ABSTRACT: Diabetic nephropathy is the single most common disorder leading to renal diseases. Reactive oxygen species (ROS) play a major role in the development of diabetic nephropathy. Nephrotic syndrome is often manifesting in progression of diabetic nephropathy. Therefore, this study was carried out to investigate oxidant and antioxidant status in diabetic nephropathy patients. The blood samples were analyzed for quantitation of malondialdehyde as index of lipid peroxide, vitamin C, total antioxidant capacity, homocysteine, lipoprotein (a) and lipid profile. Significantly increased levels of serum total cholesterol, triglycerides, low density lipoprotein, malondialdehyde as index of lipid peroxide, lipoprotein (a), homocysteine (p<0.001) and decreased levels of serum total antioxidant capacity, total protein, albumin, high density lipoprotein & plasma vitamin C (p<0.001) were noticed in the patients with diabetic nephropathy as compared to control subjects.

KEYWORDS: Malondialdehyde (MDA), Total antioxidant capacity (TAC), vitamin C (vit C), Diabetic nephropathy (DN), Lipoprotein (a), Homocysteine, Reactive oxygen species.

INTRODUCTION

DN is characterized by excessive accumulation of extracellular matrix in the kidney, reactive oxygen species (ROS) play a central role in the extracellular matrix synthesis and degradation in the glomeruli and tubulointerstitium leading to renal diseases (Ha H, et.al., 2005 a). Oxidative stress has been known to play an important role in the development and progression of diabetic nephropathy (Ha H, et.al., 2001 b). Diabetic nephropathy is a leading cause of end stage renal failure, DN has several pathways for development such as glomerular hyperfiltration, upregulation of protein kinase C, advanced glycation end products, activation of polyl pathway, increased oxidative stress and upregulation of growth factors (Ohgas, et.al., 2004). There is considerable evidence that hyperglycemia represents the main cause of complications of diabetes mellitus (DM) and oxidative stress resulting from increased generation of reactive oxygen species plays a crucial role in their pathogenesis (Davi G, et.al., 2005). Classical factors contributing to the pathology of diabetic nephropathy e.g., hypertension, hyperglycemia, hypoinsulinemia, and hyperlipidemia (Miyata T, et.al., 2009). Recent studies, mainly perform new markers such as hypoxia, advanced glycation, oxidative stress, and other bioactive molecules in the pathogenesis of DN (Miyata T, et.al., 2009). Diabetic nephropathy has several distinct phases of development and multiple mechanisms contribute to the development of the disease and its outcomes (Dronavalli S, et.al., 2008). HCY is a link between DN and both chronic inflammation and hypercoagulability increasing cardiovascular risk (Wotherspoon F, et.al., 2006, Zdemir G, et.al., 2005, Aso Y, et.al., 2004). Lp (a) is an independent risk factor for the progression of diabetic nephropathy in type 2 diabetic patients with overt proteinuria (Kramer GA, et.al., 1996).

The objective of this study was to investigate possible associations between oxidative stress and the severity of diabetic nephropathy in nephrotic syndrome patients with the estimation of the serum HCY, Lp(a), TAC, MDA, plasma ascorbic acid (vit C), interrelationship of all biochemical parameters and correlate with severity of DN.
MATERIALS AND METHODS

Location
Patients included in the present study were all admitted to the intensive care unit (ICU) or attending the OPD of medicine of M.Y. Hospital attached to M.G.M. Medical College, Indore, Madhya Pradesh, India.

The study group:- The present study was case control study conducted on 2 groups.

Group I:- Comprised with control (135).

Group II:- Comprised with adult DN patients (65).

Age of the patients group I & II ranged from 30 to 80 years, patients were from same geographical area and none was taking a special diet, untreated DN patients newly diagnosed by biopsies evidences of nephritis. Fasting blood glucose levels > 126.0 mg/dl, BMI > 24.0 kg/m2, HTN – SBP > 140 mm Hg and DBP > 90 mm Hg. Group I was judged to be free of any illness by clinical examination, DN patients were not with any other active complication medical condition or with systemic diseases. Excluded the subjects or patients taking vitamins tablet from prolonged time, alcohol abusers, smokers, acute and chronic renal failure and hemodialysis patients, other systemic diseases such as amyloid nephropathy, hepatic impairment, lupus nephritis, cardiovascular nephropathy, sickle cell anemia, amyloidosis, sarcoidosis, leukemia, lymphoma, cancer of breast, colon and stomach, reaction to drugs, allergic reactions. Fasting venous blood were drawn from all.

