# Association between Manganese Superoxide Dismutase (MnSOD Val-9Ala) genotypes with the risk of generalized aggressive periodontitis disease

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## Abstract

Generalized aggressive periodontitis (GAP) is a subtype of periodontal diseases that characterized by rapid destruction of periodontal supporting tissues. The MnSOD Val-9Ala mutation of manganese superoxide dismutase gene (MnSOD Val-9Ala) and its correlation with periodontal diseases has been studied in different populations. The purpose of this study was to investigate the possible association of MnSODVal-9Ala polymorphism with periodontitis disease in sample of GAP patients in Iran for the first time. Following a GAP examination, 50 GAP patients and 100 healthy individuals were recruited. Genomic DNA was extracted from peripheral blood leukocytes and the MnSODVal-9Ala polymorphismwas detected using PCR-RFLP method. The frequency of Ala/Ala, Ala/Val and Val/Val genotypes in healthy individuals were 25, 66 and 9%, respectively. In periodontitis patients, frequencies were as Ala/Ala (12%), Ala/Val (50%) and Val/Val (38%) genotypes. There was a significant positive association between distribution of MnSOD Val-9Ala genotypes and the risk of periodontitis disease (p<0.05). Our results indicated that MnSOD Val-9Ala gene polymorphism has a positive association with the risk of periodontitis disease.

*Key words:* MnSOD Val-9Ala, Aggressive periodontitis, Gene polymorphism, Iranian population.

## Introduction

Periodontal disease is a multi-factorial infection of the tooth supporting tissues that characterizes the indicator of gingival inflammation and which is the main cause of tooth loss in different worldwide populations (4, 18). Periodontitis has been correlated to infection diseases, chronic disease and also human cancers (23). This disease consists of two types of aggressive periodontitis and chronic periodontitis (2). The aggressive periodontitis comprises kinds of periodontal diseases which is characterized by a rapid progression rate and fast destruction of teeth supporting tissues (11). Therefore, this disease is regarded as one of the general health problems with increasing prevalence in the different worldwide populations. Time of onset, severity and development of periodontitis disease depend on immunological, genetic, environmental and their interactions (5). The genetic background seems to have a strong association with susceptibility to periodontitis disease (4, 6) . Therefore, there have been several attempts to characterize genetics factors implicated in the development of periodontitis diseases. Nowadays there is a research background on correlation between gene polymorphism and the severity of periodontitis disease (4, 9).

The inflammatory responses and oxidative stress play an important role in pathogenesis and development of periodontitis disease. Therefore, genes related to inflammatory responses and oxidative stress are the main candidates for polymorphism analysis and possible associates with GAP (8, 12). The oxidative stresses that caused by reactive oxygen species or ROS are involved in pathogenic mechanisms of the disease (16, 24). The ROS as oxygen-derived free radicals existed in normal cellular respiration during metabolism of xenobiotics, leads to damage the cell membranes, mitochondria and DNA (7, 24). The ROS can induce or enable mitochondrial permeabilization, whereas glutathione and antioxidant enzymes inhibit it. The superoxide dismutase (SOD) families as an antioxidant system are involved in the scavenging of ROS radicals. There are three types of enzymes for dismutation of the superoxide anion to form hydrogen peroxide ($H_2O_2$) in mammalian cells: SOD, catalase and glutathione peroxidase enzymes (1, 25). Among SOD enzymes, MnSOD is necessary for life that has a role as the major antioxidant in the mitochondria. The protein of MnSOD precursor is naturally synthesized in the cytoplasm that requiring post translational modifications at a cleavable N-terminal mitochondrial targeting sequence (MTS) (15). The activity of MnSOD enzymes are affected by functional polymorphisms in the gene promoter and genes encoding. A functional polymorphism in MnSOD in the name of rs4880 exists in codon 16, which is located at position 9 in the mature protein and results in the incorporation of either alanine (C allele) or valine (T allele) in the MTS. This polymorphism of MnSOD is predicted to alter the secondary structure of MnSOD and may influence the effectiveness of mitochondrial transport of MnSOD (20).

However, the association between polymorphisms of the MnSoD gene with different cancers has been extensively studied. There has been no reported data on the association between the polymorphism of MnSOD

and periodontitis disease. Thus, the current study aimed to investigating MnSOD Val-9Ala genotype polymorphism and its association with periodontitis among the samples of the Iranian population.

