ORIGINAL ARTICLE CODEN: AAJMBG

Smoking, glutathione S transferase polymorphisms *(GSTM1 & GSTT1)* and their association with selected inflammatory biomarkers

Sangeeta Singh[1], Basant Joshi[2*], Aman Saini[3] and Tapan Mohapatra[4]

[1]Department of Biochemistry, Soban Singh Jeena Government Institute of Medical Sciences and Research, Lower Mall Road, Khatyari, Almora-263601, Uttarakhand, India, [2]Department of Biochemistry, Rohilkhand Medical College and Hospital, Pilibhit Bypass Road, Bareilly-243006, Uttar Pradesh, India, [3]District Surveillance Officer, Tihari Garhwal-249001, Uttarakhand, India and [4]Department of Biochemistry, Santosh Medical College, Sector 12, Santosh Nagar, Pratap Vihar, Ghaziabad-201009, Uttar Pradesh, India

Received: 20th September 2021; ***Accepted:*** 12th March 2022; ***Published:*** 01st April 2022

Abstract: *Introduction:* In the United States (US) & other countries cigarette smoking (CS) continues to be the more preventable cause of disease and death. Glutathione S-transferase (GST) enzyme is implicated in the detoxification of carcinogens present in tobacco smoke and consequent polymorphisms in this gene may confer susceptibility to systemic diseases if DNA damage is important in various diseases. The presences of a gene-deletion polymorphism have shown by genetic studies on *GSTT1 & GSTM1*. Elevated tumor necrosis factor alpha (TNF α) & high sensitivity c-reactive protein (hs-CRP) has been increasingly used as a surrogate marker of systemic inflammation in diverse conditions. Lipoprotein (a) Lp(a) is a low-density lipoprotein (LDL)- like plasma lipoprotein composed of apolipoprotein B (apoB) and a large glycoprotein termed apolipoprotein (a) [apo (a)]. We investigated the associations of *GST (M1 or T1)* genotypes, exposure to smoking, and their interaction with selected markers of inflammation. *Materials & Methods:* The present study was carried out in the Department of Biochemistry, Santosh Medical College, Ghaziabad. Estimation was done including serum TNF-α by ELISA, hs-CRP & Lp(a) by turbidimetric immunoassay. DNA was extracted from serum and the *GSTM1* and *GSTT1* polymorphisms were determined using a simple polymerase chain reaction (PCR). *Results:* Out of total 284 cigarette smokers, *GSTT1* gene was present in 51.1% & absent in 48.9% subjects whereas *GSTM1* gene was present in 59.9% & absent in 40.1% subjects as compared to age & sex matched non cigarette smokers. These differences were found to be statistically significant ($p<0.05$). The mean serum TNF-α, Lp(a) & hs-CRP levels were significantly raised in cigarette smokers as compared to non-smokers. These differences were found to be statistically significant ($p< 0.05$). *Conclusion:* In conclusion, the finding of a significant association between inflammatory markers, *GSTM1* and *T1* with smoking status may influence systemic diseases including cardiovascular diseases via DNA damage.

Keywords: Cigarette Smoking, *GSTT1 & GSTM1,* Gene Polymorphism, Inflammatory Markers.

Introduction

In most of the world cigarettes are the most common form of tobacco used and each year 443,000 deaths occur in the United States (US). Due to rise of tobacco industry and population growth cigarette smoking and use of other tobacco products is increasing in the developing world [1].

In tobacco smoke over 5300 compounds have been identified. Compounds includes, carbon and nitrogen oxides, amides, imides, lactams, carboxylic acids, lactones, esters, aldehydes, ketones, alcohols, phenols, amines, *N*-nitrosamines, *N*-heterocyclics, aliphatic hydrocarbons, monocyclic and polycyclic aromatic hydrocarbons (PAHs), nitriles, anhydrides, carbohydrates, ethers, nitro compounds and metals included but not limited to neutral gases [2]. Nicotine a principal tobacco alkaloid in smoke is an addictive property of tobacco smoke. Nornicotine, anatabine and anabasine included as minor tobacco alkaloids. The alkaloids in

tobacco are not generally considered carcinogenic, but in each puff of smoke are accompanied by carcinogens [3].

Genetic determinants evidence affecting the smoking phenotype has steadily accumulated both from analysis of the contributions of genetics studies of substance abuse in animals and in humans. Regions of the genome in which loci affecting nicotine dependence and ever smoking may be found with further work by two recent linkage studies in humans have indicated [4].

