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ORIGINAL ARTICLE

Polymorphism in *ADH* and *MTHFR* genes in oral squamous cell carcinoma of Indians

PR Solomon, GS Selvam, G Shanmugam

Department of Biochemistry, Centre for Advanced Studies in Functional Genomics, School of Biological Sciences, Madurai Kamaraj University, Madurai, India

OBJECTIVES: Alcohol consumption is known to increase the risk for several cellular disorders like oral cancer. The risk may be reinforced by polymorphism in genes like alcohol dehydrogenase. Therefore, this study is designed to asses the polymorphic status in *ADH1B* (formerly *ADH2*), *ADH1C* (formerly *ADH3*) and *MTHFR* genes in order to correlate the susceptibility to oral squamous cell carcinoma (OSCC).

SUBJECTS AND METHODS: DNA from 126 OSCC samples were amplified using primers for *ADH1B*, *ADH1C* and *MTHFR* genes. The amplicons were analyzed for *ADH1B*1*, *ADH1C*2* and *MTHFR C677T* allelic polymorphism by restriction digestion using appropriate enzymes.

RESULTS: *ADH1B*1/*1* genotype in cancer patients who were heavy drinkers showed a negligible risk association with an odds ratio of 1.62; 95% CI = 1.08–2.14. In OSCC patients, *ADH1C*2/*2* genotypes showed a relatively higher risk (odds ratio 2.65; 95% CI = 1.78–3.53) in heavy drinkers and a less significant risk (1.6; 95% CI = 1.15–2.03) in moderate drinkers and negligible risk in light drinkers (1.23; 95% CI = 0.77–1.63). In contrast, *MTHFR 677TT* genotype showed a high risk association for OSCC in heavy drinkers (odds ratio 3.0; 95% CI = 2.02–4.0). Interestingly, the combination of *ADH1B*1/*1/ MTHFR 677TT* genotypes in alcoholic cancer patients showed a high risk (odds ratio 4.16; 95% CI = 2.78–5.53). A similar risk (odds ratio 4.16; 95% CI = 1.18–5.53) was shown by *ADH1B*1/*2/*2/*2MTHFR 677TT* genotype combination. The *ADH1C*2/*2 /MTHFR 677TT* genotype combination showed the maximum risk (odds ratio 20; 95% CI = 13.45–26.64) in the heavy drinker group. This combination showed a high risk in moderate drinkers (odds ratio 5.88; 95% CI = 4.24–7.50) and relatively lower risk in light drinkers (odds ratio 2.77; 95% CI = 1.74–3.68).

CONCLUSIONS: The *ADH1C*2/*2/MTHFR 677TT* genotype combination appears to be more susceptible for OSCC, since it showed a 20-fold increase in risk in heavy drinkers and a 5.9- and 2.8-fold increase in risk respectively in moderate drinkers and light drinkers. This study suggests the association of *ADH1C*2/*2/MTHFR 677TT* genotype combination as a risk factor for OSCC in alcoholics.

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Keywords: *ADH*; *MTHFR*; OSCC; polymorphism; odds ratio

Introduction

Although there are various types of genetic polymorphisms, the predominant are the base substitutions called single nucleotide polymorphism (Syvanen, 2001). Any population contains ~85 percent of the worldwide genetic variation, but none contains all of them (Barbujani *et al*, 1997). Oral cancer ranks number one among all cancers in male patients and number three among cancers in female patients in India (Fenley *et al*, 2001; Sinha *et al*, 2003). Several polymorphisms were reported to be associated with increased susceptibility to OSCC (Harty *et al*, 1997; Peters *et al*, 2005; Vairaktaris *et al*, 2006).

The enzyme alcohol dehydrogenase is a cytosolic, zinc-containing dimeric enzyme responsible for the oxidation of long-chain alcohols and omega-hydroxy fatty acids. There are at least seven *ADH* genes encoding enzymes that catalyze the conversion of alcohols to aldehydes (Osier *et al*, 2002). *ADH1B* and *ADH1C* are closely related; their cDNA sequences are 94 percent identical (Ikuta *et al*, 1986). Polymorphism in *ADH1B* and *ADH1C* gives rise to three β sub units and two γ subunits respectively (Duester *et al*, 1986; Hoog *et al*, 1987; Burnell *et al*, 1989). The second allele of *ADH1B,* codes for β_2 subunit of alcohol dehydrogenase. The β_2 subunit differs from both β_1 and β_3 by the presence of histidine instead of arginine at position 47. This single amino acid substitution results from the substitution of

Correspondence: G Shanmugam, School of Biological Sciences, Madurai Kamaraj University, Madurai-625 021, India. Tel: 91(452)859126, Fax: 91(452) 858228, E-mail: shan1938@yahoo.com
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a guanine in *ADH1B*1* and *ADH1B*3* by an adenine (in *ADH1B*2*) in the coding sequence of the third exon (Groppi *et al*, 1990). *ADH1B*1/*1* genotype was shown to enhance the risk of esophageal cancer in Asian populations (Yokoyama *et al*, 2002).

