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A CASE STUDY ON FACTORS INFLUENCING PROTECTIVE HEALTH ECOLOGY FOR ALCOHOLISM AMONG THE HILL BONDA TRIBAL GROUP OF ODISHA

Jayanta Kumar Nayak and P. K. Das

Every society or culture has its own belief, notion, attitude, knowledge, test and perception about drink and non-drink, superior drink and inferior drink etc., which are rooted in the framework of cultural logic of specific society or community. Positive, integrated, non-temperance cultures tend to favour more 'open' drinking environments, while negative, ambivalent, temperance cultures are associated with 'closed', insular designs. The health status of individuals and groups is influenced not only by environmental factors but also by a variety of personal attributes, including genetic heritage, psychological dispositions and behavioural patterns. The studied hill Bonda of Odisha is such a community that they use different types of alcoholic beverages as a food item and as a part of their cultural celebrations. This paper tries to focus on genetic, socio-cultural and ecological analysis of the protective aspect against alcoholism among the hill Bonda tribal group where in a health ecological context drinking alcoholic beverages is common for all from birth to death.

Introduction

Attainment of material and spiritual success of human beings cannot be realized if their basic or biological needs including eating, drinking, excreting etc. are not accomplished. At the ideological level, every society or culture has its own belief, notion, attitude, knowledge, taste and perception about food and non-food, drink and non-drink, sacred food and polluted food, superior drink and inferior drink etc. which are rooted in the framework of cultural logic of specific society or community (Nayak, 2011). An individual if consumed alcoholic beverages daily he may be affected by alcoholism. Continued alcohol use and abuse may lead to addiction. People who become addicted are preoccupied with alcohol to the extent that they place more value in it than in meeting their basic needs. They continue to have little or no control over their drinking in spite of arrests, physical harm, assault, financial problems, relationship difficulty, poor work performance, and other negative consequences. The current definition of alcoholism as defined by the American Society of Addiction Medicine states "Alcoholism is a primary, chronic disease with genetic, psychological and environmental factors influencing its development and manifestations. The disease is often progressive and fatal. It is characterized by continuous or periodic impaired control over drinking, preoccupation with the drug alcohol, use of alcohol despite adverse consequences,

Address for communication: **Jayanta Kumar Nayak,** Asst. Professor, Centre for Anthropological Studies, Central University of Orissa, Koraput, Odisha, *E-mail: jayanta.nayak@rediffmail.com*, **P. K. Das,** Professor and Head, Department of Anthropology and Tribal Development, Guru Ghasidas Vishwavidyalaya, Bilaspur, Chhattisgarh, *E-mail: pkdas123@gmail.com*

and distortions in thinking, most notably denial." As Heath (1982), "A population that drinks daily may have high rate of cirrhosis and other medical problems but few accidents, fights, homicides, or other violent alcohol associated traumas. A population with predominantly binge drinking usually shows the opposite complex of drinking problems. A group that views drinking as a ritually significant act is not likely to develop many alcohol related problems of any sort, whereas another group, which sees it primarily as a way to escape from stress or to demonstrate one's strength, is at high risk of developing problems with drinking." Anthropologists have been known for their expertise in the study of different societies, in which preparation and drinking of alcoholic beverages constitute an important and inalienable component of their socio-cultural traditions and customs. Anthropologists do not consider drinking leads to serious social problem in indigenous tribal communities and alcoholism seems to be virtually absent in many societies where drunkenness is frequent, highly esteemed and actively sought (Heath, 1975). Mary Douglas (1987) clearly stated that, "In general tenor of anthropological perspective, celebration is normal and that in most cultures alcohol is a normal adjunct to celebration. Drinking is essentially a social act, performed in a recognized social context." The drink ideology, drink ways, and drinking habits show variation across societies. Blum and Blum (1964) stated that, "In those cultures where drinking is integrated in to religious rites and social customs, where the place and manner of consumptions are regulated by traditions, and where, moreover, self-control, sociability 'knowing how to hold' ones liquor are matters of mainly pride, alcoholism problems at a minimum, recently introduced."

Human well-being is based on dynamic interplay among diverse environmental and personal factors (Stokols, 1992). The scale of environmental units relevant to individual and collective well-being ranges from multiple situations and settings. Situations are sequences of individual or group activities occurring at a particular time and place (Forgas, 1979; Pervin, 1978). Settings are geographical locations in which various personal or interpersonal situations occur on a regular basis (Barker, 1968; Stokols & Shumaker, 1981). The healthfulness of a situation is influenced by multiple factors of both the physical environment (e.g., geography, architecture, and technology) and the social environment (e.g., culture, economics and politics) (Stokols, 1992). Health ecology evaluates human wellness in regards to their total environment. It also evaluate feelings, thought, attitudes, religious and exercise practices and also diet in examining overall health. From the health ecology view point it is important to understand the person's perception towards the issue. Applications of health ecology tend to include analysis of groups, based on known interactions with the entire environment. This paper tries to focus on genetic, socio-cultural and ecological analysis of the protective aspect against alcoholism among the hill Bonda tribal group where in a health ecological context drinking alcoholic beverages is common for all from birth to death. Most ethanol elimination occurs by alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) systems via oxidation of ethanol to acetaldehyde and acetic acid (Nayak *et al.* 2008). ADH and ALDH genes are responsible for giving protection against alcoholism. Brofenbrenner (1977, 1979)'s ecological model can be implemented for understanding socio-cultural and ecological factors providing protection against alcoholism.

