

Role of MnSOD Val16Ala Gene Polymorphism in Changing the Level of Serum Metals in Workers Exposed to Heavy Metals in Al-Nasiriyah City

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Abstract

Low activity of antioxidant enzymes due to polymorphism in antioxidant genes and differences in mineral and metal levels creates oxidative stress that may play a role in advancing many diseases. The aim of this study was to determine the role of Manganese superoxide dismutase (MnSOD) gene polymorphism [rs4880 Val16Ala] in exposed workers and to compare the levels of metals and minerals among its various genotypes in control group. A total of 80 individuals including age and gender matched workers and control group were genotyped. Detection of rs4880 polymorphism was done using sequencing technique. The blood serum were tested for determination of metals and minerals using Atomic absorption spectrophotometer (AA 6600 Shimadzu). This study indicate that the frequency of T allele was higher than that of C allele in control group as well as in workers. Our results indicate no association between all genotypes in study subjects. The results of present study indicates that Pb and Cd concentration differ significantly between TT genotypes of MnSOD (rs4880) gene polymorphism as compared to CC genotypes in workers and control group ($p < 0.05$). Control group with TT, TC and CC genotypes have high concentration of Fe, Cu and Zn as compared to workers group.

Key Words: Heavy Metals, MnSOD, Polymorphism, PCR, Workers.

Introduction

Oxidative stress is responsible for a number of pathological and physiological circumstances, including atherosclerosis, diabetes, aging, osteoarthritis, types of cancer, inflammatory bowel diseases, and more^(1,2). Oxidative stress is a word used to demonstrate the cell imbalance between prooxidant manufacturing and cell antioxidant defense. It happens either owing to deficiencies in antioxidant protection structures or due to excessive reactive nitrogen species (RNS) and reactive oxygen species (ROS) manufacturing⁽³⁾.

ROS may result from inherent variables such as mitochondria, peroxisomes and inflammatory cell activation, and contact with extrinsic variables including environmental, pharmaceutical and industrial chemicals^(4,5). Oxidative stress caused by ROS which are typically balanced by antioxidant defenses of the cell⁽⁴⁾. Oxidative stress may cause protein, DNA and lipid damage, leading to an arisen chance of chromosomal aberrations, genetic mutation and alteration of cell growth that may happen in cancer⁽⁶⁾.

In the human body, numerous mechanisms protect cellular systems against oxidative damage. These processes include some intracellular enzymes such as superoxide dismutase(SOD), catalase(CAT), glutathione peroxidases(GPX), reductase of thioredoxin, and peroxidases. Some of the antioxidant enzymes which defense against oxidative damage are polymorphic⁽⁷⁾.

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Manganese superoxide dismutase(MnSOD), is one of the essential antioxidant enzymes which convert superoxide radicals to H_2O_2 and O_2 in mitochondria, and thus represents a first line defense in mitochondria against ROS (8). Therefore, the polymorphisms of the MnSOD gene have crucial role on the maintenance of ROS levels in cells^(8,9). The structure of the MnSOD gene comprises of five exons interrupted by four introns and the promoter. Several polymorphisms in distinct regions of the MnSOD gene have been recognized⁽¹⁰⁾. These polymorphisms were correlated with various illnesses. MnSOD is encoded by the nuclear MnSOD gene situated on the human chromosome 6q25 and the exon 2 of the human MnSOD gene identifies the Val16Ala polymorphism. Val16Ala (47T > C) is a commonly researched SOD2 single nucleotide polymorphism (SNP) on the MnSOD gene^(10,11). MnSOD Val16Ala polymorphism is found in the mitochondrial targeting sequence and was suggested to alter the peptide structure, affecting protein translocation and maturation in the mitochondrial matrix⁽¹²⁾. This polymorphism was linked to various pathologies including asthma⁽¹³⁾, diabetes⁽¹⁴⁾, aging⁽¹⁵⁾, cardiomyopathy⁽¹⁶⁾ and cancer⁽¹⁷⁾.

ROS production is not only influenced by polymorphism in antioxidant genes but it is also affected due to exposure of heavy metals. Heavy metals like lead (Pb), cadmium (Cd), chromium (Cr) and nickel (Ni) creates oxidative stress and contributes in the development of many human diseases which includes degenerative lung and heart conditions, Alzheimer disease and rheumatoid arthritis⁽¹⁸⁾.

