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RESEARCH ARTICLE

Association of IL-1α (-889) and TNF-α (-309) Gene Polymorphisms with Chronic Periodontitis in Hilla City

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Abstract

Background: Periodontitis is a infectious disease leading to the destruction of periodontium including periodontal ligament and adjacent alveolar bone induced by periodontal pathogens biofilm. Materials and Methods: A total of 90 patients, 50 with chronic periodontitis (CP) and 40 normal subjects0referring to the clinic of collage of Dentistry, Babylon University, Hilla City, Iraq, were evaluated, TNF-α and IL-1α genotyping was performed by PCR)and analyzed by polyacrylamide gel. Results: polymorphism of IL-1α (-889) and TNFα(-308), The results showed that there was no (significant) allele frequency) difference between(patients and)control groups. Conclusion: The current study suggest that there is no association between IL-1α (-889) and TNF-α 308 polymorphism and chronic periodontitis in this population.

Keywords: *Periodontitis, IL1α (-889), TNF-α 308, Genetic Polymorphism, PCR.*

Introduction

Periodontal-disease is a multifarious inflammatory illness affecting the tissue that surrounded and supported the teeth, periodontitis involved increasing damage of the alveolar bone round the teeth , and these affected teeth, if left without treatment ,may lead to increased mobility of these teeth,0and subsequent teeth loss [1,2,3,4]. Periodontitis is one of the most common diseases that caused by periodontal bacteria [5, 6]. Periodontal bacterial infection caused inflammatory host response. Many specific bacteria that increased in sub gingival0plaque (predominantly gram negative anaerobes) [7,8] in addition to Aggregatibacter actinomycetumcomitans, Porphyromonasgingivalis, Spirochaete, Treponema denticola and Tannerella for sythensis were the most common periodontal disease associated microorganisms [9-12].

Pathogenic0bacteris, various environmental risk factors and genetics are also involved (in the) pathogenesis of the disease [13-16].The immune response, activated by bacteria and their products accumulate in the gingival sulcus and mediate connective tissue destruction, while a large number of inflammatory mediators or biomarkers are created by numerous cellular elements. These pro inflammatory mediators are generally known as cytokines. These cytokines, when are working together to modulate cellular actions; they are identified as cytokine networks and are sharing the innate immune system [17].

Cytokines are a varied and large family of soluble mediators including, interleukins colony-stimulating factors, growth factor, and cytotoxic factors. Cytokines play a major role in various biological activities such as differentiation, proliferation regeneration, development, repair0inflammation0and 0homeostasis [18, 19].

Pro inflammatory cytokines have vital function in microbial-induced destructive inflammation which leads to initiate periodontal disease progression [20]. Kornman et al., Mc Devitt et al, they study the relationship between periodontal disease and polymorphisms of selected genes and from the time ,that the Gram-ve anaerobic bacteria and mediated by inflammatory' activation of endogenous MMP (matrix metalloproteinases) were observed to be responsible for the loss of attachment of periodontitis patients, like IL-1α and TNF-α response to the bacterial bio films; therefore

specific genes have been candidate from many pro inflammatory and regulatory cytokines and founded that IL & TNF gene polymorphisms have been related with chronic periodontal diseases[13, 21].

Materials and Methods

This study was (conducted) in the Department of period ontology at the college of Dentistry, Babylon University, Hilla City Iraq, chronic periodontal disease diagnosis by period oncologist on the basis of clinical examinations, evaluating probing attachment loss and loosening of tooth,0medical and dental history, bleeding on probing (BOP) and probe depth.

We excluded patients who taking anti-inflammatory drugs, diabetes, smoking, rheumatoid arthritis, hepatitis, HI, using orthodontic instruments, cardiovascular disease, pregnancy, oral cavity infections, and other systematic diseases with influence on periodontal condition. We collected and analyzed venous blood of 50 unrelated patients affected by chronic periodontal disease and 40 healthy control, the age group (21-62) years for patients and controls.

