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Oxidative stress in secondary nephrotic syndrome: Recent advances with homocysteine, copper and zinc

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ABSTRACT

Imbalance in oxidant/antioxidant status is well document in patients with nephrotic syndrome & secondary nephrotic syndrome. Serum total antioxidant capacity, malondialdehyde, homocysteine, copper, zinc, plasma vitamin C were estimated in 2 groups, group I comprised of 50 nephrotic syndrome patients and group II comprised of 41 secondary nephrotic syndrome patients. It was observed there were decreased level of serum total antioxidant capacity ($P<0.0001$), copper ($P<0.02$), zinc ($P<0.02$), plasma vitamin C ($P<0.0001$) and increased serum level of malondialdehyde, homocysteine ($P<0.0001$) in secondary nephrotic group II when compared to nephrotic group I. Serum homocysteine were significantly positive correlated with MDA, negative correlated with serum Cu & Zn in nephrotic group. In conclusion increased oxidative stress in secondary nephrotic syndrome than nephrotic syndrome. Hyperhomocyst(e)inemia is related to decrease concentration of copper, zinc and supported to oxidative stress & endothelial dysfunction in nephrotic syndrome and secondary nephrotic syndrome.

Key words: Nephrotic Syndrome, Total antioxidant capacity, Homocysteine, Malondialdehyde, Vitamin C, Copper, Zinc.

INTRODUCTION

In the kidney, oxygen radical production has been detected in vascular cells, juxtra glomerular cells, tubular cells, podocytes, mesangial cells and isolated glomeruli. Free radicals have a negative influence on renal tissue in nephrotic syndrome (NS) [1]. Membranous nephropathy is the major life threatening complication of diabetic nephropathy and lupus nephritis, infections can be the causative agent in secondary nephrotic syndrome and diagnostic criteria include clinicle and laboratory data and tissue molecular analysis[2]. Secondary systemic amyloidosis with NS in association with chronic inflammation disorders and chronic infections[3]. Total antioxidant activity as the most reliable factor involved in antioxidation protection with NS[4]. Peroxidation of lipid membranes raises the concentration of their by product MDA and the consequent lowering of antioxidants as a result of consumption[5]. Cysteine and homocysteine can induce oxidative modification of LDLC, NS provides an excellent model in which to study a possible link between hyperhomocyst(e)inemia and NS with atherosclerosis[6,7]. Copper and zinc deficiency in NS related to increased urinary zinc and copper losses[8]. The aim of the present study was to estimate the serum total antioxidant capacity, malondialdehyde, homocysteine, copper, zinc, plasma vitamin C and correlate with oxidative stress to all above parameters in nephrotic syndrome & secondary nephrotic syndrome.

MATERIAL AND METHODS

The present study was conducted at the Department of Biochemistry S.S. Medical College Rewa (M.P.) with collaboration of Department of

Biochemistry N.S.C.B. Medical College Jabalpur (M.P.).

The study group

The present study was conducted on 2 groups.

Group I

Comprised of 50 NS patients.

Group II

Comprised of 41 secondary NS patients.

Out of 41 group II, included[11] patients with atherosclerosis[12], patients with diabetic nephropathy[11], patients with lupus nephritis[7], amyloidosis patients with NS. Age of the patients and controls group range was from 30 to 80 years. Patients were from same geographical area and none was taking a special diet, untreated NS patients newly diagnosed by biopsies evidences of nephritis. Group I (nephrotic patients) were not with any active complication medical condition or with systemic diseases such as diabetes mellitus, hepatic impairment, heart diseases, sickle cell anemia, amyloidosis, systemic lupus erythematosus, sacroidosis, leukemia, lymphoma, cancer of breast, colon and stomach, reaction to drugs (including nonsteroidal anti-inflammatory drugs) allergic reactions, acute and chronic infection and severe high blood pressure. Group II were selected from atherosclerosis, diabetic nephropathy, lupus nephritis, amyloidosis nephritis by biopsies evidences of membranous nephropathy. Other systemic diseases, acute -chronic infections, alcohol abusers, smokers and any other complications with NS were excluded. 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.,* [9]. 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.,* [10]. Plasma ascorbic acid (Vit C) was measured by colorimetric method described by Roe and Kuether *et al.,* [11]. Homocysteine was estimated by commercially available kit "Keragen diagnostic kit" by semiautoanalyzer. Serum Zn was measured by using commercially available kit method (ELI Techlogotech) by colorimeter. Serum copper was measured by colorimetric method described by Veture and king *et al.,* [12]. All the laboratory investigations were performed in group I & group II. The ethics committee of the DAVV of M.G.M. Medical College approved the study protocol. 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 between group I and group II.

