Study of Antioxidant Enzyme - Superoxide Dismutase Activity and Lipid Profile in Diabetes Mellitus patients

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ABSTRACT

Introduction: Hyperglycemia and oxidative stress has been recognized as cardinal features of diabetes. Superoxide Dismutase Enzyme fight at the antioxidant forefront by detoxifying free radicals. In the present study our aim was to assess the antioxidant enzyme SOD activity and its correlation with Serum Glucose and lipid profile in Diabetic patients and normal healthy controls.

Material and method: In our study 45 Diabetic Patients (Type I & Type II), 20 controls were taken and study was done at S.M.S Medical college, Jaipur. S. SOD, Hb%, S. glucose, S. cholesterol, S. Triglyceride, S. HDL cholesterol, S. VLDL cholesterol and S. LDL cholesterol were measured.

Result: In our study Diabetic group have mean value of super oxide dismutase (U/ml &u/gm Hb), S Glucose & S lipid profile (S.chol., S.TG, S.HDL, S.VLDL, S.LDL,) was 73.67±15.22u/ml (638.61±128.14 u/ gm Hb), 167.58±40.39mg/dl, 220.81±27.85 mg/dl, 203.00±38.72 mg/dl, 39.09±6.02 mg/dl, 40.25±8.02 mg/dl, 141.64±28.81 as compare to control group 106±23.85u/ml (924.04±307.86u/gm Hb). 93.35±7.10,mg/dl , 161.30±16.23mg/dl , 151.12±9.39mg/dl , 41.21±4.74 mg/dl, 30.92±2.95 mg/dl, 89.11±14.06 mg/dl respectively. In Diabetic patients Duration and S. Glucose have positive correlation with S. TG, S. VLDL & S. LDL and negative with Blood SOD and S. HDL Cholesterol. Super oxide Dismutase Enzyme have negative correlation with S. Glucose, S. Chol., S. TG, S. VLDL, S. LDL and positive with S. HDL.

Conclusion: Diabetic patients have decreased level of Antioxidant Superoxide dismutase and disturbed lipid profile in compare to control subjects and indicate that there is a oxidative stress and to fight against this SOD level was decreased.

Keywords: Oxidative stress, Antioxidants, Superoxide Dismutase

Introduction:

Diabetes Mellitus (DM) is one of the most frequent chronic diseases worldwide, being among the top five main causes of death in developed countries. This endocrine disease is also becoming epidemic in developing countries [1]. The world prevalence of DM in 2010 was 6.6%, with an estimated number of 285 million carriers; by 2030, this number may reach 552 million carrier [2]. It is characterized by absolute or relative deficiencies in insulin secretion and/or insulin action associated with chronic hyperglycemia.

In Diabetes persistent hyperglycemia causes increased production of free radicals especially reactive oxygen species (ROS) from glucose auto-oxidation and protein glycation [5,6,7]. Overproduction and/or insufficient removal of these reactive species result in damage to cellular...
proteins, membrane lipids and nucleic acids. The level of antioxidant enzymes critically influences the susceptibility of various tissues to oxidative stress and is associated with the development of complication in diabetes. Beta cells are particularly affected as have the lowest level of intrinsic antioxidant defenses.[8,9]. In Diabetic patients non enzymatic Glycation of protein (SOD Enzyme) leads to its decrease activity [10] and considered to be indicator of severity of oxidative stress.

The relationship between DM and lipid profile status is of considerable interest. In oxidative stress All biomedicals may attacked by free radicals but the lipids are more susceptible. The human cells are rich sources of polyunsaturated fatty acids (PUFAS) hence are readily attacked by oxidizing radicals by a process known as “lipid peroxidation” to form lipid peroxides. This is a self perpetuating chain reaction and highly damaging [11] This causes for pathogenesis of DM and its complication. Our Aim was to assessed the Antioxidant enzyme SOD activity and its correlation with S. glucose, S. lipid profile and duration in Diabetic patient and normal healthy controls.

**Material and method:**
The present study was carried out on 45 Diabetic Patients (Type I & Type II), selected from SMS Medical College, Jaipur and ESI Hospital, Jaipur. 20 age and sex matched normal healthy subjects were taken as a control. Inclusion criteria was diagnosed case of diabetes and exclusion criteria was patients suffering from fever, infection, stress, pregnancy, malignancy, etc. Detailed personal and clinical history, dietary habit, socio economic status, life style, personal habit, family history carefully recorded. Routine lab investigation were undertaken. 6ml of fasting Blood sample were collected within EDTA Vial under all aseptic precautions. 1ml is used for estimating Blood Hb% and SOD concentration and 5 ml for estimating serum glucose and lipid profile.

