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Original paper

Single Nucleotide Polymorphisms in Two Inflammation-Related Genes and Chronic Periodontitis risk in Romanian Adults

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Abstract

Background: The existing literature showed conflicting data on the association between peridontosis and IL6 and TGFb1 polymorphisms.

Aim: to investigate the correlation between susceptibility to chronic periodontitis and TGFb1 C-509T, IL6 G-174C polymorphisms and TTV infection in Romanian population.

Materials and methods: Five hundred and fifteen subjects (260 healthy and 255 patients with chronic periodontitis) of Romanian origin were selected for this study. DNA was extracted from peripheral blood samples and the two polymorphisms and the presence of torque teno virus DNA were tested by PCR-based methods. StatsDirect was used for data analysis.

Results: Our data showed that lifestyle choices (e.g. *inadequate* personal *oral hygiene* -O.R. =7.66, $p<0.0001$, taking irregular meals -O.R. =2.82, $p<0.001$ and smoking O.R.=1.5, $p=0.01$) increase the risk of periodontitis. We also identified differences in distribution of TGFb1 C-509T and IL-6 G-174C polymorphisms in patients and control groups. However, there was no association between the distribution of TGFb C-509T variants and the risk of periodontitis in male ($p=0.85$). There was an association between the presence of IL6 -174G allele and chronic periodontitis in the overall group of subjects (O.R.=1.6, $p=0.01$) and in men (O.R.=1.82, $p=0.02$), but not in women ($p=0.2$). The prevalence of TTV DNA and association with periodontitis was consistent with previous studies.

Conclusions: Lifestyle choices, IL6 G-174C and TTV were associated with periodontitis in our lot. Further investigation conducted on a larger group with less confounding factors is required in order to validate these findings.

Keywords Chronic periodontitis, TGFb1 polymorphism, IL-6 polymorphism, TTV, Romania

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Introduction

The pathogenesis of periodontitis involves complex interaction between periodontal pocket microbiota and different other risk factors (e.g. host immune response, genetic polymorphisms in different cytokines, deficit of oral hygiene, tobacco smoking, alcohol consumption, psychological stress and depression, age, other diseases).

Periodontal bacteria can stimulate the production of transforming growth factor-beta (TGFb1) and the secretion of proinflammatory and tissue-destructive mediators. TGFb can inhibit or stimulate different cells to produce other cytokines (including IL-6), proteinase inhibitors and matrix of connective tissue components and can modulate inflammatory process and rhythm of cell growth and differentiation.

IL6, a pro- and antiinflammatory cytokine, represents a key factor in periodontitis evolution due to its role in mediating inflammatory processes, osteoclast differentiation, bone resorption and loss of tooth attachment. Its levels were found to be higher in gingival biopsies from patients with periodontal disease and seemed to decrease when periodontal therapy was effective.

In some studies transcription and circulating levels of IL6 were associated with different factors (e.g. genetic polymorphisms and certain haplotypes, methylation status of the promoter region, infections, smoking) [1-4].

Torque teno virus (TTV) is a highly prevalent virus often found in human saliva [5]. TTV has been shown to modulate the immune response by driving the production of cytokines via interaction with TLR-9 receptors [6]. In consequence, it may be speculate that TTV can interfere with inflammatory processes.

Objective. The aim of this study was to investigate the correlation between susceptibility to chronic periodontitis and TGFb1 C-509T, IL6 G-174C polymorphisms and TTV infection.

Materials and methods

The study was conducted in accordance with the Declaration of Helsinki of 1975, as revised in 2013. It was approved by the Ethics Committee of National Institute of Research and Development for Food Bioresources – IBA Bucharest (342/2014).

Subjects. For the present study, adult Romanian volunteers with chronic periodontitis (n=255) and healthy controls individuals with no evidence of clinical attachment loss (n=260) were recruited from the Bucharest metropolitan area. The subjects have been living in this region for at least two generations, spoke Romanian language and had at least 20 teeth in the buccal cavity. The diagnosis of chronic periodontitis was made on the basis of standardized clinical criteria.

