PREVALENCE OF IL-1β+3954 AND IL-1α-889 POLYMORPHISMS IN THE LEBANESE POPULATION AND ITS ASSOCIATION WITH THE SEVERITY OF ADULT CHRONIC PERIODONTITIS

G. TAWIL[1], F.A. AKL[1], M.F. DAGHER[1], W. KARAM[2], I. ABDALLAH HAJJ HUSSEIN[3], A. LEONE[4] and A.R. JURJUS[3]

[1]Department of Periodontology, Saint-Joseph University, Beirut-Lebanon; [2]Department of Biology-Lebanese University, Hadath-Lebanon; [3]Department of Anatomy, Cell Biology, and Physiology, American University of Beirut-Lebanon; [4]Sezione di Istologia ed Embriologia, BioNec, Facoltà di Medicina e Chirurgia, Palermo, Italy

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Pro-inflammatory cytokine, i.e., IL-1 mediate the inflammatory response and are genetically regulated in periodontal diseases. Strong association was found between the composite genotype allele 2 of IL-1β+3954 and IL-1α-889 and severe chronic periodontitis. The aim of this study is to determine the prevalence of IL-1β+3954 and IL-1α-889 polymorphism in a group of Lebanese individuals of homogeneous ethnicity and the possible association between genotype positive individuals and the severity of periodontal disease. One hundred and fifty-seven patients aged 53.29±13.13 years participated in the study. Subjects were classified as follows: 1) healthy subjects with no attachment loss >1mm and no clinical signs of gingival or periodontal inflammation; 2) diseased subjects with mild periodontitis (less than 15% of global periodontal bone loss); 3) subjects with moderate periodontitis (less than 4 interproximal sites with bone loss = or >50% and mean bone loss between 15 and 30%); 4) subjects with severe periodontitis (more than 7 interproximal sites with >50% bone loss and mean bone loss >35%). Blood samples were taken and analyzed for polymorphism in the IL-1α gene at position +4845 and in the IL-1β gene at position +3953. Statistical analysis was performed using *chi*-square test, Fisher Exact test, and ANOVA followed by Bonferroni multiple comparisons. The prevalence of genotype-positive subjects was 52.3% in the healthy control group and 42 % in the diseased group. Positive genotype heterozygous of allele 1 and 2 for IL-1β+3954 and IL-1α-889 did not represent in this study a major risk for chronic periodontitis (p=0.590). Only subjects homozygous for allele2 of the IL-1β+3954 and IL-1α-889 were significantly more at risk for severe periodontitis with OR of 51.42.

Periodontitis is a multi-factorial inflammatory disease initiated by oral bacteria, predominantly gram negative, which results in the loss of the supporting tooth structures and ultimately tooth loss. Chronic periodontitis is the most common clinical form of these diseases, affecting nearly 30% of the adult population. The prevalence of the severe form varies between 7% and 13% (1, 2). The large variations in the clinical appearance, distribution of the lesions and severity of the destructive process cannot be exclusively related to bacterial challenge be it qualitative or quantitative (3). Host modulating

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Mailing address:
Angelo Leone, DDS Sp. Ortho,
Department of Experimental Biomedicine and Clinical Neuroscience,
Medical and Dental School, University of Palermo,
90127 Palermo, Italy
Tel.: +39 091 6553581 Fax: +39 091 6553586

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factors seem to play an important role in disease progression. Pro-inflammatory cytokine, i.e., IL-1, TNF, are produced by many cells in response to noxious stimuli. They mediate the inflammatory response and are genetically regulated. Three interleukin-1 genes have been mapped on the long arm of chromosome 2q 13. The IL-1 gene cluster includes IL-1α, IL-1β and IL-RN genes that code for IL-1α, IL-1β and IL-1receptor antagonist. Large variations in IL-1 production have been found in a given population. Polymorphism of the host-response genes result in hyper secretion of specific cytokines in response to bacterial challenge. The genetic polymorphism of the IL-1 gene cluster was found to be associated with up to 4-fold increase in IL-1 production (4).

Polymorphism of the IL-1 gene cluster and its association with the severe form of chronic periodontitis was first investigated by Kornman (5). A strong association was found between the composite genotype allele 2 of IL-1β+3954 and IL-1α-889 and severe chronic periodontitis in non-smokers of North-European heritage. Adults carrying at least one allele2 of both IL-1β + 3954 and IL-1α-889 were considered genotype positive and were 6.8 times more likely to develop severe forms of periodontitis compared to individuals with early forms of the disease. The risk factor was found to vary between 4.7 (6) and 7.7 (7) depending on individual genetic predisposition and smoking habits.