Materials and Methods

The study area is a natural museum of scenic beauty with rolling mountains, scattered streams, meadows, and picturesque valleys, popularly known as *'Bonda Ghati'*, which lie between 180.15' to 180.30' North Latitude and 820.15' to 820.30' East Longitude. The entire area is a hilly and mountainous terrain covering about 130 square kilometres with altitudes approximately ranging from 1500 feet to 3700 feet from the mean sea level. The hill Bonda speaks a dialect of Mundari group, which is affiliated to Austro-Asiatic language family. They are known among themselves as '*Remo*' means 'Man'. In this paper, nine Bondo villages such as Chalanpada, Baunsapada, Sileiguda, Mudulipada, Bandiguda, Padeiguda, Kirsanipada, Dumuripada and Andrahal have been studied. This study is conducted in 714 households of nine villages having total population of 2700 (1240 male and 1460 female) with the help of conventional anthropological techniques such as participation observation method, in-depth interview method, genealogy method, case study and schedule method. For the collection of information on alcohol dependence status of the population, individuals were interviewed with the modified Michigan Alcoholism Screening Test (MAST) questionnaire, which is worldwide most used questionnaire to test the personality with respect to alcoholism. After stressing family history through genealogy, venous blood samples of 110 unrelated adult (18 years and above) alcohol drinkers were collected in 6ml K2EDTA vacutainer for DNA analysis after obtaining the consent as per the Institutional Ethical Review Committee of the Anthropological Survey of India, Kolkata. PCR standardised with MJ Research (PTC-200) gradient cycler PCR system and regular PCR activities done by ABI GeneAmp 9700 thermalcycler. PCR amplicons (70ng) were directly sequenced using the Big-Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). Extended products were purified by alcohol precipitation followed by washing with 70% alcohol. Purified products were then dissolved in 10% of Hi-Di formamide and analysed in ABI 3730 automated DNA analyser. Sequencing results were checked by sequence analysis v5.2. Linkage disequilibrium were estimated by use of SNP analyser web based programme-Haploview (*http://www.broad.mit.edu/mpg/haploview*).

Results and Discussion

According to the Bonda ideology, the choice of alcoholic beverages is contextual. Its meaning varies according to its context of use. No life cycle event is ever meaningful and complete without the use of alcohol. Alcoholic beverages of different kinds not only provide nourishment to the Bonda for their survival but also enliven all kinds of social interactions and cultural celebrations. In true sense of the terms it adds colour and vigour to festivity. The Bonda broadly consume three categories of alcoholic beverages, i.e. (1) alcoholic product collected from the sago palm tree (palm wine) (locally known as *salap, sapung*), (2) alcoholic products prepared from the fermentation of food grains (beer) (locally known as *pendum*), (3) distilled alcoholic products (spirit) (locally known as *sagur*). The Bonda prepare different kind of distilled alcoholic products (*Sagur)*. The terminologies are made according to the objects used for fermentation. The different types of Sagur are such as; *Bawh Sagur* (Mahua Flower), *Unkusuin Sagur* (Jack Fruit), *Titim Sagur* (Tamarind), *Gurh Sagur* (Sugar Cane), *Siager Sagur* (Amala), *Bulura Sagur* (Date Palm Nut), *Unsugdak Sagur* (Banana), *Uli Sagur* (Mango). They consider palm wine is sweet in test and tender on the stomach, but spirits burn the liver. Whereas, fermented gruels (beer) fill their belly and make them lazy. The Bonda also use these alcoholic products separately for providing refreshment in separate occasion. As per MAST score the information on the alcohol dependence status of the Bonda is presented in **Table 1**. From the Table 1, it is observed that 70% of Bonda people are normal with respect to alcohol dependence. Among the Bonda population, 58.1% consume alcoholic beverages once daily, whereas 25.6% consume 2-3 times daily. On the other hand, around 11.6% take alcoholic beverages 1-2 days in a week and 3.1% consume 3-5 days in a week. A small fraction of Bonda (1.6%) consumes alcoholic beverages only 2-3 times in a month. A majority of the Bonda population (76.7%) consume alcoholic beverages which are either homemade or locally made, whereas 23.3% consume alcohol both available locally as well as available in market. The Bonda use the alcoholic products of different kinds in rituals connected to different socio-religious, magico-religious and in other celebrations throughout the year. In Bonda society, alcohol has long been regarded as a social leveller, and the act of communal drinking as a means of communication between those of different ranks and status in society. These integrative qualities, along with its role contribute to the key function of the drinking-place as a facilitator of social bonding. The facilitation of social interaction and social bonding is one of the main functions of drinking itself. The 'drinking-place' appears to be an essential feature of almost all alcohol-using cultures. In

