

Oxidant and Antioxidant Status in Childhood Nephrosis and its Relation to Hyperlipidemia

Mohammad Osman and Magdy Ibrahim'

Departments of Pediatrics and Biochemistry, Faculty of Medicine, Zagazig University, Egypt.

ABSTRACT

Background: Oxidative damage by free radicals has been implicated in a number of clinical disorders including renal injury. An abnormality in the oxidative system in patients with nephrotic syndrome (NS) has been reported.

Objective: To assess the oxidant status (red cell MDA) and antioxidant status (red cell GSH, SOD' and plasma vitamin E, copper, zinc) in relation to hyperlipidemii in children with active nephrotic syndrome (NS) and during remission.

Methods: This study was conducted on 65 children with NS; 35 with active NS (Group 1) & 30 with NS during remission (Group 2) and 20 healthy controls. Red cell glutathione (GSH), superoxide dismutase (SOD) and malonfialdehyde (MDA) concentrations as well as plasma levels of vitamin E, copper, zinc and plasma lipids (total cholesterol "TC", triglycerides "TG", high density lipoprotein cholesterol "HDL-C" and low density lipoprotein cholesterol "LDL-C") were measured in children with NS and in controls.

Results: SOD concentrations significantly increased in both group 1 & group 2 when compared to the controls. MDA concentrations significantly increased in both group 1 & group 2 compared to the controls. GSH concentrations along with plasma vitamin E concentrations significantly decreased in both group 1 & group 2 compared to controls. A significant decrease in plasma copper and zinc concentrations was observed in group 1 compared to controls. However, a non-significant decrease in plasma copper and zinc concentrations was observed in group 2 compared to controls. Plasma TC, TG, LDL-C concentrations significantly increased while plasma HDL-C significantly decreased in both group 1 & group 2 compared to controls. Significant +ve correlation was found between MDA and each of TC, TG, LDL-C, while significant -ve correlation existed between MDA and HDL-C. Significant -ve correlations existed between each of albumin, SOD, vitamin E, zinc, copper and each of TC, TG, LDL-C. Meanwhile, significant +ve correlations existed between HDL-C and each of albumin, SOD, vitamin E, zinc and copper.

Conclusion: Increased pro-oxidant status with compensatory changes in antioxidant concentrations occur in children with NS. These changes are linked to lipid disorders in NS patients and may be related to the pathogenesis of NS.

INTRODUCTION

The generation of reactive oxygen species (ROS) is a normal steady state process in cells, although uncontrolled production results in damage to cellular molecules⁽¹⁾. Oxidative damage by free radicals has been implicated in a number of

clinical disorders including renal injury⁽²⁾. An abnormality in the oxidative system in patients with nephrotic syndrome (NS) has been reported^{3,4)}. Dyslipidemia of NS is also known to be linked to oxidative reactions and atheroseierosisc⁵⁾. ROS promote cell injury by lipid peroxidation,

which disrupts the structural integrity of the tubular epithelial cells, and increase glomerular permeability to proteins along with alteration of the glomerular hemodynamics⁽⁶⁾. Therefore, a decrease in the antioxidants like erythrocyte superoxide dismutase (SOD), glutathione (GSH) and plasma ceruloplasmin and vitamin E may play a role in the susceptibility of these patients to the disease⁽⁷⁾. Direct assessment of ROS is not feasible because of the extremely short half-life of the free radicals⁽⁵⁾. Therefore, the oxidative activity must be measured indirectly by the levels of lipid membrane peroxidation product; malondialdehyde (MDA). Also, by measuring the total antioxidant capacity, albumin (which is an antioxidant protein and its reduced thiol moiety on cysteine 34 plays a direct role of antioxidant)^(8,9), serum zinc and copper levels⁽¹⁰⁾.

AIM OF THE WORK

The aim of the present study was to assess oxidant status (represented by erythrocyte MDA levels) and antioxidant status (represented by erythrocyte levels of GSH & SOD, and plasma levels of zinc, copper and vitamin E) in relation to hyperlipidemia (in terms of plasma levels of total cholesterol "TC", triglycerides "TG", high density lipoprotein cholesterol "HDL-C" and low density lipoprotein cholesterol "LDL-C") in children with active nephrotic syndrome and during remission of steroid sensitive nephrotic syndrome.

SUBJECTS AND METHODS

Study design:

This was a prospective study carried

out on 35 newly diagnosed patients with pediatric NS (Group 1). The patients were consecutively selected from the Nephrology Unit of Zagazig University Children's Hospital during the period from February 2008 to June 2010. They were 20 males and 15 females with an age range from 3-11 years. Patients were diagnosed to have PNS according to the criteria of the International Study of Kidney Disease in Children (ISKDC)⁽¹¹⁾. None of the children enrolled in the study showed evidence of acute infection or systemic diseases, and none had received albumin, blood or nonsteroidal anti-inflammatory drugs in the last 2 weeks prior to the study. All patients received standard oral corticosteroid induction therapy for 1 month. If the patients achieved remission, corticosteroids were tapered and withdrawn over a 2-month period. Of those 35 patients with NS, 30 children had complete remission after treatment with and withdrawal of corticosteroids (Group 2). Twenty healthy children of matched age and sex were included in the study as a control group. They were selected from children referred for preoperative evaluation before elective surgery (e.g. hernia, ocular surgery etc).

