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Superoxide Dismutase (MnSOD) Gene Polymorphism in Workers Exposed to Heavy Metals in Some of Industrial Foundations

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Abstract- *The current study was aimed to study the concentrations of (Pb, Cd, Fe, Cu and Zn) workers exposed to heavy metals in some of industrial foundations with various genotypes of MnSOD Val16Ala polymorphism. It was also aimed to detection the mutation in MnSOD gene. The concentrations of Pb, Cd, Fe, Cu and Zn was analyzed by Atomic absorption spectrophotometer. DNA was extracted and MnSOD genes was amplification by polymerase chain reaction (PCR). PCR products sent to a Macrogen Company to sequencing. There was a significant difference between ion metals concentration in the serum of workers and control. The current study showed a significant differences between TC genotype and TT genotype as a reference allele in the control and oil refinery workers group (OR 1.62, 95% CI 0.36 – 7.20). In workers of brick factories, the risk ratio increased with TC genotype (OR 1.73, 95% CI 0.31 – 9.57), while the risk ratio increased by approximately two and a half times in the workers with the CC genotype (OR 2.60, 95% CI 0.51 – 13.04). The current study recorded 11 mutation in different locus of target sequence. The most common mutation was the insertion of G base at locus 74857 of the gene, which may be effect of synthesis protein at this gene locus to the end of protein. This study concluded that the majority of workers carry allele T, which is associated with diseases-related of oxidative stress. Exposure of workers with TT genotype to heavy metals is a risk factor for many diseases.*

Key words*: Heavy metals, MnSOD, polymorphism, PCR, Sequencing.*

I INTRODUCTION

Heavy metals are generally referred to as those metals which possess a specific density of more than 5 g/cm^3 and adversely affect the environment and living organisms [1], [2]. Human exposure has risen dramatically as a result of an exponential increase of their use in several industrial, agricultural, domestic and technological applications. Environmental pollution is very prominent in point source areas such as mining, foundries and

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smelters, and other metal-based industrial operations [3], [4]. Industrial sources include metal processing in refineries, coal burning in power plants, petroleum combustion, nuclear power stations and high tension lines, plastics, textiles, microelectronics, wood preservation and paper processing plants [5]. Metal ions have been found to interact with cell components such as DNA and nuclear proteins, causing DNA damage and conformational changes that may lead to cell cycle modulation, carcinogenesis or apoptosis [6]. Reactive oxygen species (ROS) production and oxidative stress play a key role in the toxicity and carcinogenicity of metals such as cadmium [7] and lead [8].

Reactive oxygen species (ROS) have been implicated in many diseases, including cancer, by causing DNA damage and spontaneous chromosomal breakage and activating procarcinogens. The body has a very effective network of antioxidants serving as scavengers of superoxide and hydrogen peroxide, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) [9].

SOD is one of the key enzymes that detoxifies the superoxide radical (O_2_) and generates hydrogen peroxide (H_2O_2), which in turn is detoxified by catalase and GPx. Of the three forms of SOD in humans, mitochondrial manganese SOD (MnSOD) may be particularly important for antioxidant defense as the mitochondria are the major site for cellular metabolism and the main site for the production of ROS [10]. MnSOD is encoded by a single gene containing five exons and it is located on chromosome 6q25.3 [11]. One of the common polymorphisms of MnSOD results in the replacement of valine (GTT) with an alanine 16 (GCT); the Vla16Ala polymorphism. This polymorphism affects the import of MnSOD into the mitochondria by altering the conformation of its leader signal [12].

The current study was aimed to compare the concentrations of selected metals among various genotypes of MnSOD Val16Ala polymorphism (rs4880) in workers exposed to heavy metals. It was also aimed to analyze the mutation in MnSOD gene in workers and control group.