TABLE 1: OBSERVED MAST SCORE AMONG THE BONDA POPULATION

MAST score	*Risk profile*	*Observed percentage*
0 – 3 points	Normal range, low risk	70%
4 – 9 points	High risk for problem in drinking, tendency to addiction alcohol is likely	30%
> 10 points	Alcoholism	0

Bonda society, there is no definite drinking place. The total Bonda-hill is considered as a drinking place. Generally, wherever they get alcoholic product, they prefer to consume instantly. The only taboo place for drinking is on *Sindibor* in every village as the Bonda consider Sindibor as the sacred place for the abode of village deity. The major amount of drinking takes place at home. Inside home, there is a particular place for keeping the containers of alcoholic products. The other drinking places for them are the *donger* or *jhola* land, under the *salap* tree and in the dormitory house. Community drinking are generally performed around the *Sindibor*, but not on it. The quantity and quality of alcoholic beverages consumed denote the higher economic status in Bonda society. *Boun Sagur* (ardent spirit of Mahua flower) is considered as a costly, superior, and prestigious drink. Brofenbrenner's ecological model (1977, 1979) describes four systems such as a) micro-system (face-to-face influences in specific settings), b) meso-system (interactions in which the individual involved), c) exo-system (forces within the larger social system), and d) macro-system (cultural beliefs and values); all these four systems are well implemented to explain the socio-cultural factors influencing alcohol drinking habits among the Bonda society and socio-cultural interactions also responsible for protecting them from alcoholism.

The Bonda mainly depends on either land or forest or upon both land and forest for the collection of the ingredients for alcoholic beverages. Fermenting material is mainly collected from forest. For the preparation of alcoholic beverages, firewood is essential, which they collect from forest. Table 2 depicts the necessary ingredients for different types of alcoholic beverages and from where the Bonda people get these materials. The adaptive dynamics of the human ecological system solely depends on the strategic behaviour of the actors or individuals who make choices or decisions in accordance with their social and cultural needs (Bennett, 1976). From system perspectives, the Bonda socio-cultural system is an integral component of the larger ecological system. The individual members of the community transform the natural order of their physical ecosystem or habitat into moral order or natural ecology to cultural ecology by means of their social means and cultural apparatus. Alcoholic beverages of the Bonda both natural and indigenously prepared signify their dependence on natural resources as well as cultural resources. The ecological prudence of the Bonda is best realized in their social and cultural system. The Bonda habitat is located at a high altitude area of Eastern-Ghat ecosystem and the temperature remains low as compared to plains. The highest temperature reported in summer season was around 30 °C, which is by any standard not high for a tropical situation. In such situation, consumption of alcoholic products provides them the required heat and energy to carry out activities in the hills and hence act as valuable means for the survival of the population. The preparation and consumption of fermented and distilled alcoholic beverages may be viewed as a great example of waste management policy of the Bonda. It is observed that the Bonda without destroying seasonal fruits, utilize a good amount of seasonal fruits in preparing alcohols since no storage facility is available neither at household level nor at community level. Consuming a little amount of alcoholic products provides the satisfaction of 'fullness' of stomach to the Bonda and consequently they do not carve for higher quantity of food. This phenomenon to some extent alleviates food crisis at household level. The adaptive dynamics of the Bonda ecological system solely depends on the strategic behaviour of the Bonda, who make choices or decisions in accordance with their social and cultural needs.