Sample collection and preparation:

Blood was collected into EDTA bottles from normal children and patients. The timing for sampling the blood from cases of active NS was just before the initiation of corticosteroid therapy when there was significant proteinuria and edema. Next samples were taken during remission, just after withdrawal of corticosteroid therapy.

The erythrocyte suspension was prepared according to the method of Beutler et

aL<". It was immediately centrifuged under refrigeration at 3000 g for 10 min. Plasma and the buffy coat were carefully removed and the separated cells were washed thrice with cold saline phosphate buffer, pH 7.4 (sodium phosphate buffer containing 0.15 mo/l NaCl). The erythrocytes were then suspended in an equal volume of physiological saline. Appropriately diluted hemolysates were then prepared from erythrocyte suspension by the addition of distilled water for the estimation of GSH, SOD and MDA. Plasma was used for estimation of TC, TG, HDL-C, LDL-C, albumin, zinc and copper by using commercially available kits from Lab kit diagnostics in semi automated auto-analyzer. GSH was estimated according to the method of Beutler et al.⁽¹²⁾. SOD was analyzed by the method of Beauchamp and Fridovich⁽¹³⁾. Plasma vitamin E was measured by the Emmerie--Engel reaction denoted by Bieri et al.⁽¹⁴⁾. MDA concentrations were determined according to the method of Jain et al.⁽¹⁵⁾. The hemoglobin content of the erythrocyte was estimated by the cyanmethemoglobin method⁽¹⁶⁾.

Statistical analysis.

Statistical analysis was done using the SPSS for windows version 11.0. Results were expressed as mean + standard deviation and comparison between the studied groups was done by student t-test, Chi-square and correlation coefficient. The difference between the groups was considered significant if the p-value was ≤ 0.05 .

RESULTS

The concentrations of GSH showed a highly significant decrease in group 1 (6.77

2.03 mg/g Hb) compared to the healthy controls (11.44 + 1.36 mg/g Hb), ($p \leq 0.01$). However, a significant decrease was observed in the GSH concentrations of group 2 (8.79 + 1.76 mg/g Hb) when compared to healthy controls (11.44 + 1.36 mg/g Hb), ($p \leq 0.01$). GSH concentrations showed an increase in group 2 (8.79 + 1.76 mg/g Hb) over those in group 1 (6.77 + 2.03 mg/g Hb), which was statistically significant ($p \leq 0.05$) (Table 1).

Significantly high concentrations of SOD were observed in both group 1 (16916.57 + 3483.31 U/g Hb) and group 2 (22099.97 + 3444.27 U/g Hb) compared to the healthy controls (6682.9 + 1716.22 U/g Hb), $p \leq 0.01$, respectively. SOD concentrations showed an increase in group 2 over those in group 1, which was statistically non-significant ($p > 0.05$) (Table 1).

Significantly high concentrations of MDA were observed in both group 1 (14.62 + 1.5 nM/g Hb) and group 2 (10.29 + 1.02 nM/g Hb) when compared to the healthy controls (7.88 + 2.29 nM/g Hb), ($p < 0.01$, $p < 0.05$) respectively. MDA concentrations showed an increase in group 1 over those in group 2, which was statistically significant ($p \leq 0.05$), (Table 1).

A highly significant decrease in plasma vitamin E concentrations was observed in both group 1 (3.37 + 0.96 mg/dl) and group 2 (4.98 + 0.79 mg/dl) compared to the healthy controls (9.55 + 1.2 mg/dl), ($p < 0.01$), respectively. However, an intergroup comparison showed a tendency of vitamin E concentrations to be higher in group 2 compared to group 1, with a significant difference between them, ($p < 0.05$) (Table 1).

A highly significant decrease in plasma zinc concentrations was observed in group 1 (79.97 ± 6.53 µg/dl) compared to the healthy controls (116.45 ± 5.9 µg/dl), ($p < 0.01$). However, a non-significant decrease was observed in plasma zinc concentrations of group 2 (110.4 ± 6.55 µg/dl) when compared to the healthy controls (116.45 ± 5.9 µg/dl), ($p > 0.05$). A highly significant trend of increase in plasma zinc concentrations was observed group 2 (110.4 ± 6.55 µg/dl) compared to group 1 (80.4 ± 6.6 µg/dl), ($p < 0.01$) (Table 1).

A highly significant decrease in plasma copper concentrations was observed in group 1 (81.63 ± 7.96 µg/dl) compared to the healthy controls (106.75 ± 5.91 µg/dl), ($p < 0.01$). However, a non-significant decrease was observed in plasma copper concentrations of group 2 (103.11 ± 6.17 µg/dl) compared to the healthy controls (106.75 ± 5.91 µg/dl), ($p > 0.05$). A highly significant trend of increase in plasma copper levels was observed group 2 (103.11 ± 6.17 µg/dl) compared to group 1 (81.63 ± 7.96 µg/dl), ($p < 0.01$) (Table 1).

