Assessment of Glutathione peroxidase and its correlation with the components of Metabolic Syndrome

Dr Rajeev Goyal¹, Dr Manoj Kumar Nandkeoliar², Dr Vartika Saxena³, Pramod Payal⁴, Dr. Rahul Saxena⁵

¹Associate Professor, Department of Biochemistry, All India Institute of Medical Sciences, Rishikesh, India
²Assistant Professor, Department of Biochemistry, School of Medical Sciences and Research; Sharda University; Greater Noida, U.P, India
³Additional Professor, Department of Community and Family Medicine, All India Institute of Medical Sciences, Rishikesh, India
⁴Department of Biochemistry, All India Institute of Medical Sciences, Rishikesh, India
⁵Assistant Professor, Department of Biochemistry, School of Allied Health Sciences, Sharda University, Greater Noida, U.P, India

*Corresponding author
Dr. Rahul Saxena
Email: rahulapril@gmail.com

Abstract: Metabolic syndrome (MS) is a cluster of cardio metabolic risk factors such as obesity, hyperglycemia, dyslipidemia, and hypertension and insulin resistance. However, the role of oxidative stress in developing components of MS needs further investigations. The objective of present study was to study the levels of antioxidant enzyme Glutathione peroxidase (GSHPx) and correlate them with components of MS. 300 participants were enrolled, which were divided into MS positive (n=150) and MS negative (n=150) groups as per NCEP ATP III criteria. Components of MS were assessed using standard methods and data from patients and controls were compared by using Student’s t-test. Correlation of GSHPx with the components of MS was carried out using Pearson correlation coefficient. Erythrocyte GSHPx activity was decreased significantly in patient group subjects. In addition, GSHPx activity was negatively correlated with the components of MS. Oxidative stress is significantly associated with MS and its components. Furthermore, components of MS should be monitored regularly with advancing of age in order to overcome the burden of cardiovascular disease risk.

Keywords: Hyperglycemia, hypertriglyceridemia, dyslipidemia, abdominal obesity, free radical

INTRODUCTION:
The metabolic syndrome refers to the co-occurrence of cardiovascular risk factors such as insulin resistance, obesity, dyslipidemia and hypertension. The incidence of metabolic syndrome (MS), which increases the future cardiovascular disease (CVD) risk, has reached epidemic proportions worldwide. Although precise etiology of this syndrome is poorly understood, probability of MS patients to develop future CVD risk appears to be associated with chronic low level inflammation and increased oxidative stress (OS) [1-3].

OS is accepted as important mediator in development and progress of hypertension, endothelial dysfunction and atherosclerosis. (4) OS is also present in conditions of insulin resistance and inflammation [5, 6]. Oxidative stress in MS is ensued by excessive production of reactive oxygen species (ROS). Glutathione peroxidase (GSHPx) is an important antioxidant enzyme which not only neutralises H₂O₂, but also transforms lipoperoxide and other organic hydro peroxide into their less reactive hydroxylated compounds. Reduced activity of antioxidant enzymes, reflecting increased oxidative stress, has been described in patients with obesity, hypertension and other components of MS [7].

Studies related to association of GSHPx and MS are scanty. Also, there are very few reports on association of oxidative stress with components of MS. In fact, to establish the relationship between MS and oxidative stress, more concrete evidences are required.
in which correlations between antioxidant enzymes and the presence of triggering factors and components of the syndrome are essential. Therefore, the aim of present study was to evaluate the erythrocyte GSHPx activity in MS patients and to determine the association of GSHPx with the components of MS.

MATERIAL AND METHODS:
A cross-sectional comparative study was carried out on a sample of 300 subjects (30-50 years old). After taking informed consent as per protocol approved by institutional ethics committee, demographic information from all participants was recorded. Participants were entered separately in Microsoft Excel sheet of windows 2007 and values were expressed as Mean ± SD. The significance of mean difference between patient and control groups was compared by using Student’s t test. The distribution of ‘t’- probability was calculated depending on ‘n’ and significance of test was obtained. P value < 0.05 and < 0.001 were considered as significant and highly significant, respectively. P value > 0.05 was considered as insignificant. In addition, correlation analysis between aforesaid parameters was performed by using Pearson correlation test.

