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The Role Of IL-32 Polymorphisms in Periodontitis Disease

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Abstract

Genetic variation is a change of DNA sequence in humans as a result of polymorphism in genes, which can detect two or more forms of genes in individuals. The polymorphism genes may impact on gene expression and lead to abnormal proteins and signaling pathways. Periodontitis disease is a chronic inflammatory disease that impacts the gingiva, periodontal ligament, and alveolar bone. Interleukin-32 (IL-32) has a significant relationship with periodontitis disease. IL-32, a gene, included eight exons that produce proteins. IL-32 is produced by natural killer (NK) cells, monocytes, and T-cells. A total of 40 periodontitis patients, including 27 female and 13 male, and 20 healthy individuals as a control group (9 female and 11 male), with an average age between 22-65 years, took part in this research. Polymorphism of the IL-32 gene assay and sequencing were done to detect IL-32 polymorphism and SNP (rs4740).

The results include a statistically non-significant association between the genotypes of patients and controls. Also, there is no significant difference ($P > 0.05$) in IL-35 from serum, saliva, and GCF between patients and control groups and the genetic variations AA, GA, and GG genotyping. These results detected a relationship between genotype and each of age and gender of patients; there is no significant difference ($P > 0.05$) in the distribution of genotypes according to age and gender. It suggests that the demographic composition of the study groups is very closed. So, these findings do not support a direct genetic link at this IL-32, but they provide valuable scientific data that help to refine future research directions.

Keywords: Genetics variation, IL-32, Periodontitis disease, Polymorphizm

1.Introduction

Genetic variation is a change of DNA sequence in humans as a result of polymorphism in genes, which can detect two or more forms of genes in individuals. The polymorphism genes may impact on gene expression and lead to abnormal proteins and signaling pathways (Jin *et al.*, 2016). These genes were associated with several diseases, including arthritis, psoriasis, ulcerative colitis, and periodontitis, by cytokines or proteins that are expressed from gene polymorphisms, which were investigated as a risk factor for developing disease, especially periodontitis, in patients (Ferrara *et al.*, 2025).

In regard to periodontitis disease, it is a chronic inflammatory disease that impacts the gingiva, periodontal ligament, alveolar bone, and all tissues surrounding the teeth. The pathogenicity of disease is a complex relationship between oral bacterial infection and the immune response system (Martínez *et al.*, 2022). In addition, some environmental factors that influence on periodontitis, such as smoking, stress, obesity, and some diseases (diabetes and arthritis). As well, genetic factors play an important role in causing or developing periodontitis (Martínez Pérez *et al.*, 2022).

Recently, International Workshop on Classification of Periodontal Diseases is classified periodontitis to two types aggressive periodontitis and chronic periodontitis. The adults are more infection by chronic periodontitis and will be slow progression. While, aggressive periodontitis was characterized inflammatory and rapid and destroyed connective tissue (Netea *et al*,2005).

However, the World Workshop on Classification of Periodontal and Peri-Implant Diseases in 2017 classifies the periodontitis disease into stages (I–IV) and grades (A–C). Staging of disease refers to severity and the extent of disease; in regard to grade, it refers to rate of progression (Ferrara *et al*., 2025). Some reports mentioned that cytokines affect periodontitis disease by devolving this disease (Brodzikowska *et al*., 2022). Interleukin-32 (IL-32) has a significant relationship with periodontitis disease. IL-32, a gene, included eight exons that produce proteins. IL-32 is produced by natural killer (NK) cells, monocytes, and T-cells. The interleukin-32 has different functions, such as proliferation and apoptosis of cells (Dede and AI, 2017).

On the other hand, the polymorphism IL-32 is associated with periodontitis and different diseases: rheumatoid arthritis and type 1 diabetes (Mazurek-Mochol *et al*., 2021). Also, it may play a role in the development of periodontitis disease. Therefore, the present study aimed to detect polymorphisms in IL-32, which may be used as a biomarker for periodontitis disease.

2.Material and methods

2.1 Sample collection

A total of 40 periodontitis patients includes 27 female and 13 male and 20 healthy individuals as control group (9 female and11 male); the average age between 22-65 years. All sample collection from clinics at faculty of dentistry, the universities of Kufa in Najaf, Iraq during the January to May 2025. The protocol was approved by consent all participants. This study excluded diabetes mellitus, pregnant or lactating women, smokers, and patients who have taken antibiotics in last three months.