Significantly high concentrations of TC were observed in both group 1 (274.92 ± 13.85 mg/dl) and group 2 (191.33 ± 14.08 mg/dl) compared to the healthy controls (147.72 ± 6.22 mg/dl), ($p < 0.01$) respectively. TC concentrations showed an increase in group 1 over those in group 2, which was statistically significant ($p < 0.05$), (Table 2). The concentrations of TG showed a highly significant increase in group 1 (169.04 ± 6.36 mg/dl) and group 2 (124.10 ± 6.38 mg/dl) when compared to the healthy controls (71.54 ± 3.74 mg/dl), ($p < 0.01$, respectively). TG concentrations

in group 1 were significantly higher than those in group 2, ($p < 0.05$) (Table 2).

The concentrations of HDL-C showed a highly significant decrease in group 1 (38.69 ± 2.24 mg/dl) compared to the healthy controls (51.83 ± 3.73 mg/dl), ($p < 0.01$). Meanwhile, a significant decrease was observed in HDL-C concentrations of group 2 (41.60 ± 3.72 mg/dl) compared to the healthy controls (51.83 ± 3.73 mg/dl), ($p < 0.05$). HDL-C concentrations showed an increase in group 2 (41.60 ± 3.72 mg/dl) over those in group 1 (38.69 ± 2.24 mg/dl), which was statistically significant ($p < 0.05$) (Table 2). Significantly high concentrations of LDL-C were observed in both group 1 (170.42 ± 7.26 mg/dl) and group 2 (125.93 ± 7.44 mg/dl) compared to the healthy controls (88.85 ± 6.76 mg/dl), ($p < 0.01$), respectively. LDL-C concentrations showed an increase in group 1 over those in group 2, which was statistically highly significant ($p < 0.01$), (Table 2).

Significant positive (+ve) correlation was found between MDA and each of TC, TG and LDL-C ($r = 0.556, 0.624, 0.623$; respectively). On the other hand, significant negative (-ve) correlation was found between MDA and HDL-C ($r = -0.556$), (Table 3). Significant -ve correlation was found between SOD and each of TC, TG, LDL-C ($r = -0.755, -0.691, -0.699$; respectively). Meanwhile, significant +ve correlation was found between SOD and HDL-C ($r = 0.874$) (Table 3).

Non-significant -ve correlation was found between GSH and each of TC, and LDL-C ($r = -0.136, -0.05$; respectively). On the other hand, non-significant +ve correlation was found between GSH and each

of TG and IID1, ¹C (r = 0.097, 0.129; respectively), (Table 3).

Significant -ve correlation was found between albumin and each of TC, TG, LDL-C (r = -0.839, -0.813, -0.846; respectively). Meanwhile, significant +ve correlation was found between albumin and HDL-C (r = 0.729), (Table 3).

Significant -ve correlation was found between vitamin E and each of TC, TG, LDL-C (r = -0.940, -0.828, -0.866; respectively). Meanwhile, significant +ve correlation was found between vitamin E and

HDL-C, (r = 0.879) (Table 3).

Significant -ve correlation was found between zinc and each of TC, TG, LDL-C (r = -0.558, -0.500, -0.551; respectively). Meanwhile, significant +ve correlation was found between zinc and HDL-C (r = 0.529) (Table 3).

Significant -ve correlation was found between copper and each of TC, TG, LDL-C (r = -0.638, -0.541, -0.587; respectively). Meanwhile, significant +ve correlation was found between copper and HDL-C (r = 0.637) (Table 3).

Table 1: Oxidants and antioxidants in studied cases.

<i>Parameter</i>	<i>Group 1</i>	<i>Group 2</i>	<i>Control</i>
<i>Albumin (mg/dl)</i>	1.98 ± 0.15 ^a	3.71 ± 0.27 ^b	4.14 ± 0.27 ^b
<i>GSH (mg/g Hb)</i>	6.77 ± 2.03 ^a	8.79 ± 1.76 ^b	11.44 ± 1.36 ^c
<i>SOD (U/g Hb)</i>	16916.57 ± 3483.31 ^a	22099.97 ± 3444.27 ^a	6682.9 ± 1716.22 ^b
<i>MDA (nM/g Hb)</i>	14.62 ± 1.50 ^a	10.29 ± 1.02 ^b	7.88 ± 2.29 ^c
<i>Vitamin E (mg/dl)</i>	3.37 ± 0.96 ^a	4.98 ± 0.79 ^b	9.55 ± 1.2 ^c
<i>Zinc (ug/dl)</i>	79.97 ± 6.53 ^a	110.4 ± 6.55 ^b	116.45 ± 5.9 ^b
<i>Copper (ug/dl)</i>	81.63 ± 7.96 ^a	103.11 ± 6.17 ^b	106.75 ± 5.91 ^b

aa, bb = p > 0.05 (non-significant),
GSH: glutathione,

ab, ac, bc = p < 0.05 (significant)
SOD: superoxide dismutase,

MDA: malondialdehyde.

Table 2: Plasma lipids in studied cases.

