RESEARCH ARTICLE

Impact of Tobacco on Glutathione S Transferase Gene Loci of Indian Ethnics

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Abstract

Background: Tobacco contains agents which generate various potent DNA adducts that can cause gene mutations. Production of DNA adducts may be neutralized by glutathione S transferase (GST) along with other phase I and phase II enzyme systems. The existence of null type of GST among the population increases the susceptibility to various disorders and diseases. The present study focuses on the impact of high tobacco usage and possible null type mutation in GST loci. Methods: Genotypes of GST were detected by multiplex polymerase chain reaction in unrelated 504 volunteers of high tobacco using natives of Gujarat. Allelic frequencies were calculated using Statistical Package for Social Studies-16 software. Hardy Weinberg Equilibrium (HWE) was calculated using Chi square test. Two sided Fisher's significance test was used to compare allelic frequencies of different populations. Results: The frequency of homozygous null genotype of GSTM1 and GSTT1 were 20% (95% CI 16.7-23.9) and 35.5% (95% CI 31.4-39.9) respectively. The GSTM1 and GSTT1 null allele frequency distribution in the Gujarat population was significantly deviating from HWE. GSTT1 null frequency of Gujaratians was significantly higher and different to all reported low tobacco using Indian ethnics, while GSTM1 was not differing significantly. Conclusion: Tobacco usage significantly influences the rate of mutation and frequency of GSTT1 and M1 null types among the habituates. The rate of mutation in GSTT1 loci was an undeviating response to the dose of tobacco usage among the population. This mutational impact of tobacco on GSTT1 postulates the possible gene - environment interaction and selection of null genotype among the subjects to prone them under susceptible status for various cancers and even worst to cure the population with GSTT1 dependent drugs.

Keywords: Tobacco usage - DNA adducts - GSTT1 - GSTM1 - mutation - susceptible - Indian Ethnics

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Introduction

Tobacco contains different carcinogens as DNA and protein adducts in its different form of usage. Polycyclic aromatic hydrocarbons (PAHs), volatile aldehydes, nitrosoamines, nitrosamino acids, phenols, volatile hydrocarbons, nitro compounds, organic and other inorganic compounds were found as carcinogenic agents in tobacco. Several compounds in tobacco like PAH, benzo (a) pyrene diol epoxide, 7-methylguanine, hydroxypyridyloxobutyl , 8-Oxo-deoxyguanosine, lipid-peroxidation derived DNA adducts were few among the tobacco-carcinogen biomarkers (Kriek et al., 1998; Tang et al., 2001; Phillips, 2002; Boysen et al., 2003; Stephen, 2003). Drug/Xenobiotic compounds metabolism is essential for biotransforming hazardous pollutants or potent carcinogenic intermediates to their non toxic forms. The continuous exposure of various DNA adducts during the life time of an individual lead to possible mutations and could end in altered or loss of function of a gene that might regulate the cell cycle, cell division, cell death or other survival factors and cause various cancers (Hecht, 1999; Lacko et al., 2009; Zhuo et al., 2009). Drug/Xenobiotic compound metabolizing enzymes are capable of regulating the removal of this exogenous and endogenous DNA adducts/carcinogens from the cell (Hecht, 2003; Lacko et al., 2009). The mutation observed in GSTM1 or T1 was a deletion mutation of null genotype and completely lost its functions to become susceptible to various cancers or diseases and further the inheritance of the same (Evans et al., 2004; Anantharaman et al., 2007; Mo et al., 2009; Sam et al., 2009; Amer et al., 2011; Chuang et al., 2011; Kumar et al., 2011). In this study, we investigated the impact of tobacco usage on drug/Xenobiotic compound metabolizing GST gene loci mutation. The age old customary habituate of high tobacco usage among Gujarat population was considered as the suitable subject for the study among Indian ethnics reported earlier (Rani et al., 2003). Further we compared the frequency rate of GST polymorphism among the subjects with the low tobacco using other populations within India, reported by various investigators (Roy et al., 1998; Buch et al., 2001; Mishra

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et al., 2004; Naveen et al., 2004; Vetriselvi et al., 2006; Konwar et al., 2010).

