.90%	

Odds ratio (Taita participants /Swahili participants)=3.144 with 95% confidence interval (1.303, 7.59).

Table 25: Comparison of the presence of plaque in the two ethnic groups

Risk factor	Cases		Controls		P-value
	N	%	N	%	
Taita	24.94	47.9	15.04	43.7	<0.001*
Swahili	25.52	52.1	18.25	56.3	

Odds ratio (Taita participants /Swahili participants) = 1.185 with 95% confidence interval (1.084, 1.296).

5.10 ORAL HYGIENE HABITS AMONGST THE TAITA PARTICIPANTS

Ninety eight percent of the participants (195) brushed their teeth with 48.5% of them doing it twice daily. Eighty eight percent (175) used commercial toothbrushes and toothpaste. Only 22 (11.1%) used chewing sticks. Majority of the participants brushed in the mornings, 195(98.5%). A large number also brushed in the evenings, 153(77.3%). 190(96%) found it necessary to brush daily. There was a significant difference between cases 82(82.8%) and controls 93(93.9%) in the use of toothbrushes, p=0.007. The frequency of those brushing in the evenings was 69(69.7%) and 84(84.8%) amongst cases and controls respectively, p=0.007, (table 26 below).

Table 26: Brushing habits of the Taita participants.

	Total	Cases	Controls	OR (95% CI)	P value
Brushed once a day	31 (15.7%)	22 (23.4%)	9(9.2%)	1.63 (0.16 - 15.59)	0.63
Brushed twice a day	96 (48.5%)	45 (47.9%)	51(52.0%)	0.59(0.07-4.60)	0.666
Brushed More than twice	58 (29.3%)	23(24.5%)	35(35.7%)	0.44(0.05-3.60)	0.64
Used tooth brush	175 (88.4%)	82(82.8%)	93(93.9%)	0.26(0.08-0.79)	0.007*
Used toothpaste	175 (88.4%)	83(85.6%)	92(95.8%)	0.30(0.04-1.71)	0.16
Used chewing stick	22 (11.1%)	17(17.2%)	5(5.1%)	Reference	
Brushed previous afternoon	77 (38.9%)	35(35.4%)	42(42.9%)	0.73(0.39-1.35)	0.279
Brushed previous evening	153 (77.3%)	69(69.7%)	84(84.8%)	0.38(0.18-0.82)	0.007*
Brushed yesterday morning	195 (98.5%)	98(99.0%)	97(98.0%)	2.02(0.14-57.26)	1
Brushed in the morning	193 (97.5%0	97(98.0%)	97(97.0%)	1.50(0.20-13.15)	1
Found it necessary to brush daily	190 (96%)	92(92.9%)	98(99.0%)	0.13(0.01-1.12)	0.064

OR = Odds Ratios, CI = Confidence Interval, * significant value

5.11 CONSUMPTION OF SUGARY FOODS AND DRINKS AMONGST THE TAITA PARTICIPANTS

Less than half of the participants 96(48.5%) consumed sugary foods. Of these, only 7(3.5%) did so daily. 73(36.9%) were willing to stop consuming sugary foods to protect their teeth. There were more participants consuming sugary drinks, 182(91.9%), than sugary foods, 95(48.5%). Those who consumed sugary drinks mainly drank tea with sugar. Of these, 153(77.3%) did so 2-3times a day. However, most of them, 167(84.3%) were willing to stop taking sugary drinks to protect their teeth. Majority of these values were not significant, except for the consumption of sugary drinks, where more controls were found to consume sugary drinks (p=0.018) (table 27 below).

Table 27: Consumption of sugary foods and drinks amongst the Taita participants

	Total	Cases	Controls	OR (95% CI)	P value
Consumed sugary foods	96 (48.5%)	51 (52.0%)	45(45.9%%)	1.28(0.70-2.33)	0.392
Sometimes	82 (41.4%)	44 (89.8%)	38 (95.0%)	Reference	
Consumed daily	5 (2.5%)	4(8.2%)	1(2.5%)	3.45(0.34-84.80)	0.374
Consumed 2-3 times a day	2 (1.0%)	1(2.0%)	1(2.5%)	0.86(0.02-32.92)	1
Found it necessary to consume sugary foods	15 (7.6%)	7(14.3%)	8(24.2%)	0.52(0.15-1.83)	0.255
Was willing to stop to protect teeth	73 (36.9%)	46 (93.9%)	27 (79.4%)	3.98(0.82-21.45)	0.082
Consumed sugary drinks	182 (91.9%)	87 (91.6%)	95 (99.0%)	0.11(0.01-0.93)	0.018*
Sometimes	5 (2.5%)	4 (4.3%)	1 (1.0%)	Reference	
Once daily	26 (13.1%)	20 (21.5%)	6 (6.2%)	0.83(0.00-11.52)	1
Consumed 2-3 times in a day	153 (77.3%)	65 (69.9%)	88 (90.7%)	0.18(0.01-1.82)	0.168
Consumed 4-6 times	5 (2.5%)	3 (2.2%)	2 (2.1%)	0.25(0.00-8.65)	0.523
Found it necessary to consume sugary drinks	167 (84.3%)	79 (83.2%)	88 (88.9%)	0.62(0.25-1.51)	0.25
Was willing to stop to protect teeth	112 (56.6%)	61 (64.2%)	51 (51.5%)	1.69(0.91-3.3)	0.074

OR = Odds Ratios, CI = Confidence Interval, * significant value

5.12 ORAL HEALTH SEEKING BEHAVIOR AMONGST THE TAITA PARTICIPANTS

Only 19(9.6%) participants had visited a dentist and none of them did so regularly. Therefore, a mixed method of reporting is used to report the findings.

When the cases were compared with controls, the health seeking behavour amongst the Taitas was the same in both groups. Thus analysis was done for both groups combined because of the few numbers who had been to a dental clinic. The main reason for hesitating to seek dental treatment for 93 of the participants was that the distance to the dental clinic was too far. 89 participants felt the treatment was too costly. The long waiting time to get treatment was reported as a hindrance in visiting a dentist by 17 participants. A few of the participants, 6, reported that they had no time to visit a dental clinic. Only four were concerned about getting HIV from a clinic, two had found unfriendly staff and one had heard bad stories about dental treatment (table 28 below).

Of the nineteen participants who had visited a dental clinic, 10 were satisfied with the visit because bleeding of the gums stopped. Six reported that the treatment being done without an injection was a source of satisfaction for them. One participant was happy because no problem was detected and 2 were satisfied because the treatment was done quickly (table 28). Of the few Taita participants (19) who had visited a dental clinic, only 12(6.06%) responded to this question on dissatisfaction with dental clinic visit. The smell in the dental clinic was a cause for dissatisfaction in 6 participants, the use of the dental injection/local anesthesia

during treatment was a problem for three participants, the noise of the equipment affected one person and the time spent waiting for treatment made two participants unhappy (table 28).

Table 28: Categorization of responses to the skip pattern questions on oral health seeking behavior amongst the Taita participants (Mixed methods[148])

Inductive categories	Participant responses
Reasons for hesitation in seeking dental treatment	• Distance to dental clinic is too long - **93 participants** • Presence of unfriendly dental workers - **2 participants** • Long waiting time at the dental clinic - **17 participants** • No time to go to the dental clinic - **6 participants** • Treatment is too costly - **89 participants** • Getting infected with HIV - **4 participants** • Heard stories about bad things happening to patients in the dental clinic - **1 participant**
Reasons for satisfaction with the dental visit	• No bleeding of gums anymore – **10 participants** • Treatment done without injection – **6 participants** • No dental problems discovered at all - **1 participant** • Treatment was done quickly - **2 participants**
Reasons for dissatisfaction with dental visit	• Don't like the smell in the surgery- **6 participants** • Don't like the local anesthesia/injection **- 3 participants** • The noise of the equipment - **1 participant** • The long time spent in the waiting room - **2 participants**

5.13 ORAL HYGIENE HABITS AMONGST THE SWAHILI PARTICIPANTS

Most of the Swahili participants, 194 (97%) reported that they brushed their teeth and did so once or twice a day. More cases, 43(46.2%) were found to brush twice a day when compared with controls, 31 (33.7%), p- value of 0.043. More than half of the Swahili participants, 48(52.2%) in the control group reported that they brushed their teeth once a day. Most Swahili participants used toothbrushes and toothpaste, 171(85.5%). Very few used chewing sticks 19(0.1%) as shown in table 29. More of the controls, 94(96.9%) were found to have brushed their teeth the previous morning, compared with cases (p- value of <0.001) (table 29).

Table 29: Brushing habits amongst the Swahili participants

	Cases	Controls	OR (95%CI)	p- value
Brushed once a day	45(48.4%)	48(52.2%)	5.63(0.63-128.90)	0.12
Brushed twice a day	43(46.2%)	31(33.7%)	8.32(0.91-192.92)	0.043*
Brushed > 2 times a day	4(4.3%)	6(6.5%)	4.00(0.25-127.24)	0.338
Brushed with a toothbrush	89(93.7%)	82 (86%)	2.35(0.79-7.32)	0.091
Brushed with toothpaste	66(69.5%)	61(64.2%)	1.27(0.66-2.43)	0.442
Used a chewing stick	6 (6.3%)	13(13.7%)	Reference	
Brushed previous evening	63(63.6%)	61(62.9%)	1.00(0.54-1.88)	0.989
Brushed previous morning	63(63.6%)	94(96.9%)	0.04(0.01-0.17)	<0.001*
Brushed in the morning	96(99%)	94(96.9%)	2.04(0.14-57.91)	0.621
Found it necessary to brush	93(93.9%)	89(91.8%)	1.39(0.42-4.75)	0.553

OR = Odds Ratios, CI = Confidence Interval

5.14 CONSUMPTION OF SUGARY FOODS AND DRINKS AMONGST THE SWAHILI PARTICIPANTS

Most of the Swahili participants consumed sugary foods at least once a day, both in cases, 83 (84.7%) and controls, 87 (89.7%). However, the difference between the two groups was not significant. Majority of the participants consumed sugary foods once a day, both in cases 35 (39.3%) and controls, 32 (38.1%). An almost equal number of participants consumed sugary foods 2-3 times a day, 37 (41.6%) and 28(33.3%) cases and controls respectively. Of the Swahili participants consuming sugary foods, 39 (44.6%) were willing to stop consumption to protect their teeth. Consumption of sugary drinks was also common but there was no significant difference when cases were compared with controls, 88(92.6%) and 87 (90.6%) respectively. Most Swahili participants consumed sugary drinks once a day. Only the cases were willing to stop for the sake of their teeth, p- value of <0.001 (table 30 below).

Table 30: Consumption of sugary foods and drinks amongst the Swahili participants

	Cases	Controls	OR (95%CI)	p-value
Consumed sugary foods	83(84.7%)	87(89.7%)	0.64(0.25-1.61)	0.297
Sometimes but not everyday	16(18.0%)	22(26.2%)	Reference	
Once a day	35(39.3%)	32(38.1%)	1.50(0.63-3.63)	0.32
Consumed 2-3 times a day	37 (41.6%)	28 (33.3%)	1.82(0.75-4.43)	0.148
Consumed 4-6 times a day	1(1.1%)	2(2.4%)	0.69(0.02-11.14)	1
Found it necessary to consume	38(42.7%)	33(37.9%)	1.22(0.64-2.33)	0.52
Was willing to stop to protect teeth	15(17.0%)	24(27.6%)	0.54(0.24-1.18)	0.094
Consumed sugary drinks	88 (92.6%)	87 (90.6%)	1.30 (0.42-4.09)	0.617
Sometimes	24 (27.0%)	22 (25.3%)	Reference	
Once daily	57 (64.0%)	59 (67.8%)	0.89 (0.42-1.85)	0.728
Consumed 2-3 times a day	8 (9.0%)	5 (5.7%)	1.47 (0.36-6.18)	0.552
Consumed 4-6 times a day	0 (0%)	1 (1.1%)	0 (0-17.15)	0.489
Found it necessary to consume	44 (48.9%)	37 (41.1%)	1.37 (0.73-2.58)	0.295
Was willing to stop to protect teeth	82 (93.2%)	0 (0%)	UD	<0.001*

OR = Odds Ratios, CI = Confidence Interval, UD = undetermined

5.15 ORAL HEALTH SEEKING BEHAVIOR AMONGST THE SWAHILI PARTICIPANTS

When asked whether they had ever visited a dentist, 193 (96.5%) participants responded to the question, with 106 (54.9%) of them reporting that they had been to a dentist.

There was no significant difference in the gender distribution of those who had visited a dentist. However, on exploring the educational level of those who had been to a dentist, most of them were of primary school education of eight years or less. Eighty four (42%), of these participants who had been to a dentist were married. 41 (20.5%) of them were of the middle income group, whereas 48 (24%) were unemployed.

The reasons for hesitating to seek dental treatment were many and there was no difference between cases and controls. Therefore, the health seeking behavior is presented as a group rather than the stratification of cases and controls. Swahili participants hesitated to seek dental treatment for the following reasons; four participants felt that the distance to the clinic was too long, five hesitated because they felt they will encounter unfriendly staff, fifteen felt that they would have to wait a long time before getting treatment, nineteen reported to have no time to go to the clinic, eighteen felt that dental treatment is costly, seven were afraid of painful treatment, three participants were afraid of getting HIV from a dental clinic. Nineteen hesistated to seek treatment because they didn't know what treatment to expect, three were afraid of the level of hygiene in the dental clinics, seven were afraid of losing a tooth, four had heard stories of bad things happening to patients in the dental clinics and three didn't know why they had not sought dental treatment (table 31).

The participants who reported to have visited a dentist regularly were only 20 (10%). Of the ones who had visited a dentist and were satisfied after the visit, six reported that they were happy with the visit because their gums did not bleed any more, five were happy because no injection was used, five reported that they were satisfied because no dental problems were detected, three reported that since only advice was given, they were happy, while three others had fillings instead of extractions and five were happy because they got relief of pain. Only one participant was satisfied because the treatment was done quickly. The respondents were however very few 28 (26.4%) (table 31)

Of the participants who had visited a dental clinic (106), seventy six (71.7%) reported being unhappy with the visit to a dental clinic and this was because three participants did not like the smell in the sugery, fifteen of them did not like the local anesthetic/injection used during treatment, four were unhappy with the unfriendly staff, twenty four were dissatisfied with the time spent waiting for treatment, six did not like it when people worked in their mouths and finally nineteen did not know why they were dissatisfied with the dental visit (table 31 below).