<i>Parameter</i>	<i>Group 1</i>	<i>Group 2</i>	<i>Control</i>
<i>TC (mg/dl)</i>	274.92 ± 13.85 ^a	191.33 ± 14.08 ^b	147.72 ± 6.22 ^c
<i>TG (mg/dl)</i>	59.04 ± 6.36 ^a	124.10 ± 6.38 ^b	71.54 ± 3.74 ^c
<i>HDL-C (mg/dl)</i>	38.69 ± 2.24 ^a	41.60 ± 3.72 ^b	51.83 ± 3.73 ^c
<i>LDL-C (mg/dl)</i>	170.42 ± 7.26 ^a	125.93 ± 7.44 ^b	88.85 ± 6.76 ^c

aa, bb = p > 0.05 (non-significant),

TC: total cholesterol,

HDL-C: high density lipoprotein cholesterol,

ab, ac, bc = p < 0.05 (significant)

TG: triglycerides,

LDL-C: low density lipoprotein cholesterol.

Table 3: Correlations of oxidants and antioxidants with plasma lipids in active NS.

	<i>TC</i>		<i>TG</i>		<i>HDL-C</i>		<i>LDL-C</i>	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
<i>MDA</i>	0.556	0.001	0.624	0.00	-0.556	0.001	0.623	0.00
<i>SOD (U/g Hb)</i>	-0.755	0.00	-0.691	0.00	0.874	0.00	-0.699	0.00
<i>GSH</i>	-0.136	0.437	0.097	0.578	0.129	0.459	-0.05	0.774
<i>Albumin</i>	-0.839	0.00	-0.813	0.00	0.729	0.00	-0.846	0.00
<i>Vitamin E</i>	-0.940	0.00	-0.828	0.00	0.879	0.00	-0.866	0.00
<i>Zinc</i>	-0.558	0.00	-0.500	0.002	0.529	0.001	-0.551	0.001
<i>Copper</i>	-0.638	0.00	-0.541	0.001	0.637	0.00	-0.587	0.00

p > 0.05 = non-significant,

p < 0.05 = significant

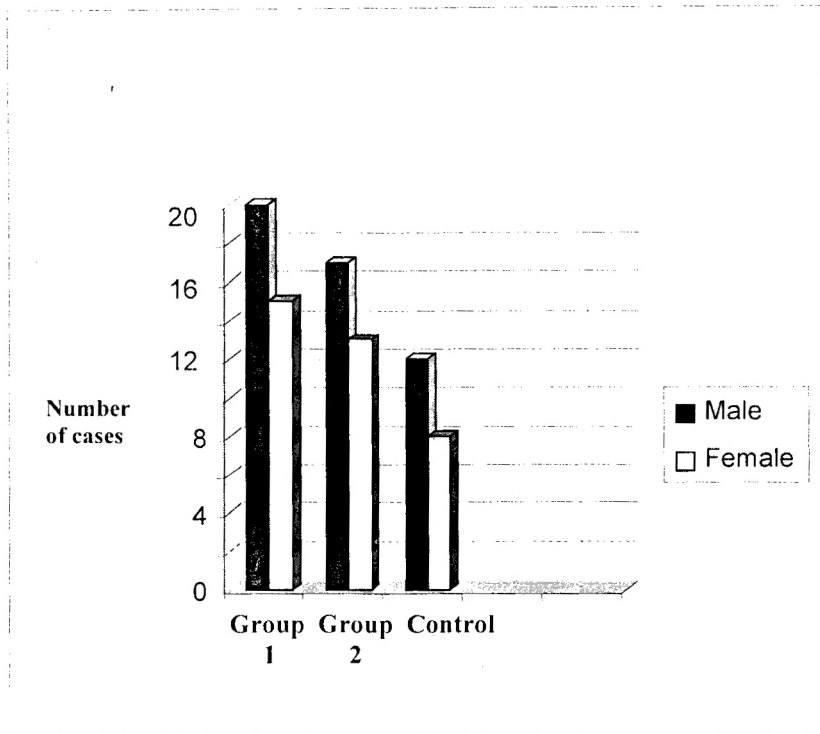


Fig. 1: Gender distribution of studied cases.