2.2 Polymorphism IL-32 Gene assay

IL-32 SNP (rs4740) gene examination was done. DNA extraction was amplification by using Genomic DNA isolation kit (iNtRON, Biotechnology, Korea) and follow the protocol provided by the company.

Genetic polymorpgizm in the IL-35 gene (rs4740) was determined by PCR and DNA sequencing. PCR assay was performed according to iNtRON biotechnology of the instructions. Total reaction volume was 20µl containing PCR Master Mix (iNtRON biotechnology), using an eppendorf thermocycler (Eppendorf, Hamburg, Germany). The primers designed using NCBI database and Primer3 (v. 0.4.0) which illustrated in table 1, to extract 800bp. PCR conditions, were optimized by gradient PCR included the range (48- 58) °C for annealing temperature.

The conditions of PCR: initial denaturation cycle (95◦C for 3 min.), then followed by 35 cycles involve denaturation (94◦C for 3 sec.), annealing (62°C for 30 sec.), elongation (72°C for 45 sec.), and final elongation cycle (72°C for 5min.). The product of PCR was assessed by electrophoresis using a 1% agarose gel. The DNA marker (100bp) was used, figure (1)

Table (1) shows the sequencing of primers

Primers	Sequence (5'->3')
Forward primer	AATATAGTGGGACCAGGGGT
Reverse primer	ATATCTCACAGTGACAGTTCAGT

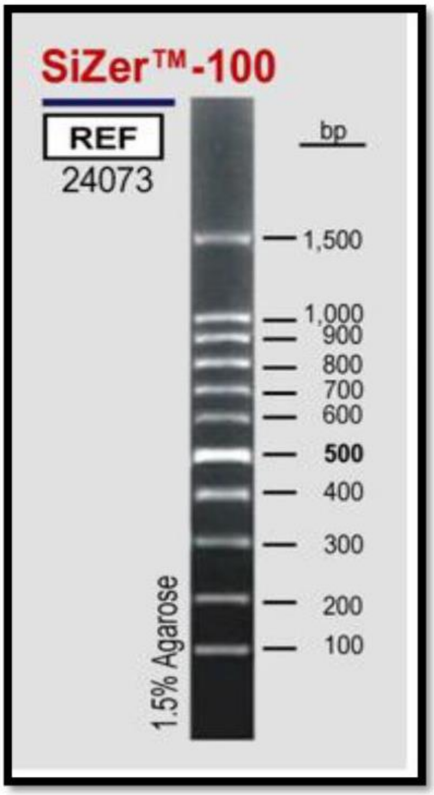

Figure (1) The DNA maker (100bp) manufactured by iNtRON(Korea)

2.3 DNA sequencing

The PCR products were commercially sequenced from both forward and reverse primers according instruction manuals of the sequencing company (Macrogen Inc. Geumchen, Seoul, South Korea). Only clear chromatographs obtained from sequence files were further analyzed by comparing the observed DNA sequences with the retrieved neighboring DNA sequences of the NCBI Blast engine, the virtual positions and other details of the retrieved PCR fragments were identified.

Analysis of sequencing results was done using BioEdit Sequence Alignment Editor Software Version 7.1 (DNASTAR, Madison, WI, USA) to determine the base transition in the sites of interest (Figure2).

