J Biochem Tech (2019) 10(2): 74-78
ISSN: 0974-2328

GST gene polymorphism in HCC cases from North East India: A pilot study

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Received: 20 December2018 / Received in revised form: 15 April 2019, Accepted: 20 April 2019, Published online: 28 April
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Abstract

Aim: The current study was designed to investigate the genetic variation of cancer critical gene namely GST in patients with HCC and healthy individuals from NE region of India. Consecutive 40 confirmed cases of HCC and 80 cases of age and sex-matched healthy individuals were included in accordance with the 1975 Helsinki guidelines. The DNA extraction was done from whole blood in all the cases by DNA extraction kit followed by PCR amplification and RFLP. Representative amplicons were sent for commercial sequencing. Results were analyzed using Mutation Taster. GSTM1 non-null genotype was found in 16 out of 40 (40%) cancer cases whereas it was found in 66 out of 80 (82.5%) control cases. GSTM1 null genotype was observed in 24 out of 40 (60%) cases and 14 out of 80 (17.5%) control cases, respectively. It was observed that people with GSTM1 null genotype had seven times higher risk of developing liver cancer compared to people harboring GSTM1 non-null type. GSTT1 non-null genotype was found in 29 out of 40 (72.5%) cancer cases whereas it was found in 72 out of 80 (90%) control cases. Again, GSTT1 null genotype was found in 11 out of 40 (27.5%) cases whereas it was 8 out of 80 (10%) control cases. It was observed that people with GSTT1 null genotype had roughly 3 times higher risk of developing liver cancer compared to people harboring GSTT1 non-null type. The study would be greatly beneficial if multiple cancer critical genes and their synergistic effect with GST gene polymorphism have been studied in a large population. Nonetheless, the present study will generate some baseline data pertaining to HCC in the northeastern population with distinct genetic makeup.

Keywords: HCC, North East India, GST, Polymorphism, PCR, sequencing.

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Introduction

Recently, it has been revealed that cancer death rate is 40% higher than the past decade (Alkhatib, Nori and Al-Ghamdi , 2017), especially in low- and middle-income countries (Abd El-Hameed and Sayed, 2018). Hepatocellular carcinoma (HCC) is the most reoccurring malignancy representing the fifth most prevalent tumor all over the world and the third cause of the death from cancer (Al-Bishri, 2017). HCC is responsible for over 26,000 deaths every year in the United States where the incidence of HCC is approximately 3 per 100,000 populations (Edwards and Myers, 2007). In the United States, HCC is observed more common in people of East Asian origin. In the U.S., a huge number of hepatocellular carcinoma cases were observed during the investigation of cases of liver disease (Deponte, 2013).

Some of the prominent proofs demonstrate that HCC in humans is a multistage process that involves multiple risk factors, remarkably the hepatitis B and C viruses and alcohol (Xie et al., 2015; Shittu et al., 2015; Hayes and Strange, 2000). The virus is reported as second hazardous carcinogen in the world after smoking (Shakeri et al., 2018). Smoking has also been considered as a possible factor for liver cancer, but the link between cigarette smoking and the possible risk of HCC occurrence is unsure (Kucukkal et al., 2014; Sturchio et al., 2008; Bhattacharjee et al., 2013). These two and other probable factors, like aflatoxin (Sirivarasai et al., 2013) and diabetes mellitus (Palmer et al., 2006), may act together to extend the risk of HCC (De Araújo et al., 2014).

Human health or development of the disease is highly influenced by the association between different gene expression and the environment (Wang et al., 2015). Among all the risk factors, the most highly studied genetic risk factors for HCC is the mutants of glutathione S-transferases (GSTs) which is an enzyme family that is a dimeric protein, seen in all kingdoms (Xie et al., 2015; Rahbar et al., 2014; David, 2011; Giménez Ortiz and Montalar Salcedo, 2010). Recent findings have revealed the significance GSTs, and its susceptibility to certain diseases where GSTs play a crucial role in Phase II detoxification enzymes pathway in humans, and protect human body against toxins by catalyzing toxin or passively binding to numerous exogenous/endogenous toxic molecules, including environmental toxins, carcinogens,

chemotherapeutic agents, or products of oxidative stress (Xie et al., 2015; Shittu, 2015; Sturchio et al., 2008). They also play a role in blocking cellular mutations and helping in antioxidant defense mechanism. Some GSTs, which undergo polymorphisms, raise an impact in the relationship between specific allelic variation and the chance of resulting a disease (Sirivarasai et al., 2013; Palmer et al., 2006; De Araújo et al., 2014; Wang et al., 2015; Rahbar et al., 2014).

