

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

Mouhammad Morad Taleb<sup>\*1</sup>, Chadi Soukkarieh<sup>2</sup> and Ali Abou Sulaiman<sup>1</sup>

<sup>1</sup>Department of Periodontology, Faculty of Dentistry, Damascus University, Syria

<sup>2</sup>Department of Biology, Faculty of Sciences, Damascus University, Syria

\*Corres.author: taleb-morad@windowslive.com, Phone No: (00) 963 947445985.

**Abstract:** Periodontitis is a multifactorial disease and its severe forms, such as aggressive periodontitis, are suggested to have a genetic basis. Among the genetic factors, polymorphisms in cytokine genes have recently been described in susceptibility to periodontitis. Interleukin-10 (IL-10) is a multi-functional cytokine thought to play a role in the pathogenesis of periodontitis. A substitution C/T polymorphism in the promoter region of the IL-10 gene at position -819 has been associated with different amounts of IL-10 production. The aim of the present study was to investigate the possible links between -819 C/T polymorphism of the IL-10 gene in patients with aggressive periodontitis in comparison with periodontitis-free controls. This study included 83 Syrian subjects suffering from aggressive periodontitis referred to American Academy of Periodontology. They were compared to 79 age matched healthy controls. DNA was 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 analyzed using the chi-squared test. There was no marked difference in genotype frequencies between the controls and aggressive periodontitis patients ( $p > 0.05$ ). Moreover, neither marked difference was found in their allele frequency nor in allele carriage ( $p > 0.05$ ). These findings suggest that the investigated polymorphism is not associated with periodontal disease in this studied sample of Syrian population.

**Keywords:** Aggressive Periodontitis, IL-10, Polymorphism, ARMS-PCR.

### Introduction

American academy of periodontology (AAP) 1999<sup>[1]</sup> defined Periodontitis as a disease comprises variety of conditions affecting the health of periodontium for people who often appear to be healthy and it tends 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 balance between proinflammatory 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<sup>[2]</sup>.

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

IL-10 is secreted by many cell types and strongly modulates the expressions of other cytokines, soluble mediators, and cell-surface molecules in cells of myeloid origin<sup>[5]</sup>. It is an anti-inflammatory cytokine synthesized by activated monocytes and T lymphocytes in response to inflammation. It has an inhibitory 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<sup>[6]</sup>. Besides this inhibitory effect on production of many cytokines, IL-10 may also promote inflammatory responses through effects on B cells, stimulating both proliferation and differentiation<sup>[7]</sup>. IL-10 is known to regulate the balance between acquired humoral response and innate inflammatory response.

Individual capacity for IL-10 production appears to be under genetic influence<sup>[8]</sup>. Genotypic variations in cytokine response have been shown *in vitro* for IL-10, and specific alleles are implicated in diseases such as systemic lupus erythematosus<sup>[9]</sup> and rheumatoid arthritis<sup>[10]</sup>. The promoter region of IL-10 gene exhibits high frequencies of single-nucleotide polymorphisms (SNP). The three IL-10 promoter region SNPs mostly described in literature are -592(C/A), -819(C/T), and -1082(G/A). Several studies have investigated IL-10 promoter polymorphisms at these positions in patients with periodontitis compared with healthy controls. The results were partially inconsistent. For instance, several previous studies<sup>[11][12]</sup> failed to demonstrate associations between periodontitis and polymorphism at -819 in the IL-10 gene. By contrast, Abou sulaiman<sup>[13]</sup>, Reichert<sup>[14]</sup> and Hu<sup>[15]</sup> have found that the allele T at position -819 was more expressed in aggressive periodontitis patients. Therefore, the aim of the present study was to examine the IL-10 promoter polymorphisms at positions -819 (C>T) in Syrian unrelated patients suffering from aggressive periodontitis.

## Materials and Methodology

### Study population

This study was approved by the Ethics Committee of Damascus University. In this study, 83 Syrian subjects suffering from AGP attending the Periodontology Department of Faculty of Dentistry at Damascus University were included. Additionally, 79 race-matched healthy subjects were 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 %) were male. (17 %) Of the 79 control subjects, were female and (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 patients must be Syrians.