Glutathione-S-transferase is a glutathione peroxidase & non-selenium dependent. 1961 it was first identified. Glutathione-S-aryltransferase was subsequently named this enzyme. Depending upon the substrate specificity later on, several other GSTs were demonstrated [5]. A difference in DNA sequence among individuals, groups, or populations (present in more than 1% of the population) called as Genetic Polymorphism. Genetic polymorphisms may have been induced by external agents (such as viruses or radiation) or may be the result of chance processes.

The gene *GSTM1* (μ) is 4.2 kb, consisting of eight exons and is located on chromosome 1p13.3, with four known alleles. *GSTM1-null* variant is widely distributed, among different populations apart from the existence of other *GSTM1* polymorphic forms *GSTM1a, GSTM1b* etc. are the major polymorphism. The gene *GSTT1* (θ) is located on chromosome 22p11.2, is flanked by two homologous regions HA3 and HA5 and is composed of six exons. This gene has two known alleles as the most frequent allele (wild) and the null allele. The recombination of the homologous regions HA3 and HA5, resulting in the deletion of 5.4 kb and, as a consequence, the loss of function of the enzyme showed this polymorphism. The presences of a gene-deletion polymorphism have shown by genetic studies on *GSTT1*. This deletion polymorphism has been reported large interethnic variability [6].

There are at least seven classes of isoezymes are known, five are cytosolic and two membrane bound. These are involved with oxidative stress, normal cellular physiology & the inflammation regulation through modulation of prostaglandin signaling pathways [7].

TNF- α is a well known inflammatory marker. TNF- α is a protein, relative molecular mass 17,000 that can be secreted by many cell types (especially monocytes and macrophages but also B- and T-lymphocytes, mast cells, neutrophils and fibroblasts). The name 'tumor necrosis factor' comes from the 1st identification of serum of mice infected with Bacillus Calmette Guerin that was cause haemorrhage and necrosis in transplanted tumors in the same mice [8]. Active inflammatory cells produce various inflammatory mediators in response to smoking, and inflammatory cytokines are the important of them.

Elevated CRP has been increasingly used as a surrogate marker of systemic inflammation in diverse conditions. hs-CRP is an annular (ring-shaped), pentameric protein found in blood plasma, the levels of which rise in response to inflammation. C-reactive protein is an acute-phase protein of hepatic origin that increases following interleukin-6 secretion from macrophages and T cells. Its physiological role is to bind to lysophosphatidylcholine expressed on the surface of dead or dying cells (and some types of bacteria) in order to activate the complement system via the C1Q complex [9].

Lp(a) is a low-density lipoprotein (LDL)- like plasma lipoprotein composed of apolipoprotein B (apoB) and a large glycoprotein termed apolipoprotein(a) [apo(a)]. Shortly after the discovery of the Lp(a) system by Berg in 1963 it was recognized that Lp(a) is a quantitative genetic trait in human plasma. The distribution of plasma Lp(a) levels is highly skewed towards lower concentrations, with more than two-thirds of the population having levels lower than 20 mg/dl. High plasma concentrations of Lp(a) are associated with the risk of premature coronary atherosclerosis and other vascular diseases [10].

Several markers of inflammation and endothelial function are associated with incident coronary disease and other systemic diseases. Based on previous research that indicates *GSTM1* and *GSTT1* modify risk of atherosclerosis, pulmonary and several

systemic diseases in smokers, it is possible that *GSTM1* and *GSTT1* reduce the potential for oxidative damage and therefore modify inflammation related pathways. Certain GST genotypes may also be associated with hemostasis, endothelial function and inflammation. So we investigated the associations of *GST (M1 or T1*) polymorphism, exposure to smoking, and their interaction with selected markers of inflammation.

Material and Methods

The present study was carried out in the Department of Biochemistry, Government Medical College, Haldwani, and Department of Biochemistry, Santosh Medical College, Ghaziabad during the period of 2016 to 2019. Institutional ethical clearance was taken prior to the study [F. No SU/2018/528 (2)].

Inclusion criteria: About 284 healthy cigarette smokers (without any systemic diseases) in the age group of 18-60 years compared with age & sex matched 284 controls (non-smokers) were included in the study.

Exclusion criteria: Patients with chronic liver diseases, tuberculosis, Pulmonary disorders, Coronary artery diseases, diabetes mellitus, renal failure, thyroid dysfunction, anaemia, malnourished individuals, person with habit of tobacco chewing along with smoking and taking other forms of smoke (bidi, hookah, cigar etc) were excluded from the study.

According to prevalence, used in previous study [11] sample size was calculated

$$n = \frac{Z^2 \times p \times q}{d^2}$$

Where n is the sample size, Z is 1.96 (5% level of significance), p is prevalence, q is 1-p and d is 0.05 (95% of c.f.). According to this formula sample size was 284 for cigarette smokers.

