RESEARCH ARTICLE

The Antioxidant Enzymes Gene Polymorphisms (GST and MnSOD Ala16Val SNP) in Poly-substance Abuse Cases

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ABSTRACT

The poly substance abuse (PSA) becomes an important problem among the Iraqi population, several factors contributed in the poly substance abuse incidence like bad lifestyle, tensions and oxidative stress, the current study was suggested to evaluate the glutathione S-transferase T1. (GSTT1) and glutathione S-transferase M1 (GSTM1) null genotyping and manganese superoxide dismutase (MnSOD Ala16Val SNP) gene polymorphisms in the poly substance abuse in some Iraqi cases, GST detected by null genotyping PCR, the results showed that the GSTM null genotyping was elevated in PSA cases 41.67% while in control 6.89% in significant association (p 0.0017) (OR 9.6429, CI95% 1.8524 - 50.1971). the null genotyping of GSTT1 also significant elevation in PSA (62.5%) while in control was (17.24%) in significant differences (p 0.0013) (OR 8.0000, CI95% 2.2480 - 28.4693), the manganese superoxide dismutase (MnSOD Ala16Val SNP) detected via tetra primers ARMS-PCR technique, the results showed that The gene polymorphism of MnSOD produced homozygote (Val/Val and Ala/Ala) and heterozygotes (Val, Ala), the homozygote (Val/Val) was more observed in PSA cases (52.38%) than control group (39.28%), in non-significant differences (OR 1.7000, CI95% 0.5416 -5.3365, p 0.3633), while (Ala/Ala) was low frequent in PSA and control (9.52, 10.71%) respectively in non-significant differences also (OR 1.1667, CI 0.1596 -8.5259, p 0.8793), The current finding concluded that there was a strong association between GSTT1 and GSTM1 null genotyping and weak assocation of the MnSOD Ala16Val SNP with Iraqi poly-substance addiction cases, its need more investigations about other antioxidant mechanisms role that contributed in the predisposed to drug abuse.

Keywords: Glutathione S transferase, MnSOD Ala16Val SNP, Null genotyping, Poly substance abuse cases, Tetra ARMS-PCR.

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INTRODUCTION

In the last decade the most important health problem in Iraq is a poly-substance abuse that needs heavy efforts to find optimal solutions to limit the contagious nature. The substance abuse is called as a chronic relapsing disease with elevation relapse rates reached (56.8–81.8)%[1,2] at 2017 the United Nations estimated 271 million people, or 5.5% of the global population have age rang (15–64 years), had used substance in the last year.[3] The poly-substance mean that individual abuses at least three combined psychoactive substance or drugs[4] that effect in the neurotransmitters, evidences found that substance abuse causes reinforcing and promote chronic relapse by stimulating the reward system of brain, that lead to an elevation in the level of dopamine in synapsis cell.[5,6]

Antioxidant enzyme is one of the developmental mechanisms in aerobic organisms to abolish oxidative stress, like superoxide dismutase (SOD) and GST, construct the secondary defense system against exogenous and endogenous

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oxidative stress. In human, three isoforms of SOD were detected including the cytoplasm isoform copper–zinc SOD, the plasma membrane isoform or its secreted in the extracellular matrix for trapping superoxide anion from both plasma membrane sides, and mitochondrial matrix isoform manganese SOD (MnSOD) that synthesized in the cytosol and transport to mitochondria after post transcriptionally modified.[7] The gene polymorphism of MnSOD has been found to be associated with different health disorder like mood disorder.[8]

Evidences found that the chronic intake of most drugs of abuse stimulated generation ROS and RNS in brain by different mechanisms like Induced Mitochondrial Dysfunction,[9] on the other hand, antioxidants mechanisms have a major role in oxidative hemostasis in the brain like glutathiones transfers enzymes family, The association between oxidative stress, antioxidant mechanisms and mood disorder have been studied in different populations, the present study aims to assess the role of MnSOD Ala16Val SNP and GSTs (M and T) in the poly-substances addiction in Iraqi cases and this due to increase this problem in Iraq and elevation oxidative stress in different mode disorder cases.[10,11]

