RESEARCH ARTICLE

Editorial Process: Submission:02/08/2022 Acceptance:08/02/2022

Genomic Pattern of *GSTM1* and *T1* Gene Null polymorphism of Head and Neck Cancer Patients in Eastern India

Richa Surit, Santosh Kumar*, Dinesh Kumar Sinha, Ravi Shekhar

Abstract

Objective: Homozygous deletion i.e., null polymorphism of the Glutathione S transferases genes hinders detoxification reactions by altering the sensitization of glutathione s transferases enzymes. Hence, we analysed the association between the *GSTM1* and *GSTT1* gene polymorphisms and head and neck cancer (HNC). **Methods:** The study consists of 238 healthy controls and 160 diagnosed cases of HNC, who attended the Regional Cancer Centre, Indira Gandhi Institute of Medical Sciences (a tertiary care hospital). DNA was extracted from whole blood of patients and control using Qiagen DNA extraction kit. *GSTM1* and *GSTT1* gene polymorphisms were examined using PCR and agarose gel electrophoresis. **Results:** GSTM0 null polymorphism was 26.25% and 15.13% in cases and control respectively. GSTT0 null polymorphism was observed in 18.13% cases and 8.82% in control groups. The GSTM0 null polymorphism was present significantly in case group as compared to control group (OR = 1.997, p = 0.006). There was also significant association of GSTT0 null polymorphism with case group as compared to control group (OR = 2.288, p = 0.006). The combined genotypes were also analysed. *GSTM0T1* genotype (n = 27) was found to be most common among HNC group followed next by GSTM0T0 double deletion (n =15). **Conclusion:** The result indicated that there was strong association of GSTM0 and GSTT0 null polymorphism in those patients. The combined genotypes i.e., GSTM0T1 and GSTM0T0 null polymorphism also showed significant association in HNC patients.

Keywords: Glutathione-s-transferase M1 gene- Glutathione-s-transferase T1 gene- Null polymorphism

Asian Pac J Cancer Prev, **23 (8)**, 2655-2659

Introduction

Head and neck cancer (HNC) is the sixth leading cancer worldwide. More than 90% of these cancers are squamous cell carcinomas with the rest 10% being lymphomas, adenocarcinomas, and sarcomas (Parkin et al., 2005). The Global Adult Tobacco Survey reported the data of tobacco use in Bihar (2016-2017). There were 25.9% adults, who were using smoke or smokeless tobacco (Global adult tobacco survey, 2016). Addiction of smoking, tobacco chewing (smokeless tobacco), or alcohol produces oxidative stress which play important role in pathogenesis of HNC. The tobacco smoke contains many carcinogens, including polycyclic aromatic hydrocarbons, mono halomethanes and nitroso compounds. The important detoxification systems like Cytochrome P-450s (CYP450s) and Glutathione-S-transferases enzymes (GSTs) play significant role in the xenobiotics. This system shows altered activation due to genetic variation, which may be responsible for differences insusceptibility of cells to chemical carcinogens. The isoenzymes of GSTs like Glutathione-s-transferases M1 (*GSTM1*), Glutathione-s-transferases T1 (GSTT1) detoxifies the reactive metabolites of benzo[a]pyrene and other polycyclic aromatic hydrocarbons. Absence of *GSTM1* enzyme activity which is due to homozygous deletion i.e., null polymorphism of the *GSTM1* gene hinders detoxification reactions (Hayes, 1995).

Studies shows that the deletion of *GSTM1* gene might contribute to the tumorigenesis and progression of nasopharyngeal cancer (Zhou et al., 2009). Few studies also suggested that the deletion polymorphism of *GSTM1* gene may increase the risk of HNC development by about twofold (Trizna et al., 1995; Kihara et al., 1997; Lafuente et al., 1993).

A similar polymorphism of the GSTT1 gene, encoding the theta class enzymes, has been described (Pemble et al., 1994). GSTT1 enzyme metabolizes various potential carcinogens such as mono halomethanes, which are widely used as methylating agents, pesticides, and solvents. Studies indicate that deletion of null polymorphism of *GSTT1* gene in about 38% of the population leads to deficiency of antioxidant enzymes. This mutation has been significantly associated with several types of tumours (Kempkes et al., 1996; Brockmoller et al., 1996; Deakin et al., 1996).

