Original Research Article

The Study of oxidant and antioxidant status with special emphasis to homocysteine, total protein and albumin in nephrotic syndrome

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Abstract

Background: Nephrotic patients show various abnormalities in protein kinetics. Plasma albumin levels and the total plasma albumin pool are reduced. Nephrotic syndrome is a consequence of an imbalance between oxidant and anti-oxidant activity. Oxidative damage has been proposed as one of the possible mechanism involved in the Nephrotic syndrome. Therefore, this study was carried out to investigate oxidant and antioxidant status with Homocysteine in Nephrotic syndrome patients. The blood samples were analyzed for quantitation of total protein, albumin, Malondialdehyde as index of lipid peroxide, vitamin C, total antioxidant capacity and Homocysteine. Significantly increased levels of serum lipid peroxide, Homocysteine and decreased levels total protein, albumin, serum total antioxidant capacity and plasma vitamin C were noticed in the patients with Nephrotic syndrome as compared to control subjects. However, significant positive correlation in lipid peroxide with Homocysteine and total protein with total antioxidant capacity, were observed in the patients of Nephrotic syndrome with age group among 30-80 years.

Keywords: Nephrotic syndrome, Total antioxidant capacity, Homocysteine, Malondialdehyde, Vitamin C.

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Received Date: 07/12/2020 Revised Date: 12/01/2021 Accepted Date: 23/02/2021
DOI: https://doi.org/10.26611/10021831

Access this article online	
Quick Response Code:	Website: www.medpulse.in
	Accessed Date: 02 June 2021

INTRODUCTION

Nephrotic syndrome (NS) is caused by increased permeability of the glomerular capillary wall for proteins[1]. Nephrotic syndrome is the common chronic disorder characterized by alteration of permeability of the glomerular capillary wall, resulting in its inability to restrict the urinary loss of proteins[2]. Nephrotic syndrome is a stressful condition for children where oxidative damage would also influence the response of these patients to therapy[3]. The production of free radicals can cause renal injury and play an important role in the pathogenesis of Nephrotic syndrome [4]. Excessive generation of reactive oxygen species is one of the incriminated mechanisms in the pathogenesis of progressive renal injury. The role of oxidant stress in acute and chronic glomerular diseases has been investigated through experimental and clinical studies[5]. Nephrotic syndrome is characterized by heavy proteinuria and hypoalbuminemia. Reactive oxygen species (ROS) seem to play an important role in the etiopathogenesis of proteinuria in NS. The potential role of reactive oxygen species in pathogenesis of NS by estimating the levels of oxidants and antioxidants in NS[6]. The atherothrombotic risk pattern of the Nephrotic

How to cite this article: G Prem Kumar, Vellanki Lakshmi Narasamma, K Satya Sri. Study of oxidant and antioxidant status with special emphasis to homocysteine, total protein and albumin in nephrotic syndrome. *MedPulse International Journal of Biochemistry.* June 2021; 18(3):

syndrome resembles that of Hyperhomocysteinemia[7]. Proteinuria and Hyperhomocysteinaemia are independently associated with increased risk of atherosclerosis and cardiovascular disease[8]. Changes in metabolism of aminothiols may have an influence on endothelial function or change the red-ox balance[9]. Various biochemical parameters that are presently determined in serum/plasma Homocysteine, total antioxidant capacity, lipid peroxidation, Vit C, total protein, albumin for the diagnosis of Nephrotic syndrome, as well as to determine the oxidative stress on the basis of above parameters in NS. The Purpose of this research is to estimate different biochemical parameters in patients of Nephrotic syndrome and to determine the Interrelationship of Homocysteine, oxidant and antioxidant status in Nephrotic syndrome.

MATERIALS AND METHODS

The present study was conducted at the Department of Biochemistry, Government General Hospital, Srikakulam with collaboration of Department of Biochemistry, Andhra Medical College, Visakhapatnam. The present 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 "ERBA" in semiautomatic auto analyzer. Total antioxidant capacity (TAC) in serum was estimated by using spectrophotometric method Koracevic *et al.*[10]. MDA, one of the aldehydic by product of lipid peroxidation in serum, was estimated by its thiobarbituric acid reactivity using, spectrophotometric method Hunter *et al.*[11]. Plasma ascorbic acid (Vit C) was measured by colorimetric method Roe and Kuether *et al.*[12]. Homocysteine was estimated by commercial "LS BIO diagnostic kit" using Bio-Rad ELISA reader. The values were expressed as mean +/- SD. Student test was done for comparison of data. The laboratory investigations were performed on groups I and II.