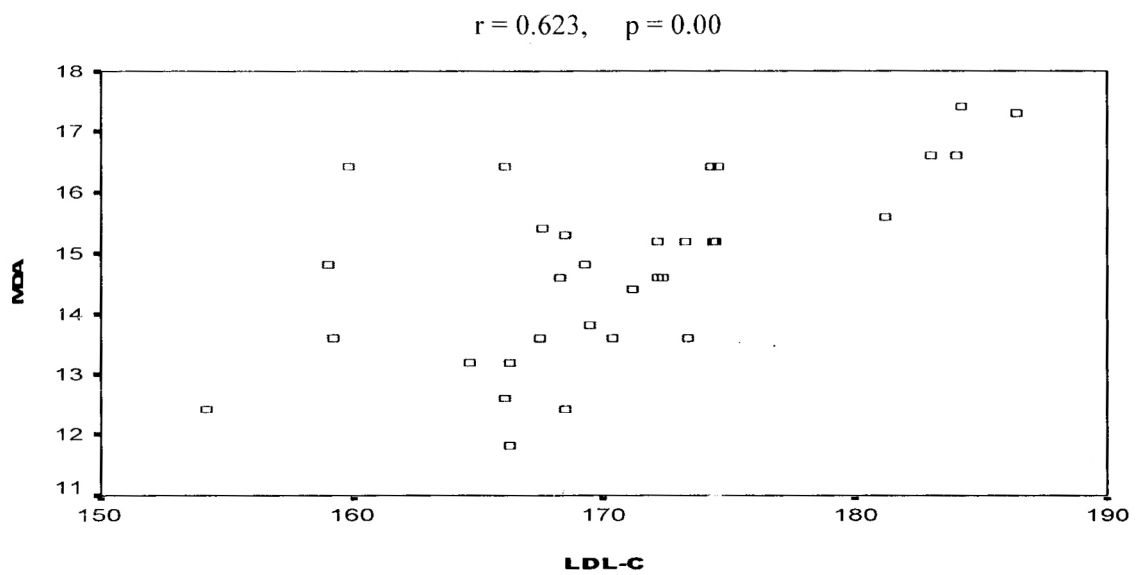


Fig. 2: Correlation between MDA and LDL-C.

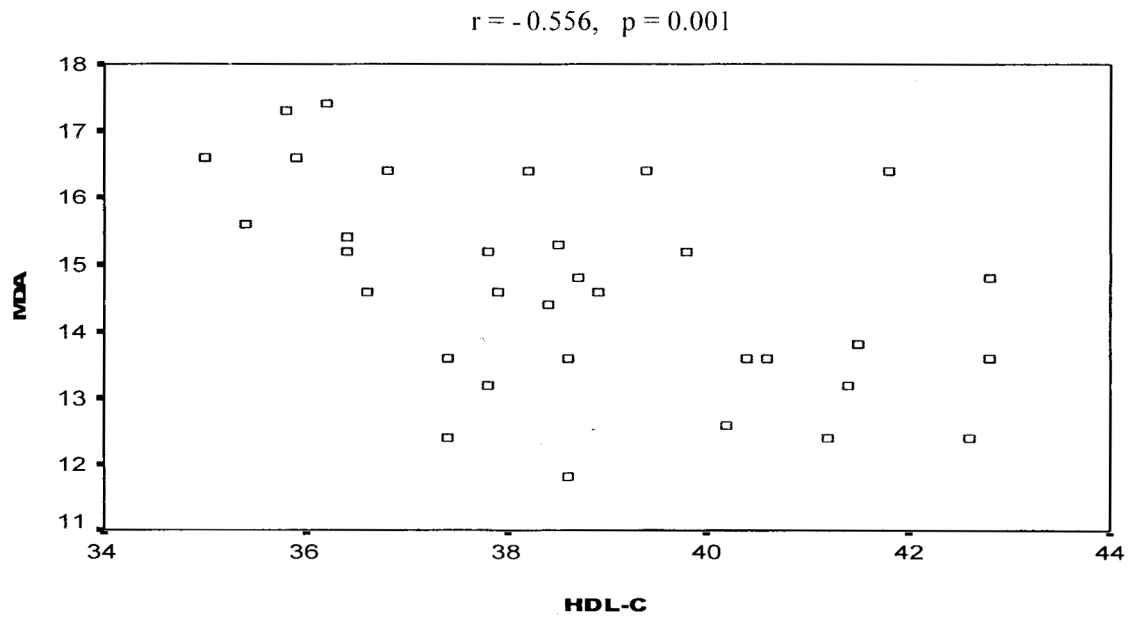


Fig. 3: Correlation between MDA and HDL-C.