Estimation of Blood SOD By using Xanthin-Xanthin Oxidase method (Ransod-SD 125, Randox lab ltd) Hb% - By Cynmethemoglobin method.
S. Glucose - By GOD-PAP Method.
S. Chol. - By CHOD-PAP
S. Triglyceride - By GPO Method.
S. HDL cholesterol - By PTA Method.
S. VLDL cholesterol - By Fieldwaldset al. Method.
S. LDL cholesterol - By Fieldwald Method.

Data obtained was subjected to statistical analysis by applying unpaired ‘t’ test at relevant levels. All the values of ‘t’ test & ‘p’ were expressed as significant if the values of p < .005. SPSS VERSION 17 was used for data analysis.
TABLE -1 : Shows distribution of subjects according to various parameters.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Particulars</th>
<th>DM patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No. of patients</td>
<td>45</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>Sex</td>
<td>Male-32,</td>
<td>Male-16,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female-13.</td>
<td>Female-4</td>
</tr>
<tr>
<td>3</td>
<td>Age in years (%)</td>
<td>30-39(11.11%)</td>
<td>30-39 (5%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40-49 (28.89%)</td>
<td>40-49 (35%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50-59 (26.67%)</td>
<td>50-59 (35%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60-69(22.22%)</td>
<td>60-69 (20%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;69 (11.11%)</td>
<td>&gt;69 (5%)</td>
</tr>
<tr>
<td>4</td>
<td>Socio-economic status</td>
<td>LIG-42.22%,</td>
<td>LIG-45%,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MIG-57.78%</td>
<td>MIG-55. %</td>
</tr>
<tr>
<td>5</td>
<td>Dietary habit</td>
<td>Vegetarian -66.44%,</td>
<td>Vegetarian -75%,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Non Vegetarian-35.55%</td>
<td>Non Vegetarian-25%</td>
</tr>
<tr>
<td>6</td>
<td>Life style</td>
<td>Active-60%,</td>
<td>Active-80%,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sedentary-40%</td>
<td>Sedentary-20%</td>
</tr>
<tr>
<td>7</td>
<td>BMI (mean value)</td>
<td>21.45</td>
<td>20.50</td>
</tr>
</tbody>
</table>

TABLE-2 : Comparison of mean value of SOD Enzyme ,S. Glucose and S. Lipid in control & Diabetic Patients.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Case</th>
<th>P value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD u/ml</td>
<td>106 ±23.85</td>
<td>73.67±15.22</td>
<td>&lt;.001</td>
<td>HS</td>
</tr>
<tr>
<td>SOD u/gm</td>
<td>924.04±307.86</td>
<td>638.61±128.14</td>
<td>&lt;.001</td>
<td>HS</td>
</tr>
<tr>
<td>S.Glucose mg/dl.</td>
<td>93.35±7.10</td>
<td>167.58±40.39</td>
<td>&lt;.001</td>
<td>HS</td>
</tr>
<tr>
<td>S.Cholesterol mg/dl.</td>
<td>161.30±16.23</td>
<td>220.81±27.85</td>
<td>&lt;.001</td>
<td>HS</td>
</tr>
<tr>
<td>S.TG mg/dl.</td>
<td>151.12±9.39</td>
<td>203.00±38.72</td>
<td>&lt;.001</td>
<td>HS</td>
</tr>
<tr>
<td>SHDL mg/dl.</td>
<td>41.21±4.74</td>
<td>39.09±6.02</td>
<td>&gt;.05</td>
<td>S</td>
</tr>
<tr>
<td>SVLDL mg/dl.</td>
<td>30.92±2.95</td>
<td>40.25±8.02</td>
<td>&lt;.001</td>
<td>HS</td>
</tr>
<tr>
<td>S.LDL mg/dl.</td>
<td>89.11±14.06</td>
<td>141.64±28.81</td>
<td>&lt;.001</td>
<td>HS</td>
</tr>
</tbody>
</table>

HS: Highly significant, S: Significant.