Table 1: Comparison of biochemical parameters in group I and group II

Parameters	Group I (controls) (Mean ± SD) N=135	Group II (Nephrotic Syndrome Cases) (Mean ± SD) N=133	Significance
Total protein(g/dL)	6.80 ± 1.6	3.26 ± 3.3	p<0.001
Albumin (g/dL)	4.14 ± 0.37	1.37 ± 0.70	p<0.001
Total Antioxidant Capacity (mmol/L)	2.37 ± 0.87	1.55 ± 0.28	p<0.0001
Malondialdehyde (nmol/mL)	1.56 ± 0.96	3.58 ± 0.42	p<0.001
Homocysteine (umol/L)	10.75 ± 3.1	17.77 ± 4.15	p<0.001
Vit C (mg/dL)	1.48 ± 0.65	0.68 ± 0.48	p<0.0001

Table 2: Correlation coefficient of different parameters in group II patients

Parameters	Correlation coefficient (r)	Significance
HCY and MDA	+0.78	p<0.001
Alb and HCY	-0.40	p<0.05
TP and HCY	-0.46	p<0.05
HCY and TAC	-0.25	p<0.0001
TP and MDA	-0.55	P<0.001

HCY-Homocysteine, MDA-Malondialdehyde, Alb-Albumin, TP-Total protein, TAC-Total antioxidant capacity.

DISCUSSION

The Nephrotic syndrome is characterized by increased urinary excretion of albumin and other serum proteins, accompanied by hypoproteinemia and edema formation[13]. The molecular mechanisms behind acquired NS are still largely unknown. One possible explanation for the development of proteinuria is oxidative damage to the glomerular cells[14]. Albumin has also emerged as a major plasma antioxidant, and recent studies have demonstrated that in patients with active focal segmental glomerulosclerosis albumin undergoes massive and stable oxidation with sulfonation of Cys34, formation of an adduct with +48 Da molecular weight, changes of the net charge due to additional negative residues, and loss of free thiol group (SH) titration. Altogether, these data suggest that oxidative stress determines selective protein damages in focal segmental glomerulosclerosis patients with formation of new adducts and fragmentation of plasma proteins. Research should now address whether oxidation of podocyte proteins is important for the maintenance of renal selectivity and is involved in proteinuria[15]. Reactive oxygen species (ROS) are reported to play a role in inducing the proteinuria of NS[16]. Albumin is a potent antioxidant as it chelates transitional metals and contains antioxidants like thiol and bilirubin[17]. In the present study increased Homocysteine level is related to endothelial dysfunction, some other study is in agreement with this concept Majumdar *et al.*[18] that Homocysteine mediated impairment of endothelial dependent vasodilation were reversed by incubating Homocysteine with nicotinamide (an inhibitor of peroxinitrate and nitrotyrosine) suggests a role of Homocysteine in redox mediating endothelial dysfunction and nitrotyrosine formation, this is supported to oxidative stress and endothelial dysfunction by Homocysteine. These findings are in agreement with the findings of Gurusharan *et al.* [19]where Homocysteine was significantly correlated with serum creatinine ($r=0.58$; $p<0.01$) and calculated GFR ($R=-0.45$; $p<0.05$). Increased Homocysteine level is due to renal failure for effective amino acids clearance. However, Margret *et al.* [20]showed significantly lower Homocysteine level in NS patients than non NS patients. Disturbances in oxidant and antioxidant status were observed by many other studies, which was in agreement of our study Warwick *et al.*[21]. The plasma ascorbate concentration was significantly lower ($p<0.001$) and decreased ratio of ascorbate: vit E ($p<0.0001$) in group of NS. Low density lipoprotein was protected for oxidation despite the severe hyperlipidemia and the low circulating Vit C. These data suggest that there may be relative deficit of oxidant/antioxidant balance in NS. This could predispose to increased oxidative stress[21]. In the present study, mean serum malondialdehyde 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 decreased total antioxidant status level is connected with abnormal intestine absorption of some antioxidants component in patients with NS. There is 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 Bulucu *et al.*[22]. 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. Reduced activities of erythrocyte and plasma GSH-Px were reported when compared to the control. Lower Se and erythrocyte Cu-Zn-SOD activity was shown in patients of NS when compared to the control. Erythrocyte and plasma level of malondialdehyde were higher in patients with NS.These results obtained in adult NS patients support the previous data indicating abnormalities in antioxidative system of NS Pawlak K *et al.*[23] During the auto oxidation of Homocysteine in plasma, reactive oxygen species are generated Coppola *et al.* The latter initiates 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 Homocysteine may be a key process in the pathogenesis of thrombosis in Hyperhomocysteinemia[24]. 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 Homocysteine. Endothelial dysfunction may cause proatherogenic effects associated with Hyperhomocysteinemia. Folicacid and Vit B_{12} deficiencies should be corrected by supplementation in Hyperhomocysteinemia. Increases in folate intake by dietary changes or fortification can also lower plasma. Homocysteine level in NS patients. In renal failure, folic acid treatment (1-5 mg/day) a-meliorate the plasma Homocysteine level in most cases but Hyperhomocysteinemia persists in the majority of patients. Primary (fasting) Hyperhomocysteinemia can be treated with folic acid (0.5-5 mg/day) Sydow *et al.*, Van Guldener *et al.*[25, 26].

CONCLUSION

We conclude that Hyperhomocysteinemia was related to oxidative stress in NS patients. Long-term follow-up in a large number of patients would be necessary to confirm these results.

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Source of Support: None Declared
Conflict of Interest: None Declared