The samples were collected from the same geographical region in 5 months (October 2016 and February 2017). 5 ml venous blood was taken and was put into tubes containing EDTA. Then, it was transferred to the Research Center and was stored at 20ºC until it was needed to be used. Extract DNA from frozen samples of blood by use favorgen0kit (FABGK 100 preps), according to the manufacturer's protocol. All DNA samples were quality checked on an agarose gel.

Genotypes for IL-1α and TNF (gene) polymorphisms were-assigned to cases and controls. These included (SNP) single (nucleotide polymorphisms) of IL-1A (-889) C/T (rs1800587) and TNF α (-308) A/G (rs1800629). DNA was amplified by PCR using the primers were presented in Table (1). PCR for IL-1A and TNF, The Master Mix is ready-made from Intron company and mixed with 16µl nucleated water, 2 primer and 2 DNA and placed in a thermo0cycling and depends on the conditions of each gene.

The PCR program on a thermal cycler in IL-1α was: a first denaturation step. At (95°C) for (1 min), followed. By (35cycles) of (57°C) for (30s), (50 °C) for (1min), (72 °C) for (7min), and a final extension0step of (5min) at (72°C). 5 µl of the amplification products was electrophoreses on a(1%) agarose gel at (100 V) for (60 min) and TNF α was: a first denaturation step at (94°C) for (3 min), followed by (35 cycles) of (94°C) for (1min), (66.5°C) for (1min), (72°C) for (1min) and a final extension step of (5min) at (72°C). (5µl) of the pcr products were analyzed by electrophoresis on 1% agarose gels at (100 V) for (60 min) containing 2 µl safe dye. Then the bands were seen under UV ray. Subsequently, Genotypes were given using RFLP technique (restriction fragment length polymorphism) by use 10 µL of the amplicon IL-1α (-889 bp fragment) and TNFα (-308 bp fragment) were digested with 00.12µl of NcoI 8 at 437°C9 for 738 hours in water bath and the created DNA fragments were analyzed by electrophoresis on a 510% Polyacrylamide Gel Figure [1 and 2].

Table 1: Primers for amplification of IL-1α (-889) and TNFα(-308)

Primers		Sequences	Amplicon size pb	References
IL-1A (-889)	F	5'-AAGCTTGTTCTACCACCTGAACTAGGC-3'	99pb	Kornman . 1997
	R	5'-TTACATATGAGCCTTCCATG-3'		
TNFA (-308)	F	5'AGG CAA TAG GTT TTG AGG GCC AT-3'	107pb	Kornman . 1997
	R	5'TCC TCC CTG CTC CGA TTC CG-3'		

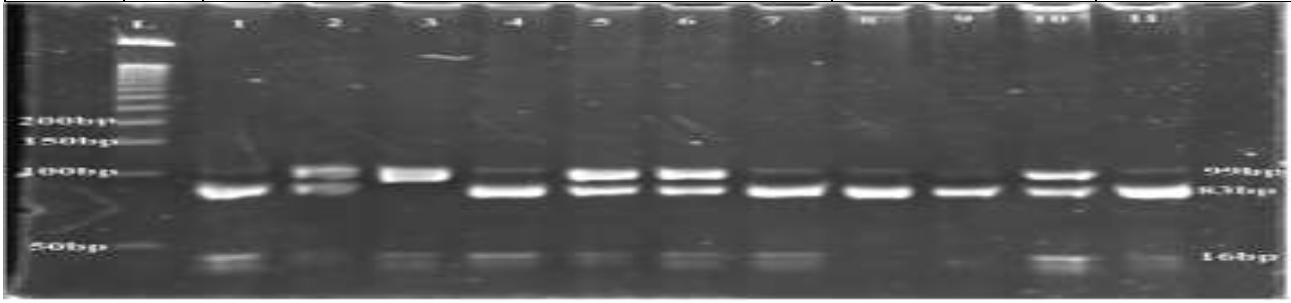

Figure 1:Electrophoresis pattern of IL1α(-889) PCR-RFLP by PAGE gel for PCR product (CC) 99bp with restriction enzyme NCO1, L: 50bp DNA ladder, Lane (1,4,7,8,9,11) homozygote(TT)genotype, Lane (2,5,6,10) heterozygote (CT), Lane(3) homozygote (CC)

