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Analysis of -819 IL-10 gene polymorphism in Syrian patients with aggressive periodontitis

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Abstract: Periodontitis is a multifactorial disease and its severe forms, such as aggressive periodontitis, are suggestedto have a genetic basis. Among the genetic factors, polymorphisms in cytokine genes have recentlybeen described in susceptibility to periodontitis. Interleukin-10 (IL-10) is a multi-functional cytokine thoughtto play a role in the pathogenesis of periodontitis. A substitution C/T polymorphism in the promoterregion of the IL-10 gene at position -819 has been associated with different amounts of IL-10production. The aim of the present study was to investigate the possible links between -819C/Tpolymorphism of the IL-10 gene in patients with aggressive periodontitis incomparison with periodontitis-free controls.This study included 83Syrian subjects suffering fromaggressive periodontitis referred to American Academy of Periodontology. They were compared to 79 age matched healthy controls. DNAwas isolated from peripheral blood cells and genotyping was performed by means of the amplification refractory mutation system polymerase chain reaction (ARMS-PCR) method. Data were analyzedusing the chi-squared test.There was no marked difference in genotype frequencies between the controls and aggressive periodontitis patients (p>0.05). Moreover, neithermarked difference was found in their allele frequency nor in allele carriage(p>0.05).These findings suggest thatthe investigated polymorphism is not associated with periodontal diseasein this studied sample of Syrian population.
Keywords: Aggressive Periodontitis, IL-10, Polymorphism, ARMS-PCR.

Introduction

American academy of periodontology (AAP) 1999 [1] defined Periodontitis as a disease comprises variety of conditions affecting the health of periodontium for people who often appear to be healthy and it trends to show familial aggregation and advanced progression.It has two forms; localized or generalized. It's considered as one of complex diseases which determined by many factors and biological pathways as palance between proinflmatory cytokines and anti-inflammatory cytokines. This balance plays roles in many complexes, autoimmune, Infectious and malignant diseases.studies have revealed that simple change in the structure of coding genes of these cytokines may alter the expression level and then type of immune response[2].

The gene of IL-10 islocalized on the chromosome (1) and containing 5 exon a long 5.2 Kb [3] . Many researchers suggested contribution of IL-10 in periodontitis in adults where it was found that T-cell in peripheral blood and gingival tissue secretsIL-10on contrary of the clones were taken from the peripheral blood and gingival tissue upon gingivitis patients [4].

IL-10 is secreted bymany cell types and strongly modulates the expressions ofother cytokines, soluble mediators, and cell-surface moleculesin cells of myeloid origin [5]. It is an anti-inflammatory cytokine synthesized by activated monocytesand T lymphocytes in response to inflammation. It has aninhibitory effect

on the production of IL-1-alpha and -beta,TNF-alpha, IL-6, IL-8, IL-10 itself, IL-12 and many others secreted proteins[6]. Besides this inhibitory effecton production of many cytokine, IL-10 may also promote inflammatoryresponses through effects on B cells, stimulating both proliferationand differentiation [7]. IL-10 is known to regulate the balance betweenacquired humoral response and innate inflammatory response.

Individual capacity for IL-10 production appearsto be under genetic influence [8]. Genotypic variationsin cytokine response have been shown *in vitro* for IL-10, and specific alleles are implicated in diseases such assystemic lupus erythematosus[9]and rheumatoid arthritis[10].The promoter region of IL-10 gene exhibits high frequenciesof single-nucleotide polymorphisms (SNP).The three IL-10 promoter region SNPsmostly described in literature are –592(C/A), –819(C/T), and –1082(G/A). Several studieshave investigated IL-10 promoter polymorphisms at these positions inpatients with periodontitis comparedwith healthy controls.The resultswere partially inconsistent.For instance, several previous studies [11][12]failed to demonstrate associationsbetween periodontitis and polymorphism at–819in the IL-10gene. By contrast, Abou sulaiman[13], Reichert[14] and Hu[15] have found that the allele T at position –819 was more expressed in aggressive periodontitis patients.Therefore, the aim ofthe present study was to examine theIL-10promoter polymorphismsat positions -819 (C>T) in Syrian unrelatedpatients suffering fromaggressive periodontitis.

Materials and Methodology

Study population

This study was approved by the Ethics Committee of Damascus University. In this study, 83Syrian subjects suffering from AGP attending thePeriodontology Department of Faculty of Dentistry at Damascus Universitywere included. Additionally, 79 race-matched healthy subjectswere recruited as a control group.The mean age of the AGP group was 25.07 years (range: 18–35) and that of the control group was 22.52 years (range: 20–52).(24 %) Of the 83 patients, were female and (59 %) weremale. (17 %) Of the 79 control subjects, were femaleand (62 %) were male. The patients were informed about the purpose and the method of the study, and their consents to participate were obtained.