Total antioxidant capacity (TAC) in serum was estimated by using spectrophotometric method described by D-Koracevic et al (Koracevic D, et.al., 2001). MDA one of the aldehydic by product of lipid peroxidation in serum was estimated by its thiobarbituric acid reactivity, spectrophotometric method described by Hunter et al (Hunter MI, et.al., 1985). Plasma ascorbic acid (vit C) was measured by colorimetric method described by Roe and Kuether et al (Roe JH, et.al., 1943). Lp(a) was estimated by ‘Turbidimetric method’ a commercially available kit from “human diagnostic kit”. HCY was estimated by a commercially available kit from a “Keragen diagnostic kit method”. Lipid profile, total protein and albumin were estimated by a commercially available kit from “AGAPPE” in auto analyzer. LDLC and VLDLC were calculated using friedwalds formula.

Present work was approved by institutional research and ethical committee. The mean and standard deviation were determined for each variable in all groups. All the results were expressed as mean +/-SD. Student “t” test was used to assess statistical significance of the results.

RESULTS

All results of group II were compared with group I. The level of all biochemical parameters were significantly changed between groups I and II. Descriptive statics of diagnostic parameters in group I & group II presented in Table I & Table II. There was a statistically significant decreased level of the serum HDLC, total protein, albumin, TAC, plasma vit C level and increased serum Tchol, TGs, LDLC, MDA, HCY, Lp(a) level in group II when compared to group I.

Table III- Description about correlation coefficient and significance with diagnosed parameters in the study group II.

DISCUSSION

In the present study DN patients had more severe oxidative stress than normal persons where oxidative stress plays an important intermediary role in the pathogenesis of diabetes complications. Diabetic nephropathy seemed to occur as a result of an interaction between metabolic and hemodynamic factors, which activate common pathways that lead to renal damage (Yamagishi S, et.al., 2007).
Table-I: Comparison of routine diagnosed parameters-lipid profile, serum proteins in group I & group II

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>135</td>
<td>65</td>
</tr>
<tr>
<td>TGs (mg/dL)</td>
<td>112.09±10.16</td>
<td>213.7±8.9*</td>
</tr>
<tr>
<td>Tchol (mg/dL)</td>
<td>173.71±15.44</td>
<td>358.06±20.5*</td>
</tr>
<tr>
<td>VLDLC (mg/dL)</td>
<td>22.40 ± 1.98</td>
<td>42.74±2.7*</td>
</tr>
<tr>
<td>HDLC (mg/dL)</td>
<td>49.15 ± 7.4</td>
<td>26.71±6.7*</td>
</tr>
<tr>
<td>LDLC (mg/dL)</td>
<td>103.68±8.24</td>
<td>288.78±21.2*</td>
</tr>
<tr>
<td>TP (g/dL)</td>
<td>6.90 ± 1.6</td>
<td>3.53±0.45*</td>
</tr>
<tr>
<td>Alb (g/dL)</td>
<td>4.34 ± 0.37</td>
<td>1.82±0.23*</td>
</tr>
</tbody>
</table>

n=No. of subjects and patients *group I compare to group II
* p<0.001; Highly Significant
All results expressed in mean and standard deviation (SD).

Table II: Comparison of diagnosed biochemical parameters between control (group I) and patients (group II) with DN

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>135</td>
<td>65</td>
</tr>
<tr>
<td>Lp(a) (mg/dL)</td>
<td>18.15 ± 9.7</td>
<td>40.55±6.2**</td>
</tr>
<tr>
<td>HCY (umol/L)</td>
<td>10.75 ± 3.1</td>
<td>26.89±7.5**</td>
</tr>
<tr>
<td>TAC(mmol/L)</td>
<td>2.37 ± 0.87</td>
<td>1.16±0.34**</td>
</tr>
<tr>
<td>MDA(nmol/mL)</td>
<td>1.56 ± 0.96</td>
<td>7.54±0.31**</td>
</tr>
<tr>
<td>Vit C(mg/dL)</td>
<td>1.48 ± 0.65</td>
<td>0.47±0.25**</td>
</tr>
<tr>
<td>p value</td>
<td>------</td>
<td>**group I compare to group II ** p&lt;0.001</td>
</tr>
</tbody>
</table>

n=No. of subjects and patients; ** group I compare to group II; p<0.001; Highly Significant
All results expressed in mean and standard deviation (SD).