II MATERIALS AND METHODS

This study was carried out in the laboratories of (Science college, College of Education for Pure Sciences, Mazaya private college and technical institute in Shatrah) for the period from November 2018 to end of April 2019. The current study included 80 persons (60 workers and 20 controls). The worker groups were randomly chosen from three industrial foundations in the center of Al-Nasiriyah city (1- Oil Refineries, 2- Car Repair Workshops and 3- Brick Factories). It included 20 workers from each organization. The control group included persons working in institutions far from direct exposure areas to pollutants. The workers and control groups distribute according to the age, smoking, place of residence and work period.

Sample collection

About 7.5 ml venous blood samples were collected from individuals of study groups. 2.5 ml put into an EDTA vacutainer tubes for used to genomic DNA extraction, while the rest of blood 5 ml were placed in a sterile plane tube and allowed to clot in order to separate the serum by centrifugation at 4000 rpm for 15 minutes. The

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genomic DNA and serum were stored at -20 c freezing, then used to amplification of genes and estimation of heavy elements.

Metal analysis

The serum sample was used for metals analysis. The samples were processed by acid digestion method described by Ji & Ren, (2002). After acid digestion, the blood samples were analyzed for determination of Pb, Cd, Fe, Cu and Zn through Atomic absorption spectrophotometer (FAAS.- Phoenix 986 AA. United kingdomUK).

Genetic analysis

Whole DNA was extracted from white blood cell, by using gSYNC^{TM}DNA Mini kit. MnSOD genes was amplification by using polymerase, chain reaction (PCR). Forward and reverse primers of MnSOD gene amplification were as follow: CAGCCCAGCCTGCGTAGACG G-3′ and reverse 5′-CTTGGCCAACGCCTCCTGGTACTT -3′) as defined by Souiden et al. [13] to amplify a 267 bp fragment.

PCR amplification was carried out with a total volume of 50 µl with templet DNA (5 µl), master mix (12.5 µl), distal water (20 µl) and 1 µl of each primers of MnSOD gene. PCR program was designed as followed an initial denaturation at 95,°C for 5 minutes, followed by 35-cycles at 94°C of denaturation for 40 sec. ,-59°C of annealing for 35 sec. , 72°C of extension for 35 sec. and a last extension at-72°C-for 5 minutes. PCR products were migrated electrophoretically on a 2% agarose gel recolored with 0.5 µl ethidium bromide. Fig.1.

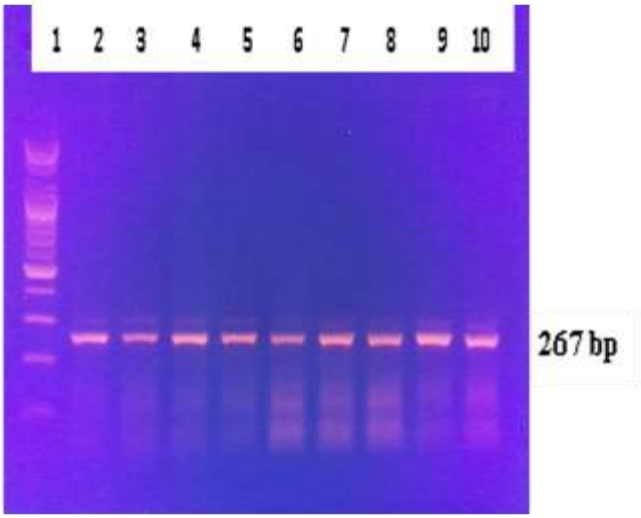

Fig.1 Products of PCR for MnSOD gene which analyzed on 2% agarose gel.

Figure 1: Products of PCR for MnSOD gene which analyzes on 2% agarose gel

30 µl of PCR products per gene were sent to a Macrogen Company to analyze the nucleotide sequence using a Genetic analyzer device. The presence of T allele alone refers to the TT genotype, and the presence of C allele

alone shows the genotype CC, while the presence of the T and C alleles together indicate the TC genotype. **Fig. 2**.

