Hypertension has multifactorial background and is a lifestyle-related disorder. Arterial hypertension is one of the most common types of hypertension and associated to oxidative stress known to be implicated in its pathogenesis. Glutathione S-transferases (GSTs) are phase II detoxification enzymes that play an important role in cellular defense against oxidative. GSTT1 and GSTM1 gene polymorphisms are reported to be associated with arterial hypertension in several populations and ethnic groups. GSTM1 and GSTT1 null genotype results in the impaired enzyme activity and consequently greater vulnerability towards various diseases such as cancer. The present study has been done to assess whether GSTT1 and GSTM1 null genotypes are associated with arterial hypertension among North Indian population. We have enrolled 99 arterial hypertension patients and 99 age and sex matched control individuals. Genotyping of GSTM1 and GSTT1 was done by multiplex PCR. We found that GSTM1 null genotype may have risk for hypertension (OR 1.75, CI 0.93-3.2), while for GSTT1 null genotypes shows protection toward hypertension (OR 0.254, CI-0.113-0.57). GSTM1 null genotypes serve as potential genetic factor and may be an independent risk factor for development of hypertension whereas GSTT1 null may have role in protection against hypertension in North Indian populations.

Keywords: GSTM1, GSTT1, Hypertension, Gene Polymorphism.

In developing and developed countries, more than 25% adults are prone to death from cardiovascular disease associated directly or indirectly to arterial hypertension\textsuperscript{1, 2}. Human arterial hypertension have complex, multifactorial and polygenic niche that interact with several genetic factors and other environmental factors\textsuperscript{3, 4}. In a meta-analysis review by Raghupathy et al it is reported that 20.6% men and 20.9% women suffer from hypertension. This ratio would be perpetually increases to 22.9% and 23.6% in men and women respectively by 2025 worldwide\textsuperscript{5}. Several studies
reported varying prevalence of hypertension in different states of India such as study done in rural Kerala reported 18% hypertension prevalence. Some studies showed hypertension increased prevalence in age groups, for instance a study reported 37% prevalence among population in the age range of 30-60; while a different study reported 55% prevalence in the age range of 40-60 . Study comparing the elderly population from urban and rural areas reported rate of hypertension to be 64% and 55% respectively. In addition to correlating the increased hypertension prevalence to age, some studies also reported the link between hypertension and socio-economic status. Mohan et al. reported 8.4% commonness of hypertension among men and women be owned to be low socio economic level. A comparative study of hypertension performed in urban areas of Chennai, India in low income and high income groups found the percentage frequency rate to be 54% and 40% whereas in a different study Mishra et al reported the prevalence to be 12 % in slums of Delhi, India. Smoking is one of the major risk factors to cause hypertension and cardiovascular disease. Cigarette smoke contains PAHs (polycyclic aromatic hydrocarbons) and hydroxilated metabolites of benzo (+) pyrene which requires bioactivation by phase I xenobiotic metabolizing enzymes (cytochrome p450s) and generates more reactive metabolites that may react with DNA and forms DNA adduct. Phase II xenobiotic enzymes glutathione S-transferase (GSTs) play an important role in detoxification process. It catalyses the conjugation of reduced glutathione with substances that contain electrophilic center. GST’s actions are known to follow phase I xenobiotic metabolism, which is elicited by cytochrome P450. CYP enzyme catalyses addition of functional groups such as epoxides which provides electrophilic center to reduced glutathione linked to phase II detoxification. Several reports suggested that some genes influence the blood pressure regulation, including IGFBP1, POR, MAWBP, XDH, GSTM1 and GSTT1. GSTM1 gene locus has been found on chromosome 1p13.3. Three variants have been reported, that differ by C to G substitution at base position 534, and gene deletion. The GSTT1 gene has been located on chromosome 22q11.2, one genotypes has been defined as GSTT-0 while genotype with one functional allele has been denoted as GSTT1. The deletion polymorphism study of GSTM1 and GSTT1 by gene deletion shows functional abnormality of enzyme in individuals with GSTM1 and GSTT1 null genotypes. Marinho et al reported that GSTT1 deletion was associated with protective effect on hypertension. However, deletion of GSTM1 has been reported in rats having cerebral stroke associated to hypertension contributing to oxidative stress. Alexey et al reported that null genotype of GSTT1 polymorphism could be a key player in susceptibility to increased risk of cerebral stroke with association to hypertension. GSTs may be related to risk of various diseases such as alzheimer, myocardial infarction, Parkinson’s, cancer and diabetes. In the present case control study, we have examined whether the loss of activity of GSTs enzyme due to deletion polymorphism in GSTT1 and GSTM1 may affect the risk in developing hypertension.