Table 31: Categorization of responses to the skip pattern questions on oral health seeking behavior amongst the Swahili participants (Mixed methods[148])

Inductive categories	Participant responses
Reasons for hesitation in seeking dental treatment	• Distance to the dental clinic is too long - **4 participants** • Presence of unfriendly dental workers - **5 participants** • Long waiting time at the dental clinic - **15 participants** • No time to go to the dental clinic - **19 participants** • Treatment is too costly - **18 participants** • Getting painful treatment **– 7 participants** • Getting infected with HIV - **4 participants** • Don't know what treatment to expect – **19 participants** • Level of hygiene is poor in the clinics – **3 participants** • Fear of losing a tooth – **7 participants** • Heard stories about bad things happening to patients in the dental clinic - **4 participants** • Don't know – **3 participants**
Reasons for satisfaction with the dental visit	• No bleeding of gums anymore – **6 participants** • Treatment done without injection **– 5 participants** • No dental problems discovered at all - **5 participants** • Only advice was given – **3 participants** • Filling done instead of extraction of tooth – **3 participants** • Got pain relief – **5 participants** • Treatment is done quickly - **1 participant**
Reasons for dissatisfaction with dental visit	• Don't like the smell in the surgery- **3 participants** • Don't like the local anesthesia/injection - **15 participants** • The noise of the equipment - **5 participant** • Unfriendly staff – **4 participants** • The long time spent in the waiting room - **24 participants** • I don't like people working in my mouth – **6 participants** • Don't know – **19 participants**

5.16 COMPARISON OF SOCIO-DEMOGRAPHIC DATA BETWEEN THE TAITA AND THE SWAHILI PARTICIPANTS

Comparison of data from the Taita and Swahili participants, showed that only in the Taita participants did marriage serve as a risk to having chronic periodontitis (p = 0.014). None of the other socio-demographic variables were significant.

When the socio-demographic data of Taita and Swahili participants who had visited a dental clinic was compared, it was found that more of the Swahili participants had visited a dentist than Taita particicpants (p- value of 0.003). Swahili participants who had been to school for <=8 years were significantly more likely to visit a dental clinic p=0.015 (table 32). More of the Swahili participants were also found to be in the high income group compared with the Taita participants, where none of them earned more than Kshs 20,000 per month.

Table 32: Comparison of participants who had visited a dentist by socio-demographic features

		Taita participants (N=198)	Swahili participants (N=200)	p- value
Gender	Male	3	55	0.003*
	Female	16	51	
Education	<=8 years	8	75	0.015*
	>8 years	11	31	
Occupation	Employed	8	35	0.440
	Self Employed	3	23	
	Unemployed	8	48	0.735
Marital status	Married	16	84	0.619
	Unmarried	3	22	
Income Level	Low	2	2	
	Middle	17	41	0.389
	High	0	19	0.023*

Comparison of data from the Taita and the Swahili participants as far as reasons for hesitating to seek dental treatment is presented in figure 24 below. Taita participants were mainly concerned about the distance to a dental clinic and the costs of treatment. The Swahili participants on the other hand were more concerned with having no time to go to the clinic and not knowing what treatment to expect.

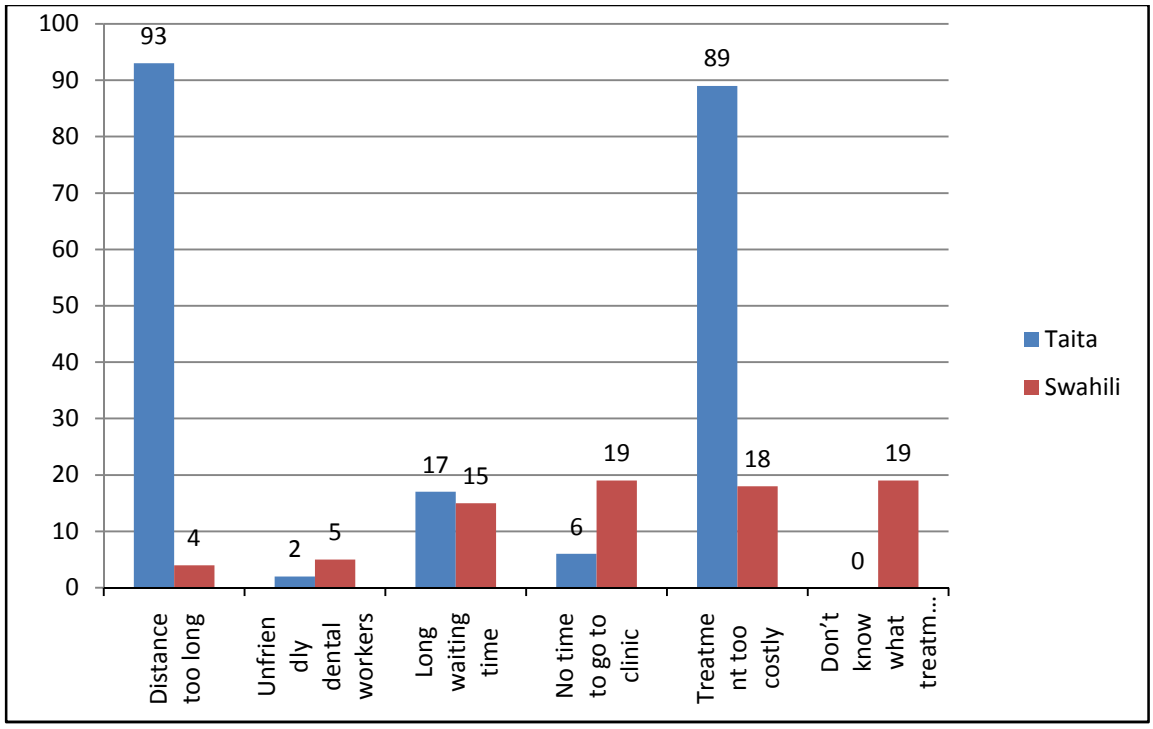


Figure 24: Reasons for hesitating to seek dental treatment amongst the Taita and the Swahili participants

When a comparison was made between Taita and Swahili participants on reasons for dissatisfaction with the visit to a dental clinic, it was found that the Swahili

participants were concerned mainly with the use of the local anaesthetic and the waiting time (figure 25 below).

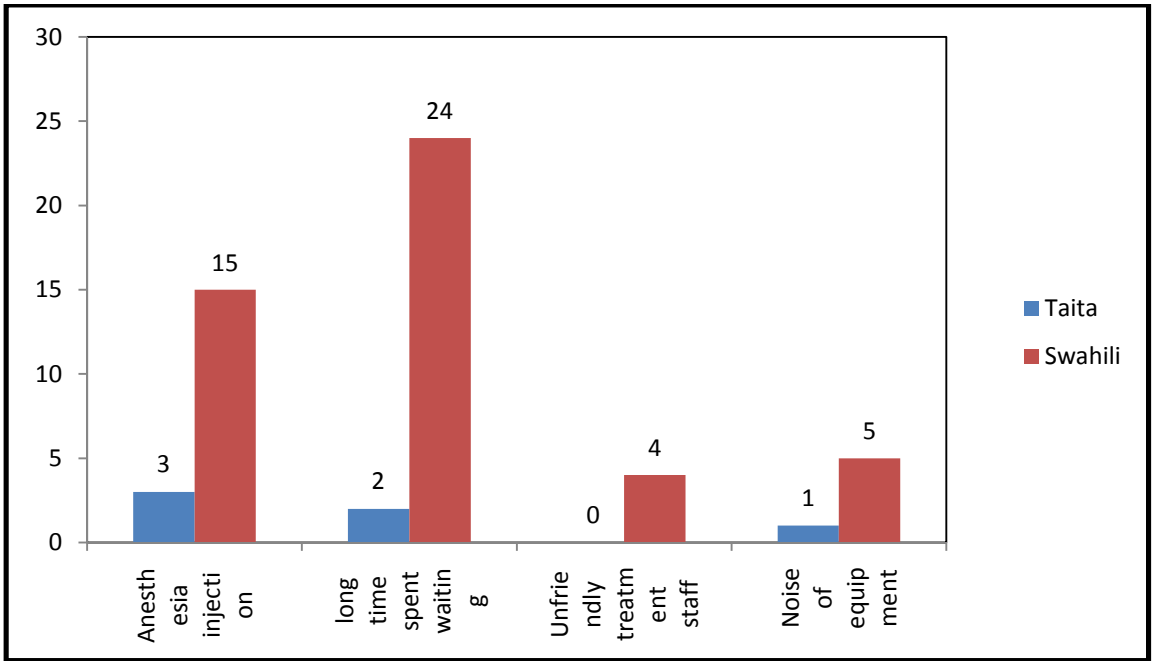


Figure 25: Comparison of Taita and Swahili participants on reasons for dissatisfaction after a dental visit

Comparison of data from the Taita and the Swahili participants on the consumption of sugary foods and drinks, it was found that more of the Swahili participants than the Taita participants consumed sugary foods/drinks, yet more of the Taita participants were willing to stop to protect their teeth (figure 26 below).

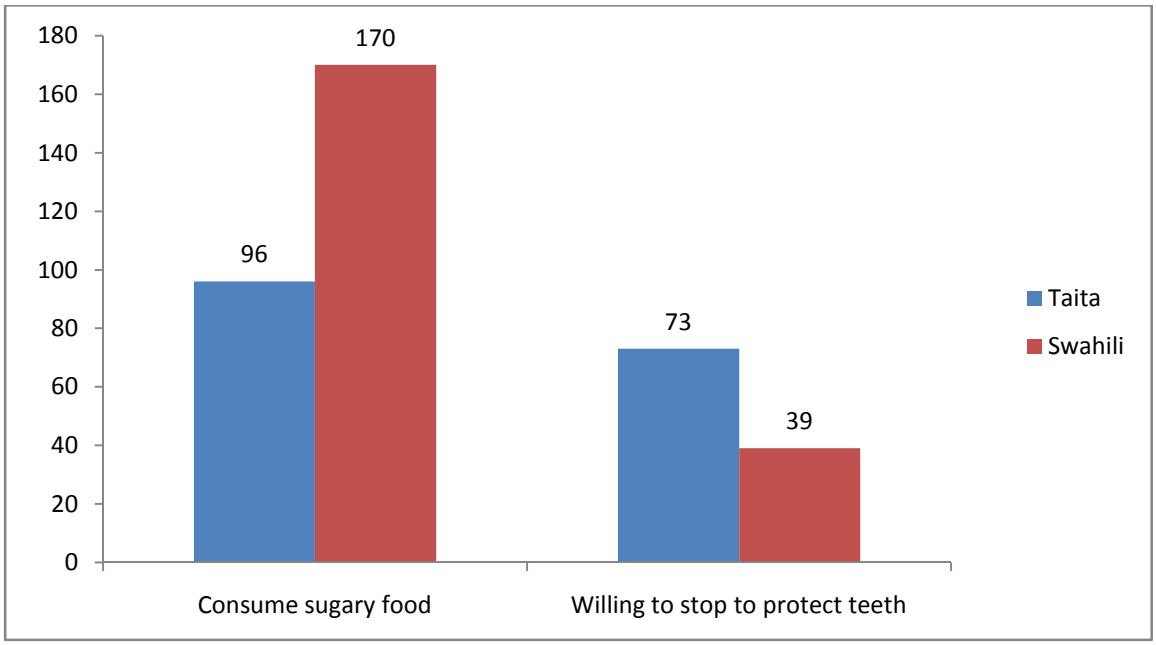


Figure 26: Consumption of sugary food and drinks amongst Taita and Swahili participants

5.17 ANALYSIS TO IDENTIFY CONFOUNDERS AND EFFECT MODIFICATION IN THE TWO ETHNIC GROUPS

When the significant variables at the bivariate analysis were explored for effect modification or confounding of the relationship between genotype and chronic periodontitis amongst the Taita participants, none of the variables were found to be significant. The variables tested were 'use of tooth brushes', 'brushed in the evenings', plaque and calculus which were significant at bivariate level between cases and controls.

Amongst the Swahili participants, the results are as presented on the following four tables, 33,34,35 and 36. Table 33, shows the test of association between genotype

-511 and chronic periodontitis before stratifying by plaque level (≤15 tooth surfaces with plaque and >15 tooth surfaces; 15 tooth surfaces because all participants had plaque on at least 15 tooth surfaces). The table (33) shows no association, p-value was greater than 0.05. Also 95% confidence interval for crude odds ratio includes 1 which is the expected value under the null hypothesis.

Table 33 : Results of Chi-square test for confounding and effect modification amongst the Swahili participants

		Control		Case				95% CI for COR	
		n	%	N	%	P-Value	COR	Lower	Upper
Genotype511_2_2	Absent	77	77	75	75	0.741	1.116	0.583	2.136
	Present	23	23	25	25				

Table 34, shows the test of association between genotype -511 and CP after stratifying by plaque level. The table provides evidence of a difference between the presence and absence of genotype -511 allele 2 for subjects with plaque on less or equal to 15 tooth surfaces. P-value for this stratum is less than 0.05 and 95% confidence interval for adjusted odds ratio since it does not include 1. The plaque level of >15 tooth surfaces stratum is not significant because it includes 1. This shows a tendency towards effect modification by plaque level in the association between CP and locus -511.

Table 34: Results of chi-square test for confounding and effect modification of plaque levels amongst the Swahili participants (Stratum specific)

Genotype-511	Plaque		Control n	Case n	P-Value	AOR	95% CI for AOR Lower	Upper
1-1	<=15	Absent	34	6	1	0	0	16.41
		Present	3	0				
1-1	>15	Absent	60	90	1	0.89	0.14	6.29
		Present	3	4				
1-2	<=15	Absent	9	4	0.06	0.16	0.01	1.4
		Present	28	2				
1-2	>15	Absent	25	29	0.304	1.47	0.71	3.03
		Present	38	65				
2-2	<=15	Absent	33	3	0.045*	8.25	1.22	55.62
		Present	4	3				
2-2	<=15	Absent	44	72	0.36	0.71	0.35	1.45
	>15	Present	19	22				

Breslow- Day test shows that there is a significant difference between the odds ratios, p=0.009 and thus there is effect modification by plaque level (table 35).

Table 35: Tests of homogeneity of the odds ratio

	Tests of Homogeneity of the Odds Ratio		
	Chi-Squared	Df	P-Value
Breslow-Day	6.851	1	0.009

Table 36 shows Mantel-Haenszel estimate of the summary odds ratio. Mantel-Haenszel method assumes exposure (genotype -511) and CP association is the same in each of the strata defined by plaque level.

