Disease Markers 28 (2010) 369–376
DOI 10.3233/DMA-2010-0717
IOS Press

Genetic polymorphism of glutathione S transferases M1 and T1 in Indian patients with hepatocellular carcinoma

Mohammad Asim[a,b], Luqman A. Khan[b], S. A. Husain[b], Sajid Husain[e], Manash P. Sarma[a], Istaq Ahmad[a], J.K. Sharma[c], Anita Sharma[c], Shashi Sharma[c], L. Thayumanavan[d] and P. Kar[a,*]
[a]*Department of Medicine, Maulana Azad Medical College, University of Delhi, India*
[b]*Department of Biosciences, Jamia Millia Islamia, New Delhi, India*
[c]*Institute of Cytology and Preventive Oncology, ICMR, Noida, India*
[d]*Department of Gastroenterology, Madurai Medical College, Madurai, Tamil Nadu, India*
[e]*Department of Microbiology, Guru Nanak Dev University, Amritsar, India*

Abstract. *Objective*: Our aim was to evaluate whether the association of GSTM1/T1 gene polymorphisms modifies the risk of Hepatocellular carcinoma (HCC) and what is its correlation with other predisposing risk factors like alcohol intake, cigarette smoking and hepatitis B and C infections.
Study design/setting: It was a case-control study, included 254 HCC cases compared with 525 hospital-based age and sex matched cases of chronic liver disease without HCC as controls from Indian population. The GSTM1 and GSTT1 genotypes were detected using conventional multiplex PCR method.
Results: In this case-control study, we observed a positive correlation between age, HBV and HCV infection, smoking habit of $>$ 20 packs/year, alcohol consumption of $>$ 100 g/day and risk of liver cancer. We found significantly increased risk associated with GSTM1 null genotype (OR = 3.49; 95% CI = 2.52–4.84) as well as GSTT1 null genotype (OR = 3.12; 95% CI = 2.19–4.45), respectively. However, an increased risk of HCC was observed among heavy drinkers with GSTM1 (OR = 2.01; 95% CI = 1.11–3.66). Further, cigarette smoking showed a non-significant association with GSTT1 (OR = 1.49; CI = 0.69–3.25).
Conclusion: Our results suggest that the variants in low penetrance gene such as GSTM1 and GSTT1 are associated with an increased liver cancer risk. Further, an influence of GSTM1/T1 null genotypes may contribute in the etiology of HCC in patients with higher cigarette and alcohol consumption.

Keywords: Hepatocellular carcinoma, GSTM1, GSTT1, smokers, alcoholics, polymorphism

1. Introduction

Hepatocellular carcinoma (HCC) is one of the most commonly occurring solid tumors worldwide, and is the most frequent cause of cancer-related death in sub-Saharan Africa and China, among other regions. The annual incidence of HCC is low in India and most Western countries (fewer than 5 cases per 100,000), whereas in Asian countries it is either intermediate (5–15 cases per 100,000) or high (above 15 cases per 100,000) [1, 2]. This variation in the global distribution of HCC is believed to be due to regional differences in a number of etiological factors.

Considerable evidence suggests that hepatocarcinogenesis in humans is a multistage process that involves multiple risk factors, particularly the hepatitis B and C viruses (HBV and HCV, respectively) and alcohol. HBV seems to play a direct role in liver cell transfor-

*Corresponding author: Dr. P. Kar, Director-Professor, Department of Medicine, Room No. 101/127, Ist floor, B.L Taneja Block, Maulana Azad Medical College, New Delhi-110002, India. Tel./Fax: +91 011 23230132; E-mail: pkar@vsnl.com.

ISSN 0278-0240/10/$27.50

mation [3]. A carcinogenic role for HCV in the presence of cirrhosis has been established, but its role in the absence of cirrhosis is controversial [4]. Alcohol is proposed to cause HCC mainly because it causes cirrhosis, whereas its association with HCC in the absence of cirrhosis has not been established [5]. Tobacco smoking has also been implicated as a risk factor for liver cancer, although the precise relationship between cigarette smoking and the relative risk of HCC development is is uncertain [6–8]. These and other risk factors, including aflatoxin [9] and diabetes mellitus [10], may act synergistically to increase the risk of HCC [11].

Genetic variations have also been proposed to influence the variations in HCC risk that occur both within and across populations. Among these, the most extensively studied genetic risk factors for HCC are the variants of glutathione S-transferases (GSTs). The phase-II isoenzymes that make up the GST superfamily participate in cellular detoxification by the neutralizing both the endogenous byproducts of oxidative stress and carcinogenic exogenous chemicals such as aflatoxins [12, 13]. Together, members of the GST supergene family encode seven classes of dimeric enzymes that are able to catalyze conjugation reactions between glutathione and chemical carcinogens, which allows epoxides to facilitate their excretion and thereby detoxify these carcinogens [14]. As a result, GSTs are believed to play an important role in opposing carcinogenesis. Among the different GST genes, only class μ1 (*GSTM1*) and class θ1 (*GSTT1*) have gene deficiency (null genotype) phenotypes that are characterized by a complete absence of enzyme activity [15]. A partial deletion in the coding region of these genes leads to complete abrogation of enzymatic function and thus an impairment in the body's ability to metabolically eliminate carcinogenic compounds [13]. It is possible that the activity of these enzymes need not be completely absent for individuals to be susceptible to the development of cancer. For example, Moyer et al. suggested that functionally significant pharmacogenomic variations, such as single nucleotide polymorphisms (SNP), might be sufficient to contribute either to carcinogenesis or to individual variations in antineoplastic drug therapy response [16].

