

Original Research

## Lack of association between the TNF- $\alpha$ <sup>-1031</sup> genotypes and generalized aggressive periodontitis disease

E. Darvishi<sup>1,2</sup>, Z. Aziziarani<sup>1,3</sup>, K. Yari<sup>1,3</sup>, M. Bagheri Dehbaghi<sup>3</sup>, D. Kahrizi<sup>1,2</sup>, H. Karim<sup>4,5</sup>, S. Vaziri<sup>4,6</sup>, J. Zargooshi<sup>4\*</sup>, K. Ghadiri<sup>7</sup>, S. Mohammadi<sup>4,5</sup>, E. Kazemi<sup>4</sup>, M. T. Moradi<sup>1,3</sup>, M. Shokrinia<sup>8</sup>, N. Mohammadi<sup>8</sup>

<sup>1</sup> Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran.

<sup>2</sup> Department of Agronomy and Plant Breeding, Faculty of Agriculture, Razi University, Kermanshah, Iran

<sup>3</sup> Zagros Bioidea Company, Razi University Incubator, Kermanshah, Iran

<sup>4</sup> Department of Sexual Medicine, The Rhazes Center for Research in Family Health and Sexual Medicine; Kermanshah University of Medical Sciences, Kermanshah, Iran

<sup>5</sup> Department of Cardiology, Kermanshah University of Medical Sciences, Kermanshah, Iran

<sup>6</sup> Department of Infectious Diseases, Kermanshah University of Medical Sciences, Kermanshah, Iran

<sup>7</sup> Nosocomial Infections Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran

<sup>8</sup> Kermanshah-Based, General Department of Taxation Affairs, Kermanshah, Iran

**Abstract:** Periodontal disease is one of the most prevalent inflammatory illnesses and is a main cause of tooth loss in human population. Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) gene is one of pro-inflammatory cytokines which has important role in pathogenesis of periodontal disease. The main purpose of this study is to determine genotype abundance of TNF- $\alpha$ <sup>-1031</sup> gene in both groups of patients and controls, and also investigation of relation of single nucleotide polymorphism (SNP) these genotypes with periodontal disease risk. DNA was extracted from blood tissue of 31 patients and 54 controls. The TNF- $\alpha$ <sup>-1031</sup> polymorphism was evaluated by polymerase chain reaction- confronting two-pair primer (PCR-CTPP) method. In the GAP group, the frequencies of TT, TC and CC genotypes were 35.48%, 61.29 and 3.23%, respectively. In controls the frequencies of TT, TC and CC genotypes were 22.22%, 72.22%, and 5.56%, respectively. Results of this study showed that there was no significant association between TNF- $\alpha$  (-1031 T/C promoter) gene polymorphisms and the risk of generalized aggressive periodontitis disease.

**Key words:** Generalized aggressive periodontitis, TNF- $\alpha$ <sup>-1031</sup>, polymorphism, PCR-CTPP.

### Introduction

Periodontal disease is one of the most prevalent inflammatory illnesses and is a main cause of tooth loss in human population. Clinically defined periodontal disease is highly widespread, has significant impacts on individuals and society and is costly to treat; the cost of dental care is the fourth highest costs of all diseases and consuming between 5 and 10% of all healthcare funds. Periodontal disease treatment costs may be as little as 500 \$, or as much as 10000 \$, depending on the severity of the disease. The cost for a regular dental prophylaxis averages between 30 \$ and 75 \$, while the average cost for periodontal scaling and root planing is about between \$140 and \$210. This disease causes tooth decay, loss of bony support, and tooth loss in all around the world and especially in developing countries (1, 2).