The *ADH1C* locus, codes for gamma-1 and gamma-2 subunit of alcohol dehyrogenase enzyme. There are two alleles at the *ADH1C* locus. They are *ADH1C*1* and *ADH1C*2* coding for $\gamma 1$ and $\gamma 2$ subunits (Hoog *et al*, 1986). The $\gamma 1$subunit differs from $\gamma 2$ subunit by two amino acid substitutions namely arginine to glutamine at position 271 (Hoog *et al*, 1986) and isoleucine to valine at position 349 (Xu *et al*, 1988). *ADH1C* plays a role in detoxification, suggesting that the above defect in this enzyme may render some individuals more susceptible to environmental toxins (Buervenich *et al*, 2005). *ADH1B*1/*1* genotype was found to be associated with oral and pharyngeal squamous cell carcinoma in Japanese men (Asakage *et al*, 2007). Individuals with *ADH1C*2/*2* genotype had an increased risk for head and neck squamous cell carcinomas (HNSCCs) than those with *ADH1C*1/*2* and *ADH1C*1/*1* genotype (Schwartz *et al*, 2001). No significant association of oral cancer with *ADH1C*1/*1* genotype was observed (Bouchardy *et al*, 2000). *ADH1C*2/*2* genotype was found associated with smoking- and drinking-related HNSCCs (Peters *et al*, 2005). The frequency of *ADH1B*1* and *ADH1C*2* alleles were found to be significantly higher in Korean men with alcohol dependence (Chai *et al*, 2005).

The enzyme 5, 10 methylenetetrahydrofolate reductase (MTHFR) plays a major role in one carbon metabolism by catalyzing the irreversible conversion of 5, 10 MTHF to 5 MTHF, the primary circulating form of folate and the carbon donor for the remethylation of homocysteine to methionine in DNA methylation. The same substrate 5, 10 MTHF is also required for thymidylate and purine synthesis. MTHFR activity and availability of folate may affect both gene expression through DNA methylation and genome integrity through DNA synthesis and repair. Hence folate intake can reduce the risk of cancer (Zhang *et al*, 1999). Deficiencies in thymidylate have been shown to increase the rate of misincorporation of uridylate into DNA and may in turn lead to an increased rate of DNA strand breaks and other chromosomal damage (Blount *et al*, 1997). The gene *MTHFR* shows a C $\rightarrow$ T substitution at nucleotide 677 resulting in the conversion of alanine to valine at codon 225. *MTHFR C677T* allelotype has a C $\rightarrow$ T substitution at 677 nucleotide position resulting in the creation of a *Hinf 1* restriction site. The contribution of *C677T* mutation in oral cancer has been investigated in a few instances (Hiyama *et al*, 2007). *MTHFR C677T* polymorphism had been found to be associated with HNSCCs (Neumann *et al*, 2005; Suzuki *et al*, 2007). This mutation was found at an increased frequency in Chinese esophageal and upper aero-digestive tract cancers (Song *et al*, 2001). In Puerto Rican populations, oral cancer showed no association with *MTHFR C677T* polymorphism (Weinstein *et al*, 2002). A minor increase of risk for oral cancer was found to be associated with *C677T* genotypes (Vairaktaris *et al*, 2006).

MTHFR may have a role in relapse and progression of chronic myelogenous leukemia, colorectal cancer, breast cancer and cervical cancer (Eichholzer *et al*, 2001; Bailey, 2003; Jeng *et al*, 2003; Stolzenberg-Solomon *et al*, 2003; Krajinovic *et al*, 2004). *ADH* and *MTHFR* gene polymorphism studies were not carried out in Indian oral squamous cell carcinoma (OSCC) patients and hence this study is important.