Carcinogens such as aflatoxin B1-8, 9-epoxide are a substrate for both GSTM1 and GSTT1 [15]. Phase-II enzymes in the liver also detoxify a wide variety of polycyclic aromatic hydrocarbons, such as benzo(a)pyrene and monohalomethanes, which are active metabolites of tobacco carcinogens [12]. Smokers with a deficiency in GSTM1 and GSTT1 were found to be predisposed to head and neck squamous cell carcinoma, and there was also a strong correlation with lung cancer in heavy smokers [17]. A study by Buch et al. showed that the GSTM1 null genotype is a risk factor for the development of oral cancer amongst Indian tobacco smokers [18].

Genetic studies of Western population have reported frequencies for the GSTM1 deletion genotype ranging between 47 and 58%, whereas the GSTT1-null genotype has been observed in 13 to 25% of white Europeans and in 27.6% of the Caucasian population of the United States [13,19]. A study based on a normal population from Shanghai observed GSTM1 as well as GSTT1 gene deletions in 49% of the subjects [20]. A North-Indian study of a healthy population showed the frequency of GSTM1 and GSTT1 gene deletion to be 33 and 18.4%, respectively [21]. A study based on healthy random volunteers from South India found the GSTM1 and GSTT1 gene deletions in 22.4 and 17.65% of subjects, respectively [22].

Although a number of published reports have shown a relationship between liver cancer and the GSTM1-null genotypes, HYL1*2 genotypes, XRCC1-399 AG genotype, or ADH3 (1-1) genotype, none of these found any significant association between the GSTT1-null genotype and HCC [7,23–26]. However, a Chinese study found that individuals with either the GSTM1 or GSTT1 null genotype had a higher susceptibility to HCC, while individuals with both null genotypes were at even greater risk [27]. A recent meta-analysis suggested that there was a slightly increased risk of HCC in individuals with the GSTT1 null phenotype, as well as with GSTM1-null genotypes in populations with high or medium incidence of HCC [28].

Although one study found no association between HCC and GSTM1/T1 gene deletion in smokers [8], another found a non significantly increased risk of HCC among light smokers (1–20 pack-years) carrying GSTT1-null and NAT2-slow-acetylator genotypes [27]. A significant association between the GSTM1-null genotype and HCC was found in individuals with high consumption of alcohol [25]. Similarly, a report by Savolainen et al., found a significant association between the GSTM1 null genotype and alcoholic liver cirrhosis [29].

A potential association between GSTM1 and GSTT1 polymorphisms and several cancers has been suggested by the conflicting results of a number of studies from the Indian subcontinent, but there is little information regarding an association with HCC [18,30–32]. The present study was designed to investigate the potential association between the GSTM1 and GSTT1 geno-

Table 1
Distribution of demographic and etiologic variables among HCC and controls

Variables	HCC cases $n = 254$ (%)	Controls $n = 525$ (%)	OR (95% CI)	P value
Gender				
Male	202 (79.52)	371 (70.66)	1	0.011*
Female	52 (20.47)	154 (29.33)	0.62 (0.43–0.90)	
Age range (*Years*)				
$\leqslant 45$	90 (35.4)	370 (70.5)	1	0.0001*
> 45	164 (64.6)	155 (29.5)	4.35 (3.13–6.06)	
HBV infection[a]				
Negative	107 (42.12%)	412 (78.47%)	1	0.0001*
Positive	147 (57.87%)	113 (21.52%)	5.01 (3.57–7.02)	
HCV infection[b]				
Negative	215 (84.64%)	485 (92.38%)	1	0.001*
Positive	39 (15.35%)	40 (07.80%)	2.20 (1.34–3.61)	
Alcohol intake (*g/day*)				
0–60	111 (43.70%)	310 (59.04%)	1	
61–100	51 (20.07%)	112 (21.33%)	1.27 (0.84–1.92)	0.276
> 100	92 (36.22%)	103 (19.61%)	2.49 (1.72–3.61)	0.0001*
Smoking status (packs-years)				
0	53 (20.86%)	153 (29.14%)	1	
1–20	65 (25.59%)	149 (28.38%)	1.26 (0.8–1.97)	0.341
> 20	136 (53.54%)	223 (42.47%)	1.76 (1.19–2.63)	0.004*

[a]HBV infection: Seropositivity for HBsAg.
[b]HCV infection: Seropositivity for HCV RNA; subjects positive for anti-HCV but negative for HCV RNA were classified among those negative for HCV infection.
OR (95% CI): estimates Odd ratios between the age and sex adjusted cases and controls for smoking habits, alcohol consumption, HBV infection and HCV infection respectively.
*Statistically significant.

types and the risk of developing HCC, taking into account the smoking habits, HBV and HCV infection status, and alcohol consumption in subjects in two Indian populations.

