Original article:

The study of total antioxidant capacity, homocysteine, lipoprotein (a), total protein and albumin with copper and zinc in nephrotic syndrome

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ABSTRACT

Background: Neprotic syndrome, the defining parameter is proteinuria with hypoalbuminemia and hyperlioproteinemia, the pathophysiological importance of reactive oxygen species (ROS) in patients with nephrotic syndrome. Patients with nephrotic syndrome (NS) have one of the most pronounced secondary changes in lipoprotein (a) and homocysteine metabolism. The zinc and copper metabolism are varied in nephrotic syndrome.

Materials and Methods: The study was carried out to investigate oxidant and antioxidant status with homocysteine, lipoprotein (a), total protein, albumin, copper and zinc in nephrotic syndrome patients. The blood samples were analyzed for quantitation of malondialdehyde as index of lipid peroxide, total antioxidant capacity, homocysteine, lipoprotein (a), copper and zinc.

Observations and Results: Significantly increased levels of serum lipid peroxide, homocysteine, lipoprotein (a) and decreased levels of serum total protein, albumin, total antioxidant capacity, copper and zinc were noticed in the patients with nephrotic syndrome as compared to control subjects. However, significant positive correlation of lipid peroxide with homocysteine and lipoprotein (a) and negative with total antioxidant capacity, total protein, albumin, copper and zinc were observed.

Conclusion: Oxidative stress is enhanced in NS patients due to hyperhomocysteinemia, hyperlipoproteinemia (a) and decreased level of TAC, total protein, albumin, copper and zinc which may contribute to the development of NS related complication with more frequency such as cardiovascular nephropathy disease and many other complications.

Keywords: Nephrotic syndrome, Total antioxidant capacity, Homocysteine

INTRODUCTION

Nephrotic syndrome is a potentially life-threatening state and persistent nephrotic syndrome has a poor prognosis with a high risk of progression to end-stage renal failure and a high risk of cardiovascular complications due to severe hyperlipidemia. The nephrotic syndrome has not been investigated in detail, even though this syndrome provides an excellent model in which to study a possible link between albuminuria, proteinuria, and hyperhomocyst(e)inemia. The oxidative defense is weakened in NS, and the focus on measurements of the oxidative-antioxidative status in the patients with nephrotic syndrome. Oxidant and antioxidant status in relation to dyslipidemia in NS. Patients with nephrotic syndrome (NS) have one of the most pronounced secondary changes in lipoprotein metabolism known, and the magnitude of the changes correlates with the severity of the disease. Various biochemical parameters that are presently determined in serum/plasma homocysteine, total antioxidant capacity, lipid peroxidation, lipoprotein (a), total protein, albumin, copper and zinc for the diagnosis of nephrotic syndrome, as well as to determine the changes that occurs in the metabolic process associated with nephrotic syndrome.
complications. The Purpose of this research is to establish biochemical parameters for the diagnosis and of nephrotic syndrome and its complication and to determine the Interrelationship of homocysteine, lipoproteine (a), copper, zinc oxidant and antioxidant through them.

MATERIALS AND METHODS

This study was conducted at the Department of Biochemistry S.S. Medical College Rewa (M.P.) with collaboration of Department of Biochemistry M.G.M. Medical College Indore (M.P.).

The study group: This study was conducted on 2 groups: group I comprised of 135 controls, group II comprised of 133 nephrotic syndrome patients in the age group of 30-80 years.

The patients were diagnosed on the basis of detailed clinical history, clinical examination and other relevant biochemical investigations. The patients suffering from other diseases, such as diabetes, inflammatory diseases, cardiac diseases, hepatic impairment, and respiratory diseases or other systemic diseases as well as smokers and alcoholics, were excluded from the study. Informed consent was obtained from each participant in the study. Fasting venous blood were drawn from all.