Subjects and methods

Sample collection and DNA isolation

Samples of OSCC were collected from the patients of Govt. Rajaji Headquarters hospital, Madurai, Tamil Nadu, India. A total of 126 OSCC samples (33 from heavy drinkers, 56 from moderate drinkers and 37 from light drinkers) were collected. Blood samples (100) from persons with no history of alcohol consumption were used for control. The institutional ethical guidelines were followed during sample collection and informed consent was obtained from the patients prior to sample collection.

The classification of the OSCC patients based upon the amount of alcohol consumption is as follows: heavy drinkers consumed >18 units/week (1 unit = 22 g of ethanol), while moderate drinkers took 9–17.9 units/week. The term light drinkers refers to passive drinkers who consumed drinks and medicine containing small quantities (<9 units) of ethanol. The average ages of the donors of the tissue samples for OSCC were 54.5 ± 8.6; 57.9 ± 6.2; 49.9 ± 9; and 55.4 ± 10 for heavy drinkers, moderate drinkers, light drinkers and control persons. All the OSCC samples were from carcinoma patients who had not undergone chemotherapy. The oral cancer samples were collected in ice cold phosphate buffered saline (PBS). The cancer tissues were processed for DNA isolation using Proteinase K method and the blood DNA was isolated using proteinase-K/phenol-chloroform-isoamylalcohol method (Sambrook *et al*, 1989).

Genotype at ADH1B *gene loci: identification of* ADH1B*1 *allele*

The $\beta 2$ differs from $\beta 1$ subunit by the substitution of guanine in *ADH1B*1* by an adenine in *ADH1B*2* allele in the codon 47 of the third exon. This creates a *Mae III* restriction site. The digestion of 63 bp amplicon from the third exon will result in the appearance of two bands (35 and 28 bp) if *ADH1B*2* allele is present. Homozygous polymorphism for this allele will result in two bands, 35 and 28 bp whereas heterozygous polymorphism will result in three bands 63, 35 and 28 bp (Groppi *et al*, 1990). Absence of *Mae III* digestion shows the presence of *ADH1B*1* allele (Figure 1).

Genotype at ADH1C *gene loci: identification of* ADH1C*2 *allele*

*ADH1C*2* allele differs from *ADH1C*1* allele by the substitution of a guanine by an adenine in codon 349

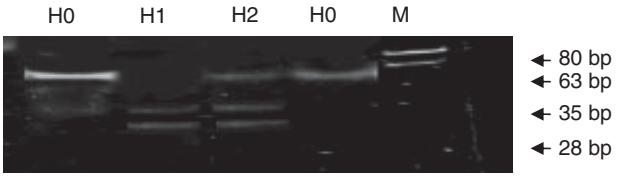

Figure 1 Amplification of exon III of *ADH1B* gene. Specific regions of the designated genes were amplified by PCR using appropriate primers. The amplicons were digested with *Mae III* and electrophoresed in 9% polyacrylamide gels. The resolved DNA fragments were visualized by ethidium bromide staining. H0 – *ADH1B*1/*1*; H1 – *ADH1B*2/*2*; H2 – *ADH1B*1/*2;* M – Marker

thereby creating an *Ssp I* site. The forward degenerate primer of the 145 bp amplicon of exon 8 was made by enclosing an additional *Ssp I* site for use as an internal control. Homozygous polymorphism for this allele would result in products of 67, 63 and 15 bp whereas heterozygous polymorphism would show products of 130, 67, 63 and 15 bp (Groppi *et al*, 1990). Positive restriction digestion indicates the presence of *ADH1C*2* allele (Figure 2).

Genotype at MTHFR *gene loci: identification of* C677T *allele*

The *MTHFR C677T* allelic variant showed a single base pair transition of a C to T resulting in the creation of a *Hinf 1* restriction site. Absence of the *C677T* variant resulted in undigested 201 bp. The homozygous presence of *C677T* polymorphic variant resulted in products of 178 and 23 bp whereas heterozygous polymorphic variant resulted in products of 201, 178 and 23 bp fragments (Frosst *et al*, 1995). Positive restriction digestion indicates the presence of *MTHFR C677T* allele (Figure 3).

