Astudy Relationship between IL 1β (+ 3954) Gene Polymorphism with Moderate and Sever Chronic Periodontitis Among Sample of Iraqians Population In Babylon Province.

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Abstract

Intoduction*:* The cytokine is aprimary activator of early chemotactic cytokines, as well as of expression of adhesion molecules that facilitate migration of leukocytes into tissues. IL-1q is also one of the most active stimulators of osteoclast cells , and lead to bone resorption. The aim of this study was to determined the prevalence of the IL 1 β (+3954) gene polymorphism in a sample of Iraqi patients with chronic periodontitis in Babylon Province and to evaluate the association of this polymorphism with the severity of disease .

Methods *:* Seventy four systemically healthy patients attending the Department of Periodontics of Faculty of Dentistry, Babylon University, Babylon Province, Iraq. Patients were classified into two groups: subjects with chronic periodontitis group (CP) (n=40) and healthy control group (C) (n=34). This study desiged a cross sectional study. Genomic DNA was obtained from venous blood(5 ml) and amplified using the Polymerase Chain Reaction (PCR) with specific primers flanking the locus +3954 of IL 1β. PCR products were submitted to restriction endonuclease digestion and analyzed by polyacrylamide gel electrophoresis, to distinguish alleles T and C of the IL 1 β gene, allowing for the determination of the genotypes and detection of the polymorphism.

Results*:* The homozygous genotype CC of the IL -1β(+3954) was dominated in control (C)group than in Chronic periodontitis (CP) group.

The heterozygous genotype CT significantly higher in Chronic periodontitis group than in control group,(*P* =0.038; OR 4.125).

The Prevalence of the TT genotype was dominated in chronic periodontitis (CP)group than in control (C)group,and statistically insignificant value was obtained when compared between these two groups(*P* =0.27). We observed that,individuals with sever chronic periodontitis group displayed high frequency of T allele(47.6 %) as compared to individuals with moderate chronic periodontitis group, at the same time no significant difference was observed between them.

The chronic periodontitis group displayed a higher percentage of the T allele (45%) when compared with control group(29.4%),and statistical insignificant in allele distribution was observed between these two groups.

Conclusion:- Our data suggested that the polymorphism in the locus +3954 of IL1 β gene could be related to chronic periodontitis in Iraqi population.

Key words: Gene polymorphism , interleukin 1 β, , chronic periodontitis, allele.

الخلاصه

يعتبر الانترلوكين 1 بيتا من اهم السايتوكينات التي تلعب دور فاعل في تحفيز الالتهابات المزمنه في الجسم ,ويعتبر عامل اولي في تفعيل جذب كرات الدم المتعددة النواة الى النسيج بالاضافه الى تحفيز عمل الخلايه المذيبه للعظم. الهدف من هذه الدراسه هو لمعرفة مدى ارتباط او علاقة التعدد الشكلي للانترلوكينون1 بيتا (3945) لمرضى التهاب اللثه بين عينه من العراقيين في محافظة بابل من المصابين بامراض اللثه المزمن العام.و مقارنته النتيجه مع مجموعه اخرى غير مصابين بالتهاب اللثه المزمن.مع بيان مدى تاثير هذا المورث مع شدة التهاب اللثه المزمن.اظهرت النتائج بان المورث او الجين الانترلوكين 1 بيتا المتعدد الاشكال (3945) يشكل خطر كبير على التهاب اللثه المزمن الشديد جدا.اي انه يلعب دور في شدة التهاب امراض اللثه المزمن.

الكلمات المفتاحية: المورث المتعدد الاشكال,انترلوكين 1 بيتا,التهاب اللثه المزمن,اليل.