Table III: - Correlation coefficient and significance in the patients group II

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Correlation coefficient(r)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lp(a) and MDA</td>
<td>+0.93</td>
<td>p&lt;0.001*a</td>
</tr>
<tr>
<td>HCY and MDA</td>
<td>+0.85</td>
<td>p&lt;0.001*a</td>
</tr>
<tr>
<td>LDL and Lp(a)</td>
<td>+0.88</td>
<td>p&lt;0.001*a</td>
</tr>
<tr>
<td>Alb and HCY</td>
<td>-0.55</td>
<td>p&lt;0.001*a</td>
</tr>
<tr>
<td>TP and HCY</td>
<td>-0.61</td>
<td>p&lt;0.001*a</td>
</tr>
<tr>
<td>Lp(a) and HCY</td>
<td>+0.80</td>
<td>p&lt;0.001*a</td>
</tr>
<tr>
<td>HCY and TAC</td>
<td>-0.42</td>
<td>p&lt;0.01*b</td>
</tr>
<tr>
<td>Lp(a) and TAC</td>
<td>-0.33</td>
<td>P&lt;0.0001*c</td>
</tr>
<tr>
<td>TP and MDA</td>
<td>-0.65</td>
<td>P&lt;0.001*a</td>
</tr>
</tbody>
</table>

*a-Highly significant,*b & *c-Significant
The oxidative stress was increased in patients with DN compared to diabetic patients without nephropathy and this increase seems to be related to the severity of microalbuminuria levels (Pan HZ, et al., 2009, Aslan M, et al., 2007). An oxidative stress was increased in diabetes and the overproduction of ROS in diabetes was a direct consequence of hyperglycemia. Various types of vascular cells including renal cells were able to produce ROS under hyperglycemic condition. Both NADPH oxidase and mitochondrial electron gradient play roles in hyperglycemia induced ROS generation. ROS mediate hyperglycemia induced activation of signal transduction cascades and transcription factors leading to transcriptional activation of profibrotic genes in the kidney (Dave GS, et al., 2007, S P Wolff, et al., 1993). Conventional and catalytic antioxidants have been shown to present or delay the onset of DN. Renal lesions were associated with increased oxidative stress and decreased renal nitric oxide availability ((Dave GS, et al., 2007, S P Wolff, et al., 1993, Prabhakar S, et al., 2007). Oxidative stress occurs as a result of the imbalance between ROS production and antioxidant defenses. Sources of ROS included the mitochondria, auto-oxidation of glucose, and enzymatic pathways including nicotinamide adenine dinucleotide phosphate reduce oxidase (Tan AL, et al., 2007, Fukami K, et al., 2008).

Oxidative stress was increased in diabetes and the overproduction of ROS in diabetes was a direct consequence of hyperglycemia. Various types of vascular cells including renal cells were able to produce ROS under hyperglycemic condition. Both NADPH oxidase and mitochondrial electron gradient play roles in hyperglycemia-induced ROS generation. In addition to their ability to directly inflict macromolecular damage, ROS can function as signaling molecules. ROS mediate hyperglycemia-induced activation of signal transduction cascades and transcription factors leading to transcriptional activation of profibrotic genes in the kidney. Furthermore, ROS-activated signaling molecules generate and signal through ROS and thus ROS act as a signal amplifier. Intensive glycemic control and inhibition of angiotensin II delay the onset and progression of diabetic nephropathy, in part, through prevention of overproduction of ROS. Conventional and catalytic antioxidants have been shown to prevent or delay the onset of diabetic nephropathy (Ha H, et al., 2008 c).

Oxidative stress has been known to play an important role in the development and progression of diabetic nephropathy, but the intracellular signal transduction pathways regulated by reactive oxygen species (ROS) have not been clearly defined. High glucose (HG) induces intracellular ROS directly via glucose metabolism and auto-oxidation and indirectly through the formation of advanced glycation end products and their receptor binding. ROS mimic the stimulatory effects of HG and up regulated transforming growth factor-beta 1, plasminogen activator inhibitor-1, and extracellular matrix (ECM) proteins by glomerular mesangial cells, thus leading to mesangial expansion. ROS activated other signaling molecules, such as protein kinase C and mitogen-activated protein kinases and transcription factors, such as nuclear factor-kappa B, activator protein-1, and specificity protein 1 leading to transcription of genes encoding cytokines, growth factors, and ECM proteins. Finally, various antioxidants inhibit mesangial cell activation by HG and ameliorate features of diabetic nephropathy. These findings qualify ROS as intracellular messengers and as integral glucose-signaling molecules in glomerular mesangial cells in diabetic nephropathy (Maryam S, et al., 2005). With this new concept, ROS assume a greater importance in the pathogenesis of diabetic nephropathy. Lower Se and GPx levels in diabetic patients may be implicated in diabetic nephropathy (Kromhauser C, et al., 2008).