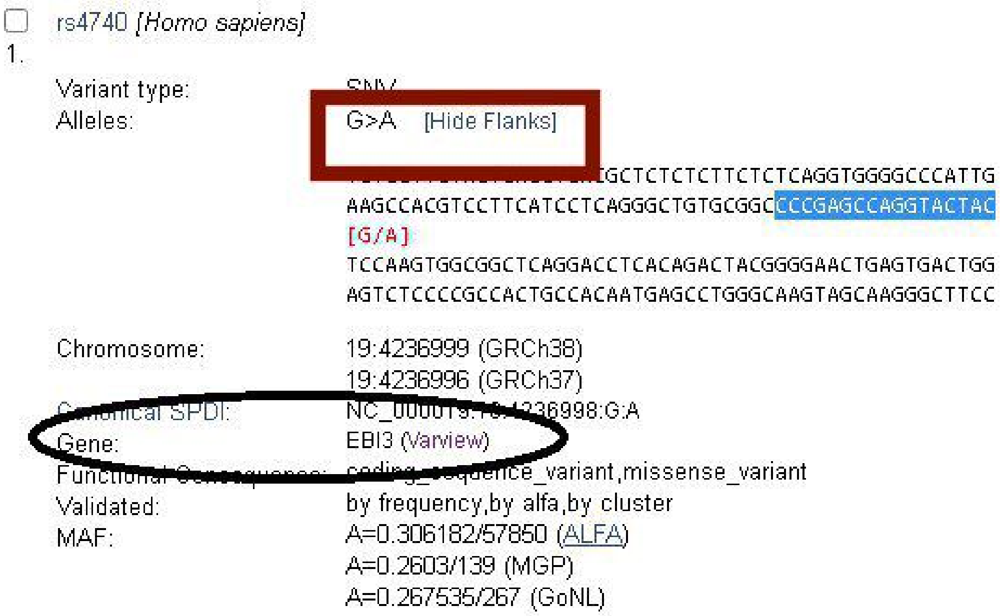

Figure 2: Detection rs4740 by NCBI Blast engine, the red box shows SNP polymorphism and black circular shows name of gene on chromosome 19.

2.4 Gingival Crevicular Fluid (GCF) sample

The sampling was selected from oral cavity in control group and patients by using paper strip (Periopaper, Oraflow Inc., NY, USA). Insert the paper strip into the Gingival Crevicular sulcus until mild resistance is felt and leave its for 30 seconds, to absorbed the fluid by capillary action method. Immediately, transferred to micro centrifuge tubes that included 200 µl phosphate buffer saline and kept its at -80Oc. Carefully remove the strip, without any contamination by blood or saliva and ensure did not touch oral tissue. Discard any strips contaminated.

2.5 Saliva and serum Sample

All saliva was collected by sterile tubes and remove cellular debris by centrifuging at 800 x g for 10 minutes, and kept at -80oC. Five ml of intravenous blood was taken from patients and control. The serum was separated and aliquot and storage in the Eppendorf tubes and kept at - 80C.

2.6 Statistical analysis

The statistical analysis was performed using the statistical software (SPSS version24), all statistical was done by student' s t-independent test for all variables that followed Chi square test, p-value 0.05.

3.Results

The genetic polymorphism in the IL-35 gene (rs4740: G>A) experiment was set up with the above primers and 20 ng gDNA of patients, as mentioned in the material and method for amplification of the expected 800 bp product. 5 µl of PCR products were run on 1% agarose gel to confirm PCR was successful, and comparison to the DNA (100 bp) marker was used to confirm the size of the product. This PCR assay was done with negative control (distilled water instead of gDNA), as illustrated in figure 3. The rest of the PCR product was sequenced to confirm the polymorphism of IL-32 (G>A) as shown in figure 4.

Figure **3**: The PCR assay was performed to detect genetic polymorpgizm in the IL-35 gene (rs4740: G>A). The PCR product produced was expected to be 800bp in length. The experiment included negative controls to exclude DNA contamination. DNA (100bp) marker was used.

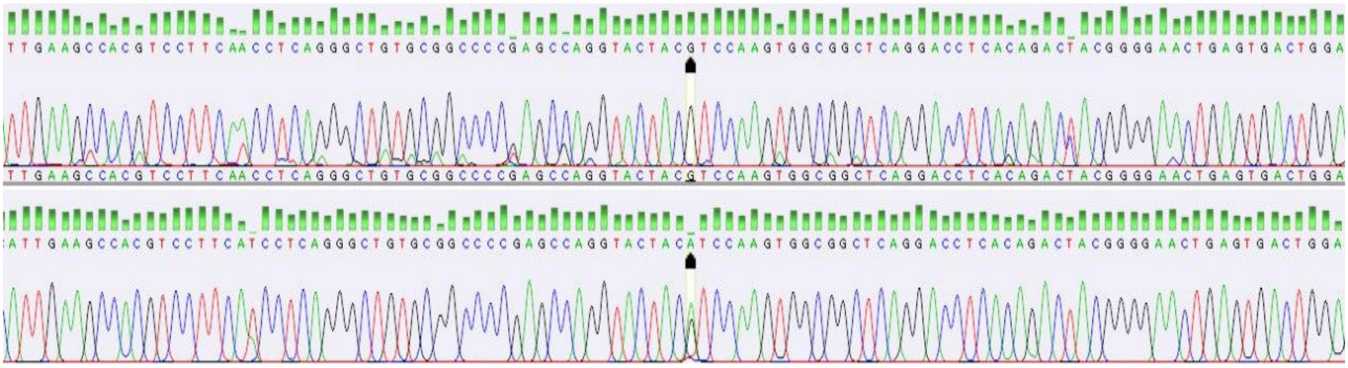

Figure **4**: Sequence data of polymorphism of genetic polymorpgizm in the IL-35 gene (rs4740: G>A). Top panel - in sequencing reactions observed only one allele (G) is indicated by a black arrow. bottom panel – in the sequencing, two alleles (G>A) are indicated by a black arrow.