## Materials and Methods

### Enzyme and Reagents

The DNA isolation kit was supplied from Zagros Bioidea Co. (Razi University Incubator, Iran). The *BSAW*I restriction enzyme was purchased from (New England Biolabs, Beverly, MA, USA). The agarose and polymerase chain reaction (PCR) materials were purchased from Fermentas (Germany). The other chemicals and reagents (analytical grade) were supplied from Merck (Darmstadt, Germany). Forward and reverse primers were synthesized by Symbion, Fruebjergvej 3 (Denmark).

### Sample collection

All individuals contributed in this study signed an informed consent in accordance with the principles of the Helsinki II declarations and were informed about their disease and nature of the research. Evaluation of each participant was based on own and family medical and dental history. Diagnosis of periodontal disease was according to the clinical and radiographic parameters including bleeding on probing *(BOP)*, probing pocket depth (PD) and clinical attachment loss (CAL). Exclusion criteria for individuals were as history of HIV and hepatitis infection, diabetes, usage of anti-inflammatory and anti immune system drugs, pregnancy or lactation and smoking. The GAP was defined by interproximal attached loss affecting at least three teeth that could be either first molars or incisors (4, 11, 13). The periodontal probe (Hu-Friedy PCP-UNC 15, Hu-Friedy, Chicago, IL, USA) was used to measuring of clinical parameters. There were a total of 50 unrelated patients suffering from periodontitis consist of 87% females and 13% males. All patients and control subjects were free of general and genetics diseases.

### DNA extraction

From each subject, 10 milliliters of venues blood were collected by venepuncture into tubes containing EDTA and then genomic DNA was isolated from whole blood using high pure DNA extraction kit. The purified genomic DNA was analyzed and verified by electrophoresis on 1% agarose gel. The DNA concentration and purity of DNA were assessed with a Nanodrop (Thermo) at wave length of 260 and 280 nm (26, 27).

### Genotyping

Three µl of extracted DNA was used as a template for polymerase chain reaction (PCR). The MnSOD Val-9Ala polymorphism was assessed by PCR amplification and then restriction fragment length polymorphism (PCR-RFLP) analysis. The specific oligo-nucleotide primers used to amplify as follows: 5'-CGGGCTG-TGCTTTCTCGTC-3' (forward) and 5'-TCAGCCTG-GAACCTACCCTT-3' (reverse). PCR amplifications were carried out in a final volume of 25 µl containing 500 ng of DNA as template stand, PCR buffer (1x), 1.3 mM of MgCl2, 0.2 mM of dNTP, 0.5 µM of each pri-

mer and 1 unit of *Taq* DNA polymerase (Fermentase, USA). The thermocycler parameters included of an initial denaturation step at 94°C for 10 min, followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 59°C for 60s, extension step at 72°C for 45s, and then a final extension at 72°C for 10 min. After PCR, 10 µl of amplified products (243 bp) were subjected to digestion with 5 units of *BsaW* I at 60°C for an overnight. The digested products were electrophoresed on a 2% agarose gel stained with Syber Safe DNA under ultraviolet light. The band patterns were as 243 bp for Ala/Ala homozygotes; 243, 196, and 47 bp for Ala/Val heterozygotes; 196, and 47 bp for Val/Val homozygotes. The samples (5%) were randomly selected and re-genotyped for quality control purposes.

### Statistical analysis

The significance difference of the frequency of heterozygosity and homozygosity for genotypes between the GAP patients and control group was evaluated by the chi-square method. The Quantitative parameters were expressed as means ±SD. The odds ratios were calculated to estimate the relative risk of disease and 95% confidence intervals were measured by logistic regression by the SPSS software (version 18). A two-tailed Student's t test analysis was used to compare quantitative results. Statistical significance was assumed at the $p < 0.05$ level (19, 26).

## Results

Fifty GAP patients and 100 healthy individuals were recruited for evaluation in this study. The control individuals consisted of periodontitis signs free male (64%) and females (36%). The average ages of control individuals were about 23±2.5 years. The patients also consisted of 87% female and 13% male with the mean age of 21±3.4. The quality of extracted DNA was analyzed by gel electrophoresis. In order to investigate of the gene polymorphism, PCR-RFLP (Kazemi et al., 2015) was carried out. After gene amplification for its verification, the PCR product was digested with *BSAW*I and electrophoresed (Fig. 1).