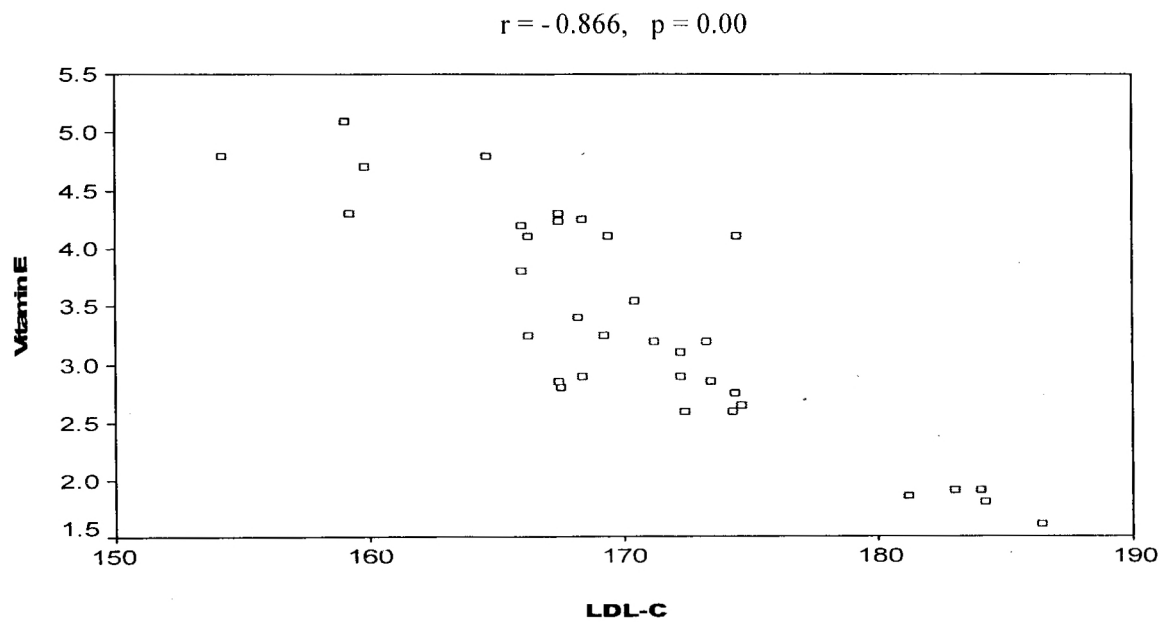


Fig. 4: Correlation between vitamin E and LDL-C.

$r = 0.879, p = 0.00$

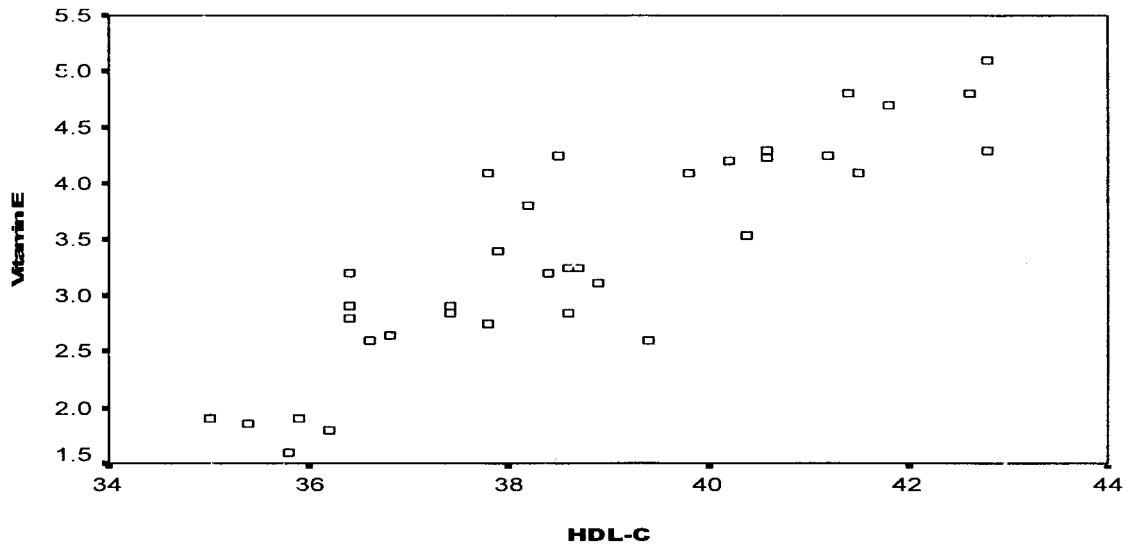


Fig. 5: Correlation between vitamin E and HDL-C.

$r = -0.846, p = 0.00$

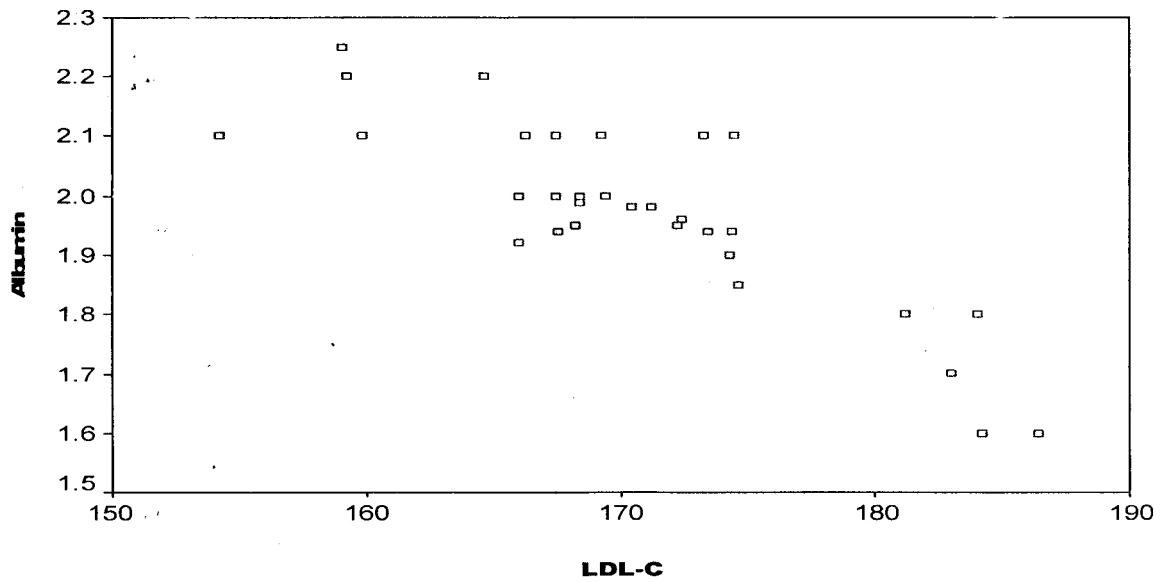


Fig. 6: Correlation between albumin and LDL-C.