The subjects were asked to self-asses their ethnic background and different lifestyle aspects.

Genotyping methods. Analysis of samples was done in a blinded fashion. DNA was extracted from peripheral blood samples (200 µl) with Axygen (Axygen® AxyPrep™ Blood Genomic DNA Miniprep Kit). The concentration and quality of the DNA were analyzed by optical density with Qubit.

The two polymorphisms were genotyped by PCR based methods. *The specificity of p*rimers used for genotyping TGFb1 C-509T (Forward GGAGAGCAATTCTTACAGGTG; Reverse TAGGAGAAGGAGGGTCTGTC) and IL6 G-174C (Reverse outer primer: 5′AGTTGGGGACACGCAAGCATGAAGGATA; Forward inner primer: 5′GCACTTTTCCCCCTAGTTGTGTCTTCCG; Reverse inner primer: 5′ATTGTGCAATGTGACGTCCTTTAGCTTG; Forward outer primer: 5′GACTTCAGCTTTACTCTTTGTCAAGACA) [7] were *verified* with Genome Artist *software [8]. The* TGFb1 *amplicons were subsequently digested* with *Dde*I restriction endonuclease digestion (Thermo Scientific). All amplicons *were resolved on PAGE 8%.* TTV DNA presence in the blood of subjects was detected by nested-PCR, as described by Ninomiya et al, 2008 [9].

Statistical analysis. Allele and genotype frequencies were obtained by direct counts. The distribution of categorical parameters was tested by Fisher's exact or Chi-Squared tests with Yates' correction. The statistical analysis was performed with StatsDirect program Version 2.8.0.

Results and Discussion

Chronic periodontitis is the result of complex interaction between different genetic and non-genetic factors. The heritability, familial aggregation, twin studies, analyses of candidate loci and GWAS reveal that the genetic component has a weak or moderate effect on disease predisposition [10, 11].

In this work we studied the association of TGFb1 C-509T and IL6 G-174C polymorphisms with chronic periodontitis. The general characterization of the subjects included in the study is shown in Table 1.

Our findings revealed that lifestyle choices - such as *inadequate* personal *oral hygiene* (O.R. =7.66, 95%, CI: 3.7-15.89, $p<0.0001$), taking irregular meals (O.R. =2.82, 95%CI: 1.92- 4.13, $p<0.001$) and smoking (O.R.=1.5, 95%CI: 1.09-2.2, $p=0.01$) - increase the risk of periodontitis. The contribution of these factors appears to be more important than that attributed to genetic factors.

We have identified some differences in distribution of TGFb1 C-509T and IL-6 G-174C polymorphisms in patients and control groups (Table 2 and 3). In the subgroup consisting of female subjects affected by periodontitis, the distribution of TGFb1 C-509T alleles deviated from the Hardy-Weinberg equilibrium. Hence, all data describing the distribution of TGFb1 C-509T alleles among women with periodontitis were cut out from further statistical analysis.

Table 1. General characteristics of the subjects. A *p*-value <0.05 was considered statistically significant; *p* <0.01 was considered highly significant.

