Abstract

Background
In metabolic syndrome, endothelial cells are directly exposed to reactive oxygen species and increase the risk of various complications. Therefore, the objective of the present study was to evaluate the markers of oxidative stress (malondialdehyde, ceruloplasmin and uric acid), endothelial dysfunction (nitric oxide) and serum magnesium levels along with the variation in waist circumference, lipid and glycemic profile among the metabolic syndrome patients and to compare it with that of healthy controls.

Methods
The parameters were estimated using standard methods in 50 healthy control group and 50 patients with metabolic syndrome. The obtained values were compared statistically by using paired t-test.

Results
It was observed that the serum nitric oxide and magnesium levels were significantly reduced \( (p<0.01) \) whereas serum malondialdehyde levels were increased \( (p<0.001) \) along with significant rise in serum uric acid \( (p<0.01) \) and ceruloplasmin levels \( (p<0.01) \) in metabolic syndrome patients.

Conclusion
These findings support that there are noticeable alterations in the level of markers of oxidative stress and components of metabolic syndrome which collectively impair the endothelial function and thereby enhances the risk of future cardiovascular complications.

Key words
Ceruloplasmin, lipid peroxidation, magnesium, nitric oxide, uric acid.
Background

Hypertension, hyperglycemia, insulin resistance, dyslipidemia and abdominal obesity are well known risk factors of cardiovascular complications. Metabolic syndrome (MetS) is a clinical condition, characterized with aforesaid factors and thereby increases the risk of future cardiovascular complications multifold [1-4]. In addition, increasing trend of sedentary life style, urbanization and burden of obesity representing the MetS as major public health and clinical challenge. Apart from the association of MetS with cardiovascular disease risk, it has been reported that MetS increases the risk of type 2 diabetes as well [5].

When metabolic syndrome components are associated, individually each factor plays a crucial role in impairing endothelial function. Misra and Khuran also reported that endothelial vasomotor dysfunction as a predictor of future coronary events in obese and insulin resistance patients [6]. Moreover, renin angiotensin aldosterone system by activation of NADPH oxidase and high levels of circulating pro-inflammatory cytokines in visceral adipose tissue also contribute ROS generation and its exposure to endothelial cell in MetS [7, 8].

Oxidative stress has also been found to be associated with decreased bioavailability of nitric oxide (NO) in endothelial cells and thereby enhances cardiovascular complications.[9] Furthermore, the study pertains to correlation between metabolic syndrome, endothelial dysfunction and oxidative stress at single platform has not been carried on in the population of the North India so far as best of my knowledge. Therefore, the aim of present study was to evaluate the marker of oxidative stress, endothelial dysfunction, dyslipidemia and glycemic profile in MetS patients and to determine the prevalence of risk of CVD among the Metabolic Syndrome patients in order to suggest the possible lifestyle changes to reduce the risk of future cardiovascular complications.

Material and Methods

Study Period

The present study was a case control study carried out for a period of one year (Feb 2015- Feb 2016).

Study design and participants

To study the comparative alterations in the level of markers of oxidative stress and endothelial dysfunction in association with abnormalities in the components of metabolic syndrome among the control and patients group of same age group i.e (20 – 55) yrs. The present study was carried out in the Departments of Biochemistry and in the Department of Medicine of School of Medical Sciences and Research and associated hospital, Sharda University. The study was conducted on 100 outdoor subjects visited at Sharda Hospital, Greater Noida. These subjects were categorized as Group I (Control group) with 50 normal healthy individuals of age group 20 – 50 years and Group II (Patient group) comprised of 50 individuals who were age and sex matched patients suffering from metabolic syndrome.

Response rate

Out of 148 subjects, only 100 subjects had given complete information required for the study as they were satisfied with us that their information were maintained with confidentiality. Thus, 100 subjects were finally recruited for the present study. In addition, confidentiality of the study was maintained by allotting unique study information number at the time of approval of the study.