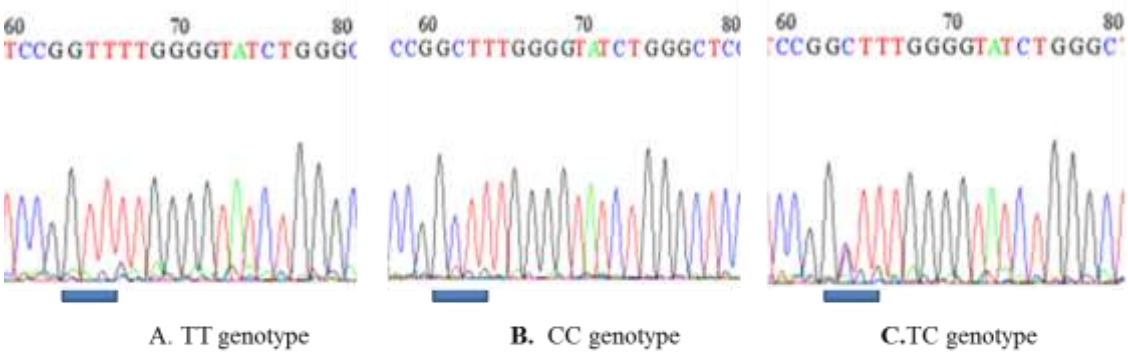

Fig. 2. Genotypes of MnSOD Val16Ala polymorphism

Figure 2: Genotypes of MnSOD val16Ala polymorphism

Statistical Analysis

Statistical analysis of this study was conducted, using the mean ± standard deviation (Mean ± SD). A chi-square and odd ratio (OR) test with 95% confidence intervals (95% CI) were used to compare genotype frequencies between the workers and control groups. ANOVA and T test were used to investigate the significant differences among the metal values between study groups. P values less than 0.05 were considered statistically significant. All statistical analyses were performed using the statistical software package SPSS V.17.

III RESULTS

The result in table (1) shows that the highest ratios for Pb, Cd and Cu were found in car repair workshops group (0.031 ±0.012, 0.007±0.002 and 0.165±0.049 ppm) respectively. A significant difference was found between this group and the control group (LSD= 0.003, 0.001 and 0.028) for Pb , Cd and Cu respectively. The highest ratio for Fe and Zn were in brick factories group (0.89 ±0.23 and 0.100 ±0.092 ppm respectively). There was a significant difference between iron and zinc concentration in the serum of workers at brick factories group and control (LSD= 0.190 and 0.029) for Fe and Zn respectively.

Table 1: The concentrations of heavy elements in workers and control groups

Study groups	Pb (ppm)	Cd (ppm)	Fe (ppm)	Cu(ppm)	Zn(ppm)
Oil Refinery	0.016^{b} ±0.003	0.004^{b} ±0.001	0.74^{ab} ±0.45	0.071^{c} ±0.035	0.062^{b} ±0.018
Car Repair Workshops	0.031^{a} ±0.012	0.007^{a} ±0.002	0.58^{b} ±0.38	0.165^{a} ±0.049	0.061^{b} ±0.034
Bricks factory	0.016^{b} ±0.003	0.003^{b} ±0.001	0.89^{a} ±0.23	0.136^{ab} ±0.067	0.100^{a} ±0.092
Control	0.013^{b} ±0.003	0.003^{b} ±0.002	0.65^{b} ±0.30	0.119^{b} ±0.055	0.095^{a} ±0.044

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P.Value	0.000*	0.000*	0.041*	0.000*	0.039*
LSD	0.003*	0.001*	0.190*	0.028*	0.029*

The different letters refer to a significant differences, the same letters refer to no significant differences.

