Association between Genetic Polymorphism of *ADH3* exon 8 and *ADH2* exon 9 Genes and Specific Enzymes with Alcoholism in Iraq

Anwar A. Abdulla

Assoc. Prof., Biology Dept. College of Science, University of Babylon, Iraq

Abstract

A retrospective cohort study of the relationship between *ADH2*- exon9 and *ADH3* exon 8 genes polymorphisms with specific enzymes SGOT, SGPT, and catalase among Iraqi individuals identified with alcohol abuse was conducted during period from December 2018 to June 2019. Blood samples of the research subjects have extracted DNA. The genetic polymorphisms analyses were conducted by Restriction Fragment Length Polymorphism (RFLP-PCR) for *ADH2*- exon9 and *ADH3* exon 8 genes. The current study shows that the presence of a catalase was significantly associated with genotype of *ADH2*- exon9 (AA, AC, CC) among cases at ($p < 0.05$). Whereas the presence of an SGOT, SGPT and catalase were non significantly associated with genotype of *ADH3* exon 8 (AA, AG, GG) among cases at ($p < 0.05$), while SGPT was significantly among control at ($p < 0.05$).

Key Words: *Alcohol, ADH2- exon9, ADH3 exon 8, SGOT, SGPT and catalase.*

Introduction

Excessive consumption of alcohol is an growing public health issue in relation to psychoactive legal use and is a global problem , affecting both an individual development, family life and social life.

Alcohol is typically absorbed by mouth, and because it is such a small water soluble substance, it can move through the walls of the stomach and intestines and surface in the bloodstream within minutes. Alcohol requires no absorption and by quick diffusion moves through the body's different membranes. Consumption of alcohol induces various medical problems including hepatic disease. Alcoholism is an important cause of terminal hepatic diseases which is world-wide in distribution [(1)].

Enzymes such as ADH and LDH play a significant role in the metabolism of alcohol in the liver. Drinking of alcohol is followed by processing with ADH1B which turned it into the highly reactive substance acetaldehyde. This toxic substance is oxidized by ALDH2 to acetic acid which through the Krebs cycle is converted into CO_2 and H_2O products which leave the body [(2)]. The three main pathways of alcohol metabolism show in Figure (1). After consumption, alcohol from the stomach and intestines is absorbed to the blood.

