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Research Article

DISTRIBUTION PATTERN OF GSTM1 AND GSTT1 GENE IN VINDHYAN REGION POPULATION

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A B S T R A C T

GST enzymes, which are encoded by GST genes, are responsible for the detoxification of chemicals found in the environment and naturally synthesized metabolites, and they play an important role in protecting tissue from oxidative damage. In this study, we aimed to investigate distribution pattern of GSTM1 (Present/Null) and GSTT1 (Present/Null) in the central Indian population of Madhya Pradesh. Sample were collected from regional institution and PCR based method were used to detect presence and null genotype of GSTM1 and GSTT1. Overall distribution pattern of GSTM1 present/null genotype suggest majority of vindhyan region population do not carry GSTM1 in either form meanwhile GSTT1 is also present in lower frequency. our finding suggest that increased burden of cancer occurance may be because of absence of these drug metabolizing genes.

INTRODUCTION

Cancer is a well known and most dangerous life threatening disease and it is results of unregulated and exponential growth of cells. The burden of cancer is increasing worldwide despite novel discoveries for diagnosis and treatment. Many life style as well as genetic factors has been found to be associated with DNA Damage but results are not consistent in different race of different ethnic origins. The prevalence of cancer is rapidly rising all over the globe at an alarming rate, it is important to note that the rise in prevalence is seen in all six inhabited continents of the globe. Associations between polymorphisms for genes encoding enzymes involved in biotransformation of xenobiotics and susceptibility to several cancers have been shown in several studies**. (**Mehmet Taspinar *et al.* 2008). The Association between carcinogen metabolic activation, DNA damage and risk of cancer has been seen. A lot of enzymes have been established as biomarkers of cancer risk such as polymorphism in GSTM1 and GSTT1 are important biomarkers of risk of cancer. PAH exposure and risk of cancer has been seen in worldwide studies. These potent carcinogens activated by CYP1A1 and its inactivation is depends on GSTM1 and GSTT1 enzymes. GST enzymes, which are encoded by GST genes, are responsible for the detoxification of chemicals found in the environment and naturally synthesized metabolites, and they play an important role in protecting tissue from oxidative damage. An increase or decrease in the tendency of certain types of cancer observed in a group of individuals is often linked to the genetic polymorphism observed in enzymes that play a role in the detoxification of xenobiotics. A significant relationship is observed between the risk of developing cancer and xenobiotic metabolism enzyme gene polymorphism. This relationship has highlighted the role of genetics in cancer etiology.12-14. In this study, we aimed to investigate distribution pattern of GSTM1 (Present/Null) and GSTT1 (Present/Null) in the central Indian population of Madhya Pradesh. As this region is having mixed population of urbans and tribals with having higher number of smokers and drinkers, Risk of cancer is increasing day by day.

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MATERIALS AND METHODS

1. Study subjects In our study, we selected the Vindhyan region, the north east part of Madhya Pradesh. This is constructed by Six district of Madhya Pradesh and is the oldest habitat of Mankind. The Rural population like Kol, Gond, and Baiga are the native tribe of this region. Modern population (Urban population) is dominated by Hindu and Mohammedan religions.
2. DNA Isolation Genomic DNA was extracted from whole blood by the modification of salting out procedure described by Miller and coworkers (Miller *et al*. 1988). Frozen blood sample was thawed at room temperature. 0.5 ml. of whole blood sample was suspended in 1.0 ml. of lysis buffer (0.32 M Sucrose, 1 mM Mgcl2, 12 mM Tris and 1% Triton-X-100) in a 1.5 ml. microcentrifuge tubes. This mixture was mixed gently by inverting the tube upside down for 1 min. The mixture was than

allowed to stand for 10 min. at room temperature to ensure proper lysis of cells. The mixture was centrifuged at 11,000 rpm for 5 min. at 4˚C to pellet the nuclei. The supernatant was discarded carefully in a jar containing disinfectant, as pellet formed is loosely adhered to the bottom of centrifuge tube. The pellet was resuspended in 0.2 ml. of lysis buffer and recentrifuge at 11,000 rpm for 5 min. The pellet was than dissolved in 0.2 ml. of deionized autoclaved water and mixed thoroughly on vortexer. The mixture was centrifuged at 14,000 rpm for 1 min. at 4˚C. Supernatant was discarded to gain an intact pellet. To the above pellet, 80 µl. of proteinase K buffer (0.375 M Nacl, 0.12 M EDTA, pH 8.0) and 10 µl. of 10% SDS (10% w/v SDS, pH 7.2) was added. Mixture was well frothed with the help of micro tip to allow proper lysis of pelleted nuclei. After digestion was complete, 100 µl. of saturated cold 5M Nacl was added and shaken vigorously for 15 sec. To the above mixture 0.2 ml. of deionized, autoclaved water and 0.4 ml. of phenol-chloroform (4:1 v/v) was added to remove most of the non nucleic acid organic molecules. Microcentrifuge tube was inverted upside down until the solution turned milky. Phases were separated by centrifuging the above mixture at 12,000 rpm for 10 min. at 4˚C. Aqueous (top) layer was saved and transferred in another microcentrifuge tube. Transferring of any interface layer was avoided. To the aqueous layer, 1 ml. chilled absolute ethanol was added and the tube was inverted several times until the DNA precipitated. DNA precipitates like thread. This was centrifuged at 14,000 rpm for 4 min. at 4˚C to pellet the DNA thread. Supernatant was discarded. The pellet was washed twice with 1 ml. of 70% alcohol. The mixture was again centrifuged at 14,000 rpm for 1 min. 4˚C. Supernatant was discarded and pellet was air dried for 10-20 min. The pelleted DNA was rehydrated in 100-200 µl. of TE buffer pH 7.4 (10 mM Tris-HCL pH 7.4, 1mM EDTA, pH 8.0). DNA was allowed to dissolve overnight at 37˚C before quantization.

PCR Amplification and Genotyping

Multiplex PCR method was used to determine GSTM1 and GSTT1 polymorphisms in the isolated DNAs. For the GSTT1 polymorphism, forward 5'-TTCCTTACTGGTCCTCACATCTC-3' and reverse 5'-TCACCGGATCATGGCCAGCA-3' primers were used. For the GSTM1 polymorphism, forward 5'-GAACTCCCT GAAAAGCTAAAGC-3' and reverse 5'-GTTGGGCTCAAATA TACGGTGG-3' primers were used. Albumin forward 5'-GCCC TCTGCTAACAAGTCCTAC-3' and reverse 5'-GCCCTAAAA AGAAAATCCCCAATC-3' primers were used as internal controls.17 Albumin 350 bp, GSTM1 219 bp and GSTT1 459 bp PCR products were formed.

RESULTS

Comparison of anthropometric parameters of urban and rural population

Characteristics	Urban study population	Rural Study population	P-value
n(Men/Women)	220 (140/80)	210 (120/90)	
Age(years)	48.6±13.5	49.1±15.2	0.7183, ns
Height(m)	162.50±12.40	163.2±13.4	0.5740, ns
Weight (Kg)			
Women	64.5 ±4.50	64.1 ± 3.3	0.2956, ns
Men	69.5±5.80	68.2±8.2	0.0575, ns
BMI (kg/m^2)			
Women	27.2±3.3	26.9± 4.1	0.4027, ns
Men	25.1±4.9	24.8± 5.5	0.4864, ns
Waist circumference (cm)			
Women	92.1±6.5	92.2±5.8	0.8666, ns
Men	90.2±7.3	89.5±6.3	0.2118, ns
Hip (cm)			
Women	96.5±5.1	97.1±2.2	0.1169, ns
Men	91.3±4.2	90.8±5.8	0.2352, ns
WHR			
Women	0.99±0.07	0.98±0.04	0.0714, ns
Men	0.95±0.06	0.94±0.08	0.1421, ns
Smoking(olive TM) Yes	140(0.58± 0.24)	135(0.57± 0.12)	
no	80(0.78± 0.93) P=0.0167*	75(0.79± 0.98) P= 0.0106*	0.9170, ns
Alcoholic habit Yes	154(0.56± 0.21)	145(0.57± 0.10)	
no	76(0.72± 0.92) P=0.0409*	65(0.74± 0.92) P=0.0287*	0.8744, ns

* denotes level of significant change between urban and rural population

GSTM1 polymorphism and distribution pattern

GSTM1 gene is a null gene some individuals carry these gene whereas some are not. Gene specific primers described in methodology were used to amplify the GSTM1 gene and 200 bp fragments were obtained in those individuals having GSTM1 in either homozygous and heterozygous form. Representative gel picture of GSTM1 is showing 200 bp length of fragments indicate presence of GSTM1.

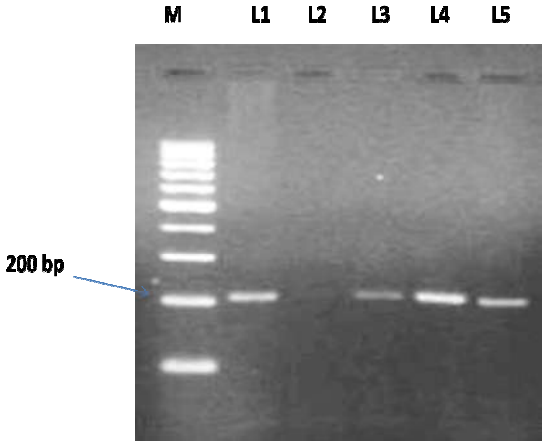

Figure No. 2 Detection of GSTM1 gene. Lane M is a ladder, Lane L1, L3, L4, L5 are representing GSTM1 gene whereas Lane L2 is a null gene

The distribution pattern of GSTM1 present/null was studied and tabulated for both group urban as well as rural population of Vindhyan region. The distribution of genotype homozygous present and heterozygous could not be discriminated so that testing for hardy-weinberg distribution has not been done. In urban population 38.18% individual carries GSTM1 gene in either homozygous or heterozygous form meanwhile 34.76% of rural population carry GSTM1 gene. 61.82% of urban and 65.24% of rural population is null type means they do not have GSTM1 gene in their genetic composition. Overall present/null genotype difference between urban and rural population was nominal and non-significant ($\chi 2$= 0.5421, P= 0.4616).

Overall distribution pattern of GSTM1 present/null genotype suggest majority of vindhyan region population do not carry GSTM1 in either form.

Distribution of GSTM1 gene in Vindhyan region using Chi Square Test

GSTM1 Genotype	Urban N= 220		Rural N=210		CHI SQUARE VALUE χ^2 (P Value)
	N	%	N	%	
Present	84	38.18	73	34.76	1.740, (0.1872, ns) Df- 1
Null	136	61.82	137	65.24	

N – Number of individuals in study group
%- Genotype allele frequency and carriage rate expressed in percentage
* denotes the level of significant association between urban and rural population

GSTT1 polymorphism and distribution pattern

GSTT1 gene is a null gene some individuals carry these gene whereas some are not. Gene specific primers described in methodology were used to amplify the GSTT1 gene and 480 bp fragment were obtained in those individuals having GSTT1 in either homozygous and heterozygous form. Representative gel picture of GSTT1 is showing 480 bp length of fragments indicate presence of GSTT1.

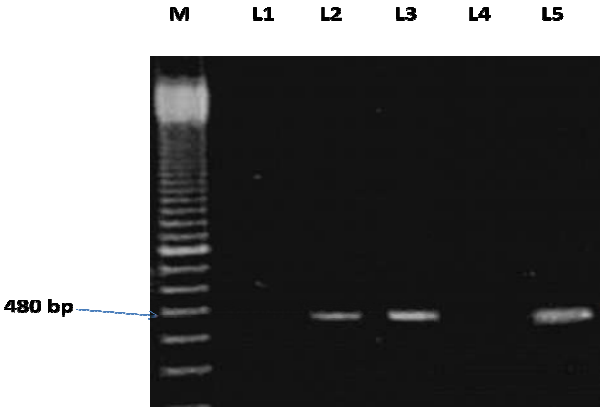

Figure No. 3 Detection of GSTT1 gene. Lane M is a ladder, Lane L2, L3, L5 are representing GSTT1 gene whereas Lane L1 and L4 is a null gene

The distribution pattern of GSTT1 present/null was studied and tabulated for both group urban as well as rural population of Vindhyan region. The present/null of GSTT1 gene is depicted in table no 5 and graph no 5.

The distribution of genotype homozygous present and heterozygous could not be discriminated so that testing for hardy-weinberg distribution has not been done. In urban population 58.63% individual carries GSTT1 gene in either homozygous or heterozygous form meanwhile 60.0% of rural population carry GSTT1 gene. 41.37% of urban and 40.0 % of rural population is null type means they do not have GSTT1 gene in their genetic composition. Overall present/null genotype difference between urban and rural population was nominal and non-significant (χ2=0.08278, P=0.7736). Overall distribution pattern of GSTT1 present/null genotype suggest majority of vindhyan region population do not carry GSTT1 in either form.

