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RESEARCH ARTICLE

Nuclear Factor-kappa B Gene Polymorphism and Interleukin-8 in Iraqi Population with Severe Chronic Periodontitis

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Abstract

Background: Periodontitis is characterized by an unbalanced host response to periodontopathogens, which lead to periodontal tissues breakdown. Genetic backgrounds play a key role in susceptibility to and protection against a spectrum of periodontal diseases. Interleukin-8 is a relevant mediator of granulocyte accumulation by attracting polymorphnuclears in the inflammatory region. Nuclear factor kappa B (NF-kB) is a key regulator during inflammatory process involving the gingiva and alveolar bone of teeth. Aim of study: case control study used to explore the correlation between polymorphisms in NF-kB-94 gene and serum level of IL-8 and the impact of polymorphisms on the degree of susceptibility to and severity of chronic periodontitis. Materials and methods: Ninety six Iraqi subjects their age range (30-50) years were included in study divided in to two groups (patients with chronic periodontitis and healthy control), venous blood were collected from each patients for immunological analysis of IL-8 and genetic analysis forNF-kB-94. Results: The present study revealed a significant decrease in the median serum level of IL-8 (p< 0.029) among patients group (78.9pg/µl) as compared to controls group (80.4pg/µl). For genetic analysis after sequencing with sanger method two polymorphisms were detected in the promoter region of NF-κB1-94 at rs28362491 and rs569599236 as there was deletion in the sequence of ATTG for rs28362491 while there was deletion in the sequence GCA for rs569599236 with non-significant association with chronic periodontitis group although significant association was reported with serum level of IL-8 and bleeding on probing index. Conclusion: The results suggest that the ins/del ATTG polymorphisms in NF-kB1-94 is associated with increased severity of developing periodontitis, since this study have documented that polymorphisms located within genes encoding cytokines regulated by NF-κB have association with disease activity.

Keywords: *Chronic periodontitis, IL-8, NF-κB, gene polymorphisms.*

Introduction

Nuclear factor-κB is a transcription factor that is involved in inflammation, cell survival, angiogenesis and apoptosis [1] and regulates a large array of genes involved in different processes of the immune and inflammatory responses [2]. Several polymorphisms have been identified in the promoter region of NF-κB1 gene, which is insertion/deletion, del/del ATTG [3]. In a study conducted on periodontal disease reported that del/del genotype of NF-κB1 gene was associated with aggressive periodontitis [4]. However, the frequency of many genetic alleles varies among ethnic groups. Interleukin-8 is one such cytokine that acts as a powerful chemo attractant for neutrophils and has been identified in crevicular fluid [5, 6]. IL-8 is an important chemokine of interest in periodontal diseases. IL-8 expression has been shown to be critically dependent on a region in its promoter, which contains binding sites for the transcription factors NF-κB [7]. Many researchers have investigated the role and interaction of these transcription factors in regulating the IL-8 gene and have shown different mechanisms of regulation depending on the cell type [7, 10]. Therefore, the aim of the current study used a case control approach to explore the correlation between polymorphisms in NF-κB and the degree of susceptibility to and severity of chronic periodontitis in Iraqi population and exploring the impact of NF-κB polymorphism on circulating IL-8 for selected populations with chronic periodontitis.

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Materials and Methods

Subject

This study was approved by the Ethics Committee of College of Dentistry / Baghdad University. Ninety six Iraqi subjects their age range (30-50) years were included in a case control study, and divided in to two groups (patients with chronic periodontitis and healthy control).

Patients group was included fifty five with severe generalized chronic periodontitis. While controls group consisted of forty one clinically healthy volunteers, all showed a clinical healthy periodontium. The patients were subjected to a questionnaire including the name, the age, ethnicity, medical history, past dental history, type and duration of treatment, medications and smoking or alcohol drinking, then clinical periodontal parameters [plaque index(PI), gingival index(GI) ,probing pocket depth (PPD), clinical attachment level (CAL)] were recorded for each subject

Sample Collection

Venous blood was collected from the antecubital vein, in 5ml vacutainer glass blood collection tubes. Two ml of venous blood was transferred into tube buffered with sodium citrate 3.2% and kept at -40C° for the genotyping of NF-κB1-94 while the other three ml were placed in gel separating tube and centrifuged to separate the serum from the cells for IL-8 analysis (with ELISA Kit) for 10 min at 3300 g. The obtained blood serum was placed into sterile Eppendorf vials and kept at -40°C until being analyzed.