A detailed history from cigarette smokers comprising of number of cigarettes per day and duration of cigarette smoking was recorded on participant proforma. Subjects were classified into different groups based on number of cigarettes per day and duration of cigarette smoking i.e. smoking 1-15 cigarette/day <5 years are mild smokers in group I , 15-20 cigarette/day <5 years in group II, 15-20 cigarette/day 5-10 years moderate, and 15-20 cigarette/day >10 years are heavy smokers [12].

Out of total 284 cigarette smokers, 129 were in group I, 42 were in group II which were in mild group, 36 were in moderate & 77 were in heavy group smokers.

All aseptic precautions were taken; with a disposable syringe about five mL of blood was drawn by veinpuncture from a peripheral vein. For the retraction of clot collected blood in clean dry glass tubes was allowed to stand for 30 minutes at room temperature. Then it was centrifuged at 3000 r.p.m. for ten minutes to obtained the serum. The serum was stored at 4°C in the refrigerator for analysis.

Estimation was done including serum TNF-α by ELISA (Elabscience, Catalog No: E-EL-H0109), hs-CRP & Lp(a) by turbidimetric immunoassay using semiautoanalyzer. Extraction of genomic DNA from serum samples was done by using Banglore Genei, India (Product No: D3396)

A simple PCR (eppondorf) method was used to detect the presence or absence of the *GSTM1* and *GSTT1* genes. Both GST primer sets taken according to Arand M *et al*., *GSTM1*-Forward (5'GAACTCCCCTGAAAA GCTAAAGC3') and Reverse (5'GTTGGG CTCAAATATACGGTG3') and *GSTT1* Forward (5'TCTTACTGGTCCTCACATC TC3') and Reverse (5'TCTCCGGATCATGG CCAGC3') in the same PCR, a third primer set for albumin (5'GCCCTCTGCTAACAAG TCCTAC3') and (5'GCCCTAAAAAG AAAATCGCCAATC3') to serve as an internal positive control for the amplification reaction.

PCR products were separated by 2% agarose gel electrophoresis using ethidium bromide stain and visualized under UV light.

Statistical Analysis: The analysis was carried out using the SPSS 19.0.2 program for windows. Chi square test, unpaired "t" test & one way ANOVA were used to analyze all the data for statistical significance.

Results

In present study, out of total 284 cigarette smokers, 272 were males and 12 were females. Mean age of the cigarette smokers and nonsmokers were 40.66±11.08 years and 37.42±9.73 years, respectively.

Table-1: Distribution of age-group among cigarette smokers (n=284)

Age group (yrs)	Male		Female		Total	
	No	%	No	%	No	%
18-29	41	15.07	02	16.67	43	15.14
30-41	75	27.57	02	16.67	77	27.11
42-53	81	29.79	04	33.33	85	29.93
54-60	75	27.57	04	33.33	79	27.82
Total	**272**	**95.77**	**12**	**4.23**	**284**	**100**

Table-2: Distribution of *GSTM1& GSTT1* gene by in cigarette smokers and non-smokers (n=568)

Parameters	*GSTT1*					*GSTM1*				
	Present	%	Absent	%	*p* value	Present	%	Absent	%	*p* value
Non-smokers (n = 284)	269	94.72	15	5.28	0.008*	257	90.5	27	9.50	0.018*
Cigarette smokers (n = 284)	145	51.1	139	48.9		170	59.9	114	40.1	

Out of total 284 non- cigarette smokers, *GSTT1* gene was present in 94.72% & absent in 5.28% subjects whereas *GSTM1* gene was present in 90.5% & absent in 9.50% subjects. Out of total 284 cigarette smokers, *GSTT1* gene was present in 51.1% & absent in 48.9% subjects whereas *GSTM1* gene was present in 59.9% & absent in 40.1% subjects as compared to age & sex matched non cigarette smokers. These differences were found to be statistically significant ($p< 0.05$).

Fig-1: *GSTM1& GSTT1* gene distribution in cigarette smokers & non smokers

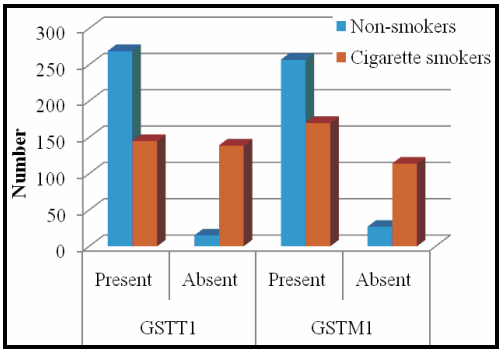

Table-3: Distribution of *GSTM1& GSTT1* gene according to duration & number of cigarette smoked in cigarette smokers (n=284)