Compared to TT genotype as a reference, risk factors of oil refinery workers with TC genotype were highly (OR 1.62, 95% CI 0.36 – 7.20), while there are no risk factors in the workers with CC genotype compared with the reference(OR 0.72, 95% CI 0.10 – 5.09). Also, the C allele was calculated in all genotypes of oil refinery workers and control groups and showed no significant difference when compared with the T allele as a reference for comparison (OR 1.00, 95% CI 0.36 – 2.75). Table (2)

Table 2: Allele frequencies of the Ala16Val polymorphism oil refineries workers and control group

Genotype	Control n (%)	Oil Refineries n (%)	OR	95 % CI	P.Value
TT	13 (65.00%)	12(60.00%)	1.00	———	———
TC	4 (20.00%)	6(30.00%)	1.62*	0.36 – 7.20	0.521
CC	3 (15.00%)	2(10.00%)	0.72	0.10 – 5.09	0.743
Total	20 (100.00%)	20(100.00%)	———	———	———
T allel	30 (75.00%)	30(75.00%)	1.00	———	———
C allel	10 (25.00%)	10(25.00%)	1.00	0.36 – 2.75	1.000
Total	40 (100.00%)	40(100.00%)	———	———	———

95% CI, Confidence Interval. OR, Odds ratio. P.value ≤ 0.05 means significant

The current study showed no significant differences between TC , CC genotypes and TT genotype (wild type) as a reference allele in the control and car repair workshops workers group (OR 0.69, 95% CI 0.13 – 3.72 , OR 0.92, 95% CI 0.15 – 5.44) for TC and CC alleles respectively. No significant differences were found when comparing C allele with T allele in all genotypes in control and car repair workshops workers groups (OR 1.00, 95% CI 0.36 – 2.75).table (3)

Table 3: Allele frequencies of the Ala16Val polymorphism in car repair workshop workers and control group

Genotype	Control n (%)	Car Repair Workshops, n (%)	OR	95 % CI	P.value
TT	13 (65.00%)	14(70.00%)	1.00	———	———
TC	4 (20.00%)	3(15.00%)	0.69	0.13 – 3.72	0.671
CC	3 (15.00%)	3(15.00%)	0.92	0.15 – 5.44	0.935
Total	20 (100.00%)	20(100.00%)	———	———	———

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T allel	30 (75.00%)	31(77.50%)	1.00	———	———
C allel	10 (25.00%)	9(22.50%)	0.87	0.31– 2.44	0.793
Total	40 (100.00%)	40 (100.00%)	———	———	———

95% CI, Confidence Interval. OR, Odds ratio. P.value ≤ 0.05 means significant

In present study, the risk ratio of brick factories workers with the TC genotype was increased by 1.73 (OR 1.73, 95% CI 0.31 – 9.57) comparing with TT genotype in control and workers groups, while the risk ratio increased by approximately two and a half times in the workers with the CC genotype (OR 2.60, 95% CI 0.51 – 13.04). With regard to C allele, this study suggests an increase in the risk ratio by two times of workers with this allele compared with workers whose had T allele (OR 2.00, 95% CI 0.76 – 5.19).

Table 4: Allele frequencies of the Ala16Val polymorphism in brick factories workers and control group

Genotype	Control n (%)	Bricks factory n (%)	OR	95 % CI	P.Value
TT	13 (65.00%)	10(50.00%)	1.00	———	———
TC	4 (20.00%)	4(20.00%)	1.73*	0.31 – 9.57	0.526
CC	3 (15.00%)	6(30.00%)	2.60*	0.51 – 13.04	0.238
Total	20 (100.00%)	20(100.00%)	———	———	———
T allel	30 (75.00%)	24(60.00%)	1.00	———	
C allel	10 (25.00%)	16(40.00%)	2.00*	0.76 – 5.19	0.152
Total	40 (100.00%)	40(100.00%)	———	———	———

95% CI, Confidence Interval. OR, Odds ratio. P.value ≤ 0.05 means significant

This study was also designed to find out the concentration of different metals in various genotypes of MnSOD gene (rs4880) in worker groups that were exposed to heavy metals. The concentrations of heavy elements (Pb, Cd, Fe, Cu and Zn) did not show any significant differences when compared between each group of workers depending on their genotypes. Table (5).