### 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

No case that produced doubt in classification was included in the study. Diagnosis of disease was made considering the patient's medical and dental histories, radiographic findings and observation of clinical signs and parameters including probing depth, assessment of clinical attachment loss (CAL), observation of tooth mobility, bleeding on probing and presence of plaque/calculus. Clinical diagnosis of periodontitis

was based on criteria established in 1999 at the International Workshop for a Classification of Periodontal Diseases and Conditions<sup>[1]</sup>. Measurements of probing depth (PD)<sup>[16]</sup> and Clinical Attachment Loss (CAL)<sup>[16]</sup> were assessed at six locations around each tooth. The severity of disease was characterized on the basis of the mean of CAL, within each clinical form. Assessment of CAL was performed by insertion of a periodontal probe in the gingival sulcus and the measurement corresponding to the distance from the cemento-enamel junction to the location of a periodontal probe tip was defined as CAL. Radiographic assurance by panorama or apical radiograph has been performed

### Genetic study

Preparation of genomic DNA from fresh human venous EDTA-anticoagulated blood was carried out using Promega Wizard® Genomic DNA Purification Kit, according to the manufacturer's manual. Extracted DNA was stored at -20 °C.

Genotyping was performed by amplification refractory mutation system polymerase chain reaction (ARMS-PCR) [17]. In this method, PCR was carried out for each of the C and T alleles separately. Amplification was performed by a thermocycler (PeQlab) on a 50  $\mu$ l mixture of the following components 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 promoter region of the IL-10 gene, a 258-base-pair (bp) fragment of DNA would be amplified. Internal control primers amplify a 429-bp product of a human growth hormone sequence [31]. The amplified products were monitored by electrophoresis on 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 $\mu$ M
Primer specific	0.5 $\mu$ M
Primer HGH forward	0.5 $\mu$ M
Primer HGH reverse	0.5 $\mu$ M
DNA	200nM
H <sub>2</sub> O	
	50 $\mu$ l

**Table No.2. specific primers used in study**

Generic (antisense)	AGGATGTGTTCCAGGCTCCT
C (sense)	CCCTTGTACAGGTGATGTAAC
T (sense)	ACCCTTGTACAGGTGATGTAAT
HGH F	GCCTTCCAACCATTCACTTA
HGH R	TCACGGATTCTGTTGTGTTTC

**Table N0.3. PCR program**

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

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

### Results

The clinical characteristics of sample groups like ratio 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 $\alpha$	
23(28.4%)	17(21.3%)	Female	Gender $\beta$
58(71.6%)	62(78.7%)	Male	
24(28.9%)	12(15.4%)	Smoker	Smoking $\gamma$
59(71.1%)	66(84.6%)	Non smoker	
$\alpha$ t-student test: P=0.001<0.05 (significant) $\gamma$ chi square test: P=0.309>0.05 (non significant) $\beta$ chi square test: P=0.058>0.05 (non significant)			

**Genetic study results**

There was no appropriateness between the observed and 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 to Hardy-Weinberg equilibrium**

P value	Expected Genotypes	Observed genotypes	Genotypes	Position
P= 0.87	23.5	24	TT	<b>-819</b>
	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	<b>-819</b>
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) (table 7).

**Table No.7. Allele carriage frequency**

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

**Alleles analysis**

We calculated 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)<sup>[19]</sup> 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<sup>[20]</sup>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<sup>[12]</sup> 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<sup>[13]</sup> 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,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(proinflammatory 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)<sup>[21]</sup>which suggests periodontitisas a result of exchange reactions between pathogen bacteria and environmental influences of individuals who have genetic predisposition for injury.

In conclusion there is no association between IL-10 polymorphism -819 and aggressive periodontitis in Syrian patients.