Materials and Methods

Subjects

The investigation comprised of 504 healthy (336 male and 168 female) unrelated volunteers of western India from Gujarat origin. The age of individuals ranged from 40-80 with the mean age of 60 years. Two ml of blood samples were collected from volunteers and informed consent was obtained. The institutional ethical committee of Sh.NP Cancer Institute, Rajkot Cancer Society; India, approved the study.

DNA isolation and Genotyping

Genomic DNA was isolated from whole blood by salting out method (Lahiri and Nurnberger, 1991). Multiplex polymerase chain reaction was performed for identification of GSTM1 and T1 null types with albumin gene as internal control (Huang et al., 2006). Primers used were GSTM1-F: 5' GAA CTC CCT GAA AAG CTA AAG C 3', GSTM1-R: 5' GTT GGG CTC AAA TAT ACG GTG G 3', GSTT1-F: 5' TTC CTT ACT GGT CCT CAC ATC TC 3', GSTT1-R: 5' TCA CCG GAT CAT GGC CAG CA 3', Albumin F: 5' GCC CTC TGC TAA CAA GTC CTA 3' and Albumin R: 5' GCC CTA AAA AGA AAA TCG CCA ATC 3'.The amplified 215 bp, 480 bp, and 350 bp were analyzed for GSTM1, GSTT1, and albumin presence respectively. Homozygous null types were identified by the absence of respective bands and the efficiency of the reaction was confirmed by the presence of the albumin band in all the samples. Amplification was done in a 25 μl reaction with 10 μl of 1:10 diluted DNA sample, 200 μM of all dNTPs, 5 pM of the forward and reverse primers for GSTM1, T1 and albumin, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$ and 2.5 U of Taq DNA polymerase.

Stratification of Tobacco Usage among the subjects

The life time tobacco usage as smokeless and smoking form was calculated using the smoking Index and chewing index, **Chewing Index (CI)**=Frequency of chewing events per day×duration in years, **Smoking Index (SI)**=Number of cigarettes/20×duration in years or Number of bidis/5×duration in years. Both smoking Index and chewing index are stratified to six levels as per the usage is concerned from no habit to ≥301, with an increment of 75 SI and CI respectively.

Statistics

Null Allele frequency of GSTM1 and GSTT1 was analyzed using Statistical Package for Social Studies (SPSS 16) software for Windows. Hardy-Weinberg Equilibrium (HWE) Testing for GST was done by using online software of Rodriguez et al. 2009. Two sided Fisher's exact test was done with statistical significance set at $p<0.05$ to compare frequency variation of null alleles among other low tobacco using ethnics reported by various investigators within India, in reference to high tobacco using ethnics of Gujarat.

Results

Genotype frequency of GST among the subjects

Among the 504 samples investigated, GSTM1 and T1 null genotype was 20% and 35.5% respectively and the stratified analysis for polymorphism based on sex revealed that there was significant difference ($P>0.05$) in the null allele frequency for GSTT1 and not for GSTM1 as shown in Table 1. The GST allele frequency distribution in the Gujarat population was not in Hardy Weinberg equilibrium according to chi square test for GSTM1 and T1 (Data not shown).

Tobacco usage among the subjects

Gender based stratified analysis for CI and SI sub population was done to identify the frequency rate for chewing and smoking respectively as shown in Table 2. No chewing was category observed in male, female and among the subjects in common with 27.1%, 33.9% and 29.4% respectively. Low chewing habit with CI of 1-75 was observed high in females (33.9%) than males (6.5%) and among the subjects in common (15.7%). High chewing habit with CI of 226-300 was observed more in males (37.5%) than females (14.3%) and among the subjects in general (29.8%). No smoking habit was observed high in females (91.1%) than to males (15.2%) and among the subjects in general (40.5%). Smoking habit of females was observed only with SI of 1-75 (8.9%); while in males it was observed in all categories.