2. Methods

2.1. Subjects

The study included 254 cases of HCC and 525 hospital based age and sex matched cases of chronic liver disease without HCC as controls, recruited from the Medical wards of Lok Nayak Hospital, and Liver clinic of All India Institute of Medical Sciences (AIIMS), New Delhi and Madurai Medical College, Tamil Nadu from February 2005 to July 2007. Data was abstracted from the medical records of participants regarding the date of diagnosis, the individual's HBV and HCV markers status, including HBV surface antigen (HBsAg) and anti-HCV, and the individual's sex and age at diagnosis. At the time of recruitment, each study subject was personally interviewed to ascertain the information on socio-demographic characteristics, lifestyle, and ethnicity, lifetime history of cigarette smoking and alcohol consumption, dietary factors, and personal and family history of various chronic diseases followed by standardized clinical examination. (Table 1). The cumulative exposure to cigarettes was calculated as "pack-years", using the following formula: (number of years smoked $\times$ average number of cigarettes smoked per day)/20 [27]. Total alcohol intake based on history of lifetime consumption was assessed. Alcohol intake was categorized at three levels (0–60, 61–100, > 100) as described earlier [27]. All the study subjects had given written informed consent for the interview and blood sample collection. The ethical committees of Maulana Azad Medical College, New Delhi and Madurai Medical College, Tamil Nadu have approved the present study.

Ten ml of blood drawn by standard venipuncture in EDTA vial for genomic study from all the study participants. The genomic DNA extracted from peripheral leucocytes, and dissolved in sterile TE buffer, pH 8.0. Another 10 ml blood drawn in plain vial to separate serum for serological testing. Blood samples were frozen immediately after collection and stored at $-70\,^{\circ}\mathrm{C}$ until use. Patients of HCC were diagnosed based on either pathological or cytological examination or an elevated $\propto$-fetoprotein level ($\geqslant$ 400 ng/ml) combined with at least one positive image on angiography, Sonography and /or computerized tomography.

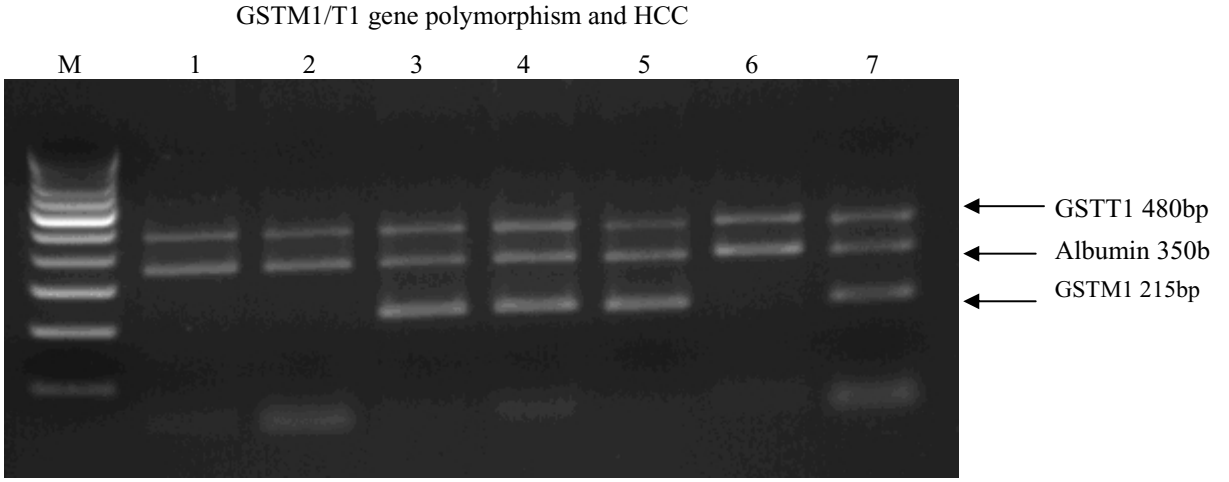

Fig. 1. Representative gel photograph showing amplification of GSTT1 (480 bp), GSTM1 (215 bp) and the control Albumin (350 bp) gene products.

2.2. Serological detection

All study participants were tested for Hepatitis B surface antigen (HBsAg) using Eliscan micro ELISA strips (Ranbaxy Diagnostics, New Delhi, India), IgG antibody to hepatitis B core antigen antibody (Melotech, Barcelona, Spain), HBeAg (Medical biological services S.R.L, Italia) and antibodies to hepatitis C virus (HCV) by Innotest HCV ABIII (Innogenetics NV, Ghent, Belgium).