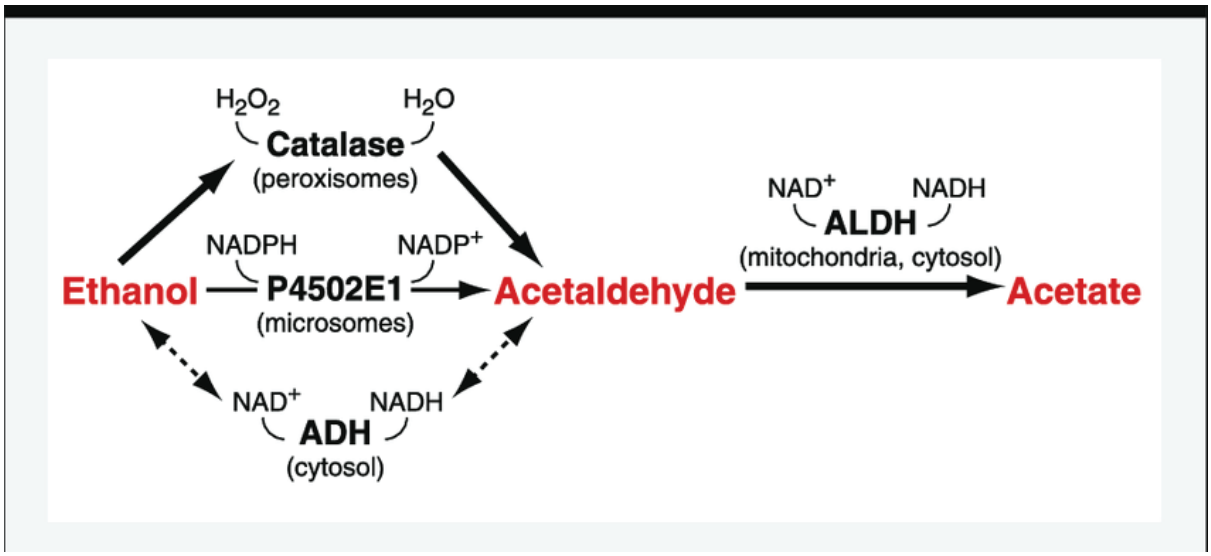

FIG. 1 Alcohol Metabolism [3].

Catalase, ADH, and ALDH may be affected through genetic polymorphisms that affect their activities in converting ethanol to acetaldehyde and then to acetate. ALDH2 is the most important alcohol- metabolizing enzyme that predisposes Asian populations to alcoholism. The prevalence of the ALDH2 * 2 allele, which codes for the physiologically inactive mitochondrial ALDH form, is lower among the alcoholic than for non-alcoholic individuals [4,5,6]. ADH enzyme in humans is a dimeric molecule, resulting from the various subunits of the seven genes. Therefore, over 20 ADH isozymes variants involved in metabolizing different types of alcohols [5].

Material and Methods

Design of the Study

A retrospective cohort study was conducted at the Institute of forensic medicine, and different hospitals in Baghdad, Iraq, during the period from December 2018 to June 2019.

Study population

A total of 50 alcoholic male subjects and 50 nonalcoholic male as a control.

Blood Sampling

Sera were collected from five milliliters of venous blood samples from each study subject. Following conventional methods

Enzymatic assay

The liver enzymes (GOT and GPT) and catalase in serum samples of alcoholic and non-alcoholic males were measured by Reflontron/ Germany as previously described [7].

Genotyping of *ADH2*- exon9 and *ADH3* exon 8 Polymorphisms

Preparation of Genomic DNA

The DNA from genome was prepared from blood samples (gSYNC™ DNA Extraction Kit) following the manufacturer's instructions. Purity and concentration of the DNA was assessed using Nano drop spectrophotometer (Apel/Germany).

Polymerase chain reaction (PCR) and Restriction Fragment Length Polymorphism (RFLP)

The primers were selected, table (1) to amplify fragments of (202 and 130) bp for the detection of alleles for each of *ADH2*- exon9 and *ADH3* exon 8 respectively. PCR mixtures was in 20 µl including 10 p mole/µl of each primer, 1x Master mix (PCR premix / Bioneer/ Korea), and 0.15µg/µl genomic DNA. The mixture was processed in thermal cycler (Cleaver Scientific, UK).

Table (1): List of primers sets

Gene	Prime sequence (5′-3′)	product Size / bp	Reference
ADH2- exon 9	F- TGGACTCTCACAACAAGCATGT R- TTGATAACATCTCTGAAGAGCTGA	202	(8)
ADH3- exon 8	F- CTTTAAGAGTAAAGAATCTGTCC R- ACCTCTTTCCAGAGCGAAGCAG	130	(8)

To detect *ADH2*- exon9 and *ADH3* exon 8 variants the PCR programs were mentioned in table (2).

Table (2): Thermal cycling conditions

Temperature/ Time	ADH2- exon 9/ No of Cycles		ADH3- exon 8/ No of Cycles	
First denaturation(ºC)	94/ 2 min	1	95/2 min	1
Second denaturation (ºC)	94/30 sec	30	95/ 1 min	35
Annealing(ºC)	54/ 30sec		53/45 sec	
Extension (ºC)	72/30sec		72/45sec	
Final extension(ºC)	72/5 min	1	72/5 min	1

The PCR product for each *ADH2- exon9* and *ADH3* exon 8 were digested with restriction endonucleases (*ALwN1* and *SspI*) respectively in a volume of 20µl including10 units of enzyme with buffers supplied by the manufacturer's instructions. The resulted products were analyzed for their molecular size on 2% agarose gel. The bands were stained with ethidium bromide and visualized under ultraviolet light. DNA molecular weight marker of 100bp (BioNeer/Korea) was included to reveal the sizes of the amplicon fragments [9].

