



ORIGINAL ARTICLE

Effect of tumor necrosis factor alpha (TNF- α) -308 and -1031 gene polymorphisms on periodontitis among Saudi subjects

Ehab Azab^{a,*}, Fathy M. Elfakhany^{a,b}

^a Department of Basic and Clinical Oral Sciences, Faculty of Dentistry, Umm Al-Qura, University, Makkah, Saudi Arabia

^b Department of Medical Biochemistry, Faculty of Medicine, Tanta University, Tanta, Egypt

Received 25 September 2021; revised 30 January 2022; accepted 8 February 2022

Available online 12 February 2022

KEYWORDS

Periodontitis;
Tumor necrosis factor- α ;
Gene polymorphism;
PCR;
Restriction enzyme

Abstract *Objectives:* Periodontitis is an infectious disorder that leads to irreversible loss of the surrounding attachment and bone destruction. Genetic polymorphism of cytokines has been suggested to play a role in periodontitis. This case-control study aimed to investigate the relationship between periodontitis and two single nucleotide polymorphisms (SNPs): rs1800629 (-308 G/A) and rs1799964 (-1031 T/C), in the TNF- α gene promoter area.

Materials and methods: Peripheral blood was used to prepare genomic DNA from 60 subjects with stage II to stage III periodontitis, as along with 65 control subjects. Polymerase chain reaction and restriction endonuclease digestion were used to genotype TNF- α SNPs.

Results: The distribution of both genotypes and alleles of TNF- α (-308 G/A) polymorphism did not vary between periodontitis subjects and the controls ($P > 0.05$). However, the CT genotype and C allele of the TNF- α (-1031 T/C) polymorphism were observed more frequently in the periodontitis subjects than in the controls, while the TT genotype and the T allele were more predominant in the control subjects than in the periodontitis patients (OR: 3.149; 95% CI: 1.494–6.639; $P = 0.002$ and OR: 2.933; 95% CI: 1.413–6.090; $P = 0.003$, sequentially).

Conclusion: The TNF- α (-308 G/A) polymorphism potentially has no correlation with periodontitis susceptibility, whereas the TNF- α (-1031) CT genotype and C allele might be related to periodontitis among Saudi subjects.

© 2022 The Authors. Production and hosting by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

* Corresponding author.

E-mail address: etazab@uqu.edu.sa (E. Azab).

Peer review under responsibility of King Saud University.



Production and hosting by Elsevier

1. Introduction

Periodontitis is a common inflammatory condition caused by pathogenic microorganisms that activate the host immunoinflammatory response (Van Dyke and van Winkelhoff, 2013). In addition, other risk factors including immunological, environmental, and genetic factors have been implicated in the

pathogenesis of periodontitis and have been reported to boost the patient's susceptibility to periodontal disease (Ronderos and Ryder, 2000; Van Dyke and Sheilesh, 2005; Kornman, 2008). In response to periodontal pathogenic attack, inflammatory cells release mediators such as metalloproteinases, proteolytic enzymes, and cytokines that are responsible for connective tissue damage, alveolar bone loss, and ultimately tooth loss (Cekici et al., 2014).

A variety of inflammatory mediators such as interleukins, TNF- α and matrix metalloproteinases have been reported to be upregulated in the periodontal tissue during periodontal disease (Heidari et al., 2019). Special precedence was given to the genetic perspectives of immunoinflammatory reactions, involving the implication of gene variations that encode the formation of inflammatory mediators and susceptibility to periodontitis (Schenkein, 2002).

TNF was first recognized as a tumor necrotizing substance. In addition to inducing cell death, TNF plays a pivotal role in pro-inflammatory and cellular communication (Pan et al., 2019). Historically, TNF was initially identified in 1984, when two cytotoxic factors were isolated: one derived from macrophages named TNF, and the other derived from lymphocytes named lymphotoxin. Both TNF and lymphotoxin exhibited 50% sequence homology in the amino acid sequences and bind to the same receptor; they are called TNF- α and TNF- β , respectively (Aggarwal et al., 2012). Previous studies have documented the involvement of the TNF superfamily in several biological processes including inflammation, apoptosis, proliferation, invasion, angiogenesis, metastasis, and morphogenesis (Aggarwal, 2003).