Conversely, several studies reported no association between the composite genotype IL-1 gene and the severity of periodontitis. Rogers et al. (8) found no association between the severity of periodontitis and the presence of positive genotype in individuals of Australian Caucasians heritage. In African-Americans, Walker et al. (9) demonstrated that IL-1 genotype was not a predictor of susceptibility to localized aggressive periodontitis.

Several studies have shown large variations in the distribution of IL-1β and IL-1α composite genotype in different ethnic groups. In Caucasians of North European heritage, approximately 36% of the individuals were genotype-positive; Of the non-smokers aged 40-60 years in this population studied by Kornman (5) suffering from severe periodontitis, 65% were genotype + compared to 22% genotype + suffering from mild periodontitis. The risk of losing teeth was found to be 2.7 times higher for subjects with positive composite IL-1 genotype compared to genotype negative individual (5). Armitage et al. (10) found a low prevalence (2.3%) of periodontitis-associated interleukin-1 composite genotype in individuals of Chinese heritage. They suggested that IL-1 genotype could not be used to determine the susceptibility to periodontitis in this population. In a study on Chilean population, Lopez et al. (11) found that genotype + individuals, with at least one allele2 present at each locus, were more at risk of developing periodontitis. It has become clear that the prevalence of IL-1 polymorphism varies widely in populations of different ethnic origin and that the susceptibility to severe periodontitis in these individuals of different genetic heritage is also variable. Smoking habits increase significantly the risk of developing severe periodontitis and tooth loss. It has been shown that the risk factor increases to 7.7 times in genotype positive smokers compared to genotype negative subjects (11). The purpose of this study is to determine the prevalence of IL-1β+3954 and IL-1α-889 polymorphism in a group of Lebanese individuals of homogeneous ethnicity and the possible association between genotype positive individuals and the severity of chronic periodontitis.

MATERIALS AND METHODS

One hundred and fifty-seven adult subjects, 76 males and 81 females aged 25 to 83 years (mean age 53.29±13.13 years), were recruited from patients attending a private periodontal practice in Beirut Lebanon, and patients visiting the department of periodontology at St Joseph University, Beirut. The study was approved by the University Ethical committee and all subjects were informed about the purpose of the study before their participation; the study was also conducted in accordance with the Helsinki Declaration of 1975, as revised in 2000.

Patients were selected based on the following inclusion criteria:

1) Over 25 years of age

2) No history or current manifestation of systemic diseases including, immunosuppressive chemotherapy, HIV, current pregnancy or lactation, chronic use of anti-inflammatory drugs, history of diabetes or hepatitis. All subjects were Lebanese including both parents and grandparents. Smoking habits were recorded and subjects were classified as current smokers or non smokers.

Clinical assessment

All subjects received a full periodontal examination and periapical radiographs. Pocket depth and clinical attachment levels were recorded at six sites of a tooth using a Hu-Friedy calibrated probe. Bleeding on probing (BOP) was assessed at 4 sites on each tooth and BOP expressed as the percentage of sites showing bleeding. Plaque index was calculated using the Loe and Silness index. Subjects were classified as diseased or healthy based on their periodontal status. Controls were periodontally healthy subjects with no attachment loss >1mm and no clinical signs of gingival or periodontal inflammation. Diseased subjects were classified as mild, moderate or severe periodontitis based on the classification of Kornman (5):

1) Mild periodontitis: less than 15% of global periodontal bone loss.

2) Moderate periodontitis: less than 4 interproximal sites with bone loss = or >50% and mean bone loss between 15 and 30%.

3) Severe periodontitis: more than 7 interproximal sites with >50% bone loss and mean bone loss >35%.