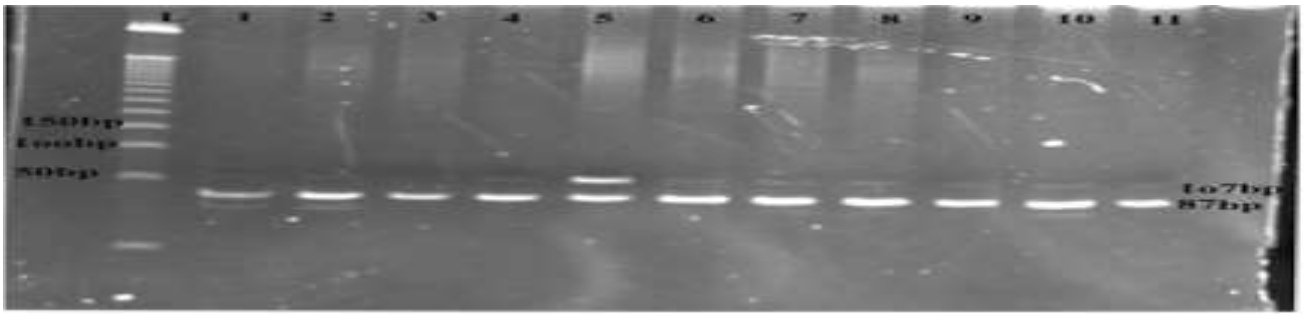

Figure 1: Electrophoresis pattern of TNF α (-308) PCR-RFLP by PAGE gel for PCR product (AA) 107bp with restriction enzyme NCO1, L:50bp DNA ladder, Lane (1,2.3,4.6,7,8,9,10, 11) homozygote (GG) genotype , Lane(5)heterozygote (AG)

Results

Demographic characteristics of case and control groups are presented in Table 2. No significant difference was observed between (IL-1, TNF) gene and periodontitis. The frequencies of genotypes and alleles of IL-1α (-889) polymorphism in both groups were compared in Table 3.

The frequencies Codominant of TT,CT, and CC genotypes in the patient and control groups were respectively, 32%, 60%,and 8%, and 40%, 50%, and 10%, and Dominant of TT 32% and 40%, and CT-CC 68% and 60%, and in Recessive TT-CT 92%, 90% and CC 8% and 10%, and with Over dominant TT-CC 40%-50% and CT 60% and 50%indicating no significant difference in distribution of genotypes. The frequencies of genotypes and alleles of TNF-α (-308 A>C) polymorphism in both groups were compared in Table 4.

And the frequencies of TNFα AA and AC genotypes in the patient and control groups were respectively 94%, 89% and 66%, and 910%, indicating no significant difference in distribution of genotypes, respectively, indicating no significant difference8between the groups and the P-value of IL-1 (0.64, 0.34, 0.74, 0.34) and TNF (p>0.05).

Table 2: They Characteristic of Study Group

Clinical Parameters	C	CP	T	P value*
Sex(M/F)	(13/27)	(37/13)		
Age range(years) (mean±SD)	30±7.7	41.3±10.8		
PI(mean±SD)	0.1±0.38	1.45±0.91	8.824	< 0.0001
%BOP	4.9±4.2	45.54±37.45	6.764	< 0.0001
PPD(mm) (mean±SD)	0.76±0.44	4.6±0.97	20.7	< 0.0001
CAL(mm) (mean±SD)	0	5.45±1.27	27.18	<0 .0001

