INTRODUCTION
Among young adults in economically developed societies, diabetes has contributed as one of the chief factor leading towards blindness. DR present in nearly all persons with duration of diabetes 20 years or more. Most retinal cells are affected by the metabolic abnormalities of diabetes, but the sight-threatening manifestations of DR are ultimately attributable to capillary damage (macular edema due to abnormal permeability of barrier capillaries, and ischemia with unregulated angiogenesis due to capillary closure). In many developing countries the incidence and prevalence of type-2 diabetes mellitus (type-2 DM) far exceed rates in the developed world but facilities for the detection and treatment of retinopathy are limited. Therefore, diabetic retinopathy has the potential to become many serious public health problems in populations. DR which is the leading cause of vision loss in adults in industrialized country, cataract, neovascular glaucoma, ocularmotor nerve palsy, lid infection etc are some of the typical complications seen in diabetes mellitus. Apart from these, symptoms like dry eyes and burning sensation were also observed in patients. Oxidative stress, induced by increased accumulation of Reactive Oxygen Species and/or lowered antioxidant capacity, plays an important role in the pathogenesis of DR. In vitro application of SOD results in better visual functions. The consumption of NADPH produces reduced glutathione which is an important scavenger of ROS; this could induce or aggravate intracellular oxidative stress. Many studies in animal models found that these antioxidants may be a logical choice for reducing diabetes-induced ROS. Oxidative stress is associated with damage to lipids, proteins, and nucleic acids. A variety of products including short chain aldehydes like MDA are produced when lipoprotein particles or membranes undergo the process of lipid peroxidation. The aim of this study was to evaluate the status of antioxidant markers and oxidative stress in diabetic retinopathy of type-2 diabetic mellitus subjects compare to non diabetic healthy subjects.

MATERIAL AND METHODS
Subjects were selected from those attending the medical outpatient department of M. D. Eye Hospital (M.L.N. Medical College, Allahabad) UP. A total number of 125 cases were included in our study, diagnosis being based on duration of diabetes mellitus in type-2. Out of these, 75 were diabetic retinopathy of type-2 diabetic patients and 50 were non diabetic healthy controls. A written consent was taken from the patients. The institutional ethics committee of M.L.N. Medical College, Allahabad (UP) provided the approval for conducting the study. Blood Pressure, fasting blood sugar (FBS), HbA1c, total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL), very low density lipoprotein (VLDL), high density lipoprotein (HDL), MDA, SOD, GPx and vitamin E level of the patients of diabetic retinopathy of type-2 diabetic were evaluated.

STATISTICAL ANALYSIS
The data was entered and analyze into Statistical packages for social science (SPSS version 21.0). Mean and standard deviation were analysed for quantitative variables like BP, FBS, HbA1c, TC, TG, LDL, VLDL, HDL, SOD, GPx, vitamin E and MDA level. Independent sample t-test was used to compare mean of all the quantitative variables between the two groups of patients.

ABSTRACT
Introduction: Diabetic retinopathy is a progressive neurological complication of diabetes. This microvascular changes accompany diabetic retinopathy and may result in blindness. Inflammation has been shown to underlie the pathogenesis of diabetic retinopathy. The aim of this study is to evaluate the status of antioxidant markers and oxidative stress in diabetic retinopathy of type-2 diabetic mellitus subjects compare to non diabetic healthy subjects.

Material and methods: The study was conducted at M.L.N. Medical College, Allahabad (UP). A total of 125 cases were included in our study. Out of these, 75 were diabetic retinopathy of type-2 diabetic patients and 50 were non diabetic healthy controls. They were evaluated by measurement of various parameters such as BP, blood parameters as FBS, HbA1c, lipid profile level, MDA, antioxidant marker- SOD, GPx and vitamin E.

Results: An increase in the BP and levels of FBS, HbA1c total cholesterol, triglycerides, LDL, VLDL level, MDA and a decrease in HDL, SOD, GPx and vitamin E were observed in diabetic retinopathy subjects. The value of all these biochemical markers were elevated in diabetic retinopathy of type-2 diabetic subjects and the difference were found to be relevant.

Conclusion: High blood pressure, hyperglycemia, Glycosylated Hb, dyslipidemia, MDA elevated and whereas decrease level SOD, GPx and vitamin E are seen in diabetic retinopathy of type-2 diabetic patients important components in the cell defense against oxidative stress.