Blood collection and DNA extraction

Two milliliters of peripheral blood were collected from each subject in EDTA vacutainer tubes. All tubes were given an identification number and the laboratory personnel responsible for genotyping did not know to which individual the sample belonged. Subjects were considered positive when they carried at least one allele 2 of both IL-β+3954 and IL-α-889. This composite genotype was suggested by Kornman (5) to be a susceptibility factor for severe chronic periodontitis. Red blood cells were lysed and genomic DNA was extracted from white blood cells using a DNA extraction kit (GFX DNA extraction kit, Amersham Pharmacia, Piscataway, NJ, USA) following the manufacturer's protocols. Samples were analyzed for polymorphism in the IL-1α gene at position +4845 and in the IL-1β gene at position +3953 following the protocol described by Engebretson et al. (12). Briefly, a 229 bp fragment of the IL-1α gene was amplified by PCR using the specified primers and amplification conditions. The PCR product was then digested with Fnu4HI. Allele 1 gave rise to a 29, a 76 and 124 bp fragments while allele 2 was identified by the resulting restriction fragments of 153 and 76 bp on agarose gel electrophoresis. A similar approach was used to identify IL-1β PCR products. In this case the 194 bp PCR product was digested using *Taq1* restriction enzyme. Allele 1 was digested into three fragments 97, 85 and 12 bp long and allele 2 resulted in two fragments 182 and 12 bp long.

Data analysis

The statistical analysis was performed using a software program (SPSS for windows version 16.0). The α error was set at 0.05, and a significance of 5% or less was considered statistically significant. Frequencies, means and standard deviations, Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. *Chi*-square test, Fisher Exact test, and ANOVA followed by Bonferroni multiple comparisons were conducted to find any statistically significant differences between the composition of each of the four groups of enrolled subjects (healthy controls, mild, moderate and advanced periodontitis). Multinomial logistic regression was applied to check for any significant association between, positive genotype, and each of the three periodontitis categories (mild, moderate and advanced) compared to the healthy controls; adjustment for age, gender and smoking status was carried out. Logistic regression was applied to check for any significant association between interleukin-1, and each of the presence or absence or periodontitis; adjustment for age, gender and smoking status was carried out.

RESULTS

Of the 157 subjects enrolled in the study, 44(28%) were diagnosed as healthy and 113(72%) as suffering from various clinical forms of chronic periodontitis: 19(12%) were diagnosed as early periodontitis, 48(31%) as moderate periodontitis and 46(29%) as severe generalized periodontitis (Fig. 1.) Subjects belonged to different socioeconomic class ranging from high to middle-low. The age ranged between 25 and 83 with a mean of 53.29±13.13 years. Healthy controls were significantly younger than all the periodontitis patients. There was no significant

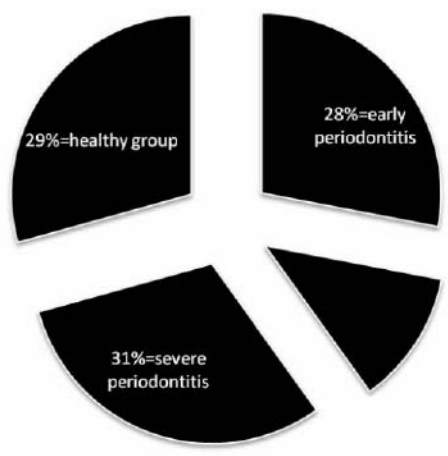

Fig. 1. *Distribution according to periodontal status.*

Table I. *Characteristics of the 4 groups of patients.*

	All patients (n=157)	**Healthy Controls (n=43)**	**Mild periodontitis (n=21)**	**Moderate periodontitis (n=49)**	**Advanced periodontitis (n=46)**	**Sig.**
Age (mean ± SD)	51.5±13.3	39.00±9.04	58.32±12.30	60.52±9.71	57.35±8.82	<0.001
Gender (% men)	76(48.4%)	23(52.3%)	10(52.6%)	24(50.0%)	19(41.3%)	0.711
Current smoker (% yes)	52(33.3%)	8(18.2%)	3(15.8%)	17(35.4%)	24(53.3%)	0.002
IL-1 (% positive)	91(58.0%)	27(61.4%)	10(52.6%)	29(60.4%)	25(54.3%)	0.850

Table II. *Distribution (%) of composite genotype of IL-1β+3954 and IL-1α-889 in each group of subjects.*

