Association between Genetic Polymorphism of 5-HTTLPR, and SGOT, SGPT and Catalase with Alcoholism of Iraqi People

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Abstract

A case- control study on the relationship of 5-HTTLPR gene polymorphism with alcohol abuse among Iraqi individuals was conducted during period from December 2018 to June 2019. DNA was extracted from the blood samples of the study subjects. The genetic polymorphism analysis was conducted by Restriction Fragment Length Polymorphism (RFLP-PCR) for SLC6A4 gene. The results raveled that the odd ratio for the *LL* genotype was 1.697 indicating that homo mutant genotype were at higher risk for alcoholism than the wild type *SS*. This result showed that L allele frequency was (0.78) in alcoholism group and (0.53) in control group whereas S allele frequency was (0.22) in the alcoholism group and (0.47) in the control group. So, the results showed there was a significant difference in allele frequencies of *SLC6A4*gene between case and control ($P<0.01$; OR=1.697; 95% CI=0.86-1.64). There was a significant positive correlation between the SGOT activity and the genotype *of 5-HTTLPR* (*SS, SL, LL*).Additionally, there was a significantly increased level of liver enzymes (GOT and GPT) and catalase in serum samples of alcoholic males compared with non-alcoholic males.

Key Words: *SLC6A4, 5-HTTLPR, SGOT, SGPT, catalase.*

Introduction

Alcohol dependence and alcohol abuse are chronic disorders comprising a wide range of clinical symptoms. Given that drinking behaviors are jointly determined by genetic and environmental risk factors, alcohol consumption and alcohol use disorders are appropriate phenotypes for investigating gene- environment interactions [(1).] Alcohol consumption is a common, complex trait, and heavy alcohol use increases the risk of alcohol use disorders, and is recognized as, a problematic global problem threatening both individual development, family life and social life of a person [(2).] There is an evidence of a causal relationship between alcohol and at least 200 diseases including gastritis, pancreatitis, cardiovascular disease, liver cirrhosis, hepato cellular carcinoma, and gastric cancer [(3).] Ethanol is not stored in the body after ingestion because it is fully ingested oxidized during metabolization in the liver [(4).] The rate of ethanol metabolism determines the concentration of ethanol and its metabolite acetaldehyde in the different tissues, which in turn influences the effects of ethanol consumption on liver and the other organs. Serotonin is a signaling molecule with a widespread effect in the CNS, and has a very important role in different aspects of mammalian life, like food intake, emotion, mood, respiration, pain sensitivity, cardiovascular regulation, sexual behavior, learning and memory, circadian rhythm, sensorimotor activity, and cognition [(5).]

The human serotonin transporter (SETR) is a monoamine transporter protein, encoded by a single gene (SLC6A4, solute carrier family 6, member 4) located on the long arm of chromosome 17 (17q11.2). The serotonin transporter (5-HTT) is an important protein responsible for the active transport of serotonin into neurons, enterochromaffin cells and platelets [(6).] Twin studies have shown alcohol dependence (AD) to have a heritability of ~50–60% [(7, 8).] Among the genetic components, many of the genes that may contribute to the risk of alcohol phenotypes encode components of the dopamine, serotonin (5-HT), and acetylcholine neurotransmitter systems.

Material and Method

Study Subjects

A case- control study on the relationship of 5-HTTLPR polymorphism with alcohol abuse among Iraqi individuals was conducted during period from December 2018 to June 2019. Fifty blood samples of alcoholic male Iraqi people (with a history of alcohol abuse for more than seven years) were collected from the Institute of forensic medicine, Al-Yarmouk Teaching Hospital, Shaikh Zayed Hospital, and Ibn-Rushed Teaching Hospital/Baghdad, Iraq. The study subjects were men of ages mean of 35.04 years ± 10.89 SD. Additionally, 50 samples were collected from non-alcoholic subjects, as control group, with mean age of 34.30 years ± 10.86.

Blood Sampling

Five milliliters of blood were collected by vein puncture, two ml was put into EDTA tubes for molecular analysis and three ml put in separating gel tube, then was allowed to clot at room temperature for 30 minutes and then centrifuged at 2000 rpm/ 15 minutes. The sera were collected and stored at -20ºC until analysis.

Enzymatic Assay

The liver enzymes (GOT and GPT) and catalase in serum samples of alcoholic and non-alcoholic males were measured by Reflontron/ Germany in this study.

Genotyping of SLC6A4 Polymorphism

Preparation of Genomic DNA

The DNA from genome was prepared from blood samples (gSYNC™ DNA Extraction Kit) according to the instructions of the manufactured. Concentrations and purity of DNA were measured by Nano drop spectrophotometer (Apel/Germany).

PCR condition and Restriction Fragment Length Polymorphism (RFLP)

Two primers were selected (F-5- GGC GTT GCC GCT CTG AAT GC -3) and (R-5- GAG GGA CTG AGC TGG ACA ACC AC -3 ′) [9] to amplify fragments of (469 &512) bp for the detection of alleles. The specific designed primers were provided by AccuOligo/ Bioneer/ Korea. The PCR reaction was performed in a total volume 20 µl containg 10 p mole/µl of each primer, 1x Master mix(AccuPower® ProFiTaq PCR PreMix/ Bioneer/ Korea), and 0.15µg/µl genomic DNA. The reaction mixture was amplified in thermal cycler (Cleaver Scientific, UK). Initial denaturation was carried out at 95°C for 5 min and the target DNA was amplified in 40 cycles. Subsequently, each cycle consisted of denaturation at 95°C for 30 sec; followed by annealing at 63°C for 30 sec. Elongation was carried out at 72°C for 1 min. The final extension step was performed at 72°C for 10 min. Aliquots of products of amplified DNA were treated with *MspI* (restriction enzyme, SibEnzyme/ Russia). The PCR product (*5-HTTLPR)* was digested with restriction endonucleases in a total volume of 20µl containing 10 units of enzyme with buffers supplied by the manufacturer's instructions. The amplified PCR products were checked for the expected size on 2% (w/v) agarose gel and visualized after staining with ethidium bromide under ultraviolet. A 100bp DNA molecular weight marker (BioNeer/Korea) was used to measure the weight of the fragments [10].

Biostatistical consideration

The [11] program was used to analyze the difference factors in study variables. Chi-square test was used to compare differences among percentage at P-values of 0.05 and 0.01 probability. Odd ratio and 95% CI were estimated.

Results and Discussion

The study subjects were men of ages mean of 35.04 years ± 10.89 SD. Additionally, 50 samples were collected from non-alcoholic subjects, as control group, with mean age of 34.30 years ± 10.86, table (1).

Table (1): Distribution of ages of alcoholic male among cases and control

Age group (year)	Group	
	Cases	Control
< 30	16 (32.00%)	14 (28.00%)
30-40	20 (40.00%)	25 (50.00%)
> 40	14 (28.00%)	11 (22.00%)
Total	50	50

Results listed in table (1) indicated that the most common age group of cases(alcohol consumption) was

of 30-40 years (40.00%) followed by the group less than 30 years (32.00%), and more than 40 years (28.00%).

Blood Chemistry Analysis

The results of testing for GPT and GOT in serum from the cases as compared to the controls are presented in figure (1: A, B).

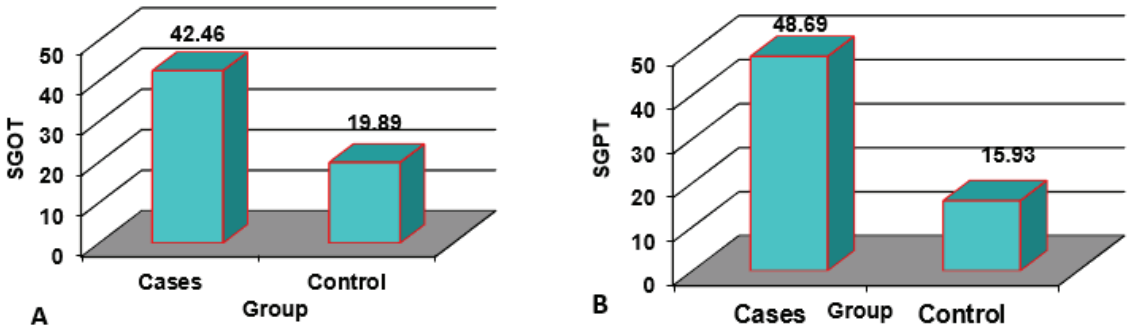

Figure 1: Comparison of liver enzymes; A (GOT) and B (GPT) in serum samples of alcoholic and non-alcoholic males

The results in the present study indicated that parameters measured (GPT and GOT), which are markers liver functions revealed significant differences between cases and controls, (42.46 ± 23.39 vs. 19.89 ± 11.31) (48.69 ± 21.31 vs. 15.93 ± 9.27) at (P<0.01) for GPT and GOT, respectively. Measurement of SGOT and SGPT becomes very important because these liver enzymes are the most important liver enzymes to represent groups or transaminase aminotransferase enzyme, which catalyze the keto acids into amino acids by transfer of amino groups. Serum enzymes are the most commonly used and sensitive biochemical markers for the assessment of liver disease. [12], who reported that alcohol is a toxin that is harmful to the liver and alcoholic liver disease and it is one of the leading causes of alcohol-related death. While [13], found consumption of alcohol causes several pathological changes in the liver. In respect to catalase there was significant difference between case and control group (363.42 ± 214.61 vs. 101.51 ± 51.64) at (P<0.01) respectively as show in figure 2.

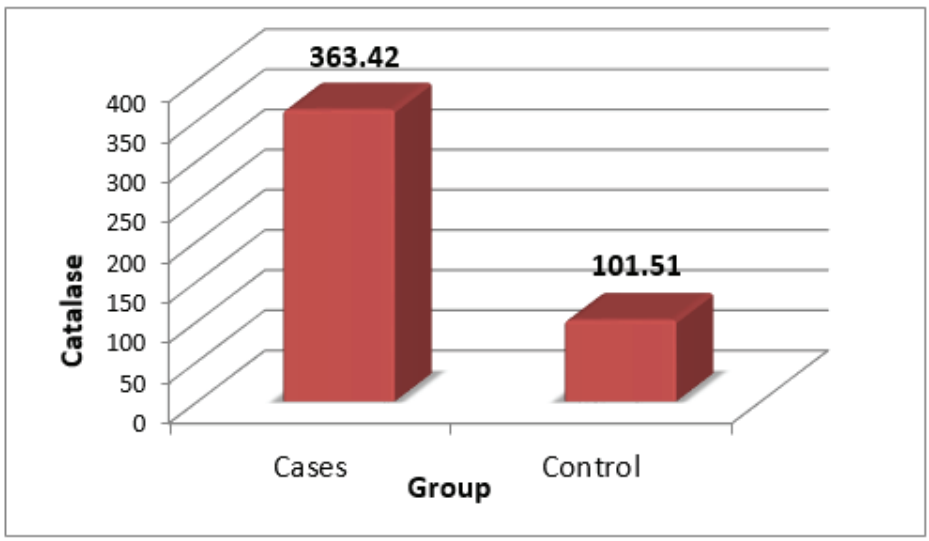

Figure 2: Comparison of catalase in serum samples of alcoholic and non-alcoholic males

The enzyme catalase has also been shown to oxidize ethanol into acetaldehyde within the peroxisomes. This process is hydrogen peroxide dependent. However, under normal physiological conditions, catalase plays only a minor role in ethanol metabolism,[14] but its contribution might be enhanced in the presence of higher amounts of hydrogen peroxide. Furthermore, catalase may be an alternative metabolic pathway for ethanol oxidation within the brain, where ADH and CYP2E1 appear to be of minor importance for ethanol metabolism [15].

[16] reported that the catalase activity increased in lower concentration of alcohol exposure, but in higher concentration of alcohol exposure catalase activity decreased compared to control groups . This finding suggested that catalase activity in the liver is changeable [17].

80-90% of alcohol breakdown in the liver results in the formation of acetaldehyde whose further metabolism in the cells leads to reactive oxygen species production (ROS) (18).

Acetaldehyde itself is a mutagenic and carcinogenic by product. It binds with DNA and interferes with DNA synthesis and repair mechanism. Furthermore, it results in tumor development [19].

Distributions of Genotypes and Allele Frequency of the 5-HTTLPR Polymorphisms in the *SLC6A4* Gene

The distribution of the observed *SLC6A4* gene genotypes and alleles frequencies in the control and cases individuals are shown in table (2).The highest genotype in the case group was homozygous *LL*(74.00%), while(18.00%) for homozygous *SS* genotype, and *LS* (8.00%).

Table (2): Distribution of genotype and allele frequency of 5-HTTLPR gene in cases and control.

Genotype of **5-HTTLPR**	**Cases No. (%)**	**Control No. (%)**	**Sig.**	**O.R. (95% C.I.)**
SS	9 (18.00%)	5 (10.00%)	0.0438 *	0.662 (0.78-1.56)
LS	4 (8.00%)	37 (74.00%)	0.0001 **	1.750 (0.86-1.71)
LL	37 (74.00%)	8 (16.00%)	0.0001 **	1.697 (0.86-1.64)
Total No.	50	50	---	---
Allele	Frequency	Frequency		
S	0.22	0.47	---	---
L	0.78	0.53	---	---
* (P<0.05), ** (P<0.01).				

The results from table (2) show significant differences in the frequencies of *SLC6A4* gene (LL) in the control and case groups (at P-value 0.01). The results raveled that the odd ratio for the LL genotype was 1.697 indicating that homo mutant genotype were a higher risk of alcoholism than the wild type SS. This result showed that L

allele frequency was (0.78) in alcoholism group and (0.53) in control group whereas S allele frequency was (0.22) in the alcoholism group and (0.47) in the control group as shown in table (2). So, the results showed there was a significant difference in allele frequencies of *SLC6A4*gene between case and control (P<0.01; OR=1.697; 95% CI=0.86-1.64).

Alleles of the 5-HTTPLR promoter have either a short (S) or long (L) copy of an imperfect repeat. The short or 'S' allele with 14 repeats was shown to have lower transcriptional activity than the long or 'L' allele with 16 repeats [20, 21]. [22] reported that the 'L' allele is associated with a predisposition to lower level of response to alcohol, which is in turn associated with the onset of alcoholism. While several previous studies that suggested an association of the S allele with alcohol and drug dependence [23, 24, 25]. [26] found out that there is an association between the 5-HTTLPR 'L' allele and the increased serotonin and platelet uptake pharmacologically; the serotonin transporter spans the plasma membrane 12 times.

Association between genotype of 5-HTTLPR and parameters

The association between 5-HTTLPR and SGOT, SGPT and catalase were investigated. The current study shows that the presence of an SGOT was significantly associated with genotype of 5-HTTLPR (*SS, SL, LL*) among cases at ($p < 0.05$), while SGPT was significantly among control at ($p < 0.05$). Regarding the association between genotype of 5-HTTLPR and catalase, there were no differences among case and control groups, table (3).

Table (3): Relationship between genotype of 5-HTTLPR and listed enzymes in cases and control.

Group	Genotype of 5-HTTLPR	Mean ± SD		
		SGOT	**SGPT**	**Catalase**
case	SS	29.16 ± 15.56 **b**	55.04 ± 15.05	389.82 ± 263.95
	SL	40.23 ± 19.95 **b**	47.84 ± 21.29	379.37 ± 243.08
	LL	61.06 ± 32.28 **a**	48.67 ± 26.12	273.13 ± 226.82
	LSD value	**20.77 ***	21.71 NS	186.97 NS
Control	SS	18.37 ± 11.25	19.42 ± 9.62 **a**	85.97 ± 24.13
	SL	20.50 ± 15.58	9.35 ± 2.15 **b**	111.22 ± 59.37
	LL	20.20 ± 11.18	15.80 ± 9.38 **ab**	104.24 ± 55.93
	LSD value	11.96 NS	**8.67 ***	55.92 NS
*** (P<0.05), NS: Non-Significant. Means having with the different letters in same column differed significantly**				

The results in the present study indicated that genetic polymorphisms of the 5-HTTLPR and SGOP in humans are linked to alcohol consumption and the incident of alcohol abuse.

Regarding the association between genotype of 5-HTTLPR and catalase, may be, duration of ethanol exposure and genetic background appear to be important variables in considering whether or not catalase changes as a response to ethanol.

Several studies have examined whether the *5-HTTLPR* polymorphism (L and S variants, LL, LS and SS genotypes) interacts with environmental risk factors to predict drinking outcomes [27, 28].

A repeat length polymorphism (5-HTTLPR) in the promoter of this gene has been shown to affect the rate of serotonin uptake and may play a role in drug dependence and other chronic neurological diseases [20, 21].

Some studies have suggested a possible involvement of the 5-HTTLPR genotype with alcoholism [22], smoking [29], suicidal behavior [30], and depression [31].

Conclusion

Researchers worldwide reported that there are

several underlying genetic factors that influence the development of alcoholism among individuals. However, to date there is only few published reports on this matter in alcoholism among Iraqi individuals. In this study there was a significant positive correlation between the SGOT activity and genotypes of 5-HTTLPR (*SS, SL, LL*). There was a significantly increased levels of liver enzymes (GOT and GPT) and catalase in serum samples of alcoholic compared to non-alcoholic males. The results in the present study indicated that genetic polymorphisms of the 5-HTTLPR and SGOP in humans are linked to alcohol consumption and conditions of alcohol abuse.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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