It is a weighted average of the odds ratios from the separate strata. A value of 0.94 implies that, after controlling for the effect of plaque level, the odds of disease are less in subjects without genotype compared to subjects with the genotype at the lower plaque level of <=15 tooth surfaces with plaque.

The crude and adjusted ORs are different (1.116 and 8.25 respectively).

Table 36: Results of Mantel-Haenszel analysis testing for confounding and effect modification

Mantel –Haenszel OR	95% CI for MHOR		P-Value
	Lower	Upper	
0.94	0.49	1.83	0.86

5.18 MULTIVARIATE ANALYSIS OF THE CLINICAL CHARACTERISTICS AND GENOTYPE AMONGST THE TAITA AND SWAHILI PARTICIPANTS

Multivariate analysis was peformed to determine the role of the significant clinical characteristics and genotype in the development of chronic periodontitis in the Taitas. Plaque level was the only factor that remained significantly associated with chronic periodontitis, OR=17.95, 95%CI=6.60 -48.79, p<0.001 as shown in table 37.

Table 37: Multivariate (Adjusted) analysis for Taita participants' clinical data

		B	S.E.	P-value	OR	95% CI Lower	95% CI Upper
Plaque	<=15	**Reference**			1		
	>15	2.888	0.510	<0.001	17.95	6.604	48.795
Uses tooth brush	No	**Reference**			1		
	Yes	-0.824	0.577	0.153	0.439	0.142	1.359
Brushed previous evening	No	**Reference**			1		
	Yes	-0.741	0.432	0.087	0.477	0.204	1.113
Consume sugary food/drinks	No	**Reference**			1		
	Yes	0.298	0.356	0.403	1.347	0.670	2.710

Further multivariate analysis were done to investigate the association between plaque levels and the significant genotypes. Table 38 shows that plaque level continued to be significantly associated with CP, OR=18.97, 95%CI=7.01 – 51.31, p<0.001. However, genotype +4845 (1-1) and +3954 (1-2) were no longer associated with CP in this multivariate model as shown.

Table 38: Multivariate analysis of Taita participants' plaque levels and significant genotype data

		B	S.E.	P-value	OR	95% C.I. for OR	
						Lower	Upper
Plaque	<=15		**Reference**		1		
	>15	2.943	0.508	<0.001	18.971	7.014	51.309
Genotype4845 1-1	Absent		**Reference**		1		
	Present	-1.108	1.209	0.359	0.330	0.031	3.530
Genotype3954 1-2	Absent		**Reference**		1		
	Present	0.630	0.476	0.186	1.878	0.738	4.777

Multivariate analysis of Swahili participants' plaque levels and genotype data.

Amongst the swahili participants, none of the clinical charateristics were significantly associated with CP.

Multivariate analysis to test the plaque levels and genotype data in the development of chronic periodontitis, demonstrated only plaque to be significantly associated with CP, OR= 7.99, 95%CI =3.16 - 20.24, p<0.001. The initially significant genotype -889 was no longer significantly associated with CP (table 39).

Table 39: Multivariate analysis of Swahili participants' plaque levels and significant genotype data

						95% C.I. for OR	
		B	S.E.	P-value	OR	Lower	Upper
Plaque	<=15		**Reference**		1		
	>15	2.079	0.474	<0.001	7.993	3.156	20.243
Genotype889 1-1	Absent		**Reference**		1		
	Present	0.266	0.731	0.716	1.304	0.312	5.462
Genotype889 2-2	Absent		**Reference**		1		
	Present	-0.760	0.531	0.153	0.468	0.165	1.325

5.19 HAPLOTYPES

There are three recognized haplotypes [161] and these are:-

- Haplotype 1: Allele 2 at IL-1A +4845 and IL-1B+3954
- Haplotype 2: IL-1B-511 allele 2
- Haplotype 3: Allele1 at the IL-1A and IL-1B markers

The first haplotype was not associated with chronic periodontitis in the two ethnic groups. The second haplotype was found to be associated with chronic periodontitis after stratifying for plaque amongst the Swahili participants only. The third haplotype is presented in table 40 below where having allele 1 in all four genotypes was significantly associated with chronic periodontitis in the Taita participants OR= 2.4, 95%CI = 1.12-5.14, p=0.022 and also amongst the Swahili participants OR= 4.2, 95% CI = 1.35-13.3, p=0.008.

Table 40: Association between chronic periodontitis and Haplotype 3: Allele1 at the IL-1A (-511 and +3954) and IL-1B (-889 and +4845) amongst the Taita and Swahili participants

Taita participants

		Control		Case				95% C.I for OR	
		N	%	N	%	P-Value	OR	Lower	Upper
Allele1 in All	Absent	84	87.5	70	74.5				
	Present	12	12.5	24	25.5	0.022	2.4	1.12	5.143
Allele2 in All	Absent	92	95.8	91	96.8				
	Present	4	4.2	3	3.2	1	0.8	0.165	3.483

Swahili participants

		Control		Case				95% C.I for OR	
		N	%	N	%	P-Value	OR	Lower	Upper
Allele1 in All	Absent	96	96	85	85				
	Present	4	4	15	15	0.008	4.2	1.353	13.26
Allele2 in All	Absent	64	64	72	72				
	Present	36	36	28	28	0.225	0.7	0.38	1.257

CHAPTER 6

DISCUSSION

6.0 STUDY POPULATION

The recruited Taita participants were mainly subsistence farmers and were exposed to limited dental services such that none of them had received periodontal therapy, restorative work or any other dental treatment apart from extractions. The nearest dental clinic was 50kms away and access was poor since they would normally have to rely on irregular public transport which was also costly. None of the Taita participants were found to earn Kshs 20,000= or more per month. Most of them earned between Kshs 2000= and less than 20,000= per month. For a Taita participant to visit a dentist, it meant missing work for that day and therefore no earnings for the day. This had implication on the family in that it would affect the household income and even food provision. They therefore preferred to visit the nearest health centre, where a community oral health worker would carry out the extraction of the offending tooth or alternatively, visit a traditional doctor for herbal medication to ease the pain or even an extraction. Thus this population was suitable to study the effects of genetic polymorphisms in a population with limited access to dental services and also with limited resources.

Swahili participants on the other hand were cosmopolitan and most of the recruited subjects lived within about 4-10 kilometers of the provincial hospital, where dental services are available. However, none were found to have had any other dental

treatment except extractions. The subjects selected were of mixed heritage of Bantus and Europeans/Arabs and thus heterogeneous.

Thus the two ethnic groups are suitable to study the association between chronic periodontitis and genetic factors in a rural homogenous population exposed to limited dental services and an urban population of mixed heritage with dental services easily available. To ensure that only Taita and Swahili participants were recruited into the study, the participants had to confirm that the grandparents on both sides were of the ethnic group under study.

6.1 SOCIO-DEMOGRAPHIC, CLINICAL CHARACTERISTICS AND GENOTYPE ASSOCIATION WITH CHRONIC PERIODONTITIS AMONGST THE TAITA AND SWAHILI PARTICIPANTS

TAITA PARTICIPANTS

The Taita participants recruited, had almost all (97%) been to school, with a distribution of 73.7% of the cases and 64.6% controls, having 8 years in school. The national adult literacy level in Kenya is reported as being 61.5%[150]. The Taitas compared well with the national averages with an average of 74.15% having primary school education.

The socio-economic status and the education level did not have a significant association with chronic periodontitis. This finding differs from that of a study carried out in India where these variables were found to be positively correlated with periodontal status. Those of higher economic status and higher level of education

were found to have better periodontal health ($p<0.0001$)[151]. In a review of socioeconomic status and its relationship with chronic periodontitis, [2] the conclusion was that socioeconomic status, including income, education levels and urban status are fairly good risk indicators for periodontal diseases. In this study, the inability to demonstrate this relationship between the economic status and education level and chronic periodontitis may be due to the fact that the participants were all from the same general location and therefore the socioeconomic status was more or less the same. Thus it is difficult to find differences in groups which are largely similar, where majority 168(84.8%) of the participants were in the middle income group. Additionally, this study was a case control study with a selective recruitment procedure aimed at fulfilling the inclusion criteria. Such associations are best investigated by cross sectional studies covering a much wider area, having large sample sizes and randomization of the recruited subjects.

Most of the participants (85.9%) were married especially those with chronic periodontitis (91.9%). This may be a reflection of the population structure of those in this age bracket in this area since these figures are higher than the national averages which report 63.5% of household heads to be married. However the figures quoted above are from the National Survey report of 2005/2006[152]. Current figures are not available. When cases were compared to the controls, being married appeared to confer a risk of developing chronic periodontitis. This finding differs from studies done on African-Americans where it was demonstrated that those who were married had less disease[117,118,119]. Amongst the Taita participants, the finding that

married individuals had more disease may be related to priorities in the families. The finances in families may be directed towards the daily sustenance of the household which will include children, rather than in the purchase of oral hygiene devices. Changing the toothbrush regularly, may be a difficult task for this population considering that most of them earn less than Kshs 20,000= per month. The effectiveness of their oral hygiene practices may also be a contributory factor in that they may not have been spending the requisite time and effectively removing plaque from most surfaces as required.

The control participants were found to significantly ($p=0.007$) use toothbrushes more than cases OR=0.26, 95% CI = 0.08-0.79. Thus using toothbrushes was protective since it also led to less plaque in the control participants, $p<0.001$. More of the participants with chronic periodontitis used chewing sticks than the controls. The fact that more of the control participants used toothbrushes and had less plaque than those with CP, meant that using tooth brushes was more effective in plaque removal. Majority of the Taita participants (68.7%) were unemployed and subsistence farmers. This compared well to the national figures of 68.8% of households reported to be engaged in crop farming[152].

The null hypothesis stating that there is no association between socio-demographic factors and chronic periodontitis was generally accepted except for martital status where a significant ($p=0.014$) association was found. Thus, there was a 3 fold more chance of a married Taita participant having CP.

Clinical characteristics and oral hygiene habits amongst the Taita participants

Plaque was present on at least one tooth surface in 100% of Taita participants with chronic periodontitis and 92% of those without the disease. This was in spite of the majority of the participants (98.5%) reporting to cleaning their teeth. Plaque levels were 89% in cases and 53.7% in controls with OR = 21, 95%CI = 7.8-56.4. This demonstrated a relationship between plaque and chronic periodontitis which was confirmed by the risk estimates where the risk of being a case when plaque was present was as high as 45 fold in the lower left central incisor.

It is difficult to study calculus in the absence of plaque since it is a plaque retentive factor and it is not possible to find one variable in the absence of the other. Therefore, the relationship between CAL and calculus was examined by assessing the risk estimates of calculus and chronic periodontitis. It was found that the presence of calculus on the tooth surface conferred a 34 fold risk of having CP (OR = 33.9, 95%CI = 13.3-86.3, $p<0.001$). The risk estimates of developing CAL in the presence of calculus on the tooth surface revealed a range as high as 23 fold on the lower left canine to 8 fold, on the upper central incisors. The effects of plaque and calculus on the development of gingivitis have been well documented and are recognized in literature[153]. This study corroborates the fact that in the presence of plaque and calculus there is a risk of developing chronic periodontitis. Kocher et al in 2005 [154] also reported plaque and calculus as being risk determinants of chronic periodontitis in their cross sectional study of 2595 subjects in Pomerania, Germany. This current study also showed that in spite of 98.5% reporting that they brushed

their teeth; plaque was present, especially in the cases. This either meant that they were just susceptible individuals or their brushing techniques were not effective in removing plaque. Presence of abundant plaque and calculus deposits in populations who claim to brush their teeth has also been reported in other studies in Kenya[13,14,39,155].

Bleeding on probing of the gingival tissues at least around one tooth was present in 90% of the sites examined in those with chronic periodontitis. The level of inflammation was 61.7%. This level of inflammation was high but is to be expected since the disease severity was also high at 43.6% of Taita participants having severe CAL. Probing pocket depths (≥4mm) were found in 3.8% of the participants. This finding where chronic periodontitis presents with mostly recession rather than probing pocket depths has been shown in other studies on Kenyans[15, 155] and seems to be the profile for destructive disease in Kenya. In this study, CAL was a more reliable measure of the level of disease. There was a masking effect on the extent of disease with the periodontal pocket depth measurements. The high level of disease in this population was due to the deliberate recruitment of those with frank disease as recommended by Shafer et al 2011, so as to increase the power of the study[137]. Therefore, in this study only those with ≥3mm attachment loss in two or more non-adjacent teeth were included.

The relationship between plaque and CAL was investigated and it was found that having plaque increased the risk of having attachment loss. The risk of being a case

in the presence of plaque ranged from as high as 45 fold on the lower first left incisor and upper first left as mentioned above to as low as 6 fold on the lower left second molar in the Taita participants. This relationship between plaque, bleeding and recession has been confirmed in a study by Muller et al in 2000[52] on 127 young adults of 17-30 years. They reported that subjects with a strong and positive association between plaque and bleeding had more gingival recession (Pearson's r=0.21, p=0.02) than those without a positive association. However there was a large variation according to tooth type, with odds ratios ranging from 1.36 in molars to 2.56 at lower premolars. This wide variation according to tooth type was also shown in the present study, where the risk estimates were varied. Another study on <65 year old men also found an association between attachment loss and bleeding, reporting odds ratio of 2.1, 95% CI = 1.5-3.1[52]. Plaque was also strongly associated with bleeding on the buccal surfaces in a different study by Muller and Heinecker 2002, [55] where the odds ratio was reported as being 1.80 and 95% CI = 1.19-2.72. This demonstrates that plaque is associated with bleeding on probing and attachment loss. The current study was able to show an association between plaque and CAL (OR=21, 95% CI=7.8-56.4); and calculus and CAL (OR=33.9, 95%CI=13.3-86.3). This can be interpreted as there being a 21 fold chance of having CAL in the presence of plaque and 34 fold chance of having CAL in the presence of calculus.

When the distribution of periodontal pockets was examined, only 3.8% of Taitas had probing pocket depths which were mainly found on the upper teeth. The upper

molars and premolars being the teeth most affected. This is an expected finding demonstrated in other studies[15,155].