**MATERIALS AND METHODS**

The institutional ethical committee approved to conduct this study. The selection of individuals in both control and cases groups was based on standard questionnaire. Blood (in EDTA vials) was collected from all subjects who have participated in the study.

**Study Population**

**Selection of normal individual**

The study groups consist of 99 normal individuals from North India and have no reported hypertension. The selection method based on those people who have normal blood pressure and normal lipid profile. Smoking habits were noted through detailed questionnaire.

**Selection of hypertensive individual**

The study group consists of 99 hypertensive individuals from Allahabad city of north India. All subjects examined under same criteria as normal individual and only those were included in the study that have elevated blood pressure and high lipid profile. Selection was based on same questionnaire as control.

**Genotyping Analysis**

DNA for genotyping was isolated from human peripheral blood (in EDTA vials) by Qaigen DNA blood mini extraction kit. Multiplex PCR
was done for analysis of GSTM1 and GSTT1 gene polymorphism (Kumar et al., 2008). 50-100 ng of isolated DNA was amplified in total volume of 25 µl reaction mixture containing 10 pmol of primers. CYP1A of Exon 7 gene were co-amplified and used as internal control to differentiate between abortive PCR and successful one. The following primer sequence was used for GSTT1, GSTM1 and CYP1A:

\[
\text{GSTT1, Forward, } 5'-\text{TTCCTTACTGGTCCTCACATCTC}3', \\
\text{Reverse, } 5'-\text{TCACCGGATCATGGCCAGCA}3', \\
\text{GSTM1, Forward, } 5'-\text{GAACTCCTGAAAGCTA}3', \\
\text{Reverse, } 5'-\text{GTTGGGCTCAAATATACGGTGG}3'.
\]

PCR reaction was carried out in thermal cycler 8800 (Agilent Technologies, Australia). Each set of PCR reaction included both positive and negative controls. Presence and absence of GSTT1 and GSTM1 genes were detected by multiplex PCR method using following PCR cycling condition: initial denaturation at 94°C for 5 min, followed by denaturation 94°C for 1 min, annealing 59°C for 1 min and extension 72°C for 10 min. 2% agarose gel prepared for electrophoresis of multiplex PCR products and visualized by ethidium bromide staining at Geldoc XR+ system. Detection of samples positive for GSTM1 and GSTT1 genotypes matched for 215 and 480bp respectively while CYP1A yield band of 315bp. The 315bp of CYP1A serve as internal control. Presence of 480bp and 215bp fragments indicates GSTT1 and GSTM1 non-null genotypes respectively.

### Statistical Analysis

Statistical analysis was interpreted with SPSS software version 20.0 (SPSS, Inc., Chicago). Descriptive measures such as mean and standard deviation were applied for normally distributed variables. Odd ratio and 95% Confidence interval (CI) were used to evaluate impact of GSTM1 and GSTT1 genotype on the risk of developing hypertension. Correlation analysis was performed for all variables which was significant at 0.01 levels (2-tailed) and 0.05 levels (2-tailed). A p-value of <0.05 considered to be statistically significant.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Control (n=99)%</th>
<th>Hypertensive patients (n=99)%</th>
<th>Odd ratio (95% confidence interval)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSTT1 Absent (null)</td>
<td>9 (9.09)</td>
<td>28 (28.29)</td>
<td>0.25 (0.11-0.57)</td>
<td>0.00054 or &lt;0.001</td>
</tr>
<tr>
<td>Present (non-null)</td>
<td>90 (90.00)</td>
<td>71 (71.72)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSTM1 Absent (null)</td>
<td>33 (33.34)</td>
<td>22 (22.23)</td>
<td>1.75 (0.93-3.2)</td>
<td>0.112</td>
</tr>
<tr>
<td>Present (non-null)</td>
<td>66 (66.67)</td>
<td>77 (77.78)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSTM1/GSTT1 Present in both (non-null)</td>
<td>90 (90.91)</td>
<td>77 (77.78)</td>
<td>2.85 (1.24-6.57)</td>
<td>0.019</td>
</tr>
<tr>
<td>Absent in both (null)</td>
<td>9 (9.09)</td>
<td>22 (22.23)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 1.** Multiplex PCR for GSTT1 and GSTM1. 2% gel electrophoresis of GSTM1 and GSTT1 genotype. Lane 1, base pair marker (ProxiO, DNA marker, 0.1-2kb). Lane 2 represents both GSTT1 and GSTM1 null genotype whereas Lane 5 shows GSTT1 null genotype whereas Lane 7 shows GSTM1 null genotype and Lane 3, 6 and 8 shows GSTM1 non-null and GSTT1 non-null genotype.
RESULTS