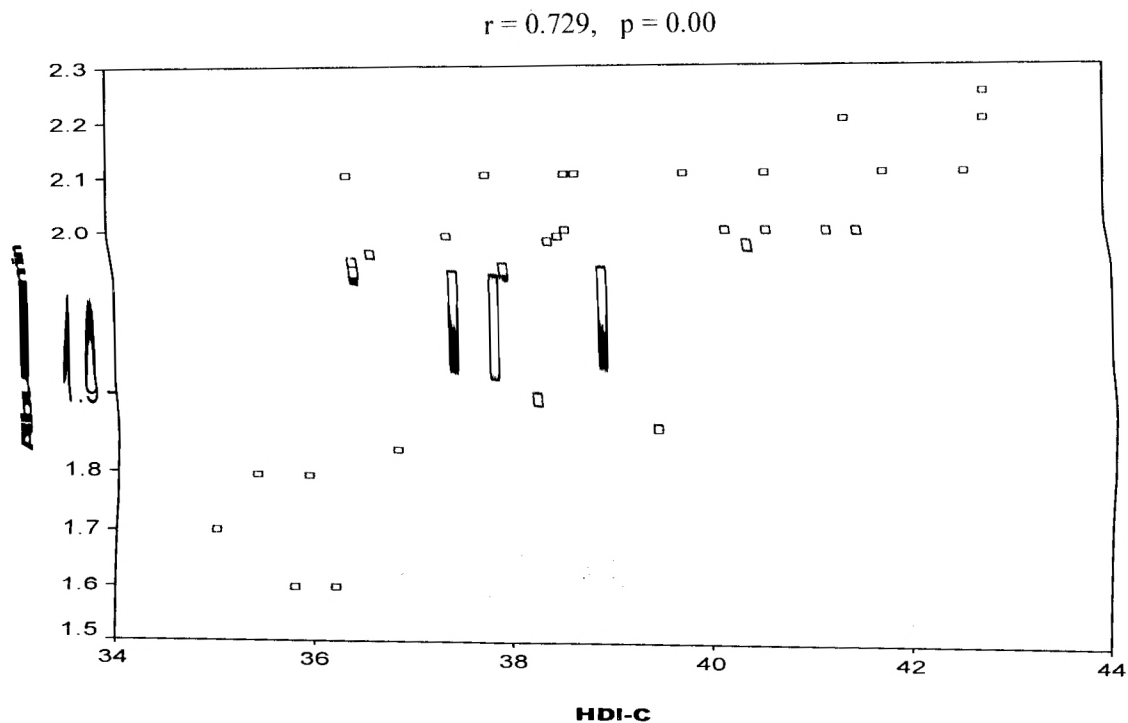


Fig. 7: Correlation between albumin and HDL-C.

DISCUSSION

An increase in free oxygen radicals and a derangement in the antioxidative system of patients with NS have been reported in many studies⁽¹⁷⁻¹⁹⁾. Antioxidant defense mechanisms involve both enzymatic and non-enzymatic strategies. Common antioxidants include the vitamins A, C and E, GSH, and the enzymes SOD, catalase, GSH peroxidase and GSH reductase. Other antioxidants include lipoic acid, mixed carotenoids, coenzyme Q10, several bioflavonoids, antioxidant minerals (copper, zinc, manganese and selenium), and the cofactors (folic acid, vitamins B1, B2, B6, B12). They work in synergy with each other and against different types of free radicals⁽²⁰⁾.

In the present study, the concentrations of erythrocyte GSH showed a significant decrease in children with NS compared to normal children; the decrease was more evident in cases of group 1 than those in group 2. GSH is a substrate for anti-oxidant

enzymes like GSH peroxidase, GSH-S-transferase and GSH reductase⁽²¹⁾. Reduced GSH is a major intracellular redox buffer that may approach levels up to $10 \text{ mO}^{\text{P}2\text{n}}$. GSH functions as a direct free-radical scavenger, as a cosubstrate for GSH peroxidase activity, and as a cofactor for many enzymes, and forms conjugates in endo- and xenobiotic reactions⁽²²⁾. The decrease in the concentrations may be due to the increased turnover of GSH in preventing oxidative damage in these cases⁽²¹⁾. Similar reports of lowered GSH concentrations in NS have been reported in other studies⁽²³⁻²⁵⁾ suggesting increased oxidative stress in nephrotic syndrome. Meanwhile, unaltered erythrocyte GSH concentrations in NS have been reported by Kinra et al⁽²⁶⁾.

In our study, the concentrations of erythrocyte SOD were significantly increased in group 1 and group 2 cases when compared to the healthy controls, more increase was evident in group 2 (NS in

remission). This may indicate a compensatory mechanism to cope up with increased pro-oxidant status in such cases. Similar observations have been reported by others^(24,27). However, other workers reported decreased SOD concentrations in NS^(23,28).