MATERIALS AND METHODS

Poly-substance abuse cases intake (diazepam, methamphetamine and amphetamine) for different periods were attending to the psychiatric clinic, to follow their treatment by Prof. Dr. Kareem N. Hessian, and 30 healthy individuals were enrolled in a case control study. Blood samples were collected with the approval of each case to contribute in our study, then store under -20ºC until DNA extracted, the GSTs null genotyping were detected by GSTM1 F 5'-GAACTCCCTGAAAAGCTAAAGC-3', R 5'-GTTGGGCTCAAATATACGGTGG -3' to produce 215 bp and GSTT1: F 5'-TTCCTTACTGGTCCTCACATCTC-3', R 5'-TCCCAGGTCACCGGATCAT-3', the amplification products were 215 bp for GSTM1 and 312 bp for GSTT1[12] at TM 60ºC for 30 sec in separated reaction. the Ala16Val SNP in the MnSOD sequence was detected using tetra-primer ARMS-PCR via primers set[13]; F1 5′ CACCAGCACTAGCAGCATGT-3′; F2 5′GCAGGCAGCTGGCTaCGGT-3′; R1 5′ ACGCCTCCTGGTACTTCTCC-3′ and; R2 5′ CCTGGAGCCCAGATACCCtAAAG-3 the amplification products were three bands (514, 366 and 189 bp for Val/Ala) in heterozygotes and two bands in homozygotes (514,189 bp for Val/Val)and (514, 366 bp for Ala/Ala). All products were separated by electrophoresis using agaros gel (1%), 0.5X TBE, 70V for 40 min with ethidum bromide staining. Statistical analysis was implemented using odd ratio (OR) (confidence intervals 95% (CI95%)) at p value less than 0.05. Percentage was used to represent the genotyping for both genes.

RESULTS AND DISCUSSION

The current study pointed to the antioxidant enzymes genes variations in poly-substances abuse in Iraqi cases, in last year's, Iraqi population suffered from addiction, for different drug abuse, current study deal with cases addict to three drugs included (methamphetamine, diazepam, and amphetamine) for different time and different doses. The drug addiction becomes the most health problem in Iraq.[14]

The results of DNA extracted from PSA and control groups have concentration ranged (80–200 ng/µL) and purity (1.8–2.1) (Figure 1).

The glutathione S transferase enzymes family is an important antioxidant molecule used to detoxify the free radicals in addition to other vital functions, the role of GSTs enzymes (GSTT, GSTM and GSTP) has been studied in different disease and health problems.[15] The Present finding shows strong association between GSTM and GSTM null genotyping with poly-substances abuse, the GSTM null genotyping was elevated in PSA cases 41.67% while in control 6.89% in significant association (p 0.0017) (OR 9.6429, CI95% 1.8524 - 50.1971) (Figure 2 and 3). the null genotyping of GSTT1 also significant elevation in PSA (62.5%) while in control was (17.24%) in significant differences (p 0.0013) OR (8.0000, CI95% 2.2480–28.4693) (Figures 2 and 4).

The null genotyping resulted from deletion mutation in GSTs encoded genes, studies found about 1500 base pairs were deleted, and others indicated that null genotyping resulted in a complete lack GSTM1 activity.[16] Previous study indicated that GSTM1 null genotype resulted in a complete lack of GSTM1 activity lead to decrease in oxidative stress.[17]

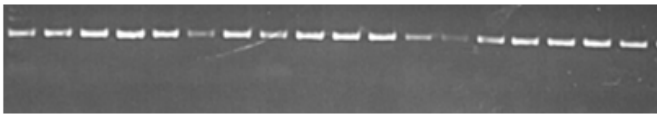

Figure 1: Electrophoresis pattern of DNA extraction from whole blood of study subjects.

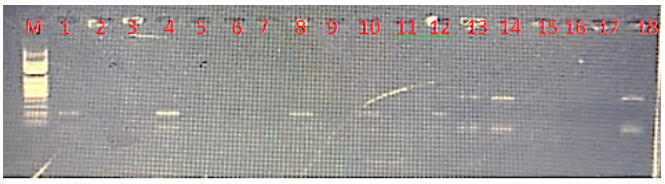

Figure 2: The GSTT1 and GSTM1 genotyping in study groups, M DNA ladder (100-1000bp), lanes 1,4,8,10,12 GSTM1, lanes 2,3,5,6,7,9,11 GSTM1 null genotyping, lanes13,14,18 GSTT1, lanes 15,16,17 GSTT1 null genotyping. 100V, 20mA, 1% agaros, 0.5X TBE buffer for 40 min.

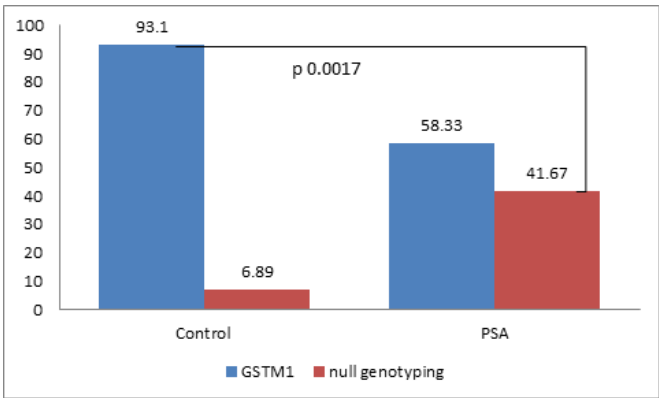

Figure 3: The GSTM1 genotyping in study groups (p value less than 0.05).