In present study, we have 99 healthy individuals who served as controls and 99 as hypertensive patients. The majority hypertensive cases were old male and have longer persistent of hypertension. In addition, they had higher systolic blood pressure, diastolic blood pressure and LDL (low density lipoprotein) compared to controls. In present study, GSTM1 null genotypes (OR 1.75, CI 0.93-3.2, Table 1) showed non-significant increase predisposition in hypertensive individuals. This association was no longer observed after adjusting age, sex, smoking, blood pressure and lipid profile. However, GSTT1 null genotypes (OR 0.25, 0.11-0.57, Table 1) showed a significant protection toward the hypertension. In combined analysis of GSTM1 and GSTT1 genotypes, both GSTM1/GSTT1 non null variants resulted as protective factor while both GSTT1null/GSTM1 null (OR 2.85, CI 1.24-6.57, Table 1) showed 2.9 times higher risk for hypertension. We found males were 1.5 times more susceptible towards hypertension than females (Table 2). Risk estimate between smokers and non-smokers deduce that smokers were 15 times more vulnerable to have hypertension (Table 3). Our results suggest that age and systolic blood pressure (SBP) were weakly correlated, whereas HDL, total lipid and triglyceride were negatively correlated. Cholesterol levels with SBP and diastolic blood pressure (DBP) were moderately correlated whereas, HDL negatively correlated with cholesterol (Table 4). On the other side, HDL level showed strong negative correlation with age, SBP, DBP, cholesterol, LDL, triglyceride, total lipid and pulse rate. Triglyceride and total lipid were negatively correlated with age and HDL whereas pulse rate showed only negative correlation with HDL.

DISCUSSION

Glutathione S-Transferases GSTM1 and GSTT1 are phase II xenobiotic metabolizing enzymes serves to catalyse glutathione conjugation and metabolize number of reactive species. GST enzymes play crucial role to prevent damage to cell against chemical carcinogens and environmental toxicants. These detoxifying enzymes have key role in protecting DNA from various endogenous oxidants; which otherwise could damage DNA and could result in development of cancer. The prevalence range of GSTM1 null genotypes in many population based studies reported it to be 16% to 60%. In our study, we found 33% individuals are GSTM1 null genotypes in controls which are similar to other reports. In case of GSTT1 polymorphism among 99 normal individuals, we found 10% frequency rate which lies in the range of reported values of European and Mediterranean population from 10.4% to 42.5%, whereas there is 13-28% deletion for GSTT1 in Caucasians. In Indian region, the frequency of null genotype for GSTM1 and GSTT1 was found to be 22.4% and 17.6% respectively in southern region whereas it was 54% and 13% respectively.

Table 2. Gender susceptibility to hypertension.

<table>
<thead>
<tr>
<th></th>
<th>Control (N=89)%</th>
<th>Hypertensive patients(N=93)%</th>
<th>Odd ratio (95% confidence interval)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>23(25.84)</td>
<td>32(34.40)</td>
<td>1.5(0.58-0.48)</td>
<td>0.258</td>
</tr>
<tr>
<td>Male</td>
<td>66(74.15)</td>
<td>61(65.59)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Smoking habits between normal and hypertensive subjects

<table>
<thead>
<tr>
<th>Smoking habits</th>
<th>Control (N=89)%</th>
<th>Hypertensive patients(N=93)%</th>
<th>Odd ratio (95% confidence interval)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non smokers</td>
<td>79(88.76)</td>
<td>32(34.40)</td>
<td>15.05(0.28-0.85)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smokers</td>
<td>10(11.23)</td>
<td>61(65.59)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4. Statistical measures including biochemical parameters in controls and hypertensive patients.

