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Genetic Polymorphism of Alcohol Dehydrogenase 3 (ADH1C) and Alcohol Dependence in Nepalese Population.

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ABSTRACT

Genetic polymorphism of the enzymes involved in alcohol metabolism is mostly ethnic and race dependent. This study sought to determine whether an association exists between *ADH1C* and alcohol dependence in Nepalese population. Blood was collected from 200 Nepalese respondents, where 100 were alcohol dependent cases and 100 were control. The *ADH3* genotype together with alleles frequencies (*ADH3*1* and *ADH3*2*) were examined by PCR-RFLP methods in blood DNA. The differences in allele or genotype frequencies between cases and controls were examined by Fisher's exact test. Chi-square tests were employed to evaluate the deviations from Hardy–Weinberg equilibrium (HWE) and statistical significance is considered at $p \le 0.05$. We found the distribution of *ADH1C* genotypes was different in alcoholics and controls. Homozygous *ADH1C*1* was significantly higher in alcohol dependence compared to control (P=0.03). However, the genotype frequency of *ADH1C*2* was more in both group than *ADH1C*1*, the heterozygous *ADH1C*1/*2* (Ile/Val) was almost equal frequency in both group. Interestingly, the frequency of fast allele γ_1 (A or *1) was significantly higher in alcohol dependence (0.215) than control (0.13) whereas slow allele γ_2 (G or *2) was marginally lower in alcohol dependence (0.785) than control (0.87) (P=0.024). Our results support for *ADH1C* as a candidate gene that affects vulnerability to alcoholism. *ADH1C* variants (*ADH1C*1*or *ADH1C*350Ile*) were associated with an increase in alcohol dependence.
Keywords: Alcohol dehydrogenase, Alcohol dependence, *ADH1C*, PCR-RFLP

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INTRODUCTION

Nepal is a multi-ethnic, multi-cultural, multi- religious and multi linguistic society with a rich repertoire of customs and traditions. It is also a geographically diverse country and a caste bound society. Alcohol consumption behavior and Nepalese society is connected from long time.[1] Nepal has been a common ground of people from both Indo-Aryan and Tibeto-Mangoloid race. However, alcohol consumption is more prevalent in Tibeto-Mangoloid races because of their traditional practice, the traditional barrier of alcohol usage is commonly existing in alcohol non-using community, known as Tagadhari, they are getting weaker in current Nepal.[2] It has deep rooted religious, cultural and traditional dimensions as well as social implications.[3] Though the trend of use of alcohol has decreased in developed world but it is increasing in developing world including Nepal.[4] The prevalence of alcohol use in Nepal was 75% in urban and 66.7% in rural area. Moreover, per capita alcohol consumption among adults (≥15 years) in Nepal , is 0.2 liters of pure alcohol.[5]

Alcohol dependence is a common disorder that causes physical, psychological, and social problems. The pathogenesis of alcohol dependence is multifactorial and includes shared genetic and environmental factors. Twin, family, and adoption studies have consistently shown that genetic factors play an important role in the development of alcohol dependence. Twin studies have estimated the heritability of alcohol dependence to be about 50–65% regardless to any gender.[6–8] In humans, the conversion of ethanol to the intermediate metabolite acetaldehyde, is catalyzed by the enzyme alcohol dehydrogenase (ADH). This is the rate-limiting step in the elimination of alcohol. Acetaldehyde is then immediately eliminated by mitochondrial aldehyde dehydrogenase (ADH2).[9] Seven SNPs in the *ADH* genes are located as a cluster on chromosome 4q22-23 and are thought to be associated with alcohol dependence, however the size of the allelic effects require clarification within different populations.[10,11] Both the *ADH1B* and *ADH1C* gene polymorphisms have been extensively studied in relation to alcohol dependence. The genetic distribution of *ADH1B* is monomorphic in Indian subcontinent. The presence of polymorphic isoenzymes ADH3 varies in different ethnic groups. *ADH1C*1* occurs in 50–60% of Caucasians and in >90% of Asian population.[12] The distribution of *ADH1C* in our neighboring country India is 50 -90% and 95% in china.[13] However, there is controversial finding regarding effect of ADH1C in alcohol related organ damage.[14–16].