Data collection

The study related data was collected manually at time of interview of the patient and after estimation the levels of study group parameters through standard methods. Overnight fasting peripheral venous blood sample was collected into plain (5ml) vials from the study group subjects. The samples were centrifuged at 2000 rpm for 15 minutes. The separated serum (plain vial) was stored at –80°C until further analysis. The main parameters estimated were serum malondialdehyde, ceruloplasmin, uric acid, nitric oxide, magnesium, fasting blood glucose and lipid profile. Serum malondialdehyde was estimated as thiobarbituric acid reactive substances by using a method of Sinnhuber et al [11]. Trimethine coloured substance was formed as a reaction of MDA with thiobarbituric acid (TBA) in acidic medium, and it was measured spectrophotometrically at 532 nm.

Ravins’s method was used to estimate serum ceruloplasmin level [12]. Ceruloplasmin catalyses the oxidation of p-phenylenediaminedihydrochloride used as substrate into purple colored oxidation product, which was measured colorimetrically at 546 nm. Serum nitric oxide was determined by the use of Vanadium III chloride and Griess reaction method which involve sulfanilamide and N-(1-naphthyl) ethylenediamine as Griess reagent. In this method, nitrate was converted to nitrite by nitrate reductase. Griess reagent act on nitrite and produces a deep purple azo compound which was measured spectrophotometrically at 540 nm [13].

Serum lipid profile contents (Total Cholesterol, Triglycerides and HDL cholesterol) were analysed enzymatically using kit obtained from (Randox Laboratories Limited, Crumlin, UK). LDL-cholesterol levels were calculated by Friedwald’s formula [14].

$$LDL-C = TC - [(TG/5) + HDL-C]$$
Caraway’s method was employed for the estimation of serum uric acid which involves the reaction of uric acid with phosphotungstic acid in alkaline medium to produce a blue color complex [15]. Fasting blood glucose levels were measured by using enzymatic kit based on glucose oxidase method. Glucose, in presence of glucose oxidase, converted into gluconic acid along with production of Hydrogen peroxide, which later oxidatively coupled with 4-aminoantipyrine and red quinoneimine dye was produced. The intensity of the color complex was directly proportional to the glucose in specimen and showed absorption maxima at 505 nm.

Results

In the present study, 50 patients of MetS and 50 healthy individuals, served as controls were included. Demographic profile of MetS patients is represented in Table 1.

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Group I N=50</th>
<th>Group II N=50</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>28.66 ± 5</td>
<td>37.4 ± 9</td>
<td>0.524^</td>
</tr>
<tr>
<td>Height (meter)</td>
<td>1.66 ± 0.9</td>
<td>1.65 ± 0.12</td>
<td>0.938^</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>63.86 ± 11</td>
<td>74.64 ± 12</td>
<td>0.001†</td>
</tr>
<tr>
<td>BMI</td>
<td>23.0 ± 4.2</td>
<td>27.74 ± 5.6</td>
<td>0.0001†</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>83.58 ± 10</td>
<td>95.80 ± 9.9</td>
<td>0.0001†</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>93.06 ± 7.2</td>
<td>103.53 ± 8</td>
<td>0.0001†</td>
</tr>
<tr>
<td>Waist-hip ratio</td>
<td>0.89 ± 0.02</td>
<td>0.92 ± 0.03</td>
<td>0.0001†</td>
</tr>
<tr>
<td>Systolic pressure (mmHg)</td>
<td>117 ± 9.13</td>
<td>126.5 ± 10</td>
<td>0.0001†</td>
</tr>
<tr>
<td>Diastolic pressure (mmHg)</td>
<td>78.46 ± 0.5</td>
<td>83.7 ± 8.8</td>
<td>0.0001†</td>
</tr>
</tbody>
</table>