Department of Biochemistry, Indira Gandhi Institute of Medical Sciences Sheikhpura, Patna, Bihar, India.
**For Correspondence: santoshjik6@gmail.com*

Hence, the aim of study was to analyse the association of *GSTM1* and *GSTT1*gene null polymorphisms in pathogenesis of head and neck cancer.

Materials and Methods

One hundred sixty cases of diagnosed HNC and 238 controls were included in this case control study. After the patient's consent, 3 ml of blood sample was drawn from each patient. A study pro forma was recorded for each patient's detail about age, sex, personal history, clinical history, and general examination. All samples were analysed for *GSTM1* and *GSTT1* gene.

This case control study was conducted for one year under Department of Biochemistry and Department of Oncology. The study was approved by the Institutional Ethics Committee for Human Research. The case group was selected from oncology department, who were diagnosed cases of HNC, which included malignancy developed in or around the throat, larynx, nose, sinuses and mouth. The control group was selected from patient attending medicine department who do not underwent any treatment for chronic illness.

DNA extraction and GSTM1 and GSTT1 analysis

Genomic DNA was isolated from whole blood using Qiagen DNA extraction kit (spin column based) according to manufacturer's instruction. The specific primer pairs of *GSTM1*, *GSTT1* and β globulin were used (Table 1). β globulin gene (299bp) was used as internal control. PCR was performed using Amplitaq Gold master mix. All genes were amplified separately on their best annealing temperature in three different set of 50µl reactions. The amplified products were pooled. For each reaction, 25µl of Amplitaq Gold master mix, 5µl (200ng approx.) of genomic DNA, 1µl of 10µM stock of each primer and 18µl nuclease free water were used. The PCR cycling conditions consisted of initial denaturation at 94°C for 5 minutes followed by 35 cycles of denaturation at 94°C for 1 minute, annealing at 60°C for 1 minute, extension at 72°C for 2 minutes and final extension at 72°C for 5 minutes. All three set of PCR reaction for each subject were run at the same time in same thermocycler (Veritiflex, Applied Biosystems). The amplified products were pooled and 10µl of each pooled product was run along with 50 bp DNA ladder on 2.5 % agarose gel containing ethidium bromide. Bands of the product were visualized under UV transilluminator. The presence or absence of 219 bp and 480 bp bands were used to group combined genotypes as M1T1, M1T0, M0T1 and M0T0.

Statistical analysis

The groups were compared and OR was calculated using the Pearson chi-square test. P value < 0.05 was considered statistically significant. Statistical analyses were performed using SPSS (IBM, version 16).

Results

A total of 160 cases of diagnosed HNC and 238 controls were included in this study. The mean age in males (n= 135) and females (n= 25) cases were 51.21±13.10 years and 59.12±8.87 years respectively. The personal history for tobacco, betel, cigarette/beedi and/or alcohol were 72% in male cases and 5% of tobacco intake in females (Table 2). The control group had 90% male and 10% females with 35% personal history of addiction in males and 2% in females. The genotyping of samples

Table 1. GSTM1, GSTT1 and β globulin Gene Specific Primers

Primer	Sequences	Base pair
GSTM1	5'-GAA CTC CCT GAA AAG CTA AAG C-3'	219 bp
	5'-GTT GGG CTC AAA TAT ACG GTG G-3'	
GSTT1	5'-TTC CTT ACT GGT CCT CAC ATC TC-3'	480 bp
	5'-TCA CCG GAT CAT GGC CAG CA-3'	
β- globin(Internal Control)	5'-ACA CAA CTG TGT TCA CTA GC-3'	299 bp
	5'-CTC AAA GAA CCT CTG GGT CC-3'	