RESULTS

In the present study all results of group II were compared with group 1. The level of all biochemical parameters were significantly changed between groups 1 and 2. Descriptive statics of all diagnostic parameters in group I & group II, presented in Table 1. There was a statistically significant decreased level of the serum TAC, Cu, Zn, plasma vit C and increased serum MDA, HCY level in group II when compared to group I. 10% NS patients (group I) had elevated serum HCY level>15 umol/l. There was significant change find out between group I & group II with HCY level ($p<0.0001$).

Table 1: Comparison of all diagnosed biochemical parameters between in group 1 and group 2 with NS

Parameters	Group I	Group II
n	50	41
TAC (mmol/L)	1.12 ± 0.04	0.76 ± 0.08*a
MDA (nmol/mL)	2.69 ± 0.22	5.07 ± 0.19*a
HCY (umol/L)	15.79 ± 0.15	21.04 ± 1.32*a
Vit C (mg/dL)	0.30 ± 0.11	0.11 ± 0.06*a
Cu (ug/dL)	70.69 ± 2.18	66.33 ± 1.26*b
Zn (ug/dL)	65.45 ± 1.46	62.49 ± 1.49*b
p value		* compare to group 1 *a – p<0.0001 *b – p<0.02

(n=No. of subjects and patients)

All results expressed in mean and standard deviation (SD).

Table 2: Correlation coefficient and significance in the patients group 2

Parameters	Correlation coefficient(r)	Significance (p)
HCY and MDA	+0.90	p<0.001
HCY and Zn	-0.34	P<0.0001
HCY and Cu	-0.36	p<0.0001
TAC and Zn	+0.56	p<0.0001
TAC and Cu	+0.50	p<0.0001

Table 2 Description about correlation coefficient and significance with diagnosed parameters in the group I. There were positive correlation between HCY & MDA (r= +0.90; p<0.001), where HCY supported to oxidative stress in study group I. HCY was negatively correlated to the serum Cu and Zn (r=-0.36; p<0.0001, r=-0.34; p<0.0001 respectively), it was related to the deficiency of Cu and Zn in NS. Total antioxidant capacity was positive correlated to serum Cu & Zn (r=+0.50; p<0.0001, r=+0.56; p<0.0001 respectively), supported for decreased antioxidant defense and oxidant/antioxidant imbalance in the study group I.

DISCUSSION

In the present study, 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 secondary NS than NS. Disturbances in oxidant and antioxidant status were observed by many other studies in agreement of the present study. Warwick GL *et al.,*[13] measured the plasma ascorbate concentration was significantly lower (p<0.001) & decreased ratio of ascorbate: vit E (p<0.0001) in group of NS. This could predispose to increased oxidative stress; LDL was protected from oxidation despite the severe hyperlipidemia and the low circulating vit C [13]. These data suggested that there may be relative defect of oxidant /antioxidant balance in NS.

Decrease total antioxidant status (TAS) connected with abnormal intestine absorption of some antioxidants component in patients with NS. There are some data in the literature showing that a diet deficient in Se and Vit C may lead to renal injury characterized by proteinuria and reduced GFR[14]. Excessive generation of reactive oxygen species is one of the incriminated mechanisms in the pathogenesis of progression renal injury. In fact the little data is available concerning SOD in NS. They reported reduced activities of eythrocyte and plasma GSH-Px when compared to the controls. They also observed lower Se and erythrocyte Cu-Zn-SOD activity in patients of NS than that of the controls. Erythrocyte and plasma level of MDA were higher in patients with NS. These results obtained in adult NS patient support the previous data indicating abnormalities in antioxidative system of NS (15).

