INTERRELATIONSHIP OF OXIDATIVE STRESS, HOMOCYSTEINE, LIPOPROTEIN (a), COPPER & ZINC IN NEPHROTIC SYNDROME: RECENT ADVANCES: RISK FOR CARDIAC DISEASES.

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Summary

The free radicals have a negative influence on renal tissue in nephrotic syndrome. Nephrotic syndrome is a consequence of an imbalance oxidant/antioxidant status. The increase risk of atherosclerosis in nephrotic syndrome is attributed in part to the association with hyperhomocyst(e)inemia. Nephrotic syndrome have one of the most pronounced secondary changes in lipoprotein (a) metabolism. Abnormality of copper and zinc metabolism are well documented in patient with nephrotic syndrome.Serum Malondialdehyde, total antioxidant capacity, homocysteine, lipoprotein(a), Copper, Zinc & plasma vitamin C were estimated in 50 patients with nephrotic syndrome along with the routine biochemical parameters and compare to 50 controls. It was observed there were decreased level of serum Total antioxidant capacity, copper, zinc, plasma vitamin C and increased level of serum Lp (a), HCY, MDA level in patients with nephrotic syndrome compared to controls. There were significant positive correlation between serum MDA&HCY($r= +0.90; p<0.001$), MDA&Lp(a) ($r=+0.80; p<0.001$), TAC&Cu ($r=+0.50; p<0.0001$), TAC&Zn ($r=+0.56; p<0.0001$), Zn&Alb ($r=+0.84; p<0.05$). Significantly negative correlation were found between serum HCY&Alb ($r=-0.42; p<0.05$), HCY&TP ($r=-0.48; p<0.05$), HCY&Cu ($r= -0.36; p<0.0001$).

Key words:-Malondialdehyde (MDA), Total antioxidant capacity (TAC), vitamin C (vit C), Homocysteine (HCY), Lp(a) ,Copper (Cu), Zinc (Zn), nephrotic syndrome (NS).

Introduction

Free radicals primary the reactive oxygen species superoxide and hydroxyl radicals which are highly reactive having an unpaired electron in an atomic or molecular orbit are generated under physiological conditions during aerobic metabolism.[1] Total antioxidant activity as the most reliable factor involved in antioxidation protection with NS.[2] Peroxidation of lipid membranes raises the concentration of their by product MDA and the consequent lowering of antioxidants as a result of consumption.[3] Disturbances of lipid metabolism are a constant features of NS. In patients with NS they compose a significant risk factors of atherosclerosis and progression of renal insufficiency.[4] Patients with NS have one of the most pronounced secondary changes in lipoprotein metabolism and the magnitude of the changes correlates with the severity of the diseases.[5] 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 NS.[6] Cysteine and homocysteine can induce oxidative modification of LDLC.This suggestion is relevant because lipoprotein oxidation is thought to play a key role in the development of atherosclerosis and in the triggering of thrombotic events.[7] NS provides an excellent model in which to study a possible link between hyperhomocyst(e)inemia with NS and cardiovascular risk factors.[8] Abnormalities of Cu and Zn metabolism are well documented in patients with NS.[9] Disturbances in oxidant status during NS leading to plasma accumulation of oxidized LDL and cholesterol oxidation products that exert cytotoxicity and known to induce atherosclerosis.[10] The aim of the present study was to estimate the serum homocysteine, Lp(a), TAC, MDA, Cu , Zn, plasma ascorbic acid (vit C) with routine biochemical parameters, interrelationship of all biochemical parameters and correlate with cardiac risk factors in NS.

Materials 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 case control study conducted on 2 groups. Each group based on 50 individuals.

GroupI: -Comprised of control.

GroupII: -Comprised with adult NS patients.

Age of the patients groupII ranged from 30 to 80 years (55.30±8.12) patients were from same geographical area and none was taking a special diet, untreated NS patients newly diagnosed by biopsies evidences of nephritis. GroupI^{st} was judged to be free of any illness by clinical examination, the age range was same 30-80 years (45.3±6.2).

Nephrotic patients were not with any active complication medical condition or with systemic diseases such as Diabetes mellitus, hepatic impairmen, 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. Fasting venous blood were drawn from all. Routine biochemical parameters-serum Tchol, TGs and HDLC, TP and Alb were estimated by using commercial available kits (AUTOPAK) by colorimeter, LDLC and VLDLC were calculated using friedwalds formula.[11] Serum Lp(a) was estimated by using quantitative latex enhanced turbidimetric method, available kit "Human diagnostic kit" by semiautoanalyzer. Total antioxidant capacity (TAC) in serum was estimated by using spectrophotometric method described by D-Koracevic et al.[12] 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.[13] Homocysteine was estimated by commercially available kit "Keragen diagnostic kit" by semiautoanalyser. Serum copper was measured by colorimetric method described by Veture and king et al.[14] Serum Zn was measured by using commercially available kit method (ELI Tech-logotech) by colorimeter. Plasma ascorbic acid (Vit C) was measured by colorimetric method described by Roe and Kuether et al.[15] Serum Na & K were measured by directly electrolyte analyzer.All the laboratory investigation were performed in groupI & groupII. The ethics committee of the university of 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 groupI and groupII.

