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International Journal of Dentistry and Oral Science (IJDOS)
ISSN: 2377-8075

Assessment of Interleukin-33 Gene Variants and Protein Level in Periodontal Disease among Saudi Population

Research Article

Fathy M. Elfasakhany[1,2,*], Zahra Alashur[3], Ebtehal H. Ali[1]

[1] Department of Basic and Clinical Oral Sciences, Faculty of Dentistry, Umm Al Qura University, Makkah, Saudi Arabia.
[2] Department of Medical Biochemistry, Faculty of Medicine, Tanta University, Tanta, Egypt.
[3] Dental intern program, Faculty of Dentistry, Umm Al Qura University, Makkah, Saudi Arabia.

Abstract

Background: Periodontal disease is an infectious disease that affects the teeth supporting structures and finally may lead to tooth loss. Interleukin-33 (IL-33) is a recently identified member of the IL-1 family and it was reported to have a role in immune response, bone homeostasis and osteoclastogenesis.
Objectives: To analyze and correlate the levels of IL-33 in the plasma and the gingival crevicular fluid(GCF) and the intronic single nucleotide polymorphism (rs1929992) in the IL-33 genein a group of Saudi individuals with moderate to severe periodontitis and a healthy control group.
Materials and methods: Seventy unrelated individuals with moderate to severe periodontitis and seventy healthy subjects were included in this study. The periodontal status was assessed based on plaque index, probing depth, bleeding on probing and clinical attachment loss. IL-33 levels were estimated in the GCF and plasma in both groups by ELISA. Peripheral blood was utilized for preparation of genomic DNA that was used for genotyping of IL-33 A/G (rs1929992) using polymerase chain reaction followed by restriction endonuclease digestion.
Results: IL-33 concentration was higher- but not statistically significant- in the GCF of the periodontitis subjects in comparison with the controls ($P > 0.05$) while the plasma level was the same in both groups ($P > 0.05$). For the IL-33 rs1929992 polymorphism, the frequencies of the IL-33 genotypes and alleles were indifferent between the control group and the periodontitis group ($P > 0.05$).
Conclusion: The levels of plasma and GCF of IL-33 could not differentiate between subjects with periodontitis and healthy controls. In addition, the results suggested that IL-33 rs1929992 polymorphism may not have a risk of periodontitis among the Saudis in Makkah environ.

Keywords: Gene Polymorphism; Periodontitis; Interleukin-33; Polymerase Chain Reaction; Restriction Enzyme; ELISA.

Introduction

Periodontitis is an infectious inflammatory state of the tissues surrounding the teeth [1]. The aetiology of this complex disease is determined by both genetic and the environmental factors (e.g., smoking and stress) [2, 3]. The most prominent clinical features of the condition are development of microbial plaque, periodontal inflammation and destruction of periodontal tissue and alveolar bone [4]. The incidence and the severity of the disease rise with the age and usually influences every gender evenly. Meanwhile, the periodontal disease is noticeably detected between family members and across different generations within the same family, suggesting that there is a genetic susceptibility to the periodontal disease [5, 6]. Besides, numerous studies reported the role of gene polymorphisms of several biomolecules especially cytokines in the host response in periodontitis, and in the progress of the disease. Such gene polymorphisms may result in a change in the protein structure or its expression and probably lead to alteration in the innate and adaptive immunity and may thus be deterministic in the outcome of the disease [7]. Cytokines are small polypeptides that have wide range of inflammatory, hematopoetic and immunomodulatory effects. They are produced by several types of cells including lymphocytes, dendritic cells, monocytes, neutrophils, fibroblasts and endothelial cells [8]. Cytokines play

***Corresponding Author**:
Fathy M. Elfasakhany,
Department of Basic and Clinical Oral Sciences, Faculty of Dentistry, Umm Al Qura University, Makkah, Abdia,715, Saudi Arabia.
E-mail: fmfasakhany@uqu.edu.sa

Received: October 07, 2020
Accepted: October 21, 2020
Published: November 10, 2020

Citation: Fathy M. Elfasakhany, Zahra Alashur, Ebtehal H. Ali. Assessment of Interleukin-33 Gene Variants and Protein Level in Periodontal Disease among Saudi Population. *Int J Dentistry Oral Sci.* 2020;7(11):997-1002. **doi: http://dx.doi.org/10.19070/2377-8075-20000198**

