J Biochem Tech (2009) 1(4):104-107
ISSN: 0974-2328

Study of oxidative stress, homocysteine, copper & zinc in nephrotic syndrome: therapy with antioxidants, minerals and B-complex vitamins

Jyoti Dwivedi*, Purnima Dey Sarkar

Received: 26 July 2009 / Received in revised form: 23 August 2009, Accepted: 15 September 2009, Published online: 22 September 2009,

Abstract

Oxidative damage has been proposed as one of the possible mechanism involved in the nephrotic syndrome. Strengthening the defense system by antioxidants may provide protection against oxidative damage. Therefore, this study was carried out to investigate oxidant and antioxidant status with copper, zinc and homocysteine in nephrotic syndrome patients and the effect of antioxidants, minerals and B-complex vitamins on oxidant and antioxidant status. The blood samples were analyzed for quantitation of malondialdehyde as index of lipid peroxide, vitamin C, total antioxidant capacity, copper, zinc, and homocysteine. Significantly increased levels of serum lipid peroxide, homocysteine and decreased levels of serum total antioxidant capacity, copper, zinc and plasma vitamin C were noticed in the patients with nephrotic syndrome as compared to control subjects. However, significant reduction in lipid peroxide, homocysteine and improvement in vitamin C, total antioxidant capacity, copper, and zinc activity were observed after treatment of antioxidants and minerals with B-complex vitamins.

Keywords: Nephrotic syndrome (NS), Total antioxidant capacity (TAC), Homocysteine (HCY), Malondialdehyde (MDA), Vitamin C (Vit C), Copper (Cu), Zinc (Zn)

Introduction

The Nephrotic Syndrome (NS) is defined by heavy proteinuria (urine total protein excretion greater than 3.5 g/d or total protein-creatinine ratio greater than 3.5 g/g) due to abnormal increase of glomerular permeability and following hypoalbuminemia, hyperlipidemia and edema (Stoycheff et al. 2009). Disorders of size selective barrier, charge selective barrier, slit diaphragm and circulating permeability factors are thought to be the causes of proteinuria. Most patients with nephrotic edema have primary salt retention. Overproduction and impaired catabolism of lipoproteins are the causes of hyperlipidemia (Togawa et al. 2004). Nephrotic Syndrome is a collection of Syndrome which occurs because the tiny blood vessels (the glomeruli) in the kidney become leaky (Deschenes et al. 2003). Peroxidation of lipid membranes raises the concentration of their by product MDA and the consequent lowering of antioxidants as a result of consumption (Sanjay et al. 2000). Total antioxidant activity as the most reliable factor is involved in antioxidation protection with nephrotic syndrome (Zachwieja et al. 2000). 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 (Zachwieja et al. 2003). Homocysteine can induce oxidative modification of LDLC (Yang et al. 2005). NS provides an excellent model to study a link between hyperhomocyst(e)inemia and NS (Coroba et al. 1996; Joven et al. 2000). Abnormalities of Cu and Zn metabolism are well documented in patients with NS (Stec et al. 1990). The prevalence in the NS of higher circulating level of homocysteine and of low levels of the B vitamins that are involved in its metabolism, plays a role in thrombosis (Podda et al. 2007). The administration of various natural or synthetic antioxidants has been shown to be of benefit in prevention and attenuation of renal scarring in kidney diseases (Leszek et al. 2003).

With this background, this study was aimed to evaluate the possible combined therapy of antioxidants, minerals with B-complex vitamins for treatment of imbalance oxidant /antioxidant status, hyperhomocyst(e)inemia and deficiency of copper and zinc in nephrotic syndrome patients.