Results

All results of group II were compared with group I. The levels of all biochemical parameters were significantly changed between controls groupI and groupII. Descriptive statics of routine diagnostic parameters in groupI & groupII, presented in TableI. Serum Tchol, TGs, VLDLC, LDLC and Na were found to be significantly higher ($p<0.001$) in groupII when compared to groupI and HDLC significantly changed but within normal range. Serum TP, Alb level were lower in groupII when compared to groupI ($p<0.001$). Significantly decreased level of TP, Alb, K and increased level of Na supported for edema in NS, with Na & water retention and proteinuria.

Table II- given comparison of special diagnosed parameters in groupI & groupII. There was a statistically significant decreased level of the serum TAC, Cu, Zn, plasma vit C level and increased serum Lp (a), MDA, HCY level in groupII when compared to groupI. Significant differences in Lp (a) level were observed between groupII & groupI ($P<0.001$). In the present study 22% NS patients had elevated serum Lp(a) level>30mg/dl and 10% NS patients had elevated serum HCY level>15 umol/l. There was significant change find out between groupI & groupII with HCY level ($p<0.0001$).

Table III- Given correlation coefficient and significance with special parameters in the study groupII. There were positive correlation between HCY&MDA (r= +0.90; p<0.001), MDA& Lp(a) (r=+0.80; p<0.001), where HCY supported to oxidative stress in study groupII.HCY was negatively correlated to the serum Alb, TP and Cu (r= -0.42; p<0.05 ,r= -0.48; p<0.05 r= -0.36; p<0.0001 respectively), where HCY was not related to proteinuria (hypoproteinemia) and albuminuria (hypoalbuminemia) in study groupII&HCY was related to the deficiency of Cu. Total antioxidant capacity was positive correlated to serum Cu&Zn (r=+0.50; p<0.0001, r=+0.56; p<0.0001 respectively), where decreased serum Cu & Zn were supported for decreased antioxidant defense and oxidant/antioxidant imbalance in the study groupII. Albumin and Zn were also positive correlated (r=+0.84; p<0.05) which was supported for decreased Zn level due to the Zn – Alb complex where albumin as a carrier protein of Zn.

Table-I: -Comparison of routine diagnosed parameters-lipid profile, serum proteins, electrolytes between controls (group I) and patients(group II) with NS:-

Parameters	**Group I**	**GroupII**
n	50	50
TGs (mg/dl)	112.89 ± 8.34	210.39 ± 6.99*
Tchol (mg/dl)	181.34 ± 8.57	335.10 ± 6.66*
VLDLC (mg/dl)	22.56 ± 1.67	42.09 ± 1.42*
HDLC (mg/dl)	42.45 ± 1.47	31.83 ± 1.35*
LDLC (mg/dl)	116.27 ± 8.35	261.22 ± 6.53*
TP (g/dl)	6.83 ± 0.39	3.08 ± 0.25*
Alb (g/dl)	4.29 ± 0.48	1.39 ± 0.14*
Na (milieq/l)	142.63 ± 2.65	169.94 ± 4.06*
K (milieq/l)	4.35 ± 0.33	2.93 ± 0.14*
p value		*group I compare to group II *p<0.001

(n=No.of subjects and patients)

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

Table II: -Comparison of special diagnosed biochemical parameters between in controls (group I) and patients (group II) with NS:-

Parameters	**Group I**	**Group II**
n	50	50
Lp(a)(mg/dl)	20.24 ± 1.13	29.35 ± 1.73*a
TAC (mmol/l)	1.68 ± 0.12	1.12± 0.04*b
MDA (nmol/ml)	0.44 ± 0.14	2.69 ± 0.22*b
HCY (umol/l)	11.27 ± 1.29	15.79 ± 0.15*b
Vit C (mg/dl)	1.11 ± 0.25	0.30 ± 0.11*b
Cu (ug/dl)	123.64 ± 23.45	70.69± 2.18*a
Zn (ug/dl)	93.90 ± 7.84	65.45 ± 1.46*a
p value		*group I compare to group II *a – p<0.001 *b – p<0.0001

(n=No.of subjects and patients)

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

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

Parameters	Correlation coefficient(r)	Significance
Lp(a) and MDA	+0.80	p<0.001
HCY and MDA	+0.90	p<0.001
LDL and Lp(a)	+0.88	p<0.001
Alb and HCY	-0.42	p<0.05
TP and HCY	-0.48	p<0.05
Alb and Zn	+0.84	p<0.05
TAC and Zn	+0.56	p<0.0001
TAC and Cu	+0.50	p<0.0001
HCY and Cu	-0.36	p<0.0001

Discussion

The diagnostic criteria for establishing NS are the presence of proteinuria, hypoalbuminemia, hypercholesterolemia and finally edema. The edema was classically thought to be a consequences of the decreased intracellular oncotic pressure because of the loss of protein, Na and water retention.[16] The common pathological Na & water retention mechanism and the tendency of inflammatory markers and dyslipidemia to decrease from hypo & hypervolemic nephrotic patients could support this hypothesis.[17] Better correlation found between log aldosterone and $U(K^+)/U(Na^+)+U(K^+)$ ratio than with other parameters measuring renal potassium handeling such as transtubular potassium gradient fractional excretion of K and urine K^+/urine Na^+ or urine K^+&creatinine ratios. In patients with renal Na retention and $U(K^+)/U(Na^+)+U(K^+)$ ratios higher than 0.60 identifies patients with increased aldosterone level and indicates functional hypovolemia.[18]

INCREASED MDA AND DECREASED TAC,Cu AND Zn INDICATE THAT INCREASED OXIDATIVE STRESS IN STUDY GROUP II:

In the present study, mean serum (MDA) level was significantly higher in study groupII as compared to groupI. 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 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. 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 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.[19, 20]

COSEQUENCES SUPPORT OF OXIDATIVE STRESS & CARDIAC RISK BY INCREASED LEVEL OF LDLC,Lp(a) & HCY:

In the present study significantly increased level of Tchol, TGs, LDLC ($p<0.001$) were observed in patients when compared to controls in agreement with the other study. EI Melegy et al[21] reported significantly higher serum level of malondialdehyde, oxidized LDL, Tchol, LDLC, TGs apolipoprotein A-I and apolipoprotein B. The serum level of albumin, glutathione peroxidase activity, vit C, vit E and HDLC were significantly lower, a significant strong relationship between the oxidant/antioxidant status and dyslipidemia is documented in nephrotic patients.

In the present study significantly higher level of Lp(a), LDLC and HCY supported by many other studies and also supported to CVD risk. Kniazewska MH et al[22] &Kuzmas et al[23] in their study found significantly higher Tchol,LDLC,HCY,apolipoprotein-B and apolipoprotein A-I level.The level of HDL was normal. Investigation indicated a positive correlation between Intima Media thickness and the no.of recurrences. These findings are in agreement of present study.Caraba A et al[24] studied endothelial dysfunction was assessed and correlated with dyslipidemia and markers of inflammation in patients with nephrotic syndrome.The endothelial function was assessed by means of flow mediated dilation on bronchial artery, using B-Mode ultrasonography. The values of flow mediated dilation were 4±1.49 % in with NS and 11.905± 0.24 % in controls $p<0.01$. There was very strong inverse correlation between flow mediated dilation and LDLC ($r=-0.96$; $p<0.001$) Tchol ($r=-0.93$; $p<0.001$) and weak correlation with TGs ($r=-0.28$,$p<0.01$) and positive correlation with respective HDLC ($r=+0.40$; $P<0.001$) the most important factors involved in the endothelial dysfunction in NS are LDLC, Tchol and their treatment is necessary to prevent atherosclerosis in patients with nephrotic syndrome. Kuge Y et al[25] showed that severe hyperlipidemia proteinuria and hypoalbuminemia, very high level of Lp(a) in the plasma suggested the nephrotic syndrome. Severe atherosclerosis was also found with nephrotic syndrome, that is abdominal aortic aneurysm (AAA) and coronary artery disease (CAD) were detected in addition to arteriosclerotic obliterans with statins and prednisone. Markedly elevated Lp(a) plasma level in patients may have played important role in the progression of atherosclerosis. Kronenberg F et al [26] the tremendously increased Lp(a) level in nephrotic syndrome are caused by primary genetic as well as disease related (pathogenetic) mechanism.

In the present study 22 % patients had elevated Lp(a) level>30 mg/dl, and 78 % patients had the Lp(a) level>25mg/dl. K S S Saibaba et al[27] reported Lp(a) levels correlated positively with severity of atherosclerosis ($p<0.001$). The Lp(a) level in healthy population reported in indian studies have taken Lp(a) level 25 mg/dl as cut of value and analyzed its association with cardiac

heart disease using logistic regression and observed significant relationship. This study confirmed positive correlation of Lp(a) with CHD at >25mg/dl.

Disturbances in oxidant and antioxidant status were observed by many other studies in agreement of the present study. Warwick G L et al[28] measured the plasma ascorbate concentration was significantly lower ($p<0.001$) & decreased ratio of ascorbate:vit E ($p<0.0001$) in group of NS. These data suggested that there may be relative defect of oxidant /antioxidant balance in NS. This could predispose to increased oxidative stress. LDL was protected from oxidation despite the severe hyperlipidemia and the low circulating vit C.

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 in females.[27] But the other study Pedreno et al[29] showed no gender differences in Lp(a) levels in both patients and controls.

In the present study found hypoproteinemia & hypoalbuminemia which is responsible for the progression of cardiovascular diseases, this findings are supported by Falaschi F et al[30] observed patients with nephrotic range proteinuria(> or=3.5 gm/24 hrs) had a significantly higher carotid initima media wall thickness than did those without($p<0.02$) patients with nephrotic range proteinuria.

In the present study HCY level was >15umol/l in 10% adults with nephrotic syndrome. Oxidative stress is supported by increased HCY level, some other study is in agreement of this concept. Majumdar V S et al [31] showed 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 is supported to oxidative stress by HCY. HCY was negatively correlated with serum TP & Alb. These findings are in agreement with the findings of Gurusharan D et al [32] found HCY was significantly correlated with serum creatinine ($r=0.58$; $p<0.01$) and calculated GFR($R=-0.45$; $p<0.05$) but not with urinary protein or serum albumin. Increased HCY level due to renal failure for effective amino acids clearance. However Margret A et al[33] showed significantly lower HCY level in NS patients than non nephrotic patients. HCY correlated significantly with serum concentration of creatinine ($r=0.53$; $p<0.050$ and albumin ($r=0.43$;$p<0.05$) GFRs($r=-0.42$; $p<0.05$) and urinary albumin excretion($r=-0.47$; $p<0.05$).

HYPERHOMOCYSTEINEMIA AND CARDIAC RISK FACTOR:

Experimental evidences suggest that an increased concentration of HCY may result in vascular changes through several mechanisms. HHCY arises from disrupted HCY metabolism. Severe HHCY is due to rare genetic defects resulting in deficiencies in cystathionine beta synthase, methylene tetrahydrofolate(MTHF) and as an activator of cystathionine beta synthase or in enzyme involved in methylcobalamine synthesis and HCY methylation. High levels of HCY induce sustained injury of arterial endothelial cells. Prolifiration of arterial smooth muscle cells and enhance expression/activity of key participants in vascular inflammation,atherogenesis, and volunerability of the established atherosclerosis plaque. These effects supported to be mediated through its oxidation and the concomitant production of reactive oxygen species. [34,35,36,37] The mechanism through which elevated circulating level of HCY cause vascular injury and promote thrombosis remain elusive. Coppola A et al[38] in homocysteinuric patients homozygotes for mutations of the gene coding for the cystathionine beta synthase enzyme abnormalities of

coagulation variables reflecting a hypercoagulable state have been reported. In vitro studies provide a biochemical background for such a state. In homocysteinuric patients in vivo platelet activation has also been reported. 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. Accumulation of adenosylhomocysteine in cells (a consequences of high circulating levels of HCY), inhibits methyl trasferase enzymes, in turn preventing repair of aged or damaged cells.

DECREASED LEVEL OF Cu & Zn ALSO SUPPORTED TO CARDIAC RISK IN NS:
Earlier study reported about the changes of Cu and Zn metabolism in NS.[9] In the present study correlated the cardiac risk with changes in serum Cu and Zn level. In the present study serum HCY is negatively correlated to the Cu. Hughes et al[39] 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 & cardiovascular disease. These datas suggested that Cu supplement helps improve cardiac function in a pressure overload dilated cardiomayopathy. This finding is in agreement of present study where decreased level of Cu due to increased level of HCY in nephrotic syndrome patients & Cu deficiency is related to risk of cardiac diseases. Ghayour M M et al [40] measured serum Cu, copper/ceruloplasmin ratio, Zn/Cu ratio and C-reactive protein were significantly different in the dyslipidemic patients groups compared to controls. 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 [41] 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. Which is supported to the positive correlation of Zn and albumin in the present study. Hughes S et al[42] observed Zn supplements have been shown in some studied to decreased Cu/Zn –SOD activel, 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 is insufficient evidence to determine the role of Zn supplementation in influencing other risk factors for CHD such as antioxidant status and thrombogenesis.

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