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a valuable role in inflammatory conditions like periodontitis and rheumatoid arthritis. Furthermore, it has been reported that IL-1 family cytokines play key roles in inflammation. Recently, it has been suggested that IL-33, an IL-1 family member, could share in the development and sequence of chronic periodontitis [9]. It acts as an alarmin, chemoattractant, and nuclear factor and has been defined to control both innate and adaptive types of immunity [10]. It is constitutively expressed as a nuclear factor in different cells such as fibroblasts, endothelial cells and epithelial cells. Within the nucleus, IL-33 behaves as an endogenous molecule that help to maintain the transcription factor NFκB and thus lowers the expression of genes that encode the inflammatory cytokines thereby guaranteeing tissue homeostasis. Once became extra cellularly upon cell damage, IL-33 works as an alarmin exhibiting proinflammatory properties [11, 12]. IL-33 performs its action through its ST2 receptors that are expressed in several cells like Th2 lymphocytes, B cells and mast cells resulting in production of pro-inflammatory cytokines [10, 13, 14]. In human, excess IL-33 expression was detected in the gingival tissue of subjects with chronic periodontitis and may work as a driving factor to recruit of B and T lymphocytes that express RANK-L [15]. However, measuring the IL-33 in the GCF showed conflicting results in subjects affected by chronic periodontitis [16-18]. Furthermore, the study of IL-33 polymorphisms and their association with periodontitis were poorly studied in different ethnic populations and to the best of our knowledge, it is not examined before in Saudi Arabia population. Therefore, we aimed in this study to estimate IL-33 concentrations in the GCF and plasma of the study group and to analyze the relationship between the IL-33 A/G (rs 1929992) single-nucleotide polymorphism (SNP) and the susceptibility to periodontitis among Saudis in Makkah environ.

Materials and Methods

Study population

This study included 140 subjects (70 unrelated subjects affected with moderate to severe periodontitis and 70 healthy controls) were recruited from the dental clinic, school of dentistry, Umm AL Qura University, Saudi Arabia. All participants were Saudis. Both groups were matched regarding the age and gender and had at least 20 teeth. The inclusion criteria for selection of the control group were the absence of both periodontal and systemic diseases. The exclusion criteria include systemic diseases, immunodeficiency diseases, previous orthodontic therapy, pregnancy, lactation and smoking. The sample size was determined based on a previous report with respect to the relationship of the IL-33 polymorphism with periodontal disease [19]. The sample size was expanded by approximately 40% to keep up the estimates at an optimal degree of precision (5%) against the expected impact of reduction of sample size because of rejections and dropouts.

Periodontal examination

The periodontal condition of all individuals was assessed based on bleeding on probing depth (PD), probing (BOP), plaque index (PI), and clinical attachment loss (CAL) by two well trained assessors. Individuals with BOP, PD ≥ 5 mm, CAL ≥ 3 mm and radiographic proof of bone damage ≥ 20% were involved in the patient group. The updated report of the American Academy of periodontology was used to characterize the periodontitis [20].

GCF samples collection

Two to four sites/individual were sampled to obtain a suitable volume of GCF in healthy subjects. For subjects affected with moderate to severe periodontitis, GCF was collected from two sites with the highest CAL and maximum bleeding score along with radiographic evidence of alveolar bone resorption. GCF samples were collected using microcapillary pipettes as described before [21]. The sample collection area was air dried and protected from saliva contamination by isolating it with sterile cotton rolls. A universal curette was used to remove the supragingival plaque to avoid contamination and obstruction of the microcapillary pipette by plaque. 10 µl volumetric microcapillary pipette (Microcapillary pipettes, Drummond Scientific Company, Broomall, USA) was inserted into the gingival sulcus for GCF collection. The collected GCF was mixed with a suitable volume of phosphate buffered saline in a new plastic tube and the dilution range was recorded to be considered during measurement. The GCF samples contaminated with saliva or blood were excluded and discarded. The GCF specimens were kept at -20°C untill the assay time.

Blood sample collection

Blood specimens were obtained from all participants in K3EDTA (tri-potassium ethylene diamine tetraacetic acid) tubes. The tubes were centrifuged at 1200 g for 5 min and the plasma was transferred to a new plastic tube. The white cells buffy coat was used for extraction of DNA. All samples were kept at -20˚C until the assay time.

IL-33 assay

IL-33 levels were measured in the GCF and plasma samples using commercially available human IL-33 enzyme-linked immunosorbent assay (ELISA) kit (ABCAM, Cambridge, USA). Analysis was performed following the manufacturer's protocol.