The analysis of the frequency and percentage of three genotyping GA, GG, and AA polymorphisms in 40 patients and 20 control groups is shown in table 2. The data includes a statistically non-significant association between the genotypes of patients and controls.

The GA genotype shows 50% is present in both the patient and control groups. The statistical test produced a p-value of 1.00, which is not statistically significant (NS). So, the GA genotype is not different from the control group. At the same time, the GG and AA genotypes also show no significant association with the patient group and control. The p-values are 0.84 and 0.81, respectively. As a result, there is no significant difference (P > 0.05) between patients and control groups regarding distribution of genotype.

Table (2): Descriptive statistics and differences in genotype for patients and control groups

Indicators	Responses	`Patients (No. = 40)		Control (No. = 20)		Chi Test	P value (Sig.)
		Feq.	%	Feq.	%		
GA	Presence	20	50	10	50	0.00	1.00 (NS) Odds Ratio : 1
	Absence	20	50	10	50		
GG	Presence	13	32.5	6	30	0.04	0.84 (NS) Odds Ratio : 1.12
	Absence	27	67.5	14	70		
AA	Presence	7	17.5	4	20	0.06	0.81 (NS) Odds Ratio : 0.84
	Absence	33	82.5	16	80		

NS: Non-significant at P value >0.05

Our data in table 3 shows analysis of a statistical table detailing Interleukin-35 (IL-35) levels in three biological fluids, serum, saliva, and GCF, in periodontitis patients according to genotype: GG, GA, and AA. The serum IL-35 levels recorded a median concentration of 19.07 pg/ml in the GG genotype, 15.14 pg/ml in the GA, while 25.67 pg/ml in the AA. In regard to the P-value, it is not statistically significant. It is indicating that the observed differences are in serum IL-35 levels and the genotype. While, for IL-35 levels in saliva, the median values were 53.71 pg/ml in the GG genotype, 45.58 pg/ml in the GA group, and 34.39 pg/ml in the AA genotype. The P value (0.65) confirmed a non-significant difference between the genotype groups and saliva sample. The data suggests that the genotype does not have a statistically significant effect on the levels of IL-35 in saliva.

In addition, IL-35 in GCF showed median concentrations of 151.17 pg/ml in GG, 177.85 pg/ml in GA, and 97.83 pg/ml in AA genotyping.Our data indicate that the observed differences in GCF IL-35 levels are not statistically significant among the genotypes in patients. It reveals the differences in IL-35 from serum, saliva, and GCF between patients classified according to genotype. According to table 2, there is no significant difference (P > 0.05) in IL-35 from serum, saliva, and GCF between patients and control groups and the genetic variations AA, GA, and GG genotyping.

Table (3): Differences in IL-35 from serum, saliva and GCF between patients' subgroups classified according to genotype

Indicators	Statistics	GG (N=**2**0)	GA (N=13)	AA (N=7)	Kruskal-Wallis H	P value (Sig.)
IL-35in serum (pg/ml)	Median (IQR)	19.07 (13.2-99.3)	15.14 (7.2-22.3)	25.67 (12.2-66.3)	2.75	0.25 NS
	SD	133.92	9.97	50.52		
	Mean Rank	21.55	16.46	25.00		

IL-35in saliva (pg/ml)	Median (IQR)	53.71 (12.8-337.6)	45.58 (12.8-235.6)	34.39 (12.3-67.6)	0.88	0.65 NS
	SD	369.65	246.05	46.91		
	Mean Rank	21.65	20.69	16.86		
IL-35inGCF (pg/ml)	Median (IQR)	151.17 (92.3-187.6)	177.85 (99.3-199.6)	97.83 (62.3-117.6)	1.25	0.54 NS
	SD	118.61	91.61	55.08		
	Mean Rank	21.00	22.08	16.14		

SD: Standard Deviation; NS: Non-significant at P value >0.05; S: Significant at P value <0.05

The data for patients in age groups between 22 and 65 years recorded a chi-square value of 2.35 with a corresponding p-value of 0.88. This indicates that the age of the participants is not significantly associated with GG, GA, and AA genotypes.