^P>0.05 statistically not significant
†P<0.01 statistically significant

There was a significant difference between the groups in terms of age (p<0.01) which indicate that MetS is more prevalent in middle age group. Among the patients 52% participants were male and 48% were females respectively. Waist circumference (p<0.01, 14.63% high), systolic blood pressure (p<0.01; 7.87%) and diastolic blood pressure (p<0.01, 6.67%) were significantly high in patient participants than normal controls as represented in Table 1. In addition, relation of drinking and smoking habit with MetS along with number of patients under medication is depicted in Table 2. The glycemic and lipid profile data showed significant abnormalities in the patients group as represented in Table 3. Fasting blood glucose level was significantly high (p<0.01; 30.91%) in patient group as compared to healthy controls. Serum total cholesterol, triglyceride, LDL and VLDL levels were found to be significantly high in metabolic syndrome patients. However, HDL was found to be reduced significantly (p<0.01) in the MetS patients as compared to healthy controls.

<table>
<thead>
<tr>
<th>Particulars</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>0.0005^</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>0.0001†</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>0.0001</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>0.0001</td>
</tr>
<tr>
<td>VLDL (mg/dl)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Fasting blood glucose (mg/dl)</td>
<td>0.0001^</td>
</tr>
</tbody>
</table>

^P<0.01 statistically significant
†P<0.05 statistically significant

Markers of oxidative stress, endothelial dysfunction and serum magnesium levels in MetS patients and healthy controls are represented in Table 4.

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Group I N=50</th>
<th>Group II N=50</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malondialdehyde (µmol /ml)</td>
<td>0.25 ± 0.04</td>
<td>0.32 ± 0.06</td>
<td>0.0001</td>
</tr>
<tr>
<td>Uric Acid (mg/dl)</td>
<td>5.0 ± 1.2</td>
<td>13.07 ± 5.5</td>
<td>0.0001</td>
</tr>
<tr>
<td>Ceruloplasmin (mg/dl)</td>
<td>44.74 ± 9</td>
<td>61.19 ± 12</td>
<td>0.0001</td>
</tr>
<tr>
<td>Nitric oxide (µM/L)</td>
<td>685 ± 268</td>
<td>98 ± 31</td>
<td>0.0001</td>
</tr>
<tr>
<td>Magnesium (mg/dl)</td>
<td>1.74 ± 0.04</td>
<td>1.55 ± 0.16</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

^P<0.01 statistically significant

Table – 4 Glycemic and lipid profile of control and patient groups

There was a significant difference between the groups in terms of age (p<0.01) which indicate that MetS is more prevalent in middle age group. Among the patients 52% participants were male and 48% were females respectively. Waist circumference (p<0.01, 14.63% high), systolic blood pressure (p<0.01; 7.87%) and diastolic blood pressure (p<0.01, 6.67%) were significantly high in patient participants than normal controls as represented in Table 1. In addition, relation of drinking and smoking habit with MetS along with number of patients under medication is depicted in Table 2. The glycemic and lipid profile data showed significant abnormalities in the patients group as represented in Table 3. Fasting blood glucose level was significantly high (p<0.01; 30.91%) in patient group as compared to healthy controls. Serum total cholesterol, triglyceride, LDL and VLDL levels were found to be significantly high in metabolic syndrome patients. However, HDL was found to be reduced significantly (p<0.01) in the MetS patients as compared to healthy controls.

Table – 5 Correlation coefficient (r) between serum Nitric oxide and markers of oxidative stress along with serum magnesium levels in MetS patients.

There was a significant difference between the groups in terms of age (p<0.01) which indicate that MetS is more prevalent in middle age group. Among the patients 52% participants were male and 48% were females respectively. Waist circumference (p<0.01, 14.63% high), systolic blood pressure (p<0.01; 7.87%) and diastolic blood pressure (p<0.01, 6.67%) were significantly high in patient participants than normal controls as represented in Table 1. In addition, relation of drinking and smoking habit with MetS along with number of patients under medication is depicted in Table 2. The glycemic and lipid profile data showed significant abnormalities in the patients group as represented in Table 3. Fasting blood glucose level was significantly high (p<0.01; 30.91%) in patient group as compared to healthy controls. Serum total cholesterol, triglyceride, LDL and VLDL levels were found to be significantly high in metabolic syndrome patients. However, HDL was found to be reduced significantly (p<0.01) in the MetS patients as compared to healthy controls.