Genomic DNA extraction

Peripheral blood leukocytes in the white coat of collected blood samples were used for preparation of DNA using DNA extraction kit (QIAamp, Qiagen, Hilden, Germany) consistent with the instructions of the manufacturer. Extracted DNA was utilized for PCR experiment.

IL-33A/G(rs1929992) genotype frequencies

IL-33 polymorphism was analyzed using the PCR-restriction fragment length polymorphism as described before [22]. The primers were forward 5'- GAAGTCATCATCAACTTGGAACC-3' and reverse 5'- GGATTGGAATCCCATGGTC-3'. The PCR program consisted of a denaturation step at 94°C for 5 min, 35 cycles: 94°C 30sec, 61 °C 30sec, 72 °C 30sec and an extensionstep at 72°C for 5 min. The PCR fragment (217 bp) was digested with SspI restriction enzyme and then visualized under ultraviolet light. Genotypes pattern were: G/G gave a single band of 217 bp; A/A gave 2 bands of 134 bp and 83 bp and AG gave 3 bands of 217 bp, 134 bp and 83 bp.

Statistical analysis

Data were assessed using SPSS version 21 (SPSS Inc., IL, USA). Continuous variables were assessed using Student's t-test, whereas the categorical data were assessed using Chi-squared test. Odds ratio (OR) was calculated with 95% confidence interval (CI) and P value < 0.05 was considered statistically significant for all analyzes.

Results

Clinical assessment

Table 1 shows that the control subjects and patients with periodontitis were matched regarding the age and gender. The mean age was 40.27 ± 4.75 and 41.35 ± 3.76 in controls and periodontitis respectively (*P* =0.136). The gender (M/F) was 40/30 and 39/31 in controls and periodontitis respectively (*P* =0.865). The clinical data showed that the mean values of BOP, CAL, PI and PD were significantly higher in subjects with periodontitis in comparison with the controls (*P* < 0.001). This confirmed that the matching between both groups was appropriate (Table 1).

GCF and plasma IL-33 levels

The results of interleukin 33 level in GCF and plasma are shown in table 1. IL-33 was detected in all GCF and plasma samples. The mean concentration of IL-33 in GCF was19.4 ± 3.55pg/ml and 20.82 ± 3.29pg/ml in controls and periodontitis subjects respectively (*P*=0.169). The mean concentration of IL-33 in plasma was 15.87 ± 3.64 pg/ml and 16.28 ± 3.79 pg/ml in the controls and periodontitis subjects respectively (*P*=0.331).

IL-33A/G (rs1929992) polymorphism

The genotypes and alleles frequencies of IL-33A/G polymorphism in both control and periodontitis groups are shown in Table 2. The distribution of IL-33 genotypes was in Hardy-Weinberg equilibrium in both groups. For the control subjects, the genotypes GG, AG and AA were 17.14 %, 32.86 % and 50 % respectively and were 12.86 %, 38.57 % and 48.57 % respectively in the periodontitis group. The percentages of G allele were 33.57 % and 32.14% while A allele was 66.43 % and 67.86% in the control group and patients with periodontitis respectively. The genotype and allele frequencies of the IL-33 showed no significant differences between the controls and subjects with periodontitis (*P* > 0.05).

GCF and plasma IL-33 levels in different genotypes

The level of the GCF IL-33 in the GG, AG, AA genotypes of the controls were 19.42 ± 3.87, 19.39 ± 3.97 and 19.4 ± 3.25 while

Table 1. Demographic, clinical date and IL-33 level in the study population.

Characteristics	Control group (70)	Periodontitis group (70)	*P* value
Age (years)	40.27 ± 4.75	41.35 ± 3.76	0.136
Gender (M/F)	40/30	39/31	0.865
BOP (%)	8.71 ± 1.12	46.04 ± 9.41	<0.001
PD (mm)	1.22 ± 0.51	5.25 ± 0.71	<0.001
CAL (mm)	0.72 ± 0.21	4.49 ± 0.59	<0.001
PI (%)	4.76 ± 0.45	47.94 ± 3.3	<0.001
GCF IL-33 (pg/ml)	19.40 ± 3.55	20.82 ± 3.29	0.169
Plasma IL-33 (pg/ml)	15.87 ± 3.64	16.28 ± 3.79	0.331

Data are shown as mean ± SD. *P*-value < 0.05 was considered as significant.

Table 2. Genotype and allele frequencies of IL-33 A/G Single Nucleotide Polymorphism in the study group.