Markers		*p* value	Mild smokers (group I)	Mild smokers (group I)	Moderate smokers	Heavy smokers	Total	%
			1-15C/D <5years	15-20C/D <5years	15-20C/D5-10years	15-20C/D5-10years		
GSTT1	Present	0.018	28	35	45	37	145	51.0
	Absent		22	41	46	30	139	48.9
GSTM1	Present	0.011	47	34	49	40	170	59.8
	Absent		18	29	39	28	114	40.1

GSTT1 gene was absent in 28 with mild smokers (group I), 41 in (group II), 46 in moderate smokers and 30 in heavy smokers were smoked 1-15C/D <5years followed by 15-20C/D <5years, 15-20C/D 5-10years &15-20C/D 5-10years respectively. Out of total 284 cigarette smokers, 48.9% *GSTT1* gene was absent. These differences were found to be statistically significant ($p<$ 0.05). *GSTM1* gene was absent in 18 with mild smokers (group I), 29 absent in (group II), 39 absent in moderate smokers and 28 absent in heavy smokers were smoked 1-15C/D <5years followed by 15-20C/D <5years, 15-20C/D 5-10years &15-20C/D 5-10years respectively. Out of total 284 cigarette smokers, 40.1% *GSTM1* gene was absent. These differences were found to be statistically significant ($p<$ 0.05).

Table-4: Serum levels of TNF-α, Lp(a) and hs-CRP in cigarette smokers and non-smokers (n=568)

Parameters	Non-smokers (n = 284) Mean ± SD	Cigarette smokers (n = 284) Mean ± SD	*t value*	*p* value	95% Confidence interval for mean		Normal value
					Lower	Upper	
TNF-α	39.01±22.22	235.88±69.50	-1.11	0.002	84.13	493.0	24-183 pg/mL
Lp(a	12.95±8.36	37.48±19.15	-1.46	0.007	2.50	48.45	<30mg/dl
hs-CRP	0.16±0.19	2.02±5.45	1.69	0.005	0.37	3.20	0.029-0.797 mg/dl

The mean serum TNF-α, Lp(a) & hs-CRP level in cigarette smokers (235.88±69.50), (37.48±19.15) & (2.02±5.45) and non-smokers (39.01±22.22), (12.95±8.36), (0.16±0.19) respectively. Unpaired 't' test was done in cigarette smokers & non-smokers. The mean serum TNF-α, Lp(a) & hs-CRP levels were significantly raised in cigarette smokers as compared to non-smokers. These differences were found to be statistically significant ($p<$ 0.05).

Table-5: Serum TNF-α, A1AT, Lp(a) and hs-CRP levels according to duration & number of cigarette smoked in cigarette smokers (n=284)

Parameters			1-15C/D <5years Mean ± SD	15-20C/D <5years Mean ± SD	15-20C/D 10years Mean ± SD	15-20C/D >10years Mean ± SD	*p* value
95% confidence interval for mean		TNF-α	241.06±76.30	224.58±61.45	238.89±61.30	240.91±41.00	
	Lower		204.79	226.80	216.38	220.54	0.037
	Upper		247.45	251.82	249.63	256.06	
		Lp(a)	36.51±20.71	37.22±15.89	39.79±17.81	44.81±21.75	
	Lower		29.15	35.06	33.92	32.28	0.021
	Upper		41.51	42.07	42.62	46.45	
		hs-CRP	1.48±0.58	1.56±0.71	1.78±0.66	5.25±15.22	
	Lower		0.25	1.33	1.46	1.48	0.007
	Upper		5.67	2.56	2.80	7.79	

The mean serum TNF-α, Lp(a) & hs-CRP levels were significantly raised in cigarette smokers when adjusted with duration and number of cigarette smoked as compared to non-smokers. These differences were found to be statistically significant ($p<$ 0.05).

Table-6: ANOVA analysis of serum TNF-α, Lp(a) and hs-CRP levels according to duration & number of cigarette smoked in studied subjects (n=284)

Parameters		1-15 C/D <5 years	15-20C/D <5 years	15-20C/D 5-10years	15-20C/D >10years	
		Sum of squares	Mean square	F value	*p* value	F Critical
TNF-α	Between groups	6684.65	2228.21	6.91	0.028	3.032
	Within groups	1298808.96	4655.22			
Lp(a)	Between groups	381.34	127.12	4.09	0.001	
	Within groups	100961.57	361.86			
hs-CRP	Between groups	81.75	27.25	7.57	0.001	
	Within groups	4197.50	15.04			

The *p* values were <0.05 which signifies significant variation in levels of serum TNF-α, Lp(a) and hs-CRP when compared among each other in terms of group mean.