Biostatistical consideration

The [10] program was used to analyze the differences in variables among cases and controls. Means, standard Error, One – sample T test was used to compare differences among percentages at a P-value of < 0.05 probability.

Results and Discussion

The present study included blood samples collected from fifty alcoholic Iraqi males registered with the Institute of forensic medicine, and several hospitals / Baghdad, Iraq. The study subjects were men of age mean of 35.04 years ± 10.89 SD. Additionally, 50 samples were collected from non-alcoholic individuals, as control group, with mean age of 34.30 years ± 10.86. All the identified frequencies of allele and genotype were in accordance with the Hardy–Weinberg equilibrium ($P<0.01$) as below.

Genotyping of ADH2

The results of present study of *ADH2* genotype were organized into three groups based on polymorphism presence or absence: AA wild homozygous (*ADH2*.1 allele) have one band expected to be 202 bp, the second genotype was CC mutant homozygous (*ADH2*.3 allele) which has both 132 bp and 70 bp bands. While the third

group AC heterozygous exhibited three bands 202 bp, 132bp, and 70bp. Differences in the distributions of the variant ADH2*1(AA) and ADH2*3(CC) alleles among case and controls were significant. Whereas the differences in the frequency distributions of the heterozygous (AC) allele were not significant. The result revealed that the risk of alcohol consumption associated with ADH2*3(CC) alleles (36% among cases vs 16% among control) was greater than the risk associated with ADH2*1(AA) (54% among cases vs 70% among control). ADH2 belongs to the family of alcohol dehydrogenase which converts alcohol into acetaldehyde. ADH2 gene changes were hypothesized to affect genetic susceptibility to *Alcoholic* liver disease (ALD)[11].

Association between genotype of *ADH2*- exon9 and parameters

The association between *ADH2*- exon9 and SGOT, SGPT and catalase were investigated. The current study shows that the presence of a catalase was significantly associated with genotype of *ADH2*- exon9 (AA, AC, CC) among cases at ($p < 0.05$), while SGPT and SGOT were non significantly among control and cases at ($p < 0.05$). Regarding the association between genotype of *ADH2*- exon9 and catalase, there were no differences among control groups, table (3).

Table (3): Relationship between genotype of *ADH2*- exon9 and parameters in cases and control

Group	Genotype of ADH2- exon9	Mean ± SD		
		SGOT	SGPT	Catalase
Case	AA	38.49 ± 24.15	50.51 ± 24.02	242.61 ± 163.84 b
	AC	33.86 ± 11.84	46.08 ± 9.78	446.80 ± 215.84 a
	CC	50.79 ± 23.06	46.70 ± 19.79	521.45 ± 324.16 a
	LSD value	18.93 NS	19.79 NS	202.79 *
Control	AA	19.34 ± 11.39	15.52 ± 10.15	97.69 ± 51.71
	AC	23.86 ± 13.94	14.10 ± 3.30	121.81 ± 58.63
	CC	18.87 ± 8.95	19.35 ± 8.61	100.45 ± 47.44
	LSD value	10.48 NS	7.57 NS	48.87 N.S
* (P<0.05), N.S: Non-Significant. Means that varied significantly with different letters in the same column				

CAT catalyzes hydrogen peroxide to water and oxygen, which are commonly present in livestock, plants and micro-organisms. CAT, which is an endogenous antioxidant enzyme, protects cell injury from ROS and plays a significant role in protection against oxidative stress [12]. Oxidant-antioxidant system may be affected by Alcohol [13].

While Catalase may scavenge hydrogen peroxide resulting from processing alcohol to water, it may decomposes alcohol which harmful that to liver, [14] which attenuates alcohol□induced acute liver injury may inhibit catalase.