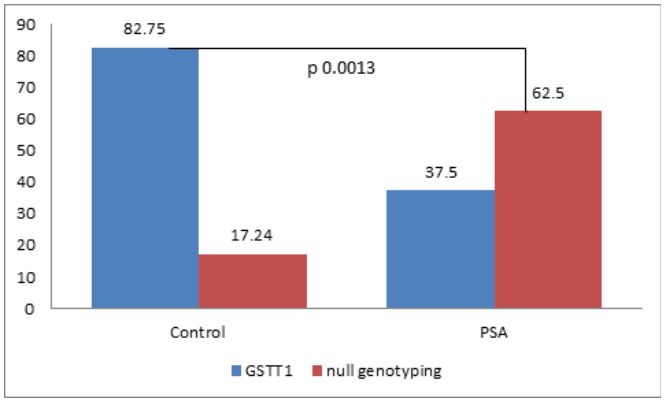

Figure 3B: The GSTT1 genotyping in study groups (p value less than 0.05).

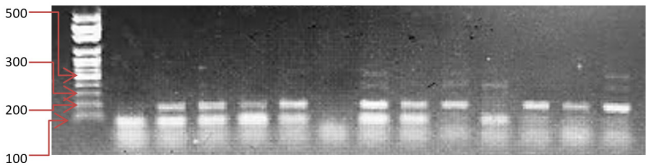

Figure 4: Electrophoresis pattern of the MnSOD Ala16Val SNP polymorphism in study groups.

Evidences suggested that, according to *GSTT1* and/or *GSTM1* function lack, the null phenotype didn't have an efficiently to conjugation reaction and eliminated the toxic products by bile and urine. The *GSTM1*null variant is particular interest, documents have explained the variations in susceptibility, variability in drug response, exposure to environmental toxicants and manifestation of several diseases.[10,12,16,18] The *GSTM1* gene located on chromosome 1p13.3 and it's highly polymorphic. About 20–67% of populations recorded variation in *GSTM1* homozygous deletion polymorphism.[19-22] Several documents deal with mood disorder found that GSTT1 null polymorphisms may be associated with the risk of schizophrenia in Chinese population, and this risk was further reduced with the combination of GSTT1 and GSTM1 null polymorphisms,[23] the present study agree with other studies proved related of GSTs null genotyping with oxidative stress in some disease like mitochondrial dysfunction,[24] cancer,[25] vitiligo,[26] diabetes mellitus,[27] and Autism spectrum disorders.[28]

The gene polymorphism of MnSOD shows homozygote (Val/Val and Ala/Ala) and heterozygotes (Val, Ala), the homozygote (Val/Val) was more observed in PSA cases (52.38%) and in control was (39.28%), in non-significant significant differences (OR 1.7000, CI 0.5416 -5.3365, p 0.3633), while (Ala/Ala) was low frequent in PSA and control (9.52%), (10.71%) respectively in non-significant differences also (OR 1.1667, CI 0.1596 -8.5259, p 0.8793) (Table 1 and Figure 4). The manganese superoxide dismutase is one of the most antioxidant enzyme contributed in the detoxification of ROS, different SNPs have been studied in the gene, the MnSOD Ala16Val SNP has been shown to enzyme localization and the mitochondrial transportation alteration which impacted in the balance of redox status.[29] This SNP is most commonly

Table 1: The MnSOD Ala16Val SNP polymorphism in poly-substances addiction and control group

Genotypes	*PSA(%)*	*Control (%)*	*Odd ratio*	*P*
Val/Val	11(52.38)	11(39.28)	1.7000 0.5416 to 5.3365	0.3633 NS
Val/Ala	8(38.09)	14(50)	1.1667 0.1596 to 8.5259	0.8793 NS
Ala/Ala	2(9.52)	3(10.71)		

studied and its substitution mutation T>C in the exon 2 codon 16 that translate Val to Alanine.[30,31]

The substitution mutation produced β-sheet secondary structure instead of α-helix structure that decreased the efficiency of enzyme transport to the mitochondria, the amino acid Ala able to transverse the membranes of mitochondrial to reach the matrix while amino acid Val causes embedded within the inner membrane that lead to defects in the enzyme activity in the ROS removing.[31,32] The association between oxidative stress and addiction abuse has been proved, like Cocaine Addiction,[33] morphine,[34] methamphetamine[35] and diazepam.[36] The elevation of ROS in addiction cases causes different diseases related with damage effects of ROS, however the antioxidant mechanisims may be had low effiency due to the long priod of ROS exposure, also other factors involvment in the oxidative stress balance in addiction cases. The current finding concluded that there was strong association of GSTT1 and GSTM1 null genotyping and weak association of MnSOD Ala16Val SNP gene polymorphism with Iraqi poly-substances addiction cases, and this don't mean the oxidative stress status in these cases was unbalanced, other antioxident mehansisms and molecules should be investigated to explaine the complex assocation among oxidative stress, addiction and other factors.

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