RESEARCH ARTICLE

The Influence of MnSOD Gene Polymorphism in the Reactive Oxygen Species and Alcohol Level in Smoker Alcoholism

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Received: 08th December, 2021; Revised: 14th January, 2022; Accepted: 29th Febraury, 2022; Available Online: 25th March, 2022

ABSTRACT

The alcohol abuse and smoker habit become the most health problems in Iraq, theses habit lead to some disease incidence and complications, the present study was conducted to estimate one of the important antioxidant enzymes gene polymorphism Manganese Superoxide Dismutase (MnSOD) in smoker and non-smoker of alcoholism individuals, and effect in the ROS and alcohol level, tetra ARMS-PCR was used for detection Ala16Val SNP, the results showed shows two alleles (T and C) and three genotyping (TT, TC and CC), the TT was more frequent in alcoholism (76.92%) than in control group (39.28%) in significant differences ($p > 0.0091$), while TC less frequent in Alcoholism (23.07%) than in the control group (50%) in non-significant differences ($p > 0.3315$), The effect of comorbidity between alcohol abuse and smoker habit in the MnSOD gene polymorphism was studied in current research, two genotypes were observed (TT and TC) and CC didn't found, non-significant differences were observed in TT and TC ($p > 0.335$), the allele frequency shows that T allele was significant association with alcoholism and smokers, The ROS level shows non-significant differences slightly increased in non-smoker group ($p = 0.555$), and in the TT genotype in the alcohol abuse ($p > 0.835$), alcohol abuse with smoker ($p = 0.787$) and low decreased in alcohol abuse non-smoker ($p = 0.667$), the alcohol level in the study groups shows increased in the smoker group than non-smoker in non-significant differences ($p > 0.250$), and its increased in TT genotype in all groups in non-significant differences of alcohol abuse ($p = 0.393$), alcohol abuse with smoker ($p > 0.326$) and alcohol abuse non-smoker ($p > 0.956$), the alcohol level in the study groups shows increased in the smoker group than non-smoker in non-significant differences ($p > 0.250$) and its increased in TT genotype in all groups in non-significant differences of alcohol abuse ($p > 0.393$), alcohol abuse with smoker ($p = 0.326$) and alcohol abuse non-smoker ($p > 0.956$), The present results concluded that there was a strong association between TT genotype and alcoholism, but the smoker shows week association with MnSOD at the Ala16Val SNP, and non-significant association of ROS and alcohol with smoker and non-smoker of alcoholism.

Keywords: Alcohol abuse, Comorbidity, MnSOD gene polymorphism, Smoker habit.

International Journal of Pharmaceutical Quality Assurance (2022); DOI: 10.25258/ijpqa.13.1.7

How to cite this article: Izzat SE, Al-husaini FK, Jabr HS, Ibrahem AA, Hasan SY, Saja M. Shareef, Hamad DA, Zabibah RS, Al-Terehi MN. The Influence of MnSOD Gene Polymorphism in the Reactive Oxygen Species and Alcohol Level in Smoker Alcoholism. International Journal of Pharmaceutical Quality Assurance. 2022;13(1):26-29.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

The oxidative stress becomes the most factors contributed in some disease and health problems, the mechanism of defense against elevation of free radicals, including antioxidant molecules and antioxidant enzymes, these enzymes have a vital role in elimination of reactive oxygen stress (ROS).[1,2] One of these enzymes is manganese superoxide dismutase (MnSOD) has major defense property against ROS in the mitochondria,

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that is a main source of ROS production, Ewa *et al*.,[3] proposed that the mutation in the Structural or functional sequences within the MnSOD encoding gene may be association with ROS detoxification. The most SNP that was more studied is MnSOD Ala16Val SNP has been shown to modify the localization of enzyme and mitochondrial transportation, which may be impacted the balance of the redox status, it has been found that it's associated with some chronic diseases.[4,5] Several studies indicated that there were numerous factors associated with redox status and diseases like the role of environmental factors in the Ala16Val genotypes in human diseases,[6,7] however it's needed to be clarified.

The interaction between Alcohol and tobacco are prominent in the maintenance phase of addiction. Studies showed that the most smokers were drunken alcohol.[8-11] This association is contributed in some vital processes alteration like oxidative stress,[12] therefor the present study aims to detect the one of an important antioxidant enzyme polymorphism with the comorbid of alcoholism and smoker habit, in addition to its effect in the ROS and alcohol levels.

MATERIALS AND METHODS

About 40 alcoholism individuals have age ranged (20–53) years and 29 apparently healthy individuals have aged (20–40) as a control group were enrolled in current work, blood samples were collected from each volunteers according to the ethical approval of environment and health ministry in Iraq, then ROS and alcohol levels were detected by chemical methods, DNA was isolated using DNA extraction kit, the Ala16Val SNP in the MnSOD sequence using tetra primers ARMS-PCR F1 5′-CAC-CAGCACTAGCAGCATGT-3′; F2 5′-GCAGGCAGCTGGC-TaCGGT-3′; R1 5′-ACGCCTCCTGGTACTTCTCC-3′ and; R2 5′-CCTGGAGCCCAGATACCCtAAAG-3,[13] three bands were produced by this technique 514 common bands , 366 bp CC, 189 bp TT and TC 366, 189 bp). The T refer to amino acid Val and C refer to amino acid Ala, statistical analysis was implemented using odd ratio (CI95%) in p value less than 0.05.