Including criteria

AGP patients must be<35 year,the amount of microbial deposits does not justify the severity of periodontal tissue destruction, have at least 20 teeth other than third molars, All patientsmust beSyrians.

Excluding criteria

Who has less than 20 teeth(other than third molars),any systemic disease may alter the immunity responses,ortho treatment,pregnancy,breast feeding,none Syrians.

Clinical examination

Nocase that produced doubt in classification was included in thestudy. Diagnosis of disease was made considering the patient'smedical and dental histories, radiographic findingsand observation of clinical signs and parameters includingprobing depth, assessment of clinical attachment loss (CAL),observation of tooth mobility, bleeding on probing and presenceof plaque/calculus. Clinical diagnosis of periodontitis

was based on criteria established in 1999 at the InternationalWorkshop for a Classification of Periodontal Diseases andConditions[1]. Measurements of probing depth(PD) [16] and Clinical Attachment Loss (CAL)[16]were assessed at six locations around each tooth. The severityof disease was characterized on the basis of the mean ofCAL, within each clinical form. Assessment of CAL wasperformed by insertion of a periodontal probe in the gingivalsulcus and the measurement corresponding to the distancefrom the cemento-enamel junction to the location of a periodontalprobe tip was defined as CAL. Radiographic assurance by panorama or apical radiograph has been performed

Genetic study

Preparation of genomic DNA fromfresh human venous EDTA-anticoagulatedblood was carried out usingPromegaWizard ® Genomic DNA Purification Kit, according to the manufacturer'smanual. Extracted DNA was stored at -20 °C.

Genotyping was performed byamplification refractory mutation system polymerase chainreaction (ARMS-PCR) [17].In this method, PCR was carriedout for each of the C and T alleles separately. Amplification was performed by a thermocycler(PeQlab) on a 50 µl mixture of the followingcomponents table(1). The primer sequences used in our study were presented in table (2)and the PCR follows program table (3).If both sense and antisense primers were attached to the promoterregion of the IL-10 gene, a 258-base-pair (bp) fragmentof DNA would be amplified.Internal control primersamplify a429-bp product of a human growth hormone sequence [31]. The amplified products were monitored by electrophoresison a 1 % agarose gel (Promega) containing ethidium bromide (0.5 mg/ml) (Roth). A 100-bp ladder (promega) was used in our study.

Table No. 1.PCR mix

Buffer	1x
dNTPs	0.2 mM
Taq polymerase	1.25 U
Primer generic	0.5 µM
Primer specific	0.5 µM
Primer HGH forward	0.5 µM
Primer HGH reverse	0.5 µM
DNA	200nM
H2o	
	50 µl

Table No.2. specific primers used in study

Generic (antisense)	AGGATGTGTTCCAGGCTCCT
C (sense)	CCCTTGTACAGGTGATGTAAC
T (sense)	ACCCTTGTACAGGTGATGTAAT
HGH F	GCCTTCCCAACCATTCACTTA
HGH R	TCACGGATTTCTGTTGTGTTTC

Table N0.3. PCR program

Cycles	**Temprature**	**period**
Heat lid	110˚C	60 sec
	95˚C	5 min
40 cycle	95˚C	30 sec
	59˚C	30 sec
	72˚C	30 sec
	72˚C	5 min
Store	4 °C	

Statistical analysis

The datawere analyzed for appropriateness between the observedand expected genotypes as well as for Hardy–Weinberg Equilibrium (HWE).All data were analyzed using SPSS version 19.0 as statistically significant when $P < 0.05$.

Chai Square was used to determine any association between alleles or genotypes and groups whereas Annova was used to compare means between groups.

Results

The clinical characteristics of sample groups likeratio male/female and distribution according to age and smoking are shown in (Table 4). There were statistical difference between groups (25.7 year in AGP versus

22.5 year in controls)while there was no difference in the ratio male/female, ratio of smokers was much more in AGP group but without statistical significance (p=0.058>0.05).

Table No.4.The descriptive study results

Aggressive	Control	Groups	
83	79	Number	
25.07(16-35)	22.52(18-34)	A α	
23(28.4%)	17(21.3%)	Female	Genderβ
58(71.6%)	62(78.7%)	Male	
24(28.9%)	12(15.4%)	Smoker	Smokingγ
59(71.1%)	66(84.6%)	Non smoker	
α t-student test:P=0.001<0.05 (significant) γ chi square test: P=0.309>0.05 (non significant) β chi square test: P=0.058>0.05 (non significant)			

Genetic study results

There was no appropriateness between the observedand expected genotypes as well as for Hardy–Weinberg Equilibrium (HWE) (P>0.05) (table 5).