For the understanding of the protecting impact of ADH and ALDH2 gene against alcoholism among the Bonda, 9 SNPs from ADH gene and 9 SNPs from ALDH2 gene are analysed. Out of 9 SNPs of ADH gene, 4 are functional in nature. For the study of the linkage and population structure, other 5 SNPs of the gene are also taken. For the understanding of the population structure, out of 9 SNPs of ADH gene, 3 SNPs are considered having different origin history, such as SNP (rs1229984) is of East Asian variety, SNP (rs2066702) is of Sub-Sahara African variety and SNP (rs698) is Black and Oriental in origin. Out of 9 SNPs of ALDH2 gene, 1 SNP (rs671) is functional in nature. It is observed from the **Table 3** that the SNPs of three ADH genes revealed nine polymorphic sites including three functional alleles i.e., ADH1B*47His [Exon3 (rs1229984)], ADH1B*369Cys [Exon9 (rs2066702)], rs2241894 and ADH1C*349Ile [Exon8 (rs698)]. The analysis of ALDH2 gene revealed nine polymorphic sites including one functional allele i.e., ALDH2*Glu487Lys [Exon12 (rs671)] (**Table 4**). In the genotype-phenotype correlation with respect to MAST score, no significant χ^2 was found in medium MAST score group; however, in low MAST score group the difference was significant. For drawing a correlation between phenotype and genotype for ADH gene, data are presented in **Table 5** and for ALDH2 gene data are presented in **Table 6**. In case of ALDH2, the mutant allele ALDH2*2 is dominant over the normal allele (ALDH2*1); individuals who have this allele has little or no enzyme activity. Homozygous Individuals with normal allele (ALDH2*1/2*1) are not protected from adverse effect of alcoholism. However, subjects with the ALDH2*2 allele, both homozygous (2*2/2*2) and heterozygous, (2*1/2*2) experience more intense reactions to alcohol compared to subjects with only ALDH2*1 (Harada et al. 1981). These individuals are alcohol sensitive and have a markedly reduced risk to develop alcoholism and alcoholic liver diseases. The genotype frequency of ALDH2*1/ALDH2*1 (GG) was 0.5 in case of higher MAST score group. The gene frequency of ALDH2*1 was 0.75 and ALDH2*2 was 0.25 in higher MAST score group, where as in lower MAST score it is 0.9019 and 0.098. The allele frequency of one intronic site of ALDH2 (rs2106696) is 0.6274 with highly significant $\chi^2 = 8.6918$ ($p < 0.01$). It is observed that though in all cases, the distinctions are not marked but for functional SNP (rs671) inference can be drawn (Nayak et al.2009). Considering the medium MAST score (high risk of problem

TABLE 2: DESCRIPTION OF SOURCE OF INGREDIENTS COLLECTED FOR THE PREPARATION OF DIFFERENT TYPES OF ALCOHOLIC BEVERAGES

Name of Alcoholic Beverages	*Main ingredients*	*Source of collection of ingredients*	*Fermenting materials*	*Source of collection of fermenting materials*
Salap	Sap of sago palm tree	Land and forest	-	-
Sarta	Sap of sago palm tree	Land and forest	Bark of Bassia latifolia and Diospyros melanoxylon	Forest
Sapung	Sap of sago palm tree	Land and forest	Bark of Bassia latifolia and Diospyros melanoxylon	Forest
Li	Rice, millets	Land	Bark of Bassia latifolia, Diospyros melanoxylon, Terminalia tomantosa, and root of Phoenix sylvestris, Baliospermum axillare	Forest
Pendam	Rice, millets	Land	Bark of Bassia latifolia, Diospyros melanoxylon, Terminalia tomantosa, and root of Phoenix sylvestris, Baliospermum axillare and leaves of Momordica charantia	Forest
Landa	Rice, millets	Land	Bark of Bassia latifolia, Diospyros melanoxylon, Terminalia tomantosa, and root of Phoenix sylvestris, Baliospermum axillare and leaves of Momordica charantia	Forest
Bawh Sagur	Mahua flower	Forest	Bark of Bassia latifolia and Diospyros melanoxylon	Forest
Unkusuin sagur	Jack fruit	Land and forest	Bark of Bassia latifolia and Diospyros melanoxylon	Forest
Titim sagur	Tamarind	Land and forest	Bark of Bassia latifolia and Diospyros melanoxylon	Forest
Gurh sagur	Sugar cane	Land	Bark of Bassia latifolia and Diospyros melanoxylon	Forest
Siager Sagur	Amla	Forest	Bark of Bassia latifolia and Diospyros melanoxylon	Forest
Buura sagur	Date palm nut	Forest	Bark of Bassia latifolia and Diospyros melanoxylon	Forest
Unsugdak sagur	Banana	Land	Bark of Bassia latifolia and Diospyros melanoxylon	Forest
Uli sagur	Mango	Land and forest	Bark of Bassia latifolia and Diospyros melanoxylon	Forest

TABLE 3: SNP LOCATION, RELATIVE POSITION IN GENE, POLYMORPHIC SITE, ANCESTRAL ALLELE, AND REFERENCE ID FOR ALL SNPS AMONG THE BONDA

SNP location	*Relative position in gene*	*Polymorphic site*	*Ancestral allele*	*db SNP ID*	*Marker*
ADH7					
Intron 6	15905	A/G AGGCTT	A	rs17529377	1
Intron 6	15969	C/G CATGGT	C	rs1154458	2
ADH1C					
Exon 5	7771	A/G GTGGTG	A	rs2241894 (functional)	3
Exon 8	Ile349Val	A/G TTTTAC	A	rs 698 (functional)	4
ADH1B					
Exon 3	Arg47His	A/G CACAGA	A	rs1229984 (functional)	5
Intron 3	3447	T/G GCTTGC	T	rs4147536	6
Intron 3	3561	A/G TTATGTA	A	rs2075633	7
Intron 3	4146	C/T ACCAAC	C	rs2066701	8
Exon 9	Arg369Cys	C/T GTACC	C	rs2066702 (functional)	9

