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Original Research Article

Polymorphisms in IL6 and IL10 genes and their implication in periodontitis on Romanian population

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Abstract

Periodontitis is a chronic inflammatory disease with multifactorial etiology. The genetic factor is the major determinant of the host predisposition. The objectives of this study were to analyze some polymorphisms in the genes encoding IL6 and IL10 (polymorphisms that control the amount of synthesized cytokines) and to correlate the influence of these polymorphisms on periodontitis in the Romanian population, thus being the first study of its kind in our country. Our study was conducted at Carol Davila University of Medicine and Pharmacy, Bucharest, Romania, between 2019-2020 and investigated the 4 gene polymorphisms in two genes known to be involved in the pathogenesis of periodontitis IL10 and IL6: IL10 – 592C/A, IL10 – 819C/T, IL10 – 1082A/G and IL6 - 174 G/C. IL10-1082 allele frequency was significantly higher in chronic periodontitis (CP) subjects in comparison to controls and the patients with at least one G allele (AG, GC genotypes) had a higher risk for chronic periodontitis. The presence of the genotype IL 10 - 819 CC (normal homozygous allele) was lower in the experimental group than in the control group and the presence of the genotype IL 10 - 819 CT (heterozygous) and not of - 819 TT, was higher in the experimental group than in the control group. Genetic varieties can affect the function of the immune system by changing the transcription of immune factors. Not all the individuals having the similar amount of plaque and calculus develop periodontitis. In Romanian population, our study showed that the subjects with at least one G allele were exposed to a higher statistically significant risk for CP.

Keywords: Epigenetics, Gene polymorphisms, Genome wide association studies, Infectogenomics, Periodontitis, SNPs

INTRODUCTION

Periodontitis is a multifactorial inflammatory disease with both environmental and genetic factors playing a major role in the progression of the disease with consequent tissue destruction around the dental roots, and alveolar bone; Periodontitis is associated with systemic alterations such as diabetes, changes in the liver, cardiovascular diseases, and even osteoporosis.

The interplay among the immune system, microbiota, and lifestyle habits like smoking, alcoholism, stress, and diet that leads to constant changes in the host is regulated by genes. These genes encode immune receptors and various molecules involved in the signal transduction pathways that play an essential role in up or down regulation of the immune response as the inflammatory

reaction in response to a stimulus (Reichert et al., 2008). Genetic research has focused on understanding how these responses work and also how these responses differ between various individuals. In addition to playing a role in health, the genetic factors also play a major role in disease susceptibility. This review focuses on the genetic aspects of periodontal diseases wherein researchers are currently focusing on genetic evidences to explain the difference in susceptibility to periodontal disease.

Although very prevalent, periodontal diseases are not evenly distributed across populations. Few people, who do not have much contributing local factors such as plaque and calculus, still develop severe destruction of bone whereas some do not develop severe forms of periodontal diseases in spite of having a very poor oral hygiene. This differential expression of periodontitis leads researchers to question if genetics and heritability play a major role (Yussif, 2020).

Dental caries is a pathologic entity that results from the interaction of endogenous and exogenous traits and scientists observed 38 like sexed monozygotic and dizygotic twin pairs in Michigan in an attempt to relate tooth decay to other factors that might be under genetic control. They reported significant heritability for the presence of several oral micro-organisms, including Streptococci, and also for salivary flow rate, salivary pH, and salivary amylase activity. Aside from hereditary factors relating directly to enamel constitution, the study established other genetically influenced factors as operative in caries etiology (Goodman et al., 1959).

While the hereditary basis for susceptibility to dental caries is rather well-founded, the situation vis-a-vis chronic inflammatory periodontal disease is considerably less so. This has been due not to any lack of investigative enthusiasm over the past eight decades, but rather to the relative complexity of the disease, continually emerging new knowledge about its pathogenesis, unclear clinical diagnosis and statistical quantitation, and the profession's own nomenclature for classifying these diseases, which keeps evolving even today.