Mean blood SOD U/ml (u/gmHb) was found to be decreased in diabetic group than control. P value was significant. Mean value of S.glucose, S.cholesterol, S.Triglyceride, SVLDL, SLDL was found to be raised in diabetic group than the control group while S.HDL value was found to be decreased in diabetic group than control.
**Table 3:** Correlation of SOD, S.Lipid Profile and S.Glucose with Duration of Disease (r value)

<table>
<thead>
<tr>
<th>Variable</th>
<th>SOD (u/ml)</th>
<th>SOD (u/gmHb)</th>
<th>S.Glu. (mg/dl)</th>
<th>S.Chol. (mg/dl)</th>
<th>S.TG (mg/dl)</th>
<th>S.HDL. (mg/dl)</th>
<th>S.VLDL. (mg/dl)</th>
<th>S.LDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration</td>
<td>-.558</td>
<td>-.633</td>
<td>+.415</td>
<td>+.532</td>
<td>+.668</td>
<td>-.458</td>
<td>+.578</td>
<td>+.443</td>
</tr>
</tbody>
</table>

Duration of Diabetes has a negative correlation with blood SOD and S.HDL level while a positive correlation with S.Glucose, S.Cholesterol, S.Triglyceride, S.VLDL and S.LDL.

**Table 4:** Correlation of Serum Glucose with SOD & Serum Lipids in control & case Group (r value)

<table>
<thead>
<tr>
<th>Glucose (mg/dl)</th>
<th>SOD (u/ml)</th>
<th>SOD (u/gmHb)</th>
<th>S.Chol. (mg/dl)</th>
<th>S.TG (mg/dl)</th>
<th>S.HDL. (mg/dl)</th>
<th>S.VLDL. (mg/dl)</th>
<th>S.LDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>In control group</td>
<td>-.264</td>
<td>-.057</td>
<td>+.027</td>
<td>+.167</td>
<td>+.251</td>
<td>+.287</td>
<td>-.102</td>
</tr>
<tr>
<td>In Diabetic group</td>
<td>-.429</td>
<td>-.481</td>
<td>+.304</td>
<td>+.480</td>
<td>-.224</td>
<td>+.369</td>
<td>+.241</td>
</tr>
</tbody>
</table>

A Negative correlation was found of Glucose with Blood SOD and S.HDL while a positive correlation with S.Cholesterol, S.Triglyceride, S.VLDL, S.LDL was found in Diabetic group.

**Table 5:** Correlation of SOD with S.Lipids in control & case Group (r value)

<table>
<thead>
<tr>
<th>SOD (u/ml)</th>
<th>S. Cholesterol (mg/dl.)</th>
<th>S.TG (mg/dl.)</th>
<th>S.HDL. (mg/dl.)</th>
<th>S.VLDL. (mg/dl.)</th>
<th>S.LDL. (mg/dl.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>In control group</td>
<td>-.194</td>
<td>+.032</td>
<td>+.096</td>
<td>+.049</td>
<td>-.265</td>
</tr>
<tr>
<td>In Diabetic group</td>
<td>-.476</td>
<td>-.319</td>
<td>+.461</td>
<td>-.395</td>
<td>-.449</td>
</tr>
</tbody>
</table>

A Negative correlation of Blood SOD with S.Cholesterol, S.Triglyceride, S.VLDL, S.LDL while a positive correlation with S.HDL observed in Diabetic group.

**Result:**
All the parameter have highly significant p value <.001, except S.HDL. i.e.>0.05 i.e significant. All above parameters were co-related with duration of the disease and with each other. Results shows that in Diabetic patients Duration and S.Glucose have positive correlation with S.TG, S.VLDL & S.LDL and negative with Bl. SOD and S.HDL Cholesterol. SOD have negative correlation with Glucose, S.Chol., S.TG, S.VLDL, S.LDL and positive with S.HDL.

**Discussion:**
In Diabetes Mellitus there is an imbalance in the antioxidant protective mechanism, leading to oxygen stress in the cells. Toxic oxygen derived products are generated eg. superoxide radical (O–2), hydrogen peroxide (H2O2) and hydroxyl radical (OH–), the latter being the most lethal. [12] One of the most important intracellular antioxidant enzyme, superoxide dismutase which scavenges free radicals...
by converting the harmful superoxide ion into stable hydrogen peroxide. Free radicals are formed disproportionately in diabetes by glucose autoxidation, polyol pathway and nonenzymatic glycation of proteins[13].

Glucose oxidation occurs through the pentose phosphate pathway leads to an excessive formation of NADPH which in turn can promote lipid peroxidation in the presence of the cytochrome P-450 system. In erythrocytes oxyhaemoglobin could act like cytochrome P-450 in the presence of NADPH and this could induce increased lipid peroxidation.[14] Lipid peroxidation appears to have a role in the development of Diabetes Mellitus. Diabetic patients with accompanied dislipidemia are soft targets of cardiovascular deaths. The present study was undertaken to explore the altered SOD, lipid, and lipoprotein, in patients of Diabetes Mellitus. We measure antioxidant enzyme superoxide dismutase, serum glucose, serum lipids like S. Cholesterol, S. Triglyceride, High density lipoprotein, Very low density lipo-protein, Low density lipoprotein in diabetic patients and correlate them with each other and also with duration of disease.