TABLE 4: SNP LOCATION, RELATIVE POSITION IN GENE, POLYMORPHIC SITE, ANCESTRAL ALLELE AND REFERENCE ID FOR ALL SNPS AMONG THE BONDA

SNP location	*Relative position in gene*	*Polymorphic site*	*Ancestral allele*	*db SNP ID*
ALDH2				
Intron 6	2798223	T/C GGGTGGC	T	rs440
Intron 6	2798243	T/C CCCAACTA	T	rs35781311
Intron 6	2798304	G/C TGAGCTGT	G	rs11613351
Intron 6	2798358	T/C CTCAAAAA	T	rs441
Intron 6	2798429	A/G ACCCCAGC	A	rs35511855
Intron 11	2810911	G/A TGGTTGCA	G	rs2158028
Intron 11	2811061	T/A TTTTTTTTA	T	rs2106696
Exon 12	2811232	G/A AGTGGCC	G	rs1064933
Exon 12	2811275 (Glu487Lys)	G/A AAGTGAAA	G	rs671 (functional)

TABLE 5: GENOTYPE-PHENOTYPE CORRELATION IN ADH GENE AMONG THE BONDA

dbSNP ID	*Genotype*	*Genotype frequency in MAST medium score*	*Genotype frequency in MAST low score*
ADH1B			
rs2066702*	CC	1.0	1.0
	CT	0	0
	TT	0	0
rs2066701	CC	0.697	0.52
	CT	0.273	0.42
	TT	0.03	0.06
rs2075633	AA	0.643	0.53
	AG	0.321	0.41
	GG	0.036	0.06
rs4147536	TT	0.241	0.2
	TG	0.552	0.54
	GG	0.207	0.26
rs1229984*	AA	0	0
	AG	0	0.15
	GG	1.0	0.85
ADH1C			
rs698	AA	0.455	0.39
	AG	0.485	0.48
	GG	0.06	0.13
rs2241894	AA	0.419	0.22
	AG	0.516	0.70
	GG	0.065	0.08**
ADH7			
rs1154458	CC	0.233	0.18
	CG	0.5	0.54
	GG	0.267	0.28
rs17529377	AA	0.844	0.76
	AG	0.156	0.24
	GG	0	0

*functional site, **$\chi^2 = 13.7812$, $p<0.001$

TABLE 6: GENOTYPE-PHENOTYPE CORRELATION IN ALDH2 GENE AMONG THE BONDA

dbSNP ID	*Genotype*	*Genotype frequency in MAST medium score*	*Genotype frequency in MAST low score*
ALDH2			
rs671*	GG	0.786	0.8
	GA	0.214	0.2
	AA	0	0
rs1064933	GG	0.842	0.95
	GA	0.158	0.05
	AA	0	0
rs2106696	TT	0.682	0.49
	TA	0.318	0.27
	AA	0	0.24**
rs2158028	GG	0.632	0.47
	GA	0.368	0.47
	AA	0	0.06
rs35511855	AA	1.0	1.0
	AG	0	0
	GG	0	0
rs441	TT	0.576	0.50
	TC	0.364	0.37
	CC	0.06	0.13
rs11613351	GG	0.719	0.47
	GC	0.281	0.53
	CC	0	0
rs35781311	TT	0.727	0.65
	TC	0.273	0.35
	CC	0	0
rs440	TT	0.594	0.51
	TC	0.344	0.37
	CC	0.062	0.12

*functional site, **$\chi^2 = 8.6918$, $p<0.01$

drinking and likely to be addict group) as case group and low MAST score (no risk group) as control group, the odds ratio of 7 ADH SNPs are calculated and mentioned in the **Table 7**. As per result shown in the Table 7, odds ratio is greater than 1 for all SNPs, whereas the lower bound of the confidence interval is below 1 for all SNPs. It is indicated that for both case and control group, the risk of alcoholism is not significant. Considering the medium MAST score (high risk of problem drinking and likely to be addict group) as case group and low MAST score (no risk group) as control group, the odds ratio of 8 ALDH2 SNPs are calculated and mentioned in the **Table 8**. As per result shown in the table-8, the odds ratio of rs2106696 shows statistically significant p-value (0.011). The odds ratio of rs671 and rs1064933 is less than 1, indicates no risk for medium MAST score group. Except these three SNPs, the other 5 SNPs though shown odds ratio above 1, but the lower bound of the 95% CI is below 1, indicates that the risk of alcoholism is not significant. For disease gene mapping and for understanding human population history, Haplotype and Linkage Disequilibrium (LD) analyses have been widely applied (Osier *et al.*, 1999 & 2002). By pair-wise linkage disequilibrium analysis of the eight SNPs of ADH genes, it is observed that genes displays significant linkage disequilibrium (LD),' However, when LD was assessed by more stringent measure of r^2, which accounts for differences in allele frequencies, these appeared more fragmented. Pair wise LD (r^2) values for ADH are mentioned in **Fig. 1** and Pair wise LD (r^2) values for ALDH2 are mentioned in **Fig. 2**. LD analysis for SNPs of ADH gene among the Bonda is shown in **Table 9** and LD analysis for SNPs of ALDH2 gene among the Bonda is shown in **Table 10.** From the LOD score and lower bound of 95% CI value shown in the Table 9, it is observed that LD of ADH gene's marker 3 with marker 4, 7, and 8; marker 4 with marker 7 and 8; marker 6 with marker 7 and 8; and marker 7 with marker 8 shows statistically significant values. From the

