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Original article

Oxidant stress in primary nephrotic syndrome in relation to dyslipidemia

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ABSTRACT

Nephrotic Syndrome is a consequence of an imbalance between oxidants and antioxidants. The present study aimed to assess oxidants and antioxidants status in relation to dyslipidemia in adults Nephrotic Syndrome patients and during remission. The study dealt with 75 adults diagnosed to have primary Nephrotic Syndrome (PNS) and all were given standard oral corticosteroid induction therapy and 50 normal healthy adults were kept for control. Blood samples were analyzed for quantification of albumin, malondialdehyde (MDA) as an index of lipid per-oxidation ,Total cholesterol(TC), Triglycerides(TG), High density lipoprotein cholesterol (HDL-C), Low density lipoprotein Cholesterol(LDL-C),Total antioxidant capacity (TAC), Copper and Zinc from control and experimental patients. Significantly increased level of MDA, TC, and TG was noted while significantly decreased levels of HDL-C TAC, Zinc and Copper were noticed in the patients with Nephrotic Syndrome as compared to healthy control. However, after standard corticosteroids induction therapy significant reduction in MDA, TC, TG, were observed but no significant change in LDL-C was observed. Although improvement in TAC, HDL-C albumin, Zinc and Copper were observed after standard corticosteroids induction therapy.

1. Introduction

The Nephrotic Syndrome is one of the best known presentations of adult or pediatric kidney disease. The term describes the association of (heavy) proteinuria with peripheral edema, Hypoalbuminemia and hypercholesterolemia. Low TAC concentrations have been reported in the proteinuric phase of SSNS during the first episode [1]. An abnormality in oxidative system in patients with Nephrotic syndrome has been reported [2, 3] .Dyslipidemia of NS is also known to be linked to oxidative reactions and atherosclerosis [4]. Direct assessment of reactive oxygen species (ROS) is not feasible because of the extremely short half-life of the free radicals [4]. Therefore, the oxidative activity must be measured indirectly by the levels of lipid membranes per oxidation by-product; malondialdehyde (MDA). Also, by measuring the Total Antioxidant Capacity (TAC), albumin (which is an antioxidant protein and its reduced thiol moiety on cystein 34 plays direct role of antioxidant) [5][6], serum zinc & copper level[7]. The present study, aimed, was to assess oxidant (in terms of serum levels of MDA) and antioxidant status (in terms of serum levels of albumin, TAC, Zinc and Copper), in relation to dyslipidemia(in terms of serum levels of TC, LDL-C, HDL-C, TG,) in adult Nephrotic Syndrome patient and during remission phase of steroid sensitive Nephrotic syndrome (SSNS).

2. Materials and Methods

2.1 Study population-Human

Present study was conducted during January 2007 to December 2009 on Nephrotic Syndrome subjects who were outdoor patients of Nephrology Department of M.G.M. Medical College and private clinical of Indore (M.P.), India.

2.2 Test-Disease: Nephrotic Syndrome.

The present study was conducted on 75 adult Nephrotic syndrome patients. Patients were diagnosed to have primary Nephrotic syndrome according to the criteria of the International Study of Kidney Disease in Children (ISKDC) 1981.The patients suffering from other diseases which may lead to oxidative stress such as diabetes, inflammatory disease, cardiac disease, hepatic impairment and respiratory diseases or other systemic disease as well as smokers and alcoholics were excluded from the study.

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study. The study was approved by the ethical committee of the D.A.V.V.Indore.

2.3 Experimental design

The present study was conducted on 50 healthy adult control subjects and on 75 adults' Nephrotic syndrome patients. Studied subjects were divided in the following three groups.

A Control Group: In this group 50 normal healthy adults were included.
B Experimental Group 1: In this group 75 adult Nephrotic syndrome patients were included.
C Experimental Group 2: In this group 75 adult Nephrotic syndrome patients which were on remission after receiving standard oral corticosteroid induction therapy for one month were included.