Total Protein and Albumin were estimated by a commercially available kit from “AGAPPE” in semiautomatic auto analyzer. Total antioxidant capacity (TAC) in serum was estimated by using spectrophotometric method (Koracevic et al. 2001). MDA, one of the aldehydic by product of lipid peroxidation in serum, was estimated by its thiobarbituric acid reactivity using, spectro-photometric method (Hunter et al. 1985). Homocysteine was estimated by commercial “Kera-gen diagnostic kit” using semiautoanalyser. Lp(a) was estimated by ‘Turbidimetric method’ a commercially available kit from “Human diagnostic kit”. Serum copper was estimated by “Dieth-ylthiocarbamate method” described by “Veture and King et al; 1951, method”.

Serum Zn was estimated by “5-Br-PAPS’ method. The values were expressed as mean +/- SD. Student test was done for comparison of data. The laboratory investigations were performed on groups I & II. The study was approved by the ethics committee of the D.A.V.V. University.

RESULTS & DISCUSSION

Descriptive statistics of all diagnostic parameters on groups I, II presented in Table I. There was a statistically significant decreased level of the serum TAC, albumin, total protein, copper and zinc and increased serum MDA, HCY and lipoprotein (a) level in group II when compared to group I. There was significant difference between group I & group II with HCY level (p<0.001).
**Table I: Comparison of all diagnosed biochemical parameters in group I and II with NS**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Mean ± SD)</th>
<th>Group II (Mean ± SD)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(control)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>n</strong></td>
<td>135</td>
<td>133</td>
<td></td>
</tr>
<tr>
<td><strong>TAC (mmol/L)</strong></td>
<td>2.37 ± 0.87</td>
<td>1.55 ± 0.28*</td>
<td></td>
</tr>
<tr>
<td><strong>MDA (nmol/mL)</strong></td>
<td>1.56 ± 0.96</td>
<td>3.58 ± 0.42*</td>
<td></td>
</tr>
<tr>
<td><strong>HCY (umol/L)</strong></td>
<td>10.75 ± 3.1</td>
<td>17.77 ± 4.15*</td>
<td></td>
</tr>
<tr>
<td><strong>Lp (a) (mg/dL)</strong></td>
<td>18.15 ± 9.7</td>
<td>28.44 ± 2.06*</td>
<td></td>
</tr>
<tr>
<td><strong>Cu (ug/dL)</strong></td>
<td>122.29 ± 12.33</td>
<td>70.96 ± 2.18*</td>
<td></td>
</tr>
<tr>
<td><strong>Zn (ug/dL)</strong></td>
<td>102.90 ± 8.02</td>
<td>66.29 ± 2.36*</td>
<td></td>
</tr>
<tr>
<td><strong>TP (g/dL)</strong></td>
<td>6.90 ± 1.6</td>
<td>3.26 ± 3.3*</td>
<td></td>
</tr>
<tr>
<td><strong>Alb (g/dL)</strong></td>
<td>4.34 ± 0.37</td>
<td>1.37 ± 0.70*</td>
<td></td>
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</tbody>
</table>

*p value* group I compare to group II

*p<0.001*

**Table II: 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>HCY and MDA</td>
<td>+0.78</td>
<td>p&lt;0.001*a</td>
</tr>
<tr>
<td>HCY and TAC</td>
<td>-0.25</td>
<td>p&lt;0.0001*b</td>
</tr>
<tr>
<td>Lp (a) and MDA</td>
<td>+0.86</td>
<td>p&lt;0.001*a</td>
</tr>
<tr>
<td>Lp (a) and TAC</td>
<td>-0.22</td>
<td>P&lt;0.0001*b</td>
</tr>
<tr>
<td>Lp (a) and HCY</td>
<td>+0.72</td>
<td>p&lt;0.001*a</td>
</tr>
<tr>
<td>Alb and Zn</td>
<td>+0.75</td>
<td>p&lt;0.001*a</td>
</tr>
<tr>
<td>TAC and Zn</td>
<td>+0.58</td>
<td>p&lt;0.0001*b</td>
</tr>
<tr>
<td>TAC and Cu</td>
<td>+0.53</td>
<td>p&lt;0.0001*b</td>
</tr>
<tr>
<td>HCY and Cu</td>
<td>-0.35</td>
<td>p&lt;0.0001*b</td>
</tr>
<tr>
<td>HCY and Zn</td>
<td>-0.31</td>
<td>p&lt;0.0001*b</td>
</tr>
</tbody>
</table>