	Healthy subjects	Diseased subjects	Total
ILα = 1:1 ; ILβ = 1:1	14 (31.8%)	42 (37.2%)	56 (35.7%)
ILα = 1:1 ; ILβ = 1:2	2 (4.5%)	3 (2.7%)	5 (3.2%)
ILα = 1:2 ; ILβ = 1:1	1 (2.3%)	4 (3.5%)	5 (3.2%)
ILα = 1:2 ; ILβ = 1:2	23 (52.3%)	43 (38.1%)	66 (42.0%)
ILα = 1:2 ; ILβ = 2:2	1 (2.3%)	1 (.9%)	2 (1.3%)
ILα = 2:2 ; ILβ = 1:2	1 (2.3%)	2 (1.8%)	3 (1.9%)
ILα = 2:2 ; ILβ= 2:1	1 (2.3%)	0 (.0%)	1 (.6%)
ILα = 2:2 ; ILβ = 2:2	1 (2.3%)	18 (15.9%)	19 (12.1%)
Total	44(100%)	113(100%)	157

difference in the age distribution in the periodontitis group. When the mean age was compared in the four different groups, it was found significantly lower in the healthy group (39±9.03 years) compared to the mild (58.32y±12.29), moderate (60.52±9.71) and advanced group (57.35±13.13) (p<0.0001). In the periodontitis groups, the mean age was not significantly different (p=0.528) (Table I).

Distribution of composite IL-1 genotype and smoking

33.3% of the subjects were smokers and 66.7% non-smokers. The frequency of smokers in the different groups is reported in Fig. 2. The percentage of current smokers is significantly greater in the

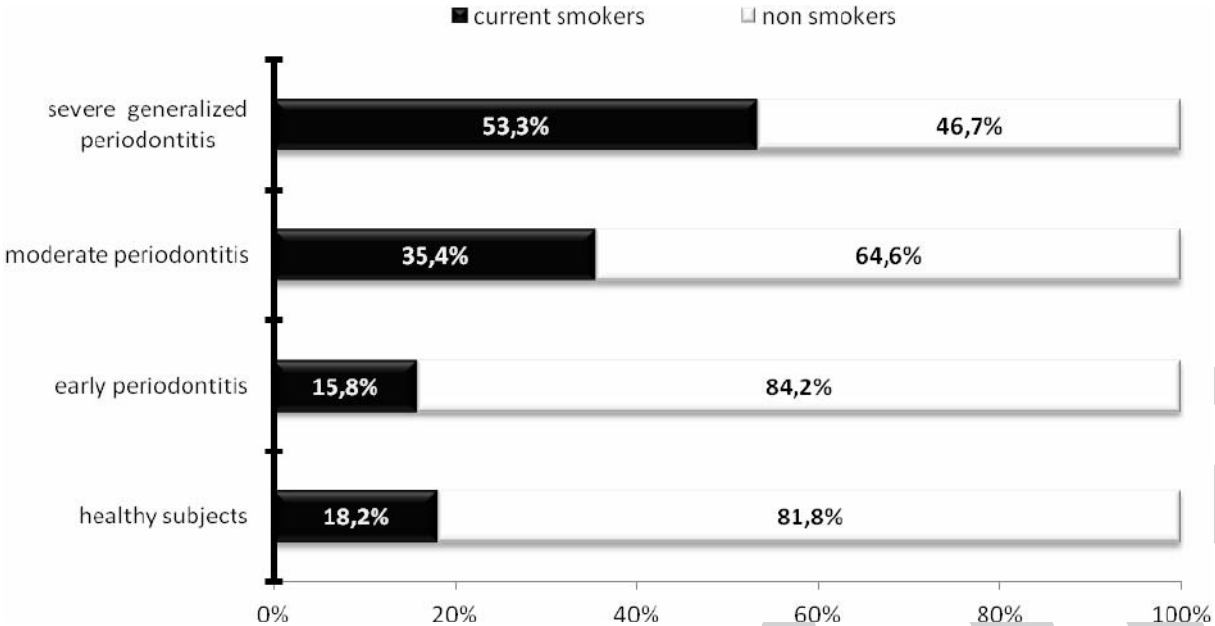

Fig. 2. *Distribution of smokers/non-smokers in the four studied groups.*

severely diseased group compared to the healthy individuals and those suffering from the early form of periodontitis (p=0.002). However, when the periodontal parameters were compared between the healthy individuals and the diseased group, smoking represented a risk factor to periodontitis with a borderline significant value (p-value = 0.056). Smokers have 3.9 times greater risk to develop periodontitis compared to healthy individuals.