The GST supergene family specify seven different classes of dimeric enzymes that are able to affect conjugation reactions between glutathione and chemical carcinogens, which allows epoxides to accelerate their excretion and thereby detoxify those carcinogens (Wang et al., 2015; David, 2011). Thereby, GSTs are considered to play a major role against carcinogenesis. Among the GST supergene family, only class µ1 (GSTM1) and class θ1 (GSTT1) have gene deficiency (null genotype) phenotypes that are specified by a complete lack of enzyme activity (Didelot et al., 2007). Complete abolition of enzymatic function is seen when a partial deletion in the coding region is done and thus the body is unable to metabolically eliminate carcinogenic compounds (Rahbar et al., 2014). The present study was designed to investigate the potential association between the GSTM1 and GSTT1 genotypes 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.

Materials and Methods

The study included 40 cases of liver disease and 80 healthy cases recruited from the downtown Hospital and NE cancer hospital, Guwahati, Assam from January 2016 to July 2017. The cases were recruited in accordance with the ethical guidelines of Helsinki, 1975 and a form was filled based on a personal interview to analyze the associated etiological factors. The healthy individuals were the volunteer blood donors from Assam down town University and the total number of cases included in this category was 80. The ethical committees of Assam down town University, Guwahati, India, has approved the present study.

Blood samples were collected in EDTA vials from all the cases. A total of 5 ml of whole blood was drawn and stored in -4 ℃ until further use. DNA extraction from whole blood was done by commercial DNA extraction Kit method (Wizard Genomic DNA extraction kit, Promega). The extracted DNA was stored at 2-8 ℃ for future use. Detection of DNA was done by gel electrophoresis and observed under the E-gel imager (Life Technologies).

Genomic DNA (50–100 ng) was amplified in a total volume of 25 µl reaction mixture consisted of 2.0 µl DNA, 2 µl Buffer, 1.0 µl dNTP, 1.0 µl $Mgcl_2$:, 0.5µl P1 (F), 0.5 µl P1 (R), 0.15 µl taq polymerase and 17.85 µl dH_2O. The reaction mixture was subjected to an initial denaturation at 94 ℃ for 10 min, followed by 30 cycles of 95 ℃ for 60 min, 55.7 ℃ for 45 min and 72 ℃ for 45 min. The final extension was done at 72 ℃ for 10 min. The amplified products were analyzed on 2% agarose gel stained with ethidium bromide and observed under E-gel imager (Life Technologies). 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 an indicator of a successful PCR (Fig. 1). The primers used are tabulated in table 1.

Table 1: Primers Used for Gst Gene Amplification

GST M1	Forward	5'-GAACTCCCTGAAAAGCTAAAGC -3'
	Reverse	5'-GTTGGGCTCAAAT ATACGGTG G-3'
GST T1	Forward	-TCTTACTGGTCCTCACATCTC-3'
	Reverse	5'-TCACCGGATCATGGCCAGC C-3'

Results:

The demographic profile of the recruited cases showed that males are more prone to the disease and male sex was found to have an increased risk of developing hepatocellular carcinoma by two fold. Although alcohol was not recorded as a risk factor, smoking was found to be associated with 1.3 times higher association in the diseased category.

Table 2: Distribution of Demographic And Etiologic Variables among Hcc And Controls

Variables	HCC cases N=40 (%)	Control N= 80 (%)	OR (95% CI)	P value
Gender				
Male	32 (80%)	51 (63.75%)	**2.275** [0.9258-5.588]	**0.035**
Female	08 (20%)	39 (36.25%)		
Age range (years)				
<= 45	7 (17.5%)	47 (58.75%)	**3.31**[1.307- 8.382]	
> 45	33 (82.5%)	33 (41.25%)		
HBV infection				
Negative	13 (32.5%)	00		
Positive	27 (67.5%)	00		
HCV infection				
Negative	36 (90%)	00		

POSITIVE	04 (10%)	00		
ALCOHOL INTAKE (ML/DAY) 0-60 61-100 >100	02 (5%) 08 (20%) 21 (37.5%)	12 (15%) 28 (35%) 39	0.2939 (0.06244 - 1.383) 0.6335 (0.2507 - 1.601) **2.154** (0.9 - 5.155)	
SMOKING STATUS (PACKS/YEAR) 0 1-20 >20	09 (22.5%) 13 (32.5%) 18 (45%)	35 (43.75%) 22 (27.5%) 23 (28.75%)	.3733 (0.1574 - 0.8853) 1.269 (0.557 -2.893) 2.028 (0.9213 - 4.463)	

GSTM1 non-null genotype was found in 16 out of 40 (40%) cancer cases whereas it was found in 66 out of 80 (82.5%) control cases. GSTM1 null genotype was observed in 24 out of 40 (60%) cases and 14 out of 80 (17.5%) control case, respectively. It was observed that people with GSTM1 null genotype had 7 times higher risk of developing liver cancer compared to people harboring GSTM1 non-null type.