* Determined by the unpaired *t* test

Table 3: Association of Interlukin IL-1α (-889) genotypes with periodontal disease under models of inheritance

Model	Genotype	Chronic	control	OR (95% CI)	P-value*
Codominant	T/T	16 (32%)	16 (40%)	1.00	0.64
	C/T	30 (60%)	20 (50%)	0.67 (0.27-1.63)	
	C/C	4 (8%)	4 (10%)	1.00 (0.21-4.71)	
Dominant	T/T	16 (32%)	16 (40%)	1.00	0.43
	C/T-C/C	34 (68%)	24 (60%)	0.71 (0.30-1.68)	
Recessive	T/T-C/T	46 (92%)	36 (90%)	1.00	0.74
	C/C	4 (8%)	4 (10%)	1.28 (0.30-5.46)	
Over dominant	T/T-C/C	20 (40%)	20 (50%)	1.00	0.34
	C/T	30 (60%)	20 (50%)	0.67 (0.29-1.54)	

*P value of Pearson's goodness-of-fitchi-square

Table4: Genotypes percentages of TNF-308 and hardy weinberg equilibrium exact test (p-value) for patient and control groups

Model	Genotype	Chronic	Control	OR (95% CI)	P-value*
---	A/A	47 (94%)	36 (90%)	1.00	0.69
	A/G	3 (6%)	4 (10%)	1.74 (0.37-8.27)	

*Exact two-tailed probability (p) of Fisher's Exact Test.

Discussion

Association of Interlukin with Periodontal Disease

IL1A is 1 of 2 structurally distinct forms of IL1, The IL1 A proteins are synthesized by a variety of cell types, including activated macrophages, keratinocytes, stimulated Blymphocytes and fibroblasts, and are potent mediators of inflammation and immunity, Interleukin-10 cytokine modulate host immune0inflammatory response. Interleukin may function as immolators of chronic periodontitis [22].

To our knowledge, this study is the first to detect of a polymorphism of IL-1α (-889) rs1800587, in patients from Hilla City suffering or not from periodontal disease were done using PCR-RFLP technique. The result showed that there was no0association between the polymorphism of IL1α (-889) polymorphism gene and chronic periodontitis (CP). Furthermore, the association keeps its no significance even when the samples segregated into male and females.

These results were0in accordance with the results were stated by Zuccarello et al, Which conducted in North-East of Italy (Caucasian) ; in Zuccarello′s study did not confirm the previously proposed relationship between periodontitis and IL1 gene cluster polymorphisms.0They collected and analyzed 206 cheek swabs of 101 patients affected by chronic PO and 105 healthy controls and found that no correlation between IL1 gene polymorphisms and chronic periodontitis [23].

The result of our study was disagreed with the results showed by Kornma et al, when sampling from 55 patient with chronic periodontitis and 44 control found associated with increase IL production, and a strong indicator of susceptibility to severe periodontal in adult [13]. Also Shirodaria et al, disagree with our result ,in their study which employ 46 patients with severe periodonta disease to these results recommend a mechanism where by this genetic polymorphismacts to modulate IL-1 alpha protein production and may lead to influence the pathogenesis of periodontal disease by disturbing the IL-1-associated by stander damage [24].

And a case control study conducted by Brett et al, which employ a huge number of case and control (57 chronic periodontal disease, and 100 controls) found this polymorphism had association with the periodontitis and achieve [odds ratio= 4.20 (95% 0CI 1.9 to 9.4)] and ($P > 0.05$), these results suggest that if there is a shared genetic etiology in the periodontal0diseases, then the phenotypic variations seen a reduce to the modification of this susceptibility by many different gene variations [25].

In a study showed by Komal et al, 60 Indian patients with 20 aggressive periodontitis, 20 chronic periodontitis and 20 healthy controls where the results0showed that the $P<0.001$ and Odds ratio for aggressive versus0chronic periodontitis was calculated as 6.20 (95% confidence0interval 6.019-7.892), This results in this there's a positive association between periodontal disease and the presence of0the0IL-1α-889 [26].