	Control (n=70)		Periodontitis (n=70)		*P value*	Odds ratio	95% CI
	No	%	No	%			
***IL-33* polymorphism**							
Genotypes:							
GG	12	17.14	9	12.86		1	
GA	23	32.86	27	38.57	0.391	1.565	0.56-4.374
AA	35	50	34	48.57	0.866	0.944	0.487-1.832
Alleles:							
G	47	33.57	45	32.14		1	
A	93	66.43	95	67.86	0.606	1.295	0.484-3.467

Chi-square analysis of genotypes between patients with periodontitis and healthy controls. **P*-value < 0.05 was considered as significant.

Table 3. Comparison between IL-33 (rs1929992) geno types with respect to GCF and plasma IL-33 concentrations in the study group.

Study group	IL-33 polymorphism (rs1929992)	Number	GCF concentration	χ2	*P*	Plasma IL-33 concentration	χ2	*P*
Control group	GG	12	19.42 ± 3.87	0.157	0.929	16.5 ± 3.09	1.127	0.569
	AG	23	19.39 ± 3.97			16.22 ± 3.25		
	AA	35	19.4 ± 3.25			15.43 ± 4.08		
Periodontitis group	GG	9	20.78 ± 4.44	1.094	0.579	16.56 ± 4.69	0.323	0.851
	AG	27	20.92 ± 2.85			16.93 ± 4.12		
	AA	34	20.32 ± 3.36			16.12 ± 3.33		

*Kruskal–Wallis test, P<0.05 considered as statistically significant. Data are shown as mean ± SD.

they were 20.78 ± 4.44, 20.92 ± 2.85 and 20.32 ± 3.36 in the periodontitis group respectively. The plasma level of IL-33 in the GG, AG, AA genotypes were 16.50 ± 3.09, 16.22 ± 3.25 and 15.43 ± 4.08 in the control group while they were 16.56 ± 4.69, 16.93 ± 4.12 and 16.12 ± 3.33 in the periodontitis group respectively. The GCF IL-33 level was indifferent between different genotypes in the controls (*P* = 0.929) and the periodontitis group (*P* = 0.579). In addition, the plasma IL-33 level was indifferent between different genotypes in the controls (*P* = 0.569) and the periodontitis group (*P*=0.851).

Discussion

Periodontitis is a chronic inflammation of the tooth surrounding tissues that results in damage of the tooth surrounding tissue and alveolar bone due to the interaction of the host immune response to the pathogenic bacteria [1]. The existence of periodontopathic bacteria in combination with elevated levels of the proinflammatory cytokines and low levels of the inflammation inhibitory cytokines and other factors cause the progression of periodontal disease [23]. It is obvious that the cytokines do not work separately but rather as a part of complex networks with diverse activities [24]. Cytokines are released during periodontitis as a result of the immune cells infiltrating the periodontal tissue, which eventually causes periodontal tissue damage [25]. IL-33 is a newly identified member of IL-1 cytokine family. It can work both as a soluble mediator and a nuclear factor. IL-33, as a cytokine, functions as an alarmin which is released upon damage of epithelial or endothelial cells and it can target multiple types of cells thus making the immune system alert to endogenous trauma like infection [26]. On the other hand, it has been shown that there was a relationship between cytokines single nucleotide polymorphisms (SNPs) and the periodontal disease. Such Polymorphisms can alter cytokines production, which in turn may lead to changes in the immune responses and can lead to chronic inflammations [27]. As the IL-1 cytokine family is associated with chronic inflammatory and autoimmune disorders such as rheumatoid arthritis and periodontitis [28, 29], we aimed to estimate the IL-33 levels in the GCF and plasma of a group of Saudi individuals with moderate to severe periodontitis and a control group. Furthermore, since IL-33 is a novel cytokine and its gene polymorphisms were poorly studied in the periodontal disease, we investigated the association of the IL-33 A/G rs1929992 SNP and the periodontitis in the study group. The GCF IL-33 level was higher-but not statistically significant-in the periodontitis subjects than the controls while the plasma level of IL-33 was similar in both groups. For the IL-33 A/G rs1929992 polymorphism, we found no association between IL-33 polymorphism and the risk of periodontitis in the studied Saudi subjects. The GCF and the plasma concentrations of IL-33 were measured in the periodontal disease by several investigators but the results showed remarkable inconsistency. Ballambettu et al (2019) could detect IL-33 in all examined samples and they found that both GCF and plasma concentrations of IL-33 were significantly higher in the aggressive periodontitis than chronic periodontitis or the controls but were indifferent between chronic periodontitis and the controls [19]. This result was consistent with our results regarding chronic periodontitis. Buduneli et al, (2012) found a significant lower level of the IL-33 in the GCF in chronic periodontitis than subjects with healthy periodontium, whereas the total amounts were similar in both groups but the plasma and salivary concentrations of IL-33 showed no difference between the studied groups. They concluded that the IL-33 concentrations in the GCF, saliva or plasma cannot differentiate between chronic periodontitis and healthy individuals [30]. Their results were consistent with our results regarding the plasma IL-33 level. They stated that the episodic nature of the periodontal disease may be a factor and indicated that it could be considered that the quiescent form of periodontal tissue damage in chronic periodontitis subjects lead to absence of significant difference between chronic periodontitis subjects and periodontally healthy subjects in terms of IL-33 concentrations in the biologic fluids. Furthermore, they found that the IL-33 concentration in the GCF was significantly lower in chronic periodontitis subjects than in control subjects. They pointed out that the lower volume of the GCF specimens in the periodontally healthy subjects may explain the significantly higher IL-33 level in the GCF in the healthy subjects than in the chronic periodontitis. Sağlam et al, (2017) found that the total amount of the IL-33 in the GCF was higher in chronic periodontitis compared to the controls while the GCF IL-33 level was lower in chronic periodontitis subjects compared to the controls. They also found that the plasma and salivary IL-33 levels were indifferent between chronic periodontitis and the control groups [16]. This result is consistent with our results regarding the plasma level of the IL-33 but the GCF IL-33 concentration differs from our results. The lower level of GCF samples in chronic periodontitis might be not only due to the GCF volume but may depend also on the sampling site. For this reason, we tried to avoid collection of GCF specimens from quiescent areas, and instead, we collected all GCF samples from the sites with the highest CAL and maximum bleeding score together with radiographic evidence of