Prevalence of IL-1α-889 and IL-1β+3954 genotypes and alleles

The distribution of IL-1β+3954 and IL-1α-889 genotypes in each group is shown in Fig. 3. 38.6% of the healthy controls were genotype negative compared to 43.4% in the diseased group. The genotype –positive healthy controls were 61.4% compared to 56.6% in the diseased group. There was no significant difference between the healthy and the diseased group when the genotype was dichotomized between positive and negative (p=0.590). The distribution of IL-1β+3954 and IL-1α-889 according to their homozygous or heterozygous status of allele 1 and 2 is shown in Table II. 31.8% of the genotype-negative subjects were homozygous allele 1 and 6.8% were heterozygous allele 1 in the healthy group compared to 37.2% and 6.2% in the diseased group. 56.9% were heterozygous for allele 1 and 2 and 2.3 % were homozygous allele 2 in the healthy group compared to 40.8% and 15.9% in the diseased group. The power to detect significant difference between the healthy and the diseased group was 30.6% given the size of the sample.

When the diseased group was separated into three subgroups of early, moderate and advanced, no significant difference for the genotype + or - for IL-1α-889 and IL-1β+3954 was found between the subgroups of diseased individuals and the healthy controls (p=0.850 *chi* square test) (Fig. 4). This study did not determine an association between the genotype and the different forms of periodontitis. When the homozygous group was compared to the heterozygous group of genotype positive subjects, the homozygous group was significantly more prevalent in the diseased group (18 subjects) compared to the healthy individuals (1 subject) (p=0.047). Among the diseased individuals, 1 individual with early form of periodontitis presented a homozygous status compared to 17 with moderate and advanced periodontitis (Table II).

Table III. *Factors associated with periodontitis.*

	-p-value	OR	95.0% C.I. of OR	
			Min	Max
Age	0.000	1.302	1.183	1.434
Gender	0.699	1.291	0.354	4.711
Current smokers	0.056	3.941	0.966	16.081
IL-1α (2 :2) IL-1β (2:2)	0.013	51.420	2.310	1145

Factors associated with periodontitis (multiple logistic regression analysis)

In the present study, several factors were investigated for their relation with chronic periodontitis. Age was associated with periodontitis independently from tobacco consumption and genotype ($p<0.0001$). Older subjects were 1.3 times more at risk for developing periodontitis compared to younger individuals (OR=1.302). Gender did not represent a risk factor for periodontitis ($p=0.699$). Positive genotype in its heterozygous form of allele 1 and 2 for IL-1β+3954 and IL-1α-889 did not represent in this study a major risk for periodontitis. However, when the different types of polymorphism were closely analyzed, those subjects with a homozygous IL-1α (2:2) and IL-1β (2:2) status were significantly more at risk to develop disease ($p=0.013$). The odds ratio (OR) in this case was estimated to be 51.42 (Table III).

DISCUSSION

The association between a specific composite genotype of IL-1β+3954 and IL-1α-889 polymorphism and various forms of periodontitis was variously reported in the literature. Since the first study of Kornman (5) that showed an increased risk for severe chronic periodontitis in a non-smoker genotype-positive Caucasian group aged 40 to 60 years, numerous studies reported the prevalence of this composite genotype in different ethnic groups and its importance as a risk factor for chronic or aggressive periodontitis. Although most of these studies reported a significant difference in the prevalence of the composite genotype between the healthy and the diseased group (12), only one case-controlled study on subjects of Maharashtrian ethnicity found no single healthy subject positive for the composite genotype compared to 30% of subjects positive for the same genotype in the severe periodontitis group (14).

In a study on the association between the IL-1 polymorphism and the severity of periodontal diseases in a Chilean population (11), the most frequent composite genotype was the homozygous for allele 1 (1-1,1-1) with no significant differences between the cases and the controls while the prevalence of the heterozygous phenotype (1-2, 1-2) was significantly higher in cases (26.06%) than in controls (9.90%) and was strongly associated with periodontitis (OR=4.09). The distribution of the other composite genotypes showed no significant differences between cases and controls. The homozygous form of allele 2 (2-2,2-2) was not found in the healthy group and in only one case of the diseased group. In the current study, the most frequent composite genotype was ,conversely, the heterozygous group(1-2,1-2) and was found in 42% of all cases with a significantly higher prevalence in the healthy group (52.3%) compared to the diseased group (38.1%).The homozygous genotype (1-1,1-1) was found in 35.7% of all cases with a slightly higher prevalence in the diseased (37.2%) compared to healthy controls (31.8%). The homozygous form of allele 2 was found in 12.1% of all cases with significantly higher prevalence in the diseased group (15.9%) compared to the healthy group (2.3%),a finding that is unusual among the studies reported in the literature. 38.6% of the healthy controls were genotype negative compared to 43.4% in the