Table 5: Relationship between heavy elements concentration (ppm) and Allele frequencies of the Ala16Val polymorphism in worker groups

Genotype	Pb (ppm)	Cd (ppm)	Fe (ppm)	Cu(ppm)	Zn(ppm)
TT	0.020±0.010	0.005±0.002	0.678±0.349	0.125±0.071	0.086±0.068
TC	0.018±0.009	0.004±0.001	0.818±0.429	0.108±0.034	0.065±0.026
CC	0.018±0.009	0.003±0.002	0.764±0.346	0.129±0.055	0.071±0.031
P.Value	**0.761**	**0. 390**	**0.379**	**0.584**	**0.378**

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LSD	0.0047	0.001	0.173	0.030	0.026

P.value ≤ 0.05 means significant

Mutation in MnSOD gene

The current study recorded 11 mutations within intron and exon 2 of MnSOD. These mutations included transition, transversion, deletion and insertion types and the sequences were recorded in gene bank with accession numbers which shown in the table below

Table 6: mutation in MnSOD gene in workers and control groups

Mutation	Location	Type	Frequency	Accession numbers
g.74659 ins. G	Intron	Insertion	4 (5.00%)	LC484967
g.74668 A>G	Intron	Transition	1(1.25%)	LC484966
g.74857*del* G	Exon	Deletion	10 (12.5%)	LC485226
g.74726 T>A	Exon	Transversion	1(1.25%)	LC484629
g.74858*del* C	Exon	Deletion	1(1.25%)	LC484629
g. 74865 A>T	Intron	Transversion	2(2. 5%)	LC484967
g.74858C>G	Exon	Transversion	3(2.5%)	LC485019
g.74664C>G	Intron	Transversion	1(1.25%)	LC485020
g.74817 C>A	Exon	Transversion	1(1.25%)	LC485021
g.74857G>C	Exon	Transversion	1(1.25%)	LC485225
g.74864ins.G	Exon	Insertion	1(1.25%)	LC485225

IV DISCUSSION

In the present study, the rustles indicate that the Pb concentration are higher in workers of car repair workshops (0.031 ±0.012) when compared to control subjects (0.013 ±0.003) with a significant difference (P=0.000, LSD=0.003). But this study did not find significant differences in the exposed level of lead between the workers of oil refinery, brick factories and the control group. In a previous studies Moshchil, [14], Al-Rudainy [15] and Mohammed, [16] found an elevated blood lead level among benzene workers, which are greater than the results of present study. A part of this rise may be due to frequent daily inhalation of leaded benzene fumes and neglect of protective measures.

The highest ratio of Cd were in workers of car repair workshop group (0.007 ±0.002) with significant differences compared to a control group (P=0.000, LSD=0.001). It is believed that continuous work in environments contaminated with high molecular weight hydrocarbons was the cause of high exposure to

cadmium in this group of workers. Also welding coins are the most important sources of exposure to cadmium[17]. In a study done in Karbala province, Hamdan et al.,[18] observe that the highest ratio of Cd were in workers of liquid battery workshops (0.082±0.031).

The highest ratio of Fe were in workers of brick factories group (0.89 ±0.23) with significant differences compared to a control group (P=0.000, LSD=0.028). In studies done in Karbala province, Al-Saadi, [19] found the Fe concentration in serum of workers of industrial field was higher than control group, also Hamdan et al.,[18] observe that the highest ratio of Fe were in workers exposed to pesticides. While Al-Shamri et al., [20] who done his study on workers of fuel station at Al–Najaf city found a significant differences of Fe concentration between exposed workers and control group (P<0.0001).