To our knowledge, there were no study conducted to study the association between heavy metal concentration and Val16Ala Ala16Val polymorphism in exposed workers. Therefore, in the present study, the aim was to investigate the association between the genetic polymorphism MnSOD Val16Ala and exposed workers to heavy metals in Al-Nasiriyah city.

Materials and Method

This research was conducted in laboratories for the period from November 2018 to the end of April 2019 (Science College, College of Education for Pure Sciences, Mazaya Private College and Technical Institute in Al-Shatrah). 80 people (60 workers and 20 controls) were included in the present research. Worker groups were selected randomly from three industrial foundations in Al-Nasiriyah town center (1- Oil Refinery, 2- Car

Repair Workshops, and 3- Brick Factories). It included from each organisation 20 workers. The control group included people working in institutions that were far from being directly exposed to pollutants.

Sample collection

Approximately 7.5 ml of venous blood specimens were gathered from study group people. 2.5 ml were placed in the EDTA vacutainer tubes for genomic DNA extraction, while the remainder of the 5 ml blood was placed in a sterile plane tube and permitted to coagulate to distinguish the serum at 4000 rpm for 15 minutes by centrifugation. The genomic DNA and serum were stored at -20 c freezing , then used to amplification of MnSOD genes and estimation of heavy elements.

Metal analysis

The serum sample was used to analyze metals. The samples were processed by acid digestion method described by Ji & Ren,⁽¹⁹⁾. After acid digestion, the blood serum was evaluated by the atomic absorption spectrophotometer(FAAS.- Phoenix 986 AA. United kingdomUK.) to determine Pb, Cd, Fe, Cu and Zn.

Genetic analysis

DNA isolation and Genotyping.

Whole DNA was obtained through the use of gSYNCTMDNA Mini kit from white blood cells. MnSOD Val16Ala polymorphism was examined using sequencing method. Forward and reverse primers of MnSOD gene amplification were as follow: CAG CCC AGC CTG CGT AGA CGG -3' and reverse 5'- CTT GGC CAA CGC CTC CTG GTACTT -3') as defined by Souiden et al.⁽²⁰⁾ to amplify a 267 bp fragment as shown in Fig.1. The PCR program was initial denaturation at 95°C for 5 min followed by 30 cycles of 95°C for 40 sec. min, 59°C for 35 sec. (annealing) and 72°C for 35 sec. (extension). The reaction was completed by a final extension cycle at 72°C for 7 min. Amplified product was sent to a Macrogen Company to analyze the nucleotide sequence using a Genetic analyser device.

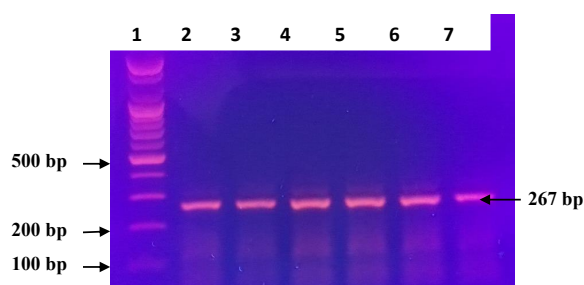


Fig.1. PCR products of MnSOD gene which analyzed on 2% agarose gel.

Statistical Analysis

All statistical analysis was conducted using version 17 of SPSS. Chi square test was used to compare the frequencies of genotype between workers and control group. Mean \pm standard deviation (Mean \pm SD) was used to explore the important distinctions between the metal values of the research groups. $P < 0.05$ were considered

statistically significant.

Results

The current study showed a high frequency of (TT) alleles compared to the frequency of other alleles (TC and CC) in other study groups, with no significant differences between the frequency of each allele in different groups. In the control group, 13 (65%) had TT genotype, 4 (20%) were heterozygote and had TC genotype and 3 (15%) had CC genotype. Among oil refinery workers, 12 (60%) items had TT genotype, 6 (30%) had TC genotype and 2 (10%) had CC genotype. The highest alleles frequency for group of car repair workers was TT genotype 14 (70%), while the other genotypes (TC and CC) were repeated at an equal rate of 15%. Out of 20 workers of brick factories, 10 (50%) had TT genotype, while 4 (20%) had TC genotype. The rest (30%) had CC genotype.