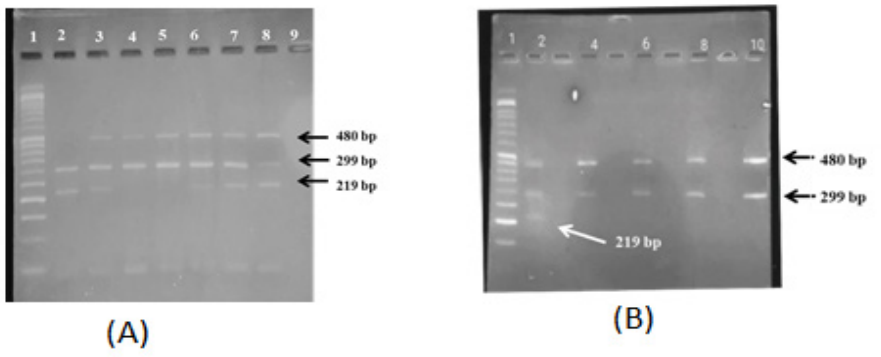

Figure 1. Showing the Polymorphic GST Gene PCR Bands on the Agarose Gel Electrophoresis. GSTM1 (219 bp), GSTT1(480 bp) and β-globulin (299 bp) fragments. A, gel run of controls-Lane 1 -50bp DNA Ladder; Lane 2 - GSTM1T0; Lane 3 ,6,7,8 - GSTM1T1; Lane 4 & 5- GSTM0T1. B, gel run of cases; Lane 1 - DNA Ladder; Lane 2 - GSTM1T1; Lane 4,6,8,10 - GSTM0T1.

Table 2. Demographic Data of Patients (Cases)

Characteristics	Cases n (%)	Control n (%)
Gender		
Male	135 (84.4)	214 (89.9)
Female	25 (15.6)	24 (10.1)
Age		
Mean± SD	52.13 ± 1.30	50.28 ± 9.54
Range (years)	27 - 77	23 - 68
Addiction		
No addiction	32 (20.0)	155 (65.1)
Tobacco and betel	38 (23.8)	40 (16.8)
Alcohol	24 (15.0)	10 (4.2)
Cigarette/ beedi	44 (27.5)	25 (10.5)
Mixed (of above three)	22 (13.8)	8 (3.4)
Tumour site		
Oral cavity	80 (50.0)	….
Nasal cavity	13 (8.1)	….
Pharynx	26 (16.3)	….
Larynx	25 (15.6)	….
Glands	16 (10.0)	….

Table 3. Comparative Evaluation of GST Genotypes Showing Frequency, Odd Ratio, Confidence Interval, and p values between GSTM0/GSTM1, GSTT0/GSTT1, GSTM0T0/ Presence of any *GST* Gene.

Genotype	Case	Control	OR	95%CI	P value
GSTM0 (null)	42	36	1.99	1.21-3.29	0.006
GSTM1	118	202			
GSTT0 (null)	29	21	2.288	1.25-4.17	0.006
GSTT1	131	217			
GSTM0T0 (null)	15	10	2.75	1.19-6.35	0.014
GSTM1/GSTT1/ Both	104	191			

showed genetic polymorphisms of *GSTM1* and *GSTT1* genes (Figure 1). All samples had well amplified β globin gene band which was used as internal control. The length of bands w as compared from DNA ladder.

The *GSTM0* gene null polymorphism was 26.25% and 15.13% in cases and control respectively whereas distribution of *GSTM1* gene was 73.75% in cases and 84.87% in control groups. There was a strong association of GSTM0 null polymorphism in case group as compared to control group (OR = 1.997, p= 0.006).

Similarly, GSTT0 gene null polymorphism was observed in 18.13% cases and 8.82% in control groups. *GSTT1* gene was 81.87% in cases and 91.18% in controls. There was weak but significant association (Phi Cramer's V measure = 0.138) of *GSTT0* gene null polymorphism in case group as compared to control group (OR = 2.288, p=0.006). The *GSTM1* and *GSTT1*, both genes were absent in 9.38% and 4.2% in cases and control respectively. There was almost 2.8 times risk of developing head and neck cancer in null polymorphism for both GSTM and GSTT compared to controls with both *GSTM1* and GSTT1 (OR = 2.75, p= 0.014) (Table 3).

Table 4. Cases and Control in Combined Genotype Groups. M0T1 - GSTM1 absent & GSTT1 present; M1T0 - GSTM1 present & GSTT1 absent; M0T0 - GSTM1 absent & GSTT1 absent; M1T1 - GSTM1 present & GSTT1 present.

GST	M0T1	M1T0	M0T0	M1T1	P Value
CASES (n=160)	27	14	15	104	0.016
CONTROLS (n=238)	26	11	10	191	

A frequency distribution of combined genotype *GSTM0T1, GSTM1T0, GSTM1T1* and *GSTM0T0* genes were summarized in (Table 4). *GSTM0T1* genotype (n=27) was found to be most common among HNC group followed by *GSTM0T0* gene double deletion (n= 15) (Table 4).