Although alcohol use is ambivalent in Nepalese population, there are no data on genes/polymorphisms that confer susceptibility to AD in this population. This study aimed to assess the nucleotide polymorphisms the *ADH1C* genes in alcohol dependent and non-alcohol dependent (control) subjects of Nepalese population in order to explore their genotypic influence on alcohol dependence.

MATERIALS AND METHODS

Subjects for participants

This was a case control study conducted in the Department of Biochemistry with collaboration of department of Psychiatry and Internal medicine of BPKIHS, Dharan, Nepal. A total of 100 alcohol dependence and 100 healthy controls were enrolled in the study. Subjects visiting Psychiatry OPD and ward diagnosed as alcohol dependence by Psychiatrist using ICD-10 and who meet AUDIT related problem were included as case in this study. The control subjects were recruited from both the community and the hospital. All subjects were interviewed using the same clinical interview tool for alcohol dependence. Subjects who did not have any alcohol related problem (either never drank or occasionally) included as control. The subjects between 25-65 years of age willing to participate in the study and with no history of undergoing long-term medical condition like diabetes, hypertension, cancer, renal failure etc. were recruited in the study. This study was carried out after getting clearance from IERB. Consent was obtained from every subject.

DNA isolation and genotyping of ADH1C

Blood samples were collected from subjects in EDTA vial and stored at -20°C until DNA extraction. Genomic DNA was isolated from whole blood using QIAmp DNA blood mini kit (Qiagen, Germany). The *ADH3* genotype and the frequency of *ADH3* alleles were determined in all the patients. Genotyping of the *ADH3* (*ADH1C*) was performed using PCR–RFLP methods. The primers for amplification were: ADH1C F1 (5'-GCTTTAAGAGTAAATAATCTGTCCCC-3') and ADH1CR1 (5'- CTACCTCTTTCCAGAGC-3'). The PCR total reaction

mixture of 25µL contained 1X PCR buffer with 1.5mM $MgCl_2$, 0.5mM $MgCl_2$, 200µM each dNTP, 0.4 µM of each primers, 0.5 U Hot Start Taq Plus (Qiagen) and 5 µL of template DNA. The PCR reaction was carried out in Mastercycler Eppendorf ProS (Germany) under the following conditions: initial denaturation at 95 °C for 5 min; 35 cycles of denaturation at 94 °C for 1min, primer annealing at 55°C for 45 sec, extension at 72 °C for 1min; followed by final extension at 72 °C for 10 min. The amplified PCR was precipitated with 0.5M sodium acetate and 100% ethanol followed by washing with 70% ethanol. The concentrated amplified product was digested with 1U of enzyme SspI (Promega) per 20 µL of reaction mixture at 37 °C for 4 hour. The digested products were visualized by electrophoresis in 3% agarose gel at 70 V for 180 min, and DNA bands pictures were taken in UV chamber after staining with ethidium bromide. The genotypes identified were named according to the presence or absence of the enzyme restriction sites. Therefore, A/A=*1/*1, A/G=*1/*2, G/G=*2/*2 are homozygotes for the absence of site (146 bp), heterozygotes (63/83/146 bp), and homozygotes for the presence of site (63/83 bp)

Statistical analysis

Data were expressed as mean±SD, median (IQR), frequency and percentage. An independent t-test was used to compare the parametric data and a Mann-Whitney U test for the non-parametric data. Chi-square test (χ^2) was for the categorical data. The Hardy-Weinberg equilibrium was tested compare the observed genotype by χ2 analysis with Yates' continuity correction frequencies to the expected among the control subjects and alcohol dependence subjects. The differences in allele or genotype frequencies between cases and controls were examined by Fisher's exact test. Data were analyzed by using IBM SPSS Statistics version 20. Statistical significance is considered at $p \leq 0.05$.

RESULTS AND DISCUSSION

A total of 100 alcohol dependence and 100 controls were included in this study. The mean differences of age, height, weight, BMI and sex distribution among alcohol dependence and control are depicted in Table 1. The mean age, height and sex distribution were not statistically significant among alcohol dependence cases and controls. Alcohol dependence subjects had significantly lower weight and BMI compared to control ($p<0.001$).