Genotyping of NF-κB1-94 Polymorphisms

The QIAamp DSP DNA Blood Mini Kit uses well-established QIAamp technology for purifying genomic DNA. The primers were employed for detection of rs398063876 and for rs569599236 were designed by extracting the promotor sequence NF-κB1-94 and obtained from the Bioneer Company in lyophilized form. The primer sequences was: Forward primer NF-κB1-94 5`T GG GCACAAGTCGT TTATGA -3, Reverse primer NF-κB1-945-CT GG AG CC GG TA GGGAAG-3` Polymerase Chain Reaction amplifications were performed with PCR Express (Thermo Cycler, BioRad, USA) with the following temperature program: denatured at 94°C for 4 min followed by 30 cycles of denaturation at 94°C for 30 sec; annealing at 55°C for 45 sec; and extension at 72°C for 60 sec.

A final extension incubation of 7 min at 72°C was included, followed by a10 min incubation at 4°C to stop the reactions. Then the samples were loaded on agarose gel to perform electrophoresis using AgaroPower™. After the emergence of the gene fragments in the PCR with the expected size, PCR product were send for Sanger sequencing for the detection of the nucleotide sequences of these fragments. The sequences of these samples were compared with the source sequence and analyzed by using Basic Local Alignment Search Tool Program (BLAST) which is available at the NCBI information site.

Results

The demographic characteristics of two study groups were summarized in Table (1), the present study showed non-significant differences between two groups according to age and gender.

Table 1: The demographic characteristics of groups

			Chronic periodontitis group n=55	Control group n =41	p-value
Age	Range		35-50	30-50	0.502
	Mean		38	36	
	SD		7.24	8.24	
Gender	Male	n	38	27	0.111
		%	69.09%	65.85 %	
	Female	n	17	14	0.131
		%	30.90 %	34.14%	
Family history	Positive	n	9	-	0.164
		%	16.36%	0%	
	Negative	n	46	41	
		%	83.64%	100%	

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The present study revealed a significant decrease in the median serum level of IL-8 at p-value < 0.029 among chronic periodontitis group (78.9 pg/µl) as compared to control group (80.4 pg/µl), Table (4).

Table 2: Serum level of IL-8 in pg/µl between chronic periodontitis and control

	Study groups			
Serum IL-8	chronic periodontitis	control subjects	z- test	p- value
n	47	41	-1.889	0.029*
Median	78.971	80.472		
Mean	79.03	83.751		
SD	10.36	6.111		
Range	75.03-143.03	76.03-94.05		

The genetic analysis was done for NF-κB1-94 gene polymorphisms and two polymorphisms were detected according to primer design, the results of PCR for NF-κB1-94 were shown in Figure (1) in gel electrophoresis.

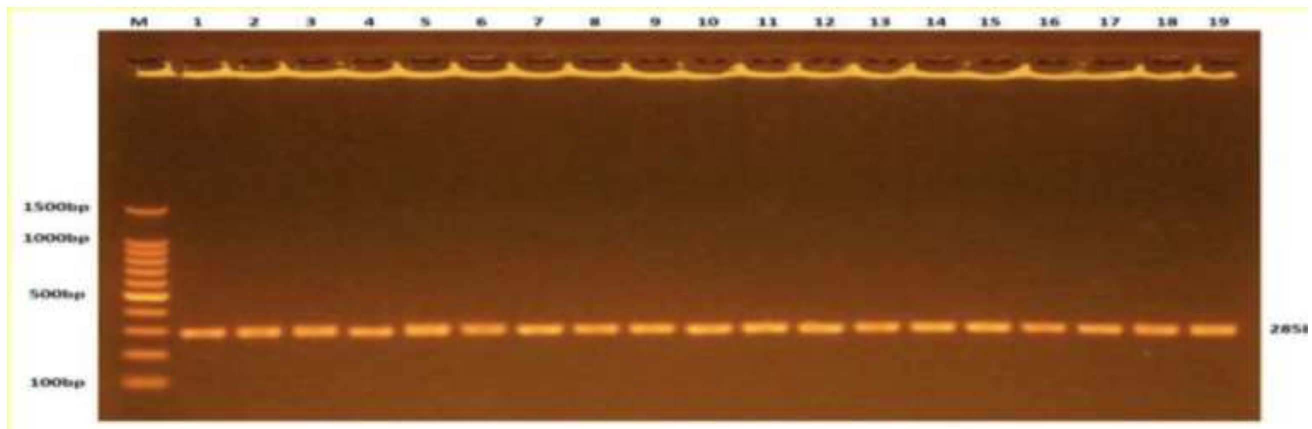

Fig 1: Electrophoresis of the PCR products for NF-κB1-94 at 285 bp for all samples