The investigations on factors of susceptibility to periodontitis have been gaining focus on genes of immunoregulatory molecules, such as cytokines, chemokines, membrane surface receptors, and antigen recognition proteins (Hart, 1996). Cytokines such as interleukins (IL-1A, IL-1B, IL-6, and IL-10, among others), surface receptors such as the Fcγ family (FCGRs), and cyclooxygenase- (COX-) 2 and matrix metalloproteinase (MMP) are considered key factors in the progression of periodontitis recruitment, differentiation and activation of B lymphocytes, inflammatory infiltrate, and stimulation of osteoclasts (Hart et al., 2000).

Interleukin-6 is produced during inflammation by T cells. It is encoded by the IL-6 gene located on chromosome 7p21. Interleukin-6 is a potent bone resorbing cytokine. It activates and regulates the osteoclasts. Thus, this plays a major role in the susceptibility and progression of periodontal destruction. Various studies found significant association between IL-6 –1363 G/T and IL-6R +48,892 A/C polymorphisms with periodontitis in specific populations.

Interleukin-10 is an anti-inflammatory cytokine expressed by T helper cells. It is encoded on gene at 1q31-q32. The major regions of interleukin-10 single nucleotide polymorphisms studied were –1082, –819, and –592. However, conflicting results have been obtained. Berglundh et al. (1999) found positive associations between IL-10 SNP and periodontitis in Swedish and Brazilian population. Scarel-Caminaga et al. did not find any significant association in the Caucasian population.

MATERIALS AND METHODS

Our study was conducted at Carol Davila University of Medicine and Pharmacy between 2019-2020 and investigated the 4 gene polymorphisms that were previously associated with periodontal disease susceptibility in different studies. IL-10 single nucleotide polymorphisms (SNPs) were analyzed in 32 patients with chronic periodontitis (CP) and 15 individuals without CP.

The DNA was extracted from peripheral blood using standard techniques (Promega kits). The amplification of the sequences was performed by PCR (polymerase chain reaction)with restriction fragment length polymorphism (RFLP). The primers used for genotyping IL-10 and IL-6 polymorphisms are listed in Table 1.

RESULTS

The prevalence of AG and GG genotypes of IL10-1082 were significantly different between CP and control groups in comparison to AA genotype. Furthermore, IL10-1082 allele frequency was significantly higher in CP subjects in comparison to controls (P value<0,0001) and the patients with at least one G allele (AG, GC genotypes) had a higher risk for CP (OR=2,157; CI = 1.531-3.038). The distribution of IL10-819 and IL10-592 alleles was not different in subjects with CP and controls (Table 3).

IL 10 - 592 A (0.32 versus 0.25) and C (0.67 versus 0.75), -1082 G (0.60 versus 0.64) and A (0.40 versus 0.36)) allele frequencies were similar between the control and patients groups. On the contrary, the frequency of the IL 10 - 819 C allele (0.81 versus 0.67) was higher in the control group than in the experimental group (Table 3).

The presence of the genotype IL 10 - 819 CC (normal homozygous allele) was lower in the experimental group than in the control group (p = 0.003; OR = 0.33; 95% CI = 1.16 - 0.67) and the presence of the genotype IL 10 - 819 CT (heterozygous) and not of - 819 TT, was higher in the experimental group than in the control group (p = 0.004; OR 2.95; 95% CI = 1.43 - 6.08) (Table 3).