Albandar and Rams defined that approximately 25% to 30% subjects more than 30 years of age in United States had suffered from onerous periodontitis (1). Unfortunately in Iran it has not been investigated yet and there isn't any complete statistical study. Periodontitis is categorized in to two types- chronic periodontitis (CP) and aggressive periodontitis (AP) (3, 4). AP attacks gingival tissue rapidly and severely in people with age of 20 to 35 years old (3). AP is divided in to two types general aggressive and local aggressive. Local aggressive occurs in mature and damages the molar alveolar bone. All the teeth are attacked by general type. AP has two active and inactive phases. In the active phase, gingival

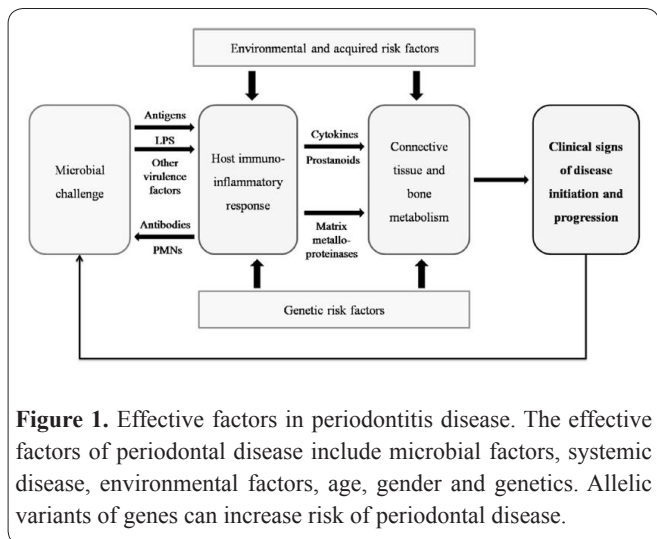
tissue becomes inflamed, but in the inactive phase, this event stops with or without signs of inflammation. The effective factors of periodontal disease may include: 1- microbial factors 2- systemic diseases *viz.*, human immunodeficiency virus (HIV) infection, diabetes, cardiovascular disease and etc 3- environmental factors, for example stress, heavy smoking and socioeconomic status 4- age (adults especially 20 to 35 years old) 5- gender (males are affected more frequently than females) 6- genetics. Many reports show that allelic variants of genes can increase risk of this illness (Figure 1) (2).

Inflammatory gene and cytokines are important genetic factors. Cytokines are known as mediators to immune system. The main biological roles of cytokines include proliferation, development, homeostasis, inflammation, gene regulation and repair (5). Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) gene is one of pro-inflammatory cytokines which has important role in pathogenesis of periodontal disease. This gene is in class III region of the major complex on the short arm of human chromosome 6 (4). The TNF- $\alpha$  as cytokine gene has been found

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\* **Corresponding author:** Javaad Zargooshi, Professor of Urology, Chair, Department of Sexual Medicine, Muhammad Zakariya Razi (Rhazes) Boulevard, Kermanshah University of Medical Sciences, Kermanshah, Iran. Email: zargooshi@gmail.com

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**Figure 1.** Effective factors in periodontitis disease. The effective factors of periodontal disease include microbial factors, systemic disease, environmental factors, age, gender and genetics. Allelic variants of genes can increase risk of periodontal disease.

at high expression levels in gingival tissues or gingival crevicular fluid from periodontal diseases. TNF- $\alpha$  was reported as a powerful inducer for destruction of tissue in different types of periodontal disease (6).

Reported studies confirmed that TNF- $\alpha$  is a highly polymorphic gene, and also genotypes of this gene are correlated with an increased possibility risk of cancers and different diseases. The association between genetic polymorphism of the related candidate genes with different types of periodontal disease in different ethnicities has been reported; but, there has been no reported study to examine possible association between the polymorphism of TNF- $\alpha^{1031}$  and generalized aggressive periodontitis disease among Iranian populations. Therefore, the main purpose of this study is to determine genotype abundance of TNF- $\alpha$  gene in both groups of patients and controls, and also investigation of polymorphism related of this genotypes with periodontal disease risk.

**Materials and Methods**

DNA *Taq* polymerase, agarose and polymerase chain reaction (PCR) materials were provided from Zagros Bioidea Co. (Iran). The other chemicals and components were purchased from Merck. All needed primers were synthesized by Sinaclon Co. (Iran). Double-distilled water was used to prepare the solutions.