Introduction

Periodontal disease is defined as inflammatory destruction of periodontal tissue and alveolar bone supporting the teeth. Severe and prolonged periodontal inflammation leads to loss of teeth, thereby affecting oral functions (e.g., mastication, speech and facial esthetics) (Nunn, 2003).Bacterial pathogens are the primary etiologic factors in the initiation of periodontitis (Haffajee & Socransky, 1994).In addition to environmental factors are believed to initiate and modulate periodontal disease progression, there now exists strong supporting data that genetic and environmental risk factors play a role in the progression of periodontal diseases.(Kornman, 1997; Stabholz *et al* 2010, Nibali *et al* 2008)

Since 1997, when professor Korn man observed 19-fold higher risk of observing bone loss in non smoking genotype-positive patients when compared to genotype-negative (Kornman *et al.*, 1997), numerous papers confirmed the influence of IL-1 polymorphism on the destruction and outcome of periodontal disease (Sujata *et al*.2012; Archana *et al*., 2012; Persson *et al*., 2003; Meisel *et al*., 2001; Meisel *et al*., 2003; Faizuddin *et al.*, 2003; Kowalski *et al*.2006, Armingohar *et al*., 2014 and; Magali *et al.*, 2016].

IL-1 is a pro inflammatory cytokine that plays a pivotal role in several chronic diseases. This cytokine is a primary activator of early chemotactic cytokines, as well as of expression of adhesion molecules that facilitate leukocytes migration into tissues. IL-1 is also known to be one of the most active stimulators of osteoclastic bone resorption (Lang *et al* ., 2000).

Rare allele of IL-1 appears to develop a susceptible phenotype , in which during inflammatory response macrophages secrete greater amounts of this cytokine, hich effect is an increased inflammatory response.These results in greater damage of periodontal tissues. In vitro studies there seemed to be a straight Mendelian correlation between IL-1 genotype and the amount of cytokine secreted by cultured macrophages (di Giovine FS *et al*. 1997)

The IL-1 family containe three homologous proteins; interleukin-1 α and interleukin- 1β, which are pro -inflammatory proteins, and interleukin-1ra , an antagonist protein. These proteins are encoded on chromosome 2q13 -21 and are polymorphic at several loci. ((Nicklin *et al*., 1994).

Single nucleotide polymorphisms(SNP) in the interleukin-1 locus, their functional consequences, and their association with susceptibility to and severity of various chronic inflammatory diseases have been described in the literature. (Greenstein & Hart , 2002)

Some reports found that polymorphisms in the IL -1 gene cluster may influence the variations in the synthesis of cytokines, and thus modify the individual responses to bacterial stimuli .With regard to the interleukin-1 polymorphism, it has

been suggested that a haplotype comprising at least one single nucleotide polymorphism in each of the genes encoding the interleukins IL -1α and IL-1β increases the susceptibility for periodontal diseases. (Kornman *et al.*, 1997)

Polymorphism in the +3954 locus (due to nomenclature change, the polymorphism at IL 1β +3953 is now referred to as IL 51β +3954 (Kornman *et al*., 1998)), of the IL 1β gene has been associated with an increased production of this cytokine. The T allele Homozygous individuals for produce a four -fold higher amount of IL-1 β compared to individuals displaying the CC genotype (Walker *et al*., 2000). It has recently been suggested that this polymorphism may explain why some people have a more sever response than others to the same stimulus (Lang *et al.*, 2000)

Several studies have evaluated gene polymorphisms in individuals with periodontitis in distinct populations. Kornman *et al*. found that the occurrence of IL 1α
(–889) and IL 1 β (+3954) polymorphisms was associated with a severity of chronic periodontitis in non-smoker Caucasians. (Kornman *et al.*, 1997), Walker et al observed a high prevalence of IL1β (+ 3954) allele C in the African American population and concluded that this polymorphism would provide little diagnostic or predictive information for periodontal diseases and specifically for localized aggressive periodontitis. (Pociot *et al*., 1992).