Discussion

Many chemicals in cigarette smoke have toxic effects on the cells and require metabolic detoxification to prevent their toxicological responses. Many genes are involved in the metabolism of a large number of potentially mutagenic chemicals in cigarette smoke. Many of these genes which are involved in chemical metabolism are polymorphic. Glutathione S-transferases (GSTs) are a large family of enzymes participating in detoxification of endogenous and exogenous toxic substrates including tobacco derived toxins; they also exhibit peroxidase activity and thus might play an important role in oxidative stress.

Previous studies have differed on whether *GST* polymorphisms modify the association between cigarette smoking and related disease risks. Smaller studies have reported that smoking did not modify associations between *GSTT1*, or *GSTM1* polymorphisms [13] and related diseases & breast cancer, whereas former smokers with *GSTT1* null were found to have an increased risk of breast cancer compared with never smokers with *GSTT1* present [14].

In present study out of total 284 non- cigarette smokers, *GSTT1* gene was present in 94.72% & absent in 5.28% subjects whereas *GSTM1* gene was present in 90.5% & absent in 9.50% subjects. Out of total 284 cigarette smokers, *GSTT1* gene was present in 51.1% & absent in 48.9% subjects whereas *GSTM1* gene was present in 59.9%& absent in 40.1% subjects as compared to age & sex matched non cigarette smokers (Table 2 & Fig. 1).

A cohort study performed in Swiss general population showed a large proportion of Caucasians to carry one or both *GST* gene deletions (around 20% of *GSTT1* gene deletion, around 50% *GSTM1* gene deletion and around 10% of *GSTT1/GSTM1* gene deletion carriers). In the Korean and Slovak populations [15], no association between *GSTM1* and *GSTT1* genetic polymorphism was found.

In all municipalities of Goias, the frequency of the *GSTT1* null genotype was 38.2%, and of the *GSTM1* null genotype 50.3%. In the Northeast of India, some authors (Thoudam *et al*., 2010) demonstrated a frequency of 32.7% for null *GSTT1* and 41.9% for the null *GSTM1* genotype [16]. Chiurillo *et al*. (2013) analyzed 120 urban smoker samples from a Venezuelan population and 188 Amerindian samples, showing a null *GSTT1* genotype frequency of 11% and a null *GSTM1* genotype of 51% in the urban population [17].

Previous report suggested that both the *GSTM1* and the *GSTT1* null variants result in the lack of enzyme production [18]. The polymorphic form of *GSTM1* and *GSTT1* genes are deletion variants that produce either a functional enzyme (non-deletion alleles or heterozygous deletion) or complete absence of

the enzyme (homozygous deletion alleles). However, these GSTs enzyme individually (*GSTM1* and *GSTT1*) or in combination (*GSTM1* + *GSTT1*) of the null genotypes of *GSTM1* and *GSTT1* genes increases the risk of lung, gastric, colon, bladder cancers [19]. Smoke exposure induces local and systemic elevations in primary inflammatory cells. Inflammatory cytokine release is evident in the airways and lungs in the hour following smoking (Flouris *et al*. 2009). However, smoke exposure suppresses the effectiveness of the immunological response [20].

The direct link between smoking and chronically increased inflammation is starting to be revealed with further understanding of gene expression and modification in habitual smokers. The interaction between smoking and the IL-6 promoter gene has been observed and is related to the systemic elevation in CRP and IL-6 in smokers in a dose-dependent manner (Sunyer *et al.* 2009) [21]. The finding of our study was the high serum level of TNF-α, hs-CRP & Lp(a) levels in healthy cigarette smokers compared to nonsmokers. The mean serum TNF-α, hs-CRP & Lp(a) when adjusted with duration & number of cigarette smoked in cigarette smokers were significantly raised (Table 4,5 & 6).

These findings were in agreement with Y. Higashimoto *et al*. who reported the increased levels of serum TNF-α, IL-6 and tissue inhibitors of metalloproteinase-1 in asthma and COPD patients with highest levels in current smoker with COPD patients and controls [22]. Moreover, our results were in consistent with those of V.M. Pinto-Plata *et al*., Marevic *et al*., 2008,who reported a significant increase in serum inflammatory markers TNF-α, hs-CRP and interlukin-6 in different stages of cigarette smokers and this increment was significantly higher than those of the controls [23].

In contrast to our findings, Vernooy *et al*. reported an increase in the concentration of soluble TNF-a receptors, while the concentration of TNF-α was within the reference range for healthy individuals [24]. Possible explanation for unchanged concentration of TNF-α is its local and short term effects, its degradation and its half life of approximately 6–7 min, as well as its binding to receptors and renal clearance.

