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Genetic polymorphisms in IL-1A and IL-1B isoforms and their associations with chronic periodontitis in the Swahili people of Kenya

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Abstract: *Genetic polymorphisms in interleukin-1 (IL-1A and IL-1B) isoforms have been associated with Chronic Periodontitis (CP) in Caucasians, Asians and Arabs but little is known about their role in Africans. Therefore, this study was to resolve the association between genetic polymorphisms in IL-1A and IL-1B isoforms and chronic periodontitis in a Kenyan community.*

Methods: *This was a case-control study. After informed consent, a clinical examination was conducted. Buccal swab samples were then obtained. Deoxyribonucleic acid was isolated from the swabs using QIAamp DNA purification protocol followed by polymerase chain reaction 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. Association analyses of the RFLP and clinical data were carried out.*

Results*: After screening 523 Swahili participants from old town Mombasa, 100 cases and 100 controls were included in the study.*

There was more plaque present in cases than controls with OR = 9.2 (95%CI = 3.7-23.1), p<0.001. Mild Chronic Periodontitis 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 amongst the Swahili participants was associated with Chronic Periodontitis (OR = 3.16, 95%CI=1.644-6.083, p<0.001). 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). Furthermore, plaque level was an effect modifier in the association between IL-1B-511 polymorphism and CP.

Conclusions*: Increased susceptibility to Chronic Periodontitis was found in Swahili participants with allele 1 at IL-1A-889.*

Keywords*: Chronic Periodontitis, IL-1 polymorphisms, Kenya, plaque, Swahili.*

I. Introduction

Genetic variation is considered as one of the risk factors, which bring about differences in periodontal disease progression [1]. 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 [1-3].

A meta-analysis investigation to determine whether IL-1A (-889) and IL-1B (+3954) composite genotype was associated with chronic periodontitis confirmed this association in Caucasians [4]. The prevalence of IL-1 composite genotype is low among Asian populations. The prevalence reported in Chinese subjects is 2.3%, [5] 2% in Thai subjects, [6] 0.2% in Japanese persons, [7] and 14% in Indians [8]. Other Indian studies reported no association of IL-1B (+3954) and chronic periodontitis [9, 10]. 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.

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 [11]. 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.

Data on IL-1 polymorphisms in native African populations are limited. The only study to the best of our knowledge was carried out amongst the Xhosa in South Africa and reported a prevalence of IL-1A (+4845) allele 2 at 46.9 % in cases and 22 % in controls and IL-1B (+3954) at 15.8 % in cases and 14.3 % in controls [12]. This study showed that IL-1 composite polymorphism was not associated with severity of periodontitis in this South African population [12].

To expand on this study in the only known African population, we sought to investigate the association of interleukin-1 polymorphisms with chronic periodontitis in a Kenyan population focusing on the Swahili tribe. The Swahili people are an interesting group because their genetic make-up is a mosaic with African, Caucasian and Arab heritage.

II. Material And Methods

The study was conducted in the old town section of Mombasa town in Mombasa County where most of the Swahili people are found. Mombasa County lies along the Kenyan coastline. The city has a population of 939,370, as per the 2009 census.

This was a case-control study design. The design allowed for selection of cases and controls without randomization as long as the inclusion criteria were adhered to [13]. The test was, whether the marker genotypes distributed differently between the cases and controls. The selection of cases involved matching by age and gender of the cases with unaffected controls. Cases comprised individuals with chronic periodontitis selected on the basis of having clinical loss of attachment of ≥ 3mm on several teeth but a minimum of at least 2 non-adjacent teeth with proximal attachment loss of ≥ 3mm [14, 15] and controls were individuals with a clinically healthy gingiva that did not bleed on probing and had no probing depth of >3mm. Controls were individuals who would have been designated study cases if they had developed the disease. Study participants were adults of ages 35-44 years since this is the age at which cumulative effects of Chronic Periodontitis will present according to the World Health Organization Basic Methods Criteria. These participants were selected from subjects who presented themselves to the various recreational centers and health centers and met the inclusion criteria. Controls were recruited from the same area and were used to characterize the distribution of the genotype.