Moreira *et al* found that a high percentage of the T allele among Brazilian individuals with chronic periodontitis when compared with aggressive periodontitis, and suggest that the polymorphism in the locus +3954 of IL 1β gene could be a risk factor for chronic periodontitis. (Moreira *et al.*, 2005).In India Kaarthikeyan *et al* concluded, that there was insignificant association between IL 1β (+3954) gene polymorphism and chronic periodontitis in the south Indian population. (Kaarthikeyan *et al.*, 2009). In India also Prakash P & Victor D. found in their study that a high percentage of the T allele among chronic periodontitis, and suggest that the polymorphism in the locus +3954 of IL 1 β gene could be a risk factor for chronic periodontitis in south Indian population. (Prakash, 2010)

Offenbacher *et al.*, found in his study of chronic periodontitis cases &controls from four ethnic group (Caucasians, African Americans, Hispanic and Asians) were recruited in USA ,Chile and China and supported the association between IL -1 genotype pattern and moderate to sever periodontitis .(Wu X. *et al*., 2015)

The aim of this study was to determined the prevalence of the IL1β (+3954) gene polymorphism in a sample of Iraqi patients in Babylon Province with chronic periodontitis and to evaluate the association of this polymorphism with the severity of disease.

Materials and Methods

Selection of Subjects

Seventy four systemically healthy patients attending the department of Periodontics of Faculty of Dentistry ,Babylon University, Babylon city, Iraq, of both sexes, between April 2015 and September 2015. The aim of the study was explained to each patient before the study. The patients were classified into two groups: subjects with chronic periodontitis group (CP) (n=40) and healthy control group (C)(n=34). According to severity of periodontitis by stratifying the groups according to clinical attachment loss,Chronic periodontitis group were

classified int moderate chronic periodontitis group and sever chronic periodontitis group.

Patients in the chronic periodontitis group were 26 – 62 years old and exhibited inflammation, loss of clinical attachment due to break down of periodontal ligament and loss of the adjacent supporting bone . All patients in the chronic periodontitis group had at least three teeth exhibiting sites of clinical attachment loss in at least two different quadrants.

Diagnosis of disease was made considering the patient's medical and dental histories, radiographic findings and observations of clinical signs and parameters, including probing depth, assessment of clinical attachment loss ,bleeding on probing and presence of plaque .

Measurements of probing depth and attachment level were assessed at six sites per tooth from the test sites (distofacial , mid- facial, mesiofacial , mesiolingual, distolingual and mid-lingual) with a periodontal Williams probe calibrated in millimeters by the same clinician. The severity of disease was characterized on the basis of the amount of clinical attachment loss , within each clinical form. Patients exhibiting clinical attachment loss≥ 5mm were considered with severe and those exhibiting clinical attachment loss ≥ 3mm to <5 mm were considered with moderate periodontitis.

Clinical diagnosis and determination of disease severity were based on criteria established in 1999 at the International Workshop for Classification of Periodontal Diseases and Conditions (Armitage , 1999).

The Control individuals included in the study were 21 – 44 years old and did not have disease at the time of sample collection and also did not present history of previous periodontal disease ; as determined by absence of clinical attachment loss and no sites with probing depth > 3 mm.

A questionnaire was applied to all individuals enrolled in this study , in order to obtain information regarding dental history, family history of periodontal disease, smoking habit, as well as general health concerns .Use of orthodontic appliances, chronic usage of anti -inflammatory drugs, history of diabetes, bleeding disorders, pregnancy or lactation and postmenopausal women and smokers were regarded as exclusion criteria.

Collection of blood samples

A 5 mL volume of venous blood was collected from the cubital fossa of each subject . These blood samples were sent to the laboratory in a tube containing ethylene diamine tetra acetic acid(EDTA) for DNA extraction was performed as described by Boom *et al*., 1990,by using the kite(Favorgen Biotech Corp.), then DNA Amplification by polymerase chain reaction(PCR).

DNA amplification by polymerase chain reaction

The sequences of PCR primers were Forward : 5´-CT CAG GTGTCC TCGAAGAA ATCAAA-3´ and Reverse:5´-G CTTTTTTGCTGTGAGTCCCG3´- with expected PCR product size of 194 bp, as described previously (Kornman *et al*., 1997). The horizontal gel electrophoresis system was employed for the detection of total DNA . Gels were stained with ethidium bromide (0.5 ug/ml) ,and DNA was visualized under UV (ultraviolety) light. The products were digested with 5 U of Taq I(Restriction enzyme) at 65°C for 4 h and obtained 97+ 85 + 12 bp DNA products for allele C and 182+ 12 bp DNA products for allele T. The visualization was performed in a 110 % polyacrylamide gel electrophoresis .