The distribution of dental caries, roots and missing teeth was not different between cases and controls. This is an expected finding since the participants were recruited from the same geographical area and would therefore be subject to similar habits and diet. As far as the consumption of sugary items was concerned, 48.5% of the Taita participants consumed sugary foods and there was no difference in cases and controls. Whereas 91.9% consumed sugary drinks and there was a significant difference between cases and controls, OR= 0.11, 95% CI = 0.01-0.93, p=0.018 with the reference being those who consume sometimes. The sugary drink that was consumed by the Taita participants was tea with sugar. Sugary foods/drinks are associated with dental caries and the later is associated with chronic periodontitis as a plaque retentive factor. However, in this study, more of the controls consumed sugary drinks but this did not lead to more dental caries. The distribution of dental caries was similar in cases and controls. It appears that the Taita participants were not very susceptible to the development of dental caries. The fact that more of the control participants used tooth brushes may also contribute to the lack of contribution of the sugary drinks to caries formation or even to plaque development. Controls amongst the Taita participants were probably more effective in using the tooth brushes to remove plaque (OR= 0.26, 95% CI=0.08-0.79).

6.1.1 FREQUENCY OF INTERLEUKIN-1B AND 1A POLYMORPHISMS AMONGST THE TAITA PARTICIPANTS AND THEIR ASSOCIATION WITH CP

Interleukin-1 gene variations have been shown to influence the risk of disease progression in many chronic illneses like rheumatoid arthritis, inflammatory bowel disease, cardiovascular disease, osteoporosis and periodontitis[147]. They do not cause the disease but influence the development and progression of the disease by amplifying the body's response to disease challenge[156]. Interleukin-1 genetic variations have been reported to influence the transcription of IL-1 and thus the susceptibility and outcome of disease[147] Interleukin-1B, the SNP variant -511 is at the promoter region and +3954 at the fifth exon site with both the polymorphisms caused by C to T transitions[87, 143]. These polymorphisms are associated with higher levels of IL-1β cytokines produced by the peripheral blood mononuclear cells. Thus there is a higher concentration of IL-1β cytokine in plasma and GCF[27,157,158]. The increase in IL-1β cytokine may lead to increase in inflammation since this is a pro-inflammatory cytokine.

In the present study, the association between interleukin-1 and chronic periodontitis amongst Taita participants was seen in heterozygous (C/T) IL-1B at position [rs1143634] +3954, p=0.045 and allele 2 (T) at position +3954. Heterozygotes Interleukin-1 B +3954 C/T carriers were found to be at an increase risk of having chronic periodontitis amongst the Taita participants, (p=0.045). This is similar to a case control study done on Chilean subjects where heterozygotes of the IL-1B+3954 C/T were significantly higher in cases than in controls and were associated

with periodontitis (p= 0.001)[89]. Other studies on Caucasians also found a significant association between IL-1B+3954 C/T and periodontitis as reviewed by Laine et al 2010[92].

The association of IL-1B with CP amongst the Taita participants may be explained by the reported finding in other studies [27,157,] in that this polymorphism leads to higher production levels of cytokine IL-1 β in plasma and GCF which in turn leads to amplification of the inflammatory response. A high level of inflammatory response was observed amongst the Taita participants where 61.8% of sites per individual were found to bleed on probing and 88% had at least one site with BOP. Interleukin-1 β cytokine is produced by activated monocytes, macrophages and epithelial cells[92]. Pociot et al 1992, showed a 2-4 fold increase in production of cytokine IL-1β levels in response to bacterial challenge in heterozygous subjects[87]. More recently, Ferriera et al 2008 showed an association between IL-1B +3954 T/T (allele 2), Porphyromonas gingivalis, Treponema denticola and Tannerella forsythia and an increase in cytokine IL-1β levels in the diseased periodontal tissues[156]. Interleukin-1B [rs1143634] SNP +3954 at exon 5 results in coding synonymous where there is no change in the amino acid (phenylalanine) produced. However the increase production in cytokine IL-1β may be due to the effect this allele [rs1143634] may have in the splicing mechanism[159]. This allele may enhance the splicing process and thus make the production of cytokine IL-1β easier. The Taita participants had a positive association between allele 2 at +3954 and CP and a high inflammatory response of the gingival tissues. A higher inflammatory response leads to more

destruction of the periodontal tissues thus more disease. Inflammation has also been shown to have a synergistic effect on plaque formation[156]. The presence of more plaque in the Taita cases corroborates the fact that there is more plaque in the presence of more inflammation.

Interleukin-1A, the SNP at position [rs1800587] -889 C/T is in the transcriptional regulatory region and [rs17561] +4845 G/T is in the coding region. IL-1A -889 T (allele 2) which is in linkage disequilibrium with +4845 alters the transcriptional ability of IL-1A thus an increased production of cytokine IL-1α[157]. Amongst the Taita participants, allele 2 at [rs17561] +4845 which is associated with increased production of cytokine IL-1 α was largely evenly distributed in cases and controls. It was therefore not associated with disease. Allele 2 of IL-1A [rs17561] +4845 T/T transcription has been shown to result in a change in the amino acid produced[159]. The amino acid change observed is a shift from alanine production to serine[159]. This change otherwise known as misssense, did not appear to have an association with CP in the Taita participants although allele 2 +4845 TT was more prevalent in the cases, (20.3% compared to 15.9% in controls, p=0.367).

Homozygous allele1 at IL-1A position +4845 (G) was significantly different in cases (1.4%) and controls (11.6%) p=0.018 amongst the Taita participants. This suggested that in this study, IL-1A [rs17561] +4845G/G (homozygous allele1) was protective. It could be that the presence of allele 1 (G/G) confers protection. However, the numbers were very small. A larger sample is required to explore this association.

There have been no studies so far reporting a significant association between periodontitis and IL-1A position +4845 as a single risk factor. Most of the studies have reported on IL-1A +4845 in combination with IL-1B +3954, otherwise known as the composite genotype. The reports have been mixed with some showing an association with chronic periodontitis while others did not find a positive correlation [160,161,162,164,165]. The current study demonstrated a probable protective effect of allele 1 of +4845 against chronic periodontitis but only amongst the female participants, (p=0.018). IL-1A+4845G (allele 1) (exon 5) has been linked to an altered IL-1α cytokine production[157]. This altered production did not correlate to more disease in the Taita participants. The explanation maybe that IL-1α was not released into the tissues and therefore did not cause increased destruction. IL-1α production is intracellular and requires cell death to be released extracellular[166]. The male Taita participants did not have homozygous allele 1 of IL-1A +4845 G/G polymorphism. This finding of the male Taita participants not having homozygous allele 1 at locus +4845 requies further exploration in a much larger sample size.

The positive composite genotype (allele 2 of IL-1A -889 and allele 2 of IL-1B +3954) distribution in cases and control amongst the Taita participants was basically equally distributed. This differs from studies done on Caucasians, Chileans and Indians where positive association with chronic periodontitis has been reported for composite genotype[16,89,95,102]. The composite genotype +3954 and +4845 was not associated with CP in the Taita participants. This finding is similar to a study done on Xhosas in South Africa where they also did not find an association between the

composite genotype and CP[109]. The explanation for both these findings could be that the composite genotype does not have an effect on CP in Africans.

When IL-1A +4845 and IL-1B +3954 were examined in Maharastrian Indians[102], there was a positive association with chronic periodontitis when assessed together which is a different finding compared to the current study where only IL-1B+3954 was independently associated with chronic periodontitis and IL-1A+4845 appeared to confer a protective effect. The amino acid serine which is produced instead of alanine[159] in carriers of this allele (+4845) may be less active.

There was no relationship between all the four allele (-511, +3954, -889, +4845) distributions and severity of disease amongst the Taita participants. Although the numbers were small, the percentage distributions were more or less the same. Additionally, when the allele distributions were compared in mild, moderate and severe chronic periodontitis and controls, there still was no significant difference. In this study, Interleukin IL-1A and IL-1B genotypes as mentioned were not associated with the severity of chronic periodontitis as reported in other studies[16, 89, 102]. This finding appears to be unique to the Taita, an African group that has not intermixed much with people from non Bantu origin. This lack of association could also be attributed to the age of the participants. The age range was relatively evenly distributed between 35-44 years. Severity of chronic periodontitis increases with age especially in the absence of treatment. An older age group may reveal a different picture.

The **null hypothesis** stating that there is no association between genetic polymorphism of IL-1 [IL-1B (-511, +3954) and IL-1A (-889, +4845)] and chronic periodontitis amongst the Taitas was rejected only in as far as heterozygous IL-1B locus [rs1143634] +3954 (C/T) and allele 2 (T/T) at the same locus are concerned. A significant association (p=0.045) was found between IL-1B +3954 and chronic periodontitis amongst Taita participants. There was a 2 fold increase risk of developing CP in those with any allele 2 (1-2 and 2-2) at IL-1B+3954.

Further analysis revealed that in the Taita participants, plaque remained associated with chronic periodontitis at bivariate and multivariate levels. The genotype of IL-1B +3954, IL-1A +4845 and using a toothbrush which were initially associated with chronic periodontitis, no longer conferred a risk. This suggests that the more important risk factor of chronic periodontitis is plaque. Chronic periodontitis may actually be a disease of multiple genes and IL-1 is just one of the several gene polymorphisms involved in the genetic risk factors. Plaque initiates the disease process and in those with the positive genotype in this case IL-1B +3954, there is a hyper sensitive reaction to the pathogenic bacteria that may be present. This will eventually lead to CP.

SOCIO-DEMOGRAPHIC VARIABLES IN THE SWAHILI PARTICIPANTS

The Swahili participants were age and sex matched and all (100%) had been to school. The National literacy level for urban areas is 90%[150]. The current study

population posts a much higher level of 100% having gone to school compared to the national figures of 90 %. The higher levels of education may be explained by the fact that in this study, the figure was obtained from the mere fact that the individual attended school upto 8 years. In the national survey, the literacy assessment test was performed on those who had been to school. This test assesses the understanding of an individual in reading and following instructions. The selected individuals had to read and understand instructions or read and make use of the information provided[150]. Such a test may lower the literacy level.

Nationally, 63.5 % of households' heads are married into monogamous unions as was mentioned earlier[152]. The figure of 75% found in this study was higher than the national average. This may be a regional and cultural characteristic or an attribute of this age group.

Most of the Swahili participants were of middle income level and unemployed. This contradiction of being unemployed yet of middle income level, may arise from the finding that most of the female participants were married housewives without gainful employment, yet were dependent on their middle income earning husbands. Thus they recorded themselves as being of middle income level and unemployed.

Amongst the Swahili participants, the socio-demographic characteristics were not statistically significant. The explanation may be that since this study was a case control in design, the selection of subjects from the same general area might have

led to recruitment of individuals of similar socio-demographic features where majority of participants who divulged their level of income were of the middle income group. A large scale cross sectional study would be best suited to explore the effect of socio-demographic features on chronic periodontitis.

The first **null hypothesis** which states that there is no association between socio-demographic characteristics and chronic periodontitis amongst the Swahili participants was accepted. There was no significant association found between socio-demograhic characteristics and CP amongst the Swahili participants.

Clinical characteristics and oral hygiene habits amongst the Swahili participants

Plaque on at least one tooth surface was present in all cases and all controls in the Swahili participants. The minimum number of tooth surfaces found with plaque in the controls was 5 and in the cases it was 14. This was a high level of plaque in subjects reporting to brush their teeth. 195(98%) reported to brushing their teeth and 48.5% said that they did it twice a day. Plaque levels were 91% in cases and 65% in controls with OR = 9.2 and 95%CI = 3.7-23.1. The reason for this discrepancy in plaque levels and brushing habits may be that the technique used may be faulty or the time spent may be inadequate. The affordability of cleaning devices may also have an impact on the frequency of changing the tooth brush. For whatever reason, it is obvious that there is a need to improve oral hygiene in this population.

In terms of brushing frequency, more of the Swahili participants with chronic periodontitis cleaned their teeth twice a day (p=0.043) when compared to controls. The explanation for this may be that in those with the positive genotype (allele 1 at -889 for Swahili participants), require a much smaller bacterial load to initiate disease compared to those without the genotype. Therefore, although Swahili participants with CP brushed more frequently, the low bacterial load was enough to initiate the disease process. This is because allele 1 of IL-1A -889 made the Swahili participants 3 fold more susceptible to developing CP. SNPs in the genes for interleukin-1, may lead to an overproduction of cytokine interleukin-1, a proinflammatory mediator of inflammation of the immune system. Even if only a few bacteria are present, an over activation of osteoclasts by the proinflammatory cytokine IL-1 results in an increased degradation of the periodontal tissues including bone[167].

Gingivitis or bleeding on probing of the gingival tissues around at least one tooth was present in 100% of the individuals with chronic periodontitis. This high prevalence was an expected finding because of the inclusion criteria. When all the gingival tissues around the teeth were examined, 89% were found to bleed on probing in the cases. Only 29(<1%) sites in control individuals were found to bleed on probing. These areas with this slight bleeding may have been inadvertently included in the control group.

Pocket depths of ≥4mm were found in 5.0% of the subjects. Pockets of less than 4mm are considered to be within normal range[153]. Clinical attachment loss was reported as ≥4mm being present in 21.9%, ≥5mm in 15% and ≥6mm in 9.8%. This demonstrated a high degree of disease which was expected because of the inclusion criteria. It is clear that in the Kenyan population, the measure of disease that should be used in detecting chronic periodontitis is CAL. Probing pocket depths mask the level of disease since the character of disease progression in the Kenyan population appears to be via recession rather than pocket formation[15, 155].

The level of dental caries and roots was low amongst these participants of Swahili origin, with only one surface being affected in less than 10% of the subjects and there was no difference between cases and control. There were also very few missing teeth with no significant difference in cases or controls. These findings are supported by there being no difference in sugar consumption between cases and controls. The sugar consumption was similar to the Taita participants in that it was attributed to the taking of tea with sugar in the morning for breakfast. It has been shown that dietary sugar influences composition of dental plaque and development of dental caries but this finding has been not been demonstrated in this study[168]. The caries experience was low.

The association between plaque and CAL was variable at each tooth and ranged from 14 fold at the upper left lateral and canine region to as low as 1 fold in the first lower molar areas both left and right. The values for the Swahili participants were

lower than those for the Taita participants, who had values ranging from 45 fold in the lower anterior incisor region. The explanation for this may be that other factors like the differences in the genotype found to be associated with CP in the two ethnic groups may have contributed to the varied results. The relationship between calculus and CAL was not as strong as that of plaque since it is a plaque retentive factor. It was significant in the upper and lower anterior region.

Brushing in the evenings conferred protection to Swahili participants. It would therefore assist these individuals if oral hygiene programs were introduced into the public health programs and individuals encouraged to brush daily especially in the evenings. This would lead to reduction in plaque levels and ultimately to less disease and tooth loss.