Table 1. Genotype Frequency of GSTM1 and T1 Among the Subjects

Genotype	*General n (%)	95%CI	Male n (%)	95%CI	Female n (%)	95%CI
GSTM1	504		336		168	
Present	403(80)	76.1-83.3	273(81.2)	76.7-85.3	130 (77.4)	70.3-83.5
+/+	185(36.7)	32.5-41.1	129(38.4)	33.2-43.8	56 (33.3)	26.3-41.0
+/-	218(43.3)	38.9-47.7	144(42.9)	37.5-48.3	74 (44.0)	36.4-51.9
Null	101(20.0)	16.7-23.9	63(18.8)	14.8-23.4	38 (22.6)	16.5-29.7
GSTT1						
Present	325(64.5)	60.1-68.6	196(58.3)	52.9-63.7	129 (76.8)	69.7-82.9
+/+	149(29.6)	25.7-33.8	81(24.1)	16.5-29.7	68 (40.5)	33.0-48.3
+/-	176(34.9)	30.8-39.3	115(34.2)	26.3-41.0	61 (36.3)	29.0-44.1
Null	179(35.5)	31.4-39.9	140(41.7)	36.4-51.9	39 (23.2)	17.1-30.3

*Includes both male and female subjects, GSTT1 null genotype of Male is significantly different to Female at $P<0.001$ level, ($P=0.006353$), GSTM1 null genotype of Male is significantly not different to Female at $P<0.05$ level, ($P=0.6029$), Homozygous wild type=+/+; Heterozygous= +/-; Homozygous null type=Null; Present=either +/+ or +/-, CI-Confidence Interval at 95%

Table 2. Distribution of Tobacco Usage Among the Subjects

Tobacco Usage	*General (504) n (%)	95% CI	Male (336) n (%)	95% CI	Female (168) n (%)	95% CI
Chewing Index						
No Chewing	148 (29.4)	25.5-33.6	91 (27.1)	22.5-32.2	57 (33.9)	26.8-41.6
1-75	79 (15.7)	12.7-19.2	22 (6.5)	4.2-9.9	57 (33.9)	26.8-41.6
76-150	20 (4.0)	2.5-6.2	12 (3.6)	1.9-6.3	8 (4.8)	2.1-9.2
151-225	64 (12.7)	10.0-16.0	47 (14.0)	10.6-18.3	17 (10.1)	6.0-15.7
226-300	150 (29.8)	25.8-34.0	126 (37.5)	32.3-42.9	24 (14.3)	9.4-20.5
≥301	43 (8.5)	6.3-11.4	38 (11.3)	8.2-15.3	5 (3.0)	1.0-6.8
Smoking index						
No Smoking	204 (40.5)	36.2-44.9	51 (15.2)	11.6-19.6	153 (91.1)	85.7-94.9
1-75	192 (38.1)	33.9-42.5	177 (52.7)	47.2-58.1	15 (8.9)	5.1-14.3
76-150	17 (3.4)	2.0-5.5	17 (5.1)	3.1-8.1	0	0.0-2.2
151-225	34 (6.7)	4.8-9.4	34 (10.1)	7.2-14.0	0	0.0-2.2
226-300	35 (6.9)	5.0-9.6	35 (10.4)	7.5-14.3	0	0.0-2.2
≥301	22 (4.4)	2.8-6.6	22 (6.5)	4.2-9.9	0	0.0-2.2

*Includes both male and female subjects; CI-Confidence Interval at 95%

Frequency of GSTT1, M1 Genotypes with Tobacco usage among subjects

Genotypes of homozygous wild type (+/+), heterozygous (+/-), homozygous null type (-/-) and either homozygous or heterozygous (+/+ or +/-) were categorized among the subjects for GSTM1 and GSTT1 gene loci. Distribution pattern of GSTM1 and GSTT1 frequency among the subjects was stratified by chewing index and smoking index to calculate the significant influence of tobacco usage among the subjects (Table 3). The GSTT1 null frequency was significant to the usage of tobacco in both chewing and smoking forms, while GSTM1 null frequency was found significant to smoking alone among the subjects. Distribution pattern of GSTT1