In the present study, the rustles indicate that the Cu concentration are higher in workers of car repair workshops (0.165 ±0.094) when compared to control subjects (0.119 ±0.055) with a significant difference (P=0.000 , LSD=0.028), also there was a significant difference in Cu concentration in blood serum of brick factories workers compare with control group. The lowest ratio of Cu was in workers of brick factories group (0.071 ±0.035). In a previous studies Moshchil, [14] and Al-Shamri et al., [20] found an elevated blood Cu level among all workers compare with control group. However, Hamdan et al., [18] observed that the Cu ratio was elevated in workers exposed to pesticides compare with other groups. Azize, [21] suggested that the concentration of copper was lower in workers group rather than control group.

Low concentration of Cu in oil refinery workers may be due to hydroquinone interaction (the one benzene metabolite), with Cu and Zn components of the SOD enzyme, and Cu release of the enzyme. Then the interaction between the released Cu and H_2O_2 produces ROS and activate lipid oxidation chain reactions and Cu deficiency contributes to increase in the processes of peroxide [21].

In this study there was a significantly lower level of antioxidant trace elements in the oil refinery and car repair workshops workers compared as the control group. This finding was consent with Azize,[21] who found that the value of Zn concentration was lower in serum of workers rather than control individuals. This may be due to the trace elements (Zn and Cu) were make as cofactors of the antioxidant enzymes and they are utilized to production of antioxidant enzymes which involved in the ROS detoxification [22]. The Zn and Cu are essential components of SOD and are related with antioxidant functions, therefore, the deficiency of these elements may lead to reduce mechanisms of free-radical scavenging thereby rising oxidative damage in the workers group [21]. Other studies showed that the value of Zn concentration was higher in blood serum of workers rather than control group [20] , [19] However , Hamdan et al., [18] observed that the Zu ratio was elevated in workers exposed to pesticides and farmers using chemical fertilizers compare with other groups.

MnSOD Val16Ala polymorphism

MnSOD Val16Ala is a prevalent polymorphism and related with various diseases such as lung cancer, diabetes microvascular complications, diabetic nephropathy[23] , [24] , [25]. In this study, The TT genotype was the most common in all study subjects followed by TC, then CC genotypes. Statistical analysis showed a significant differences between wild TT and heterozygous TC genotypes (OR= 1.62) in workers of oil refinery.

However in car repair workshop workers there were no significant differences between all genotypes. In brick factories workers, the frequencies of the TT, TC and CC genotypes were determined to be 50, 20 and 30%, respectively with (OR=1.73 and OR=2.60) for TC and CC, respectively. There are no significant difference of elements concentration between workers and control group according to genotypes (P>0.05).

Irfan et al., [26] found that the levels of Pb may causes development of rheumatoid arthritis in the presence of SOD1 (rs2070424) gene polymorphism. In study of Chinese population, Qian et al.,[27] showed that CYP1A1 genetic polymorphism, rs1048943, is related with an increased risk of Pb poisoning. It may have considered as a biomarker for Pb-exposed workers.

Huang et al., [28] found that diabetic patients with TT genotype have a high risk of developing retinopathy. In other study done in Tehran, Abbasi et al., [29] showed that patients of chronic kidney disease with the Val/Val genotype exhibited higher CKD risk than those with the other genotypes. Also Abdelrauf et al.,[30] showed that the Val allele and the VV genotype are related with the occurrence of acute myocardial infarction in Egyptians. Reduced resistance to oxidative stress and a decline in MnSOD activity was noted more frequently in homozygous Val / Val than in patients of diabetic neuropathic with other MnSOD alleles[31].

The disruption of the protein by valine explained this finding. The α-helix structure is crucial for the enzyme's translocation to the mitochondrial matrix where its function is exercised. This explained suggested by Sutton et al.,[32] who mentioned that the disruption by Val allele causes the protein to be retained at inner membrane of mitochondrial and associated with increased susceptibility to oxidative damage. Therefore, Val allele considered a risk factor for many diseases [32].