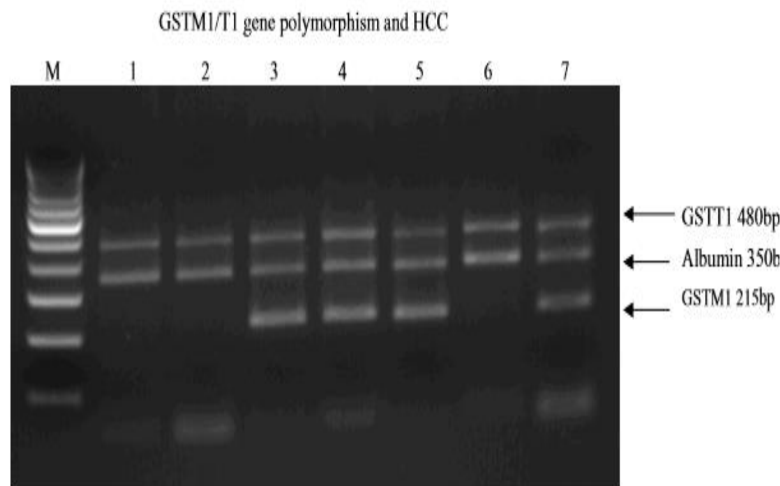

Figure 1: Gel Photograph Showing Amplicons For Gstt1, 480 Bp [Well 1-7]; Gst M1, 215 Bp [Well 3,4,5,7] And Housekeeping Gene, Albumin, 350 Bp [All Wells].

GSTT1 non-null genotype was found in 29 out of 40 (72.5%) cancer cases whereas it was found in 72 out of 80 (90%) control cases. Again, GSTT1 null genotype was found in 11 out of 40 (27.5) cases whereas it was 8 out of 80 (10%) control cases [Table 1]. It was observed that people with GSTT1 null genotype had roughly 3 times higher risk of developing liver cancer compared to people harboring GSTT1 non-null type.

Table 3: Distribution of Various Genotypes

Genotypes	Cases (%) n = 40	Controls (%) n = 80	OR (95%CI)
GSTM1 non-null	16 (40)	66 (82.5)	Reference
GSTM1 null	24 (60)	14 (17.5)	**7.0714** [3.0043 to 16.6446]
GSTT1 non-null	29 (72.5)	72 (90)	Reference
GSTT1 null	11 (27.5)	08 (10)	**2.9923** [1.1218 to 7.9821]

- OR estimates for each gene, in all subjects, adjusted for age, sex, area of recruitment, and alcohol consumption.

The allelic distribution showed a high prevalence of AA genotype both in HCC (90%) and control cases (66.25%). The heterozygous AB allele was observed in 30% of control and 10% of HCC cases while BB genotype was observed in 3 cases from control cases. [Table 2]. Allele A was found to have a 4 times higher association than allele B with respect to the disease.

Table 4: Allelic Distribution of Gst Gene

Groups	N	Genotype distribution			Allele frequencies (%)		OR	P value
		AA	AB	BB	A	B		
Control	80	53 (66.25)	24 (30)	3 (3.75)	130 (81.25)	30 (18.75)	**4.3846** [1.4875 to 12.9241]	0.0074
HCC	40	36 (90)	4 (10)	0	76 (95)	4 (5)		

Discussion

Male sex has been observed as a risk factor in the current study which is in line with almost all previous studies where the range is between 1.4-3.3 (Ferlay et al., 2001). The study findings of an increased risk of 2.154 was recorded with respect to heavy alcohol consumption cases which is in accordance with numerous previous reports which are summarized in a study by Chun et. al. (2015) Older age was found to be a risk factor which was previously reported by Asim et. al. (2010) and is found to increase the risk by roughly 3 fold. However, smoking was found to be positively correlated with a two-fold risk which has already been documented in a previous study (Asim et. al., 2010).

The current study confirms the role of GSTM1 null genotype as a risk factor for the development of HCC and was found with 7 fold increased risk in patients harboring the genotype. On the other hand, GSTT1 null genotype was associated with a three-fold increase in risk for the disease. Both these findings are well in support with numerous previous reports on GST gene polymorphism. (Asim et. al., 2010; Ladero et al., 2006; Munaka et al., 2003; Covolo et al., 2005; Long et al., 2005; Boccia et al., 2015; De Mattia et al., 2017) This is a case-control study from North-Eastern part of India and boost to be the only study carried out from this part of the country and the slight variation in the OR than the study carried out by Asim et. al., may be attributed to the fact that the current study population has completely different genetic makeup which has been documented in previous studies (De Mattia et al., 2017).

Conclusion

However, the current pilot study reports male sex, heavy alcohol consumption, smoking and older age as the risk factors for HCC. GST M1 and T1 null genotypes were found to be independent risk factors. The study would have been greatly benefitted if multiple cancer critical genes and their synergistic effect with GST gene polymorphism would have been studied in a large sample. Nonetheless, the present study will generate some baseline data pertaining to HCC in the northeastern population with distinct genetic makeup.

Acknowledgment

The authors acknowledge the laboratory facility provided by Assam down town University and NE cancer hospital and down town hospital for providing samples and clinical data.

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