Included persons had to have most of their teeth but 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 multi-rooted teeth because progression of chronic periodontitis may differ in the different tooth types [16]. Only those who consented to participate in the study were recruited. Those excluded were persons with a history of periodontal treatment, six months prior to the study since this would have interfered with disease definition by the inclusion of cases with a reduced but healthy periodontium as control subjects. Those on any medication or with any systemic illness were also excluded.

The required minimum sample size for case control studies was calculated using a formula developed by Kirkwood and Sterne [17]. The exposure rate reported in the study by Kornman et al., [18] was used since there are no known Kenyan African studies on genetic polymorphism and chronic periodontitis. The specifications 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 ensured proper utilization of resources since sample size is often determined by logistic and financial considerations [15].

A modified WHO questionnaire on oral health seeking behavior, oral health practices and sugar consumption was used [19]. A Kiswahili version was used for participants who did not understand English. 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 carried out on the same six sites per tooth. Bleeding on probing, calculus and plaque were recorded as present or absent. DNA collection was carried out using the isohelix buccal swabs (Boca Scientific, Isohelix, Kent, England) as per the manufacturer's instructions.

Labeled swabs were used to collect buccal cells from the cheeks of the participants from which DNA samples were obtained. DNA was purified from cells adhering on the swabs using QlAmp DNA Minikit spin protocol (Qiagen, Turnberry Lane, Valencia, CA) following the manufacturer's instructions. Centrifugation steps were carried out at room temperature (15-25^{0}C). Purity and concentration of isolated DNA was determined using NanoDrop 2000c spectrophotometer (Thermo Scientific, Waltham, Mass., USA). Genomic DNA from cases and controls were analyzed for polymorphisms within the IL-1A gene loci -889 [20] and +4845 [21] and the IL-1B gene loci -511 [22], and +3954 [23]. PCR reaction conditions were as previously described [24, 25]. Briefly, a reaction mix excluding Taq polymerase was prepared with template DNA added prior to heating at 95°C for 15 min. Taq polymerase (Invitrogen Corporation, Grand Island NY, USA) was added and PCR

initiated. $MgCl_2$ and primer concentrations were varied in each type of reaction for the different loci ([rs1800587] -889, [rs17561] +4845, [rs16944] -511 and [rs1143634] +3954). Restriction fragment length polymorphism (RFLP) assays of PCR-amplified gene fragments were carried out as previously described [25] before subjecting the digests to polyacrylamide gel electrophoresis followed by staining with ethidium bromide (0.2pg/ml) and visualized under ultraviolet light. 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, Breslow-Day 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) was tested for genotype frequency by chi square test, with 1 degree of freedom.

III. Results

All the participants were of the Swahili ethnic group and 108 were males and 92 females. There was no difference in the distribution of male and female participants OR = 1.00(95% CI = 0.57-1.74); p=1.000. Seventeen (17) tooth surfaces had plaque in controls compared to 26 in cases per individual. When cases were compared to controls, the mean number of teeth with plaque was 26 (SD=4) tooth surfaces per individual in cases and 18 (SD=6) tooth surfaces per individual in controls with OR = 9.2 (95%CI = 3.7-23.1), p<0.001 (Fig 1). The mean number of teeth in cases 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 observed on 29 (1.07%) sites out of 2800 sites examined. When the gingival tissues surrounding 27 teeth out of a possible 28 were examined, it was found that approximately 64% of the teeth in cases had tissues that bled on probing (Fig 2) .

Less than 50% of the control individuals had calculus. When presence of calculus was examined in cases compared to controls, we found an OR = 114.6, 95%CI = 33.1-397.2, p<0.001 showing that calculus was most likely in cases than controls.

On probing the periodontal pockets, it was found that cases had probing depths of more than 3mm but none of the controls had pockets of more than 3mm (normal probing depths range from 0-3mm). Periodontal probing depths of more than or equal to 4mm were found on 8.33 (5.0%) sites per individual in cases. The mean pocket depth in all participants was 1.93 (SD=1.07).

Mean clinical attachment loss (CAL) ≥4mm was present in 37 (SD = 30.6) of the sites per individual in cases. None of the controls had CAL. When CAL of more than or equal to 5mm and more than or equal to 6mm were considered, 25 (SD = 8.6) and 16 (SD = 24.8) sites per individual were affected respectively. The mean total CAL was 3.13 (SD = 1.07). Amongst the Swahili participants, the distribution of disease according to the CDC/AAP definitions (Page and Eke 2007) revealed a high prevalence of severe chronic periodontitis with 56 (56%) cases having severe periodontitis, 35(35%) moderate periodontitis and only 9 (9%) with the mild form.