Table 3. Distribution Pattern of GSTT1 and M1 Genotypes with Tobacco Usage Among Subjects

	GSTT1, n (%)				GSTM1, n (%)			
	Present[a]	+/+[b]	+/-[b]	Null[a,b]	Present[a]	+/+[b]	+/-[b]	Null[a,b]
Chewing Index								
No Chewing	120 (39.9)	77 (51.7)	43 (24.4)	28 (15.6)	119 (29.5)	55 (29.7)	64 (29.4)	29 (28.7)
1-75	56 (17.2)	31 (20.8)	25 (14.2)	23 (12.8)	61 (15.1)	27 (14.6)	34 (15.6)	18 (17.8)
76-150	16 (4.9)	6 (4.0)	10 (5.7)	4 (2.2)	19 (4.7)	9 (4.9)	10 (4.6)	1 (1.0)
151-225	30 (9.2)	13 (8.7)	17 (9.7)	34 (19.0)	52 (12.9)	24 (13.0)	28 (12.8)	12 (11.9)
226-300	77 (23.7)	15 (10.1)	62 (35.2)	73 (40.8)	121 (30)	57 (30.8)	64 (29.4)	29 (28.7)
≥301	26 (8.0)	7 (4.7)	19 (10.8)	17 (9.5)	31 (7.7)	13 (7.0)	18 (8.3)	12 (11.9)
Smoking index								
No Smoking	163 (50.2)	95 (63.8)	68 (38.6)	41 (22.9)	162 (40.2)	76 (41.1)	86 (39.4)	42 (41.6)
1-75	131 (40.3)	47 (31.5)	83 (47.2)	62 (34.6)	143 (35.5)	61 (33.0)	82 (37.6)	49 (48.5)
76-150	11 (3.4)	3 (2.0)	8 (4.5)	6 (3.4)	15 (3.7)	7 (3.8)	8 (3.7)	2 (2.0)
151-225	11 (3.4)	2 (1.3)	10 (5.7)	22 (12.3)	32 (7.9)	19 (10.3)	13 (6.0)	2 (2.0)
226-300	6 (1.8)	1 (.7)	5 (2.8)	29 (16.2)	34 (8.4)	13 (7.0)	21 (9.6)	1 (1.0)
≥301	3 (0.9)	1 (.7)	2 (1.1)	19 (10.6)	17 (4.2)	9 (4.9)	8 (3.7)	5 (5.0)

*GSTT1: Chewing Index: [a]Chi Square-41.613, DF-5, P<0.001; [b]Chi Square-85.545, DF-10, P<0.001; Smoking Index: [a]Chi Square-96.765, DF-5, P<0.001; [b]Chi Square-115.902, DF-10, P<0.001, GSTM1: Chewing Index: [a]Chi Square-4.987, DF-5, P>0.05; [b]Chi Square-5.325, DF-10, P>0.05; Smoking Index: [a]Chi Square-15.165, DF-5, P<0.05; [b]Chi Square-19.859, DF-10, P<0.05

Table 4. Distribution Pattern of GSTT1 and M1 Genotypes with Tobacco Usage Among Male Subjects