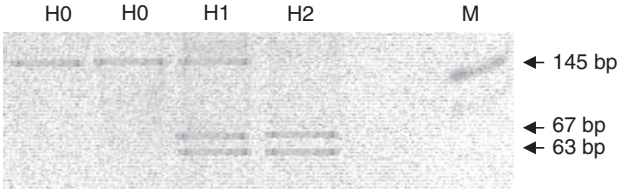

Figure 2 Amplification of exon VIII of *ADH1C* gene. Specific regions of the designated genes were amplified by PCR using appropriate primers. The amplicons were digested with *Ssp I* and electrophoresed in 9% polyacrylamide gels. The resolved DNA fragments were visualized by ethidium bromide staining. H0 – *ADH1C*1/*1*; H1 – *ADH1C*1/*2*; H2 – *ADH1C*2/*2*; M – Marker

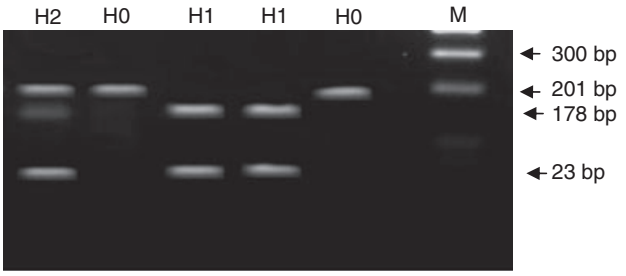

Figure 3 Amplification of *MTHFR* gene. Specific regions of the designated genes were amplified by PCR using appropriate primers. The amplicons were digested with *Hinf I* and electrophoresed in 2.5% agarose gel. The resolved DNA fragments were visualized by ethidium bromide staining H2 – *677CT*; H0 – *677CC*; H1 – *677TT*; M – Marker

Statistical analysis

Odds ratio was calculated using logistical regression. Age and sex adjusted 95% CI (confidence interval) was also calculated for all cases of OSCC. All statistical analysis were done using SAS statistical package (Version 6.12, SAS Institute Inc., Cary, NC 27513–2414, USA). The Hardy-Weinberg formula is used in this study to determine allelic frequency.

Results

In all, 126 genetically unrelated oral cancer samples (including 33 from heavy drinkers, 56 from moderate drinkers and 37 light drinkers were analyzed for the presence of *ADH1B, ADH1C* and *MTHFR* gene polymorphism. Heavy drinking OSCC patients showed little risk association with an odds ratio of 1.6; 95% CI = 1.08–2.14 for *ADH1B*1/*1* genotype whereas moderately drinking patients showed an odds ratio of 1.5; 95% CI = 1.17–1.83. In light drinkers, *ADH1B*1/*1* genotype (odds ratio of 1; 95% CI = 0.63–1.33) showed no significant risk for OSCC. The frequency of *ADH1B*1* allele is 0.27 in control population, 0.29 in light drinkers and 0.33 in both heavy and moderate drinkers (Table 1).

*ADH1C*2/*2* genotype showed an increased risk (odds ratio 2.65; 95% CI = 1.78–3.53) in heavy drinking cancer patients and a less significant risk (odds ratio 1.6; 95% CI = 1.15–2.03) in moderate drinkers group. The light drinking OSCC patients showed negligible association with *ADH1C*2/*2* genotype (1.23; 95% CI = 0.77–1.63). *ADH1C*2* allele frequency was 0.17 in control groups followed by 0.33 and 0.26 in heavy drinking and moderate drinking groups respectively.

Table 1 *ADH1B* genotype variant analysis in oral squamous cell carcinoma

Group	*no*	**1/*1 n(%)*	**2/*2 n(%)*	**1/*2 n(%)*	**2/*2 or*1/*2 n(%)*	*Odds ratio for *1/*1 95% CI*	**1 allele frequency*
Control	100	8 (8)	54 (54)	38 (38)	92 (92)	1 Reference	0.27
Heavy drinkers	33	4 (12.1)	15 (45.4)	14 (42.4)	29 (87.8)	1.62 (1.08–2.14)	0.33
Moderate drinkers	56	6 (10.7)	24 (42.8)	26 (46.4)	50 (89.2)	1.5 (1.17–1.83)	0.33
Light drinkers	37	3 (8.1)	18 (48.6)	16 (43.2)	34 (91.8)	1 (0.63–1.33)	0.29

*ADH1B*2/*2* or**1/*2* of the control used as reference.
CI, confidence interval; *n*, number of alleles.