TABLE 7: ODDS RATIO IN STUDIED SNPS OF ADH GENE

SNP	*ODDS RATIO*	*95% CI*	*p VALUE*
rs2066701	1.875	0.905 to 3.876	0.121
rs2075633	1.48	0.694 to 3.147	0.356
rs4147536	1.212	0.655 to 2.244	0.636
rs698	1.314	0.7 to 2.464	0.431
rs2241894	1.6	0.861 to 2.971	0.165
rs1154458	1.154	0.62 to 2.156	0.749
rs17529377	1.595	0.571 to 4.425	0.461

TABLE 8: ODDS RATIO IN STUDIED SNPS OF ALDH2 GENE AMONG THE BONDA

SNP	*ODDS RATIO*	*95% CI*	*p VALUE*
rs671	0.906	0.247 to 3.27	1.0
rs1064933	0.111	0.016 to 0.81	0.056
rs2106696	3.139	1.296 to 7.563	0.011
rs2158028	1.823	0.708 to 4.671	0.256
rs441	1.448	0.737 to 2.843	0.256
rs11613351	2.222	0.999 to 4.928	0.063
rs35781311	1.343	0.585 to 3.076	0.539
rs440	1.434	0.718 to 2.861	0.388

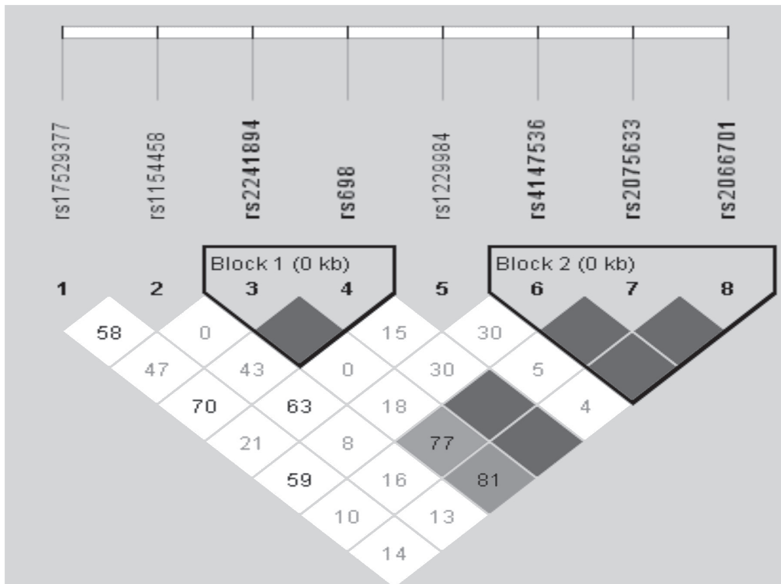

Figure 1: Pair-wise linkage disequilibrium between the SNP markers for ADH gene among the Bonda. Values in cells are r^2 (multiplied by 100)

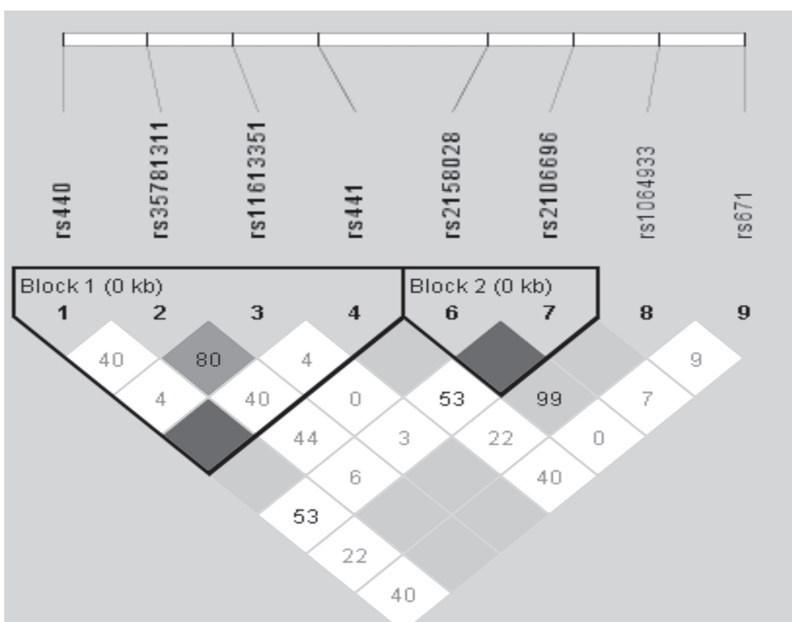

Figure 2: Pair-wise linkage disequilibrium between the SNP markers in ALDH2 gene among the Bonda. Values in cells are r^2 (multiplied by 100)