TNF has been reported to participate in bone metabolism. Although TNF does not directly stimulate osteoclast differentiation, it catalyzes the expression of receptor activator of nuclear factor κ B ligand in osteoblastic precursors (Walsh and Choi, 2014). Moreover, TNF also reduces the expression of osterix and runt-related transcription factor 2 in osteoblasts (Osta et al., 2014; Algate et al., 2016). Therefore, these conclusions suggest that TNF triggers bone destruction by stimulating osteoclastic activity and decreasing osteoblastic activity. TNF has been shown to be involved in periodontitis pathogenesis (Deo and Bhongade, 2010), wherein TNF levels were increased in the GCF and serum of patients with chronic periodontitis (Górska et al., 2003; Madureira et al., 2018). Interestingly, it has been reported that TNF induces apoptosis in gingival epithelial cell as well as fibroblast and blocks gingival fibroblastic extracellular matrix production (Arancibia et al., 2013; Basso et al., 2016).

In recent years, single nucleotide polymorphisms (SNPs) and their association with disease susceptibility have been widely studied with great interest. SNPs are considered a suitable tool for identifying genetic factors associated with periodontal disease initiation and progression (Tarannum and Faizuddin, 2012). Several polymorphisms within TNF- α promoter area including (-308 G/A), (-1031 T/C), (-857 C/T), (-238 G/A), and (-376 G/A) have been identified and studied in various populations. Some of these SNPs were found to be associated with periodontitis, while others were not (Kato et al., 1999; Craandijk et al., 2002; Soga et al., 2003; Ho et al., 2015; Majumder et al., 2018).

The most frequently studied TNF- α gene polymorphisms were (-308 G/A) and (-1031 T/C) which have not been exam-

ined previously in the Saudi population with periodontitis. Therefore, this case-control study aimed to examine TNF- α gene polymorphisms in a sample of a Saudi population with periodontitis, in comparison to a sample of healthy individuals.

2. Methods

2.1. Study subjects

A total of 125 Saudi subjects (60 unrelated systemically healthy subjects with stage II or stage III periodontitis, constituting the majority of our periodontally checked patients, and 65 healthy controls) were selected from the dental teaching hospital, Faculty of Dentistry at Umm AL-Qura University, Saudi Arabia. The research was conducted in the research laboratory, Faculty of Dentistry, Umm AL-Qura University, Saudi Arabia. Ethical approval was obtained from the Institutional Review Board of Umm Al Qura University, Makkah, Saudi Arabia. Furthermore, signed informed consent was obtained from all participants prior to their enrolment in the study. The controls were periodontally healthy and free from any systemic ailments. Age and gender were matched in both groups, apart from also ensuring that subjects possessed at least 20 teeth. Individuals with systemic diseases, lactation, pregnancy, orthodontic treatment, immunodeficiency diseases, chemotherapy, and smoking were excluded from this study. The sample size of 125 participants was calculated with 80% power and 5% alpha error using the Raosoft software, according to a previous study (Menezes and Colombo, 2008).

2.2. Clinical examination

The periodontal condition of all subjects was assessed by skilled investigators using the following parameters: plaque index (PI), probing depth (PD), bleeding on probing (BOP), and attachment loss (CAL) (Ramfjord et al., 1968; O'Leary et al., 1972; Ainamo and Bay, 1975). Subjects with BOP, CAL \geq 3 mm, PD \geq 5 mm, and bone loss \geq 20% on radiographic assessment were included in this investigation. A recent published report was used to characterize periodontitis (Tonetti et al., 2018).

2.3. DNA preparation

Peripheral venous blood was collected from all participants in tri-potassium ethylene diamine tetraacetate coated tubes and subsequently used for DNA preparation utilizing a salable kit (Qiagen, Germany). Polymerase chain reaction (PCR) was carried out utilizing the purified DNA.