2.3. Laboratory analysis

Genomic DNA was extracted by a salting-out method [33]. Briefly, buffy coats of nucleated cells obtained from anticoagulated blood (EDTA) were resuspended in 15 ml polypropylene centrifugation tubes with 3 ml of nuclei lysis buffer (10 mM Tris-HCl, 400 mM NaCl and 2 mM Na_2EDTA, pH 8.2). The cell lysates were digested overnight at 37°C with 0.2 ml of 10% SDS and 0.5 ml of a protease K solution (1 mg protease K in 1% SDS and 2 mM Na_2EDTA). After digestion was complete, 1 ml of saturated NaCl (approximately 6M) was added to each tube and shaken vigorously for 15 seconds, followed by centrifugation at 2500 rpm for 15 minutes. The precipitated protein pellets were left at the bottom of the tube and the supernatant containing the DNA was transferred to another 15 ml polypropylene tube. Exactly 2 volumes of room temperature absolute ethanol was added and the tubes inverted several times until the DNA precipitated. The precipitated DNA strands were removed with a plastic spatula or pipette and transferred to a 1.5 ml microcentrifuge tube containing 100–200 μl TE buffer (10 mM Tris-HCl, 0.2 mM Na_2EDTA, pH 7.5). The DNA was allowed to dissolve for two hours at 37°C. Genomic DNA (50–100 ng) was amplified in a total volume of 50 μl reaction mixture consisted of 50 pM of each primer (Quiagen, USA), 200 μM dNTPs (Bangalore Genei, India), 25 mM $Mgcl_2$ (Bangalore Genei, India), and 0.5 U Taq DNA polymerase (New England Biolabs, England). The reaction mixture was subjected to initial denaturation at 95°C for 5 min, followed by 35 cycles of 94°C for 1 min, 58°C for 1 min and 72°C for 1 min. The final extension was done at 72°C for 7 min. The amplified products were analyzed on 2% agarose gel stained with ethidium bromide and observed under UV light. DNA samples positive for GSTM1 and GSTT1 genotypes yielded bands of 215 bp and 480 bp, respectively. The presence of 350 bp albumin fragment was indicator of a successful PCR (Fig. 1). A multiplex PCR method was use to detect the presence or absence of the GSTM1 (5'-GAACTCCCTGAAAAGCTAAAGC -3' and 5'-GTTGGGCTCAAAT ATACGGTG G-3') and GSTT1 (5'-TCTTACTGGTCCTCACATCTC-3' and 5'-TCACCGGATCATGGCCAGC C-3') genes simultaneously in the same tube according to Arand et al. [34]. For those cases that were having GSTM1 or GSTT1 null genotypes, a pair of primers specific to albumin gene (5'-GCCCTCTGCTAACAAGTCCTAC-3' and 5'-GCCCTAAAAAGAAAATCGCCAATC -3') was used as an internal control. Generation of albumin gene specific PCR products with the help of these internal control primers necessarily helped in ruling out the possibility of any type of PCR artifact.

2.4. Statistical analysis:

Differences between cases and controls in the distribution of GSTM1 and GSTT1 genotypes, which were considered as dichotomicus variables, were analyzed using Mantel-Haenszel chi-square test. The odd ratios with their corresponding 95% confidence intervals were calculated at the same time. The calculations were made with the SPSS 11.5 statistical software for windows and the EpiInfo V.6 software of the Atlanta Centers for Disease Control. Differences considered significant for Pvalues less than 0.05.

3. Results

The study population comprised of 254 (202 male and 52 female) HCC cases and 525 (371 male and 154 female) controls (Table 1). Male predominance observed in HCC cases as well as in controls (Table 1). The mean age of cases and control subjects were 64.9 $\pm$ 3.8 years and 44.6 $\pm$ 7.6 years, respectively. The age of the HCC cases and controls enrolled in the study has been stratified into two arbitrary groups of $\leqslant$ 45 and $>$ 45 years to understand the frequency in each group. The age group $>$ 45 years showed significant difference amongst the HCC cases and controls (P = 0.0001).

Table 1 showed the collective distribution of etiologic variables like Hepatitis B and C virus infections, cigarette smoking and alcohol consumption for HCC and controls along with the corresponding ORs and 95% CIs. Both HBsAg and HCV RNA positivity were associated with HCC. A five fold increased risk of HBV positivity was observed with HCC [OR = 5.01, 95% CI (3.57–7.02)]. On the other hand, HCV positivity poses a two fold increased risk with HCC (OR = 2.2; 95% CI = 1.34–3.61; Table 1). Considering alcohol consumption of 0–60 g/day as reference range, the calculated risk for consumption of 61–100 g/day among the HCC cases, showed a non-significant increase (OR = 1.27, 95% CI 0.84–1.92). However, 2.5 fold increased risk of HCC was observed for alcohol consumption in patients having the higher dose of alcohol consumption, i.e. $>$ 100 g/day (OR = 2.49; 95% CI = 1.72–3.61; Table 1). Hence, a dose-response association observed. Amongst light smokers with history of 1–20 packs-year of lifetime cigarette smoking, a 1.26 fold increased risk of HCC observed (OR = 1.26; 95% CI = 0.8–1.97), when compared with non-smokers. However, there is 1.8 fold increased risk with smoking habit of $>$ 20 packs per year (OR = 1.76; 95% CI = 1.19–2.63), which shows an increasing trend of risk for heavy smokers (Table 1).