Characteristic	Patients	Controls	*p*-value
Female	128	132 (50.8%)	>0.05
Age	48.36±7.86	44.45±5.83	< 0.001
Smokers	119 (46.67%)	94 (36%)	0.01
Alcohol consumption	58 (22.75)	43(16.5%)	>0,05
IMC	25.36±2.38	24.33±1.67	n.a.
Arterial hypertension	68 (26.67%)	0	n.a.
Cerebrovascular accidents	36 (14.12)	0	n.a.
Type 2 diabetes mellitus	69 (27.06%)	0	n.a.
Breast cancer	16 (6.27%)	0	n.a.
Hepatitis A	71 (27.84%)	67 (25.8%)	>0,05
Glycaemia	99.8±8.75	95.25±8.8	n.a.
Total cholesterol	127.45±24.47	126.83±21.47	n.a.
Triglycerides	107.16±23.57	112.74±21.96	n.a.
TTV	169/ 86	148/ 112	0.03
Last dental visit (<6 / 6-12 / >12 months)	70/88/97	109/99/52	<0.001
Tooth brushing frequency (≥2 times/day / 1 time/day / occasionally)	62/138/55	141/110/9	<0.001
Caries or dental restorations	219 (85.88%)	215 (82.69%)	0.3
Number of missing teeth	2.05±1.40	0.72±0.94	n.a.
Physical activity (sedentary/ occasional / regular)	99/117/39	78/130/52	>0.05
Residential area (urban=1, rural=2, commuters=3)	111/91/53	130/78/52	>0.05
Regular meals/day (unable to specify/ 2 meals / 3 or more meals)	114/53/88	58/56/146	<0.001

Table 2. TGFb1 in men with periodontitis and other pathologies; T2DM = type 2 diabetes mellitus, BC = breast cancer, CVA = cerebrovascular accident, AHT = arterial hypertension.

Investigated lots vs. healthy controls	TGFb			*p*-value
	CC	CT	TT	
Healthy controls male	66	53	9	-
Periodontitis	64	50	13	>0.05
Periodontitis only	30	27	4	>0.05
Periodontitis and other conditions (T2DM, BC, CVA, AHT)	34	23	9	>0.05
Periodontitis and AHT	22	15	5	>0.05
Periodontitis and CVA	9	5	3	>0.05
Periodontitis and T2DM	19	12	5	>0.05

TGFb1 polymorphisms were associated with an increased risk of chronic periodontitis in some (e.g. Han Chinese, Japanese, Turkish, Macedonian, German), yet not all populations [12-17]. Some TGFb1 polymorphisms can create a consensus sequence in the gene region or may interfere with the production and secretion levels, activation or activity of this cytokine [18]. Consequently, they were considered functional candidate polymorphisms for susceptibility to different diseases. TGFb1 variants have only a limited association with gingival inflammation in the Brazilians with chronic periodontitis [19] but were not associated with chronic periodontitis in Japanese [17] and Czech [20] populations.

There was no association between the distribution of TGFb C-509T variants and the risk of periodontitis in men enrolled in this study. This result support previous findings.

protein concentrations [31] and were more likely to get periodontopathogenic bacteria [32]. These discordant results may reflect ethnic differences in the studied populations or a confounding factor (e.g. difference in study design, disease severity, impact of environmental factors and modifier genes, differences in bacterial profile and levels associated with disease, redundant inter-changeable roles of investigated factors and compensatory mechanisms).

The findings of the present study showed an association between the presence of IL6 -174G allele and chronic periodontitis in the overall group of subjects (O.R.=1.58, 95%CI: 1.11-2.25, p=0.01) and in men (O.R.=1.82, 95%CI: 1.10-3, p=0.02), yet not in women (Table 3).

Table 3. IL-6 in periodontitis and other pathologies; T2DM = type 2 diabetes mellitus, BC = breast cancer, CVA = cerebrovascular accident, AHT = arterial hypertension.

Investigated lots vs. healthy controls	**IL6 G-174C**			**Statistical significance for GG genotype**
	GG	GC	CC	
Healthy controls	133	109	18	-
Periodontitis	159	83	13	O.R. =1.58, 95%CI: 1.11-2.25, p=0.01
Periodontitis only	80	29	8	O.R.=2.06, 95% CI: 1.30-3.27, p=0.001
Periodontitis and other conditions (T2DM, BC, CVA, AHT)	79	54	5	p>0.05
Periodontitis and AHT	24	37	7	O.R.=0.52, 95% CI: 0.3 - 0.9, p= 0.02
Periodontitis and CVA	19	16	1	p>0.05
Periodontitis and T2DM	41	24	3	p>0.05
Periodontitis and BC (vs. healthy women)	6/ 70	9/ 52	1/ 10	p>0.05
Periodontitis male (vs. healthy men)	81/ 63	41/ 57	5/ 8	O.R. =1.82, 95%CI: 1.1-3.0, p=0.02