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

Jyoti Dwivedi

Dept. of Biochemistry, S.S. Medical College Rewa, Madhya Pradesh, India, Pin-486001

Purnima Dey Sarkar

Dept. of Biochemistry, N.S.C.B. Medical College Jabalpur, Madhya Pradesh, India, Pin-482003

*Tel: 0091-7662-257424, Fax.: +91-7662-251167
Email: jyoti.bioc@rediffmail.com

The study group: This study was conducted on 3 groups: group I comprised of 50 controls, group II comprised of 50 nephrotic syndrome patients and group III comprised of 50 remissions 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, which may lead to oxidative stress, 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 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, spectrophotometric method (Hunter et al. 1985). Plasma ascorbic acid (Vit C) was measured by colorimetric method (Roe and Kuether et al. 1943). Homocysteine was estimated by commercial "Keragen diagnostic kit" using semiautoanalyser. Serum Zn was measured using commercial protocol (ELI Tech-logotech) by colorimeter. Serum copper was measured by colorimetric method (Veture and king et al. 1951). All patients were given 3 months supply of commercial available tablet Zincovit C (Vit C = 75 mg, Niacinamide = 50 mg, Alpha tocopherol = 15 mg, Thiamine mononitrate = 10 mg, Riboflavin = 10 mg, Calcium pentothenate = 10 mg, Pyridoxine hydrochloride = 2 mg, Folic acid = 1 mg, Vit A = 5000 IU, Vit D_3 = 400 IU, Vit B_{12} = 7.5 mcg, Zinc sulphate = 63 mg, Magnisium oxide = 30 mg, Magnesium sulphate monohydrate = 2.8 mg, Copper sulphate = 2 mg, Colloidal silicon dioxide equivalent to silica = 1 mg, Iodine = 150 mcg, Sodium borate equivalent to boron = 150 mcg, Selenium dioxide monohydrate = 70 mcg, Chromium piconilate equivalent to chromium = 25 mg, Sodium molybdate dehydrate equivalent to molybdenum = 25 mcg, Carbohydrate = 200 g) under medical supervision. Blood samples were collected after the third month. The values were expressed as mean +/- SD. Student test was done for comparison of data. The laboratory investigations were performed on groups I, II & III. The study was approved by the ethics committee of the D.A.V. University.

Results

Descriptive statistics of all diagnostic parameters on groups I, II, & III is presented in Table I. 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 had elevated serum HCY level (>15 umol/L).There was significant difference between group I & group II with HCY level ($p<0.0001$). The level of serum HCY and MDA were significantly decreased whereas activity of TAC, Cu, Zn, plasma Vit C were significantly increased in post treated group after 3 months of Zincovit prescription.

Table 1. Comparison of all diagnosed biochemical parameters in group I, II, and III with NS

Parameters	Group I (control)	Group II (pre-treatment)	Group III (post-treatment)
n	50	50	50
TAC (mmol/L)	1.68 ± 0.12	1.12± 0.04*a	1.34 ± 0.08**c
MDA(nmol/mL)	0.44 ± 0.14	2.69 ± 0.22*a	0.93 ± 0.17**c
HCY (umol/L)	11.27 ± 1.29	15.79 ± 0.15*a	14.66 ± 0.36**c
Vit C (mg/dL)	1.11 ± 0.25	0.30 ± 0.11*a	0.71 ± 0.14**c
Cu (ug/dL)	123.6 ± 23.4	70.69± 2.2*b	75.42 ± 0.91**d
Zn (ug/dL)	93.90 ± 7.84	65.45 ± 1.4*b	72.96 ± 1.48**d
p value		* compare to group I *a: p<0.0001 *b: p<0.001	** compare to group II **c: p<0.0001 **d: p<0.05

n = No. of subjects and patients
All results are expressed in mean and standard deviation (SD)

Table 2. Correlation coefficient and significance in the patients group II

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

Discussion

Increased MDA and decreased TAC, Cu and Zn indicate that increased oxidative stress in group II

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 NS. The decreased total antioxidant status (TAS) 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. 2000). 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 MDA 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. 2005).