In the present study, a significant decrease in plasma vitamin E levels was observed in group 1 when compared to the healthy controls. The decrease in the concentrations may be due to increased turnover of vitamin E in preventing oxidative damage in these cases. Other workers have also reported decreased vitamin E concentrations in such cases^(24,26,28). A rising trend in plasma vitamin E concentrations was observed in group 2 which showed non-significant decrease compared to the control. This may indicate a compensatory mechanism to make up for increased pro-oxidant status in such cases. Vitamin E suppresses the propagation of lipid peroxidation, inhibits hydroperoxide formation; metal complexing agents such as penicillamine; bind transition metals involved in some reactions in lipid peroxidation and scavenges free radicals⁽²⁰⁾.

In our study, the concentrations of the lipid peroxidation product, erythrocyte MDA, showed a significant increase in group 1 when compared to the healthy controls. These results denote increased lipid peroxidation which exceeded the antioxidant capacity in such cases. MDA causes the cross linking and polymerization of membrane components and also reacts with DNA nitrogenous bases⁽¹⁾. Elevated plasma MDA levels in NS were reported to be strongly associated with

the severity of NS and renal injury²⁹⁾. Other workers^(24,26,28,30), reported similar observations where there has been an increase in the MDA concentrations in NS. On the other hand, unaltered MDA concentrations in children with NS have been reported by Rajani et al.⁽³¹⁾. They explained their results by the probability that, the compensatory increase in the concentrations of the antioxidants like SOD, resulted in unaltered MDA concentrations.

In our study, we also found that concentrations of Copper and Zinc were significantly decreased in group 1 when compared to the healthy controls. These findings are in agreement with the findings of previous studies⁽³²⁻³⁴⁾. Zinc deficiency was probably a consequence of reduced absorption of zinc in conjugation with excessive urinary loss⁽²⁶⁾. NS patients may be associated with excretion of ceruloplasmin; a protein which is normally not found in urine. Urinary copper loss is in direct proportion to the amount of proteinuria⁽²⁸⁾. Both ceruloplasmin and copper levels decrease in NS patients⁽²⁷⁾.

Peroxidation of lipid membrane raises the concentration of the MDA that results in lowering of the concentration of antioxidants because of consumption⁽⁵⁾. Albumin is a leading preventive but not a chain breaking antioxidant of serum⁽³⁴⁾. In the present study, significantly lower level of mean plasma albumin was observed in group 1 in comparison with those of the healthy controls. It is reported that even at very low concentration, albumin has a high antioxidant activity³⁴⁾.

Dyslipidemia is a contributing factor in the progression of initial glomerular injury

in NS⁽³³⁾. In the present study, there were significantly increased levels of TC, TG, LDL-C and significantly decreased levels of HDL-C in group 1 when compared to the healthy controls. Meanwhile, significant +ve correlation was found between MDA levels and each of TC, TG, LDL-C, while significant —ve correlation existed between MDA and HDL-C. This indicates that lipid peroxidation is higher with hyperlipidemia and HDL-C being an antioxidant inhibits lipid peroxidation. On the other hand, each of SOD, vitamin E, albumin, zinc and copper showed significant —ve correlations with TC, TG, LDL-C as well as significant +ve correlations with HDL-C. Increased levels of TC could be attributed to impaired metabolism of mevalonate by the nephrotic kidney. This allows a greater cholesterol availability that; coupled with an enhanced hydroxymethylglutaryl-CoA (HMO-CoA) reductase activity; leads to increased hepatic cholesterol synthesis and unbalanced lipid homeostasis⁽³³⁾. HDL-C is an effective antioxidant with the capacity to inhibit oxidative modification of LDL-C. HDL-C also possesses anti-inflammatory properties. These antioxidant and anti-inflammatory properties of HDL-C may be as important as its cholesterol efflux function in terms of protecting against the development of atherosclerosis⁽³⁸⁾.

In the present study, we observed normalization of plasma zinc, copper and albumin levels in group 2 after the use of

corticosteroid induction therapy. On the contrary, there was no normalization in plasma levels of TC, TG, LDL-C, HDL-C, MDA, vitamin E, GSH and SOD in group 2 despite the use of corticosteroids. These findings are in agreement with previous studies^{36,37}. Considering the results of our study as well as some other experimental studies, it is believed that steroids directly or indirectly impair the antioxidant reactions and lead to over-production of ROS. The use of anti-oxidant therapy in NS opens a promising field in prevention of oxidative stress-related pathologies in renal patients. Maryam et al.⁽³⁸⁾ reported that vitamins C, and E and also combinations of magnesium, zinc, vitamin C and E supplements were effective in improvement of glomerular but not tubular renal function in type 2 diabetic patients. The beneficial effects of antioxidants, minerals and B-complex vitamins on oxidative stress in NS were also reported by Dwivedi et al.⁽³⁹⁾.

Conclusions and recommendations: Oxidative stress and decreased antioxidant response are evident in steroid sensitive NS and are tightly linked to hyperlipidemia. Despite the use of corticosteroids and occurrence of clinical remission, no normalization of biochemical indices was observed. Regular lipid monitoring during follow up of NS patients is indicated to identify high risk patients. The use of antioxidant therapy in addition to corticosteroids may be beneficial in NS.

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