Table 1: Allele frequencies of the Ala16Val polymorphism among study groups

Study groups	TT n (%)	TC n (%)	CC n(%)	Total
Control	13 (65.00%)	4 (20.00%)	3 (15.00%)	20 (100.00%)
Oil Refinery	12 (60.00%)	6 (30.00%)	2 (10.00%)	20 (100.00%)
Car Repair Workshops	14 (70.00%)	3(15.00%)	3 (15.00%)	20 (100.00%)
Brick factories	10 (50.00%)	4 (20.00%)	6 (30.00%)	20 (100.00%)
Total	49 (61.25%)	17 (21.25%)	14 (17.50%)	80 (100.00%)
P.Value	0.870	0.327	0.319	

P.value \leq 0.05 means significant

This study was also intended to determine the concentration of distinct metals and minerals in separate MnSOD gene polymorphism genotypes. in workers exposed to heavy metals as compared to control. The mean concentration of Pb in TT genotype was 20 μ g/L and 17 μ g/L, in TC it was 18 μ g/L and 16 μ g/L, and in CC it was 18 μ g/L and 16 μ g/L respectively in workers and control group (Fig. 2). There was significant difference between TT and CC in exposed workers ($P < 0.05$). No significant difference was present between CC and

TC genotypes of MnSOD gene [rs4880 (Val16Ala)] polymorphism in control group as compared to exposed workers ($P > 0.05$).

The mean concentration of Cd was found to be 5 μ g/L, 4 μ g/L and 3 μ g/L in TT, TC and CC genotypes in workers and 4 μ g/L, 3 μ g/L and 3 μ g/L in TT, TC and CC genotypes in control group. Present finding indicated that significant difference occurred for Cd concentration between TT and other genotypes in workers and control group ($P < 0.05$). Control group with TT, TC and CC genotypes have high concentration of Fe, Cu and Zn as compared to workers group.