Keywords: DR (diabetic retinopathy), HbA1c (Glycosylated hemoglobin), MDA (Malondialdehyde), SOD (Superoxide dismutase), GPx (Glutathione Peroxidase),

Status of Oxidative Stress and Antioxidant in Diabetic Retinopathy of Type-2 Diabetic Subjects

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Antioxidants are substances that buffer free radicals in the body. SOD and glutathione peroxidase (GPx) are antioxidant defense enzymes responsible for scavenging free radicals and maintaining redox homeostasis such as SOD, GPx, and vitamin E. In the retina, the activities of antioxidant defense enzymes - SOD, GPx and vitamin E and HDL were negatively correlated with diabetic retinopathy. MDA and other serum biochemical parameters except HDL, antioxidant- SOD, GPx and vitamin E were elevated in diabetic retinopathy patients as compared to healthy subjects and the differences were found to be statistically significant (P value <0.001). Oxidative stress marker MDA and Antioxidant markers – (SOD, GPx and vitamin E) and HDL were negatively correlated with diabetic retinopathy. MDA and other serum biochemical parameters such as FBS, HbA1c, Total Cholesterol, Triglyceride, LDL, and VLDL etc were positively correlated with diabetic retinopathy.

DISCUSSION

The prevalence of retinopathy is high in conditions associated with type-2 diabetes mellitus. The presence of DR correlates significantly with Glycosylated hemoglobin (HbA1c) and antioxidant markers - SOD, GPx and vitamin E. In our study systolic blood pressure was found higher in DR patients 166.85±3.89 mm/Hg as compared with healthy control subjects 104.6±2.28 mm/Hg. Our study showed FBS levels in type-2 diabetic retinopathy subjects group (mean 188.0±37.7) were higher than healthy control group (mean 86.4±12.46), which confirmed the obvious dysglycemia in these patients. P-value less than 0.001. Significantly increased amounts of glucose amplify the physiological process of non-enzymatic protein glycosylation (glycation). For example, glycated hemoglobin (HbA1c) is a recognized indicator of time-integrated glycaemia. We also found that increased HbA1c levels (mean 11.2±3.26) in DR group as compared to control healthy group (4.7±2.24) and the results were statistically significant (P value <0.001). HbA1c is a useful indicator of how the blood glucose level has been kept in check in the recent past. The indication of glycemic control is better provided by HbA1c than blood or urinary glucose determinations. Studies have already shown that poor long term glycemic control can contribute to complications like diabetic retinopathy in subjects.

In this study we found that lipid profile- serum total cholesterol, triglyceride and LDL were higher in diabetic retinopathy patients and whereas HDL lower in these diabetic patients than healthy control and the results were statistically relevant (P value <0.001). Similar results were reported by others studies. Early treatment diabetic retinopathy study and the Wisconsin epidemiologic Study of diabetic retinopathy (WESDR) pointed out a significant association between elevated serum lipid levels and increased risk of retinal hard exudates. The severity of retinopathy was positively associated with triglycerides as well as with VLDL-cholesterol and negatively associated with HDL-cholesterol.

The present study showed that the erythrocyte antioxidant defense enzymes SOD, GPx and vitamin E levels were significantly lower in diabetic retinopathy patient as compared to healthy control group. In diabetes, the activities of antioxidant defense enzymes responsible for scavenging free radicals and maintaining redox homeostasis such as SOD, glutathione reductase, glutathione peroxidase, and catalase are diminished in the retina. Antioxidants are substances that counteract free radicals or their actions. Each cell has its own appropriate protective mechanisms against any harmful effects of free radicals. SOD and glutathione peroxidase (GPx) are buffering systems in every cell. Also, vitamin E is also part of the body's natural antioxidants. It is better provided by HbA1c than blood or urinary glucose determinations. Studies have already shown that poor long term glycemic control can contribute to complications like diabetic retinopathy in subjects.

RESULTS

The study was included 75 patients of diabetic retinopathy of type-2 diabetic and 50 were non diabetic healthy controls. The mean age of the patients was 52 ± 6 years (Ranging from 34 to 75). Comparison of means of serum biochemical markers between diabetic retinopathy of type-2 diabetic and healthy groups is presented in Table-1. The values of all these biochemical parameters except HDL, antioxidant- SOD, GPx and vitamin E were elevated in diabetic retinopathy patients as compared to healthy subjects and the differences were found to be statistically significant (P value <0.001). Oxidative stress marker MDA and Antioxidant markers – (SOD, GPx and vitamin E) and HDL were negatively correlated with diabetic retinopathy. MDA and other serum biochemical parameters such as FBS, HbA1c, Total Cholesterol, Triglyceride, LDL, and VLDL etc were positively correlated with diabetic retinopathy.