Table No. 5.Frequencies of expected and observed Genotypes according toHardy-Weinberg equilibrium

P value	Expected Genotypes	Observed genotypes	Genotypes	Position
P= 0.87	23.5	24	TT	**-819**
	72.9	72	CT	
	56.5	57	CC	

Genetic analysis

Genotypes analysis

We determined the distribution of each genotypes among groups; there was no statistical significance(p>0.05) in association between the genotypes -819CC, -819CT, -819TT and groups(table 6).

Table No.6.The genotypes frequency

AgP vs C			C	AGP	Study groups	
P	O.R	CI			genotype	Position
0.332	0.64	0.23-0.73	13(19%)	10(13.5%)	TT	-819
0.7	1.13	0.55-2.32	27(39%)	32(42.1%)	CT	
0.7	1.12	0.55-2.27	29(42%)	34(54.4%)	CC	

Alleles carriage analysis

We calculated the distribution of alleles carriage (C carriage=CT or CC) and (T carriage=CT or TT) between groups; there was no significant difference between groups(p value>0.05)(table7).

Table No.7.Allele carraige frequency

AgP vs C			C	AGP	Study Groups	
P	O.R	CI			Allele carraige	Position
0.4	1.46	0.54-4.04	56(81.1%)	63(83%)	C carrige	-819
0.79	0.92	0.45-1.86	40(58%)	43(57%)	T carrige	

Alleles analysis

We caculated the frequencies of each alleles upon groups CC=2(C allele), TT=2(T allele),CT=1(C allele)+1(T allele);there was no significant difference between groups(p value>0.05)(table8).

Table NO.8.Allele frequency

AgP vs C			C	AGP	Syudy groups	
P	O.R	CI				
0.45	0.83	0.5-1.39	53	52(34%)	T	-819
			85	100(66%)	C	

Discussion

Sample contain 162 patient divided between two groups (AGP) and (C).There wasno statistical difference in ratio male/female between groups (p=0.309)referring to consistency of sample. Smokers in AGP group were much more than control group but without statistical difference (p=0.058>0.05) that it's common in medical literature which assure prevalence of periodontitis among smokers [7, 18].Analyzing age shows statistical difference where it was 25.07 year in AGP it was 22.52 years in control and, this may agrees with (LoeandBrown)[19] about age as a risk factor for periodontitis but this difference doesn't exclude any of groups from definition of aggressive periodontitis about point of age which is "under 35 years" and keep them in the range.However recent studies began to separate disease from age to be happening in older ages.

Many studies could not revealed any correlation between (SNP) single nucleotide polymorphism and AGP as Moreira et al[20]on Brazilians even various diagnostic criteria was used and performed on a mix of two distinct races (white and black)and other differences in sample size and range of age for each group(control 20-70 years)(AGP 15-45 years).Erciyas[12] found in his study no correlation between SNP and disease,may be sample volume was less than ours (35 AGP and 85 years for controls) but it contained only non-smokers.on the other side many researches revealed positive correlation between SNP and aggressive periodontitis as Abou sulaiman[13] in his study on British Caucasians containing 75 patients in AGP group 30.2 years and 75 case in control group 28.4 year in addition to resistant control group 67 individual 54.6 year. In total mean age is still older comparing to our study and variant race may alter the results toward correlation between disease and SNP.Researchers also found correlation between disease and SNP -819*T,study performed on 32 patient AGP,36 year mean age and control 32 individual with mean age 42 years, the different results may refer to role of the various race and different including criteria.

Results of the study support that we can't summarize occurrence the disease because of only one SNP in one position for particular gene (IL-10 gene)because complicated net of immunity mediators(proinflamatory and inhibitory)and their specific receptors so it's logical that any little change in a definite cytokine coding gene structure as SNP in promoter regain may alter the amount of protein expression and consequently the immune response. These changes in genes varies due to human races and may the role of SNP be canceled or altered by another genetic change in elsewhere, and we can explain results in accordance to theory of (Seymour and Gemmell)[21]which suggests periodontitisas a result of exchange reactions between pathogen bacteria and environmental influences of individuals who have genetic predisposition for injury.

In conclusion thereis no association between IL-10 polymorphism -819 and aggressive periodontitis in Syrianpatients.

Abbreviations

AgP: Aggressive Periodontitis;
PD: Probing Depth;
GI: Gingival Index;
PI: Plaque Index;
CAL: Clinical Attachement Level ;
IL: Interleukine;
IFN-γ: Interferon Gamma;
TNF: Tumor Necrosis Factor;
ARMS-PCR: Amplification Refractory Mutation System-Polymerase Chain Reaction;
TNF-α:Tumor Necrosis Factor Alpha;
SNP: Single Nucleotide Polymorphism;
HWE: Hardy–Weinberg Equilibrium.

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