Smoking is a major risk factor for atherosclerotic cerebro- and cardiovascular diseases (CVD) through leading to dyslipidemia. Smolders *et al*, reviewing 31 cross-sectional and prospective studies involving approximately 50,000 individuals, have suggested that high Lp(a) levels can be associated with the risk for ischemic cerebral vascular accident in smokers [25]. A cohort study involving 2,365 smoker individuals with CAD, 284 with ischemic cerebral vascular accident and 596 with peripheral arterial disease has shown an association of increased Lp(a) levels with future events of arterial diseases, but not with ischemic cerebral disease. It is worth noting that such association was independent of LDL-cholesterol levels [26].

Previous studies confirmed that in general population, current smoking increased Lp(a) levels and prevalence of abnormal total cholesterol (TC), heavy smoking was associated with increased Lp(a) and higher risk for abnormal TC and smoking cessation was associated with decreased Lp(a) and decreased risk for abnormal TC.

Cigarette smoking has been associated with increases in CRP and previous investigations have demonstrated that increased CRP levels are a secondary effect of cigarette smoking and reflect tissue injury [27]. Studies in adolescents have shown a positive association between active smoking and hs-CRP [28]. Interestingly, non significant higher hs-CRP concentrations associated with increased severity of airway obstruction and an increased degree of dyspnea were observed in their study [29].

In a study conducted by Broekhuizen *et al*., cigarette smokers had increased levels of inflammatory markers like CRP [30]. F. Karadag *et al*., found out that serum CRP was significantly higher in stable COPD patients with heavy smokers than in control subjects ($p < 0.001$) [31]. This study confirms that circulating CRP levels are higher in stable COPD patients and may thus be regarded as a valid biomarker of low-grade systemic inflammation.

In our study, hs-CRP was found to be significantly higher in smokers as compared to non-smokers. Our work duplicates the previous finding of Juan P de Torres *et al*. regarding higher CRP levels in those who are active smokers compared with those who are not [32]. A study by Fro¨hlich *et al*. has one of the longest follow-up periods, with levels of CRP that were as low as a mean of 1.25 mg⁄l following 30–55 years of cessation in male subjects, compared with 1.92 mg⁄l in regular smokers [33].

Epidemiological studies have demonstrated tobacco smoke to be a major cause of both cancer and inflammatory diseases. However, the mechanism by which exposure leads to disease is better understood in the former case. There are many identified carcinogens in tobacco smoke that induce DNA damage by direct binding to form DNA adducts [34].

Unrepaired DNA damage can result in the induction of somatic mutations in genes regulating cell growth, thus providing a mechanism for inflammation & carcinogenesis. Glutathione S-transferases are the main enzymes responsible for the detoxification of carcinogenic compounds such as polycyclic aromatic hydrocarbon and aromatic amines, which are largely present in cigarette smoke. *GSTM1* and *GSTT1*, which are important members of the GST multigene family, are polymorphic in humans. Homozygous deletion of part of these genes (null genotype) results in enzyme deficiency and, thus, an impaired ability to eliminate carcinogenic compounds metabolically [35].

Conclusion

Cigarette smoking may induce inflammation measured as TNF- α, hs-CRP, Lp(a) level, and combinations of the *GSTM1* and *GSTT1* polymorphisms may modify the deleterious effect of smoking on these markers, indicating genetic susceptibility to smoking-related diseases. It would be beneficial to know if the patterns observed in this study could be replicated in other study populations and if the results could be strengthened by repeated measurement of these markers. A better understanding of intermediate mechanisms is imperative to help resolve the importance of GSTs and selected inflammatory markers in the etiology of certain cancers or cardiovascular and other systemic diseases.

Limitations: The study was limited to the population residing in and around Haldwani and their involvement in the study especially in case of females. Lack of fund, time and manpower prevented the inclusion of a large study group and other sensitive biochemical markers of cigarette smoke. Future cross-sectional, longitudinal and mechanistic studies are needed to determine how CS, inflammatory markers & GST gene (*GSTT1* & *GSTM1*) are useful in large populations of cigarette smokers with the inclusion of clinically relevant endpoints are needed to extend these findings.

Acknowledgement

In the name of the lord we pray and receive. Before proceeding further, I thank the almighty for all the kindness and grace he has showered upon me. I thank all the teaching staff of Department of Biochemistry, Santosh Medical College, Ghaziabad for their suggestions and co-operation. I wish to offer my thanks to Department of Medical Education for their valuable information and support.

Financial Support and sponsorship: Nil

Conflicts of interest: There are no conflicts of interest.