<table>
<thead>
<tr>
<th>Study Variables</th>
<th>Healthy Control n=50</th>
<th>Type-2 Diabetic Retinopathy n=75</th>
<th>r-Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP Systolic (mm/Hg)</td>
<td>104.6±2.28</td>
<td>166.85±3.89</td>
<td>5.048**</td>
</tr>
<tr>
<td>BP Diastolic (mm/Hg)</td>
<td>76.6±1.18</td>
<td>110.85±4.85</td>
<td>2.052*</td>
</tr>
<tr>
<td>FBS</td>
<td>86.42±12.46</td>
<td>188.0±37.7</td>
<td>6.642**</td>
</tr>
<tr>
<td>HbA1c</td>
<td>4.7±2.24</td>
<td>11.2±3.26</td>
<td>5.263**</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dl)</td>
<td>156.8±21.09</td>
<td>252.4±41.9</td>
<td>4.234**</td>
</tr>
<tr>
<td>TG</td>
<td>118.89±19.49</td>
<td>212.7±45.05</td>
<td>8.549**</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>48.4±6.14</td>
<td>34.2±7.02</td>
<td>3.267**</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>114.7±20.20</td>
<td>158.7±39.3</td>
<td>7.384**</td>
</tr>
<tr>
<td>VLDL (mg/dl)</td>
<td>28.58±4.09</td>
<td>45.89±11.3</td>
<td>2.125*</td>
</tr>
<tr>
<td>MDA (n mol/ml)</td>
<td>4.12±0.60</td>
<td>12.0±2.94</td>
<td>4.542**</td>
</tr>
<tr>
<td>SOD(U/gm Hb)</td>
<td>112.88±59.16</td>
<td>552.4±44.08</td>
<td>9.842**</td>
</tr>
<tr>
<td>GPx (mg/dl)</td>
<td>68.44±2.26</td>
<td>45.56±1.20</td>
<td>7.902**</td>
</tr>
<tr>
<td>Vitamin E (mg/dl)</td>
<td>24.8±2.56</td>
<td>18.69±2.32</td>
<td>3.364**</td>
</tr>
</tbody>
</table>

* Significant at P<0.01, ** significant at P<0.001
membranes in the human body by acting as a chain breaker.\cite{18}

Results of this study indicate that oxidative stress are increasing over the time course of the disease and might lead to the development of diabetic retinopathy. The increased oxidation stress may or may not be effectively compensated by the present antioxidants. This may be a reason for divergent results obtained in various studies. Further research is needed to find a definitive association between intake of antioxidant nutrients and reduction in the development of diabetic retinopathy.

**CONCLUSION**

This study concludes, relatively high levels of diabetic retinopathy in all ethnic groups in Indian, including asymptomatic subjects with diabetes diagnosed during screening. Independent risk factors identified were duration of diabetes, systolic blood pressure, fasting plasma glucose, HbA1c (Glycosylated hemoglobin), lipid profile, oxidative stress marker- MDA, Antioxidant markers- SOD, GPx and Vitamin E level. As in other populations of the developing world with high rates of type-2 diabetes mellitus, effective primary prevention, early detection of diabetes, and quality clinical care and education, focusing particularly on blood pressure glycemic and oxidative stress control, provide the key to averting an epidemic of blindness and other diabetic complications.

Within the near future, pharmacologic treatment will probably be available for treating and preventing the progression of diabetic retinopathy. Antioxidant administration may help to reduce the oxidative stress from diabetes and hyperglycemia, but further studies are needed to determine whether retinopathy progression can be reduced. Further, more studies are needed to determine whether retinopathy progression can be reduced. The increasing use of medical therapies such as pharmacologic agents will also require greater communication between ophthalmologists, diabetologists and primary care physicians.

**ACKNOWLEDGEMENT**

The authors are grateful to the Principal, Director (M.D.Eye Hospital) and Head of Department of Ophthalmology and Biochemistry as well as Librarian Mr. K. P. Singh who has given prompt and proper reference services for this study and whole M.L.N. Medical College, Allahabad (U.P.) for providing facilities for research.

**REFERENCES**