Interleukin-1 polymorphisms were tested at loci-511, and +3954 for IL-1B and loci -889 and +4845 for IL1-A. 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 reported (table 1).

An insignificantly higher frequency of heterozygous IL-1B (-511) was found in cases 67 (69.8%) compared to controls 66 (69.5%), p=0.881. The frequency of homozygous allele 1 at this locus 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. Overall, the frequency distribution of allele 2 at -511 locus 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.

Polymorphisms at the +3954 locus of interleukin-1B 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 at 115 (65.5%) and at 99 (58.2%) in controls, with p value of 0.174.

Homozygous frequency for IL-1A allele 1 at locus-889 was 15 (16.3%) for cases and 5 (5.1%) for controls, p = 0.018, indicating significantly higher frequency in cases than controls. 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 1). Heterozygous frequency was 6 (6.5%) and 4 (4.1%) for cases and controls respectively with a p value of 0.516.

The carriage rate of allele 2 polymorphism at -889 in cases was 148 (80.4%) and 182 (92.9%) in controls, 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 IL-1A locus+4845, the frequency of the homozygous allele 1 was the lowest, with cases at 5 (5.7%) and controls at 6 (6.7%), p=0.756. Homozygote frequency for allele2 was 24 (27.3%) and 28 (31.1%) in cases and controls respectively with a p=0.519. Heterozygote frequency for allele1 at locus +4845 was 59 (67%) and 56 (62.2%), p=0.668 in cases and controls respectively. 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 1).

When Hardy Weinberg Principle was tested, χ^2 distribution with 1df (3.84) showed that equilibrium exists for IL-1A loci -889 (χ^2 = 85.22, p<0.05) and +4845 (χ^2 = 23.80, p<0.05) and IL-1B at locus -511 (χ^2 = 38.66, p<0.05) and +3954 (χ^2 = 14.66, p<0.05) in the Swahili participants.

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 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.

Severity of chronic periodontitis was subjected to analysis by comparing the frequency of IL-1A and IL-1B genotypes in mild, moderate and severe chronic periodontitis with controls. We found that allele 1 frequency in IL-1A at locus -889 was 4 (28.6%) for cases with mild chronic periodontitis and 14 (7.1%) in the controls, (p=0.005 and OR=5.2, 95%CI=1.445-18.7). The frequency for allele 1 at locus -889 in cases with moderate chronic periodontitis was 17 (25.8%) and 14 (7.1%) for controls, (p=<0.001, OR=4.51, 95%CI=2.08-9.79). In cases presenting with severe form of chronic periodontitis, the distribution frequency was 15 (14.4%) in cases and 14 (7.1%) in controls, (p=0.042, OR=2.19, 95% CI = 1.013 – 4.738). There was no significant correlation between the other alleles and chronic periodontitis.

Breslow- Day test showed that there was a significant difference between the odds ratios, p=0.009 and thus there is effect modification by plaque level (table 4). The crude and adjusted ORs are different (1.116 and 8.25 respectively).

Mantel-Haenszel method assumes exposure (genotype -511) and chronic periodontitis 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 Mantel-Haenszel estimate value of 0.94 implied that, after controlling for the effect of plaque level, the odds of disease were less in subjects without genotype compared to subjects with the genotype at the lower plaque level of <=15 tooth surfaces with plaque (table 5).

IV. Discussion

Plaque levels were 26 (91%) tooth surfaces in cases and 17 (65%) tooth surfaces in controls with OR = 9.2 and 95%CI = 3.7-23.1. 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 (Newman 2006). 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. Recording >3mm attachment loss ensured that all cases actually had chronic periodontitis and of a moderate or severe form as recommended by Schafer et al 2011[15]. In this paper, Schafer et al 2011[15] 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 individuals was used. This high level of chronic periodontitis improves the statistical power of the study [13]. Therefore, the severity of chronic periodontitis in this study was high. It is clear that in this 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 [26].