2.4 Biochemical investigation

Fasting venous blood were drawn from all subjects. The blood was allowed to clot at room temperature and centrifuged at 5000 rpm for 10 minutes and then the serum was kept frozen at -70°C in aliquots until the time of assay of the parameters. The prospective study was carried out at Biochemistry laboratory of the Government Holkar Science College Indore(M.P.).

Biochemical parameters selected for present study included. Total Cholesterol, Triglyceride, HDL-Cholesterol Albumin, Zinc, and Copper were determined by using commercially available kit from Lab Kit diagnostics from Span in semi automated auto analyzer . LDL-Cholesterol level was calculated by using Friedewald's equation.

2.5 Measurement of serum TAC

Serum TAC was measured according to the method described by Koracevic *et al*. [8] in which the determination of Total antioxidant capacity is based on the reaction of Antioxidants in the sample with a defined amount of exogenously provided Hydrogen peroxide. The antioxidants in the sample eliminate a certain amount of the Hydrogen peroxide and the residual hydrogen peroxide is determined by an enzymatic reaction (the conversion of 3, 5 dichloro-2-hydroxy benzene sulphonate) produces colored product. Measure the absorbance at 532 nm wavelength.

2.6 Measurement of serum MDA

The colorimetric method described by Ohkawa *et al.* [9] was used to measure serum MDA level. The reaction of Thiobarbituric acid with MDA in an acidic medium at 95°C for 30 min produces pink colored thiobarbituric acid reactive product. The absorbance of the pink colored product was measured at 534 nm wavelength.

2.7 Statistical analysis

The SPSS software programmed ver.15 (SPSS, Chicago, IL) was used to analyze the data statistically. Laboratory characteristics of patients were expressed as mean ± standard deviation (SD).ANOVA was used to compare these data between patients and controls. P values <0.05 was considered to be significant.

3. Results

Results obtained were summarized in Table's 1-3.Table 1 shows Comparison of all diagnosed biochemical parameter in healthy control group and experimental group 1 with SSNS. Significantly increased mean serum level of MDA, TC, LDL-C, TG ($p<0.000$) and significantly decreased level of HDL-C ($p<0.001$) in group 1 were observed when compared to healthy control. In serum level of MDA overall 185.55 % increase was observed in group 1. In serum level of Total cholesterol overall 79.22 % increase was observed in group1. In serum level of triglyceride overall 195.15 % increase was observed in group 1. In serum level of LDL-C overall 100 % increase was observed in group 1. In serum level of HDL-C overall 8.99 % decrease was observed in group 1. Significantly decreased mean serum level of albumin, Zinc, Copper, TAC, were observed in group 1 when compared to healthy control ($p<0.000$). In serum level of Albumin overall 42.95 % decrease was Observed in group 1. In serum level of Zinc overall 21.47 % decrease was observed in group 1. In serum level of Copper overall 17.0 % decrease was observed in group 1. In serum level of TAC overall 32.98 % decrease was observed in group 1. In serum level TAC overall 32.98% decrease was observed

Table 2 shows comparison of all diagnosed biochemical parameter in healthy control group and experimental group 2 with SSNS. Significantly different mean serum level of MDA, TC, LDL-C, and TG ($p<0.000$) were observed in experimental group 2 and insignificant difference was observed in serum HDL-C level of experimental group 2 when compared to healthy control. In serum level of MDA overall 75.94 % difference was observed in group 2. In serum level of total cholesterol overall 58.40 % difference was observed in group 2. In serum level of triglyceride overall 57.45 % difference was observed in group 2. In serum level of LDL-C overall 87.34 % difference was observed in group 2. In Serum level of HDL-C overall 3.59 % difference was remained in group 2.Significantly decreased mean serum level of albumin and TAC, were observed in group 2 when compared to healthy control ($p<0.000$). In serum level of Albumin overall 20.55 % difference was observed in group 2. In serum level of TAC overall 13.91 % difference was observed in group 2. Insignificant difference was observed in serum level of Zinc and copper of experimental group 2 when compared to healthy control. In serum level of Copper overall 4.53 % difference was observed in group 2. In serum level of Zinc overall 0.72 % difference was observed in group 2.