Table 1. Primers used for genotyping IL-10 and IL-6 polymorphisms

Polymorphism	Primers	Amplicons
-174 G/C IL6 (rs 1800795)	F:5'GGAGTCACACACTCCACCT3' R:5'CTGATTGGAAACCTTATTAG3'	527 bp
-592 C/A IL10 (rs 1800872)	F 5' GCTCACTATAAAAATAGAGACGG 3' R 5' GACTGGCTTCCTACAGT allele C R 5'CTGGCTTCCTACAGG allele A	223 bp

Table 2. Characteristics of enzymatic digestion for the studied polymorphisms (G- guanine; T- thymine; A – adenine; C – cytosine)

Polymorphism	Restriction enzyme	Restriction site	Restriction fragments	Interpretation
-174 G/C IL6 (rs 1800795)	Nla III	CATG↓	331 bp, 29 bp, 122 bp, 45 bp	- When the **G** allele is present, there are three segments with the following lengths 331 bp, 29 bp, 167 bp. - When the **C** allele is present, there are four segments with the following lengths 331 bp, 29 bp, 122 bp, 45 bp. In gel, there can be seen just the 331 bp and the 122 bp fragments.
-592 C/A IL10 (rs 1800872)				- The combination 592 F+R amplify the **C** allele**.** - The combination 592 F+0 amplify the **A** allele.
-819 C/T IL10 (rs 1800871)	SspI.	AAT ↓ATT	219 bp; 24 bp	- If present, the **C** allele stops the enzyme from recognizing the restriction site. -If present, the **T** allele, makes the enzyme cut and generate 2 fragments of 219 bp and 24 bp (the last one cannot be seen in gel)
-1082 G/A IL10 (rs 1800896)	BSeLI,	CCNNNNN↓N NGG	290 bp; 25 bp	- When present, the **A** allele hides the restriction site. - When present, The **G** allele, makes the enzyme cut and generate 2 fragments: 290 bp and 25 bp (invisible in gel).

Table 3. Demographic parameters of chronic periodontitis patients and control group

	Genotypes			Allele frequency	
IL10 – 592C/A	**CC**	**CA**	**AA**	**C**	**A**
Patients	30(69)	33(69)	6(69)	0,673	0,326
Controls	35 (64)	26 (64)	3(64)	0,750	0,250
P value	0,22	0,48	0,49		
Odds Ratio	0,637 (0,321-1,264)	1,33(0,674-2,66)	1,93 (0,463-8,091 6)	0,688	1,477
IL10 – 819C/T	**CC**	**CT**	**TT**	**C**	**T**
Patients	28 (69)	37(69)	4 (69)	0,673	0,326
Controls	43 (64)	18(64)	3 (64)	0,812	0,187
P value	**0,003**	**0,0046**	1,00		
Odds Ratio	0,33(0,16-0,67)	2,95 (1,435-6,080)	1,253(0,269-5,820)	1.25	2.09
IL10 – 1082A/G	**AA**	**AG**	**GG**	**A**	**G**
Patients	7(53)	28 (53)	18 (53)	0,40	0,60
Controls	11(64)	24(64)	29 (64)	0,36	0,64
P value	0,61	0,13	0,257		
Odds Ratio	0,73 (0,26-2,04)	1,86 (0,89-3,91)	0,62 (0,29-1,31)	1,18	0,84

DISCUSSION

Many studies have assessed a genetic association between genetic variation and periodontitis; however, there is still no agreement regarding the genetic bases of this disease. Indeed, conflicting results have been reported (McGuire and Nunn, 1999). These are mainly related to the different ethnicities of the studied populations and also to the small sample size. Indeed, small studies – with less than 100 patients and 100 controls–cannot provide adequate statistical power to detect a moderate genetic effect. Another source of variability could be related to the study design, being different studies focused on specific phenotypes, such as aggressive periodontitis, chronic periodontitis or response to treatments (Laine et al., 2012).

The prevalence of AG/GG genotypes were significantly different between CP patients and controls, in comparison with AA genotype. In addition, IL10 – 1082G allele was significantly more frequent in CP subjects, comparing to controls and the subjects with at least one G allele had a higher risk for CP (OR = 2.157; CI = 1.531-3.038). The distribution of IL10-819 și IL10-592 alleles was not different in subjects with CP and controls (Table 3).