The increased activity of catalases following ingestion of ethanol and its impact in the CNS is correlated with low activity of ADH. This increase in CNS catalase activity may be due to adaptive processes induced by hydrogen peroxide increase by animals consumption of high concentrations of ethanol in the CNS [15].

Genotyping of ADH3

ADH3 genotypes were distributed in three classes

based on the existence or absence of the polymorphism: AA wild homozygous (*ADH3*.1 allele) contain restriction site for *SspI* have both 67 bp and 63 bp bands; the second genotype was GG mutant homozygous (*ADH3*.2 allele) with have one band expected 130 bp. While the third group AG heterozygous exhibited three bands 130 bp, 67bp, and 63bp.

The ADH3 genotype for the homozygous AA was not present in samples from cases and control. While the heterozygous AG has genotype frequency as (40%) for case and (36%) for control, while the GG genotype frequency was (60%) and (64%) for case and control respectively, as shown in table (4.7). The current results showed that the ADH3 frequency distributions have not varied significantly. Although there was no significant variations between case and control on ADH3 polymorphism, the result show protective role for ADH3 among individuals with alcoholism, (O.R: 0.084; C.I. 95%:0.77-1.48) for both AG and GG genotypes. Several studies have shown that ADH3 enzyme genetic polymorphism reduces the alcohol risk, and ADH3 * 1/*1 enzyme polymorphism raises the risk of colorectal adenoma [16, 17]. In addition, further studies have reported by [16] and [17] who indicated that genetic polymorphism of ADH2 * 2 and ADH3 * 1 enzymes reduces the risk of alcoholism, and that ADH3 * 1/*1 enzyme polymorphism raises the risk of colorectal adenoma.

Association between genotype of *ADH3* exon 8, and parameters

The association between *ADH3* exon 8 and SGOT, SGPT and catalase were investigated. The current study shows that the presence of an SGOT, SGPT and catalase were non significantly associated with genotype of *ADH3* exon 8 (AA, AG, GG) among cases at ($p < 0.05$), while SGPT was significantly among control at ($p < 0.05$), table (4).

Table (4): Relationship in case and control between the ADH3 exon genotype 8 and the enzymes identified.

Group	Genotype of ADH3 exon 8	Mean ± SD		
		SGOT	SGPT	Catalase
Case	AG	47.82 ± 23.10	52.46 ± 22.46	391.40 ± 279.22
	GG	38.88 ± 23.28	46.18 ± 20.49	344.76 ± 241.97
	LSD value	12.37 NS	12.94 NS	130.59 NS
Control	AG	21.63 ± 11.93	20.37 ± 9.87 a	94.21 ± 31.90
	GG	18.92 ± 11.02	13.43 ± 8.03 b	105.62 ± 60.08
	LSD value	6.94 NS	5.02 *	32.39 N.S
* (P<0.05), N.S: Non-Significant. Means that varied significantly with different letters in the same column				

Serum enzymes are the most widely used and reliable biochemical indicators for liver disease evaluation. [18], who has reported that alcohol is a liver-destroying toxin, one of the leading causes of death due to alcohol disease. While [19], found consumption of alcohol causes several pathological changes in the liver. A percentage distribution of abnormal (GPT, GOT, ALP) in alcohol users was reported to be greater than that of non-alcohol users; (GPT, GOT, ALP) normal levels in non-alcoholic users were lower than those in alcohol users [18].

Conclusion

Excess alcohol intake is a significant concern of public health, especially given the severe damage done by chronic or prenatal alcohol exposure affecting various physiological processes. The current study suggests that genotypes of both ADH2 and ADH3 influence the metabolic rate of alcohol, and are susceptible to alcohol development. The current study shows that the presence of a catalase was significantly associated with genotype of *ADH2*- exon9 (AA, AC, CC) among cases at ($p < 0.05$). The present research indicates the presence of an SGOT, SGPT and catalase were non significantly associated with genotype of *ADH3* exon 8 (AA, AG, GG) among cases at ($p < 0.05$), while SGPT was significantly among control at ($p < 0.05$).