2.4. Genotyping of tumor necrosis factor alpha

A 25 μ L PCR mixture containing 100 ng of purified DNA and 2 \times master mix (Thermo Fisher Scientific, Inc., USA) was used for PCR amplification. For TNF- α (-308 G/A) (rs1800629) genotyping, PCR was carried out as described previously (Guzmán-Flores et al., 2011). The primers used were: 5'- AGG CAA TAG GTT TTG AGG GCC AT -3' and 5'- TCC TCC CTG CTC CGA TTC CG -3'. The PCR cycling

conditions consisted of one step at 94 °C for 3 min, 60 °C for 1 min, and 72 °C for 1 min; then 35 cycles of 1 min at 94 °C, 1 min at 60 °C, and 2 min at 72 °C; and a terminal step of 1 min at 94 °C, 1 min at 60 °C, and 5 min at 72 °C. The PCR product was cut with *NcoI* (Thermo Fisher Scientific, Inc., MA, USA) according to supplier directives, separated on a 3.5% agarose gel, and visualized by UV light. The size of the final PCR fragment so obtained was 107 bp. Restriction cutting of the GG genotype yielded 80 and 27 bp fragments, while the AA genotype produced a 107 bp fragment (Fig. 1).

With regard to the TNF- α (-1031 T/C) (rs1799964) polymorphism, PCR was performed as previously described (Yun et al., 2011). Primers used were: 5'-TAT GTG ATG GAC TCA CCA GG -3' and 3'- CCT CTA CAT GGC CCT GTC TT -3'. The PCR conditions were as follows: initial step at 96 °C for 5 min; then 30 cycles of 94 °C for 30 s, 63 °C for 40 s, and 72 °C for 1 min; and a terminal step at 72 °C for 10 min. The amplified PCR fragment size was 264 bp. Ten μ L PCR product were cut with 1 μ L *BbsI* (Thermo Fisher Scientific) at 37 °C for 14 h and separated on a 2% agarose gel. The TT genotype yielded two bands of 251 and 13 base pairs, while the CC genotype produced three bands of 80, 71, and 13 base pairs each (Fig. 1).

2.5. Data analysis

Data analysis was carried out using SPSS version 21. Continuous variables were assessed using an independent *t*-test, whereas categorical data were assessed using the Chi-square

analysis. Odds ratios were determined with a 95% confidence interval, and $P < 0.05$ was considered statistically significant.

3. Results

3.1. Clinical measurements

Both clinical and demographic data are presented in Table 1. The average measurements of PI, CAL, BOP, and PD were significantly more in periodontitis patients than control group ($P < 0.001$). This proved that both groups were well-matched.

3.2. TNF- α (-308) genotyping

The genotypes and alleles of the TNF- α (-308) polymorphism which were in Hardy-Weinberg equilibrium for both groups have been presented in Table 2. In the control group, the GG, AG, and AA genotypes were 86.15%, 13.85%, and 0%, respectively, while they were 78.33%, 20%, and 1.67%, respectively, in the patient group. Moreover, the proportion of the G allele was observed to be 93.08% and 88.33%, while the A allele was 6.92% and 11.67% in the control and periodontitis subjects, respectively. The genotype and allele distribution of TNF- α (-308) did not vary between both groups ($P > 0.05$).

3.3. TNF- α (-1031) gene polymorphism

The genotypes and alleles of the TNF- α (-1031) polymorphism, which were in Hardy-Weinberg equilibrium for both groups, have been tabulated as depicted in Table 2. The TT, CT, and CC genotypes had a predominance of 67.69%, 29.23%, and 3.08%, respectively, in the control group, while they were 41.67%, 56.67%, and 1.66%, respectively, in the patient group. The CT genotype was significantly more in subjects with periodontitis than in control individuals (OR: 3.149, 95% CI: 1.494–6.639, $P = 0.002$) and TT genotype (used as a reference) appeared to be more protected (Table 2). In addition, the proportion of T allele was 82.31% and 70%, whereas C allele was 17.69% and 30% in the controls and periodontitis subjects, respectively. The T allele (used as a reference) was significantly more common in the controls than in the subjects with periodontitis, while the C allele was significantly more common in periodontitis patients than the control individuals (OR: 2.933, 95% CI: 1.413–6.09, $P = 0.003$) (Table 2). This finding implies that individuals with the CT genotype and those with the C allele are more likely to develop periodontitis than those with the TT genotype.