	GSTT1, n (%)				GSTM1, n (%)			
Chewing Index	Present[a]	+/+[b]	+/-[b]	Null[a,b]	Present[a]	+/+[b]	+/-[b]	Null[a,b]
No Chewing	69 (20.5)	50 (14.9)	19 (5.7)	22 (6.5)	73 (21.7)	36 (10.7)	37 (11.0)	18 (5.4)
1-75	13 (3.9)	7 (2.1)	6 (1.8)	9 (2.7)	17 (5.1)	6 (1.8)	11 (3.3)	5 (1.5)
76-150	11 (3.3)	3 (0.9)	8 (2.4)	1 (0.3)	11 (3.3)	6 (1.8)	5 (1.5)	1 (0.3)
151-225	19 (5.7)	5 (1.5)	14 (4.2)	28 (8.3)	39 (11.6)	18 (5.4)	21 (6.2)	8 (2.4)
226-300	62 (18.5)	9 (2.7)	53 (15.8)	64 (19.0)	104 (31.0)	51 (15.2)	53 (15.8)	22 (6.5)
≥301	22 (6.5)	7 (2.1)	15 (4.5)	16 (4.8)	29 (8.6)	12 (3.6)	17 (5.1)	9 (2.7)
Smoking index								
No Smoking	43 (12.8)	31 (9.2)	12 (3.6)	8 (2.4)	40 (11.9)	21 (6.2	19 (5.7)	11 (3.3)
1-75	122 (36.3)	43 (12.8)	78 (23.2)	55 (16.4)	135 (40.2)	60 (17.9)	75 (22.3)	42 (12.5)
76-150	11 (3.3)	3 (0.9)	8 (2.4)	6 (1.8)	15 (4.5)	7 (2.1)	8 (2.4)	2 (0.6)
151-225	11 (3.3)	2 (0.6)	10 (3)	23 (6.8)	32 (9.5)	19 (5.7)	13 (3.9)	2 (0.6)
226-300	6 (1.8)	1 (0.3)	5 (1.5)	29 (8.6)	34 (10.1)	13 (3.9)	21 (6.2)	1 (0.3)
≥301	3 (0.9)	1 (0.3)	2 (0.6)	19 (5.7)	17 (5.1)	9 (2.7)	8 (2.4)	5 (1.5)

*Homozygous wild type=+/+; Heterozygous=+/-; Homozygous null type=Null; Present=either +/+ or +/-, GSTT1: Chewing Index: [a]Chi Square-27.468, DF-5, P<0.001; [b]Chi Square-83.130, DF-10, P<0.001; Smoking Index: [a]Chi Square-74.576, DF-5, P<0.001; [b]Chi Square-101.209, DF-10, P<0.001, GSTM1: Chewing Index: [a]Chi Square-1.984, DF-5, P>0.05; [b]Chi Square-3.831, DF-10, P>0.05; Smoking Index: [a]Chi Square-13.417, DF-5, P<0.05; [b]Chi Square-17.969, DF-10, P>0.05.

Table 5. Distribution Pattern of GSTT1 and M1 Genotypes with Tobacco Usage Among Female Subjects

	GSTT1, n (%)				GSTM1, n (%)			
Chewing Index	Present[a]	+/+[b]	+/-[b]	Null[a,b]	Present[a]	+/+[b]	+/-[b]	Null[a,b]
No Chewing	51 (30.4)	27 (16.1)	24 (14.3)	6 (3.6)	46 (27.4)	19 (11.3)	11 (6.5)	11 (6.5)
1-75	43 (25.6)	24 (14.3)	19 (11.3)	14 (8.3)	44 (26.2)	21 (12.5)	13 (7.7)	13 (7.7)
76-150	5 (3.0)	3 (1.8)	2 (1.2)	3 (1.8)	8 (4.8)	3 (1.8)	NA	NA
151-225	11 (6.5)	8 (4.8)	3 (1.8)	6 (3.6)	13 (7.7)	6 (3.6)	4 (2.4)	4 (2.4)
226-300	15 (8.9)	6 (3.6)	9 (5.4)	9 (5.4)	17 (10.1)	6 (3.6)	7 (4.2)	7 (4.2)
≥301	4 (2.4)	NA	4 (2.4)	1 (0.6)	2 (1.2)	1 (0.6)	3 (1.8)	3 (1.8)
Smoking index								
No Smoking	120 (71.4)	4 (2.4)	56 (33.3)	33 (19.6)	122 (72.6)	55 (32.7)	31 (18.5)	31(18.5)
1-75	9 (5.4)	NA	5 (3.0)	6 (3.6)	8 (4.8)	1 (0.6)	7 (4.2)	7 (4.2)