Table 2
Distribution of GSTM1 and GSTT1 genotypes among HCC cases and controls

Genotypes	Cases (%) $n = 254$	Controls (%) $n = 525$	OR (95% CI)[a]
GSTM1			
Non-null	102 (40.1)	368 (70.1)	Reference
Null	152 (59.8)	157 (29.9)	3.49 (2.52–4.84)
GSTT1 Non-null	156 (61.4)	437 (83.2)	Reference
Null	98 (38.6)	88 (16.8)	3.12 (2.19–4.45)

[a]OR estimates for each gene, in all subjects, adjusted for age, sex, area of recruitment, HBsAg, HVC RNA positivity and alcohol consumption.

Table 2 showed the distribution of GSTM1 and GSTT1 genotypes with the corresponding ORs and 95% CIs. GSTM1 null genotypes was observed in 58.9% HCC cases compared to 29.9% in controls, showing 3.5 fold increased risk of HCC (OR; 3.49, 95% CI 2.52–4.84). GSTT1 null genotype was found in 38.6% HCC cases compared to 16.8% in controls, showing 3.1 fold increased risk (OR; 3.12, 95% CI 2.19–4.45; Table 2). Furthermore, we have calculated the odd ratio for data, classified by age, smoking and alcohol consumption to evaluate the effect of GSTM1 and GSTT1 in association with age, smoking and drinking habits along with the 95% CI (Table 3). However, we have clubbed the different categories of smoking habit and alcohol consumption described in Table 1, as only smokers / non-smokers and alcoholics / non–alcoholics for convenience, to study their interaction with the risk genotypes. The cases were of nearly one decade higher in age, as compared to controls in this study. No significant association of GSTM1 null genotype observed with respect to age and smoking habit. Although not statistically significant, an increased risk was observed among alcoholics carrying the GSMT1 null genotype (OR = 2.01, 95% CI 1.1–3.66). A protective effect was observed for GSTT1 null genotype with respect to age and alcohol consumption, although smoking habit poses a slightly non-significant increased risk of HCC (OR = 1.49, 95% CI 0.69–3.25; Table 3).

4. Discussion

We observed the gender ratio among the HCC patients included in our study to be skewed 3.9:1 in favor of males, a ratio comparable previously reported gender-specific incidence rates ranging from 1.4 to 3.3

Table 3
Risk due to polymorphism in GSTM1 and GSTT1 genotypes according to age, smoking and drinking habits in HCC patients

Variables	GSTM1 null			GSTT1 null		
	HCC $n = 152$ (%)	Controls $n = 157$ (%)	OR (95% CI)	HCC $n = 98$ (%)	Controls $n = 88$ (%)	OR (95% CI)
Age						
$\leqslant 45$	27 (17.8)	29 (18.5)	1	26 (26.5)	18 (20.5)	1
> 45	120 (78.9)	128 (81.5)	1.01 (0.54–1.87)	72 (73.5)	70 (79.5)	0.71 (0.34–1.49)
Alcohol intake						
Non-Alcoholics	24 (15.8)	43 (27.4)	1	22 (22.4)	16 (18.2)	1
Alcoholics	128 (84.2)	114 (72.6)	2.01 (1.11–3.66)	76 (77.6)	72 (81.8)	0.77 (0.35–1.67)
Smoking habit						
Non-smokers	35 (23.0)	29 (18.5)	1	17 (17.3)	21 (23.9)	1
Smokers	117 (77.0)	128 (81.5)	0.76 (0.42–1.36)	81 (82.7)	67 (76.1)	1.49 (0.69–3.25)

in favor of males worldwide [35]. The major etiological factor among our subject population was a positive HBV infection status, which was associated with a 5.01-fold increased risk for HCC compared to controls. In contrast, HCV infection conferred a 2.2-fold increased risk for HCC. These findings are in agreement with a previous Indian study that showed HBV infection, followed by HCC infection, to be the major etiological factors associated with HCV worldwide [36]. Similarly, studies based in Asia and Africa found HBV appeared to play a major role, and HCV a minor one, in HCC development. This lies in contrast to the results of a number of reports from North America, Japan and Europe, which have provided growing evidence that HCV infection is at least partly responsible for the increasing incidence of HCC in these low-to-intermediate-risk regions [1].

In our study, heavy alcohol consumption was significantly correlated with the risk of HCC (odds ratio [OR] = 2.49), which is in good accordance with the previous findings of Sakamoto et al. [37]. However, it is not certain whether alcohol is a true carcinogen or if; rather, it may act as a cofactor in the presence of coexistent infection with HBV or HCV. Several epidemiologic studies among alcoholics have reported a high prevalence of HBV and HCV markers (16–70% and 10–20%, respectively), compared to background prevalence rates of approximately 5% and less than 1%, respectively. These prevalences are even higher in primary liver cancer patients who are also alcoholics (27–81% for HBV markers and 50–77% for HCV), suggesting that there may exist a complex interaction between alcohol and viral infections in the etiology of primary liver cancer [38].

Franchis et al. reported cigarette smoking to be a causal risk factor for HCC [6], although subsequent studies failed to support this correlation [7,8]. However, given the strongly positive correlation between the use of tobacco and alcohol in virtually all populations studied, the latter interview-based epidemiologic data are considered by many to be inconclusive in ruling out a causal role for tobacco in the pathogenesis of HCC. In our study, a slightly but nonsignificantly increased risk of HCC was observed among heavy smokers (OR = 1.76), which is in agreement with the findings of Munaka et al. [8].