Table 2 *ADH1C* genotype variant analysis in oral squamous cell carcinoma

Group	*no*	**2/*2 n(%)*	**1/*1 n(%)*	**2/*1 n(%)*	**2/*1* or**1/*2 n (%)*	*Odds ratio for *2/*2 95% CI*	**2 allele frequency*
Control	100	9 (9)	74 (74)	17 (17)	91 (91)	1 References	0.17
Heavy drinkers	33	7 (21.2)	18 (54.5)	8 (24.2)	26 (78.7)	2.65 (1.78–3.53)	0.33
Moderate drinkers	56	8 (14.2)	34 (60.7)	14 (25.0)	48 (85.7)	1.6 (1.15–2.03)	0.26
Light drinkers	37	4 (10.8)	24 (64.8)	9 (24.3)	33 (89.1)	1.23 (0.77–1.63)	0.22

*ADH1C*1/*1* or**2/*1* of the control used as reference.
CI, confidence interval; *n*, number of alleles.

The light drinking group showed an allelic frequency of 0.22 for *ADH1C*2* (Table 2).

MTHFR 677TT genotype was found to have high risk association with heavy drinking population (odds ratio 3.0; 95% CI = 2.02–4.0). Moderate drinking population showed less significant association with *677TT* genotype (odds ratio of 1.7; 95% CI = 1.24–2.19). The light drinking population showed no significant association (odds ratio 1.1; 95% CI = 0.68–1.46). The frequency of *MTHFR C677T* allele in the control population was 0.31. Heavy drinking and moderate drinking groups showed a frequency of 0.50 and 0.39 respectively whereas the light drinking group showed 0.32 (Table 3).

*ADH1B*1/*1/MTHFR677TT* genotype combination showed a higher risk (odds ratio 4.16; 95% CI = 2.78–5.53) in heavy drinking population for susceptibility to OSCC than *ADH1B*1/*2* or **2/*2/*MTHFR677CC or 677CT genotype combination. A similar risk association (odds ratio 4.16; 95% CI = 1.18–5.53) was shown by *ADH1B*1/*2* or **2/*2MTHFR677TT* genotype combination. Moderate drinking population showed relatively similar risk (odds ratio 2; 95% CI = 1.44–2.55) in *ADH1B*1/*1/MTHFR 677TT* genotype combination whereas the light drinking population showed the absence of that genotype combination (Table 4). All the alleles analyzed were found to be in agreement with Hardy-Weinberg equilibrium.

Interestingly, the *ADH1C*2/*2/MTHFR 677TT* genotype combination showed the maximum risk (odds ratio 20; 95% CI = 13.45–26.64) in heavy drinking group for OSCC, which is a significant finding of this study (Table 5). Moderate drinking population showed a moderate risk (odds ratio 5.88; 95% CI = 4.24–7.50) in *ADH1C*2/*2/MTHFR 677TT* whereas the light drinking population showed a minimal risk (Odds ratio 2.77; 95% CI = 1.74–3.68) in that genotype combination (Table 5).

Discussion

Alcohol abuse is a risk factor for cancer in the upper aero-digestive tract (oral cavity, pharynx, hypopharynx, larynx, esophagus) (Seitz *et al*, 2004). Several epidemiological studies have demonstrated a correlation between alcohol ingestion and the occurrence of cancers (Doll *et al*, 1999; Stickel *et al*, 2002). These studies show that consumption of alcoholic beverages is associated

Table 3 *MTHFR* C677T genotype variant analysis in oral squamous cell carcinoma

Group	*no*	*677TT n (%)*	*677CC n (%)*	*677CT n (%)*	*677CT or 677CC n (%)*	*Odds ratio for 677TT 95% CI*	*Valine allele frequency*
Control	100	10 (10)	48 (48)	42 (42)	90 (90)	1 Reference	0.31
Heavy drinkers	33	10 (30.3)	10 (30.3)	13 (39.3)	23 (69.6)	3.0 (2.02–4.0)	0.50
Moderate drinkers	56	9 (16.0)	21 (37.5)	26 (46.4)	47 (83.9)	1.72 (1.24–2.19)	0.39
Light drinkers	37	4 (10.8)	17 (45.9)	16 (43.2)	33 (89.1)	1.1 (0.68–1.46)	0.32

MTHFR 677CT or*677CC* of the control used as reference.
CI, confidence interval; *n*, number of alleles.