*a- Highly significance, *b-Significance*
In the present study shown that, mean serum (MDA) level was significantly higher in study group II as compared to group I. This result showed the presence of oxidative stress in adult with NS. The lower total antioxidant status (TAS) level connected with abnormal intestine absorption of some antioxidants component in patients with NS. There were some data in the literature showing that a diet deficient in Se and Vit C may lead to renal injury characterized by proteinurias and reduced GFR. Excessive generation of reactive oxygen species was one of the incriminated mechanisms in the pathogenesis of progression renal injury. In fact the little data was available concerning SOD in NS. They reported reduced activities of erythrocyte and plasma GSH-Px activities when compared to the controls. They also reported lower erythrocyte Cu-Zn-SOD activity in patients of nephrotic syndrome than that of the controls. Erythrocyte and plasma level of MDA were higher in patients with NS. Plasma Se level of the patients were lower than that of the controls. These results obtained in adult nephrotic syndrome patients support the previous data indicating abnormalities in antioxidative system of NS. 9, 10, 11

In the present study showed higher Lp(a) level in females when compared to males in NS patients and is in agreement with other reports though influence of sex on Lp(a) in females than in males may be due to lowering effect of testosterone in males and presence of menopausal status. 12 But the other study Pedreno et al 13 showed no gender differences in Lp(a) levels in both patients and controls. It has been found that the relation between Lp(a), mesangial cells and HDLc with NS. 14, 15, 16 In the present study shown that significantly higher level of Lp(a) LDLc and HCY supported by many other studies and also supported to CVD risk.

Kniazewska MH et al 17 & Kuzmas et al 18 It has been demonstrated recently that Lp(a) abnormalities in NS. The atherogenic serum lipoprotein (a) [Lp(a)] was significantly elevated in patients with nephrotic syndrome. The primary causes became apparent by a markedly elevated number of low-molecular-weight apo (a) phenotypes which were usually associated with high Lp(a) levels. In addition, secondary causes by the pathogenetic mechanisms of the nephrotic syndrome itself resulted in a different increase of Lp(a) in the various apo(a) isoform groups. The tremendously increased Lp(a) levels in nephrotic syndrome were caused by primary genetic as well as disease-related mechanisms. 19 In same patients lipid profile disturbances persist during nephrotic syndrome remission, evaluation of genetic polymorphisms of proteins involved in lipoprotein metabolism in children and adolescents with nephrotic syndrome. 20

Some datas found that nephrotic syndrome (NS) was a stressful condition for patients where oxidative damage would also influence the response of these patients to therapy. Antioxidant concentrations change considerably, indicating a compensatory mechanism to cope up with increased pro-oxidant status in such cases. 21 Antioxidant status and reliable factors involved in antioxidant protection in children with nephrotic syndrome (NS). There was increased lipid peroxidation and insufficient antioxidant defence in nephrotic syndrome. 22

In the present study HCY level was >15μmol/l in adults with nephrotic syndrome. Oxidative stress was supported by increased HCY level; some other study is in agreement of this concept. Majumdar VS et al 23 showed that HCY mediated impairement of endothelial dependent vasodilation were reversed by coincubation of HCY with nicotinamide (an inhibitor
of peroxinitrate and nitrotyrosine) suggesting a role of HCY in redox mediating endothelial dysfunction and nitrotyrosine formation which was supported to oxidative stress by HCY. HCY was negatively correlated with serum TP & Alb These findings were in agreement with the findings of Gurusharan D et al. 24