Previously, some Chinese studies had suggested a strong association of GSTM1 and GSTT1 null genotypes with an increased risk of HCC. Further, the risk of HCC was essentially doubled in individuals carrying both null genotypes [26]. A recent meta-analysis by White et al. reported an association between HCC and the GSTM1 and GSTT1 null genotypes in populations having a medium or high incidence of the disease [28]. Ours is the first case-control study on the role of GST gene polymorphism in hepatocarcinogenesis amongst Indian population. This study also supports an association of GSTM1 and GSTT1 genetic polymorphisms and the risk of HCC in the Indian population. However, few studies have found an association between the GSTM1/T1 null genotypes and the risk of HCC [7,8, 25,27].

We observed no association between age and the putative risk genotypes in this study. In addition, the study revealed a statistically non-significant increase in HCC risk among smokers carrying the GSTT1 null genotype (OR = 1.49), whereas a protective role was observed for the GSTM1 null genotype (OR = 0.76). Similarly, Gelatti et al., showed a small degree of association between the GSTT1-null genotype and HCC [27]. Further, this study observed a significant interaction between alcohol intake and the GSTM1-null genotype in those subjects with heavy alcohol intake. These findings suggest that absence of GSTM1 gene activity might reduce an individual's capacity to conjugate cytotoxic lipid peroxidation products, as well as the free rad-

icals generated during alcohol metabolism, and hence increase the risk of liver damage due to alcohol. Covolo et al. reported an association between HCC risk and the GSTM1 null genotype and alcohol, but observed no association with the GSTT1 null genotype [25].

The hospital based case-control design has been regarded as a useful strategy for investigating gene-environment interactions, particularly where control subjects are not affected by the diseases studied, whereas population-based studies may be more prone to selection bias where requirements such as the need to provide blood samples may lead to a poor response rate [39]. However, for metabolic genes, such as the genes considered in the present study, a large pooled analysis showed no difference regarding genotype frequencies among controls when hospital- and population-based studies were compared [23]. In this study, cases and controls matched accurately with respect to age, gender, and ethnicity. The sample size had sufficient statistical power to detect a gene-environment interaction.

Members of the GST family are important candidates for conferring susceptibility to commonly occurring forms of cancer, because they may regulate an individual's ability to metabolize environmental carcinogens. Normal or increased GST enzyme activity may protect susceptible tissue from somatic mutations in DNA by facilitating the conjugation and subsequent elimination of electrophilic carcinogens. Absent or deficient GST enzyme activity may result in poorer elimination of electrophilic carcinogens, particularly in the presence of very active electrophilic activation by phase-I enzymes. The results of the present study suggest that the variants in low-penetrance gene such as GSTM1 and GSTT1 are associated with an increased risk of liver cancer. In addition, a possible dose-dependent risk was associated with smoking and alcohol consumption. In conclusion, our findings suggest a potential role for GSTM1/T1 null genotypes in the etiology of HCC in patients with higher cigarette and alcohol consumption.

Acknowledgements

This study was supported by a research grant from Department of Science and Technology (DST). The authors are also grateful to all the members of PCR Hepatitis Laboratory, Department of Medicine, MAMC, New Delhi for their support and participation in this research work. We also thank Mrs. Sarita Sardana and Shashi Sharma for statistical analysis.

References

[1] F.X. Bosch, Global epidemiology of hepatocellular carcinoma, in: *Liver cancer*, K. Okuda and E. Tabor, eds, New York: Churchill Livingstone, 1997, pp. 13–27.

[2] K.M. Mohandas and V. Dhir, Epidemiology of hepatocellular carcinoma in India, in: *Hepatitis B and C: carrier to cancer*, S.K. Sarin and K. Okuda, eds, Harcourt Publishers, 2002, pp. 279–288.

[3] R. Idilman, N. De Maria, A. Colantoni and D.H. Van Thiel, Pathogenesis of hepatitis B and C-induced hepatocellular carcinoma, *J Viral Hepat* **5** (1998), 285–299.

[4] M.S. De Mitri, K. Poussin, P. Baccarini, P. Pontisso, A. D'Errico, N. Simon, W. Grigioni, A. Alberti, M. Beaugrand, E. Pisi, et al., HCV-associated liver cancer without cirrhosis, *Lancet* **345** (1995), 413–415.

[5] W.T. London and K.A. McGlynn, Liver cancer, in: *Cancer Epidemiology and Prevention*, D. Schottenfeld and J.F. Fraumeni, Jr., eds, New York: Oxford University Press, 1996, pp. 772–793.

[6] S. Franceschi, M. Montella, J. Polesel, C. La Vecchia, A. Crispo, L. Dal Maso, P. Casarin, F. Izzo, L.G. Tommasi, I. Chemin, C. Trepo, M. Crovatto et al., Hepatitis viruses, alcohol, and tobacco in the etiology of hepatocellular carcinoma in Italy, *Cancer Epidemiol Biomarkers Prev* **15**(4) (Apr 2006), 683–689.