<table>
<thead>
<tr>
<th>Particulars</th>
<th>MDA</th>
<th>Uric acid</th>
<th>Ceruloplasmin</th>
<th>Mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO</td>
<td>-0.562</td>
<td>-0.614</td>
<td>-0.576</td>
<td>-0.308</td>
</tr>
<tr>
<td>P value</td>
<td>0.058^</td>
<td>0.032*</td>
<td>0.056†</td>
<td>0.075^</td>
</tr>
</tbody>
</table>

^P>0.05 statistically not significant
†P<0.05 statistically significant
Serum MDA levels were significantly increased (p<0.01, 26% high) in patients group than controls. Similarly, serum ceruloplasmin levels were also higher (p<0.001, 36.76%) in patients group as compared to healthy group. Serum uric acid levels were also increased significantly (p<0.01, 161.20%) in patient group as compared to control group.

Serum NO levels in the patients group were decreased significantly (p<0.01,) as compared to the healthy group. Similarly, serum Mg levels were reduced (p<0.05, 10.69%) in the patients group as compared to healthy group.

In addition, we observed a negative correlation between NO and markers of oxidative stress such as MDA, uric acid and ceruloplasmin whereas serum magnesium levels were positively correlated with NO levels, as shown in Table 5. Similarly, marked correlation exist between serum NO and components of MetS, as presented in Table 6. Serum NO level was negatively correlated with waist circumference, blood pressure, fasting blood glucose, total cholesterol, triglycerides, LDL and VLDL levels (p < 0.05); whereas serum HDL levels were positively correlated with NO levels in MetS patients. These results clarify the role of NO reduction in enhancing the CVD risk in MetS patients most probably by its relation with dyslipidemia, elevated sugar, oxidative stress and waist circumference including BMI.

### Discussion

Incidence of CVD has been found to be increased dramatically with increase in body weight and metabolic alteration. In addition, abdominal obesity also acts as a driver of cardio-metabolic risks. Our findings suggested that there was a significant increase in the waist circumference in the MetS patients as compared to the healthy controls which increases the risk of future CVD in MetS group. A growing body of evidence supported our study and suggested that increased oxidative stress in white adipose tissue through the activation of NADPH oxidase and depletion of anti-oxidative enzymes plays a crucial role in establishing cardiac complication in MetS [16].

Interestingly, our study showed increase in plasma MDA levels as compared to healthy individuals, which was an indication of marked oxidative stress. Similarly, previous studies have documented the increased oxidative stress status in diabetes mellitus characterized by increased lipid peroxidation and fall in antioxidant enzymes in plasma [17-19].

In order to protection against deleterious effect of ROS, non-enzymic antioxidants contribute significant role in the body. Our study showed a marked elevation in the serum uric acid level in the MetS which was probably to get rid of the ROS by virtue of its role as non-enzymic anti-oxidant. However, Hisalkar et al. observed decreased level of serum uric acid in MetS patients probably due to its consumption in lowering the effect of ROS [20].

Apart from that, consistent high level of serum uric acid as observed in our study could also be explained on the basis of its dual nature as the oxidative environment milieu tends to act more like a proxidant under the oxidative stress because hydrophobic environment created by lipids was unfavorable for the antioxidant effects of uric acid and oxidized lipids could convert uric acid into an oxidant which predominantly target LDL and cell membrane [21-23]. Moreover, evolutionary loss of the uricase gene and concomitant increase in serum uric acid in non-human and human primates also provides a mechanism for its antioxidant capacity to compensate the loss of other antioxidant such as ascorbic acid, utilized during oxidative stress [24].