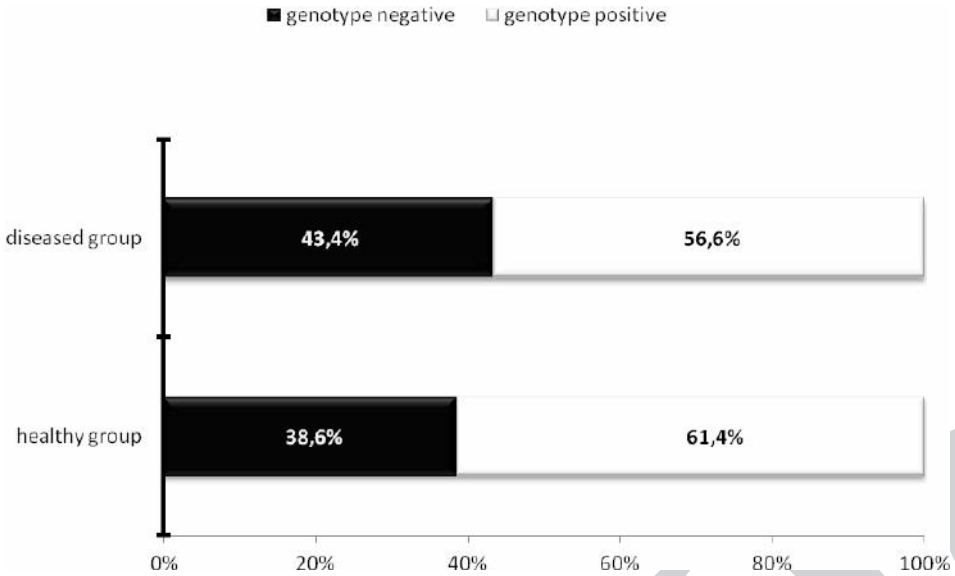

Fig. 3. *Distribution of genotype positive and negative in the healthy and diseased groups.*

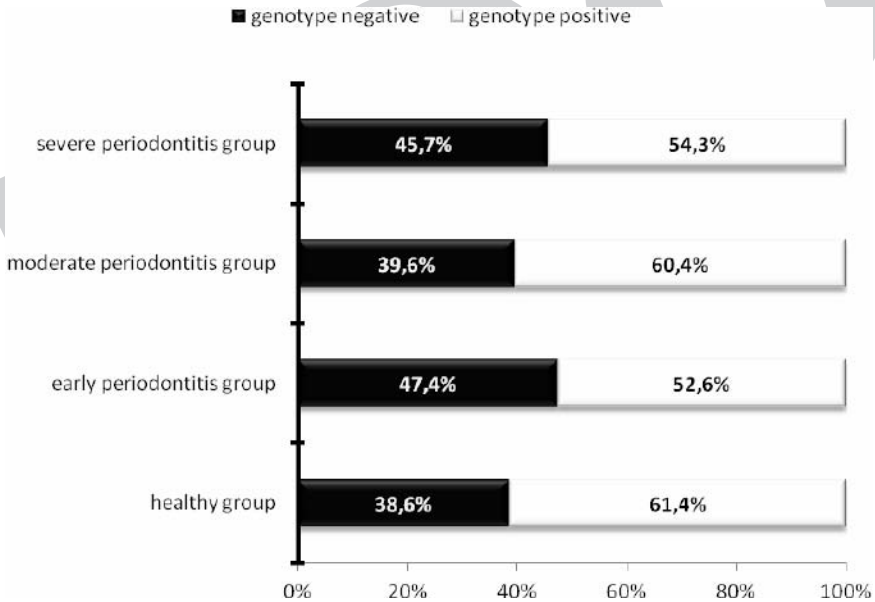

Fig. 4. *Distribution of the genotype + and - individuals in the four studied groups.*

diseased group. The heterozygous (1,2.1,2/1.2,2.1/2.2,2.1/2.2,1.2) genotype positive healthy controls were 59.1% compared to 40.7% in the diseased group, with a dominance of allele 1 (52.3% for the healthy controls compared to 42% for the diseased group) and a low prevalence of allele 2 carriers (7.2% in the healthy group compared to 3.3% in the diseased group) indicating a high prevalence of IL-1 genetic polymorphism in the Lebanese population. However, positive composite genotype for IL-1β+3954 and IL-1α-889 in its heterozygous form of allele 1 and 2 did not represent in this study a major risk for chronic periodontitis. Only subjects with a composite homozygous IL-1α-889 (2:2) and

IL-1β+3954 (2:2) status were significantly more at risk to develop disease (p=0.013). The OR in this case was estimated to be 51.42. The differences in the various form of polymorphisms reported in the current study may be attributed to ethnic variations in the Lebanese population .However, the size of the population may not be sufficient to reach robust conclusions.