Table 4 *ADH1B* and *MTHFR* genotype combination and susceptibility to oral squamous cell carcinoma

ADH1B genotype	*MTHFR genotype*	*Control n (%)*	*Heavy drinkers*		*Moderate drinkers*		*Light drinkers*	
			n (%)	*Odds ratio/95% CI*	*n (%)*	*Odds ratio/95% CI*	*n (%)*	*Odds ratio/95% CI*
**1/*2* or **2/*2*	*677CC* or *677CT*	83 (83)	20 (60.6)	1 Reference	42 (75.0)	1 Reference	30 (81.0)	1 Reference
	677TT	9 (9)	9 (27.2)	4.16 (1.18–5.53)	8 (14.2)	1.77 (1.27–2.25)	4 (10.8)	1.23 (0.76–1.63)
**1/*1*	*677CC* or *677CT*	7 (7)	3 (9.0)	1.78 (1.18–2.37)	5 (8.9)	1.42 (1.02–1.80)	3 (8.1)	1.19 (0.74–l.57)
	677TT	1 (1)	1 (3.0)	4.16 (2.78–5.53)	1 (1.7)	2 (1.44–2.55)	–	–

*ADH1B*1/*2* or **2/*2* and *MTHFR 677CC* or *677CT* used as the reference.
CI, confidence interval; n, number of individuals with that genotype combination.

Table 5 *ADH1C* and *MTHFR* genotype combination and susceptibility to oral squamous cell carcinoma

ADH1C genotype	*MTHFR* genotype	*Control n (%)*	*Heavy drinkers*		*Moderate drinkers*		*Light drinkers*	
			n (%)	*Odds ratio/95% CI*	*n (%)*	*Odds ratio/95% CI*	*n (%)*	*Odds ratio/95% CI*
**1/*2* or **1/*1*	*677CC* or *677CT*	82 (82)	21 (63.6)	1 Reference	42 (75.0)	1 Reference	30 (81.0)	1 Reference
	677TT	9 (9)	5 (15.1)	2.22 (1.48–2.95)	6 (10.7)	1.30 (0.93–1.65)	3 (8.1)	0.92 (0.57–1.21)
**2/*2*	*677CC* or *677CT*	8 (8)	2 (6.0)	(0.52–1.33)	5(8.9)	1.22 (0.88–1.55)	3 (8.1)	1.04 (0.64–1.37)
	677TT	1 (1)	5 (15.1)	20 (13.45–26.64)	3(5.3)	5.88 (4.24–7.50)	1 (2.7)	(1.74–3.68)

*ADH1C*1/*2* or **1/*1* and *MTTFR 677CC* or *677CT* used as the reference.
CI, confidence interval; n, number of individuals with that genotype combination.

with increased cancer risk. However, it should be taken into account that drinking no alcohol at all may expose the individual to coronary heart disease (Rehm *et al*, 1997).

Several studies suggest that the effect of alcohol is modulated by polymorphisms in genes encoding enzymes for ethanol metabolism (alcohol dehydrogenase, aldehyde dehyhdrogenase and cytochrome P4502E1), folate metabolism and DNA repair. The mechanism by which alcohol consumption exerts its carcinogenic effect has not been fully defined, although plausible events include: a genotoxic effect of acetaldehyde (the main metabolite of ethanol), increased estrogen concentration, production of reactive oxygen- and nitrogen species and changes in folate metabolism (Bailey, 2003; Boffetta and Hashibe, 2006).

The carcinogenic effect of ethanol appears to be on account of acetaldehyde, rather than alcohol itself (Seitz *et al*, 2001). Genomic polymorphism of alcohol dehydrogenase *ADH1B* and *ADH1C* are reported to modulate acetaldehyde levels (Poschl and Seitz, 2004). The *ADH1B*2* allele encodes an enzyme, which is approximately 40 times more active than the enzyme encoded by the *ADH1B*1* allele.

The *ADH1B*2* allele frequency is significantly higher in Asians and lower in Caucasians. Our study shows that heavy drinking persons are at relatively higher risk for OSCC with an odds ratio of 1.6; 95% CI = 1.08–2.14 for **1/*1* genotype than moderately drinking persons who showed an odds ratio of 1.5; 95% CI = 1.17–1.83. The light drinking persons had no significant risk association with *ADH1B *1/*1* genotype (Table 1). This genotype was shown to enhance the esophageal cancer risk in Asian populations (Yokoyama *et al*, 2002; Asakage *et al*, 2007).