Distribution of GSTT1 gene in Vindhyan region using Chi Square Test

Gstt1 Genotype	Urban N= 220		Rural N=210		CHI Square Value χ^2 (P Value)
	N	%	N	%	
Present	129	58.63	126	60.0	0.08278, (0.7736)
Null	91	41.37	84	40.0	

(* denotes the level of significant association between urban & rural population
N – Number of individuals in study group
%- Genotype allele frequency and carriage rate expressed in percentage

DISCUSSION

Genetic association study as well as Functional genomics has now opened the door of new era to understand the possible role of genes in many disorders including diabetes, cancer, many other autoimmune and life threatening diseases. The disorders in metabolism could be due to lack of normal expression of gene and their functional proteins. This abnormality could arise in the genes due to the presence of specific polymorphic allele of particular gene. Many genetic studies already conducted which clearly indicate the role of genetic factors in the susceptibility of disease causation. Genes have widely studied and found to be associated with vast range of metabolic disorders such as many type of cancers. The deficient GST genotypes seem thus to be important risk modifiers for lung cancer and related histological subtypes, especially in combination in Indian population Sharma *et al*., 2015).

Genetic polymorphisms in metabolizing enzymes play a crucial in host susceptibility. Glutathione-S transferases (GSTs, EC 2.5.1.18), the second largest studied enzyme family, are genetically polymorphic and widely expressed in mammalian tissues with broad substrate specificity (Mannervik and Danielson, 1988; Daniel, 1993; Hayes and Pulford, 1995). On the basis of their primary structure, they have been characterized into five classes. Polymorphism in GSTM1 and GSTT1 is the most extensively studied among human population with major ethnic differences. Five mu class genes are situated (GSTM1–GSTM5) on chromosome 1 (Xu *et al*., 1998). There are two classes of theta genes GSTT1 and GSTT2, located on chromosome 22 and 14 respectively.

GSTM1-null genotype has attracted interest because its frequency varies from 45% to 60% in different population (Pemble *et al*., 1994; Capoluongo *et al*., 2007). The role of genetic polymorphism in susceptibility to specific genotoxic exposure can be revealed by analyzing level of biomarkers in exposed and control subjects. Board *et al*. (1990) showed that GSTM1 gene is lacking in about 38–65% of ethnic population. The distribution of genotype homozygous present and heterozygous could not be discriminated so that testing for hardy-weinberg distribution has not been done. In urban population 38.18% individual carries GSTM1 gene in either homozygous or heterozygous form meanwhile 34.76% of rural population carry GSTM1 gene. 61.82% of urban and 65.24% of rural population is null type means they do not have GSTM1 gene in their genetic composition. Overall present/null genotype difference between urban and rural population was nominal and non-significant (χ2= 0.5421, P= 0.4616).

Overall distribution pattern of GSTM1 present/null genotype suggest majority of vindhyan region population do not carry GSTM1 in either form. Our findings show little lower frequency of GSTM1 occurrence in central Indian population as compared to others. The analyses for associations between GSTM1 genotypes and the Olive TM values did not show any association. Our study is consistent with Chinese study done by Chen *et al*., 2006. GSTT1 gene is represented by two alleles: a functional or wild allele (GSTT1_1) and a nonfunctional or null allele (GSTT1_0). The GSTT1_0 (null type) frequency ranges from 16% to 38% in different population (Capoluongo *et al*., 2007). In urban population 58.63% individual carries GSTM1 gene in either homozygous or heterozygous form meanwhile 60.0% of rural population

carry GSTM1 gene. 41.37% of urban and 40.0 % of rural population is null type means they do not have GSTM1 gene in their genetic composition. Overall present/null genotype difference between urban and rural population was nominal and non-significant ($\chi 2$=0.08278, P=0.7736). The frequency of GSTT1 null genotype was found to vary from 16% to 38% depending on the size and ethnicity of population (Pemble *et al*., 1994; Abdel-Rahman *et al*., 1996; Capoluongo *et al*., 2007, Agrawal *et al*., 2010). However, we found slightly higher frequency of GSTT1 null type (41.37–40.0%) among studied subjects of urban and rural population. Overall distribution pattern of GSTM1 present/null genotype suggest majority of vindhyan region population do not carry GSTM1 in either form meanwhile GSTT1 is also present in lower frequency. our finding suggest that increased burden of cancer occurance may be because of absence of these drug metabolizing genes.

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