The IL6 gene polymorphisms seemed to increase the risk for chronic periodontitis in different populations (e.g. German [16], Han [21], Brazilian [22], and South Indian [23]). However, conflicting results were found in other populations, such as Italian [24, 25], Chinese [26, 27] and Japanese populations [13, 28]. The SNP in IL6 -174 position was correlated with chronic periodontitis susceptibility among Brazilians and Caucasians [29], but not in Japanese [28]. A recent meta-analysis reported that the IL6 G-174C polymorphisms was associated with periodontitis in samples from Brazilian populations (O.R. G allele=2.39, p=0.03), whereas the IL6 -572G allele increases the risk for chronic periodontitis (O.R.=1.58, p=0.04) and periodontitis in Europeans (O.R.=2.11, p=0.005) [30]. Patients with severe periodontitis carrying the IL6 -174G variant displayed significantly higher serum IL6 and C-reactive

TTV is a highly prevalent virus in humans across the globe with a controversial involvement in human pathology (reviewed in [5]). However, studies showed that the presence of TTV DNA in the gingival tissue is associated with periodontitis [33]. TGFb and IL6 polymorphisms were also associated with higher risk for other chronic diseases, while TTV is believed to lead to or worsen pre-existent conditions related to chronic inflammation [34].

The prevalence of TTV DNA in the subjects tested in this study was 61.6%. Also, the distribution differed significantly between the patients and matched controls (66.5% vs. 56.9%, p=0.03).

The presence of TTV DNA in the blood of tested subject was not associated with any lifestyle choice (drinking and smoking habits, oral hygiene, physical activity, diet or living environment). The results from the

present paper revealed an association between TTV DNA and periodontitis in the overall group of patients (O.R.=1.48, 95%CI: 1.04-2.12, p=0.03). The split analysis revealed that the percent of TTV DNA was higher only in female patients (O.R.=1.86, 95%CI: 1.11-3.12, p=0.02) and are consistent with previous studies [34]. The analysis performed on couples of variables showed a significant association between the presence of TTV DNA and the IL6 -174G allele, both in the overall study group, as well as in subgroups of men and women (Table 4). This finding may be explained by the modulating effect of TTV on regulation and production of cytokines, including IL6 [6].

Table 4. Distribution of couples of variables in patients and controls enrolled in this study.

Investigated risk factors	Patients	Controls	Statistical significance
IL6 GG and TTV	111/144	65/195	O.R.=2.31, 95% CI: 1.59-3.36, p=0.001
IL6 GG and TTV (men)	58/70	35/97	O.R=2.29, 95% CI: 1.36-3.86, p=0.001
IL6 GG and TTV DNA (women)	53/74	30/98	O.R.=2.34, 95%CI: 1.36- 4.01, p=0.002

The minor allele frequency of the polymorphisms at the TGFb and IL6 promoters varies greatly among populations. Overall, the minor allele IL6 -174C is either absent or very rare in some samples from Asia and Africa populations, whereas in Caucasians its frequency can reach 54% [35, 36]. The frequency of the minor allele for both polymorphisms in the control lot was comparable with results published previously for other populations.

Study limitations. Two limitations of the present study are the restricted size of case samples and lack of data regarding periodontal pathogens present in plaque.