In regard to gender, it also examined the distribution of male and female patients within the genotype group. The P-value for gender resulted in 0.85. So, the finding is not statistically significant for the association between genotype and gender distribution among the patient genotypes, as illustrated in table (4).

These results detected a relationship between genotype and each of age and gender of patients; there is no significant difference (P > 0.05) in the distribution of genotypes according to age and gender. It suggests that the demographic composition of the study groups is very closed.

Table (4): Relationship between genotype and each of age and gender of patients

Indicators	Subgroups	GG (No. = 20)		GA (No. = 13)		AA (No. = 7)		Chi Test	P value (Sig.)
		Feq.	%	Feq.	%	Feq.	%		
Age	22-32	6	0.3	2	0.15	2	0.28	2.35	0.88 (NS)
	33-43	3	0.15	3	0.23	2	0.28		
	44-54	7	0.35	5	0.38	1	0.14		
	55-65	4	0.20	3	0.23	2	0.28		
Genotype (GA)	Male	6	0.3	5	0.38	2	0.29	0.31	0.85 (NS)
	Female	14	0.7	8	0.62	5	0.71		

4. Discussion

The inflammatory cytokines that played an important role within the periodontitis disease were investigated. Researchers detected serum IL-32 levels with gingival tissue in rats, and they found the other cytokines associated with periodontitis disease, such as IL-6 and IL-10 (Ferrara *et al*., 2025). IL-32 has an essential role in enhancing adhesion cells, controlling inflammatory processes, and regulating apoptotic cell pathways. As a result, it is protecting periodontal tissue from any bacterial pathogens. Also, IL-32 is an antimicrobial that decreases the development of periodontitis in the oral cavity, so it is involved as a barrier and defense in periodontal tissue from disease. Previous studies indicated that the polymorphism of sequence DNA such as mutation (SNP) may impact on pathogenesis of periodontal tissue (Brodzikowska *et al*, 2021).

In our results, after detecting the size of the product of the IL-32 gene (800 pb) by PCR assay, the sequence is done to evaluate our data. The data reported here appear to show no significant association with the patient group and control according to the rs4740: G>A genotype and the results in the GG and AA genotypes. Both gender and age recorded that there is no significant difference ($P > 0.05$) in IL-35 level polymorphism and periodontitis disease.

It is also found that the patients with periodontitis disease did not appear to have a strong relationship between serum, saliva, and GCF samples and variation genetics AA, GA, and groups. It seems possible that these results are due to genetic polymorphisms with robust associations with disease. Recent research has mentioned that VDR and FCGR3B genetic profiles were found to be significantly more associated with early-stage periodontitis disease than genotype polymorphism (Kaur, Rajinder, *et al*., 2018 & Zhao *et al*., 2016).

Although, de Albuquerque *et al*. (2021) found IL-32 levels in the GCF and saliva were detected in the periodontitis patients compared with healthy patients. In addition, IL-32 in serum was indicated as a marker for periodontitis disease (Abood and Fadhil, 2024). Our results showed that IL-32 polymorphisms may influence periodontitis in the population and that there is no significant relationship between genetic variation (SNP) and increased levels of IL-32 in periodontitis patients. A possible explanation for this might be that there are six subtypes of IL-32, but their role in periodontitis disease is not clear and requires more research in this field.

Our results are similar to those reported by Abood and Fadhil (2024); they found in 187 patients who have EBV infection no significant difference between the control and patients with allele T frequency, which has a P value of 0.160. Several studies have investigated that IL-17 (rs2275913) and IL-10 (819 and 592 SNPs) was not associated with periodontitis when compare with healthy individual (Brodzikowska and Górski,2022&Mazurek *et al*,2021). Although IL-32 can play an important role as an anti-inflammatory in healthy gingival tissue, the nucleotide polymorphism (SNP) is not related to the development of disease (Rasool *et al*., 2018). Taken together, these findings could help to further define periodontal disease and understand the background of genetics. The limitations pointed out in this paper are that a small sample of patients was not enough to evaluate the relationship between periodontitis and polymorphism IL-32.

5.Conclusion

The polymorphism of IL-32 did not show a statistically significant association between the GG, GA, and AA genotypes and the periodontitis disease. The observed differences in median values were not statistically meaningful due to high intra-group data variability and limited statistical power. While these findings do not support a direct genetic link at this IL-32, they provide valuable scientific data that help to refine future research directions. This paper serves as a detailed and authoritative scientific analysis of the provided data, guiding further inquiry into the complex regulation of IL-32

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