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

Conflict of Interest: None

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References

1. Walter A, Mohammed Ashraf, A. study correlating the quantity and duration of alcohol consumption with liver function tests, IOSR Journal of Dental and Medical Sciences. 2014; 13(3), 2279-0861.
2. Lee SL, Lee YP, Wu ML, Chi YC, et al. Inhibition of human alcohol and aldehyde dehydrogenases by aspirin and salicylate: assessment of the effects on first-pass metabolism of ethanol. Biochem. Pharmacol. 2015; 95: 71-79.
3. Deitrich R, Zimatkin SMD, and Pronko S. Oxidation of Ethanol in the Brain and Its Consequences. Alcohol Research & Health J. 2006; Vol. 29, No. 4.
4. Chao YC, Liou SR, Chung YY, et al. Polymorphism of alcohol and aldehyde dehydrogenase genes and alcoholic cirrhosis in Chinese patients. Hepatology. 1994; 19:360–366.
5. Agarwal DP. Genetic polymorphisms of alcohol metabolizing enzymes. Pathologie-Biologie (Paris) 2001; 49:703–709.
6. Bailey SM. A review of the role of reactive oxygen and nitrogen species in alcohol-induced mitochondrial dysfunction. Free Radical Research. 2003;37:585–596.
7. Abdulla AA. Association between Genetic Polymorphism of 5-HTTLPR, and SGOT, SGPT and Catalase with Alcoholism of Iraqi People. Indian Journal of Forensic Medicine & Toxicology. 2020;Vol. 14, No. 2.
8. Eltahir H B. Genetic Polymorphsims in ALcohol and Xenobiotic Metabolizing Enzymes as Risk Factors of Esophageal Cancer in Sudan. 2007. Thesis. University of Khartoum.
9. Sambrook J, Russell D. Molecular cloning: A laboratory Manual 3rd Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York. 2001.
10. SAS. Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA. 2012.
11. Zintzaras E., Stefanidis I., Santos M., Vidal F. Do alcohol-metabolizing enzyme gene polymorphisms increase the risk of alcoholism and alcoholic liver disease? Hepatology. 2006;43: 352-361.
12. Goth L, Rass P, Pay A. Catalase enzyme mutations and their association with diseases. *Mol Diagn* .2004; 8:141–149.
13. Das SK, Nayak P, and Vasudevan, DM. Biochemical markers for alcohol consumption. ind. J. Clin. Biochem. 2003; 18(2), 111-118.
14. Ai Ge P, Dai J, Liang TC, Yang Q, Lin L, Zhang L. The catalase inhibitor aminotriazole alleviates acute alcoholic liver injury. Sheng Li Xue Bao. 2015; 67: 97–102.
15. Fukui Y, Sakata-Haga H. Intrauterine environment-genome interaction and children's development (1): ethanol: a teratogen in developing brain. *Journal of Toxicological Sciences*. 2009; 34(supplement 2):SP273–SP278. doi: 10.2131/jts.34.sp273.
16. Groppi A, Begueret J, Iron A. Improved methods for genotype determination of human alcohol dehydrogenase (ADH) at ADH2 and ADH3 loci by using polymerase chain reaction-directed mutagenesis. Clin Chem. 1990; 36:1765–1768.
17. Tiemersma EW, Wark PA, Marga C, et al. Alcohol consumption, alcohol dehydrogenase 3 polymorphism, and colorectal adenomas. Cancer Epidemiol Biomarkers Preview. 2003; 12:419–25.
18. Okonkwo P., Oluchukwu BE,Raphael JO. Enzymes as markers of liver damage in apparently healthy alcohol drinkers resident in Vom community, International Journal of Biosciences (IJB). 2012; 2

(4), 90-95.

19. Rehm J, Samokhvalov AV, Shield KD.Global burden of alcoholic liver diseases, Journal of Hepatol. 2013; 59, (100)160–168.