Conclusions

Our data showed that lifestyle choices (e.g. *inadequate* personal *oral hygiene* -O.R. =7.66, p<0.0001, taking irregular meals -O.R. =2.82, p<0.001 and smoking O.R.=1.5, p=0.01) increase the risk of periodontitis. We also identified a significant association between periodontitis and IL6 -174G allele and TTV in the overall group of patients (O.R.=1.58, p=0.01). Split analysis revealed that IL6 -174G allele was more likely to confer risk in men (O.R. =1.82, p=0.02), while TTV was more likely associated with periodontitis in women (O.R. =1.86, p=0.02). Further investigation conducted on a larger group with less confounding factors is required in order to validate these findings.

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References

1. J.M. SAMUEL, D. KELBERMAN, A.J.P. SMITH, S.E. HUMPHRIES, P. WOO, Identification of a novel regulatory region in the interleukin-6 gene promoter. *Cytokine*, 42(2), 256-264 (2008).
2. M.S. FIFE, E.M. OGILVIE, D. KELBERMAN, J. SAMUEL, A. GUTIERREZ, S.E. HUMPHRIES, P. WOO, Novel IL-6 haplotypes and disease association. *Genes Immun*, 6(4), 367-70 (2005).
3. W. GU, D.Y. DU, J. HUANG, L.Y. ZHANG, Q. LIU, P.F. ZHU, Z.G. WANG, J.X. JIANG, Identification of interleukin-6 promoter polymorphisms in the Chinese Han population and their functional significance. *Crit Care Med*, 36(5), 1437-43 (2008).
4. C.B. FOSTER, T. LEHRNBECHER, S. SAMUELS, S. STEIN, F. MOL, J.A. METCALF, K. WYVILL, S.M. STEINBERG, J. KOVACS, A. BLAUVELT, R. YARCHOAN, S.J. CHANOCK, An IL6 promoter polymorphism is associated with a lifetime risk of development of Kaposi sarcoma in men infected with human immunodeficiency virus. *Blood*, 96(7), 2562-7 (2000).
5. S. SPANDOLE, D. CIMPONERIU, L.M. BERCA, G. MIHAESCU, Human anelloviruses: an update of molecular, epidemiological and clinical aspects. *Arch Virol*, 160(4), 893-908 (2015).
6. J. ROCCHI, V. RICCI, M. ALBANI, L. LANINI, E. ANDREOLI, L. MACERA, M. PISTELLO, L. CECCHERINI-NELLI, M. BENDINELLI, F. MAGGI, Torquetenovirus DNA drives proinflammatory cytokines production and secretion by immune cells via toll-like receptor 9. *Virology*, 394(2), 235-42 (2009).
7. S. YE, S. DHILLON, X. KE, A.R. COLLINS, I.N. DAY, An efficient procedure for genotyping single nucleotide polymorphisms. *Nucleic Acids Res*, 29(17), E88-8 (2001).
8. A.A. ECOVOIU, I.C. GHIONOIU, A.M. CIUCA, A.C. RATIU, Genome ARTIST: a robust, high-accuracy aligner tool for mapping transposon insertions and self-insertions. *Mob DNA*, 7(3 (2016).