A significant association was detected between allele 2 of the IL-1β+3954 and the severity of chronic periodontitis but not with IL-1α-889 or the composite IL-1β+3954 and IL-1α-889 by Laine and co-workers (15). A significant association was found between allele2 of the composite genotype of IL-1β+3954/IL-1α-889/IL-1RN and severe periodontitis in non-smokers but not with the composite genotype of IL-1β+3954 /IL-1α-889. It has been shown that monocytes and polymorphonucleocytes (PMN) from subjects with allele 2 of IL-1β+3954 produce significantly more IL-1β compared to allele 1 carrier (6). Also, Shirodaria et al. (16) showed that patients suffering from severe periodontitis and carrying allele 2 of IL-1α-889 presented a four-fold increase in IL-1α production in their crevicular fluid compared to allele1 carriers. Thus, genetic loci of the polymorphism whether on allele1 or 2 or on IL-1α or IL-1β seem to play an important role in regulating the inflammatory response, and consequently in modulating periodontal breakdown. These polymorphisms vary widely with the populations studied and their genetic heritage.

In fact, a low prevalence of the genotype positive individuals was found in a Chinese population (2.3%) (10) compared to Caucasians (36%) (5). Likewise, a low prevalence of genotype-positive individuals was found in a Thai population (1.6%) (17). The majority of these subjects presented homozygous allele 1 of the IL-1β+3954 and the IL-1α-889 with no subjects presenting homozygous allele 2 at these loci. No allele 2 homozygous for IL-1β+3954 and IL-1α-889 were found in Japanese (18) or in Chinese subjects (10). This clearly means that these polymorphisms may not be adequate to predict the severity of periodontal disease in the Asian population. In a group of African-American aggressive periodontitis patients, 8% of the diseased individuals were tested positive compared to 14.5% in the control group. None was found homozygous for both IL-1α-889 and IL-1β+3954 gene (2,2/2,2). It has been suggested that the composite (1,2/1,2) genotype may be "protective" based on its lower prevalence in the diseased group compared to the healthy controls (9), a finding that is in accordance with our results and in conflict with the results reported by Lopez et al. (11).

In the current study, age was significantly correlated with periodontal disease severity. (OR=1.30.p<0.0001). This finding was similarly reported by Mc Devitt (19), with an OR of 1.26.

Smoking was found to be a significant confounding factor in relation to the severity of periodontitis (20). Smoking status, whether current or former, the age at which smoking was started, the dose smoked measured in terms of packs/year, the frequency and duration of smoking and smoking cessation have been variously investigated in the reported studies. Smoking was found to be associated with an increased risk of attachment loss independent of the IL-1 genotype. The genotype was only found to be a risk factor in smokers, the two factors acting synergistically with a risk of 4.5 and 2.4 in genotype-positive and genotype-negative smokers [21]. IL-1 negative former moderate smokers were found to be at increased odds of 7.43 to have moderate to severe periodontitis compared to IL-1 negative non-smokers or former light smokers [19], thus underlying the importance of smoking on disease progression and the possibility of this factor, when present, to mask other important risk factors. In our study, a current smoking habit was the only parameter that was accounted for. This could be considered a limitation in view of the results that showed that smoking represented a risk factor to periodontitis with only a borderline significant value (p=0.056). However, it is interesting to note that the percentage of current smokers was significantly greater in the severely diseased group compared to the healthy individuals and those suffering from the early form of periodontitis (p=0.002).

In conclusion, the prevalence of genotype-positive subjects in the Lebanese population was high with 52.3% of the healthy controls and 42% of the diseased group showing a positive composite IL-1β+3954/IL-1α-889. However no association could be found between this genetic polymorphism in its heterozygous form and the severity of chronic periodontitis (p=0.590). Only subjects homozygous

for allele2 of the IL-1β+3954 and IL-1α-889 were significantly more at risk for severe periodontitis with OR of 51.42.

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