RESULTS:
In the present study, 150 MS positive (MS+) and 150 MS negative (MS-) participants were included. Demographic profile of both groups is given in Table 2. Among the MS positive group, 54% were male and 46% were females. There was significant difference in BMI, waist circumference, blood pressure (systolic as well as diastolic) of MS positive and MS negative groups. While BMI of MS positive group was 25% higher than MS negative group, there was increase of 17% and 15% in waist circumference and blood pressure, respectively, of MS positive group as compared to MS negative group.

Erythrocyte GSHPx activity was estimated by commercial kit (Ransel, Randox Laboratories). In this method, GSHPx catalyses the oxidation of Glutathione (GSH) by Cumene Hydro peroxide. In the presence of Glutathione Reductase (GR) and NADPH the oxidised Glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP+. The decrease in absorbance at 340 nm is measured. All colorimetric analyses were performed on fully automatic analyzer (Beckman Coulter AU 680) with 2 levels of quality control. Participants were diagnosed as with MS (n=150) or without MS (n=150), as per National Cholesterol Education Program Adult Treatment Panel III criteria (NCEP- ATP III) guidelines (Table 1). [9]

Statistical Analysis:
The data collected from patients and control were entered separately in Microsoft Excel sheet of
Table 1: NCEP-ATP III criteria for Metabolic Syndrome (Any 3 of the given 5 parameters are required for a diagnosis of MS)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waist circumference</td>
<td>&gt; 40 inch (male)/ &gt; 35 inch (female)</td>
</tr>
<tr>
<td>Blood Pressure</td>
<td>&gt; 130 mmHg systolic or &gt; 85 mmHg diastolic</td>
</tr>
<tr>
<td>Fasting Triglycerides</td>
<td>&gt; 150 mg/dl</td>
</tr>
<tr>
<td>HDL</td>
<td>&lt; 40 mg/dl in males</td>
</tr>
<tr>
<td>Fasting blood sugar</td>
<td>&gt; 100 mg/dl</td>
</tr>
</tbody>
</table>

Table 2: Demographic profile of MS negative and MS positive groups (Mean ± SD)

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Particulars</th>
<th>MS negative (N=150)</th>
<th>MS positive (N=150)</th>
<th>% Increase</th>
<th>% Decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Age (years)</td>
<td>41.66 ± 7.4</td>
<td>42.4 ± 8.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Height (meter)</td>
<td>1.65 ± 0.8</td>
<td>1.66 ± 0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Weight (kg)</td>
<td>64.80 ± 5.8</td>
<td>72.52 ± 6.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>BMI</td>
<td>22.9 ± 2.0</td>
<td>28.71 ± 2.6***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Waist Circumference (cm)</td>
<td>82.54 ± 8.8</td>
<td>96.62 ± 9.2**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Hip (cm)</td>
<td>93.06 ± 7.2</td>
<td>103.53 ± 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Waist-hip ratio</td>
<td>0.87 ± 0.08</td>
<td>0.94 ± 0.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Systolic pressure (mmHg)</td>
<td>108 ± 9.5</td>
<td>124.8 ± 8.2**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Diastolic pressure (mmHg)</td>
<td>74.6 ± 5.2</td>
<td>86.4 ± 6.8**</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Where,
* p < 0.1, **p<0.05, *** p < 0.001

Table 3: Glycemic and lipid profile of study group subjects (Mean ± SD)

