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ASSOCIATION OF IL-17A POLYMORPHISMS IN TYPE-2 DIABETES WITH CHRONIC PERIODONTITIS PATIENTS

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IL-17A Polymorphism, Chronic Periodontitis, Type-2 Diabetes Mellitus.

Abstract

Introduction: The association of genetic polymorphisms with periodontitis has been studied extensively. The IL-17 is a group of cytokines which comprises six different molecules (IL-17A, B, C, D, E & F). Among these IL-17A & F are the most commonly understood cytokine which plays a critical role in inflammatory diseases and periodontal inflammation. Objective: To evaluate whether Interleukin -17A gene polymorphism has any influence on the development of periodontitis in type 2 diabetes patients. Materials and Methods The study was carried out in 60 subjects and consists of 4 groups which included, Group A: 15 Type 2 diabetes patients with chronic periodontitis, Group B: Type 2 diabetes patients without periodontitis, Group C: 15 Nondiabetic patients with periodontitis, Group D: Nondiabetic patients without periodontitis. Blood samples were drawn from the subjects and analyzed for IL-17A polymorphism by using the conventional polymerase chain reaction method. Results: No statistically significant differences seen in the genotype distribution among chronic periodontitis patients with or without diabetes and healthy controls. The odds ratio indicated that increased risks for CP were associated with the rs2275913 allele (IL-17A) in patients with or without diabetes. This allele was correlated with worse clinical parameters of chronic periodontitis in diabetes patients. Conclusion: These results showed that the IL-17A (rs2275913) polymorphism is associated with increased risk for chronic periodontitis in type 2 diabetes patients.

Introduction

The periodontitis is an inflammatory disease resulting in loss of soft and hard tissues around the tooth. It is caused by multiple factors by like microbial infection and risk factors such as diabetes, plaque control, host response and genetics[1]. The presence of gram negative bacteria is mandatory to initiate and progress the periodontal diseases[1]. The inflammatory mediators are released from host cells which is a part of host defense mechanism leads to the host-microbial interaction. The role of genetic factors in the immune response is important for maintenance and progression of diseases[2]. The IL-17 is a group of cytokines which comprises six different molecules (IL-17A, B, C, D, E & F). IL-17 has pleiotrophic activities like activation of T cells, fibroblasts, osteoclast and dendritic cells maturation by inducing the expression of pro- inflammatory cytokines and metalloproteinases[3]. In this regard, IL-17A & F are the most commonly understood cytokine which plays an important role in inflammatory diseases and periodontal inflammation[4]. The association of genetic polymorphisms with periodontitis has been studied extensively[5,6,7,8]. The IL-17A polymorphism was associated with chronic periodontitis (CP) and aggressive periodontitis and are more frequent in type 2 diabetes mellitus (T2DM) patients and can worsen its metabolic control[9,10,11]. Nevertheless, molecular mechanisms responsible for periodontal disease and its progression in T2DM patients remain unknown. Despite the important role of IL-17 cytokine in T2DM and CP pathogenesis, so far no study has investigated IL-17 gene polymorphism inT2DM patients[12,13]. Only a few studies have reported a relationship between IL-17A polymorphisms and periodontitis that showed contradictory results. The present study was designed to evaluate whether Interleukin -17A gene polymorphism has any influence on development of periodontitis in type 2 diabetes patients.

Materials

The study was performed with the approval of the Committee for Ethics, JKKN Dental College and Hospital and written informed consent was obtained from all the participants in line with the ethical declaration before inclusion in the study. The study was carried out in 60 subjects and consists of 4 groups which included as, Group A: 15 Type 2 diabetes patients with periodontitis, Group B: Type 2 diabetes patients without periodontitis, Group C: 15 Non

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diabetic patients with periodontitis, Group D: Non diabetic patients without periodontitis. The inclusion Criteria for Type 2 Diabetes were both male or female patients, Age group between 35-48 years, those who are already diagnosed with Type 2 diabetes, (both uncontrolled and controlled diabetic), T2DM both undergoing or not undergoing treatment were included in the study. Inclusion Criteria for Periodontal parameters were based on AAP criteria of Generalized Chronic periodontitis: Presence of clinical attachment loss more than 3 mm in at least six sites, Pocket probing depth more than 4 mm at least six sites, bleeding on probing must be there in more than 30% of sites. The exclusion Criteria were Smokers, Existing orthodontic therapy, Aggressive Periodontitis, General Health problems - Hepatitis, HIV infection, Chemotherapy, Pregnancy and lactation, Non –Indian races.

Methodology- Clinical Parameter & DNA Extraction

Priodontal parameters were measured using Williams probe and the gingival Index was measured. Diabetic Parameters were monitored with HbA1c and Fasting Glucose level. The Sample of 5 ml venous blood was collected from cubital fossa of each subject and transferred to the laboratory in a falcon tube containing ethylene diamine tetraacetic acid (EDTA) which stored it -20^0 C for DNA Separation. The 200 µl of blood sample collected into micro centrifuge tube and 600 µl of RBC cell lysis solution was added.

Picture 1, 2: Test tubes with RBC lysis followed on centrifuging

The uniformity in sample mix is obtained by inverting the tube several times and flicking the bottom of the tube. The sample is incubated in room temperature for 5 minutes and shaken briefly. Again it is incubated in room temperature for 5 minutes followed by brief agitating the tube. The centrifuging was done for 25 seconds with 4000 rpm at 4º C. The supernatant was removed leaving approximately 25 µL of liquid in the tube, followed by adding of 300 µl of tissue and cell lysis solution by pipetting the cells several times. Then 1µ L RNase A solution added and mixed thoroughly and incubated at 37º C for 30 minutes. The samples were cooled by placing on the ice for 3to 5 minutes. Then 150 µl of MPC protein precipitation reagent was added to 300 µl of lysed sample. Again the test tube is agitated briefly. The centrifugation is done at 4º C for 10 minutes at 10,000 rpm. If the resultant pellet is clear or loose, again add 25 µl of MPC protein precipitation reagent mix, and remove the debris. Transfer the supernatant to a clean micro centrifuge tube and discard the debris. Then Add 500 µl of Isopropanal to the recovered supernatant. The tube was inverted for 30-40 times. Then keep it for centrifugation at 4º C for 10 minutes. Carefully pace off the isopropanol without dislodging the DNA pellet. The final pellet was rinsed twice with 70 percent ethanol without dislodging. If the pellet is dislodged, Centrifuging is done briefly. Remove all the residual ethanol with pipette and resuspend the DNA in 35 µl of TE buffer.

DNA Amplification by Polymerase Chain Reaction

The primer pair (Bioserve, Beltsville,USA) used for this study to check IL-17A polymorphism was,
Sense: IL-17AF
5´-AACAAGTAAGAATGAAAAGAGGACATGGT-3´
Antisense:IL-17AR
5´-CCCCCAATGAGGTCATAGAAGAATC-3´

The amplification was performed with a conventional PCR system with cycle consists of an initial denaturation step at 95º C for 15 minutes, a denaturation step of 35 cycles at 94º C for 30 s, and annealing step of 35 cycles at 57º C

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for 30 s. Then, extension step was on 35 cycles at 72º C for 30 s and final extension step at 72º C for 10 minutes. The PCR products were incubated overnight at 37º C with XagI (Waltham, USA). The bands were viewed in a 6.5% polyacrylamide agarose gel electrophoresis system stained with silver.

Picture 3: DNA Standardization by Gel Electrophroresis

Statistical Analysis

The distribution of the IL-17A genotype and Allelic variations in the groups were measured by using the Chi-Square test. The comparisons of periodontal parameters between the groups (Mean ± SD) were analyzed by One way ANOVA test.

Results

The genotype frequency distribution of IL-17A polymorphism in each groups were within Hardy-Weinberg equilibrium. The clinical parameters of three types of genotypes (AA,AG,GG) were compared in T2DM with chronic periodontitis patients and only periodontitis. The genotype and allelic distributions of the IL-17A polymorphisms are shown in table (Table 1, 2). In this, IL-17A polymorphism showed no statistical significant difference among the study groups (Control (D) vs Diabetes with CP (A) and Control (D) Vs Chronic Periodontitis (C)) based on genotype distribution. (Table 1)

Table 1: Distribution of the IL-17A genotypes in the groups

IL-17A	Group A n=15	Group B n=15	Group C n=15	Group D n=15	D vs A (p value)	D vs B (p value	D vs C (p value	A Vs B (p value
AA (%)	0	0	1	2	0.0072	0.0154	0.0096	0.234
AG (%)	5	6	6	6				
GG (%)	10	9	8	7				
H-W (P value)	0.5643	0.3987	0.1276	0.9764				

P < 0.05. The chi-square test was used. H-W: Hardy-weinberg equilibrium

Control vs diabetes with periodontitis: OR=1.080, CI=0.0434- 1.1051
Control vs diabetes without periodontitis: OR=0.0506, CI=0.0674-0.8051
Control vs chronic periodontitis: OR=1.040, CI=0.0344-1.095
Diabetes with CP vs diabetes without CP OR=0.0613, CI=0.0564-0.7051

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We found high frequency of AA genotype in CP (P= 0.00154) when compared to the healthy group (D). No statistical different was found among the groups in genotype variation (AA, GG, and AG). Considering the allelic distribution of the IL-17A gene, the higher frequency of A allele was observed in group C (p=0096) and also in group A when compared with group D.

Table 2: Distribution of the IL-17A alleles in the groups

IL-17A	Group A	Group B	Group C	Group D	D vs A (p value)	D vs B (p value)	D vs C (p value)	A vs B (p value)
A(%)	5	6	4	7	0.0042	0.0125	0.0076	0.563
G(%)	10	9	11	8				

P < 0.05. The chi-square test was used

Control vs diabetes with periodontitis: OR=1.0306, CI=0.0374-1.2051
Control vs diabetes without periodontitis: OR=0.0631, CI=0.0521-0.8351
Control vs chronic periodontitis: OR=1.1426, CI=0.0574-1.2951
Diabetes with CP vs diabetes without CP OR=0.0736, CI=0.084-0.751

There was no statistically significant result when considering the genotype and clinical form of CP but when dealing of allelic distribution, the A allele was correlated with worsened clinical form of chronic periodontitis. There was a no significant different seen in the genotype distribution among the chronic periodontitis patients with or without diabetes and healthy controls. The Odds ratio indicated that marginal risks for CP were associated with rs2275913 allele (IL-17A) in patients with or without diabetes. This allele was correlated with worse clinical parameters of chronic periodontitis in type 2 diabetes patients.

Discussion

Regulation of inflammation is a complex process, tightly controlled by signaling messengers of the immune system, such as cytokines. IL-17A and IL-17F, produced mainly by Th17 cells, have been found to be involved in the pathogenesis of autoimmune diseases including diabetes and chronic inflammation, such as periodontitis[11]. IL-17A is a potential inflammatory cytokine which contributes to several auto immune and inflammatory diseases including T2DM [9]. The previous study revealed that the serum levels of IL-17A were increased in the Iranian T2DM patients[14]. Interestingly previous literature has shown that the ratio of T regulatory/Th17cells was decreased in patients with T2DM[12]. The results suggested that IL-17A cytokine played important role in the pathogenesis of T2DM. We evaluated IL-17A −197A/G (rs2275913) polymorphism in a group of T2DM patients and/or CP from the population. The present study revealed there was no association of this polymorphism with CP.

Although no significant differences were found in the genotype frequencies between the healthy subjects and T2DM with or without CP patients, the A allele was marginally associated with an increased risk of T2DM ($P < 0.05$). Three Brazilian studies examined variability in theIL-17A (rs2275913) gene in relation to periodontal disease with controversial results[6,7,8]. In the study by Saraiva et al, the A allele was associated with the absence of periodontitis, but Correa et al. and Zacarias et al. found the AA genotype and the A allele as a risk factor for CP[8,15]. Gursoy et al. recently described the association between pocket depth (PD) and IL-17 levels in saliva of type 2 diabetic patients, but independently of glycemic status[13]. Increased expression of IL-17AmRNA was seen in the gingival biopsies of patients with T2DM suffering from chronic periodontitis[16]. In addition, we found no statistical significant differences in the IL-17A (rs2275913) genotype frequencies between the healthy subjects and T2DM patients with CP but the A allele was correlated with worse clinical parameters of chronic periodontitis in type 2 diabetes and chronic periodontitis patients without diabetes.

Conclusion

The present study evidenced that association of IL-17A expression and A allele frequency in IL-17 A rs2275193 gene considered to be a risk factor for development of chronic periodontitis in Type 2 Diabetes Mellitus Patients. Further studies needed to be done in large sample with inclusion of different ethnic groups and in proper selection method of polymorphism.

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