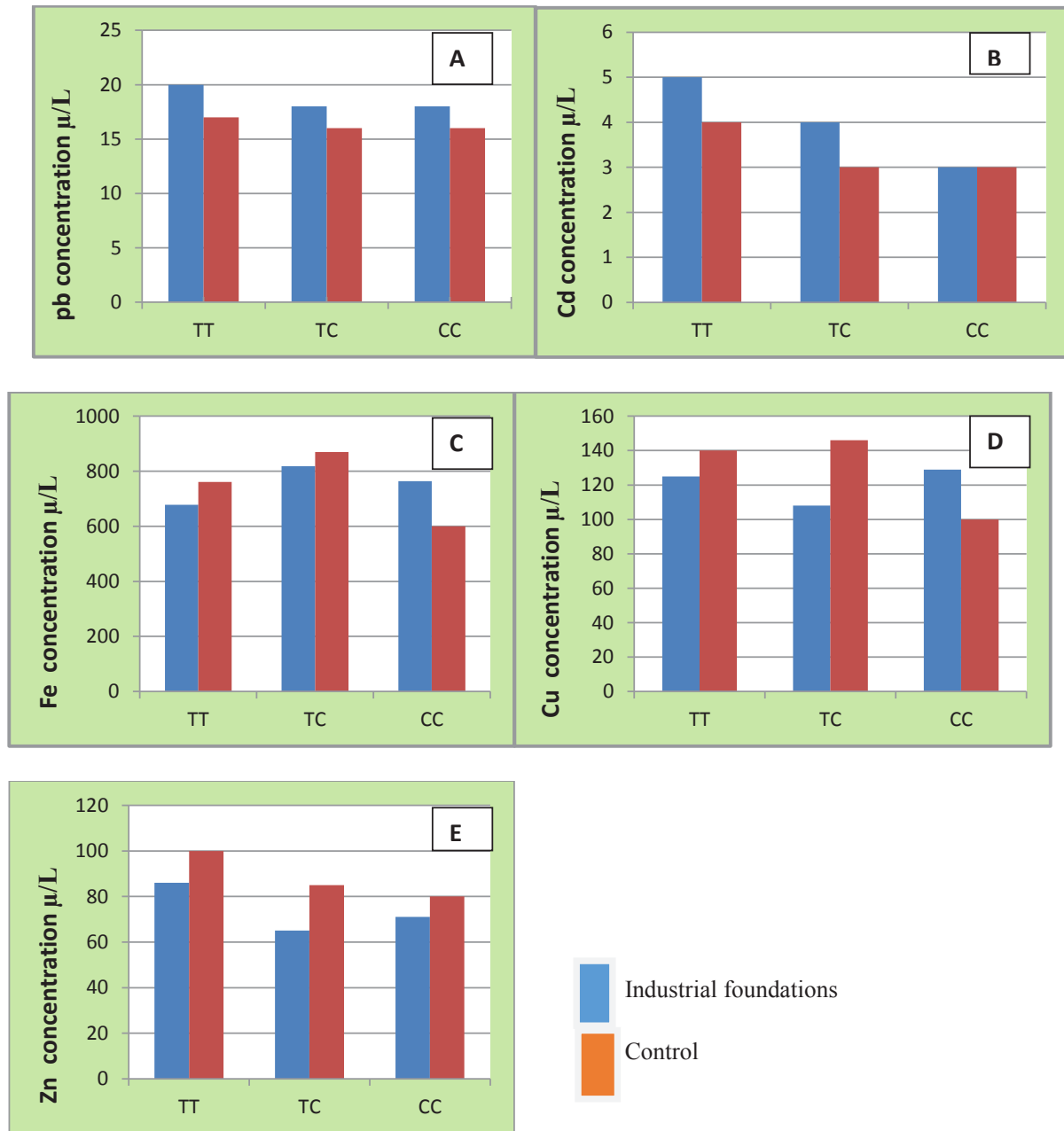


Fig. 2. Concentration comparison of A, Pb; B, Cd; C, Fe; D, Cu and E, Zn in different genotype carrier of MnSOD gene [rs4880 Val16Ala] polymorphism in workers and control group.

Discussion

Until now, no study has been revealed for study of association between MnSOD Val16Ala polymorphism and concentration of heavy metals. The findings of this research show that T allele frequency is greater than that of C allele in control group as well as in workers. Our results indicate no association between all genotypes in study subjects. Metal ion toxicity mechanism is partly

known, but it is obvious that they can generate ROS that involves nitrogen oxide (NO), superoxide ions (O₂⁻), hydroxyl (OH) and H₂O₂ with the assistance of Fenton / Haber-Weiss response⁽²¹⁾. No one has studied the association between MnSOD gene polymorphism and heavy metals in exposed workers. It is not fully understood the role of genetic variations in the MnSOD gene in altering the concentration of heavy metals. This aspect has also been attempted by the present research.

Concentration of Pb has been observed to change due to genotypes. In TT carriers in the workers and control group, the concentration of Pb was significantly high compared to the CC genotype. Similarly the finding of this study indicates that Cd concentration differ significantly between TT and CC genotypes of MnSOD1 gene [rs4880 (Val16Ala)] polymorphism in study subjects. A significant difference of Cd was present between TT and TC genotypes carriers in workers and control group as compared to CC genotype. These results indicate a complicated interaction of polymorphism of the MnSOD gene [rs4880 (Val16Ala)] with the concentration of Pb and Cd in the human body homeostasis. Although the result was observed that Fe, Cu and Zn concentration changed in all genotypes carriers in control group and workers but this difference was non-significant ($P>0.05$).

There is a gap in understanding of how metals and minerals interact in the antioxidant gene with MnSOD1 Val16Ala polymorphism (rs4880) which may affect progress of oxidative stress and development different diseases. Present information can assist to understand this polymorphism interaction with concentration of ion metals. Detailed study of exposure to minerals and metals will assist to confirm the relationship between genotypes and oxidative stress. In order to create the real picture of the association of MnSOD1 Val16Ala polymorphism (rs4880) and heavy metal concentration, further investigation is needed.

Conclusion

The present finding indicates that level of Pb and Cd increase in individual with TT genotypes. Regarding of Fe, Cu and Zn, this study suggested that the concentration of these mineral was changed slightly in all genotypes carriers in control group and workers but this difference was non-significant.

Ethical Clearance: The research and laboratory tests were approved by the Ethics Committee of the College of science for Pure Sciences / Thi-Qar University.

Source of Funding : Self

Conflict of Interest : Nil

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