9. M. NINOMIYA, M. TAKAHASHI, T. NISHIZAWA, T. SHIMOSEGAWA, H. OKAMOTO, Development of PCR assays with nested primers specific for differential detection of three human anelloviruses and early acquisition of dual or triple infection during infancy. *J Clin Microbiol*, 46(2), 507-14 (2008).
10. H. YOSHIE, T. KOBAYASHI, H. TAI, J.C. GALICIA, The role of genetic polymorphisms in periodontitis. *Periodontol 2000*, 43(102-32 (2007).
11. M. TABA, JR., S.L. SOUZA, V.C. MARIGUELA, Periodontal disease: a genetic perspective. *Braz Oral Res*, 26 Suppl 1(32-8 (2012).
12. X.Z. ZHAO, Z.M. GUAN, Y.M. ZHANG, [Relationship between transforming growth factor beta-1 gene-509C/T polymorphism and severe chronic periodontitis]. *Zhonghua Kou Qiang Yi Xue Za Zhi*, 45(10), 4 (2010).
13. T. KOBAYASHI, T. NAGATA, S. MURAKAMI, S. TAKASHIBA, H. KURIHARA, Y. IZUMI, Y. NUMABE, H. WATANABE, M. KATAOKA, A. NAGAI, J. HAYASHI, H. OHYAMA, Y. OKAMATSU, Y. INAGAKI, H. TAI, H. YOSHIE, Genetic risk factors for periodontitis in a Japanese population. *J Dent Res*, 88(12), 1137-41 (2009).
14. G. ATILLA, G. EMINGIL, T. KOSE, A. BERDELI, TGF-beta1 gene polymorphisms in periodontal diseases. *Clin Biochem*, 39(9), 929-34 (2006).
15. A. ATANASOVSKA-STOJANOVSKA, D. TRAJKOV, M. POPOVSKA, M. SPIROSKI, Analysis of transforming growth factor-beta1 gene polymorphisms in Macedonian patients with chronic periodontitis. *Macedonian Journal of Medical Sciences*, 2(1), 30-35 (2009).
16. N. BABEL, G. CHEREPNEV, D. BABEL, A. TROPMANN, M. HAMMER, H.D. VOLK, P. REINKE, Analysis of tumor necrosis factor-alpha, transforming growth factor-beta, interleukin-10, IL-6, and interferon-gamma gene polymorphisms in patients with chronic periodontitis. *J Periodontol*, 77(12), 1978-83 (2006).
17. Y. KOMATSU, J.C. GALICIA, T. KOBAYASHI, K. YAMAZAKI, H. YOSHIE, Association of interleukin-1 receptor antagonist +2018 gene polymorphism with Japanese chronic periodontitis patients using a novel genotyping method. *Int J Immunogenet*, 35(2), 165-70 (2008).
18. C. STOLL, S. MENGSTEAB, D. STOLL, D. RIEDIGER, A.M. GRESSNER, R. WEISKIRCHEN, Analysis of polymorphic TGFB1 codons 10, 25, and 263 in a German patient group with non-syndromic cleft lip, alveolus, and palate compared with healthy adults. *BMC Med Genet*, 5(15 (2004).
19. A.P. DE SOUZA, P.C. TREVILATTO, R.M. SCAREL-CAMINAGA, R.B. DE BRITO, S.R. LINE, Analysis of the TGF-beta1 promoter polymorphism (C-509T) in patients with chronic periodontitis. *J Clin Periodontol*, 30(6), 519-23 (2003).
20. L.I. HOLLA, A. FASSMANN, P. BENES, T. HALABALA, V. ZNOJIL, 5 polymorphisms in the transforming growth factor-beta 1 gene (TGF-beta 1) in adult periodontitis. *J Clin Periodontol*, 29(4), 336-41 (2002).
21. H.Y. ZHANG, L. FENG, H. WU, X.D. XIE, The association of IL-6 and IL-6R gene polymorphisms with chronic periodontitis in a Chinese population. *Oral Dis*, 20(1), 69-75 (2014).
22. P.L. CASADO, R. VILLAS-BOAS, W. DE MELLO, M.E. DUARTE, J.M. GRANJEIRO, Peri-implant disease and chronic periodontitis: is interleukin-6 gene promoter polymorphism the common risk factor in a Brazilian population? *Int J Oral Maxillofac Implants*, 28(1), 35-43 (2013).
23. N.B. KALBURGI, A. BHATIA, S. BILICHODMATH, S.R. PATIL, S.B. MANGALEKAR, K. BHAT, Interleukin-6 promoter polymorphism (-174 G/C) in Indian patients with chronic periodontitis. *J Oral Sci*, 52(3), 431-7 (2010).
24. M. IANNI, G. BRUZZESI, D. PUGLIESE, E. PORCELLINI, I. CARBONE, A. SCHIAVONE, F. LICASTRO, Variations in inflammatory genes are associated with periodontitis. *Immun Ageing*, 10(1), 39 (2013).
25. L. SCAPOLI, A. GIRARDI, A. PALMIERI, F. CARINCI, T. TESTORI, F. ZUFFETTI, R. MONGUZZI, D. LAURITANO, IL6 and IL10 are genetic susceptibility factors of periodontal disease. *Dent Res J (Isfahan)*, 9(Suppl 2), S197-201 (2012).
26. W.H. FAN, D.L. LIU, L.M. XIAO, C.J. XIE, S.Y. SUN, J.C. ZHANG, Coronary heart disease and chronic periodontitis: is polymorphism of interleukin-6 gene the common risk factor in a Chinese population? *Oral Dis*, 17(3), 270-6 (2011).
27. W.T. LOO, C.B. FAN, L.J. BAI, Y. YUE, Y.D. DOU, M. WANG, H. LIANG, M.N. CHEUNG, L.W. CHOW, J.L. LI, Y. TIAN, L. QING, Gene polymorphism and protein of human pro- and anti-inflammatory cytokines in Chinese healthy subjects and chronic periodontitis patients. *J Transl Med*, 10 Suppl 1(S8 (2012).
28. Y. KOMATSU, H. TAI, J.C. GALICIA, Y. SHIMADA, M. ENDO, K. AKAZAWA, K. YAMAZAKI, H. YOSHIE, Interleukin-6 (IL-6)--373 A9T11 allele is associated with reduced susceptibility to chronic periodontitis in Japanese subjects and decreased serum IL-6 level. *Tissue Antigens*, 65(1), 110-114 (2005).
29. P.C. TREVILATTO, R.M. SCAREL-CAMINAGA, R.B. DE BRITO, JR., A.P. DE SOUZA, S.R. LINE, Polymorphism at position -174 of IL-6 gene is associated with susceptibility to chronic periodontitis in a Caucasian Brazilian population. *J Clin Periodontol*, 30(5), 438-42 (2003).
30. J.Y. YU, L. LI, H. MA, K. LIU, X. CHENG, Y.L. LI, X.L. SONG, Tumor necrosis factor-alpha 238 G/A polymorphism and gastric cancer risk: a meta-analysis. *Tumour Biol*, 34(6), 3859-63 (2013).