Wang et al.,[33] found the MnSOD Val16Ala polymorphism may disturbed antioxidant balance and contribute to cancer development. Moradi et al.,[34] showed that there was a significant association between the polymorphism of MnSOD gene and gastric cancer. Caple et al., [35] noted that higher iron consumption was associated with increased risk of aggressive prostate cancer cases with Val / Val genotype by more than two fold increases. This finding was similar with Choi et al.,[36] who interpreted that the Val variant, with reduced activity of MnSOD enzyme, may increase O_2- levels in mitochondria, causing release of ferric acid H_2FeO_4 from iron-containing enzymes and enhanced Fenton reactions which was known as a cause of cancer[37]. Similarity with this study, Valenti et al.,[38] suggested that patients with Val allele had low activity of MnSOD gene and they had higher incidence of cardiomyopathy related with hereditary hemochromatosis, which is features by excessive deposit of iron and is the result of produced ROS due to overload of hepatic iron. Also Pérez & Cederbaum, [39] noted that MnSOD overexpression prevents iron-related oxidative stress in vitro.

In contrast, a study of 162 cases with alcoholic cirrhosis suggested that patients with Ala allele and high-activity of MnSOD gene have a high risk of hepatocellular carcinoma and develop hepatic iron accumulation [40]. The inconsistent results may be due to the various profile of factors in the hepatocellular carcinoma study that effect iron accumulation such as age (younger progressive accumulation) and gender (female loss of iron through menstruation) [36]. Choi et al.,[36]theorized that the higher activity of Ala allel would raise risk of prostate carcinogenesis with high iron intake. Explaining it, since Ferric acid activates the production of ROS with H_2O_2 via Haber-Weiss chemistry including Fenton reaction.

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Other studies suggested an association between the MnSOD Ala allele and increased risk of prostate cancer in smoker individuals with low vitamin consumption [41]. Authors suggested that the Ala allele related with cancer mediated by low consumption of antioxidants may be due to excessive of H_2O_2 production. The accumulation of H_2O_2 may react with metal ions producing highly carcinogenic ROS, such as (HO•). Research has found an influence of the Ala16Val SNP in the response to these prooxidant molecules, which could raise the risk of developing dysfunctions or diseases [42].

Mutations in MnSOD gene

The current study recorded 11 mutations in different locus of target sequence. These mutation included substitution, nonsense and frameshift mutations. Some of mutation repeated in more one sample, whereas some of them occurred in same sample. The most common mutation was the insertion of G base at locus 74857 of the gene, which may be effect of synthesis protein at this gene locus to the end of protein.

The current study recorded six variable sites of MnSOD gene in oil refinery workers (g.74659 ins G, g.74668 A>G, g.74857 del G, g.74726 T>A, g.74858 del C and g.74865 A>T). g.74659 ins. G was frequented in four samples. The (g.74857 del G) variation was shared between workers of oil refinery, car repair shops. It was frequented in 3 individuals of oil refinery workers and in 7 individuals of car repair shop workers. Also g.74659 ins G was shared between two groups of workers. In addition, the current study recorded two other variations in of car repair shop workers (g.74858 C>G and 74664 C>G). g.74858 C>G was repeated in two workers and one individual of control group. The frequency of the same mutations in both workers of oil refinery and car repair shops may be due to their exposure to similar chemicals such as hydrocarbons and heavy metals. The present study did not record variations in the MnSOD gene of the brick factory workers. This may be due to the nature of their rural residence which are lower pollutants compared to urban areas and consumption food that rich in antioxidants such as dates and fresh animal products. As for the control group, the current study recorded three variations (g.74817 C>A, g.74857 G>C and g.74864 ins.G) two of which occurred in the same sample.

V CONCLUSION

This study concluded that the majority of workers carry allele T, which is associated with diseases-related of oxidative stress. Exposure of workers with TT genotype to heavy metals is a risk factor for many diseases. This study also recorded various variations in studied region of MnSOD gene due to exposure to heavy metals.

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