The only polymorphism that was found to be significantly associated with chronic periodontitis was at the IL-1A-889 locus. At this locus, homozygous allele 1(1-1), 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 locus has been associated with chronic periodontitis in some populations and not others [27-29]. Hence amongst the Swahili participants, the association between [rs1800587] IL-1A -889 and chronic periodontitis was similar to studies in Caucasians, probably reflecting that the genetic make-up is a mosaic with Caucasoid contribution. The increased destruction of the periodontium in those genotype-positive for IL-1A-889 allele1, are probably due to reduced production of IL-1α cytokine [30]. This leads to increased destruction of the periodontium due to low levels of IL-1α protein resulting in lowered recruitment of

inflammatory cells. Variations in IL-1α also affect the production of IL-1β [30, 31]. The distribution of allele 2 at IL-1A locus -889 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 study where allele 2 at IL-1A locus -889 was highest in cases than controls hence associated with severe periodontitis [32]. Additionally, it was also shown by Hulkkonen et al [31] that homozgyous allele 2 IL-1A at locus -889 was associated with high IL-1α transcription and these high levels then induced IL-1β production only in the presence of allele 2 in IL-1B at locus -511 [31]. In the Swahili participants, allele 2 in IL-1B at locus -511was 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 -(91%) of the teeth affected per individual amongst the Swahili participants- were affecting these relationships. 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 chronic periodontitis.

Regarding the effects of composite genotype amongst the Swahili, the known composite genotype, allele 2 in IL-1A at locus -889 and allele 2 in ILA-1A at locus+4845,was not associated with chronic periodontitis. This finding is similar to that found in Xhosas of South Africa [12] indicating the African genetic heritage of this Swahili group. However, this finding contrasts studies, which have shown a positive correlation of these alleles (-889, 2-2 and +4845, 2-2) with chronic periodontitis [18, 33, 34] in Caucasians or Arabs or Asians. The severity of chronic periodontitis and association with the tested genotypes (-889, +4845, -511 and +3954) also differed from other populations because amongst the Swahili participants, there was no correlation, whereas other studies report a positive relationship [18, 35].

Amongst the Swahili participants, homozygous allele 1 of IL-1A-889 was found to be more frequent in those with chronic periodontitis. Although the frequency was low (16.3% in chronic periodontitis and 5.1% in controls), this allele was associated with chronic periodontitis. Other studies have reported frequencies for homozygous allele 1 at locus -889 as follows: D'Aiuto et al 2004 [36] (Caucasians) reported 39% in those with chronic periodontitis, Lopez et al 2005 [35] (Caucasians) reported 53.9% in chronic periodontitis and 64.35% in controls and Al-hebshi et al 2012 [37] (Yemenis) reported 20.3% in chronic periodontitis and 32.5% in controls. In all these studies, homozygous allele 1 of IL-1A-889 was not associated with chronic periodontitis. Hence amongst the Swahili participants the association of allele 1 at IL-1A-889 with chronic periodontitis is unique. There was a 3-fold odds of developing chronic periodontitis in the presence of allele 1 [rs1800587] IL-1B-889. 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 [38]. Thus this may affect their genetic makeup. 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 vary widely in Caucasians. According to Asian [6], Japanese [39] and Brazilian [40] studies, the carriage rate in these populations was found to be lower than that reported for the Caucasian [35, 41]. The lower carriage has also been reported in the Arab population where in Syrian Arabs, the rate was 12.5% and 8.6% in chronic periodontitis and controls [42]. However, in Yemeni Arabs, the carriage rates were 30% and 10% in chronic periodontitis and controls [37]. The frequency distribution of alleles appears to be different in various ethnic groups.

Amongst the Swahili participants, the frequency of allele 2 at IL-1A-889 was high but was not associated with chronic periodontitis. The negative correlation observed implies that this more common allele is not associated with chronic periodontitis. The levels in the Swahili participants are comparable to the study by Wagner et al [43] amongst Caucasians.

Amongst the Swahili participants, the distribution of polymorphism in IL-1A at locus +4845 was most frequent in heterozygote individuals where 67% in the chronic periodontitis 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 also 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 [5]. It is difficult to compare our findings with other studies since most have studied -889 rather than +4845. The fact that -889 and +4845 are in linkage disequilibrium and only -889 was found significant can only be explained by the finding that in the African population, there is a increased genetic diversity that is not seen in other populations [44].