Disturbances in oxidant and antioxidant status were observed by many other studies, which is in agreement of our study (Warwick et al. 2000). The plasma ascorbate concentration was significantly lower ($p<0.001$) & decreased ratio of ascorbate: vit E ($p<0.0001$) in group of NS. Low density lipoprotein was protected from 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.

Consequences support of oxidative stress by increased level of HCY

We found that HCY level was >15umol/l in 10% adults with NS. Increased HCY level is related to endothelial dysfunction, some other study is in agreement with this concept (Majumdar et al. 2001). That HCY mediated impairment of endothelial dependent vasodilation were reversed by incubating HCY with nicotinamide (an inhibitor of peroxinitrate and nitrotyrosine) suggests a role of HCY in redox mediating endothelial dysfunction and nitrotyrosine formation, this is supported to oxidative stress and endothelial dysfunction by HCY. These findings are in agreement with the findings of (Gurusharan et al. 2001) where HCY was significantly correlated with serum creatinine ($r=0.58$; $p<0.01$) and calculated GFR ($R=-0.45$; $p<0.05$). Increased HCY level is due to renal failure for effective amino acids clearance. However, (Margret et al. 2001)

showed significantly lower HCY level in NS patients than non NS patients.

During the auto oxidation of HCY in plasma, reactive oxygen species are generated (Coppola et al. 2000). 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 HCY may be a key process in the pathogenesis of thrombosis in HHCY.

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 HCY. Endothelial dysfunction may cause proatherogenic effects associated with HHCY. Folicacid and Vit B_{12} deficiencies should be corrected by supplementation in HHCY. Increases in folate intake by dietary changes or fortification can also lower plasma HCY level in vitamin repleted subjects with normal plasma HCY level. In renal failure, folic acid treatment (1-5 mg/day) a-meliorate the plasma HCY level in most cases but HHCY persists in the majority of patients. Primary (fasting) HHCY can be treated with folic acid (0.5-5 mg/day) (Sydow et al. 2001; Van Guldener et al. 2001).

Decreased level of Cu & Zn related to HCY

Earlier study reported about the changes of Cu and Zn metabolism in NS (Stec et al. 1990). We observed serum HCY is negatively correlated to the Cu. 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 with the study where the level of Cu is decreased due to increased level of HCY in nephrotic syndrome patients. (Hughes et al. 2008). Low activity of GSH-Px, SOD and Zn concentration are associated with HHCY (Kenkeni et al. 2008). In some study, Zn supplements have shown to decrease Cu/Zn –SOD activity, primarily due to the antagonistic relationship between high Zn intakes and Cu absorption (Hughes et al. 2006).

Antioxidant therapy for nephrotic syndrome

The use of antioxidant therapy in NS opens a promising field in prevention of oxidative stress, related pathologies in renal patients. Vit C, Vit E and also combination of magnesium, zinc, Vit C & Vit E supplement effect on improvement of glomerular but not tubular renal function in type 2 diabetic patients in clinical investigation (Maryam et al. 2005). Significant reduction in lipid peroxide, homocysteine and improvement in vitamin C, total antioxidant capacity, copper, and zinc activity were observed after treatment of antioxidants and minerals with B-complex vitamins (Zincovit). Our study suggests a protective effect of antioxidants, Minerals & B-Complex vitamins against lipid peroxidation and hyperhomocyst(e)inemia.

Conclusion

We conclude that oxidative stress is enhanced in NS patients due to hyperhomocysteinemia and deficiency of Cu, Zn, which may contribute to the development of NS related complication with more frequency such as diabetic nephropathy, lupus nephritis, cardiovascular diseases, acute and chronic infection and many other complications. The study also reports beneficial effects of antioxidant, minerals, and B-complex vitamins on oxidative stress in NS patients. Treatment with zincovit improves renal function in these patients and may prolong need for NS patients. Long-term follow-up in a large number of patients would be necessary to confirm these results.

Acknowledgements

We sincerely thank to the M.G.M. Medical College, Indore (M.P.) and D.A.V. University for study support.

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