RESULTS AND DISCUSSION

Alcoholism becomes, the more social problems in Iraq in the last decade, the stress, unhealthy lifestyle and the absents of laws contributed in the alcoholism habit, that associated with some health disorders, as a result of unaffected of ROS and total antioxidant level by alcohol level in alcoholism in previous study implemented on the Iraqi drunks that showed non-significant differences in ROS level between drunks and,[12] the present study was suggested to detect the role of one of the most antioxidant enzyme Manganese Superoxide Dismutase (MnSOD) Ala16Val SNP in alcoholism, current genotyping shows two alleles (T and C) and three genotyping (TT,TC and CC) (Figure 1), the TT was more frequent in alcoholism (76.92%) than in control (39.28%) in significant differences ($p = 0.0091$), while TC less frequent in Alcoholism (23.07%) than in the control group (50%) in non-significant differences ($p = 0.3315$), the allele frequency shows of T allele with alcoholism ($p = 0.001$) (Table 1). The role of MnSOD gene polymorphism has been studied in other population, Sun *et al*.,[14] found an interesting correlation between genotype of MnSOD and alcohol and tobacco habits that lead to conclude complex interaction between gene MnSOD and the phenotype of alcohol misuse, they found that 50% of heavy drinkers have genotype TC in non-significant differences, However, the association between MnSOD polymorphism and alcohol abuse still unclear, a study conducted by Larosche *et al*.,[15] found that the prolonged ethanol administration of animal model was caused depletes in mitochondrial DNA in transgenic mice

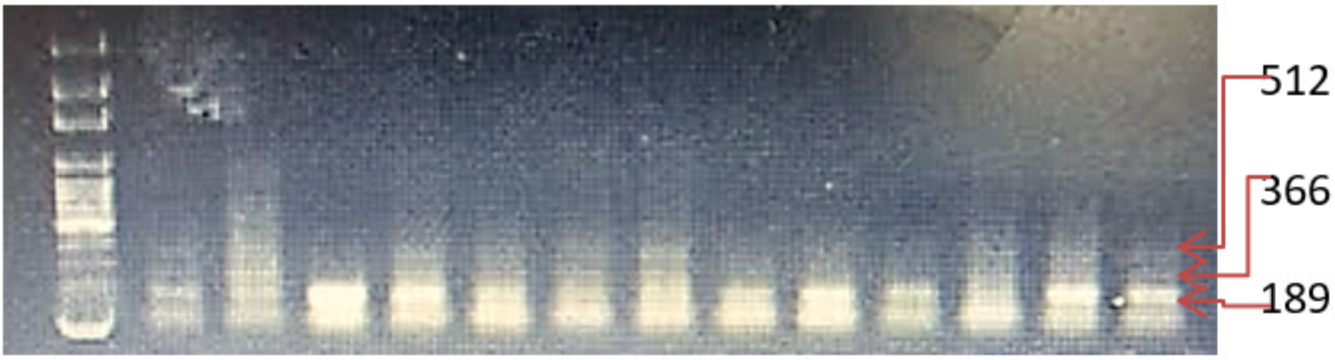

Figure 1: Agaros gel electrophoresis of (TT,CC and TC) genotyping of MnSOD that produced (514, 366 and 189 bp) using tetra ARMS-PCR, 100 v, 20 mA, 0.5x TBE buffer for 40 minutes.

Table 1: The MnSOD gene polymorphism in alcoholism and control groups (NS non-significant, *significant at p less than 0.05).

Genotypes	*Alcoholism*	*Control*	*Odd ratio*	*p-value*
TT	30 (76.92%)	11(39.28)	4.2424 1.4327 to 12.5628	0.0091*
CT	9 (23.07%)	14(50)	4.5862 0.2120 to 99.2019	0.3315 NS
CC	0	3(10.71)		
T	0.88	0.64	0.2286 0.1080 to 0.4839	0.0001*
C	0.11	0.35		

Table 2: The MnSOD gene polymorphism in smoker and non-smoker in alcoholism (NS non-significant, *significant at p less than 0.05).

Genotypes	*Smoker*	*Non-smoker*	*Odd ratio*	*p-value*
TT	21 (70%)	7 (87.5%)	0.3333	0.3356
CT	9 (30%)	1 (12.5%)	0.0356–3.1189	NS
T	0.85	0.93	2.7353	0.0467*
C	0.15	0.06	1.0149–7.3716	

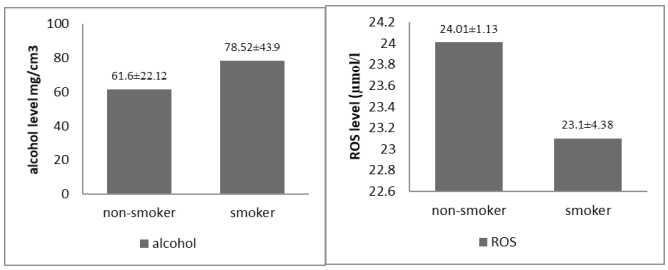

Figure 2: The alcohol and ROS level in the smoker and non-smoker in alcohol abuse