Kromhauser C *et al.,*[16] reported lower Se and GSH-Px levels in diabetic patients may be implicated in diabetic nephropathy. Bhatia S *et al.,*[17] reported serum MDA concentration was significantly higher value with diabetic nephropathy (p<0.05) than without diabetic nephropathy. Catalase & SOD activity in group of diabetic nephropathy being significantly lower than group without diabetic nephropathy (p<0.005). Erythrocyte GSH contents were significantly lower in group of diabetic nephropathy as compared to controls (p<0.005) (17). Results of present study indicate the oxidative stress was increased and oxidant – antioxidant defense was imbalance with diabetic nephropathy. These dearrangements are of higher magnitude in patients of type 2 diabetes mellitus with nephropathy. The plasma level of Cu/ Zn SOD was significantly higher in secondary nephrotic syndrome and Cu/Zn SOD was positively correlated to MDA. Increased SOD and CAT activity were found in patients with lupus nephritis[18,19].

Addition of Cu^{2+} or Zn^{2+} to amyloid B-peptides in a negatively charged lipid environment caused a conformational change from B-sheet to alpha helix, accompanied by peptide oligomerization and membrane penetration. These results suggest that metal binding to amyloid B-peptides generated an allosterically ordered membrane penetrating oligomer linked by SOD-like binding-histidine residues[20].

We found that HCY level was >15umol/l in 10% adults with NS (group I) and in secondary

nephrotic syndrome patients (group II) were all >15umol/l. Oxidative stress is supported by increased HCY level; some other study is in agreement with this concept. Majumdar V S *et al.,*[21] showed HCY mediated impairment 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 is supported to oxidative stress by HCY[21]. During the autooxidation of HCY in plasma, reactive oxygen species are generated. The latter initiate lipid peroxidation in cell membranes (potentially responsible for endothelial dysfunction) and in circulating lipoprotein, oxidized LDLC may trigger platelet activation as well as some of the homeostatic abnormalities reported in such patients. Thus the oxidative stress induced by HCY may be a key process in the pathogenesis of thrombosis in HCY noted by Coppola A *et al.,*[22].

Earlier study reported about the changes of Cu and Zn metabolism in NS (8). We observed serum HCY is negatively correlated to the Cu. Hughes *et al.,*[23] showed elevated level of HCY are involved in dilated cardiomayopathy, HCY chelates copper and impairs Cu dependent enzymes, Cu deficiency has been linked to HHCY. This finding is in agreement of present study where decreased level of Cu due to increased level of HCY in nephrotic syndrome patients.

Kerkeni M *et al.,*[24] indicated that low activity of GSH-Px, SOD and Zn concentration are associated with HHCY. Hughes S *et al.,*[25] observed Zn supplements have been shown in some studied to decreased Cu/Zn –SOD activity, primarily due to the antagonistic relationship between high Zn intakes and Cu absorption. High plasma HCY may be a link in diabetic nephropathy & lupus nephritis between chronic inflammation and hypercoagulability, increasing cardiovascular risk[26-29].

The amyloidogenic protein transthyretin (prealbumin) undergoes homocysteinylation at its single cysteine residue (Cys10) both in vivo & vitro in HHCY burden. This in turn may contribute to the pathological consequences of amyloid disease[30]. Injury appears to be involved in either the amyloid formation process or in post fibrillar modification in several types of amyloidosis. The role of oxidative stress in pathogenesis of secondary amyloidosis, propose radical scavenger treatment for such amyloidosis[31]. Laboratory data showed severe hyperlipidemia with lipoproteine and nephrotic syndrome in primary systemic amyloidosis[32].

CONCLUSION

In conclusion, it was observed that decreased level of serum total antioxidant capacity, copper, zinc, plasma vitamin C and increased serum level of malondialdehyde, homocysteine are important estimation to assess the increased oxidative stress in secondary nephrotic syndrome than nephrotic syndrome patients. Hyperhomocysteinemia is related to decrease concentration of copper, zinc and supported to oxidative stress. It may be also responsible for endothelial dysfunction in nephrotic syndrome and secondary nephrotic syndrome.

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