Statistical analysis

Statistical analysis was performed using the SPSS version 22 software ,to determine the mean & standard deviation for periodontal parameters in addition ,unpaired *t* tests were used to compare differences in scores and measurements in clinical parameters. Genotype and allele frequencies of IL 1B (+53954) gene polymorphism were compared between control and chronic periodontitis groups using Fisher exact test. Odd ratio with 95 % confidence intervals (CI) in 2×2 comparisons were calculated to determine the strength of the association. A *p*-value < 0.05 was considered significant .

Results

The characteristics of the study groups are mentioned in table 1.The values of the plaque index were compared between Control group(C) and chronic period-ontitis group (CP) by using unpaired Student *t* test, a value of 2.58 was obtained, this difference is considered to be statistically significant.

The mean values & standard deviation of %BOP for group C and CP were 6.06±9.37,38.26±32.09 respectively ,on comparison between groups we obtained an extremely statistically significant value 5.64(*P* value is less than 0.0001) .

The mean values & standard deviation of PPD for group C was1.9±0.58 and CP was, 4.8±1.15, on comparison between groups we obtained an extremely statistically significant value 13.32(*P* value is less than 0.0001)

Table 1. The characteristics of study groups.

Clinical Parameters	C	CP	t	P value*
Sex(M/F)	(30/10)	(10/24)		
Age range(years) (mean±SD)	25.8±7.52	42.48±9.51		
PI(mean±SD)	0.88±0.69	1.43±1.07	2.58	0.012
%BOB	6.06±9.37	38.26±32.09	5.64	< 0.0001
PPD(mm) (mean±SD)	1.9±0.58	4.8±1.15	13.32	< 0.0001
CAL(mm) (mean±SD)	0	5.14±1.70		

*Determined by the unpaired *t* test

The homozygous genotype CC of the IL -1β + 3954 was dominated in control (C)group than in Chronic periodontitis (CP) group [Table 2.]. The heterozygous genotype CT significantly higher Chronic periodontitis group than in control group,(*P* =0.038; OR 4.125). The Prevalence of the TT genotype was dominated in Chronic periodontitis (CP) group than in control (C) group, and statistically insignificant value was obtained when compared between these two groups(*P* =0.27).

Table 2.Distribution of IL - 1β(3954) in study groups.

Genotype or allele IL-1β Genotype	Control No.(%)	Chronicqperiodontitis No.(%)	*P* Value*	OR	CI
CC(%)	22(64.7)	16(40)			
CT(%)	4(11.8)	12(30)	0.038	4.1250	1.1219-15.1664
TT(%)	8(23.5)	12(30)	0.27	2.0625	0.6850-6.2103
Allele					
C(%)	48(70.6)	44(55)			
T(%)	20(29.4)	36(45)	0.06	1.9636	0.9921- 3.8866

*Determined by Fisher exact test.
OR, odd ratio, CI, confidence interval.