6.1.2 FREQUENCY OF INTERLEUKIN-1B AND 1A POLYMORHISMS AMONGST SWAHILI PARTICIPANTS AND THEIR ASSOCIATION WITH CP

The only polymorphism that was found to be significantly associated with chronic periodontitis was IL-1A-889. Homozygous allele 1(C/C), p=0.018 and carriage of total allele 1 where the OR = 3.16 95%CI=1.644-6.083 p< 0.001 were both associated with chronic periodontitis amongst Swahili participants. Interleukin-1A -889 has been associated with chronic periodontitis in some populations and not others[16, 88, 91,96,165]. Hence amongst the Swahili participants, the association between [rs1800587] IL-1A -889 and chronic periodontitis was similar to other studies in Caucasians. The increased destruction of the periodontium in those genotype positive for IL-1A-889 allele1, could be due to the reduced production of IL-1α

cytokine[158]. This leads to increased destruction of the periodontium. This could be due to lowered recruitment of inflammatory cells as a result of the lower IL-1α. Variations in IL-1α also affect the production of IL-1β[158]. The distribution of allele 2 in Swahili participants was 80.4% in cases and 92.9% in controls, OR=0.32, 95%CI=1.16 – 0.61, p<0.001. This is in contrast to other studies where allele 2 -889 was associated with severe periodontitis due to strong binding of Jun-family transcription factors of AP-1 family which is associated with a high IL-1α transcription and therefore more disease[169]. Additionally, it was also shown by Hulkkonen et al 2000 that homozgyous allele 2 IL-1A-889 was associated with high IL-1α transcription and these high levels than induced IL-1β production only in the presence of IL-1B -511[157]. In the Swahili participants, allele 2 -511 was associated with chronic periodontitis but this effect was modified by the presence of plaque. In the presence of low plaque levels, the association between [rs16944] IL-1B-511 was observed but this association was lost when there was a high plaque level. It may be that the high levels of plaque (25.52(91%) teeth affected per individual) amongst the Swahili participants were affecting these relationships.

Interleukin-1B was not associated with chronic periodontitis amongst the Swahili participants. However, when bivariate analysis was performed, plaque was found to modify the effect at IL-1B -511 homozygous allele 2 (2-2). On further multivariate analysis, this effect did not remain. Thus the effect of the genotype is expressed when there is less plaque. When there is more plaque, the genotype effect is masked by the strong association between plaque and CP.

As far as the composite genotype is concerned, amongst the Swahili, none of the controls were heterozygous for the composite genotypes. That is, 1-2/1-2 was found to be 0% for the controls. Additionally, the frequency in those with CP was only 3.6%. Heterozygosity for the composite genotype in the Swahili participants appears to be rare. This may be a unique finding in the Swahili participants. The known composite genotype, allele 2 at -889 and allele 2 at +4845 were not associated with chronic periodontitis amongst the Swahili participants. This finding is similar to that found in Xhosas of South Africa [109] but differs from other studies which have shown a positive correlation of these alleles (-889, 2-2 and +4845, 2-2) with CP[18,106, 160,163]. The severity of chronic periodontitis and association with the tested genotypes also differed from other populations in that amongst the Swahili participants, there was no correlation, whereas other studies report a positive relationship[18,89, 93,94,95,97].

The **null hypothesis** that there is no association between genetic polymorphism of IL-1 [IL-1B (-511, +3954) and IL-1A (-889, +4845)] and chronic periodontitis amongst the Swahili participants was partially accepted in that IL-1B was not found to contribute to chronic periodontitis amongst the Swahili participants. Only allele 1 of IL-1A at loci -889 was significantly ($p<0.001$) associated with chronic periodontitis.

6.2 CARRIAGE RATES AND COMPARISON OF INTERLEUKIN-1A AND 1B GENOTYPE AND ALLELE FREQUENCIES AMONGST TAITA AND SWAHILI PARTICIPANTS

The distribution of IL-1B genotype at site -511 showed that allele 1 was largely equally distributed between cases and controls in both ethnic groups. Allele 2 frequency distribution was also equally distributed in cases and controls in both ethnic groups. Very few studies are available to compare with for this locus of -511. The few available studies that have reported on locus -511 have been on Caucasians and one on Japanese[142, 170, 171,172]. In a study by Gore et al 1998, allele 1 distribution or carriage rate in Caucasians was reported as 89.6% and 90% in cases and controls. The distribution of allele 2 for Caucasians was 59% in patients and 59% in controls (n=32)[93]. In a different study, the reported frequency was 53% in patients and 49% in controls by Brett et al 2005[170]. In the Japanese, the distribution of allele 1 was 67% in patients and 78% in controls, [172] and allele 2 was reported as 84% in cases and 75% in controls. As shown in table 41, allele 1 distribution amongst the Taita participants was low compared with Caucasians and Japanese. IL-1A -511 was not associated with chronic periodontitis amongst the Taita participants.

Table 41: Distribution of IL-1B -511 in various studies including the present study

	Allele 1		Allele 2	
	Cases –CP	Controls	Cases –CP	Controls
Current study (Taita)	38.5%	38.3%	61.5%	61.7%
Current study (Swahili)	39.1%	41.1%	60.9%	58.9%
Gore et al 1998 (Caucasian) [93]	89.9%	90%	59%	59%
Brett et al 2005 (Caucasian)[170]	92.9%	90%	53%	49%
Soga et al 2003 (Japanese) [172]	84%	75%	67%	78%

Amongst the Swahili participants, the distribution of IL-1B-511 was more or less the same as that amongst the Taita participants, but lower than in Caucasians and Japanese (table 41). When the Swahili participants were compared with the Taita participants, it was found that heterozygous (1-2) IL-1B-511 (C/T) was more prevalent amongst the Swahili $p<0.001$. Swahili partcipants with chronic periodontitis also had a higher prevalence of heterozygous IL-1B-511 (C/T) than Taita participants ($p=0.016$). It is known that allele 1 homozygotes have reduced cytokine IL-1β levels and any form of heterozygotes have increased levels of cytokine IL-1β[88]. Therefore, heterozygotes will have a higher level of this proinflammatory cytokine. This will lead to more inflammation as seen in the Swahili participants who were found to have a 3 fold increase in bleeding when compared to the Taita participants.

This genetic polymorphism was not associated with chronic periodontitis as found in other populations in different parts of the world[142,170,172]. However, amongst the Swahili participants, on further analysis, plaque was found to modify the effect of the [rs16944] IL-1B -511 genotype in individuals with lower levels of plaque (≤15 tooth surfaces). The explanation could be that when plaque levels are low, the gene expression is observed but when there is more plaque, the effect of plaque on CP overrides the genetic effect.

Interleukin-1B at +3954 amongst the Taita participants demonstrated statistically significant difference in the frequency distribution of allele 1 in CP and controls. Homozygous allele 1 of the interleukin-1B+3954(C/C) was the most frequent genotype found amongst the Taita participants (CP and controls), 54% in CP and 70.8% in controls. More of the controls were homozygous for allele 1 but no significance was detected. This finding was similar to other studies[164, 165,166, 173,174]. Heterozygotes for IL-1B+3954(C/T) were significantly higher amongst Taita participants with CP and was associated with the disease. This contrasts with a study by Parkhill et al 2000,[174] who reported heterozygotes at this locus as being the most frequent genotype in controls. The difference may be attributed to the populations under study. The current study was on an African population of Bantu origin, whereas Parkhill et al 2000 studied a Caucasian group[174]. Literature shows that there are differences between racial groups[175]. A study by Lopez et al 2005 found heterozygous IL-1B to be strongly associated with periodontitis[89]. This finding is similar to this study. Lopez et al 2005 also reported homozygous allele 1 of IL-1B

to be protective, which was also in agreement with the finding of this study, where allele 1 of IL-1B+3954 was more prevalent amongst the healthy controls, signifying a protective effect[89]. A study done in India, also reported that the homozygous genotype allele 1 (CC) of IL-1B +3954 was the most frequent genotype in control subjects than in cases, whereas the heterozygous (CT) genotype dominated in cases than in controls[104].

The carriage rate for allele 2 at IL-1B +3954 (29.4% in those with CP and 17.7% in healthy controls) amongst the Taita participants was much lower than that reported for Brazilians[176]. Other similar studies have been on Caucasians, Asians, Japanese, Brazilians and Indians. Out of 24 studies which have compared this genotype with CP, 6 have reported positive associations[92]. However, in the Japanese population,[172] homozygous for allele 2 was absent. Allele 2 of IL-1B +3954 was reported to be 3.3% in the Chinese population[99]. Hence, allele 2 carriage rate amongst the Taita participants compares well with that of a Brazilian population,[176] where the reported carriage rate was 28% in those with periodontitis and 18% in controls. Amongst the Taita participants and the Brazilians, allele 2 of IL-1B+3954 was associated with CP.

Amongst the Swahili participants, IL-1B+3954 was not associated with CP. Homozygous allele 1 (C/C) of IL-1B+3954 was more frequent in those with CP amongst the Swahili participants (48.9% in CP and 41.2% in controls). Homozygous allele 2 (T/T) of the same genotype was more prevalent in controls (18.2% in CP and

24.7% in controls), although the difference in cases and controls were not significant. This differs with results of other studies, [89, 93, 95, 96, 98, 108, 163, 164] where homozygous (C/C) allele 1 of IL-1B was more frequent in controls. It also differed with the results from the Taita participants. This polymorphism was not however associated with CP and the difference with other populations in the carriage rate is a unique finding.

The carriage rate for the rare allele 2 IL-1B+3954 amongst the Swahili participants was exactly as that reported for a Caucasian population by Thomson et al 2001[177]. In their study, they examined 61 Caucasians and reported a carriage rate of 34% in the patients and 41% in the controls. Rogers et al in 2002[166] reported a carriage rate of 35% in Caucasian patients and 40% in controls. However, in their study, [166] there was an association with CP. Drozdzik et al in 2006[178] reported a carriage rate of 34% in patients and 40 % in controls in a Caucasian group. Swahili participants being of mixed heritage had a similar carriage rate as the Caucasian population. The lack of association with CP was also similar to other studies. However, in the study by Rogers et al in 2002,[164] an association with CP was found. This difference could be attributed to the study design in that Rogers et al investigated genotype frequencies in patients undergoing treatment[164].

The Taita participants when compared to the Swahili participants, it was found that the frequency at IL-1B+3954 was higher in heterozygous individuals (1-2) as well as homozygous allele 2 (2-2) subjects. Carriage rate was also higher in allele 2

amongst Swahili participants (24.3% in Taita and 38.2% in Swahili participants respectively).

Interleukin-1A-889 amongst Taita participants was more or less evenly distributed between all the alleles 1-1 (C/C), 1-2(C/T) and 2-2 (T/T). Homozygous allele 1 (1-1) was 41.4% in CP and 34.1% in controls. Homozygous allele 2 (2-2) was 19.5% in CP and 22% in controls and there was no significant difference. This finding contrasts with other studies in that homozygous allele 1 has been reported as the most frequent genotype and found at a higher frequency in controls[89, 96, 108,162].

Amongst Arabs, reports show homozygous allele 1(C/C) at Il-1A-889 to be 37.5% in cases and 57.1% in controls in Syrians[179]. Yemeni Arabs on the other hand have been reported to have a frequency of 20% and 32.5% in cases and controls respectively[180]. These results show that there was a higher frequency in controls. In this study, the frequency of homozygous allele 1 was higher in those with CP.

Amongst the Swahili participants, homozygous allele 1 of IL-1A-889 was found to be more frequent in those with CP. Although the frequency was low (16.3% in CP and 5.1% in controls), this allele was associated with CP. Other studies have reported frequencies for homozygous allele 1 at -889 as follows: D'Aiuto et al 2004[163] reported 39% in those with CP, Lopez et al 2005 [89] reported 53.9% in CP and 64.35% in controls and Al-hebshi et al 2012[180] reported 20.3% in CP and 32.5% in controls. In all these studies, homozygous allele 1 of IL-1A-889 was not associated with CP. Hence amongst the Swahili participants the association of allele 1 at IL-1A-889 with CP is unique. There was a 3 fold chance of developing CP in the presence

of allele 1 [rs1800587] IL-1B-889. It is also unique when compared with the Taita participants. This uniqueness may be attributed to the Swahili participants being of mixed heritage. They are Bantu in origin but have over the years intermarried with Arabs and Europeans especially of Persian heritage[126]. Thus this may affect their genetic make up. This finding requires further investigations with a mapping of their genome to find out how their genes have changed, if at all, over the years.

Carriage rate for allele 2 of IL-1A-889 has been reported to demonstrate a wide range for Caucasians (table 42). According to Asian [100], Japanese [181] and Brazilian studies, [176] the carriage rate was found to be lower than that reported for the Caucasian[89,97,171]. The lower carriage has also been reported in the Arab population. In a study on Syrian Arabs, the rate was 12.5% and 8.6% in CP and controls. In Yemeni Arabs, [180] the carriage rates were 30% and 10% in CP and controls. In this study, the higher frequency was in controls amongst the Taita participants as opposed to other studies where a higher frequency was in CP group (table 42). This finding amongst the Taita participants is the same as that for an Asian group,[100] a Japanese group, [182] and a Brazilian group[176]. The frequency distribution of alleles appears to be different in various ethinic groups. Table 42 also shows a difference in allele -889 distributions in two Japanese groups[181,182].

Amongst the Swahili participants, (table 42) the frequency of allele 2 at IL-1A-889 was high and was not associated with CP. A higher frequency was found in controls just as reported amongst Asians, [100] Japanese, [181] and Brazilian groups[176].

However, the levels were much higher in the Swahili participants and are comparable to the study by Wagner et al 2007[183].

When Taita participants were compared with Swahili participants, the frequency of IL-1A -889 was found to be higher amongst the Taita participants at all levels $p<0.001$. However, the association with chronic periodontitis was seen in Swahili participants. This finding may be attributed to their genetic make up.