The frequency of Ala/Ala, Ala/Val and Val/Val genotypes in healthy individuals were 25, 66 and 9% respectively. In periodontitis patients, frequencies were found as Ala/Ala (11%), Ala/Val (50%) and Val/Val (38%) genotypes (Table 1). There was a significant positive cor-
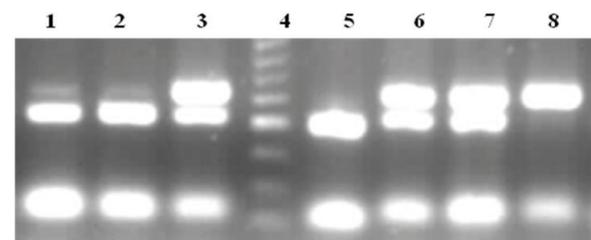


**Figure 1.** The 2% agarose gel electrophoresis for amplified MnSOD gene that digested by *BSAW* I restriction enzyme; lane 1, 2 and 5 show the resulting product of 196 and 47 bp for Val/Val homozygotes, 243 bp for Ala/Ala homozygotes; lane 3, 6 and 7 show 243, 196, and 47 bp for Ala/Val heterozygotes; lane 8 shows 243 bp for Ala/Ala homozygotes; Lane 4 shows 50 bp DNA molecular size markers.

**Table 1.** Distribution of MnSOD Val-9Ala genotypes for periodontitis disease patients and control group.

| MnSOD | Control | | GAP | |
|---|---|---|---|---|
| | N | % | N | % |
| Ala/Ala | 25 | 25 | 6 | 12 |
| Ala/Val | 66 | 66 | 25 | 50 |
| Val/Val | 9 | 9 | 20 | 38 |
| Total | 100 | 100 | 50 | 100 |

relation (P<0.05) between MnSOD Val-9Ala genotypes with the risk of periodontitis disease.

## Discussion

The periodontitis disease as a subtype of periodontal disorder is an inflammatory infection that affects the supporting tissue of teeth (24). Generalized aggressive periodontitis, which can occur locally or generally, is a complex disease attacking adult person in the ages between 20 to 35 years old with the damage of the first incisive and molar alveolar bone (4, 6). Although the microbial and some environmental risk factors are necessary for initiation and pathogenesis of the periodontal disease, but several documented studies demonstrated that genetic background seems to have significant influence on susceptibility of periodontal disease in various ethnic groups (10, 22). Therefore, searching to establish an association between polymorphism and genetic backgrounds of candidate genes with periodontitis diseases have been investigated (4, 14). The periodontal disease is more susceptible to an imbalance of antioxidant oxidative stress situation (24). Reactive oxygen species (ROS) generated during metabolism of xenobiotics and in normal cellular respiration causes damage to membranes, mitochondria and macromolecules including DNA. The MnSOD is the most important antioxidant gene in the mitochondria that catalysis's the dismutation of superoxide radicals to hydrogen peroxide form and detoxification of mitochondrial ROS (3, 21, 25). A number of polymorphisms have been reported in the MnSOD gene that some of them are associated with the increased risk of human cancers. Recently, documented studies confirmed the single functional polymorphism of C/T at nucleotide 47, changing the encoded amino acid from Ala (GCT) to Val (GTT). The Val allele-containing residue protein with beta-sheet structure exhibited impaired transportation, but Ala allele-containing residue with alpha helical structure showed normal transportation. Polymorphic alleles of MnSOD gene is widely dependent to ethnicity. The frequency of Ala allele has been reported about 12% among Japanese (20) and 14% in Chinese , while it is more frequent with 41-55% in the population of Caucasian (1) and also 41% in Jordanian breast cancer population.

There are several available data to investigate the role of Val-9Ala MnSOD variants in susceptibility to human cancers such as breast, gastric and prostate cancer. Moradi et al (2015) reported a positive association between MnSOD Val-9Ala gene polymorphism and risk of gastric cancer disease in Iranian population

(17). In meta analysis that reported by Wang suggest the MnSOD Val-9Ala polymorphism may contribute to cancer development through a disturbed antioxidant balance (23). Wei et al., have reported among Chinese population that lipid peroxidation was higher in the periodontal region, with total oxidative status and superoxide dismutase increasing both locally and peripherally (24). Some reported studies indicated that GAP disease was common in females than in males individuals that is in accordance with our results in which of 87% of patients were females. In agreement with our study, Guzeldemir et al have reported that the 29 of 31 patients were females among Turkish population (11). The reasons for more frequency of female GAP patients could be explained by hormonal alteration through menstrual cycle, pregnancy and also earlier age of puberty (4).

In conclusion, results of current study showed that there was a significant positive correlation between MnSOD genotypes and periodontitis disease. Also our results show that the MnSOD genotype is an important risk factor associated with GAP in Iranian population. However, future studies with a larger sample size are required to confirm these results. The study presented here is the first report on association between MnSOD gene polymorphism and the risk of periodontitis disease in sample of Iranian population. This study can be used as a basis for studying polymorphisms of other important genes in correlation to periodontitis disease.

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