31. F. D'AIUTO, M. PARKAR, P.M. BRETT, D. READY, M.S. TONETTI, Gene polymorphisms in pro-inflammatory cytokines are associated with systemic inflammation in patients with severe periodontal infections. *Cytokine*, 28(1), 29-34 (2004).
32. L. NIBALI, N. DONOS, S. FARRELL, D. READY, J. PRATTEN, Y.K. TU, F. D'AIUTO, Association between interleukin-6 -174 polymorphism and Aggregatibacter actinomycetemcomitans in chronic periodontitis. *J Periodontol*, 81(12), 1814-9 (2010).
33. R. ROTUNDO, F. MAGGI, M. NIERI, L. MUZZI, M. BENDINELLI, G.P. PRATO, TT virus infection of periodontal tissues: a controlled clinical and laboratory pilot study. *J Periodontol*, 75(9), 1216-20 (2004).
34. S. SPANDOLE-DINU, D.G. CIMPONERIU, A.-M. CRĂCIUN, I. RADU, S. NICA, M. TOMA, O.A. ALEXIU, C.S. IORGA, L.-M. BERCA, R. NICA, Prevalence of human anelloviruses in Romanian healthy subjects and patients with common pathologies. *BMC Infectious Diseases*, 18(1), 334 (2018).
35. M.L. LAINE, B.G. LOOS, W. CRIELAARD, Gene polymorphisms in chronic periodontitis. *Int J Dent*, 2010(324719 (2010).
36. P. SODSAI, J. NAKKUNTOD, P. KUPATAWINTU, N. HIRANKARN, Distribution of cytokine gene polymorphisms in Thai population. *Tissue Antigens*, 77(6), 593-7 (2011).