Table 42: IL-1A -889 allele 2 gene polymorphism carriage rates in various populations

References	Ethnic group	Cases	Controls
Lopez et al, 2005 [89]	Caucasian	44%	35%
Wagner et al, 2007 [183]	Caucasian	90%	79%
Struch et al, 2008[97]	Caucasian	54%	49%
Geismar et al, 2008[171]	Caucasian	71%	60%
Sakellari et al, 2006[162]	Caucasian	54%	49%
Anusakasathien et al, 2003 [100]	Asian (Thai)	8%	23%
Kobayashi et al, 2007[181]	Japanese	14%	16%
Kobayashi et al, 2007[182]	Japanese	20%	16%
Gonzales et al, 2006[176]	Brazilian	14%	23%
Moreira et al, 2007[184]	Brazilian	60%	41%
Current study	Taita	39%	44%
Current study	Swahili	80%	93%

Table adapted from review by Laine et al 2010[92]

Interleukin-1A +4845 homozygous for allele 1 was significantly different between cases and controls amongst the Taita participants. However, the numbers were small (1.4% in CP and 11.6% in healthy controls) p=0.018. This finding demonstrated a possible protective effect and was only present in the female subjects. None of the male Taita participants had IL-1A+4845. The most frequent genotype was heterozygous for IL-1A+4845, and no significance was detected p=0.181. It is difficult to compare with other studies because most have only studied IL-1A -889 (which is in linkage disequilbium with +4845) and not IL-1A+4845. It was important to study both these loci separately because there are no known African studies to show their distribution. This will avoid assumptions being made that they do not exist. This has been demonstrated in this study in that the male members of the Taita group have been found not to have homozygous allele 1 at IL-1A+4845.

Amongst the Swahili participants, the distribution of IL-1A+4845 was most frequent in heterozygote individuals where 67% in the CP group had the allele and 62.2% in controls but the difference was not significant p=0.668. The carriage rate of allele 1 was 39.2% in cases and 37.8% in controls but the difference was not significant. The carriage rate for allele 2 was almost evenly distributed between cases and controls at 60.8% and 62.2% respectively.

Very few studies have tested +4845 alone. Armitage et al in 2000 reported a carriage rate of 17% (51/300) for allele 2 amongst Chinese, with only 2 subjects being homozygous[99]. Interleukin-1A +4845 amongst Chinese males was reported as

9% and 2.2% for CP and controls respectively[185]. It is difficult to compare with other studies since most have studied -889 rather than +4845.

When the Taita participants were compared with the Swahili participants, it was found that the Swahili had a significantly higher frequency of homozygous allele 2(2-2) of IL-1A+4845. Allele 1 at IL-1A+4845 was not associated with CP but appeared to be protective to female Taita participants.

The **null hypothesis** that states that the alleles of interleukin-1polymorphisms that are associated with chronic periodontitis are the same in both the Taita and Swahili participants is rejected. Amongst the Taita participants, allele 2 at IL-1B+3954 was significantly associated with CP, whereas allele 1 at IL-1A-889 was significantly associated with CP amongst the Swahili participants.

6.2.1 GENOTYPE ACCORDING TO GENDER

The only statistically significant difference between cases and controls according to gender amongst Taita participants was observed in the distribution of homozygous allele 1(1-1) at locus +4845 in the female participants but the odds ratio was not significant OR=0.137, 95%CI=0.016-1.14. Amongst the males, when cases were compared to controls, none of the frequency distributions of the genotypes were significant.

The frequency distribution of homozygous allele 2 at IL-1A-889 was high in the control subjects of the male Swahili participants p=0.01. It appears that allele 2 of -

889 was protective to the male Swahili participants, OR=0.228, 95%CI=0.069-0.748. However, frequency levels of homozygous allele 1 -889 amongst the female Swahili participants, was higher in cases and there was a 6 fold chance of having chronic periodontitis in the presence this genotype.

In summary, amongst the Swahili participants, IL-1A-889 allele 2 appeared to be protective in the male participants and allele 1 was associated with chronic periodontitis amongst the female participants. Further, it is difficult to compare these findings since there are no studies that compare genotype distribution in chronic periodontitis amongst males with females. The available studies are in individuals with generalized aggressive periodontitis, where the prevalence has been reported to be higher in male subjects.[178,184].

6.3 SEVERITY OF CP AND GENOTYPE CARRIAGE

The interleukin-I genotypes tested appeared not to have significantly contributed to the different levels of disease severity in the Taita participants even when they were compared to controls. Amongst the Swahili participants, IL-1A-889 allele 1 was significantly associated with the severity of disease when compared with controls. Thus having allele 1 IL-1A-889 conferred a 5 fold risk of developing mild CP. The presence of allele 1 at locus -889 also conferred a 4.5 risk of developing moderate CP and a 2 fold risk of developing severe CP. Studies reporting on other populations reported an association between severity of CP and presence of this genotype[18,93,94].

Haplotypes

Haplotypes are a set of closely linked alleles at adjacent loci on the chromosome that are inherited together. In this study, only the third haplotype (allele 1 at the IL-1A and IL-1B markers) was significantly associated with chronic periodontitis amongst both the Swahili and Taita participants. This means that having allele 1 simultaneously at -511, +3954, -889 and +4845 is likely to make an individual more susceptible to chronic periodontitis. This haplotype being common in both ethnic groups may be one that is transmitted in people of Bantu origin. A study of other Bantu groups in the region would enlighten on the role this haplotype has in Bantus.

6.4 ORAL HEALTH SEEKING BEHAVIOR AMONGST TAITA AND SWAHILI PARTICIPANTS

Most of the Taitas 90.4% had never visited a dental clinic. The main reason being that the dental clinic was too far and the treatment too costly. This finding was similar in both the cases and controls. Amongst Swahili participants, 45.1% had never visited a dental clinic. This is a much lower frequency when compared to the Taita participants. The explanation is that in Mombasa, dental clinics were available in most places and the government hospital is within walking distance.

The finding that more of the male Swahili participants of high income level and with ≤ 8 years of education had visited a dentist is probably an indication that they had more disposable income and hence it was easier for them to make the decision to visit the dentist. The finding that those of <8years of education having visited the

dentist more frequently is more difficult to explain because the reported findings in other studies are that the more educated individuals are the ones who visit the dentist since they have more disposable income[154]. This finding in the Swahili participants defers with other studies.

Although Swahili participanst visited a dentist, it was mostly for extractions. This may be a reflection of their not being regular attenders. It has been shown that dentists tend to extract teeth more easily in erratic attenders[154]. In this country, unavailability of restorative materials as well as costs of restorations may also contribute.

Multivariate analysis

In the Taita participants, the multivariate analysis confirmed that plaque was the main independent factor that was significantly associated with chronic periodontitis. This finding is in tandem with literature since it is known that the genes will contribute the susceptibility but plaque will initiate the disease process. In the Swahili participants, plaque was also confirmed as the main independent factor significantly associated with chronic periodontitis at the multivariate level. Hence controlling plaque will ultimately lead to better control of CP and its destructive effects.

6.5 IMPLICATIONS OF THE STUDY

The implications of this study are that the relationship between plaque and chronic periodontitis in both African tribes suggests that it initiates the disease process against a background of genetically susceptible individuals. Hence plaque is an independent risk factor that has implications in the clinical and public health sectors.

This study contributed to the knowledge of genetic polymorphisms of interleukin-1 and their association with chronic periodontitis. IL-1B+3954 allele 2 was independently associated with chronic periodontitis in the Taita participants. This was unique because other studies have tended to demonstrate an association when there is a combination of IL-1A+4845 and IL-1B+3954.

This study demonstrated that allele 1 at IL-1A-889 contributed to the susceptibility to CP in the Swahili participants. Of note is the fact that none of the studies on Caucasians and non Caucasians have found a significant association between CP and IL-1A-889 as a single risk factor. This could therefore mean that, this finding may be a reflection of a locus that is in linkage disequlibrium with other biologically significant polymorphisms in an adajacent region of the genome.

Genetic polymorphism at IL-1B-511 has not been associated with chronic periodontitis in many populations studied. However, in this study, this genetic polymorphism was associated with CP amongst the Swahili participants when there was less plaque. Plaque level was found to have a tendency towards effect modification in the association between allele 2 at -511 and CP. This implies that when there was less plaque, the effect of the genotype polymorphism was observed, but in the presence of abundant plaque of >15 tooth surfaces involved, the effect plaque has on CP overrides the effect of the genotype.

The carriage rates of the various genetic polymorphisms of interleukin-1 gene differed in the two African tribes studied. This implies that genetic polymorphisms may be affected by intermarriage since the genotype distribution in the Swahili participants was similar to Caucasians as compared to the Taita participants.

6.6 CONCLUSIONS

1. The two ethnic groups are different despite having a common Bantu origin. Intermarrying appeared to bring about differences in the carriage rates of genes in that the Taita who do not generally marry outside their ethnic group had a lower frequency of three of the loci studied (-511, +3954 and +4845). As for allele 1 at IL-1A-889, the Taita participants had a higher frequency distribution compared to the Swahili participants. Inspite of the Taita participants having a higher prevalence of this genotype (-889), it was not associated with chronic periodontitis. IL-1A-889 was instead associated with CP in the Swahili participants. IL-1B+3954 was independently associated with chronic periodontitis in the Taita participants.
2. The male Taita participants did not have the homozygous allele 1 of IL-1A+4845 polymorphism. Amongst the male Swahili participants, allele 2 at IL-1A-889 appeared to provide protection and allele 1 at locus-889 was associated with a 6 fold risk of CP in female Swahili participants.
3. The Swahili participants with chronic periodontitis had a higher frequency distribution of heterozygous IL-1B-511 than the Taita participants ($p=0.016$).

Plaque level was found to modify the association between IL-1B -511 and CP in the Swahili participants.

4. Haplotype 3 where a combination of allele 1 at loci -511, +3954, -889 and +4845 was associated with CP in both ethnic groups may be an indication of the commonality of the origins of both groups.
5. Plaque and calculus were associated with clinical attachment loss in both ethnic groups. The Swahili paricipants had significantly more plaque and bleeding on probing when compared to the Taita participants.
6. Brushing in the evenings was protective for the Taita participants whereas brushing in the mornings was protective for the Swahili participants.
7. Oral health seeking behavior in both ethnic groups was poor. This may be an indication of the lack of knowledge on oral health matters in the general population in Kenya. Unaffordability of treatment and the long distances to the nearest dental clinic may also lead to poor utilization of the facilities.

6.7 CLINICAL APPLICATION

It is now a well known fact that only 10-15% of any given population will develop destructive chronic periodontitis[1]. Certain microorganisms have been shown to initiate the process of development of inflammation but the progression in susceptible individuals from gingivitis to periodontitis and destructive periodontitis has not been clearly defined to date. The study of genomics has recently provided a tool to test the genetic contribution of the host to the progression of destructive disease. An individual's propensity to develop chronic periodontitis will depend on

their response to the microorganisms. This response is dependent on many factors including the genetic makeup, smoking, diet, systemic illness, behavior and psychological stress among other things as was indicated earlier. Identifying the genetic susceptibility, environmental factors and behavioral habits of an individual will assist in improving periodontal management. Currently, proven genetic testing is available for the syndromic forms of periodontitis like Papillon Lefevre, Chediak Higashi, Lazy leukocyte syndrome and Ehlers-Danlos syndrome.

Genetic testing for chronic periodontitis is also available for the composite genotype of allele 2 of IL-1A at -889 and allele 2 of IL-1B at +3954. This test would not be applicable in those of Bantu origin since the findings do not support the association between the composite genotype and CP. This study has shown that in the African population of Bantu origin, the two polymorphisms that produced significant p-values in this study were IL-1B+3954 amongst the Taita and IL-1A-889 amongst the Swahili as single risk factors in each ethnic group. Additionally haplotype 3, where the wild type allele at loci -511, +3954, -889 and +4845, was associated with CP in both ethnic groups. Hence the development of genetic testing for susceptible individuals would need to include the testing of the various loci singly as well as testing for haplotype 3 in the African population. However, the cost implication may not allow testing of a majority of individuals in this country since dental treatment is largely financed by out of pocket expenses. Finally, plaque control as a well tested mode of prevention of CP has been proven in this study.

6.8 RECOMMENDATIONS

1. There is need to carry out similar studies in different Bantu groups, in Cushites and in Nilotes resident in Kenya to assess whether the same gene polymorphisms are associated with chronic periodontitis and if haplotype 3 will remain significant in the other African groups.
2. Similar studies with larger sample sizes are needed to assess whether the genetic polymorphisms associated with chronic periodontitis remain significant.
3. Inclusion of oral health education in public health programs to create awareness and improvement in the oral health status of the general population as it has been clearly shown in this study that plaque is the main factor associated with chronic periodontitis. In time, this could lead to an alteration in the phenotype and enhanced periodontal disease protection.

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7.0 LIST OF APPENDICES

APPENDIX 1: CLASSIFICATION OF PERIODONTAL DISEASES AND CONDITIONS

1.Gingival Diseases
- Plaque-induced gingival diseases (these diseases may occur on a periodontium with no attachment loss or on one with attachment loss that is stable and not progressing)
- Non-plaque- induced gingival diseases

2.Chronic Periodontitis
- Localized
- Generalized

3.Aggressive Periodontitis
- Localized
- Generalized

4.Periodontitis as a Manifestation of Systemic Disease

5.Necrotizing Periodontal Diseases
- Necrotizing ulcerative gingivitis (NUG)
- Necrotizing ulcerative periodontitis (NUP)

6.Abscesses of the Periodontium
- Gingival abscess
- Periodontal abscess
- Pericoronal abscess

7.Periodontitis Associated with Endodontic Lesions
- Endodontic-periodontal lesion
- Periodontal-endodontic lesion
- Combined lesion

8.Developmental or Acquired Deformities and Conditions
- Localized tooth-related factors that predispose to plaque induced gingival diseases or periodontitis
- Mucogingival deformities and conditions around teeth
- Mucogingival deformities and conditions on edentulous ridges
- Occlusal trauma

APPENDIX 2: CONSENT FORM

Title of the study: A Comparative Study to Determine Interleukin 1 Polymorphisms of Chronic Periodontitis among Two Kenyan Coastal Communities.

Part A

You are invited to participate in a study that seeks to find out whether gum disease runs in families. You have been selected as a participant for this study. However, you are requested to read and understand the contents of this form before accepting to take part. You are free to ask any questions or demand for clarification in regard to your participation in the study.

Procedure of the study: After consenting to participate in this study, the questionnaire will be administered to the participants. The questionnaires will be used to collect socio-demographic information. This will be followed by an examination of the mouth and some cell samples will be taken from the cheeks by rubbing a small cotton swab. The sample collected will be stored at the National Influenza Centre - Kenya Medical Research Institute laboratory next to Kenyatta National Hospital where the genetic part of the study will take place. Free emergency dental treatment will be provided where needed and referral to the nearest hospital when treatment cannot be provided.