After sequencing with Sanger method two polymorphisms were detected in the promoter region of NF-κB1-94 at rs28362491 and rs569599236 as there was deletion in the sequence of ATTG for rs28362491 while there was deletion in the sequence GCA for rs569599236. The Hardy- Weinberg equilibrium for study groups and total samples were done for rs28362491 and the results were non-significant between the observed and expected polymorphisms in the study groups and total sample at p-value <0.05 Furthermore the Hardy-Weinberg equilibrium for rs569599236 was non-significant in both groups and total sample between the observed and expected polymorphisms as illustrated in Table (3).

Table 3: Hardy-Weinberg equilibrium for study groups in rs28362491and rs569599236 polymorphism

	Chronic periodontitis n=55		Control n=41		Total n=96	
rs28362491	Observed n	Expected n	Observed n	Expected n	Observed n	Expected n
Ins/Ins	25	26.25	19	19.12	44	45.38
Ins/Del	26	23.49	18	17.76	44	41.28
Del/Del	4	5.25	4	4.12	8	9.38
Hardy-Weinberg equilibrium	0.63		0.01		0.43	
	p-value= 0.427		p-value=0.920		p-value=0.511	
rs569599236	observed	expected	observed	expected	observed	expected
Ins/Ins	54	54	41	41	95	95
Ins/Del	1	0.99	0	0	1	0.99
Del/Del	0	0	0	0	0	0
Hardy-Weinberg equilibrium	0		-		0	
	p-value=1		-		p-value=1	

In the present study, the polymorphisms number and distribution was illustrated in table (4) and there was non-significant difference between groups although there

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were 30 polymorphisms for rs28362491 in chronic periodontitis group and 23 in control group while there was only one polymorphism for rs 569599236 in chronic periodontitis group. The effect of polymorphisms on disease distribution was assessed by odd ratio and it was 0.939 for rs 28362491.

Table 4: The number of polymorphisms for NF-κB1-94 in chronic periodontitis patients and control samples

	Chronic periodontitis		control		Fisher test	p–value	OR	CI 95%
NF-κB1-94	n.	%	n.	%				
rs28362491	30	56.60	23	43.33	1	NS	0.93	0.41- 2.11
rs569599236	1	-	0	-	-	-	2.28	0.09-57.5

Furthermore, the frequency of genotype distribution in polymorphisms of NF-κB1-94 in both study groups showed non-significant association between them in each polymorphisms as described in Table (5) Moreover the effect of each genotype on disease distribution was also discussed and there was high risk to have a disease with genotype (Ins-Del) for both polymorphisms since the odd ratio was 1.597 and 2.280 for rs rs28362491 and rs 569599236 respectively.

Table 5: Analytic statistics of genotype distribution of NF-κB1-94 polymorphism

	genotype	Chronic periodontitis		control		Fisher test	p	OR	CI 95%
		n	Freq.	n	Freq.				
rs28362491	Ins- Ins	25	0.454	18	0.439	1	0.31	0.736	0.31- 1.70
	Ins- Del	25	0.454	18	0.439	1	0.31	1.597	0.68- 3.74
	Del- Del	5	0.090	5	0.121	0.7	0.42	0.720	0.19- 2.67
rs569599236	Ins- Ins	54	0.981	41	1.00	1	0.31	0.437	0.01-11.0
	Ins- Del	1	0.019	0	0.00	1	0.31	2.280	0.09-57.5
	Del- Del	0	0.00	0	0.00	-	-	-	-

The spearman correlation for NF-κB polymorphism and cytokines (IL-8) was described in table [11] and there was significant correlation between IL-8 with rs 28362491 in chronic periodontitis group at p-value (0.017, 0.049) while there was non-significant correlation between rs569599236 and IL-8 in both groups