Serum MDA concentration was significantly higher value with diabetic nephropathy (p<0.001) than control, catalase & SOD activity in group of diabetic nephropathy being significantly lower than group without diabetic nephropathy (Bhatia S, et al., 2003). Erythrocyte GSH contents was significantly lowers in group of diabetic nephropathy as compared to controls (Bhatia S, et al., 2003). Results of present study indicate the oxidative stress was increased and oxidant-antioxidant defense was imbalance with DN. These dearrangements were of higher magnitude in patients of type 2 diabetes mellitus with nephropathy (Bhatia S, et al., 2003). No independent correlation between proteinuria (or albuminuria) and HCY levels, this study improves the external of previous negative finding (Friedman AN, et al., 2002 a). Therefore it is unlikely that the observed positive association between proteinuria and CVD was directly related to HHCY (Buysschaert M, et al., 2001, L Martinez CA, et al., 2002). In diabetic nephropathy, oxidant injury and renal tubular damage accompany and may even precede microalbuminuria. The presence of these abnormalities in the absence of glomerular proteinuria favours the hypothesis that alterations first occur in the peritubular microcirculation, which by causing oxidant injury and tubular damage, may initiate diabetic nephropathy (M. Yaqoob, et al., 1994). There were evidences for increased oxidative stress due to hypoproteinemia in DN patients.

Elevated blood levels of homocyst(e)ine represent a known independent risk factor for macrovascular disease; the link between hyperhomocyst(e)ine and diabetic microvascular complications, hyperhomocysteinemia related to endothelial dysfunction and supported to oxidative stress (Hofmann MA, et al., 1997).
Total serum homocysteine has been shown to predict de novo and recurrent cardiovascular events in many studies. However, results in diabetic populations with minimal nephropathy are mixed. The independent relationship between tHCY and arteriosclerotic outcomes and congestive heart failure (CHF) events in a population with high cardiovascular risk and diabetic nephropathy (Friedman AN, et.al., 2005 b). Patients with both types of diabetes and nephropathy had higher plasma homocysteine concentrations than those without nephropathy. Increases of homocysteine in plasma were related to increases in the severity of the nephropathy, increases in fasting homocysteine in diabetic patients were associated with increased albumin excretion rate, especially in those with NIDDM, thus providing a potential new link between microalbuminuria, diabetic nephropathy and cardiovascular disease (A Chico, et.al., 1998). Increased plasma homocysteine concentrations may contribute to increased morbidity and death from cardiovascular disease in adolescents and young adults with diabetic retinopathy and nephropathy (Chiarelli F, et.al., 2000).

The underlying pathogenic mechanism that links diabetic nephropathy (DN) to a high risk for CVD (Cardiovascular disease) remains unclear. In addition to traditional risk factors, including hypertension, hyperglycemia and dyslipidemia, hyperlipoproteinemia, hyperhomocysteinemia identification of novel modifiable risk factors was important in preventing CVD in people with diabetes & DN (L Martinez CA, et.al., 2002, Iwasaki T, et.al., 2008). Inflammation/oxidative stress were known to be associated with an increased risk for CVD in patients with DN (Iwasaki T, et.al., 2008). Moreover homocysteine advanced glycation end products asymmetric dimethylarginine and anemia may play a role in the development and progression of atherosclerosis in patients with DN (Iwasaki T, et.al., 2008, Aso Y, et.al., 2008).

CONCLUSION
We conclude that oxidative stress is enhanced in DN patients due to hyperhomocysteinemia, hyperlipoproteinemia & hypoproteinemia which may contribute to the development of DN related complication with more frequency such as cardiovascular diseases and end stage renal diseases and many other complications. Several evidences suggest that patients with DN had imbalance oxidant/antioxidant status and increased subsequent oxidative stress is due to oxidation of LDL and lipoprotein, low intake of antioxidants in diet, HHCY, hyperlipoproteinemia & hypoproteinemia. We can only hypothesize that in patients at the acute phase of the disease, decreased total antioxidant capacity may lead to abnormal lipid peroxidation, resulting in a high rate of glomerular injury. On the other hand prolonged lipid oxidation may lead to diminished antioxidant activity. Long term follow up in a large number of patients would be necessary to confirm these results. Antioxidant supplements for oxidative stress can achieve excellent long term results in the treatment of DN.

ACKNOWLEDGEMENT
We sincerely thank to the University for Study Support.

ABBREVIATIONS
CHD - Cardiac heart diseases, tHCY - Total homocysteine,
HHCY - Hyperhomocyst(e) inemia, DM - Diabetes mellitus,
CVD - Cardiovascular diseases, Tchol - Total cholesterol,
TGs - Triglycerides, BMI - Body mass index
VLDLC - Very low density lipoprotein cholesterol
HDLCL - High density lipoprotein cholesterol
LDLC - Low density lipoprotein cholesterol
TP - Total protein, Alb - Albumin, SOD - Superoxide dismutase,
HTN - SBP - Hypertension systolic blood pressure
DBP - Diastolic blood pressure, HG - High glucose,
NADP - Nicotinamide adenine dinucleotide phosphate,
ECM - Extracellular matrix, GPx - Glutathione peroxidase,
GSH - Glutathione, Se - Selenium, CHF - Congestive heart failure,
NIDDM - Non insulin dependent diabetes mellitus
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