Consistent Hardy–Weinberg equilibrium (HWE) was obtained (using Chi squared test) for the *ADH1C* polymorphism in the healthy controls. However, the observed genotype counts deviated significantly from those expected according to the HWE in the alcohol dependence group for the *ADH1C* polymorphism (p value=0.0001) as depicted in Table 2.

Table 3 shows distribution of *ADH1C* genotypes was different in alcoholics and controls. Faster homozygous *ADH1C**1 was significantly more in alcohol dependence compared to control (P=0.03). Slow metabolizing genotype frequency *ADH1C**2 was more in both group than *ADH1C**1.Ditribution of heterozygous *ADH1C**1/*2 (Ile/Val) was almost equal frequency in both group. Similarly, the frequency of fast allele γ_1 (A or *1) was 0.215 in alcohol dependence and 0.13 in control and slow allele γ_2 (G or *2) was 0.785 in alcohol dependence and 0.87 in control which were statistically significant (P=0.024). The pattern of electrophoretogram of *ADH1C* genotype is shown in figure 1.

Alcohol metabolism occurs mainly in the liver. Alcohol dehydrogenase (ADH; EC 1.1.1.1) and aldehyde dehydrogenase (ALDH; EC 1.2.1.3) have important roles in the elimination of ingested ethanol. The major polymorphisms of alcohol dehydrogenase unit are β and γ. The gamma subunit encoded by *ADH1C* plays a key role in the oxidation catabolism of a wide variety of substrates, including ethanol, retinol, other aliphatic alcohols, hydroxyl steroids, and lipid peroxidation products.[12] *ADH1B* appears to play the greatest role in modulating alcohol dependence risk among the *ADH* loci.[15] The *ADH1C* gene (formerly called *ADH3*), located on chromosome 4q21-q23, is adjacent to *ADH1B* and in the region of a gene cluster of the alcohol dehydrogenase subunits 6, 1A, 1B, 1C, and 7. The common form of a single nucleotide polymorphism (SNP: rs698, Ile350Val in exon 8,formerly known as *ADH1C* *1/*2) at the *ADH1C* gene locus is (γ_1) 350Ile (*1) and other350Val (*2) is γ_2.[16] The isoenzyme $\gamma_1\gamma_1$ (kcat = 87/ min) is moderately more active than the $\gamma_2\gamma_2$ isoenzyme (kcat = 35/ min).[17] They are distinguished using a restriction enzyme digestion with SspI as the restriction enzyme. The genotype distribution *ADH1C* in Indian subcontinent is more than other, so *ADH1C* is

chosen for the study. Polymorphisms in *ADH1C* have been associated with both alcohol dependence[18–22] and alcoholic liver disease.[23,24]

Table 1: Demographic and anthropometric variables among alcohol dependence cases and controls

Parameter		Case (N=100)	Control (N=100)	P value
Age (yrs)		43.22±10.23	44.12±9.43	0.51
Sex	Male (N)	78	76	0.73
	Female (N)	22	24	
Weight (Kg)		58.23±8.19	65.22±10.27	0.001
Height (M)		1.64±0.074	1.62±0.079	0.14
BMI (Kg/m^2)		21.66±2.90	24.69±3.28	0.001

Table2: Ditribution of genotype *ADH1C* with Hardyn-werg equilibrium goodness of fit.

Genotype of ADH1C	Group			
	Case (n=100)		Control (n=100)	
ADH1C*1 *1/*1 (Ile/Ile)	Observed	11	Observed	2
	Expected	4.6	Expected	1.8
ADH1C *1/*2 (Ile/Val)	Observed	21	Observed	22
	Expected	33.8	Expected	22.3
ADH1C*2 *2/*2 (Val/Val)	Observed	68	Observed	76
	Expected	61.6	Expected	67.8
χ 2 and P Value (df=1)	χ 2 = 14.27, P=0.0001		χ 2 = 0.019, P=0.888	

Table3: Distribution of genotype *ADH1C* and its allele in alcohol dependence and control.