Some categories were with zero values and Chi Square significant is Invalid, NA-Not available among the subjects, Homozygous wild type=+/+; Heterozygous=+/-; Homozygous null type=Null; Present=either +/+ or +/-, GSTT1: Chewing Index: [a]Chi Square-10.290, DF-5, P>0.05; [b]Chi Square-17.468, DF-10, P>0.05*; Smoking Index: [a]Chi Square-2.603, DF-1, P>0.05*; [b]Chi Square-2.815, DF-2, P>0.05*, GSTM1: Chewing Index: a Chi Square-7.286, DF-5, P>0.05; [b]Chi Square-8.320, DF-10, P>0.05; Smoking Index: [a]Chi Square-5.442, DF-1, P<0.05*; [b]Chi Square-7.750, DF-2, P<0.05*.

Table 6. Frequencies of Homozygous Deletions at GSTM1 & GSTT1 Loci and Their Comparison with other Indian Population

Location	Sample No.	GSTM1 Null%	P value	GSTT1 Null%	P value	Literature
Gujarat	504	20	Reference	35.5	Reference	Present Study
Karnataka	110	36.4	0.01183*	19.1	0.01091*	Naveen et al., 2004
Lucknow	200	36.5	0.01773*	14	0.0005253***	Konwar et al., 2010
Andhra Pradesh						
	115	33	0.05395	18.8	0.01091*	Naveen et al., 2004
North India	370	33	0.05395	18.4	0.006471**	Mishra et al., 2004
Kerala	122	31.9	0.07561	15.6	0.002018**	Naveen et al., 2004
South India	772	27.7	0.2463	17.09	0.003691**	Naveen et al., 2004; Vetriselvi et al., 2006
Western Central India						
	883	26.6	0.317	13	0.0002481***	Buch et al., 2001
Tamilnadu-Pondicherry						
	170	23.5	0.6089	13	0.0002481***	Naveen et al., 2004
Orissa	72	23.8	0.6089	NA	NA	Roy et al., 1998

*Significance at P<0.05, **Significance at P<0.01, ***Significance at P<0.001, NA-Data Not available

and M1 Genotypes with Tobacco usage among male and female subjects was observed separately to differentiate the level of tobacco influence in mutation (Table 4 and 5 respectively). In male the distribution pattern was found almost similar to the observation seen previously among the subjects (Table 3). But the distribution pattern for GSTT1 and M1 frequency with respect to stratified analysis for CI and SI in female could not predict the significant influence of tobacco usage in null frequencies, as more than one zero values were observed in the frequencies among female subjects (Table 5).

Null type frequency of GSTM1 and GSTT1 among Indian populations

The frequency of GSTT1 and M1 null type of various populations were analyzed and compared to the subjects for their tobacco usage (Rani et al., 2003). GSTM1 null allele frequency of Gujaratians (20%) was not significantly different to other Indian ethnic's frequency of 23% to 33% but GSTT1 null frequency (35.5%) was significantly different (Table 6) from 13% to 19.1% of various Indian populations reported earlier (Roy et al., 1998; Buch et al., 2001; Mishra et al., 2004; Naveen et al., 2004; Vetriselvi et al., 2006; Konwar et al., 2010).