**Sample collection**

All procedures of current study were approved by the ethics committee in Kermanshah University of Medical Sciences (Iran). This study was carried out in Razi University Incubator, Zagros Bioidea Co., Biotechnology Lab (Kermanshah-Iran). The informed consent based on the principles of the Helsinki II was signed by contributing Individuals. Medical and dental history of subjects and their families were informed based on their oral information. As mentioned in our pervious study (4), clinical and radiographic parameters such as, probing pocket depth (PD), bleeding on probing (BOP), and clinical attachment loss (CAL) were used to identify periodontitis. Exclusion parameters of the study were any history of HIV and hepatitis infection, diabetes, consumption of anti-inflammatory and anti-immune system drugs, lactation or pregnancy, and heavy smoking. Selection

of GAP patients and calculation of sample size for this study was based on our pervious study (4, 7). 54 healthy individuals as control group and 31 GAP patients studied in this research. The control group were 62% men and 38% women (mean age of about 25 $\pm$ 1.5 years), didn't have periodontal disease signs. Further, the GAP group consisted of 87% women and 13% men with the mean age of 21 $\pm$ 3.4 years.

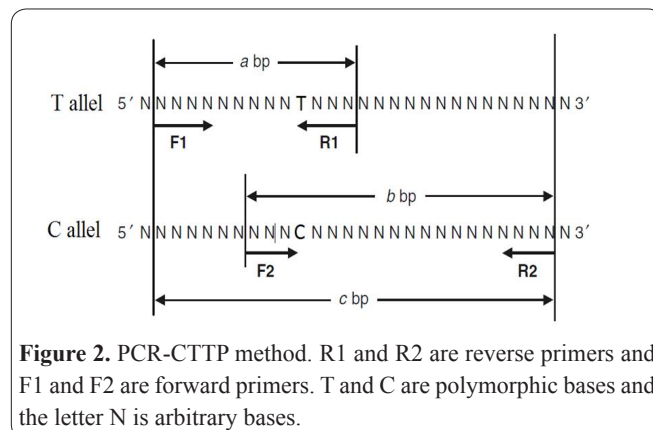
**DNA extraction**

DNA was extracted from blood cells of 31 patients and 54 controls according to Moradi and et al. protocol, 2014 (8). Then quantity of extracted DNA was checked by electrophoresis on 0.8% agarose gel, while purity and concentration of extracted DNA was analyzed with a Nano-drop with 260/280 measurement ratio and at the wavelength of 260 and 280 nm.

**Genotyping**

In this study, PCR with confronting two-pair primer (PCR-CTPP) method was used. In this method, allele-specific primers are designed for each allele opposing at the base of a single nucleotide polymorphism. PCR-CTPP is a suitable method for finding single nucleotide polymorphisms at position TNF- $\alpha^{1031}$  (Figure 2) (9, 10). Four primers were used in this method that F1 and R1 are designed for one allele, F2 and R2 are designed for the other allele, and F1 and R2 amplify another band (Figure 2). All primers sequences include:

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**Figure 2.** PCR-CTTP method. R1 and R2 are reverse primers and F1 and F2 are forward primers. T and C are polymorphic bases and the letter N is arbitrary bases.

sion at 72 °C for 1 min and final extension at 72 °C for 5 min. The resulting PCR-CTPP products were visualized by 1.5% agarose gel electrophoresis containing gel red and the predicted size of was confirmed using 100 bp molecular weight markers (11).