### Abbreviations

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

### References:

1. Armitage, G.C., *Development of a classification system for periodontal diseases and conditions*. Ann Periodontol, 1999. 4(1): p. 1-6.
2. Bidwell, J., et al., *Cytokine gene polymorphism in human disease: on-line databases*. Genes Immun, 1999. 1(1): p. 3-19.
3. Kim, J.M., et al., *Structure of the mouse IL-10 gene and chromosomal localization of the mouse and human genes*. J Immunol, 1992. 148(11): p. 3618-23.
4. Gemmell, E. and G.J. Seymour, *Cytokine profiles of cells extracted from humans with periodontal diseases*. J Dent Res, 1998. 77(1): p. 16-26.
5. McGuirk, P. and K.H. Mills, *Pathogen-specific regulatory T cells provoke a shift in the Th1/Th2 paradigm in immunity to infectious diseases*. Trends Immunol, 2002. 23(9): p. 450-5.
6. de waal Malefyt, R.Y., H.de Vries, J.E. *Direct effects of IL-10 on subsets of human CD4+T cell clones and resting T cells . specific inhibition of il-2 production and proliferation*. J Immunol, 1993. 150: p. 4754-4765.
7. Tonetti, M.S., *Cigarette smoking and periodontal diseases: etiology and management of disease*. Ann Periodontol, 1998. 3(1): p. 88-101.
8. Hart, T.C., et al., *Localisation of a gene for prepubertal periodontitis to chromosome 11q14 and identification of a cathepsin C gene mutation*. J Med Genet, 2000. 37(2): p. 95-101.
9. Mok, C.C., et al., *Interleukin-10 promoter polymorphisms in Southern Chinese patients with systemic lupus erythematosus*. Arthritis Rheum, 1998. 41(6): p. 1090-5.
10. Crawley, E., et al., *Polymorphic haplotypes of the interleukin-10 5' flanking region determine variable interleukin-10 transcription and are associated with particular phenotypes of juvenile rheumatoid arthritis*. Arthritis Rheum, 1999. 42(6): p. 1101-8.
11. Moreira, P.R., et al., *TNFA and IL10 gene polymorphisms are not associated with periodontitis in Brazilians*. Open Dent J, 2009. 3: p. 184-90.
12. Erciyas, K., et al., *Association between TNF- $\alpha$ , TGF- $\beta$ 1, IL-10, IL-6 and IFN- $\gamma$  gene polymorphisms and generalized aggressive periodontitis*. Clin Invest Med, 2010. 33(2): p. 85-91.
13. A. ABOU SULAIMAN, *Interleukin-10 Gene Polymorphisms in Patients with Aggressive Periodontitis in periodontology*. 2006, University of Manchester,: Manchester.
14. Reichert, S., et al., *The interleukin-10 promoter haplotype ATA is a putative risk factor for aggressive periodontitis*. J Periodontal Res, 2008. 43(1): p. 40-7.
15. Hu, K.F., et al., *Interleukin-10 (-592 C/A) and interleukin-12B (+16974 A/C) gene polymorphisms and the interleukin-10 ATA haplotype are associated with periodontitis in a Taiwanese population*. J Periodontal Res, 2009. 44(3): p. 378-85.
16. Loe, H., *The Gingival Index, the Plaque Index and the Retention Index Systems*. J Periodontol, 1967. 38(6): p. Suppl:610-6.
17. Perrey, C., et al., *ARMS-PCR methodologies to determine IL-10, TNF-alpha, TNF-beta and TGF-beta 1 gene polymorphisms*. Transpl Immunol, 1999. 7(2): p. 127-8.

18. Johnson, G.K. and M. Hill, *Cigarette smoking and the periodontal patient*. J Periodontol, 2004. 75(2): p. 196-209.
19. Loe, H. and L.J. Brown, *Early onset periodontitis in the United States of America*. J Periodontol, 1991. 62(10): p. 608-16.
20. P. R. Moreira, J.E.C., R. S. Gomez , K. J. Gollob and W. O. Dutra, *TNFA and IL10 Gene Polymorphisms are not Associated with Periodontitis in Brazilians*. The Open Dentistry Journal, 2009. 3: p. 184-190.
21. Seymour, G.J. and E. Gemmell, *Cytokines in periodontal disease: where to from here?* Acta Odontol Scand, 2001. 59(3): p. 167-73.

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