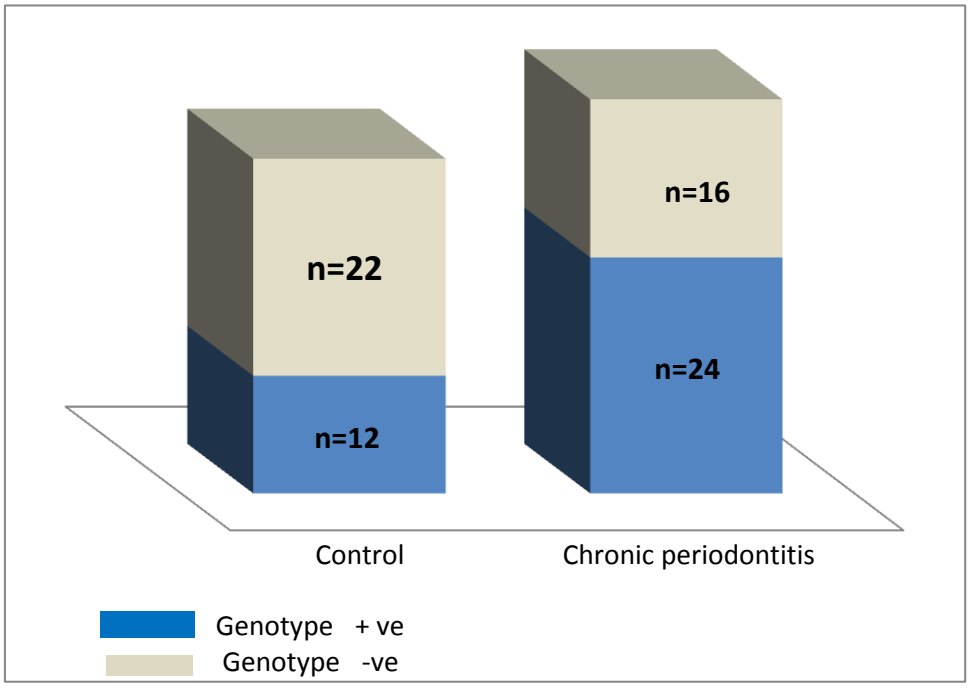

Figure 1 Analysis of T+ and T- genotype in the study groups.(T allele)

The presence of T allele in a group was referred to T+ (genotype positive) and the absence of T allele was referred to as T- (genotype negative).The chronic periodontitis group displayed a higher percentage of the T allele (45%) when compared with control group (29.4%), and statistical insignificant in allele distribution was observed between these two groups. The analysis was made by utilizing the Fisher exact test (P =0.06). [Figure 1].

When we evaluated the severity of periodontitis by stratifying the groups according to clinical attachment loss (Table 3), we observed that individuals with sever chronic periodontitis group displayed high frequenc of T allele (47.6%) as compared to individuals with moderate chronic periodontitis group, at the same time no significant difference was observed between them. The homozygous genotype CC of the IL 1β + 3954 was dominated in sever chronic periodontitis group than in moderate chronic periodontitis group .

The heterozygous genotype CT insignificantly higher in moderate chronic periodontitis group than in sever chronic periodontitis group, (P =0.3).

The Prevalence of the TT genotype waodominated in sever qchronicq periodontitiq group than in moderat chronic periodontitis group, and statistically insignificant value was obtained when compared between these two groups (P =0.7).

Table 3.Distribution of IL- 1β(3954) in study groups, considering the severity of disease .

IL-1β Genotype	Chronic periodontitis		*P* Value*	OR	CI
	Moderate No.(%)	Sever No.(%)			
CC(%)	7 (36.8)	9 (42.86)			
CT(%)	8 (42.1)	4 (19.05)	0.3	0.3889	0.0822 to 1.8404
TT(%)	4 (21.1)	8 (38.09)	0.7	1.5556	0.3287 to 7.3615
Allele					
C(%)	22(57.9)	22(52.4)			
T(%)	16(42.1)	20(47.6)	0.66	1.2500	0.5164-3.0256

Patients exhibiting clinical attachment loss ≥ 5mmqwere considered with severe and those exhibiting clinical attachment loss ≥ 3mm to < 5 mm were considered with moderate periodontitis .

*Determined by Fisher exact test.

OR, odd ratio, CI, confidence interval

Discussion

From our reviewing of literatures, we concluded that , there's no previous study that determined the prevalence of the IL 1β (+3954) gene polymorphism in a sample of Iraqi patients with different classification of chronic periodontitis.

It is widely accepted that genetic factors may predispose to periodontal diseases ,several studies had been conducted to determined whether IL-1β (3954) gene polymorphysim predispose to periodontitis .The frequency of many geneti alleles varies between ethnic groups and several studies have found contradictory results when comparing among distinct populations. (Greenstein & Hart , 2002)

Thus, the analysis of gene polymorphysims in a sample of the Iraqi population in Babylon province represents an important in the study of periodontal disease in Iraq. In our study ,the subjects which included were from Babylon provence only, and recruited from periodontal department, the predictability of the genetic test is of no practical use.