[7] J.M. Ladero, C. Martínez, E. García-Martin, P. Ropero, O. Briceno, A. Villegas, M. Diaz-Rubio and J.A. Agundez, Glutathione S-transferase M1 and T1 genetic polymorphisms are not related to the risk of hepatocellular carcinoma: a study in the Spanish population, *Eur J Cancer* **42**(1) Jan 2006 73–77. Epub 2005 Nov 28.

[8] M. Munaka, K. Kohshi, T. Kawamoto, S. Takasawa, N. Nagata, H. Itoh, S. Oda and T. Katoh, Genetic polymorphisms of tobacco- and alcohol-related metabolizing enzymes and the risk of hepatocellular carcinoma, *J Cancer Res Clin Oncol* **129**(6) (Jun 2003), 355–360.

[9] C.J. Chen, Y.J. Zhang, S.N. Lu and R.M. Santella, Aflatoxin B1 DNA adducts in smeared tumor tissue from patients with hepatocellular carcinoma, *Hepatology* **16** (1992), 1150–1155.

[10] H.B. El-Serag, T. Tran and J.E. Everhart, Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma, *Gastroenterology* **126** (2004), 460–468.

[11] J.A. Marrero, R.J. Fontana, S. Fu, H.S. Conjeevaram, G.L. Su and A.S. Lok, Alcohol, tobacco and obesity are synergistic risk factors for hepatocellular carcinoma, *J Hepatol* **42** (2005), 218–224.

[12] J.D. Hayes and D.J. Pulford, The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprevention and drug resistance, *Crit Rev Biochem Mol Biol* **30** (1995), 445–600.

[13] T.R. Rebbeck, Molecular epidemiology of the human glutathione S-transferase genotypes GSTM1 and GSTT1 in cancer susceptibility, *Cancer Epidemiol Biomarkers Prev* **6** (1997), 733–743.

[14] H. Esaki and S. Kumagai, Glutathione-S-transferase activity toward Aflatoxin epoxide in livers of mastomys and other rodents, *Toxicon* **40** (2002), 941–945.

[15] J.K. Wiencke, K.T. Kelsey, R.A. Lamela and W.A. Toscano, Jr., Human glutathione S-transferase eficiency as a marker of susceptibility to epoxide-induced cytogenetic damage, *Cancer Res* **50** (1990), 1585–1590.

[16] A.M. Moyer, O.E. Salavaggione, S.J. Hebbring, I, Moon, M.A. Hildebrandt, B.W. Eckloff, D.J. Schaid, E.D. Wieben and R.M.

Weinshilboum, Glutathione S-transferase T1 and M1: gene sequence variation and functional genomics, *Clin Cancer Res* **13**(23) (1 Dec 2007), 7207–7216.

[17] L. Cheng, E.M. Sturgis, S.A. Eicher, D. Char, M.R. Spitz and Q. Wei, Glutathione-S-transferase polymorphisms and risk of squamous-cell carcinoma of the head and neck, *Int J Cancer* **84** (1999), 220–224.

[18] S.C. Buch, P.N. Notani and R.A. Bhisey, Polymorphism at GSTM1, GSTM3 and GSTT1 gene loci and susceptibility to oral cancer in an Indian population, *Carcinogenesis* **23**(5) (2002), 803–807.

[19] S. Garte, L. Gaspari, A.K. Alexandrie, C. Ambrosone, H. Autrup, J.L. Autrup, H. Baranova, L. Bathum, S. Benhamou, P. Boffetta, C. Bouchardy, K. Breskvar et al., Metabolic gene polymorphism frequencies in control populations, *Cancer Epidemiology, Biomarkers and Prevention* **10** (2001), 1239–1248.

[20] J. Shen, G. Lin, W. Yuan, J. Tan, H.M. Bolt and R. Thier, Glutathione transferase T1 and M1 genotype polymorphism in the normal population of Shanghai, *Arch Toxicol* **72**(7) (Jun 1998), 456–458.

[21] D.K. Mishra, A. Kumar, D.S. Srivastava and R.D. Mittal, Allelic variation of GSTM1 and GSTT1 genes in North Indian population, *Asian Pac J Cancer Prev* **5**(4) (Oct–Dec 2004), 362–365.

[22] V. Vettriselvi, K. Vijayalakshmi, F.D. Paul Solomon and P. Venkatachalam, Genetic Variation of GSTM1, GSTT1 and GSTP1 Genes in a South Indian Population, *Asian Pacific J Cancer Prev* **7**(2) (Apr–Jun 2006), 325–328.

[23] X.D. Long, Y. Ma, Y.P. Wei and Z.L. Deng, The polymorphisms of GSTM1, GSTT1, HYL1*2, and XRCC1, and aflatoxin B1-related hepatocellular carcinoma in Guangxi population, China, *Hepatol Res* **36**(1) (Sep 2006), 48–55.

[24] X.D. Long, Y. Ma, Y.P. Wei and Z.L. Deng, A study about the association of detoxication gene GSTM1 polymorphism and the susceptibility to aflatoxin B1-related hepatocellular carcinoma, *Zhonghua Gan Zang Bing Za Zhi* **13**(9) (Sep 2005), 668–670.