<table>
<thead>
<tr>
<th></th>
<th>Control (n=89)</th>
<th>Hypertensive patients (n=93)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>Mean 51.78±10.92</td>
<td>Mean 53.67±12.51 Bessa et al.</td>
</tr>
<tr>
<td><strong>Systolic blood pressure</strong></td>
<td>121.87±10.92</td>
<td>159.94±15.44 Abbas et al.</td>
</tr>
<tr>
<td><strong>Diastolic blood pressure</strong></td>
<td>81.33±1.92</td>
<td>95.13±7.74 Polimati et al.</td>
</tr>
<tr>
<td><strong>Cholesterol (mg/dl)</strong></td>
<td>179.98±16.28</td>
<td>240.46±7.24 Bessa et al.</td>
</tr>
<tr>
<td><strong>HDL (mg/dl)</strong></td>
<td>70.13±1.92</td>
<td>100.46±1.54 Bessa et al.</td>
</tr>
<tr>
<td><strong>LDL (mg/dl)</strong></td>
<td>170.94±1.54</td>
<td>240.46±7.24 Bessa et al.</td>
</tr>
<tr>
<td><strong>Triglyceride (mg/dl)</strong></td>
<td>100.46±15.44</td>
<td>240.46±7.24 Bessa et al.</td>
</tr>
<tr>
<td><strong>Total Lipid (mg/dl)</strong></td>
<td>125.17±16.26</td>
<td>227.91±95.16 Bessa et al.</td>
</tr>
<tr>
<td><strong>Pulse rate (min)</strong></td>
<td>72.91±3.04</td>
<td>99.35±25.97 Ettore et al.</td>
</tr>
</tbody>
</table>

The present study stated a prevalence of hypertension were 22% GSTM1 null genotype and 28% GSTT1 null genotype polymorphism respectively as determined in North Indian population. We found GSTM1 null genotypes represent independent risk factor for development of hypertension. Our findings were in accordance to Daniel and co-workers as they reported the association of null genotypes of GSTT1 and GSTM1 to hypertension in patients with type 2 diabetes in Caucasians. Ettore and co-workers reported that GSTM1 null variant was independent factor with association to hypertension in Italian region which was accordance to our result. Our result suggests that GSTM1 gene may be considered as major gene associated with susceptibility of hypertension in North Indian population. LDL (low density lipoprotein) shows positive correlation with systolic blood pressure, so it may serves as marker to primary detection of hypertension whereas HDL (high density lipoprotein) shows negative correlation. In contrast to present study, Alexy et al. concluded that GSTT1 null genotype was higher in patients suffering from essential hypertension and cerebral stroke. Abbas and co-workers demonstrated the significant association of null genotypes of GSTM1 and GSTT1 for the hypertension risk. Similarly a recent meta-analysis published by Eslami and coworkers shows that GSTM1 and GSTT1 null genotypes serve as markers for hypertension. However, Kosar et al. reported that null genotype of GSTM1 and GSTT1 were not significantly associated with hypertension. Polimati et al. stated that GSTT1 null genotypes were significantly associated with high risk of hypertension (OR-2.24). In present study males were 1.5 times more susceptible to hypertension than females in contrast a study by Polimati et al. showed the risk was higher in female hypertensive patients (OR-3.25). Recent study by Bessa and coworkers also reported that null genotypes of GSTM1/GSTT1 were main risk factors for the development of hypertension and can be served as a marker in primary detection.

**CONCLUSION**

Present study suggest that GSTM1 null genotypes may depict as crucial genetic factor to predict development of hypertension, whereas...
GSTT1 null may have role in protection against hypertension. There is significant association between systolic blood pressure and LDL which may serves as marker to primary detection of hypertension and indicates the pathogenic role in its development among different populations.

ACKNOWLEDGMENT

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REFERENCES