Risks of the study: There are no risks involved in the study.

Confidentiality: Every aspect of this study will be treated with strict confidentiality. Names will not be revealed in any way. All data will be kept in a secure place and no one will be allowed to access it apart from the research team.

Voluntary nature of the study: Your participation in this study is voluntary. You have the right to withdraw from the study at any time. In case you so wish, there will be no intimidation for you to remain in the study.

Patient Information: This work may be published but no use of names and strict confidentiality is maintained.

Benefits of the study: The study results will be used to inform policy – makers on the need to set up rural dental clinics where susceptible individuals may be identified and treated before tooth loss begins to affect their nutritional status and general health. The information gathered will be used to help in identifying individuals who may develop the disease and therefore early preventive measures can be instituted. Others will also benefit because the information gathered will be used in future to develop new ways of managing the condition. Individuals will also benefit from emergency dental treatment and oral health education.

Contacts and questions:

Any questions may be directed to Dr Evelyn G. Wagaiyu of School of Dental Sciences, College of Health Sciences, University of Nairobi, P.O.Box 19676, 00202, Nairobi. Tel: +254722672567. Email: evelynwagaiyu@uonbi.ac.ke.

In case you need to contact somebody else regarding the study apart from the researcher, feel free to contact the following person:

Prof K M Bhatt, Chairperson, KNH/UON-ERC

Kenyatta National Hospital

Hospital Rd, along Ngong Rd.

P.O.Box 20723, 00202, Nairobi.

Tel: 726300-9, Fax: 725272

Email: KNHplan@Ken.Helathnet.org

Participant's statement: I agree to cooperate and participate in this study

Name of participant…….

Signature……..

Date ……

APPENDIX 3: FOMU YA MAKUBALIANO

Kichwa cha utafiti: Upimaji wa Interleukin 1 Polymorphisms na vile vinavyo kusiana na ugonjwa wa ufizi in Two Kenyan Coastal Communities.

Part A

Umealikwa kujiunga na utafiti huu wa magonjwa ya sine. Lengo lake ni kubaini kama haya magonjwa ni ya ukoo.Umechaguliwa kama mshiriki wa utafiti huu lakini unaombwa usomo kwa uangalifu na uelewe yaliyomo kabla ya kukubali kushiriki. Uko huru kuuliza masawali yoyote au maelezo zaidi kuhusu kushiriki kwako katika utafiti huu.

Utaratibu wa utafiti: Baada ya makubaliano washiriki watapewa hojaji. The questionnaires will be used to collect socio-demographic information. Hojaji hii itatumika kukusanya takwimu ya kijamii.Hii itafuatiwa na ukaguzi wa mdomo na kutolewa kwa sampuli za chembechembe kwa kutumia pamba Matibabu ya dharura bila malipo yatatolewa na watakao hitaji matibabu maalumu watepewa barua za kuenda hospitali.

Hatari za utafiti: Utafiti huu hauna madhara yoyote.

Usiri: Kila kipengele cha utafiti huu kitatekelezwa kwa usiri. Majina ya wahusika hayatatolewa kwa vivyote vile. Matokea yote yatahifadhiwa kwa uangalifu na kutumika na wahusika pekee.

Kujitolea kwa utafiti: Kushiriki kwa utafiti huu ni kwa hiari yako mwenyewe. Uko na uhuru wa kijiuzulu wakati wowote. Hutalazimishwa kuendelea na utafiti huu ijapo utabadili nia.

Habari ya wagonjwa: Matokea yatachapishwa bila kutaja majina ya wahusika.

Umuhimu wa utafiti: Matokea ya utafiti yatachangia muongozo wa sera za ujenzi wa zahanati za matibabu ya meno katika maeneo ya mashambani kwa minajili ya kuzuia kuzorota kwa lishe na afya kwa jumla. Uzinduzi wa maradhi mapema utawezesha wahasiriwa kupokea usaidizi utakaozuia madhara ya baadaye. aidha wengine watasaidika kwa matokeo yatakayo patikana kwa uvumbuzi wa njia mpya za kukabiliana na maradhi. Wahusika watafaidi kutokana na matibabu ya dharura ya meno na masomo ya afya ya kinyua kwa jumla.

Anwani na maswali

Maswali yatumwe kwa Dr Evelyn G. Wagaiyu of School of Dental Sciences, College of Health Sciences, University of Nairobi, P.O.Box 19676, 00202, Nairobi.Tel:+254722672567. Email: evelynwagaiyu@uonbi.ac.ke

Anwani badala ya mtafiti mkuu:

Prof K M Bhatt, Chairperson, KNH/UON-ERC

Kenyatta National Hospital

Hospital Rd, along, Ngong Rd.

P.O.Box 20723, 00202, Nairobi.

Tel: 726300-9, Fax: 725272

Email: KNHplan@Ken.Helathnet.org

Tamko la mshirika: nakubali kushiriki na kuambatana na maagizo yote ya utafiti

Jina la muhusika…….

Sahihi……..

Tarehe ……

APPENDIX 4: QUESTIONNAIRE

S/NO. _____-

35-44 YEAR OLDS

Sex_____

Age in years as at last birthday_____

Occupation _____

Ethnicity_____

Household Income per month/week/daily in Kshs_____

Education Level _____

Religion_____

Marital status_____

GeographicLocation: Province_____

District_____

Division_____

Location_____

Sub-Location_____

Village_____

Are your two sets of grandparents all of Taita origin? Yes ☐ No ☐

The following questions are related to cleaning one's teeth:

1. Do you clean your teeth?

   (1) no (go to question 25)

   (2) yes (Continue with question 18)

2. How often do you clean your teeth?

   (1) don't know

   (2) sometimes

   (3) once a day

   (4) twice a day

   (5) more than 2 times a day

3. With what do you clean your teeth?

   (1) with a toothbrush

   (2) with a chewing stick

   (3) others, specify…….

4. What do you put on your cleaning device?

   (1) nothing

   (2) toothpaste

   (3) others, specify……

5. Did you clean your teeth yesterday afternoon?

   (1) no

   (2) don't know or remember

   (3) yes

6. Did you clean your teeth yesterday evening?

(1) no

(2) yes

(3) don't know or remember

7. Did you clean your teeth yesterday morning?

(1) no

(2) yes

(3) don't know or remember

8. Did you clean your teeth this morning?

(1) no

(2) yes

(3) don't know or remember

9. I find having to brush my teeth everyday

(1) necessary

(2) unnecessary

(3) don't know

The following questions are related to some things you may or may not eat:

10. Do you eat foods which contain sugars such as buns, scones,

toffees, chocolate, biscuits, sweets, ice cream, cakes, honey, jam, etc?

(1) no (go to question 30)

(2) yes (continue with question 27)

11. How often do you eat these sweet types of foods?

    (1) Sometimes but not every day (go to question 30)

    (2) Once a day

    (3) 2 to 3 times a day

    (4) 4 to 6 times a day

    (5) more than 6 times a day

12. Do you find it necessary to consume these sweet types of food daily?

    (1) no

    (2) yes

13. Would you stop consuming these sweet types of food if that would be better for your teeth?

    (1) no

    (2) yes

    (3) if no, why not?.....

_____

_____

The following questions are related to things you may or may not drink:

14. Do you drink drinks which contain sugar such as sodas, tea with lots of sugar, juices, etc?

    (1) no (go to question 34)

    (2) yes (continue with question 31)

15. How often do you drink these sugary drinks?

(1) sometimes but not everyday

(2) once a day

(3) 2 to 3 times a day

(4) 4 to 6 times a day

(5) more than 6 times a day

16. Do you find it necessary to consume these sugary drinks daily?

(1) no

(2) yes

17. Would you stop consuming these sugary drinks if that would be better for your teeth?

(1) no

(2) yes

(3) If no, why not?……

_____

_____

The following questions are related to seeking dental care:

18. Have you ever visited a dental clinic to seek dental care?

(1) no (go to question 24)

(2) yes (continue with question 19)

19. Are you visiting the dentist or trained dental personnel on a regular basis, for example,

twice a year.

(1) no (go to question 22) ☐

(2) yes (continue with question 20)

20. You are a regular visitor to the dentist or trained dental personnel. The following question is related to how satisfied you are about your experiences in the clinic:

Do you usually leave the dental clinic in a satisfied manner?

(1) no (go to question) ☐

(2) yes (continue with question 21)

21. What makes your visit to the dental clinic satisfying?

Read the following carefully!

There is more than one answer possible.

(1) no bleeding gums anymore ☐

(2) treatment done without injection ☐

(3) no dental problems discovered at all ☐

(4) only advice was needed ☐

(5) fillings done instead of extraction ☐

(6) get relief from pain get painless treatment ☐

(7) treatment is quickly done

(8) explanation of what is going to happen

(9) don't know

(10) other, specify ……..

22. What kind of things don't you like about receiving dental care?

Read the following carefully!

There is more than one answer possible.

.

(1) the smell in the surgery

(2) the local anaesthesia/injection

(3) the noise of the equipment

(4) the unfriendly treatment of the staff

(5) the long time spent in the waiting room

(6) don't like people working in my mouth

(7) other, specify…….

(8) don't know

23. Are there things which make you hesitate to seek dental care?

(1) no (end of questionnaire)

(2) yes (continue with question 24)

24. Since you have not visited the dental clinic and are hesitant to do so in the future, Would you tell us reasons why you are hesitant in seeking dental care?

Read the following carefully!

There is more than one answer possible.

(1) distance to dental clinic is too long ☐

(2) presence of unfriendly dental workers ☐

(3) long waiting time at the dental clinic ☐

(4) no time to go to the dental clinic ☐

(5) treatment is too costly ☐

(6) getting painful treatment ☐

(7) getting infected with HIV ☐

(8) don't know what kind of treatment to expect ☐

(9) dental clinic is not clean ☐

(10) for fear of losing a tooth ☐

(11) heard stories about bad things happening to patients in the dental clinic ☐

(12) Other, specify …… ☐ …

(13) don't know

25. Are there things which make you hesitate to seek dental care on

a regular basis?

(1) no (end of questionnaire) ☐

(2) yes (go to question 26)

26. Since you have visited the dental clinic but are not going on a regular basis and you are hesitant in seeking further dental care, would you tell us the reasons why you are hesitant?

There is more than one answer possible.

(1) distance to dental clinic is too long ☐

(2) presence of unfriendly dental workers ☐

(3) long waiting time at the dental clinic ☐

(4) no time to go to the dental clinic ☐

(5) treatment is too costly ☐

(6) getting painful treatment ☐

(7) getting infected with HIV ☐

(8) dental clinic is not clean ☐

(9) for fear of losing a tooth ☐

(10) heard stories about bad things happening to patients in the dental clinic ☐

(11) other, specify …… ☐

(12) don't know ☐

Thank you for filling in the questionnaire.

APPENDIX 5: HOJAJI

S/NO. _____-

RIKA LA UMRI 35-44

Uana_____

Umri_____

Ajira_____ Ukoo_____

Mapato kwa mwezi/wiki/siku Kshs_____

Kiwango cha elimu_____

Dini_____

Una mke au mume_____

Mkoa_____

Wilaya_____

Eneo_____

Kata_____

Kata Ndogo_____

Kijiji_____

Je babu zako kutoka pande zote ni wa Taita? Ndio ☐ La ☐

Maswala yafuatayo yanahusu usafi wa meno.

1. Je wewe husafisha meno? ☐

(1) la (enda swala la 25)

(2) ndio (endelea na swala la18)

2. Unasafisha meno mara ngapi?

(1) sijui

(2) mara kwa mara

(3) mara moja kwa siku

(4) mara mbili kwa siku

(5) zaidi ya mara mbili kwa siku

3. Unasafisha meno na nini?

(1) mswaki

(2) kijiti

(3) chengine, eleza…….

4. Je unaweka nini kwa mswaki?

(1) situmii chochote

(2) dawa ya mswaki

(3) chengine eleza……

5. Je ulisafisha meno jana adhuhuri?

(1) la

(2) sikumbuki

(3) ndio

6. je ulisafisha meno jana jioni?

(1) la

(2) ndio

(3) sikumbuki

7. Je ulisafisha meno jana asubuhi?

   (1) la

   (2) ndio

   (3) sikumbuki

8. je ulisafisha meno leo asubuhi?

   (1) La

   (2) ndio

   (3) sikumbuki

9. Naona kupiga mswaki kila siku

   (1) ni lazima

   (2) si lazima

   (3) sijui

Maswali yafuatayo yanahusu vyakula mbali mbali ambavyo huenda wala au huli:

10. Je wala vyakula vya sukari kama mikate peremende kaimati biskuti asali mahamri keki?

    (1) la

    (2) ndio

11. Je wala hivi vyakula vya sukari mara ngapi?

    (1) mara kwa mara lakini sio kila siku.

(2) mara moja kwa siku

(3) mara mbili au tatu kwa siku

(4) mara nne au sita kwa siku

(5) zaidi ya mara sita kwa siku

12. Je kuna unahitaji kula vyakula hivi viatmu kila siku?

(1) la

(2) ndio

13. je waweza kuwacha kula vyakula hivi kama ingekuwa bora kwa meno yako??

(1) ndio

(2) la

Maswala yafuatayo yanahusu vinywaji ambavyo huenda wanywa au hunywi:

14. Je wanywa vinywaji vya sukari kama coca cola, fanta, schweppes orange, sprite, pepsi cola, chai ya sukari nyingi, maji ya machungwa ……..na kadhalika?

(1) la

(2) ndio

15. Je wanywa vinywaji hivi mara ngapi?

(1) mara kwa mara lakini sio kila siku. Mara moja kwa siku

(2) mara mbili au tatu kwa siku

(3) mara nne au sita kwa siku

(4) zaidi ya mara sita kwa siku

16. Je unahitaji kunywa vinywaji hivi vya sukari kila siku ?

(1) la

(2) ndio ☐

17. Je waweza kuwacha kunywa vinywaji hivi kama ingekuwa bora kwa meno yako

(1) la ☐

(2) ndio

Maswala yafuatayo yanahusu utunzi na usalama wa meno:

18. Je umewahi kwenda kwa zahanati ya meno?

(1) la (enda swala 24) ☐

(2) ndio (endelea na 19)

19. Je wewe huenda kuona daktari wa meno kama mara mbili kwa mwaka? ☐

(1) la (enda 22)

(2) ndio (endelea 20)

20.Kama wewe huenda kwa daktari wa meno mara kwa mara maswala yafuatayo yanahusu huduma unayoipata .