Endothelial dysfunction has been found to be associated with oxidative stress. However, the mechanism behind the incidence of endothelial dysfunction in the metabolic syndrome is still in dark. Serban et al. showed that impaired endothelial dysfunction was associated with a reduced release of nitric oxide in MetS [25]. Consistent findings have been observed in present study which could be explained on the basis of decreased synthesis of NO due oxidation and depletion of the endothelial NO synthase (eNOS) cofactor, tetrahydrobiopterin by ROS. Further, the reduction in serum NO level in patients might be specifically due to oxidative modification of LDL to oxidized LDL. It has been reported that oxidized LDL is transformed to foam cells by macrophages which is responsible for inactivation of NO and thereby inducing endothelium destruction [26]. However, Soydine et al. in their study observed a marked increase in serum nitric oxide levels as one of the risk factor of MetS patients, probably, due to high adipose tissue in MetS which contains NO synthetase enzyme that lead to excessive NO production [27, 28].

Moreover, activated macrophages induce the production of ceruloplasmin which has a ferroxidase activity and act as a

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Waist circumference</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>NO</td>
<td>-0.502</td>
<td>-0.678</td>
<td>-0.592</td>
<td>-0.374</td>
<td>-0.445</td>
<td>0.642</td>
<td>-0.598</td>
<td>-0.473</td>
<td>-0.306</td>
</tr>
<tr>
<td>P value</td>
<td>0.059*</td>
<td>0.022*</td>
<td>0.037*</td>
<td>0.075*</td>
<td>0.070*</td>
<td>0.025*</td>
<td>0.054*</td>
<td>0.064*</td>
<td>0.088*</td>
</tr>
</tbody>
</table>

*P<0.05 statistically not significant
*P<0.05 statistically significant
P<0.01 statistically significant
marker of inflammation as acute phase reactant. Our study showed a significant rise in serum ceruloplasmin levels in the MetS group as compared to the healthy control group. These findings are in consistent with the findings of Virgolici et al. According to them, an increase in serum ceruloplasmin levels could generate an excess of oxidized LDL which causes atherosclerosis [29]. 

Apart from dyslipidemia and oxidative stress in the MetS, our study showed aberration in the serum magnesium levels which was supported by study of Jinsong et al., conducted in Metabolic Syndrome patients participated in a dietary magnesium trial [30]. Hypomagnesemia, as observed in present study, could indicate a higher possibility of critical situations associated with MetS such as diabetes because magnesium is required for tyrosine kinase activity at the insulin receptor level to exert blood-sugar-lowering effects of insulin [31]. Serum magnesium has a crucial role in maintaining healthy lipid profile by decreasing the activity of lecithin and HMG-CoA, and increasing lipoprotein lipase activity [32]. Depletion in serum magnesium levels along with its positive correlation with serum Nitric oxide levels makes the MetS patient more susceptible to develop future CVD.

**Conclusion**

Thus, MetS patients are more susceptible to develop future CVD due to their uncontrolled metabolic profile. In addition, elevated level of oxidative stress incorporation with components of MetS contribute significantly in developing high blood pressure, characterized by endothelial dysfunction and thereby leading to develop cardiac complications. Therefore, in order to lower the risk of cardiovascular complications in MetS, primary goal should be to control dyslipidemia, adoption of healthy life style along with improving the glycemic profile, incorporation of antioxidant and mineral rich diet; and proper exercise with timely monitoring of blood pressure.

**Limitations & future scope of the study**

The main drawback of this study was the sample size, which was less and it is recommended to perform a multicenter study in future, with a larger population. Long term follow up and inclusion of more investigations even at molecular basis also needed to get accurate result.

**Abbreviations**

Cardio vascular disease (CVD), endothelial Nitric oxide synthase (eNOS), high density lipoprotein (HDL), low density lipoprotein (LDL), Malondialdehyde (MDA), metabolic syndrome (MetS), nitric oxide(NO), reactive oxygen species (ROS), very low density lipoprotein (VLDL)

**Competing interests**

The authors declare that there have no competing interests.

**Authors’ contribution**

Biochemical analysis, data collection and interpretation was done by Shrestha S. Formulation of study, facilitating the sample collection, data interpretation, drafting, revising and editing the manuscript done by Saxena R. Srivastava S facilitate the sample collection, data collection and data interpretation. Thakur RK, assist in data collection and biochemical analysis.

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**References**