References

1. U.S. Environmental Protection Agency. Quality Criteria for Water 1986. Washington: U.S. Environmental Protection Agency, Office of Water Regulations and Standards, 1986. *Publication No. EPA 440/5-86-001.*
2. Rodgman A, Perfetti TA. Alphabetical Component Index. In: The Chemical Components of Tobacco and Tobacco Smoke. *BocaRaton, FL: CRC Press,* 2009; 1483-1784.
3. Hukkanen J, Jacob P, Benowitz NL. Metabolismand disposition kinetics of nicotine. *Pharmacol Rev.* 2005; 57:79-115.
4. Noble EP, St. Jeor ST, Ritchie T, Syndulko K, St. Jeor SC, Fitch RJ et al. D2 dopamine receptor gene and cigarette smoking: a reward gene?. *Med Hypotheses* 1994; 42:257-260.
5. Booth J, Boyland E and Sims P. Concordance between enzymeactivity and genotype of

glutathioneS-transferase theta (GSTT1). *Biochem. J.,* 2001; 79:516.
6. Rodríguez M, Mejia F, Lecourtois M, Domínguez, V, Castillo J. Influence of GSTT1, GSTM1 and GSTP1 polymorphisms on the development of breast cancer. *J. Cancer Ther.* 2014; 5:552-559.
7. Elsby R, Kitteringham NR, Goldring CE et al. Increased constitutive c-Jun N-terminal kinase signaling in mice lacking glutathione S-transferase Pi. *J Biol Chem.* 2003; 278:22243-22249.
8. Battaglia S, Mauad T,van Schadewijk AM, Vignola AM, Rabe KF, Bellia V et al, Differential distribution of inflammatory cells in large and small airways in smokers. *J. Clin. Pathol*. 2007; 60:907-911.
9. Vanderschueren S, Deeren D, Knockaert DC et al. Extremely elevated C-reactive protein. *Eur J Intern Med*. 2006; 17:430-433.
10. Fulcher G. Lipoprotein(a): a new independent risk factor or atherosclerosis. *Australian and New Zealand Journal of Medicine*. 2002; 22:326-328.
11. Imtiaz D, Kandpal SD, Juyal R, Shrotriya VP, Singh AK. A study on prevalence and pattern of smoking among rural population in dehradun district of Uttarakhand. *Natl J Community Med.* 2014; 5(4):440-443.
12. Nagaraj, Satish Kumar D, Paunipagar PV. Study of serum malondialdehyde and vitamin C in smokers. *Journal of Scientific & Innovative Research.* 2014; 3(6):569-571.
13. Vogl FD, Taioli E, Maugard C, Zheng W, Pinto LF, Ambrosone C et al. Glutathione S-transferases M1, T1, and P1 and breast cancer: a pooled analysis. *Cancer Epidemiol Biomarkers Prev*. 2004; 13(9):1473-1479.
14. Van der Hel OL, Bueno-de-Mesquita HB, van Gils CH, Roest M, Slothouber B, Grobbee DE et al. Cumulative genetic defects in carcinogen metabolism may increase breast cancer risk (The Netherlands). *Cancer Causes Control*. 2005; 16(6):675-681.
15. Zidzik J, Slabá E, Joppa P, Kluchová Z, Dorková Z, Skyba P, Habalová V, Salagovic J, Tkácová R. Glutathione S-transferase and microsomal epoxide hydrolase gene polymorphisms and risk of chronic obstructive pulmonary disease in Slovak population. *Croat Med J.* 2008; 49(2):182-191.
16. Thoudam RD, Yadav DS, Mishra AK, Kaushal M, Ihsan R, Chattopadhyay I, Chauhan PS, Sarma J, Zomawia E, Verma Y, Nandkumar A. Distribution of glutathione S-transferase T1 and M1 genes polymorphisms in north east Indians: a potential report. *Genet. Test. Mol. Biomark.* 2010; 14:163-169.
17. Chiurillo MA, Griman P, Santiago L, Torres K, Moran Y, Borjas L. Distribution of GSTM1, GSTT1, GSTP1 and TP53 disease-associated gene variants in native and urban Venezuelan populations. *Gene*. 2013; 531:106-111.
18. Mannervik B. Evaluation of glutathione S-transferase T1 deletion polymorphism on type 2 diabetes mellitus risk in Zoroastrian females in Yazd, Iran *Adv. Enzymol.* 1995; 57:357.
19. Levi AJ, Gatmoitan Z and Arias IM. Genetic polymorphism of epoxide hydrolase and glutathione S-transferase in COP. *J. Clin Invest*. 1969; 48, 21-56.
20. Flouris AD et al. Acute and short-term effects of secondhand smoke on lung function and cytokine production. *American Journal of Respiratory and Critical Care Medicine.* 2009; 179(11):1029-1033.