Discussion

The glutathione-S-transferases (GSTs) are a family of multifunctional enzymes that play a vital role in the neutralization of carcinogenic electrophiles. This family of enzymes encoded by eight gene families -alpha, kappa, mu, omega, pi, sigma, theta and zeta. *GSTM1* gene encoded the GSTs enzymes that are involved in phase II detoxification reaction of compounds, including xenobiotics, pesticides, environmental carcinogens and some chemotherapeutic drugs. Homozygous deletion of *GSTM1* gene (locus 1p13.3) and GSTT1gene (locus 22q11.23) result in null polymorphism and compromise the function of GSTs enzymes (Curtin et al., 2012; Singh et al., 2009; Chen et al., 2008). Charles Lu et al told in his studies that the variant genotypes affect the activity of GSTs enzymes, which in turn may lead to decreased ability to detoxify carcinogenic and mutagenic electrophiles (Charles et al., 2006).

Homozygous deletion of *GSTM1* gene leads to stifle the GSTs enzymes activity towards detoxification of the reactive metabolites like benzo[a]pyrene and other polycyclic aromatic hydrocarbons. We analysed the *GSTM1* gene and its null genotype and found null genotype GSTM0 was strongly associated with HNC. It was found that there was two fold risk of HNC in *GSTM1* gene null polymorphism. Some case-control studies in HNC have also suggested that the deletion polymorphism of GSTM1 gene may increase the risk by about two fold (Mathais et al., 1998; Gonzalez et al., 1998; Cheng et al., 1999). In contrast, few studies deny it (Ophuis et al., 1998).

GSTT1 enzyme related gene polymorphism also show effect on antioxidant activities. GSTT1 enzyme metabolizes various potential carcinogens such as mono halomethanes, which are widely used as methylating agents, pesticides and solvents (Khan et al., 2014). A study demonstrated that the effect of *GSTT1* gene showed a modest, but not significant elevation in the odds ratio of HNC tumours development among cases (Trizna et al., 1995). Another study failed to justify any association of the GSTT1 enzyme deficiency with oral cancer (Deakin et al., 1996). We also analyse *GSTT1* gene null polymorphism and found that that it has significant but weak association with HNC cases.The combined null polymorphism

(GSTM0T0) showed a strong association with HNC cases with almost 2.8 times increased risk (Table 3).

We also analyse the frequency distribution of combined genotype groups of *GSTM1* and *GSTT1* genes (Table 4). Result showed significant association in null polymorphism and HNC. This result was found to be similar with a study that concluded that patients with larynx cancer present more *GSTM1* and *GSTT1* gene null polymorphisms (Sánchez-Siles et al., 2020). In contrasts other studies found no significant association (Hung et al.,1997). Polymorphisms of these genes leads to differences in the level of susceptibility of individuals to the potential adverse effects of environmental influences, particularly to tobacco smoke, products of oxidative stress and other toxic agents.

In conclusion, there is strong association of *GSTM1* gene null polymorphism with HNC patients. GSTs enzymes have an essential role in protection of DNA from genotoxic damage by inhibiting the formation oxidants. These enzyme groups play an important role in protecting tissue from oxidative reaction and its subsequent damages. These genetic parameters open wider window that how a cell act to any insult. These can be utilized to detect more vulnerable groups among population so that, they can be screened out and primary prevention can be taken.

This is the first study of its type, done in a tertiary care centre in this area. All patients who smoke or chew tobacco do not develop cancer. This may be due to the individual's genetic susceptibility. Genetic detailed studies can become a more important path for primary prevention.

Author Contribution Statement

RS, SK and RS performed the experimental work and data analysis. DKS studied the clinical subjects and collected the related data. All authors participated in the design of the study. RS and SK wrote the manuscript. All authors read and approved the final manuscript.

Ethical Approval

This study was approved by Institutional Ethical Committee, IGIMS, Patna.

Availability of data

The data related to current study are available from the corresponding author on request.

Conflict of interest

The authors declare that no conflict of interests was present during the study.

References

Brockmoller J, Cascorbi I, Kerb R, et al (1996). Combined analysis of inherited polymorphisms in aryl amine N-acetyltransferase-2, glutathione S-transferases M1 and T1, microsomal epoxide hydrolase and cytochrome P450 enzymes as modulators of bladder cancer risk. *Cancer Res*, **56**, 3915–25.

Charles L, Margaret RS, Hua Z et al (2006). Association between Glutathione-S-transferase II polymorphism and survival in patients with advanced non-small cell lung carcinoma (NSCLC). *Cancer*, **106**, 441-7.

Chen HC, Hu WX, Liu QX, et al (2008). Genetic polymorphisms of metabolic enzymes CYP1A1, CYP2D6, GSTM1 andGSTT1 and leukemia susceptibility. *Eur J Cancer Prev*, **17**, 251–8.