As well as in Arabic studies in Jordan by Karasneh et al, this case-control study consisted of 260 unrelated individual's and0included 100 CP cases, 80 AgP cases and 80 controls. There were 126 males and 134 female, this is the first study investigating IL-1α association with periodontitis in Jordani an population, where allele frequencies and haplotype patterns were determine, she explained the IL-1 were association0with chronic periodontitis0and not association with aggressive0periodontitis [27]. Generally, all above studies founded that there's a correlation of IL-1(-889) gene polymorphism with periodontitis conditions.

The inconsistent of our study results observed in the literature could be attributed to several factors related to the definition of chronic periodontal disease (multifactorial), population heterogeneity (race), environmental and demographic confounding as risk factors. In addition to that, small sample size, Since most0researchs used a few samples, So this may cause lack of association between genotypes and clinical status of periodontal disease. Finally, the genetic basis for periodontitis0may not be related to a single genetic variant, but may be influenced by multiple genes acting synergistically with environmental factors to increase or decrease the likelihood of developing a disease.

Association of Tumor Necrosis Factor with Periodontal Disease

In the current study, the detection of polymorphism of TNF 1-308 rs 1800629 in a DNA sample of the Babylon population suffering or not from periodontal disease were done using PCR-RFLP technique. Our results were disagree with the results showed by Kornman and AL-Waeli, Kornman et al, where they found that there's a correlation of TNFα-308 gene polymorphisms with periodontal disease. In Kornman et al study they demonstrated specific genetic markers, that have been associated with increased TNF- production are a strong indicator of susceptibility to periodontal disease[13].

And AL-Waeli, genotype odds ratio was 4.68; compared with 1.74 odds ratio of the current study; and he found that there was an association between the polymorphism of TNF-308 polymorphism gene and chronic periodontitis. The reasons for these results may be attributed to the frequent wars in our country and the impact of weapons used and radiation, which led to a weakness in the genetic structure of the Iraqi people and increase its sensitivity to bacterial infections [28]. Very few studies have reported some 0correlation between TNF-α polymorphism and periodontitis. In our current study was documented a risk for chronic periodontitis of TNF for mutant AG genotype according to odds ratio 1.74, this result indicated that there was no association between TNF polymorphism and0chronic periodontitis n Hilla population.

These results was , agreed with the results that stated by Solhjoo et al, they found that TNFα-308 was not considered as a risk0factor for0periodontitis [29]. Also Brett et al, agreed with our result, their study which employ 570 patients with chronic disease and1000 healthy controls, the results show statistically significant ($p \leq 0.05$) differences between genotype frequencies in chronic periodontitis and controls.

These results suggest that there are in fact both shared and unique genetic associations in chronic periodontitis [25]. As well as Natascha et al, study evaluated the frequency of the tumor necrosis factor-alpha (TNF-α)-308 in Brazilians with periodontal health (PH=51), chronic periodontitis (CP=74), suggested that the TNF-α-308 was not associated with periodontitis in this Brazilian population [30]. Furthermore, in our study, the association keeps it's no significance even when the samples segregated into male and females.

The inconsistent to four study results observed in the0literature couldbe0attributed to several factors0relatedtothedefinition of chronic periodontal disease (multifactorial), population heterogeneity (race), environmental and demographic confounding as risk factors. In addition to that, small sample size, Since most researches used a few samples.

So this may cause lack of association between genotypes and clinical status of periodontal disease. Finally, the genetic basis for periodontitis may not be related to a single genetic variant, but may be influenced by multiple genes acting synergistically with environmental factors to increase or decrease the likelihood of developing a disease. Further researchers are required to establish the role of host genes in the etiology and pathogenesis of the periodontal diseases.

Conclusion

The current study suggest that there is no association between IL1α (-889) and TNF-α 308 polymorphism and chronic periodontitis in Babylon population.

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