The weakness in this study is the sample size. A larger sample size would have given a better representation. Nevertheless, the information obtained is unique in that four alleles were examined and for the first time in an African ethnic group of mixed descent. These findings confirm the genetic mosaic heritage of the Swahili as well as that our immune response to disease is greatly dependent on our genetic heritage.

V. Conclusions

IL-1A-889 was associated with chronic periodontitis in the Swahili participants. Plaque level was found to modify the association between IL-1B -511 and chronic periodontitis in the Swahili participants. Plaque and calculus were associated with clinical attachment loss.

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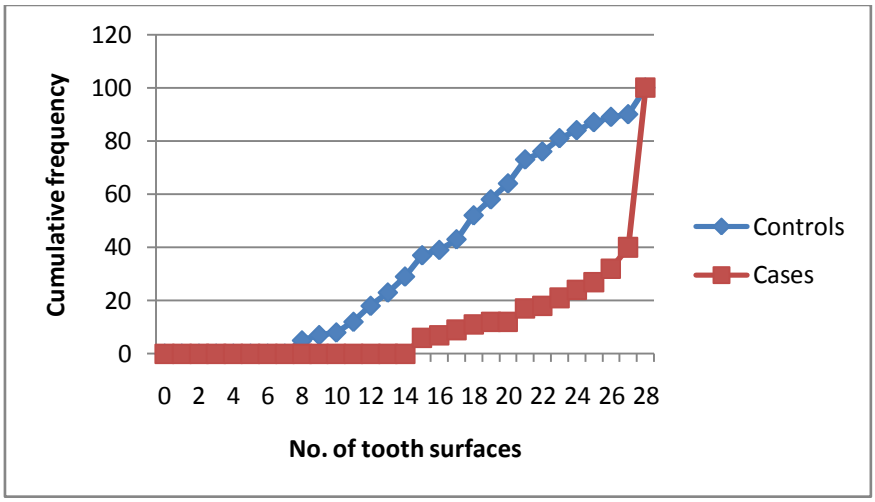

Figure 1: Cumulative frequency distribution of tooth surfaces with plaque amongst the Swahili participants by cases and controls

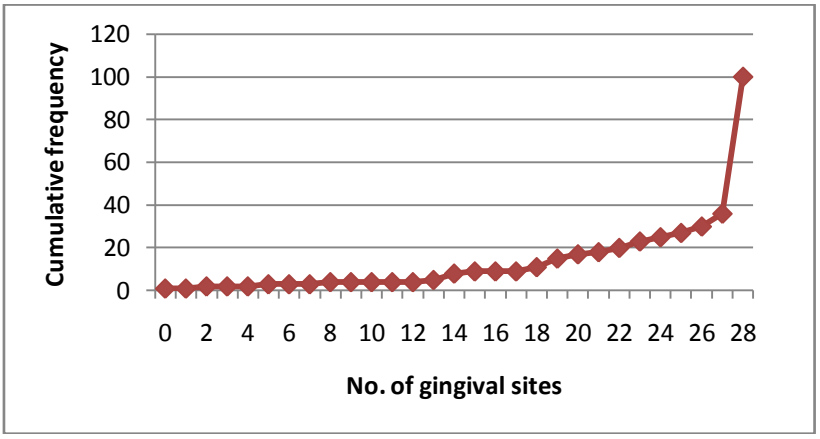

Figure 2: Cumulative frequency distribution of bleeding on probing of the gingival tissues around the teeth amongst the Swahili participants

Table 1 : 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

Table 2: This table 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 (2) 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.

		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 3: Chi-square test for confounding and effect modification of plaque levels amongst the Swahili participants (Stratum specific). This table shows the test of association between genotype -511 and chronic periodontitis after stratifying by plaque level. Subjects with plaque on less or equal to 15 tooth surfaces displayed a significant difference between the presence and absence of genotype -511 allele 2 (P<0.05; 95% CI 1.22-55.62; table 5). 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 chronic periodontitis and locus -511.

			Contro	Case	P-Value	AOR	95% CI for AOR	
Genotype-511	Plaque		n	n			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				

Table 4: Tests of homogeneity of the odds ratio. 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

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

Table 5: Mantel-Haenszel analysis testing for confounding and effect modification. 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.

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