In the present study decreased concentration of SOD is there. Loss of SOD activity in the erythrocytes appear due to the absence of protein synthesizing machinery in the erythrocyte. One Study in NIDDM patients found that SOD deficiency is seen within 2 years and further with development of complications[15]. Diminished activity of SOD points out to an exhausted antioxidant reserve which further exacerbates the oxidative stress. Excessive peroxidation is associated with reduced SOD activity in diabetes. Previous studies demonstrate a consistent association amongst risk factors and Diabetes Mellitus. Duration of Disease also had an important role in development of vascular complications[16]. In our study mean duration of disease was 4.1 years. Table -3 shows correlation of duration with different parameters. The S. Glucose mean value in diabetic group is 167.58±40.39 as compare to control group 93.35±7.10 p value <.001 and highly significant. In Diabetes Mellitus, Hyperglycemia causes Glucose autoxidation and causes non enzymatic glycation of protein that is shown to be source of free radicals and causes oxidative stress [17] and have a important role in development DM and associated complication.[18].

Regarding SOD our study shows that diabetic group have decreased mean value of super oxide dismutase 73.67±15.22u/ml (638.61±128.14 u/gm Hb) as compare to control group 106±23.85u/ml (924.04 ±307.86u/gm Hb). P value was<.001 ie highly significant. In the context of Antioxidant enzymes activity, SOD was shown to be increased in individuals with Type-2 DM compared to controls in a broad range of studies [19,20] On the other hand, many studies shows diminished SOD activity.[21,22]

Our study shows that there is a negative correlation of Duration with SOD while some studies found highest activity at the onset of disease and then subsequently decrease in SOD activity. The increase in SOD activity may be interpreted as compensatory activation due to free radical generation and subsequent decrease level suggest that with longer disease duration SOD induction and activity progressively decreases[23]. As shown in table 4&5A strongly negative correlation was found in Diabetic patients between blood SOD concentration u/ml (u/gm Hb) and serum glucose level ( mg/dl ) than controls. An another study shows that decrease in activity of
SOD is more in patients with higher glucose level and revealed that glucose could be considered as modifier of the activity of SOD.[24,25] Antioxidant defenses are reduced even during the Oral glucose tolerance test in normal and NIDDM subjects and supports the hypothesis that hyperglycemia may even acutely decrease the activity of SOD is patient with higher oxidative stress.[26]

The table-5 shows that In Diabetic patients lipid profile have a significant increased level of S. cholestrol, S. Triglyceride ,S.VLDL and S. LDL level and HDL level is decreased in DM Patients than the control group . p value is < .001 ie. Highly Significant. Our study match with other study in Asian Indians[27]. Duration of the disease ( DM) is important factor for the development of vascular complications. Our Results shows that in diabetic patients duration have a positive correlation with S.TG, S.VLDL & S.LDL and negative with SOD and HDL Cholesterol . Another study shows that duration of disease did not affect the plasma cholesterol. and triglyceride level [28,29]

SOD have negative correlation with glucose, S.cholesterol,S.TG, S.VLDL ,S.LDL and positive with S.HDL. In DM hyperglycemia can stimulate free radical production and LDL particles are more prone to oxidation and causes atherogenetic vascular disease. susceptibility of these particles to oxidation is related to fatty acid composition of its cholesterol fraction . we found in our study that HDL cholesterol levels were significantly low and other parameters of lipoproteins were significantly high as compare to control group. HDL Cholesterol is the main carrier of LDL Particles in the reverse cholesterolmetabolism and thereby protect against oxidative damage.[30] HDL is known to have a strong inverse and independent association with clinical atherosclerotic disease.[31] The low levels of HDL cholesterol is a key feature for oxidative stress status.[32]

CONCLUSION: Antioxidant Enzyme SUPER -OXIDE DISMUTASE level is significantly decreased and there is a disturbed lipid profile in Diabetic Patients. HDL level decreased while serum Cholesterol,TG,VLDL&LDL level found increased.

REFERENCES:
6. Bonnefont rousselot, d., j.p.bastard.m.c.jaudon,j.delattre, consequences of the diabetic status on the
oxidant/antioxidant balance, diabetic metab.,2000,26,163-176
23. Michiels C, Raes M Toussaint O et al. Importance of serum Glutathione peroxidase, Catalase, Cu/Zn SOD for
27. Bhoraskar AS, Dyslipidemias in middle and upper socio economic urban population with or without NIDDM. Jr. Diab. Assoc. India, 1993;33:84-88

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