## Results

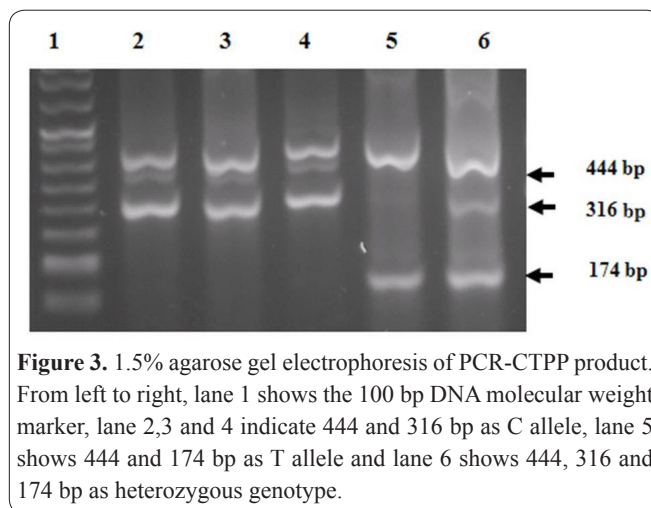
In patients, the mean of PD was 4.2 – 1.6mm (7 >PD> 1.7); Mean CAL was 5.9 – 1.4mm (8 >CAL> 3.9); the mean of BOP was 52.9% – 21.6% (30 >BOP> 85). In the control group, mean CAL, BOP, and PD were 1.2 – 0.09mm, 14.3% – 6.34%, and 2.1 – 0.19 mm, respectively. Based on the results, quality of the extracted genomic DNA is high and there is no contamination in all extracted samples. The A260/A280 ratio of isolated DNA is 1.80 to 2 (Results not shown). TNF- $\alpha$ <sup>-1031</sup> gene polymorphism was investigated by PCR-CTPP. Based on designed primers, three different sizes with 444, 316 and 174 bp of DNA were obtained. Figure 3 shows the gel electrophoresis of PCR-CTPP products of TNF- $\alpha$ <sup>-1031</sup>. Figure 3 shows the bands corresponding to a 444 bp as common bands, 316 bp as C allele and 174 bp as T allele following of gel electrophoresis of PCR-CTPP method.

In the GAP group, the frequencies of TT, TC and CC genotypes were 35.48%, 61.29 and 3.23%, respectively. In controls the frequencies of TT, TC and CC genotypes were 22.22%, 72.22%, and 5.56%, respectively (Table 1). Therefore, all three TNF- $\alpha$ <sup>-1031</sup> genotypes were found in both the patient and healthy individuals.

## Discussion

There are few studies about TNF- $\alpha$  gene and its connection with periodontitis, in Iran. But in other countries there are many researches about this gene and its relation with this disease and also other illnesses which show importance of this gene. Lu *et al.*, (2005) studied TNF-alpha-1031 and -863 promoter single nucleotide polymorphisms in *H. pylori* infection (12). TNF-alpha-1031 and -863 promoter SNP are new host factors to define the gastric inflammation and risk of peptic ulceration upon *H. pylori* infection. Cay *et al* (2011) determined Polymorphism in the TNF-alpha gene promoter at position -1031 in primary Sjögren's syndrome (13). Polymorphism in the TNF-alpha gene promoter at position -1031 is related to increased circulating levels of TNF-alpha which is associated with increased plasma MPO activity and protein nitration in Sjögren's syndrome.

Yun *et al.*, (2011) observed that 1031(T/C) poly-



**Figure 3.** 1.5% agarose gel electrophoresis of PCR-CTPP product. From left to right, lane 1 shows the 100 bp DNA molecular weight marker, lane 2,3 and 4 indicate 444 and 316 bp as C allele, lane 5 shows 444 and 174 bp as T allele and lane 6 shows 444, 316 and 174 bp as heterozygous genotype.

morphism of TNF-alpha gene is related with polycystic ovary syndrome (PCOS) in a Korean population (14) which corresponds with the results of Soga *et al.*, (2003) in Japan (15). According to the results of Menezes and Colombo (2008) in Brazil and those of Heidari *et al.*, (2014) in Iran determined that there were no significant differences associated between Chronic Periodontitis Patients and TNF- $\alpha$  (-308G/A) gene polymorphisms (6, 16).

In conclusion, the current research indicates that there is no significant association between TNF- $\alpha$  (-1031 T/C promoter) gene polymorphisms with the risk of generalized aggressive periodontitis disease in Iranian population. However, results of this study may be confirmed and extended in future studies with larger sample size

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**Table 1.** Frequency of TNF- $\alpha$ <sup>-1031</sup> gene polymorphism determined with PCR-CTPP method.

Genotype	AgP		Control	
	N	%	N	%
TT	11	35.48	12	22.22
TC	19	61.29	39	72.22
CC	1	3.23	3	5.56
Total	31	100	54	100

(AgP- generalized aggressive periodontitis, N- number of candidates, TNF- Tumor Necrosis Factor, PCR-CTPP - polymerase chain reaction- confronting two-pair primer).

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