21. Sunyer J et al. Interaction between smoking and the interleukin-6 gene affects systemic levels of inflammatory biomarkers. *Nicotine & Tobacco Research: Official Journal of the Society for Research on Nicotine and Tobacco,* 2009; 11(11):1347-1353.
22. Higashimoto Y, Yamagata Y, Taya S, Iwata T, Okada M, Ishiguchi T et al. Systemic inflammation in chronic obstructive pulmonary disease and asthma: similarities and differences. *Respirology.* 2008; 13:128-133.
23. Pinto-Plata VM, Mullerova H, Toso JF, Feudjo-Tepie M, Soriano JB, Vessey RS et al. C-reactive protein in patients with COPD, control smokers and non-smokers. *Thorax*. 2006; 61:23-28.
24. Vernooy JH, Kucukaycan M, Jacobs JA, Chavannes NH, Buurman WA, Dentener MA et al. Local and systemic inflammation in patients with chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* 2002; 166:1218-1224.
25. Wannamethee SG, Lowe GD, Shaper AG et al. Associations between cigarette smoking, pipe / cigar smoking, and smoking cessation, and haemostatic and inflammatory markers for cardiovascular disease. *Eur Heart J*. 2005; 26:1765-1773.
26. Gurdasani D, Sjouke B, Tsimikas S, Hovingh GK, Luben RN, Wainwright NW, et al. Lipoprotein(a) and risk of coronary, cerebrovascular, and peripheral artery disease: the EPIC-Norfolk prospective population study. *Arterioscler Thromb Vasc Biol.* 2012; 32(12):3058-3065.
27. Miller M, Zhan M and Havas S. High attributable risk of elevated C-reactive protein level to conventional coronary heart disease risk factors: the third National Health and Nutrition Examination Survey. *Archives of Internal Medicine.* 2005; 165(18):2063-2068.
28. Pirkola J, Vääräsmäki M, Ala-Korpela M et al. Low-grade, systemic inflammation in adolescents: Association with early-life factors, gender, and lifestyle. *Am J Epidemiol.* 2010; 171:72-82.
29. Samy N, Abd El-Maksoud MD, El Khayyal A, Imam A. Clinical utility of biomarkers as predictors of lung function in chronic obstructive lung disease. *New York Sci. J.* 2010; 6(3):25-32.
30. Broekhuizen R, Wouters EF, Creutzberg EC, Schols AM: Raised CRP levels mark metabolic and functional impairment in advanced COPD. *Thorax*. 2006; 61(1):17-22.
31. Karadag F, Kirdar S, Karul AB, Ceylan E: The value of C-reactive protein as a marker of systemic inflammation in stable chronic obstructive pulmonary disease. *Eur J Intern Med*. 2008; 19(2):104-108.
32. De Torres JP, Cordoba-Lanus E, López-Aguilar C, De Torres JP, Cordoba- Lanus E, López-Aguilar C, de Muros Fuentes M, de Montejo Garcini A, Aguirre-Jaime A, Celli BR, Casanova C: C-Reactive Protein levels and Clinically Important Predictive Outcomes in Stable COPD Patients. *Eur Respir J*. 2006; 27(5):902-907.

33. Fro¨hlich M, Sund M, Lowel H et al. Independent association of various smoking characteristics with markers of systemic inflammation in men - results from a representative sample of the general population (MONICA Augsburg Survey 1994 / 95). *Eur Heart J*. 2003; 24:1365-1372.
34. Hemminki K, Diple A, Shuker DEG, Kadlubar FF, Segerback D, Bartsch H. DNA adducts: Identification and biological significance. *Oxford University Pres, Oxford*. 1994; 136(4):441-449.
35. Rebbeck TR. Molecular epidemiology of the human glutathione S-transferase genotypes GSTM1 and GSTT1 in cancer susceptibility. *Cancer Epidemiol Biomarkers Prev*. 1997; 6:733-743.

Cite this article as: Singh S, Joshi B, Saini A and Mohapatra T. Smoking, glutathione S transferase polymorphisms (GSTM1 & GSTT1) and their association with selected inflammatory biomarkers. *Al Ameen J Med Sci* 2022; 15(2): 113-122.

*All correspondences to: Dr. Basant Joshi, Assistant Professor, Department of Biochemistry, Rohilkhand Medical College and Hospital, Pilibhit Bypass Road, Bareilly-243006, Uttar Pradesh, India. E-mail: bjoshi86@gmail.com