Cheng L, Sturgis EM, Eicher SA, Char D (1999). Glutathione-S-transferase polymorphisms and risk of squamous-cell carcinoma of the head and neck. *Int J Cancer*, **84**, 220–4.

Curtin NJ (2012). DNA repair, dysregulation from cancer driver to therapeutic target. *Nat Rev Cancer*, **12**, 801–17.

Deakin M, Elder J, Hendrickse C, et al (1996). Glutathione S-transferase GSTT1 genotypes and susceptibility to cancer: studies of interactions with GSTM1 in lung, oral, gastric and colorectal cancers. *Carcinogenesis*, **17**, 881–4.

Global adult tobacco survey fact sheet, Bihar 2016-2017. http://tiss.edu/uploads/files/15_BH.pdf Last accessed 29 Dec 2021.

Gonzalez MV, Alvarez V, Pello MF, et al (1998). Genetic polymorphism of N-acetyltransferase-2, glutathione S-transferase-M1, and cytochromes P450IIE1 and P450IID6 in the susceptibility to head and neck cancer. *J Clin Pathol*, **51**, 294–8.

Hayes JD, Pulford DJ (1995). The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. *Crit Rev Biochem Mol Biol*, **130**, 445-600.

Hung HC, Chuang J, Chien YC, et al (1997). Genetic polymorphisms of CYP2E1, GSTM1, and GSTT1; environmental factors and risk of oral cancer. *Cancer Epidemiol. Biomarkers Prev*, **6**, 901–5.

Kempkes M, Golka K, Reich S, et al (1996). Glutathione S-transferase GSTM1 and GSTT1 null genotypes as potential risk factors for urothelial cancer of the bladder. *Arch Toxicol*, **71**, 123–6.

Khan SU, Mahjabeen I, Malik FA, Kayani MA (2014). Prognostic Significance of Altered Blood and Tissue Glutathione Levels in Head and Neck Squamous Cell Carcinoma Cases. *Asian Pac J Cancer Prev*, **15**, 7603-9.

Kihara M, Kihara M, Kubota A, et al (1997). GSTM1 gene polymorphism as a possible marker for susceptibility to head and neck cancers among Japanese smokers. *Cancer Lett*, **112**, 257–62.

Lafuente A, Pujol F, Carretero P, et al (1993). Human glutathione S-transferase mu (GST mu) deficiency as a marker for the susceptibility to bladder and larynx cancer among smokers. *Cancer Lett*, **68**, 49–54.

Matthias C, Bockmuhl U, Jahnke V, et al (1998). Effect of gene polymorphism on detoxifying glutathione-S-transferase enzymes on chromosomal stability of squamous epithelial carcinomas in the area ofthe head-neck. *Laryngorhinootologie*, **77**, 201-6.

Ophuis MBO, Lieshout EM, Roelofs HM, et al (1998). Glutathione S-transferase M1 and T1 and cytochrome P4501A1 polymorphisms in relation to the risk for benign and malignant head and neck lesions. *Cancer*, **82**, 936–43.

Parkin DM, Bray F, Ferlay J, Pisani P (2005). Global cancer statistics, 2002. *CA Cancer J Clin*, **55**, 74-108.

Pemble S, Schroeder KR, Spencer SR (1994). Human glutathione-S-transferase (GSTT1): cDNA cloning and the characterization of a genetic polymorphism. *Biochem J*, **300**, 271–6.

Sánchez-Siles M, Pelegrín-Hernández JP, Hellin-Meseguer D, et al (2020). Genotype of Null Polymorphisms in Genes GSTM1, GSTT1, CYP1A1, and CYP1A1*2A (rs4646903 T>C)/CYP1A1*2C (rs1048943 A>G) in Patients with

Larynx Cancer in Southeast Spain. *Cancers*, **12**, 2478.
Singh MS, Michael M (2009). Role of xenobiotic metabolic enzymes in cancer epidemiology. *Methods Mol Biol*, **472**, 243–64.
Trizna Z, Clayman GL, Spitz MR, et al (1995). Glutathione s-transferase genotypes as risk factors for head and neck cancer. *Am J Surg*, **170**, 499–501.
Zhuo WL, Wang Y, Zhuo XL, et al (2009). Polymorphisms of CYP1A1 and GSTM1 and laryngeal cancer risk: evidence-based meta-analyses. *J Cancer Res Clin Oncol*, **135**, 1081-90.