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Particulars</th>
<th>MS negative N=150</th>
<th>MS positive N=150</th>
<th>% Increase</th>
<th>% Decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Total cholesterol(mg/dl)</td>
<td>153.5 ± 28.7</td>
<td>186.0 ± 32.4**</td>
<td>21.17</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Triglycerides(mg/dl)</td>
<td>85.7 ± 16.3</td>
<td>142.6 ± 20.8***</td>
<td>66.39</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>LDL (mg/dl)</td>
<td>97.8 ± 14.2</td>
<td>122.5 ± 16.7**</td>
<td>25.25</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>HDL (mg/dl)</td>
<td>47.4 ± 5.5</td>
<td>38.7 ± 6.2***</td>
<td>-</td>
<td>18.35</td>
</tr>
<tr>
<td>5</td>
<td>VLDL (mg/dl)</td>
<td>18.2 ± 4.8</td>
<td>31.4 ± 5.0***</td>
<td>72.52</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Fasting blood glucose</td>
<td>85.6 ± 12.5</td>
<td>112.0 ± 18.4**</td>
<td>26.4</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 4: Correlation coefficient (r) between Glutathione peroxidase and components of MetS

<table>
<thead>
<tr>
<th>Particular</th>
<th>SBP</th>
<th>DBP</th>
<th>TC</th>
<th>TG</th>
<th>HDL</th>
<th>LDL</th>
<th>VLDL</th>
<th>FBG</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSHPx</td>
<td>-0.402</td>
<td>-0.378</td>
<td>-0.563</td>
<td>-0.474</td>
<td>0.645</td>
<td>-0.608</td>
<td>-0.518</td>
<td>-0.670</td>
</tr>
</tbody>
</table>

Where,
* p < 0.1, **p<0.05, *** p < 0.001
DISCUSSION:
Metabolic syndrome is a major and escalating public-health challenge worldwide. Due to increasing urbanization, surplus energy intake, increasing obesity, and sedentary life habits, the prevalence of MS in increasing. In India, prevalence of MS varies from 20-30% [10, 11]. MS confers a 2-fold the risk of developing cardiovascular disease and 5-fold increase in the risk of type 2 diabetes mellitus over the next 5 to 10 years. Increased visceral adiposity, reflected by high BMI and waist circumference, is now known to be central to development of MS. This phenomenon is seen in our study participants also. Our findings show that there is a significant increase in BMI and blood pressure in the MS patients as compared to the participants without MS, which exposes them to increased risk of future cardiovascular disease. Here, we also show that high blood pressure is an important component of MS, as both systolic and diastolic BP were significantly elevated in MS group compared to control group. A recent study also found that high blood pressure is most important factor linked to OS in elderly [12].

Though insulin resistance is considered to be the main underlying mechanism in metabolic derangements in MS, low grade chronic inflammation and oxidative stress are also known to be associated with components of MS. Also, it has been shown that inflammation as well as oxidative stress is associated with insulin resistance [13-15]. Increased visceral adiposity is related to the establishment of insulin resistance and low-grade chronic proinflammatory state of patients, through inflammatory cytokines release. In the present study, we show that oxidative stress, as indicated by reduced erythrocyte GSHPx is significantly associated with MS. This finding is corroborated by several other studies [16, 17]. Moreover, there is a strong relationship between oxidative stress and altered metabolic profile leading to MS. Further, erythrocyte GSHPx was inversely correlated with components of MS. This could be explained either by their decreased synthesis or rapid consumption in protecting the cells from H2O2 mediated oxidative damage in MS patients. Such as dyslipidemia, endothelium dysfunction, hypertension and insulin resistance.

Recently, Shrestha et al.; on their study on metabolic syndrome patients reported the MS patients are more susceptible to develop future cardiovascular disease due to their uncontrolled metabolic profile and elevated level of oxidative stress [18]. Goyal et al.; also observed the low GSHPx activity in individuals with components of MS and its association with increased oxidative stress and proinflammatory state [6]. It has also been found that decrease in GSHPx activity is associated with increased body mass index (BMI) and waist circumference, as observed in our study, and thereby reinforcing the establishment of pro-oxidant condition in MS patients which may impair insulin signal pathway and lead to harmful action on the endothelium.

CONCLUSION:
On the basis of findings of the present study and consistent support of previous evidences, it is
obvious that oxidative stress plays a crucial role in MS etiopathology. Further, high blood pressure is an important derangement associated with MS. Study of oxidative stress in MS patients can help in getting better knowledge regarding pathways which contribute to complications of MS.

REFERENCES: