RESEARCH ARTICLE

Substitution Mutation of eNOS gene (rs1799983 T>G) Effect in the ROS and TAO Levels in Alcohol Abuse Cases

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ABSTRACT

The current study aims to validate the association between eNOS gene variation at SNP rs1799983 and alcoholism regarding to the increment in the alcohol abuse and comorbidities in a high percent in Iraqi population in last years, and its effect in the reactive oxygen species (ROS), total antioxidant capacity (TAO) and Alcohol level. A tetra ARMS-PCR technique used for detection T>G mutation. The results show three genotypes (TT, TC and CC) and two alleles (T and C), the genotyping distribution shows that GG was closely recurrent in alcoholism and control groups (55.26%, 54.16%) in non-significant differences ($p > 0.932$), the GT was low frequent in alcoholism than the control group (34.21%, 41.66%) in non-significant differences ($p > 0.346$), the TT was low frequent than other genotypes in both groups. The allele frequency shows non-significant closely frequent for T and C in both groups ($p > 0.622$), the ROS level was low in all genotypes in non-significant differences GG ($p > 0.541$), GT ($p > 0.502$) and TT ($p > 0.175$) in alcoholism than control group and non-significant differences among genotyping within the group for alcoholism and control groups ($p > 0.756$, $p > 0.171$) respectively, the TAO level was non-significant decreased in the GT ($p > 0.135$) and significant elevation in the TT in alcoholism ($p > 0.00$), while in the GG ($p > 0.911$) was approximately similar in non-significant between alcoholism and control group and non-significant variation within the alcoholism genotyping ($p > 0.807$) and in control group ($p > 0.098$). Our study concluded that a weak association of eNOS gene mutation at SNP rs1799983 and alcoholism, ROS and TAO level.

Keywords: Alcoholism, ARMS-PCR. ROS, Endothelial Nitric Oxide Synthase, Gene polymorphism, TAO

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INTRODUCTION

The association between the genetic biotechnology and disease etiology has been developed, a wide range of diseases etiology were detected by gene SNPs, the endothelial nitric oxide synthase (eNOS) gene has been documented to be associated with different disease and health disorder.[1,2] The Nitric oxide synthase 3 (NOS3) stimulates to generate nitric oxide (NO) which is an important in different physiological and pathophysiological processes, it is made the NO from the amino acid l-arginine, this production implemented by a family of NOS enzymes, there are three isoforms of NOS included the NOS generated in macrophages and vessel walls induction by endotoxin lipopolysaccharides and cytokines during pathological conditions.[3,4] The neuronal NOS produced in the

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peripheral, central nervous systems and kidney macula densa, it has major roles in physiological[5,6] and pathophysiological processing.[7] The endothelial NO synthase (eNOS) produced in endothelium, generated NO from l-arginine, then it transfers to the vascular smooth muscle cells, to increase cGMP concentration via trigger the soluble guanylate cyclase for vascular relaxation.[8]

Alcohol consumes individuals or alcoholism become the most health disorder problem that leads to social, economic and health complications in population, different diseases have been detected that be associated with alcohol abuse.[9] The association of eNOS with alcoholism and other disease has been studied in other like cardiovascular disease like hypertension and cancer.[1,10] The present study aims to detect the eNOS rs1799983 SNP and it's associated with alcoholism using tetra-primer in one step.

METHODOLOGY

The current study consists of 40 alcoholism and 29 healthy individuals as a control group with written consent of them, DNA was extracted from whole blood samples that collected of each volunteer, concentration and purity were detected then tetra primer PCR-ARMS were used to detect eNOS rs1799983 as a following F1: 5'-AGCCTCGGTGAGATAAAGGATG-3' R1: 5'-CCTGGACCTGCTCTGATTGTC-3' F2: 5'-GCTGCTGCAGGCCCCAGATAAG-3' R2:5' GCAGAAGGAAGAGTTCTGGGAGA-3' to produce G allele (475 bp) T allele (271 bp) Control band (701 bp)[11] at 60ºC and extension for 40 seconds. the PCR products were separated using electrophoresis pattern. Data were analysis using odd ration (CI95%) and p-value less than 0.05, the allele frequency was detected by Hardy-Weinberg law.

RESULTS AND DISCUSSION

The current study was conducted with the alcoholism cases has age (30.43 ± 9.61) years and control group has an age (28.20 ± 5.57) years, all alcoholism cases have the high percent of alcohol in blood, the genotyping of eNOS rs1799983 SNP in alcoholism and control groups shows three genotypes (TT, TC and CC) and two alleles (T and C) (Figure 1), the genotyping distribution was shown in Table 1, the GG was closely recurrent in alcoholism and control (55.26, 54.16%) in non-significant differences ($p > 0.932$), the GT was low frequent in alcoholism than the control group (34.21, 41.66%) in non-significant differences also ($p > 0.346$), the TT was low frequent than other genotyping in both groups. The allele frequency shows non-significant closely frequent for T and C in both groups ($p > 0.622$) (Table 1).

The effect of alcohol uptake in the vascular actions of NO and eNOS has been studied in human and animal lab, the Chronic exposure of alcohol lead to endothelial function decline; harmful effects of a symmetric dimethylarginine and superoxide anions. In human, low alcohol level elevates the production of NO in endothelial cells, while high level causes endothelial dysfunction and apoptosis, as a result of these effects it impacted in the eNOS also, via excessive in eNOS protein and mRNA expression by high concentration of alcohol and NO generation in animal endothelial cells, the Endothelium-dependent, NO-mediated cerebral arteriolar dilatation, as well as vasodilatation mediated by nNOS-derived NO, is declined by chronic alcohol uptake; increased ROS production and depletion of tetrahydrobiopterin may be contributed in these effects (Figure 2).[12,13] the present study found a weak association between eNOS gene polymorphism and alcoholism, this didn't agree with Hong *et al*.,[14] who found that the mutant genotype of eNOS Glu298Asp polymorphism is affected by smoking, drinking and the interactions between these habits in comparing with the wild genotype in male hypertensive cases. Kang *et al*.,[15] agree with our results as they didn't find an association between, Glu298Asp: rs1799983; T-786C: rs2070744, rs7830; rs3918188 and uptake

Figure 1: electrophoresis pattern of genotyping distribution and allele frequency of eNOS rs1799983 SNP in alcoholism and the control group (G allele (475bp) T allele (271 bp) common band (701 bp).

Table 1: The genotyping distribution and allele frequency of eNOS rs1799983 SNP in alcoholism and control groups (NS; non-significant)

Genotyping	*Alcoholism*	*Control*	*Odd ratio CI%*	*sig*
GG	21(55.26%)	13(54.16%)	1.0452 0.3745 to 2.9175	0.9327NS
GT	13(34.21%)	10(41.66%)	0.3250 0.0313 to 3.3783	0.3468NS
TT	4(10.52%)	1(4.16%)		
T	0.56	0.52	1.1503 0.6583 to 2.0101	0.6228NS
G	0.44	0.47		

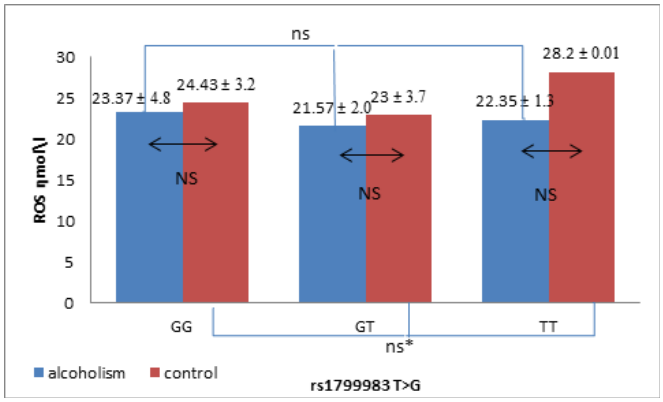

Figure 2: The ROS level according to the rs1799983 T>G genotyping in study groups (NS non-significant differences between group, independent t test, ns non-significant among genotyping in the alcoholism, ns* non-significant among genotyping in the control group, ANOVA one way)

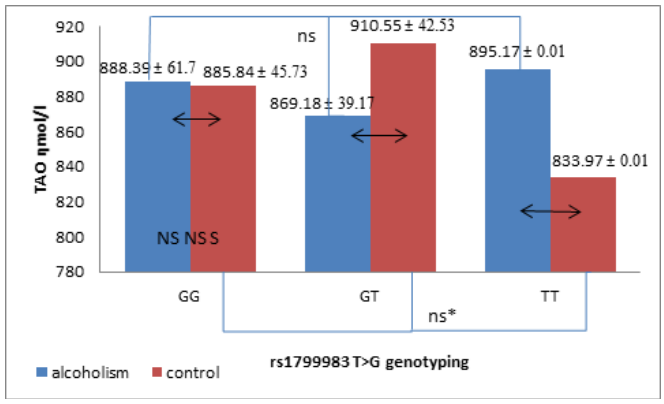

Figure 3: The TAO level according to the rs1799983 T>G genotyping in study groups. (NS non-significant differences between groups, independent t test, ns non-significant among genotyping in the alcoholism, ns* non-significant among genotyping in the control group, ANOVA one way)

of alcohol and red wine, this disassociation regarding to low concentrations of alcohol to induce genetic mutation, other found a remarkable gene-environment relation between eNOS exon 7 894GG genotypes and behavioral risk factors like alcohol uptake for the risk of hypertension.[16] The present study needs to instigate other sites of eNOS gene to prove an association with alcoholism, the limitation of our study was sample size and difficult in obtained it.

The ROS and TAO level have been studied in alcohol abuse.[13,17] in present study, the ROS and TAO levels were detection according to the eNOS gene mutation at SNP rs1799983, the findings show that ROS level was low in all genotypes in non-significant differences GG (p 0.541), GT ($p > 0.502$) and TT ($p > 0.175$) in alcoholism than control group (Figure 3). The multiple comparisons among genotyping in alcoholism and control group show non-significant differences also ($p > 0.756$, $p > 0.171$), respectively.

The TAO shows disparate levels, the TAO level was non-significant decreased in the GT ($p > 0.135$) and significant elevation in the TT in alcoholism ($p < 0.00$), while in the GG ($p > 0.911$) was approximately similar in non-significant between alcoholism and control group (Figure 4). The multiple comparisons among genotyping in alcoholism shows non-significant variation ($p > 0.807$) and in control group ($p < 0.098$).

The assertion between antioxidant enzyme gene polymorphism and ROS production was studied by Silvia *et al*.,[18] that found the *eNOS* asparagine allele has been related to low ROS levels in rectal cancer patients after radiotherapy, and this also proved by Veldman *et al*.,[19] that found the Glu298Asp polymorphism in eNOS 3 gene is the baseline production of nitric oxide and low level of free radicals. The weak association of eNOS gene polymorphism with TAO in present study may be because that the total antioxidant dependent on the different factors including nutrition like vitamins, antioxidant molecules and minerals, other antioxidant enzymes genes polymorphism and other diseases, all these factors are varied among population.[20-23] In conclusion, the our study concluded that weak association of eNOS gene polymorphism at SNP rs1799983 with alcoholism and with the ROS and TAO level in alcohol abuse cases.

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