The mean age of the chronic periodontitis group was slightly higher than control group. In many previous studies, age matching between patients and controls have not been more important because the genetic patterns don't changed with age.

The homozygous (CC) genotype of IL-1β(3954) was the most frequent genotype in control subjects than in chronic periodontitis. Whereas the heterozygous (CT) genotype dominated in chronic periodontitis than in controls subjects and statistically significant value was obtain when compared between these two groups (*P*-value =20.03 and odd ratio= 4.1250). This finding is in accordance with results were obtained by study conducted in Brazil (Moreira *et al* 2005; Samuel *et al.,* 2008 and Magali *et al.,* 2016), in Chile (López *et al*., 2005),in India (Sujata *et al*., 2012, Kaarthikeyan *et al.,* 2009& Prakash and D.J. 2010). However , contradictory results were obtained by study conducted in India (Gayathri *et al.,* 2011), Where they have found that CC genotype was the more frequently found in chronic periodontitis than inqcontrolqgroup. In our study The homozygousy genotype CC of the IL-1β + 3954 was dominated in sever chronic periodontitis group than in moderate chronic periodontitis group, (*P*-value =0.271).

The prevalence of TT genotype of the IL -1β(3954) was higher in the chronic periodontitis (30%) than in control group (23.5%).This results observed in various studies conducted in Brazil (Moreira *et al.,* 2005 & Gore *et al.,* 1998 and Magali *et al.,* 2016) in addition to studies in India (Sujata *et al*., 2012; Prakash and DJ 2010& Gayathri *et al.,* 2011). We found in this study, the prevalence of the TT genotype was dominated in sever chronic periodontitis group than in moderate chronic periodontitis group , and statistically insignificant value was obtained when compared between these two groups (*P*-value = 0.48).

The chronic periodontitis group displayed a higher percentage of the Tallele (45%) when compared with control group(29.4%),and statistical insignificant in allele distribution was observed between these two groups .

In chronic periodontitis group 45% of subjects were T+ and 55% were T-. In control group individuals the percentage of frequently of T+ were 29.4% and T- were 70.6%.When comparing between study groups,the frequency of T+ subject were insignificantly higher in chronic periodontitis group than in control group,

our study results matched with studies**,** where an observed association of IL -1 β + 3954 with chronic periodontitis in Brazilian (Moreira *et al.,* 2005;

Magali *et al.*, 2016) and Indian population (Prakash and D.J. 2010 & Sujata *et al.*, 2012) was present.

However, single polymorphisms has been associated with severity of periodonti- tis, as reported by Gore *et al* 1998, who found that the frequencies of the T allele of the IL 1β (+3954) gene polymorphism was significantly increased in sever periodontitis patients as compared to mild periodontitis patients, but not increased as compared to healthy individuals and (Moreira *et al.*, 2005)observed that there's no association of this polymorphism with sever chronic periodontitis.

In our results observed an evident association of this polymorphism with sever periodontitis when a higher frequency of T+ genotype was detected when compared with moderate periodontitis.

Some possible limitations consist that some individuals who were classified, as healthy control at the time of study.They might develop signs of periodontal disease in the future, in addition to that the sample is very small as to provide for any generalized conclusions, and collected from one provence in Iraq (Babylon), and due to the phenomenon of genetic heterogeneity, the periodontal phenotype may be due to several different genotypes.

In periodontal diseases ,there's many risk factors ,the best method to study the genetic effects would be to start before the disease sets in and following it up through the natural history.

As recommended ,more researches are needed to evaluate the role of IL-1genotypes in periodontal diseases among more number of sample than our study and from different cities in Iraq, to evaluate the ability of IL -1 genotypes to predict disease initiation.

Conclusion

The chronic periodontitis group displayed a higher percentage of the T allele (40%). And we observed an evident association of this polymorphism with sever periodontitis when a higher frequency of T+ genotype (47.6%) was detected when compared with moderate periodontitis (42.1%). Our data suggested that the polymorphism in the locus +3954 of IL1β gene could be related to chronic periodontitis in Iraqi population.

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