TABLE 9: LD ANALYSIS FOR SNPS OF ADH GENE AMONG THE BONDA

L1	*L2*	*D'*	*LOD*	*r^2*	*CIlow*	*CIhi*
rs17529377	rs1154458	0.582	0.7	0.04	0.1	0.85
rs17529377	rs2241894	0.47	0.33	0.042	0.05	0.78
rs17529377	rs698	0.7	0.77	0.03	0.12	0.91
rs17529377	rs1229984	0.212	0.37	0.026	0.02	0.5
rs17529377	rs4147536	0.592	0.29	0.037	0.06	0.89
rs17529377	rs2075633	0.106	0.07	0.005	0	0.44
rs17529377	rs2066701	0.148	0.18	0.01	0.01	0.43
rs1154458	rs2241894	0.001	0	0	0	0.32
rs1154458	rs698	0.431	1.44	0.096	0.15	0.63
rs1154458	rs1229984	0.63	0.48	0.029	0.08	0.9
rs1154458	rs4147536	0.089	0.1	0.007	0	0.31
rs1154458	rs2075633	0.161	0.12	0.009	0.01	0.49
rs1154458	rs2066701	0.133	0.07	0.005	0.01	0.49
rs2241894	rs698	1	8.62	0.326	0.83	1
rs2241894	rs1229984	0	0	0	0.02	0.81
rs2241894	rs4147536	0.186	0.3	0.024	0.02	0.41
rs2241894	rs2075633	0.777	4.87	0.26	0.53	0.9
rs2241894	rs2066701	0.816	6.83	0.297	0.61	0.92
rs698	rs1229984	0.158	0.02	0.001	0.02	0.74
rs698	rs4147536	0.301	0.63	0.042	0.05	0.54
rs698	rs2075633	1	3.56	0.135	0.59	1
rs698	rs2066701	1	4.65	0.145	0.67	1
rs1229984	rs4147536	0.302	0.11	0.005	0.03	0.77
rs1229984	rs2075633	0.05	0.01	0	0	0.45
rs1229984	rs2066701	0.045	0.01	0	0	0.45
rs4147536	rs2075633	1	8.22	0.292	0.81	1
rs4147536	rs2066701	1	9.36	0.322	0.83	1
rs2075633	rs2066701	1	30.32	0.944	0.93	1

Details for each column of the table are explained below:

- L1 and L2 are the two loci in question, referenced by their number or name (if marker info file is provided)
- D' is the value of D prime between the two loci.
- LOD is the log of the likelihood odds ratio, a measure of confidence in the value of D'
- r^2 is the correlation coefficient between the two loci
- CIlow is 95% confidence lower bound on D'
- CIhi is the 95% confidence upper bound on D'

TABLE 10: LD ANALYSIS FOR SNPS OF ALDH2 GENE AMONG THE BONDA

L1	*L2*	*D'*	*LOD*	*r^2*	*CIlow*	*CIhi*
rs440	rs35781311	0.404	0.35	0.012	0.05	0.75
rs440	rs11613351	0.044	0	0	0.01	0.64
rs440	rs441	1	36.95	1	0.95	1
rs440	rs2158028	1	1.16	0.084	0.19	0.99
rs440	rs2106696	0.533	1.13	0.046	0.15	0.78
rs440	rs1064933	0.226	0.05	0.006	0.02	0.75
rs440	rs671	0.4	0.12	0.008	0.03	0.86
rs35781311	rs11613351	0.8	7.81	0.43	0.61	0.91
rs35781311	rs441	0.404	0.35	0.012	0.05	0.75
rs35781311	rs2158028	0.441	0.86	0.127	0.1	0.69
rs35781311	rs2106696	0.066	0.03	0.002	0	0.41
rs35781311	rs1064933	1	0.45	0.009	0.07	0.98
rs35781311	rs671	1	0.47	0.019	0.08	0.98
rs11613351	rs441	0.044	0	0	0.01	0.64
rs11613351	rs2158028	0.008	0	0	0	0.43
rs11613351	rs2106696	0.03	0.01	0.001	0	0.32
rs11613351	rs1064933	1	0.71	0.016	0.11	0.99
rs11613351	rs671	1	0.32	0.039	0.06	0.98
rs441	rs2158028	1	1.16	0.084	0.19	0.99
rs441	rs2106696	0.533	1.13	0.046	0.15	0.78
rs441	rs1064933	0.226	0.05	0.006	0.02	0.75
rs441	rs671	0.4	0.12	0.008	0.03	0.86
rs2158028	rs2106696	1	12.86	0.937	0.87	1
rs2158028	rs1064933	0.995	0.11	0.027	0.04	0.97
rs2158028	rs671	0	0	0	0.03	0.96
rs2106696	rs1064933	1	0.4	0.027	0.07	0.98
rs2106696	rs671	0.077	0.01	0.001	0.01	0.59
rs1064933	rs671	0.098	0	0	0.03	0.96