Discussion

India is one of the top ten polluted nations in world and the study subjects were recruited from one of the most polluted states of India alongside high tobacco habituated populations (Rani et al., 2003; Singh and Kohli, 2012). Among the study population, higher null types of GST were observed in high tobacco using individuals of male than female as shown in Table 1. The distribution pattern of GST genotypes with stratified tobacco usage as CI and SI among the subjects was found with an interaction between the dose of tobacco use and null type frequency as illustrated in Table 2. GSTT1 null genotype frequency was significant to tobacco use in both the forms, while GSTM1, significant to smoking tobacco and not for chewing. The distribution patterns for GSTT1 and M1 genotypes were observed to be similar in general and male subjects as in Table 3 and 4, but not for female subjects as shown in Table 5. This difference in female subject's observation was possibly due to less and non frequent tobacco usage. The frequency of GSTT1 null genotype in Gujaratians was high among the other population with low tobacco usage (Rani et al., 2003). And it might be due to high tobacco usage in both chewing and smoking forms among the subjects than the other populations in India (Roy et al., 1998; Buch et al., 2001; Mishra et al., 2004; Naveen et al., 2004; Vetriselvi et al., 2006; Konwar et al., 2010). GSTM1 null frequency among different population in India was not significantly different to Gujaratians, as the influence was moderate due to smoking tobacco form alone and not by chewing form.

Both GSTT1 and M1 genotypes were deviating from HWE among the subjects. This might be due to the existence of recent somatic mutation, which has not attained equilibrium or null alleles (Liu, 2012; Xu, 2012), non-random mating selection (Lauren, 2012), overrepresentation of a certain allele in long time survivors (Nordfors, 2012). We claim this non existence of HWE in GSTT1 and M1 was due to all of the above mentioned reasons among the subjects. We claim that in Gujarat, the high tobacco usage and its related DNA adducts mediated the mutation in GSTT1 and M1 null alleles. The population of Gujarat were monogamy and marriages were done within the same community as non-random mating (Gadgil and Malhoera, 1983) and therefore the overrepresentation of null alleles among the population. In addition, under certain environmental conditions, natural selection of certain phenotypes allow for increased fitness among the populations (Barreiro, 2008; Lordelo, 2012).

In this study a probable gene-environment interaction was observed between high tobacco using subjects and their null type frequency in GST gene loci and in particular GSTT1 than GSTM1. This hypothesizes the impact of tobacco dependent mutation in GST gene loci and provides background for the tobacco dependent DNA adducts effect on null type frequency existence among the subjects. This observation of null type mutation in GSTT1 might have susceptible status or protective role to the individual for various diseases related to tobacco or their respective drug substrates. GSTT1 null type mutation leading to susceptibility for various cancers and diseases among the high frequency populations, reported earlier by various investigators (Lacko et al., 2009; Mo et al., 2009; Sam et al., 2009; Chuang et al., 2011; Kumar et al., 2011) were not yet uniformly demonstrated. In our observation, the DNA adducts of tobacco were causing mutation in GSTT1 gene loci and nullifying its detrimental function in carcinogenic compound biotransformation ability. In due course, the null type frequency was recorded high among the individuals with more habituate of tobacco pronounced in Gujarat. We claim that the tobacco dependent DNA adducts impact on GSTT1 null type mutation frequency among the population is a matter of concern in clinical aspects for therapeutic purposes.

In conclusion, GSTT1 null type frequency observed high among the habituates of more tobacco usage and this might be due to the possible gene-environment interactions. These null type mutations could be caused by DNA adducts of tobacco, as the allelic frequency was

high with respect to more habitual of tobacco. The GSTT1 null type mutation might influence the susceptibility nature of the Gujarat population for various diseased status and cancerous conditions as mentioned by various investigators (Evans et al., 2004; Anantharaman et al., 2007; Mo et al., 2009; Sam et al., 2009; Amer et al., 2011; Chuang et al., 2011; Kumar et al., 2011). In addition these populations were not able to utilize the GSTT1 dependent drugs for their treatment. Further molecular studies are needed in this aspect of tobacco related mutation in drug/xenobiotic compound metabolizing genes to understand the multifaceted pathways involved in induction of various disorders or unhealthy conditions. The data reported in this study would add another vital reason for existing difficulties to cure the cancer patients with drugs that were not accustomed to the gene-environment interactions like habits of tobacco and GSTT1 metabolism. The study also reports the more habitual nature of Gujaratians for tobacco and their susceptibility to GSTT1 null type allele related disorders, which is a matter of concern to design drugs that could overcome the difficulties of these disease prone statuses of the ethnics too.

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