[25] L. Covolo, U. Gelatti, R. Talamini, S. Garte, P. Trevisi, S. Franceschi, M. Franceschini, F. Barbone, A. Tagger, M.L. Ribero, G. Parrinello, V. Donadon, L. Covolo, U. Gelatti, R. Talamini, S. Garte, P. Trevisi, S. Franceschi et al., Alcohol dehydrogenase 3, glutathione S-transferase M1 and T1 polymorphisms, alcohol consumption and hepatocellular carcinoma (Italy), *Cancer Causes Control* **16**(7) (Sep 2005), 831–838.

[26] U. Gelatti, L. Covolo, R. Talamini, A. Tagger, F. Barbone, C. Martelli, F. Cremaschini, S. Franceschi, M.L. Ribero, S. Garte, G. Nardi, V. Donadon et al., N-Acetyl transferase-2, glutathione S-transferase M1 and T1 genetic polymorphisms, cigarette smoking and hepatocellular carcinoma: a case-control study, *Int J Cancer* **115**(2) (10 Jun 2005), 301–306.

[27] X.D. Long, Y. Ma, Y.P. Wei and Z.L. Deng, Study on the detoxication gene GST M1- GSTT1-null and susceptibility to aflatoxin B1 related hepatocellular carcinoma in Guangxi, *Zhonghua Liu Xing Bing Xue Za Zhi* **26**(10) (Oct 2005), 777–781.

[28] D.L. White, D. Li, Z. Nurgalieva and H.B. El-Serag, Genetic Variants of Glutathione S- Transferase as Possible Risk Factors for Hepatocellular Carcinoma: A HuGE Systematic Review and Meta-Analysis, *Am J Epidemiol* **167**(4) (15 Feb 2008), 377–389. Epub 2007 Dec 7.

[29] V.T. Savolainen, J. Pjarinen, M. Perola, A. Penttila and P.J. Karhunen, Glutathione-S- transferase GST M1 "null" genotype and the risk of alcoholic liver disease, *Alcohol Clin Exp Res* **20** (1996), 1340–1345.

[30] D.S. Srivastava, D.K. Mishra, A. Mandhani, B. Mittal, A. Kumar and R.D. Mittal, Association of genetic polymorphism of glutathione S-transferase M1, T1, P1 and susceptibility to bladder cancer, *Eur Urol* **48**(2) (Aug 2005), 339–344. Epub 2005 Mar 7.

[31] D.S. Srivastava, A. Mandhani, B. Mittal and R.D. Mittal, Genetic polymorphism of glutathione S-transferase genes (GSTM1, GSTT1 and GSTP1) and susceptibility to prostate cancer in Northern India, *BJU Int* **95**(1) (Jan 2005), 170–173.

[32] M. Jain, S. Kumar, N. Rastogi, P. Lal, U.C. Ghoshal, A. Tiwari, M.C. Pant, M.Q. Baiq and B. Mittal, GSTT1, GSTM1 and GSTP1 genetic polymorphisms and interaction with tobacco, alcohol and occupational exposure in esophageal cancer patients from North India, *Cancer Lett* **242**(1) (8 Oct 2006), 60–67. Epub 2005 Dec 7.

[33] S.A. Miller, D.D. Dykes and H.F. Polesky, A simple salting out procedure for extracting DNA from human nucleated cells, *Nucleic Acids Research* **16**(3) (1988), 1215.

[34] M. Arand, R. Muhlbauer, J. Hengstler, E. Jager, J. Fuchs, L. Winkler and F. Oesch, A multiplex polymerase chain reaction protocol for the simultaneous analysis of the Glutathione S transferase GSTM1 nd GSTT1 polymorphisms, *Anal Biochem* **236** (1996), 184–186.

[35] J. Ferlay, F. Bary, P. Pisani and D.M. Parkin, GLOBOCAN 2000: cancer incidence mortality and prevalence worldwide, version 1.0. Lyon: IARC Press, 2001.

[36] M. Kumar, R. Kumar, S.S. Hissar, M.K. Saraswat, B.C. Sharma, P. Sakhuja and S.K. Sarin, Risk factors analysis for hepatocellular carcinoma in patients with and without cirrhosis: a case- control study of 213 hepatocellular carcinoma patients from India, *J Gastroenterol Hepatol* **22**(7) (Jul 2007), 1104–1111. Epub 2007 Jun 7.

[37] T. Sakamoto, M. Hara, Y. Higaki, M. Ichiba, M. Horita, T. Mizuta, Y. Eguchi, T. Yasutake, I. Ozaki, K. Yamamoto, S. Onohara, S. Kawazoe et al., Influence of alcohol consumption and gene polymorphisms of ADH2 and ALDH2 on hepatocellular carcinoma in a Japanese population, *Int J Cancer* **118**(6) (15 Mar 2006), 1501–1507.

[38] A.M. Di Bisceglie, R.L. Carithers, Jr. and G.J. Gores, Hepatocellular carcinoma, *Hepatology* **28** (1998), 1161–1165.

[39] S. Wacholder, N. Chatterjee and P. Hartge, Joint effect of genes and environment distorted by selection biases: implications for hospital-based case-control studies, *Cancer Epidemiol Biomarkers Prev* **11**(9) (Sep 2002), 885–889.