Interleukin-6 is a pro-inflammatory cytokine that is also an important regulator of the immune response and has been associated with periodontal disease. IL-6 has been examined in several patient populations with varying results. Brett *et al*. (2005) examined 10 SNPs in 7 candidate genes from Caucasians living in the UK, including alleles of IL-1, IL-6, and IL-10 as well as other non-IL genes. The IL-6 (–174) genotype was found to be not significantly related to aggressive periodontitis (AgP), although there was a significant association between AgP + CP, as well as a significant finding involving CP when compared to controls.

The variant allele rs1800795-C (also known as IL6 -174G >C) was significantly less represented among periodontitis patients. This indicated that carriers of rs1800795-C allele had a lower risk of developing periodontitis, as previously reported by other researchers.

Interleukin-10 is a regulatory IL which is strongly involved in the downregulation of the inflammatory response. Multiple studies, most of which were published referring to the European populations (Fiebig et al., 2008), like this paper, have examined a possible relationship between different IL-10 polymorphisms and AgP, but showed mostly insignificant associations. AgP can be either generalized (GAgP) or localized to the first molars and incisors localized (LAgP). A 1999 study on patients in Glasgow with diagnosed GAgP showed no significant association for two microsatellites (known polymorphisms) in IL-10. G or IL-10.R. IL-10 (–627 and – 1082) were examined in a UK Caucasian populations, and no significant association was found with AgP (Lang et al., 1999). In a German Caucasian populations, one study showed no association with SNPs of IL-10 – 824 and – 597, whereas another study looking at IL-10 (–1082, –819, and – 590) did conclude that certain alleles of IL-10 – 819 and IL-10 – 590 occurred more frequently in AgP (not significant) and that the haplotype ATA/ATA was significantly associated with GAgP, although after adjusting for age and presence of Aa and Pg, this was not significantly different (Maney and Owens, 2015). Scapoli et al. (2005) examined polymorphisms of IL-10 (–819, –592, –1082) as three of the 28 SNPs studied in 14 candidate genes in an Italian Caucasian populations, and found no significant association with AgP.

Outside of Europe, there have also been multiple studies of the IL-10 gene polymorphisms that show differing results, based on region and ethnicity. In a Turkish populations diagnosed with GAgP, there was no significant association with SNPs of IL-10 (–1082, –819, –592).

A polymorphism of IL-10 (–1082) was examined in an Iranian populations diagnosed with GAgP, and no association was seen with either allele or genotype frequency. SNPs of IL-10 (–592, –819, and – 1082) were evaluated in a Taiwanese (Han Chinese ethnicity) populations in which individual genotype frequencies were not significantly associated with GAgP.

In this study, we tested 3 polymorphisms of the IL10 promoter region and showed that the variant allele rs1800872-A increased the risk of developing periodontitis, with an observed OR of 1.38 (95% CI 1.01-1.86). Interestingly, Reichert et al. demonstrated that this allele is functional in chronic periodontitis, being associated with a lower expression level of IL10.

CONCLUSIONS

Infections such as CP can be influenced by host's genetic factors. The aim of the current study is to provide prognostic genetic markers for detection of the susceptibility of an individual to the chronic periodontitis. Genetic varieties can affect the function of the immune system by changing the transcription of immune factors. In Romanian populations, our study showed that the subjects with at least one G allele were exposed to a higher statistically significant risk for CP. Complex diseases, such as inflammatory bowel disease, type 2 diabetes and many other immune-mediated diseases, including chronic periodontitis, share a number of genetic risk variants. In this regard, if a candidate gene has an effect on multiple phenotypes, the pleiotropy occurs. Recently, the effects of pleiotropy in the pathogenesis of complex diseases have been studied. In the future, we pursue the idea of having a multidisciplinary team to assess a larger group of subjects. The results could help clinicians improve the management of patients and also we could develop a series of prophylactic genetic tests based on these polymorphisms. The team should consist of dentists, periodontists, clinical geneticists and bioinformaticians.

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