Genotype of ADH1C		Group		P value
		Case (n=100)	Control (n=100)	
*ADH1C**1 *1/*1 (Ile/Ile)		11	2	0.033
ADH1C *1/*2(Ile/Val)		21	22	
*ADH1C**2 *2/*2 (Val/Val)		68	76	
Allele (frequency)	*1γ_1	43 (0.215)	26 (0.13)	0.024
	*2 γ_2	157 (0.785)	174 (0.87)	

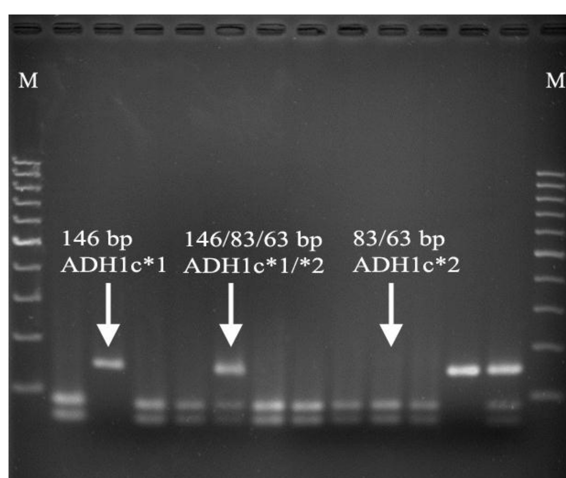

Figure 1: Genotype of ADH1C on Agarose gel electrophoresis.

To our knowledge, this is the first report to investigate *ADH1C* and alcohol dependence among Nepalese population. The distribution of *ADH1C* in control is consistent with Hardyn-Weinberg equilibrium which confirms genotyping is correct. However, the observed genotype counts deviated significantly from those expected according to the HWE in the alcohol dependence group for the *ADH1C* polymorphism (p

value=0.0001). These results strongly suggest that a selection pressure may be involved in the test group for the *ADH1C* polymorphism.

There are more contradictory and ambiguous finding among whites regarding *ADH1C**1. Some studies show no correlation [12,25], protection against alcohol dependence [12] or inconclusive results.[26,27] Some study reported that the *ADH1C**1 allele was more frequent in alcohol-dependent subjects than among non-drinkers.[28–30] The present study also show that *ADH1C**1 allele and *ADH1C**1/*1 genotype were detected significantly more frequently in the alcohol-dependent group than in the control group. Different results were obtained in studies of Asian populations,[31–33] which showed that the *ADH1C**1 allele has protective effects against excessive alcohol consumption. *ADH1C**1 has been found to occur at lower frequencies in alcohol-dependent individuals than in nonalcohol dependent controls in eastern Asian samples.[21,23,34,35] However study in Taiwanese Chinese alcohol dependents showed the lowered frequency of *ADH1C**1 as compared to controls was most likely due to linkage disequilibrium with *ADH1B**2. Therefore, it has been suggested that studies of *ADH* polymorphisms and alcohol-related phenotypes should be studied on a population- by-population basis.[20,36]

Our result show both group had more *ADH1C**2 allele than *ADH1C**1. It has been suggested that the presence of the *ADH1C**2 allele, that codes for a less active enzyme, may increase the risk for alcoholism by delaying the formation of acetaldehyde, perhaps leading to a less intense response to alcohol and/or less alcohol-induced negative side effects, ultimately resulting in higher levels of drinking.[16,24] Both group are bearer of gene for susceptible alcoholism. It seems, however, that whether the genetically predisposed individual becomes an alcoholic or not is determined by interactions between genetic factors and promoting or protecting environmental effects.[37] It would be important to find the genes responsible for the susceptibility to alcohol addiction and to conduct the screening examinations defining whether a particular individual is genetically loaded, which might reduce this phenomenon. The genetically loaded individuals would be able to choose consciously between increased risk of alcohol consumption and abstinence.

CONCLUSION

It may be concluded that the role of *ADH3* polymorphism is different in various races and varies markedly from one population to another *ADH1C* variants (*ADH1C**1or *ADH1C*350Ile*) were associated with an increase in alcohol dependence. Furthermore, coexisting environmental factors are also likely to be involved. Future research on this topic would be benefited from larger samples that would disclose more explicit details on intra/inter-group variations in alcohol consumption, alcohol dependence, and their relationship to *ADH1C*.

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