In the present study showed that the serum Cu & Zn concentration were decreased in NS & nephropathic patients. In the present study correlated the cardiac risk and imbalance antioxidant status with changes in serum Cu and Zn level. Many other studies supported to this evidence. In the present study found that the serum HCY was negatively correlated to the Cu & Zn. Hughes et al 25 showed elevated level of HCY were involved in dilated cardiomyopathy HCY chelates copper and impairs Cu dependent enzymes, Cu deficiency has been linked to HHCY & cardiovascular disease. These data suggested that Cu supplement helps improve cardiac function in a pressure overload dilated cardiomyopathy. These findings is in agreement of present study where decreased level of Cu due to increased level of HCY in nephrotic syndrome patients & Cu deficiency was related to risk of cardiac diseases. Kenkeni M et al reported low activity of GSH-Px, SOD and Zn concentration were associated with HHCY. 26

Ghayour MM et al 27 measured serum Cu, copper/ceruloplasmin ratio, Zn/Cu ratio and C-reactive protein were significantly different in the dyslipidemic patients groups compared to control. These findings are supported to the imbalance in Cu & Zn metabolism in dyslipidemic patients with NS. The imbalance in Zn & Cu metabolism may either contribute to the CHD risk or be a consequence of an acute phase response. Bovio G et al 28 found the serum Cu and Zn level was below the normal range in patients with nephrotic proteinuria. Serum Zn was directly correlated with proteinuria and urinary Zn, but negatively correlated with testosterone levels in both sexes. This is supported to the positive correlation of Zn and albumin in the present study. Hughes S et al 29 observed Zn supplements have been shown. In some studied to decreased Cu/Zn–SOD actively, primarily due to the antagonistic relationship between high Zn intakes and Cu absorption. Besides the demonstrated adverse effect of Zn supplementation on plasma HDLC concentration in apparently healthy men, there was insufficient evidence to determine the role of Zn supplementation in influencing other risk factors for CHD such as antioxidant status and thrombogenesis. Free radicals should be implicated in the pathogenesis of proteinuria in NS 30

The increased Lp(a) levels are mainly related to hypoalbuminemia, probably through a mechanism involving apoB overproduction, which leads to an increased number of LDL particles to be converted into Lp(a). 31 Proteinuria and plasma compositional changes contribute to defective lipoprotein catabolism in the nephrotic syndrome by separate mechanisms. 32 Hyperhomocysteinaemia is a frequent cardiovascular risk factor present in patients with nephrotic syndrome and renal failure, but it is not directly associated with proteinuria. 2 Proteinuria and hyperhomocysteinaemia are independently associated with increased risk of atherosclerosis and cardiovascular disease. 33 Reactive oxygen species (ROS) are reported to play a role in inducing the proteinuria of nephrotic syndrome (NS). 34 Proteinuria as the most important pathophysiological change can reduce serum colloid osmotic pressure, which leads to an increase in the synthesis of serum proteins including lipoproteins in the liver for export to the serum. Thus,
the severity of lipid abnormalities may correlate with the degree of proteinuria. The patients with NS positive correlation between serum lipids and proteinuria is presented. 35 findings suggest that a decrease in serum albumin led to increased hepatic Lp(a) synthesis. It is well known that thrombotic disease supervenes on hypercoagulability in the nephrotic syndrome, so the determination of Lp(a) levels in these patients may provide information which is useful for preventing thrombotic complications. 36 Although lipoprotein abnormalities of the nephrotic syndrome are assumed to be related to the presence of proteinuria. 37 A significant inverse correlation was found between the total plasma cholesterol concentration and both the plasma albumin concentration and the plasma oncotic pressure but not the plasma viscosity. Enhanced hepatic synthesis of lipoprotein lipids may be stimulated by a decreased plasma albumin concentration or oncotic pressure but does not appear to be due to changes in plasma viscosity. 38

CONCLUSION
We conclude that oxidative stress is enhanced in NS patients due to hyperhomocysteinemia, hyperlipoproteinemia (a) and decreased level of TAC, total protein, albumin, copper and zinc which may contribute to the development of NS related complication with more frequency such as cardio-vascular nephropathy diseases, acute and chronic infection and many other complications. Long-term follow-up in a large number of patients would be necessary to confirm these results.

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REFERENCES:


