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Study of homocysteine, lipoprotein (a), lipid profile with oxidative stress in nephrotic syndrome and lupus nephritis

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ABSTRACT

Homocysteine and lipoprotein (a) are important markers for oxidative stress in nephrotic syndrome and lupus nephritis. Oxidative stress contributes to the pathophysiology of kidney injury. Membranous nephropathy is the most important cause of the nephrotic syndrome in elderly patients. Reactive oxygen species play an important role in the pathogenesis of different glomerulopathies. The purpose of this study was to examine selected markers of oxidative stress and antioxidant defense in nephrotic syndrome & lupus nephritis. Therefore, this study was carried out to investigate oxidant and antioxidant status in nephrotic syndrome and lupus nephritis patients. The blood samples were analyzed for quantitation of malondialdehyde as index of lipid peroxide, vitamin C, total antioxidant capacity, homocysteine, lipoprotein (a) & lipid profile. Significantly increased levels of serum total cholesterol, low density lipoprotein, malondialdehyde, homocysteine, lipoprotein (a) ($p<0.001$) and decreased levels of serum high density lipoprotein, total antioxidant capacity, total protein, albumin, & plasma vitamin C ($p<0.001$) were noticed in the patients with lupus nephritis as compared to nephrotic syndrome and control subjects. In conclusion the oxidative stress is enhanced in nephrotic syndrome & lupus nephritis patients due to hyperhomocysteinemia, hyperlipoproteinemia and hypoproteinemia. Lupus nephritis patients had more Oxidative stress than nephrotic syndrome patients.

Key words: Malondialdehyde, Total antioxidant capacity, Nephrotic syndrome, Lupus nephritis, Homocysteine, Lipoprotein (a).

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INTRODUCTION

The nephrotic syndrome (NS) is defined by heavy proteinuria due to abnormal increase of glomerular permeability and following hypoproteinemia, hypoalbuminemia, hyperlipidemia and edema [1]. Homocysteine (HCY) is independent risk factors for atherosclerosis in several clinical settings in which renal function is impaired but its prevalence in the nephrotic syndrome has not been investigated in details [2]. Even though this syndrome provides an excellent model in which to study a link between hyperhomocyst (e) inemia with NS and cardiovascular risk factors [2]. Abnormalities of homocysteine and vitamins in adult patients with NS have multiple risk factors for thrombosis [2,3]. Serum/plasma levels of lipoprotein (a) [Lp (a)] an atherogenic particles are elevated in Kidney disease which suggest a role of this organ in the metabolism of Lipoprotein (a) [4]. Lipoprotein abnormalities of the Nephrotic syndrome are assumed to be related to the presence of proteinuria [5]. Total Antioxidant Status as the most reliable factor involved in anti oxidation protection with Nephrotic syndrome [6]. Peroxidation of lipid membranes raises the concentration of their by product MDA and the consequent lowering of antioxidants as a result of consumption. [7-9].

The lupus nephritis (LN) is classified according to WHO classification and is correlated with response to therapy and prognosis [10]. Membranous lupus nephritis with nephrotic syndrome remains controversial, while the risk of progressive renal deterioration may be small, persistent heavy proteinuria leads to the complications of edema, hypoalbuminemia, hyprlipidemia, hypercoagulability and venous thrombosis [11]. LN patients with the abnormal tubular study results more often present with nephritis or nephritic sediment and peripheral edema [12,13]. Oxidative stress plays an important intermediary role in the pathogenesis of lupus nephritis [14]. Increase oxidative stress is a hallmark of autoimmune disease of lupus nephritis [14].

Lupus nephritis is characterized by increased expression of oxidative markers [15]. Oxidative stress can be correlate with glomerular injury [16]. Lp (a) is known to be an independent risk factors of atherosclerosis in patients with lupus nephritis [17]. Lipoprotein (a) concentration in patients with lupus nephritis, further increasing the risk of athero-thrombotic cardiovascular complications [18]. Early atherosclerosis risk factor in patients with systemic lupus erythematosus in respect to the presence of lupus nephritis and Lp(a), HCY & oxidative stress [14, 17-19].

The objective of this study was to investigate possible associations between oxidative stress and the severity of lupus nephritis in NS with the estimation of the serum lipid profile, total protein, albumin,TAC, MDA, Lp(a), HCY, plasma ascorbic acid (vit C) and correlate with severity of lupus nephritis in other complications of LN with cardiovascular diseases.

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.

The study group: The present study was conducted on 3 groups.

Group I: Comprised with controls (135).
Group II: Comprised with adult NS patients (133).
Group III: Comprised with adult LN patients (58).

Age of the patients group I, II & III ranged 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. LN patients also diagnosed by biopsies evidences of nephritis. Group Ist was judged to be free of any illness by clinical examination, group II (NS patients) were not with any other active complication medical condition or with systemic diseases. Group III included only LN patients, were not included with any other systemic diseases with NS. Excluded other acute and chronic infections with NS, liver diseases with nephritis, taking vitamins tablet from prolonged time, Alcohol abusers, smokers, Acute and chronic renal failure and hemodialysis patients, other systemic diseases such as Diabetes mellitus, hepatic impairment, heart diseases, sickle cell anemia, amyloidosis, sacroidosis, leukemia, lymphoma, cancer of breast, colon and stomach, reaction to drugs, allergic reactions. Fasting venous blood were drawn from all.

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. 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". Total antioxidant capacity (TAC) in serum was estimated by using spectrophotometric method described by D-Koracevic et al [20]. Malondialdehyde (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 [21]. Plasma ascorbic acid (Vit C) was measured by colorimetric method described by Roe and Kuether et al [22].

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

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Table III Description about correlation coefficient and significance with diagnosed parameters in the study group III. There was positive correlation between Lp (a) & MDA, HCY was positively correlated to the serum MDA & Lp (a) where HCY supported to oxidative stress in study group III. HCY was negatively correlated to the serum TAC, TP & Alb it was related to the decreased defense system of antioxidant protection of the body, which is related to increased oxidative stress in study group III while proteinuria and albuminuria was not related to the HHCY in study group III. Total antioxidant capacity was negative correlated to serum Lp (a), supported for decreased antioxidant defense and oxidant/antioxidant imbalance in the study group III. Total protein was negative correlated to MDA, where decreased concentration of total protein supported to increased lipid peroxidation in the patients group III.

Table I: Comparison of routine diagnosed parameters-lipid profile, serum proteins in group I, II & III

Parameters	Group I	Group II	Group III
n	135	133	58
TGs (mg/dL)	112.09 ± 10.16	196.64±23.89*	202.95 ± 6.81**
Tchol (mg/dL)	173.71 ± 15.44	297.14±25.92*	343.25 ± 18.6**
VLDLC (mg/dL)	22.40 ± 1.98	39.34 ± 3.7*	40.59 ± 3.3**
HDLC (mg/dL)	49.15 ± 7.4	39.63 ± 1.28*	29.39 ± 8.3**
LDLC (mg/dL)	103.68 ± 8.24	217.38±19.36*	273.25 ± 19.2**
TP(g/dL)	6.90 ± 1.6	3.26 ± 3.3*	4.0 ± 0.78**
Alb (g/dL)	4.34 ± 0.37	1.37±0.70*	2.36 ± 0.28**

(n=No. of subjects and patients), *group I compare to group II, * p<0.001; Highly Significant, **group II compare to group III, * p<0.001; Highly Significant

Table II: Comparison of diagnosed biochemical parameters between control (group I) and patients (group II & III) with NS & LN

Parameters	Group I	Group II	Group III
n	135	133	58
Lp(a) (mg/dL)	18.15 ± 9.7	28.44 ± 2.06*	37.59 ± 8.3**
HCY (umol/L)	10.75 ± 3.1	17.77 ± 4.15*	24.48 ± 6.0**
TAC(mmol/L)	2.37 ± 0.87	1.55 ± 0.28*	1.33 ± 0.51**
MDA(nmol/mL)	1.56 ± 0.96	3.58 ± 0.42*	5.90 ± 0.76**
Vit C(mg/dL)	1.48 ± 0.65	0.85 ± 0.48*	0.50 ± 0.32**
p value		*group I compare to group II * p<0.001	**group II compare to group III * p<0.001

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

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

Parameters	Correlation coefficient (r)	Significance
Lp(a) and MDA	+0.90	p<0.001*a
HCY and MDA	+0.82	p<0.001*a
LDL and Lp(a)	+0.86	p<0.001*a
Alb and HCY	-0.52	p<0.001*a
TP and HCY	-0.58	p<0.001*a
Lp(a) and HCY	+0.76	p<0.001*a
HCY and TAC	-0.38	p<0.0001*b
Lp(a) and TAC	-0.32	P<0.0001*b
TP and MDA	-0.62	P<0.001*a

*a-Highly significant,*b-Significant

DISCUSSION

In the present study represent that LN patients had more oxidative stress than NS & normal persons where oxidative stress may play an important intermediary role in the pathogenesis of lupus nephritis.

Proteinuria alters the apolipoprotein content of lipoproteins. Proteinuria also alters the concentrations of oxidized lipids within lipoprotein density fractions. Proteinuria increased the total oxylipid amounts in the HDL and VLDL fractions. Nephrotic syndrome alters the lipoprotein oxylipid composition independently of an increase in total lipoprotein levels [23].

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 [24] 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 [24].

In the present study, mean serum (MDA) level was significantly higher in study group II & III as compared to group I. This result showed the presence of oxidative stress in NS and LN. 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 supported to the previous datas indicating abnormalities in antioxidative system of NS [25-27]. EI Melegy et al [28] 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 patients with steroid sensitive nephrotic syndrome, especially among relapsers. No normalization of the biochemical indices was observed despite the use of glucocorticoids. Therefore the combined use of steroid, antioxidant therapy and lipid lowering therapy can be recommended in such patients. Scrzep-Polozek B et al [29] showed higher amounts of electronegatively charged (oxidized) LDL particles as well as different oxysterol in patients have also been reported significant disturbances in oxidant/antioxidant status during NS leading to plasma accumulation of oxidized LDLC and cholesterol oxidation products that exert cytotoxicity and are known to induce atherosclerosis. Warwick G L et al [30] 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. LDLC was protected from oxidation despite the severe hyperlipidemia and the low circulating vitC.

In the present study HCY level was >15umol/l with nephrotic syndrome and LN. Oxidative stress is supported by increased HCY level; some other study is in agreement of this concept. Majumdar VS 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 nonnephrotic 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($r=-0.47$; $p<0.05$). 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 are supported to be mediated through its oxidation and the concomitant production of reactive oxygen species [34-37]. Several studies have demonstrated that dietary supplementation with folic acid and vit B_{12} and vit B_6 is an efficient means to decrease plasma HHCY [38,39].

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 [40] & Kuzmas et al [41] in their study found significantly higher Tchol, LDLC, HCY, apolipoprotein-B (apo-B) and apolipoprotein A-I level. 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 [42] 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. 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 the NS are LDLC, Tchol and their treatment is necessary to prevent atherosclerosis in patients with nephrotic syndrome [42]. The atherogenic serum lipoprotein (a) [Lp(a)] is 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 are 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 [43]. In same patients lipid profile disturbances persist during nephrotic syndrome remission, Evaluation of genetic polymorphisms of proteins involved in lipoprotein metabolism in nephrotic syndrome [44].

Lupus nephritis was severe in patients with predominance of proliferative forms, hypertension, nephrotic syndrome and initial renal failure were statistically associated with deterioration of renal function [45]. Significantly increased Lp (a) concentration in patients with lupus nephritis as compared to the absence of lupus nephritis group, in a good correlation of these observations patient with nephritis suffered more frequently from deep venous thrombosis and ischemic heart diseases [17]. Present results showed the importance of elevated oxidative stress & LP (a) concentration in patients with lupus nephritis; further increasing the risk of atherothrombotic cardiovascular complications [46,47]. A positive correlation was found between plasma HCY concentration and renal involvement based on the positive of renal biopsy, abnormalities of urine sediment and serum creatinine.[18] Increased oxidative stresses was represented increased susceptibility for glomerulonephritis & end stage renal diseases with LN [48,49]. Despite an inordinately high risk of CV disease in SLE, assessment of CV risk factors was surprisingly uncommon among the practices assessed, greater attention needs to be paid to CV disease risk factor screening in patients with lupus.[50,51]. In general patients with systemic lupus erythematosus (particularly with lupus nephritis and cardiovascular diseases) had more oxidized epitopes on LDL compared with controls [46]. Increased oxidative stress with systemic lupus erythematosus and LN may be of importance for the development of end stage renal diseases premature cardiovascular diseases and possibly also for other autoimmune phenomena observed [46]. The degree of lipid peroxidation seemed to be correlated with the extent of proteinuria in lupus nephritis [52]. As compared with the normal values the activity of the three enzymes superoxide dismutase, catalase, glutathione peroxidase were decreased and did not correlate with the level of proteinuria [52]. Early atherosclerosis risk factor in patients with systemic lupus erythematosus in respect to the presence of lupus nephritis and antiphospholipid antibodies [53]. Intravenous immunoglobulin might be effective in treatment resistant membranous or membranoproliferative lupus nephritis [54]. Patients with LN showed impaired oxidative status, even without clinical signs of renal activity [52]. ROS production may be counterbalanced by adequate antioxidant capacity in some patients with quiescent LN [52]. The association of hyperhomocysteinemia and

antiphospholipid antibodies positivity may increase the risk of cardiovascular and/or thrombotic events in LN patients [55].

Intensive immunosuppression with steroids, statin based drug for hypercholesterolemia and antioxidant supplements for oxidative stress can achieve excellent long term results in the treatment of systemic lupus with lupus nephritis.

CONCLUSION

We conclude that oxidative stress is enhanced in NS & LN patients due to hyperhomocysteinemia, hyperlipoproteinemia and hypoproteinemia which may contribute to the development of LN related complication with more frequency such as cardiovascular diseases and end stage renal diseases, acute and chronic infection and many other complications.

Several evidences suggest that patients with LN had imbalance oxidant/antioxidant status and increased subsequent oxidative stress than nephrotic syndrome patients is due to oxidation of LDL and lipoprotein, low intake of antioxidants in diet, hyperhomocysteinemia, hyperlipoproteinemia and 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 lupus nephritis.

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