Table 6: Correlations of NF-kB and serum cytokines in study groups

Spearman correlation		IL-8	
		r	p
rs 28362491	Chronic periodontitis	-0.321	0.017
	control	0.253	0.062
rs 569599236	Chronic periodontitis	0.032	0.732
	control	-	-

*significant at p-value <0.05 Furthermore, the correlation between clinical periodontal parameters with NF-κB1-94 revealed significant correlation between NF-κB1-94 with bleeding on probing at p-value (0.049) while there was non-significant association for all remaining clinical periodontal parameter

Discussion

The present study showed significant decrease in the serum levels of IL-8 in the chronic periodontitis patients in comparison with control group, similarly with other results reported by [11, 12, 19], The reason for this result may be due to a multifunctional role of IL-8 in the pathogenesis of periodontal disease and health, the result of the meta-analysis of studies done by Finoti *et al* in (2017) showed that the GCF of patients with chronic periodontitis presented significantly lower IL-8 levels than the GCF of healthy control subjects, Zhang *et al* [13]. Demonstrated that both gingival and oral epithelial cells infected with *P. gingivalis* produced IL-8, and after infection these cells continued to express IL-8 mRNA, although the accumulation of the secreted protein could not be detected. Finoti *et al* [12] suggested that IL-8 could be degraded locally by *P. gingivalis* proteinases and their conclusions might confirm the results of the present study. The chemokine expression in local tissues may be suppressed

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due to mechanisms related to microbial challenge, and this may decrease its level in the circulatory system [14, 15]. Hence, low levels were detected in the sera of periodontitis patients in this study. A number of studies have investigated the relationship of INS allele of -94 ins/ del polymorphism with various inflammatory diseases. According to our Knowledge no previous study carried out to evaluate the association between NF-kB polymorphism and periodontitis risk in Iraq.

Current study showed there was deletion in the sequence GCA for rs569599236 with non-significant difference between study groups although it show high odd ratio which give indication about association with periodontitis risk and unfortunately there is no available references to compare current result with it. The results of Hardy-Weinberg equilibrium for rs28362491 were non-significant for both chronic periodontitis and control groups as well as total samples and this correspondence with [16] Moreover the present result shown high association of polymorphisms with periodontitis although non-significant difference was found between the study groups at rs28362491 as there was deletion in the sequence of ATTG and this was in correspondence with [4, 16], who found a similar, apparent non-significant, influence of the ins/del and del/ del genotype in chronic periodontitis group.

The del allele was associated with decreased promoter activity of NF-kB. Furthermore, the induction of NF-kB activity as a result of stimulation with bacteria, including periodontopathogens, has been reported to play a crucial role in host defense. In accordance with this notion, a del/del dependent impaired immune response to periodontopathogens could be assumed. Schulz and colleagues [4] found a significant association of the del/del genotype and the occurrence of *A. actinomycetemcomitans*. When investigating putative correlations of the genotype with sub gingival bacterial colonization. Interestingly, there were a significant correlation between polymorphisms at rs28362491 and IL-8 this could be explained by: that activation of NF-κB may maintain the normal cellular defenses against periodontal bacteria by the innate immune system the mechanism behind NF-kB1 in relation to disease susceptibility remains unclear, -94 del ATTG has been showed reduce activation of NF-κB1 transcription. The -94 del ATTG allele may result in decreased NF-kB1 message and hence decreased p50/p105 NF-κB protein production [16].

Concerning the correlation with periodontal parameters the present results show significant correlation with BOP index which suggest that this polymorphisms as indicator of disease activity that may lead to further extent and severity of disease and this could be explained by decrease NF-kB1 transcriptional activity probably by interfering with binding sites for some crucial transcription factors. Given the important role of NF-κB signaling in the maintenance of integrity of the epithelial barrier [17].

Previous studies suspect that decreased transcriptional activity of NF-kB1 resulting in lower p50 concentration in epithelium which may favor development of intestinal inflammation; defect of the NF-κB allows bacteria to cross the barriers and escape the clearance by immune system, and hence contribute to on-going inflammation [18]. The conclusion of the current study suggest that the NF-kB1 promoter -94 ins/del ATTG polymorphisms is associated with increased severity of developing periodontitis, since our study have documented that polymorphisms located within genes encoding cytokines regulated by NF-κB have association with disease activity.

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