alveolar bone resorption. Another study done by Papathanasiou et al, (2014) measured the IL-33 in the GCF using multiplex assay and they could not detect IL-33 in the GCF of all studied samples from chronic periodontitis subjects and subjects with healthy periodontium and they concluded that there is no association between IL-33 and the periodontal disease and they indicated that the presence of auto antibodies may produce non specific bindings which can prevent the identification of the cytokines in the biological specimens using multiplex assay [17].

Kurşunlu et al, (2015) measured the IL-33 and other cytokines in the GCF in the individuals with gingivitis, chronic periodontitis, generalized aggressive periodontitis and healthy group and they found that the levels of IL-33 in the GCF were indifferent between all studied groups [18]. Their results were also consistent with our results.

Gümüş et al, (2017) showed that the salivary concentration of IL-33 was higher in the chronic periodontitis group even than aggressive periodontitis while serum IL-33 level was indifferent and they could not definitely explain their results but thought that both diseases may have separate pathways and so, they suggested further research [31].

Regarding IL-33 A/G rs1929992 polymorphism, it was studied in several chronic diseases such as systemic lupus erythematosus [32], systemic sclerosis [33], peptic ulcer [34], Behçet's disease [35] and ischemic stroke [36] but not in periodontitis according to our knowledge. We found no association between IL-33 A/G rs1929992 genotype or allele frequencies and the periodontitis in Saudis in Makkah environ. To our knowledge, this is the first report that studied the association between IL-33 A/G rs1929992 polymorphism and the periodontal disease among Saudi subjects.

However, this study is not without limitations especially the small sample size. So, further research will be required using a large periodontitis cohort with clinical data, other methods like evaluation of gene expression of the IL-33 in the gingival tissues or intervention studies are needed to understand the role of the IL-33 in periodontal disease.

Conclusion

Our findings suggested that the GCF and the Plasma IL-33 cannot differentiate between the subjects with healthy periodontium and those with periodontal disease. Also, we concluded that the IL-33 A/G rs1929992 polymorphism may not be associated with the risk of periodontitis among Saudis in Makkah environ, however more genetic analysis with larger sample size and other SNPs and haplotype analysis are required to elucidate the role of IL-33 in the pathogenesis of periodontitis.

Ethical Statement

The institutional review board of the Faculty of Dentistry, Umm Al Qura University, Saudi Arabia approved the study. These guidelines of the IRB follow the Saudi and International guidelines that follows the Declaration of Helsinki in 1995 (as revised in Fortaleza, Brazil, October 2013). Informed consents were obtained from all participants.

Acknowledgment

We thank Dr. Abd Elrahman Sabry for his help in the research unit, Um Al-Qura University, Saudi Arabia. Also, we thank Prof. Abd Elaziz Y. for his help in the section of statistics.

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