TABLE 11: DISTRIBUTION OF ALDH2*2 (RS 671) PROTECTIVE ALLELE AMONG DIFFERENT CLAN GROUPS AMONG THE BONDA POPULATION

Clan Group	*Male*	*Female*	*Total*
Sisa	10 (45.5%)	1 (4.5%)	11 (50.0%)
Kirsani	1 (4.5%)	6 (27.4%)	7 (31.9%)
Batri	2 (9.1%)	1 (4.5%)	3 (13.6%)
Challan		1 (4.5%)	1 (4.5%)
Total	13 (59.1%)	9 (40.9%)	22 (100%)

LOD score of the Table-10, it is observed that ALDH2 gene's marker 1 with marker 2 and 4; marker 2 with marker 3, 4, 6, 8, and 9; marker 3 with marker 8 and 9; and marker 6 with marker 7 forms strong LD. Considering both LOD score and lower bound of 95% CI index, it is observed that marker 1 with marker 4; marker 2 with marker 3 and marker 6 with marker 7 forms very strong LD. As per Paterson and Petronis (1999), the excess number of statistically significant LD values of SNPs of ADH and ALDH2 gene indicates that migrants attribute these SNPs to the studied population recently from other source population.

The Bonda in heterozygous state for ALDH2*2 are found protected against alcoholism, who have the distinction of perpetual alcohol drinkers. Two explanations may be offered to explain possible occurrence of the gene. First, like ADH gene variant, the ALDH2*2 gene variant might have come into the Bonda population as result of independent mutation, in the ancestral population in the remote part to serve as an inhibitory factor to alcoholic dependence. Secondly, the gene might have entered into the Bonda population as an offshoot of the ancestral mongoloid population of East-Asian origin during Neolithic period. Morphologically some of the Bondo are found with presence epicanthic eye folds. When the distribution of the allele was made clan wise (**Table 11**), it is observed that the allele is comparatively greater among the individuals belonging to *Sisa* clan. From the Table-11, it is seen that 50% of individuals with ALDH2*2 allele are from *Sisa* clan. Even the *Kirsani* clan members possessing the allele have inherited it from their parents (mothers) who belonged to *Sisa* clan prior to marriage. On enquiry, it was ascertained from the respondents of *Sisa* clan that they had gone to Manipur and Northeast provinces to work as tea garden labour and brought women from Northeast area as wives. Therefore, the presence of the allele ALDH2*2 in the Bonda population may be attributed to people of the East-Asian region come during Neolithic phase or to marriage of women belonging to North-East region.

When the distribution of both ADH and ALDH2 alleles in the Bonda population are taken together, one observes that protection of Bonda individuals from adverse effect of alcoholism has been naturally enforced on the population due to dual action of functional variants of ADH and ALDH2*2 alleles.

Conclusion

In most societies, drinking is essentially a social act and as such, it is embedded in a context of values, attitudes, and other norms. These values, attitudes, and other norms constitute important socio-cultural factors that influence the effects of drinking, regardless of how important biochemical, physiological, and pharmacokinetic factors may be in that respect. The drinking of alcoholic beverages tends to be hedged about with rules concerning who may and may not drink how much of what, in what context, in the company of whom, and so forth. Often such rules are the focus of exceptionally strong emotions and sanctions. The Bonda population do not reveal any sign of clinico-pathological disorders of alcoholism despite the fact that they (adult Bonda) consume different varieties of alcoholic beverages throughout the year profusely. Not a single case of alcoholism was reported. The habit of consumption of alcoholic beverages in different seasons among the Bonda signifies a remarkable means of cultural adaptation of the people to seasonal food scarcity and nutritional insecurity. The contribution of ecological niche to the processes of alcohol preparation and consumption of the Bonda has been substantial, and the Bonda portray a high degree of indigenous skill, ingenuity, institutional arrangements and technical out fit in transforming the natural resources to cultural resources and alcohol is one such cultural resource. The genetic heritage of the Bonda population such as the important functional variants of gene family ADH (**ADH1B*47His, ADH1C*349Ile**) and ALDH2 (**ALDH2 Glu487Lys**), rendering protection from alcoholism.

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