4. Discussion

The presence of periodontopathic bacteria induces the increased production of proinflammatory cytokines from the host, resulting in periodontal disease progression (Gemmell and Seymour, 2004). TNF- α has been shown to participate in bone metabolism and is elevated in the GCF of patients with periodontitis. Hence, the TNF- α encoded gene is a candidate gene that might be involved in periodontitis susceptibility. Multiple polymorphisms in the TNF- α gene have been detected, which may have an impact on genetic predisposition to periodontal disease (Pan et al., 2019).

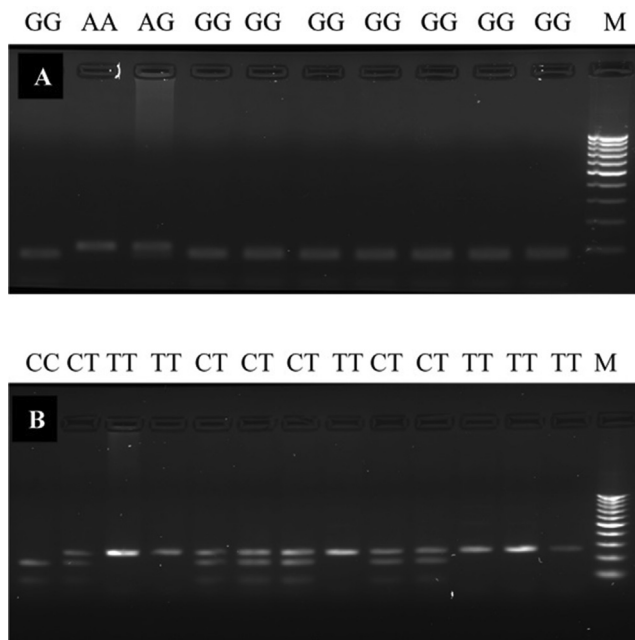


Fig. 1 Agarose electrophoresis of TNF -308 and -1031 genotypes. The 264 bp PCR products of the TNF gene were digested with *NcoI* (A) and *BbsI* (B) restriction enzymes, followed by their resolution in an agarose gel and visualization with UV light. Lane M = DNA molecular ladders (100–1000 bp). The remaining lanes correspond to the genotypes labeled at the top of each photo.

Table 1 Demographic and laboratory data for control subjects and patients with periodontitis.

Characteristics	Control group (65)	Periodontitis group (60)	P value
Age (years)	41.48 \pm 5.68	42.13 \pm 5.65	0.519
Gender (M/F)	38/27	32/28	0.564
BOP (%)	8.67 \pm 1.05	49.43 \pm 7.11	<0.001
PD (mm)	1.18 \pm 0.52	5.06 \pm 0.8	<0.001
CAL (mm)	0.71 \pm 0.19	4.87 \pm 0.91	<0.001
PI (%)	4.75 \pm 0.45	48.41 \pm 3.47	<0.001

Data are shown as mean \pm SD. NS = non-significant.

TNF- α gene polymorphisms have been studied in periodontitis in various populations, but the results are inconsistent (Laine et al., 2010)(Laine et al., 2012). This case-control study examined two SNPs in TNF- α promoter area: rs1800629 (-308 G/A) and rs1799964 (-1031 T/C), along with their relationship with periodontitis. The GG genotype of the TNF- α (-308) SNP was observed to be more common in the investigated samples than in the AA genotype; however, there was no significant variation in genotype and allele frequencies between both studied groups ($P > 0.05$). For the TNF- α (-1031) SNP, the TT genotype was more common in Saudi participants than the CC genotype; however, the CT genotype and C allele were more in periodontitis group, whereas the TT genotype and T allele were lower in comparison with the control individuals ($P = 0.002$ and 0.003 , respectively).

Several investigators have obtained concurrent results. Menezes and Colombo (2008) as well as Moreira et al. (2009) demonstrated that the TNF- α (-308) polymorphism is not linked to periodontal disease among Brazilians. In addition, Donati et al. (2005) did not find any link between chronic periodontitis and the TNF- α -308 polymorphism in Swedish Caucasian subjects of a northern European origin. Furthermore, similar results were obtained in several ethnic populations that also exhibited no correlation between TNF- α -308 genotypes and periodontal diseases in Germans (Folwaczny et al., 2004), Czech population (Fassmann et al., 2003),

Netherlandish subjects (Craandijk et al., 2002), Greek population (Sakellari et al., 2000), Japanese population with severe periodontitis (Soga et al., 2003), Colombian, population (Amaya et al., 2013), Turkish population (Özer Yücel et al., 2015), and Taiwanese population (Ho et al., 2015). All the above results are in agreement with our findings.

In contrast, other investigators obtained contrary results that reported an association between TNF- α (-308) polymorphism and periodontitis. Sharma et al. (2014) revealed that the TNF- α (-308) A allele is linked to a higher incidence of chronic periodontitis in the Malayalam-speaking Dravidian population in India. Moreover, Majumder et al. (2018) reported that TNF- α (-308 G/A) genotype was linked with both aggressive and chronic periodontitis in the eastern Indian population.

With regard to the TNF- α (-1031) polymorphism, several investigators have evaluated these polymorphisms in their ethnic populations. Yang et al. (2013) investigated four TNF- α gene polymorphisms with susceptibility to aggressive periodontitis and chronic periodontitis in Chinese subjects. They demonstrated that the TNF- α (-1031) CC genotype is associated with chronic periodontitis, while TNF- α (-308) AA genotype is associated with aggressive periodontitis. Furthermore, Soga et al. (2003) examined the relationship between severe periodontitis in Japanese and five SNPs in the TNF- α promoter area and discovered that periodontitis patients had a greater incidence of at least one allele in TNF- α -1031, -863, or -857 SNPs, compared to healthy controls. In addition, an association between TNF- α gene (-1031) gene polymorphism and chronic periodontitis has been reported in the Indian population (Majumder et al., 2018). These results were consistent with our observations. However, Endo et al. (2001) obtained contrary results and did not find any association between the TNF- α gene (-1031) polymorphism and early onset periodontitis.

In accordance with the above, the findings of the present investigation on the link between TNF- α genotypes and the risk of periodontal disease varied across ethnic groups. The reasons for these conflicting results may be attributed to several factors, such as the differences in the inclusion criteria

Table 2 Genotype distribution and allele frequencies of TNF- α -308 (G/A) and -1031 (T/C) polymorphisms in subjects with periodontitis and the control group.

TNF- α polymorphism	Control group (N = 65)		Periodontitis group (N = 60)		^a χ^2	P value	Odds ratio	95% CI
-308 (G/A) genotypes:	N	%	N	%				
GG	56	86.15	47	78.33			1	
AG	9	13.85	12	20	0.927	0.350	1.589	0.616–4.097
AA	0	0	1	1.67	1.178	0.462	0.979	0.94–1.02
Alleles:								
G	121	93.08	106	88.33	1.316	0.347	1	
A	9	6.92	14	11.67			1.721	0.676–4.380
-1031 C/T genotypes:								
TT	44	67.69	25	41.67			1	
CT	19	29.23	34	56.67	9.356	0.002	3.149	1.494 – 6.639
CC	2	3.08	1	1.66	0.01	0.919	0.88	0.076–10.199
Alleles:								
T	107	82.31	84	70	8.546	0.003	1	
C	23	17.69	36	30			2.933	1.413 – 6.090

^a Chi-square analysis of genotypes and alleles between patients with periodontitis and healthy controls.

of subjects in the patients and control groups, the variations in genetic backgrounds of the populations (so the ethnic origin of subjects must be considered), the definition of disease, population heterogeneity, exposure, as well as presence of different environmental and demographic confounding risk factors. Another important point for consideration is that an allele in a certain position (e.g., TNF- α (-308) A allele) has been established to be very rare in the Saudi population, while this allele was more frequently represented in other populations, such as in Brazilian (Menezes and Colombo, 2008) and Indian populations (Majumder et al., 2018).

As per our understanding, this is the first study in Saudi Arabia to investigate the relationship between TNF- α (-308 G/A) as well as (-1031 T/C) polymorphisms and periodontitis risk. However, there are some limitations most notably the small sample size. Further research involving a larger cohort of subjects with various stages of periodontitis is necessary, along with more detailed clinical information and serological analyses, in order to clarify the role of TNF- α and its genetic modification in periodontitis risk and progression.

5. Conclusion

The results obtained in this investigation indicated that TNF- α (-308 G/A) polymorphisms might not be associated with the risk of periodontitis, whereas the CT genotype and the C allele of the TNF- α (-1031) T/C polymorphism are potential risk factors for periodontitis in Saudi subjects.

6. Source of funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical statement

This study was approved by the Institutional Review Board of Umm Al Qura University, Makkah, Saudi Arabia.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The authors would like to thank Dr. Abdulrahman Yousuf for his technical support in the research laboratory in the Faculty of Dentistry, Um Al-Qura University, Saudi Arabia. They also thank Dr. Abd Elaziz Yasin for his help with the statistical analyses.

References

Aggarwal, B.B., 2003. Signaling Pathways of The TNF Superfamily: A Double-Edged Sword. *Nat. Rev. Immunol.* 3 (9), 745–756.

- Aggarwal, B.B., Gupta, S.C., Kim, J.H., 2012. Historical perspectives on tumor necrosis factor and its superfamily: 25 years later, a golden journey. *Blood* 119 (3), 651–665.
- Ainamo, J., Bay, I., 1975. Problems and proposals for recording gingivitis and plaque. *Int. Dent. J.* 25, 229–235.
- Algate, K., Haynes, D., Bartold, P., Crotti, T., Cantley, M., 2016. The effects of tumour necrosis factor- α on bone cells involved in periodontal alveolar bone loss; osteoclasts, osteoblasts and osteocytes. *J. Periodontol. Res.* 51, 549–566.
- Amaya, M.P., Criado, L., Blanco, B., et al, 2013. Polymorphisms of pro-inflammatory cytokine genes and the risk for acute suppurative or Chronic nonsuppurative apical periodontitis in a Colombian population. *Int. Endod. J.* 46 (1), 71–78.
- Arancibia, R., Oyarzún, A., Silva, D., Tobar, N., Martínez, J., Smith, P.C., 2013. Tumor necrosis factor- α inhibits transforming growth factor- β -stimulated myofibroblastic differentiation and extracellular matrix production in human gingival fibroblasts. *J. Periodontol.* 84, 683–693.
- Basso, F.G., Pansani, T.N., Turrioni, A.P., Soares, D.G., de Souza Costa, C.A., Hebling, J., 2016. Tumor necrosis factor- α and interleukin (IL)-1 β , IL-6, and IL-8 impair in vitro migration and induce apoptosis of gingival fibroblasts and epithelial cells, delaying wound healing. *J. Periodontol.* 87, 990–996.
- Cekici, A., Kantarci, A., Hasturk, H., Van Dyke, TE., 2014. Inflammatory and immune pathways in the pathogenesis of periodontal disease. *Periodontol.* 2000. 64(1), 57–80.
- Craandijk, J., van Krugten, M.V., Verweij, C.L., van der Velden, U., Loos, B.G., 2002. Tumor necrosis factor-alpha gene polymorphisms in relation to periodontitis. *J. Clin. Periodontol.* 29 (1), 28–34.
- Deo, V., Bhongade, M.L., 2010. Pathogenesis of periodontitis: role of cytokines in host response. *Dent. Today.* 29(9), 60–2, 64–6, quiz 68–9.
- Donati, M., Berglundh, T., Hytönen, AM., Hahn-Zoric, M., Hanson, LA., Padyukov, L., 2005. Association of the -159 CD14 gene polymorphism and lack of association of the -308 TNFA and Q551R IL-4RA polymorphisms with severe chronic periodontitis in Swedish Caucasians. *J. Clin. Periodontol.* 32(5), 474–9.
- Endo, M., Tai, H., Tabeta, K., Kobayashi, T., Yamazaki, K., Yoshie, H., 2001. Analysis of single nucleotide polymorphisms in the 5' flanking region of tumor necrosis factor-alpha gene in Japanese patients with early-onset periodontitis. *J. Periodontol.* 72 (11), 1554–1559.
- Fassmann, A., Holla, L.I., Buckova, D., Vasku, A., Znojil, V., Vanek, J., 2003. Polymorphisms in the +252(A/G) lymphotoxin-alpha and the -308(A/G) tumor necrosis factor alpha genes and susceptibility to chronic periodontitis in a Czech population. *J. Periodontol. Res.* 38 (4), 394–399.
- Folwaczny, M., Glas, J., Török, H.P., Mende, M., Folwaczny, C., 2004. Lack of association between the TNF alpha G -308 A promoter polymorphism and periodontal disease. *J. Clin. Periodontol.* 31 (6), 449–453.
- Gemmell, E., Seymour, G.J., 2004. Immunoregulatory control of Th1/Th2 cytokine profiles in periodontal disease. *Periodontology* 2000 (35), 21–41.
- Górska, R., Gregorek, H., Kowalski, J., Laskus-Perendyk, A., Syczewska, M., Madaliński, K., 2003. Relationship between clinical parameters and cytokine profiles in inflamed gingival tissue and serum samples from patients with chronic periodontitis. *J. Clin. Periodontol.* 30, 1046–1052.
- Guzmán-Flores, Juan M., Muñoz-Valle, Jose F., Sánchez-Corona, Jose, Cobián, Jose G., Medina-Carrillo, Leopoldo, García-Zapién, Alejandra G., et al, 2011. Tumor necrosis factor-alpha gene promoter -308G/A and 238G/A polymorphisms in Mexican

- patients with type 2 diabetes mellitus. *Dis. Markers* 30 (1), 19–24. <https://doi.org/10.3233/DMA-2011-0759>.
- Heidari, Z., Moudi, B., Mahmoudzadeh-Sagheb, H., 2019. Immunomodulatory factors gene polymorphisms in chronic periodontitis: an overview. *BMC Oral Health*. 19(1), 29.
- Ho, Y.P., Lin, Y.C., Yang, H.Y., Chou, Y.H., Ho, K.Y., Wu, Y.M., Tsai, C.C., 2015. Analysis of tumor necrosis factor- α -308 and lymphotoxin- α +252 gene polymorphisms in Taiwanese patients with periodontitis. *Journal of Dental Sciences* 10, 316–322.
- Kato, T., et al., 1999. Novel polymorphism in the promoter region of the tumor necrosis factor alpha gene: No association with narcolepsy. *American journal of medical genetics*. 88(4), 301–4.
- Kornman, K.S., 2008. Mapping the pathogenesis of periodontitis: a new look. *J. Periodontol.* 9 (8 Suppl), 1560–1568.
- Laine, M.L., Loos, B.G., Crielaard, W., 2010. Gene polymorphisms in chronic periodontitis. *Int. J. Dentistry*. 2010, 324719.
- Laine, M.L., Crielaard, W., Loos, B.G., 2012. Genetic susceptibility to periodontitis. *Periodontol.* 2000 (58), 37–68.
- Madureira, D.F., De Abreu, L., Lima, I., Costa, G.C., Lages, B.E.M., Martins, C.C., Aparecida Da Silva, T., 2018. Tumor necrosis factor-alpha in gingival crevicular fluid as a diagnostic marker for periodontal diseases: a systematic review. *J. Evid. Based Dent. Pract.* 18, 315–331.
- Majumder, P., Thou, K., Bhattacharya, M., Nair, V., Ghosh, S., Dey, S.K., 2018. Association of tumor necrosis factor-alpha (TNF-alpha) gene promoter polymorphisms with aggressive and chronic periodontitis in the eastern Indian population. *Biosci. Rep.* 38(4), BSR20171212.
- Menezes, N.G., Colombo, A.P., 2008. Lack of association between the TNF-alpha -308 (G/A) genetic polymorphism and periodontal disease in Brazilians. *Braz Oral Res.* 22 (4), 322–327.
- Moreira, P.R., Costa, J.E., Gomez, R.S., Gollob, K.J., Dutra, W.O., 2009. TNFA and IL10 gene polymorphisms are not associated with periodontitis in Brazilians. *Open Dent. J.* 3, 184–190.
- O'Leary, T.J., Drake, R.B., Naylor, J.E., 1972. The plaque control record. *J. Periodontol.* 43 (1), 38.
- Osta, B., Benedetti, G., Miossec, P., 2014. Classical and paradoxical effects of TNF- α on bone homeostasis. *Front. Immunol.* 5, 48.
- Özer Yücel, Ö., Berker, E., Mesci, L., Eratalay, K., Tepe, E., Tezcan, İ., 2015. Analysis of TNF- α (-308) polymorphism and gingival crevicular fluid TNF- α levels in aggressive and chronic periodontitis: A preliminary report. *Cytokine* 72 (2), 173–177.
- Pan, W., Wang, Q., Chen, Q., 2019. The cytokine network involved in the host immune response to Periodontitis. *Int. J. Oral Sci.* 11 (3), 30.
- Ramfjord, S.P., Emslie, R.D., Greene, J.C., Held, A.J., Waerhaug, J., 1968. Epidemiological studies of periodontal diseases. *Am. J. Public Health Nations Health.* 58, 1713–1722.
- Ronderos, M., Ryder, M.I., 2000. Risk assessment in clinical practice. *Periodontol.* 34, 120–135.
- Sakellari, D., Katsares, V., Georgiadou, M., Kouvatsi, A., Arsenakis, M., Konstantinidis, A., 2000. No correlation of five gene polymorphisms with periodontal conditions in a Greek population. *J. Clin. Periodontol.* 33 (11), 765–770.
- Schenkein, H.A., 2002. Finding genetic risk factors for periodontal diseases: is the climb worth the view? *Periodontol.* 30, 79–90.
- Sharma, N., Joseph, R., Arun, R., Chandni, R., Srinivas, K.L., Banerjee, M., 2014. Cytokine gene Polymorphism (interleukin-1beta + 3954, Interleukin-6 [-597/-174] and tumor necrosis factor-alpha -308) in chronic periodontitis with and without type 2 diabetes mellitus. *Indian J. Dent. Res.* 25 (3), 375–380.
- Soga, Y., Nishimura, F., Ohyama, H., Maeda, H., Takashiba, S., Murayama, Y., 2003. Tumor necrosis factor-alpha gene (TNF-alpha) -1031/-863, -857 single-nucleotide polymorphisms (SNPs) are associated with severe adult periodontitis in Japanese. *J. Clin. Periodontol.* 30 (6), 524–531.
- Tarannum, F., Faizuddin, M., 2012. Effect of gene polymorphisms on periodontal diseases. *Indian J. Hum. Genet.* 18 (1), 9–19.
- Tonetti, M.S., Greenwell, H., Kornman, K.S., 2018. Staging and grading of periodontitis: Framework and proposal of a new classification and case definition. *J. Periodontol.* 89 (Suppl. 1), S159–S172.
- Van Dyke, T.E., Sheilesh, D., 2005. Risk factors for periodontitis. *J. Int. Acad. Periodontol.* 7 (1), 3–7.
- Van Dyke, T.E., van Winkelhoff, A.J., 2013. Infection and inflammatory mechanisms. *J. Periodontol.* 84 (4 Suppl), S1–S7.
- Walsh, M.C., Choi, Y., 2014. Biology of the RANKL–RANK–OPG system in immunity, bone, and beyond. *Front. Immunol.* 5, 511.
- Yang, W., Jia, Y., Wu, H., 2013. Four tumor necrosis factor alpha genes polymorphisms and periodontitis risk in a Chinese population. *Hum Immunol.* 74 (12), 1684–1687.
- Yun, Ji H., Choi, Jin W., Lee, Kyung J., Shin, Joong S., Baek, Kwang H., 2011. The promoter -1031 (T/C) polymorphism in tumor necrosis factor-alpha associated with polycystic ovary syndrome. *Reprod. Biol. Endocrinol* 9, 131. <https://doi.org/10.1186/1477-7827-9-131>.