Je huduma unaiyoipokea inatosheleza ?

(1) la (enda swala la 22) ☐

(2) ndio (endelea swala la 21)

21.Ni kitu gani kinachokuridhisha unapoenda kumwona daktari wa meno?

Soma maelezo yafuatayo kwa uangalifu!

Kuna zaidi ya jibu moja. Orodhesha majibu yako kulingana na umuhimu.

(1) sine zimepona hazitoi damu tena ☐

(2) matibabu bila kudungwa sindano ☐

(3) hakuna shida yoyote iliyojitokeza ☐

(4) ushauri pekee ulihitajika ☐

(5) kuziba ufa pekee badala ya kungolewa jino ☐

(6) afueni ya uchungu ☐

(7) tiba kwa wakati unaofaa ☐

(8) maelezo ya shida na tiba ☐

(9) sijui ☐

(10) chengine, eleza …….. ☐

☐

22.Je ni mambo gani ambayo hupendelei kuhusu tiba hii?

Soma yafuatayo kwa maakini!

Kuna zaidi ya jibu moja. Orodhesha majibu yako kulingana na umuhimu

(1) harufu ya hospitali ☐

(2) sindano ya ganzi ☐

(3) kelele za vifaa ☐

(4) ujeuri wa wafanyikazi ☐

(5) kungoja mda mrefu kabla kupata matibabu ☐

(6) sipendelei watu kugusa mdomo wangu ☐

(7) lengine, eleza…… ☐

(8) sijui ☐

23.Je kuna mambo ambayo yafanya usite kutafuta tiba ya kinywa?

(1) la (mwisho wa hojaji) ☐

(2) ndio (endelea na swala la 24)

24.tupe sababu zako za kutotaka kuenda kupokea ushauri kuhusu afya ya kinywa.

Soma yafuatayo kwa maakini!

Kuna zaidi ya jibu moja. Orodhesha majibu yako kulingana na umuhimu

(1) zahanati iko mbali sana ☐

(2) wafanya kazi wajeuri ☐

(3) muda mrefu wa kusibiri matibabu ☐

(4) ukosefu wa wakati wa kutembelea zahanati ☐

(5) matibabu ni ya ghali sana

(6) matibabu yenye chungu ☐

(7) kuambukizwa maradhi ya HIV ☐

(8) kutojua nitarajie tiba ipi ☐

(9) zahanati chafu

(10) hofu ya kungolea jino ☐

(11) nimesikia hadithi za kuogofya humo kwa zahanati ya meno ☐

(12) lengine,fafanua …… ☐ …..

(13) sijui

25.Je kuna kitu kinachokuzui kutafuta ushauri wa afya ya kinyua

(1) la (mwisho wa hojaji)

(2) ndio (enda kwa 26) ☐

26.Wewe umeshawahi kuhudhuria zahanati lakini unasita kurudi tena, je unasababu zipi?

Kuna zaidi ya jibu moja. Orodhesha majibu yako kulingana na umuhimu

zahanati iko mbali sana ☐

(1) wafanya kazi wajeuri ☐

(2) muda mrefu wa kusibiri matibabu ☐

(3) ukosefu wa wakati wa kutembelea zahanati ☐

(4) matibabu ni ya ghali sana

(1) matibabu yenye chungu ☐

(2) kuambukizwa maradhi ya HIV

(3) kutojua nitarajie tiba ipi ☐

(4) zahanati chafu ☐

(5) hofu ya kungolea jino ☐

(6) nimesikia hadithi za kuogofya humo kwa zahanati ya meno

(7) sababu nyengine, eleza ☐

(8) sijui ☐

Asante kwa kujibu maswali..

APPENDIX 6: CLINICAL EXAMINATION FORM

Participant no…….. Date……

Have you lost any teeth which became loose then had to be removed or fell out themselves: Yes……. No……

Smoker: Yes…… No……..

Other local factors like (developmental/anatomical, overhangs, crowding, PD, Ortho appliance etc) specify …….

PLAQUE CALCULUS GINGIVAL RECESSION

	B	L		B	L		DB	F	MB	MP	P	DP
17						17						
16						16						
15						15						
14						14						
13						13						
12						12						
11						11						
	B	L		B	L		MB	F	DB	DP	P	MP
21						21						
22						22						

23						23						
24						24						
25						25						
26						26						
27						27						
	B	L		B	L		DB	F	MB	ML	L	DL
37						37						
36						36						
35						35						
34						34						
33						33						
32						32						
31						31						
	B	L		B	L		MB	F	DB	DL	L	ML
41						41						
42						42						
43						43						
44						44						
45						45						
46						46						
47						47						

PERIODONTAL POCKET DEPTHS

BLEEDING ON PROBING

CARIES

	DB	F	MB	MP	P	DP		Yes	No		M	D
17							17					
16							16					
15							15					
14							14					
13							13					
12							12					
11							11					
	MB	F	DB	DP	P	MP		Yes	No		M	D
21							21					
22							22					
23							23					
24							24					
25							25					
26							26					
27							27					
	DB	F	MB	ML	L	DL		Yes	No		M	D
37							37					
36							36					

35							35					
34							34					
33							33					
32							32					
31							31					
	MB	F	DB	DL	L	ML		Yes	No		M	D
41							41					
42							42					
43							43					
44							44					
45							45					
46							46					
47							47					

APPENDIX 7: ETHICAL APPROVAL

KENYATTA NATIONAL HOSPITAL
Hospital Rd. along, Ngong Rd.
P.O. Box 20723, Nairobi.
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP", Nairobi.
Email: KNHplan@Ken.Healthnet.org
4th April 2011

Ref: KNH-ERC/ A/75

Dr. Evelyne Wagaiyu
School of Dental Sciences
University of Nairobi

Dear Dr. Wagaiyu,

RESEARCH PROPOSAL: **"A COMAPARATIVE STUDY TO ACCESS INTERLEUKIN 1 POLYMORPHISMS AND THEIR RELATIONSHIP WITH CHRONIC PERIODONTITIS IN TWO KENYAN COASTAL COMMUNITIES" (P56/2/2011)**

This is to inform you that the KNH/UON-Ethics & Research Committee has reviewed and **approved** your above revised research proposal for the period 4th April 2011 – 3rd April 2012.

You will b
e required to request for a renewal of the approval if you intend to continue with the study beyond the deadline given. Clearance for export of biological specimens must also be obtained from KNH/UON-Ethics & Research Committee for each batch.

On behalf of the Committee, I wish you a fruitful research and look forward to receiving a summary of the research findings upon completion of the study.

This information will form part of the data base that will be consulted in future when processing related research study so as to minimize chances of study duplication.

Yours sincerely,

PROF A N GUANTAI
SECRETARY, KNH/UON-ERC

c.c. The Deputy Director CS, KNH
The HOD, Records, KNH
Supervisors: Prof. Jacob T. Kaimenyi, Deputy V/Chancellor, Academic and Professor of Periodontology, UON
Dr. Wallace D. Bulimo, Dept of Biochemistry, UON
Dr. Peter Wanzala, Centre for Public Health, KEMRI

APPENDIX 8. LETTER OF APPROVAL TO CONDUCT THE RESEARCH FROM DISTRICT COMMISSIONER

OFFICE OF THE PRESIDENT

Telephone: 020 – 3558398
043-42210

E-Mail: dctaita1@yahoo.com
When replying please quote:
Ref .No. ADM.17/VOL.V/167

District Commissioner's office
Taita District
Private Bag
WUNDANYI

8th February 2010

The Chiefs
TAITA DISTRICT

RE: RESEARCH PROJECT BY EVERLYN G. WAGAIYU

The above named is a member of staff of the Department of Periodontology, Community and Preventive Dentistry, College of Health Sciences, University of Nairobi.

She is undertaking her research project for her PhD entitled **"A case control study to assess genetic risk factors of chronic periodontitis in two population: the Taita and Swahili groups in Kenya."**

This is therefore to direct you to ensure that she is accorded the necessary assistance for the period she will be in the community.

D. K. BOEN
FOR: DISTRICT COMMISSIONER
TAITA DISTRICT

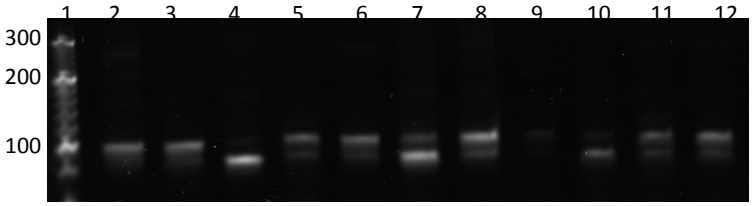
c.c

District Medical Officer of Health
TAITA

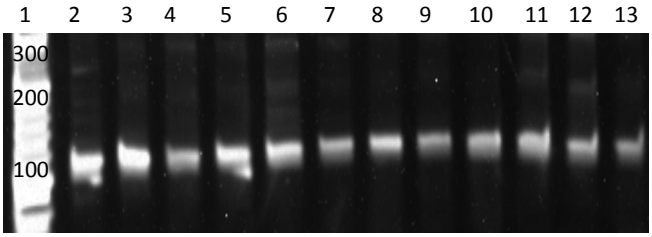
Dr. Evelyn G. Wagaiyu
NAIROBI UNIVERSITY

1

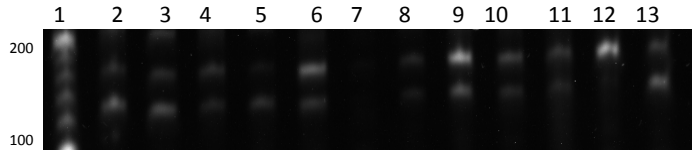
APPENDIX 9: SAMPLE PICTURES OF THE VARIOUS LOCI AFTER THE GENOTYPING REACTIONS



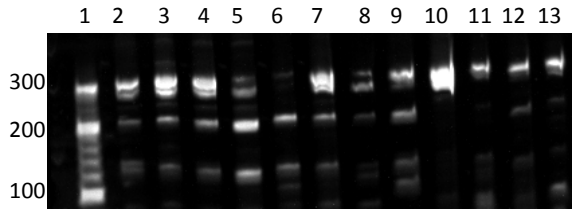
Lane 1 = 20bp DNA ladder (O'RangeRuler), Samples were amplified using allele -889-specific primers and digested with *Nco 1* restriction enzyme.
Lane 2 = sample 43, lane 3 = sample 44, lane 4 = sample 45, lane 5 = sample 47, lane 6 = sample 48, lane 7 = sample 49 , lane 8 = sample 50, lane 9 = sample 53, lane 10 = sample 53, lane 11 = sample 54, lane 12 = sample 55. Samples are from individuals of Taita origin



Lane 1 = 20bp DNA ladder (O'RangeRuler), Samples were amplified using allele -889-specific primers and digested with *Nco 1* restriction enzyme.
Lane 2 = sample 729, lane 3 = sample 722, lane 4 = sample 712, lane 5 = sample 710, lane 6 = sample 706, lane 7 = sample 734 , lane 8 = sample 735, lane 9 = sample 736, lane 10 = sample 737, lane 11 = sample 738, lane 12 = sample 740, lane 13 = sample 741. Samples are from individuals of Swahili origin.

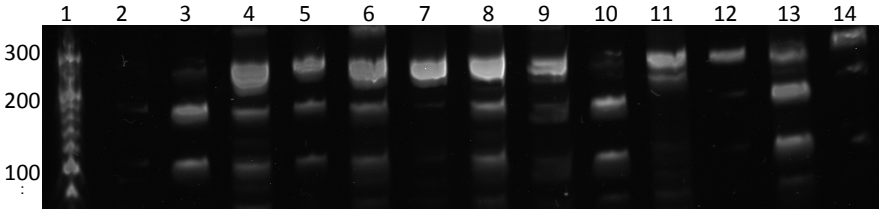


Lane 1 = 20bp DNA ladder (O'RangeRuler), Samples were amplified using allele +4845-specific primers and digested with *Fnu* restriction enzyme. Lane 2 = sample 153, lane 3 = sample 558, lane 4 = sample 559, lane 5 = sample 560, lane 6 = sample 563, lane 7 = sample 564 , lane 8 = sample 565, lane 9 = sample 566, lane 10 = sample 567, lane 11 = sample 569, lane 12 = sample 570. Samples are from individuals of Swahili origin



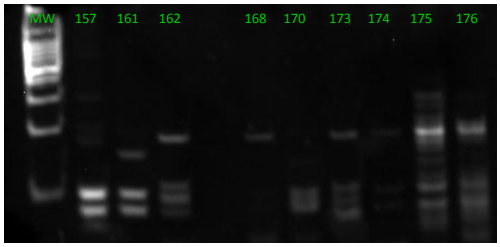
Lane 1 = 20bp DNA ladder (O'RangeRuler), Samples were amplified using allele -511-specific primers and digested with *Avu1*restriction enzyme.

Lane 2 = sample 1, lane 3 = sample 2, lane 4 = sample 4, lane 5 = sample 5, lane 6 = sample 6, lane 7 = sample 7 , lane 8 = sample 8, lane 9 = sample 9, lane 10 = sample 10, lane 11 = sample 11, lane 12 = sample 12, lane 13 = sample 13. Samples are from individuals of Taita origin.



Lane 1 = 20bp DNA ladder (O'RangeRuler), Samples were amplified using allele -511-specific primers and digested with *Avu1* restriction enzyme.

Lane 2 = empty ladder, lane 3 = sample 731, lane 4 = sample 732, lane 5 = sample 733, lane 6 = sample 734, lane 7 = sample 735 , lane 8 = sample 736 , lane 9 = sample 737 , lane 10 = sample 738 , lane 11 = sample 739 , lane 12 = sample 740 , lane 13 = sample 741 , lane 14 = sample 743. Samples are from individuals of Swahili origin.



Restriction enzyme analyses of PCR amplified fragments to detect allelic polymorphisms among subjects from Taita.

Samples in lanes 1-9 were amplified using allele +3954-specific primers and digested with *TaqI* restriction enzyme

Figure 12 Lane 1 = 100bp DNA ladder (Sigma), Samples were amplified using allele +4845-specific primers and digested with *Fnu* restriction enzyme. Lane 2 = sample 153, lane 3 = sample 558, lane 4 = sample 559, lane 5 = sample 560, lane 6 = sample 563, lane 7 = sample 564 , lane 8 = sample 565, lane 9 = sample 566, lane 10 = sample 567, lane 11 = sample 569, lane 12 = sample 570. Samples are from individuals of Swahili origin