Our studies show that persons with *ADH1C*2/*2* genotype may have an increased risk for OSCC (odds ratio 2.65; 95% CI = 1.78–3.53) in heavy-drinking population and a less significant risk (odds ratio 1.6; 95% CI = 1.15–2.03) in moderate-drinking persons. Light drinkers with *ADH1C*2/*2* genotype had negligible risk association (Table 2). *ADH1C*2* allele is a relatively less active allele present at frequencies ranging from 0.12 to 0.39 depending upon ethnicity (Olshan *et al*, 2001). On the contrary, the more common and fully functional *ADH1C*1* allele has been identified as a protective factor in cancer associated with alcoholism (Shen *et al*, 1997). Bouchardy *et al*, (2000) showed no significant association of oral cancer with *ADH1C*1/*1* genotype in a study carried out among French population. *ADH1C*2/*2* genotype was found to be associated with smoking- and drinking-related HNSCCs (Peters *et al*, 2005). Individuals with *ADH1C*2/*2* genotype had an increased risk of HNSCCs than those having *ADH1C*1/*2* and *ADH1C*1/*1* genotypes (Schwartz *et al*, 2001).

A relationship has been reported between the *MTHFR 677CT* polymorphism and folate or alcohol consumption and risk of colorectal cancer (Sharp and Little, 2004). In particular, the *TT* genotype seems to be protective only in individuals who are non-alcoholic or moderate-alcohol consumers. There are two main ways by which low folate deficiency may increase the cancer risk. One prominent way is through mediating the transfer of one-carbon moieties, as folate is critical for the synthesis of S-adenosyl methionine, an important compound for DNA methylation. DNA methylation is an epigenetic determinant in gene expression, DNA stability and mutagenesis. Secondly folate is important for normal DNA synthesis and repair. Folate deficiency may lead to an imbalance in DNA precursors, misincorporation of uridylate for thymidylate in DNA synthesis and chromosome breakage (Fowler *et al*, 1998). Folate intake reduces the risk of oral and pharyngeal cancers (Pelucchi *et al*, 2003).

The amino-acid affected by the *MTHFR C677T* polymorphism lies at the base of the binding site for flavin adenine dinucleotide (FAD), a cofactor for the MTHFR enzyme. The thermolabile form of the MTHFR enzyme (*677TT* genotype) has been shown to dissociate with the FAD cofactor more readily than the wild-type enzyme, resulting in decreased enzyme activity (Guenther *et al*, 1999).

Our studies show a significant association of *677TT* genotype in heavy-drinking population (odds ratio 3.0; 95% CI = 2.02–4.0). Moderately drinking group showed a less significant association with 677TT genotype (odds ratio 1.7; 95% CI = 1.24–2.19). A similar case of minor increase in the risk of oral cancer was found among *C677T* genotypes (Vairaktaris *et al*, 2006). Earlier observations on oral cancer among Puerto Rican populations report that this genotype is not associated with risk for oral cancer (Weinstein *et al*, 2002). However, our studies are in agreement with the observations in Chinese population in which the polymorphism in *MTHFR* gene was shown to increase the risk of

esophageal squamous cell carcinoma (Song *et al*, 2001). Similarly, several studies had pointed out the association of *MTHFR* gene polymorphism with HNSCC (Neumann *et al*, 2005; Suzuki *et al*, 2007).

The consumption of alcohol by women is considered by and large to be a taboo in India and hence data regarding this is scarce. The *ADH1B*1/*1/MTHFR 677CC/677CT* genotype combination did not show a clear association of susceptibility to oral cancer. However, the *ADH1C*2/*2/MTHFR677TT* genotype combination depicted a maximum (20-fold) risk for OSCC in heavy-drinking population. Moderate-drinking and light-drinking patients also showed a 5.9- and 2.8-fold increase in risk for the above genotype combination (Table 5). Heavy-drinking population showed a significant sevenfold increase in risk when compared to the light-drinking population of this genotype combination. Moderate-drinking population too showed a minor twofold increase in risk in comparison to the light-drinking *ADH1C*2/*2/MTHFR 677TT* genotype. This is the notable finding of this study.

Conclusions

The *ADH1B*1/*1* genotype along with *MTHFR 677TT* and *677CT* combinations did not show any significant risk for OSCC in heavy drinkers, moderate drinkers and light-drinking populations. However, the *ADH1C*2/*2 MTHFR677TT* genotype combination implies maximum risk for OSCC in all populations. This study indicates the importance of the *ADH1C*2/*2/MTHFR 677TT* genotype combination in alcoholics as a risk factor for the genesis of OSCC.

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