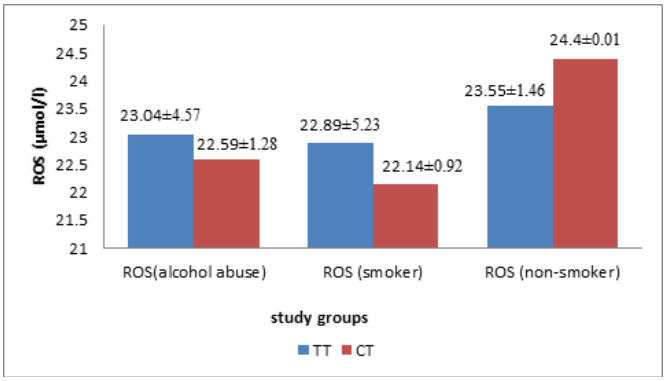

Figure 3: The ROS (µmol/L) level in the study groups according to MnSOD Ala16Val polymorphism (alcohol abuse, alcohol abuse with smoker and alcohol abuse non-smoker).

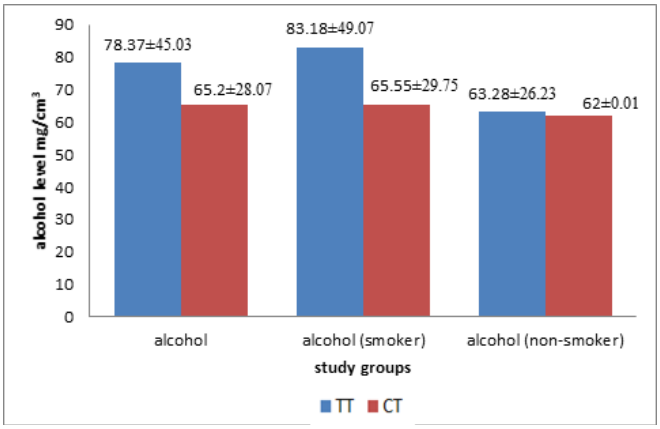

Figure 4: The alcohol level (mg/cm^3) in the study groups according to MnSOD Ala16Val polymorphism, in alcohol abuse, alcohol abuse with smoker, and alcohol abuse non-smoker.

have overexpressing of MnSOD but not in their wild-type littermates.

The effect of comorbidity between alcohol abuse and smoker habit in the MnSOD gene polymorphism was studied in current research, two genotypes were observed (TT and TC) and CC didn't found, non-significant difference was observed in TT and TC (p = 0.335), the allele frequency shows of T allele with alcoholism (p = 0.046) (Table 2), the present study shows a weak association between MnSOD gene polymorphism and smoker in drunk individuals. The relation of MnSOD mutation with a smoker was poor studied; meanwhile this relation was further studied in smoker patients of different cancer types like prostate cancer.[16,17] it can be speculated that Ala/Ala genotypes may cause lower MnSOD expression that don't cause deleterious effects as well as oxidative stress,[14] this didnot agree with present results.

investigations found low levels in an activity of SOD enzyme in chronic alcoholic patients[18] and alcoholics in withdrawal,[19] this reduction regarding to the decreased in the gene expression levels or by protein structure alteration; however, these may be induced or inherited like missense variant (Ala16Val) that putatively reduces the SOD2 enzyme activity.[8,9,20-22]

The ROS level shows slightly increased in non-smoker group (p = 0.555) (Figure 2) and slightly increased also in the TT genotype in the alcohol abuse (p = 0.835), alcohol abuse with smoker (p = 0.787) and low decreased in alcohol abuse non-smoker (p = 0.667) in non-significant differences (Figure 3).

the alcohol level (mg/cm^3) in the study groups shows increased in the smoker group than non-smoker in non-significant differences (p 0.250) (Figure 2), and its increased in TT genotype in all groups in non-significant differences of alcohol abuse (p 0.393), alcohol abuse with smoker (p 0.326) and alcohol abuse non-smoker (p 0.956) (Figure 4).

The association of MnSOD Ala16Val polymorphism with ROS has been studied in different disease, the MnSOD has main role in the oxidative stress balance and decreased ROS level,[23] the polymorphism may be effected in the alcohol abuse, smoking and exposure to environmental factors that lead to other disease like cancer.[24]

The compromises between alcohol and smoker have harmful effects in the cells and this lead to disruption in the oxidative hemostasis,[25-27] and anti-oxidative molecules like enzymes, the antioxidant enzyme gene polymorphism contributed with these factors in the cell functions, the present study. The present results concluded that there was a strong association between TT genotyping and alcoholism ROS in smoker group and alcohol level, but the smoker shows weak association with MnSOD at the Ala16Val SNP, nevertheless to prove the theory of a causal link between alcohol intake and MnSOD genotyped analysis of groups with heavy drinkers with limited tobacco smoke versus heavy smokers and minimal drinkers would need more investigations.

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