` Table 3 shows comparison of all diagnosed biochemical parameter in experimental group 2 and group 1 with SSNS. Significantly decreased mean serum levels of MDA, TC, and TG in group 2 were observed when compared to group1 ($p<0.000$). Significant increased level of HDL-C in group 2 was observed when compared to group1 ($p<0.006$). In serum level of MDA overall 39.04% decrease was observed in group 2. In serum level of Total cholesterol overall 11.61 % decrease was observed in group2. In serum level of triglyceride overall 46.65 % decrease was observed in group 2. In serum level of LDL-C overall 6.32 % decrease was observed in group 2. In serum level of HDL-C overall 5.93 % increase was observed in group 2. No significant difference in serum LDL-C was observed in group 2 when compared to group1 ($p<0.077$). Significantly increased mean serum level of albumin, Zinc, Copper, TAC, were observed in group 2 when compared to group 1 ($p<0.000$). In serum level of Albumin overall 39.27 % increase was observed in group 2. In serum level of Zinc overall 26.41 % increase was observed in group 2. In serum level of Copper overall 15.18 % increase was observed in group 2. In serum level of TAC overall 28.46 % increase was observed in group 2.

Tables. Results obtained were summarized in Table's 1-3.

Table 1. Comparison of all diagnosed biochemical parameter in Control and group 1 with SSNS.

Parameter	Control	Group 1	Difference	% change	p-Value
Albumin(gms/dl)	4.33±0.33	2.47±0.57	1.86	42.95	0.000***
MDA (nmol/L)	2.91±0.67	8.40±2.18	5.49	185.55	0.000***
TAC (mmol/L)	1.94±0.16	1.30±0.20	0.64	32.98	0.000***
Zinc (µg/dl)	118.11±29.43	92.75±20.14	25.36	21.47	0.000***
Copper (µg/dl)	106.19±18.37	88.01±20.92	18.18	17.00	0.000***
TC (mg/dl)	170.68±23.87	305.90±63.00	135.22	79.22	0.000***
TG (mg/dl)	90.69±28.89	267.68±69.63	176.99	195.15	0.000***
HDL-C (mg/dl)	48.35±11.16	44.00±07.00	4.35	8.99	0.001***
LDL-C (mg/dl)	104.19±26.52	208.36±61.42	104.17	100.00	0.000***

*** Extremely significant, ** highly significant,

Table 2. Comparison of all diagnosed biochemical parameter in Control and group 2 with SSNS.

Parameter	Control	Group 2	Difference	% change	p-Value
Albumin(gms/dl)	4.33±0.33	3.44±0.48	0.89	20.55	0.000***
MDA (nmol/L)	2.91±0.67	5.12±1.98	2.21	75.94	0.000***
TAC (mmol/L)	1.94±0.16	1.67±0.24	0.27	13.91	0.000***
Zinc (µg/dl)	118.11±29.43	117.25±20.94	0.086	0.72	0.243NS
Copper (µg/dl)	106.19±18.37	101.37±17.43	4.82	4.53	0.055NS
TC (mg/dl)	170.68±23.87	270.37±44.26	99.69	58.40	0.000***
TG (mg/dl)	90.69±28.89	142.80±23.26	52.11	57.45	0.000***
HDL-C (mg/dl)	48.35±11.16	46.61±6.56	1.74	3.59	.175NS
LDL-C (mg/dl)	104.19±26.52	195.19±44.41	91.00	87.34	0.000***

*** Extremely significant, ** highly significant, NS- Insignificant

Table 3. Comparison of all diagnosed biochemical parameter in group 1 and group 2 with SSNS.

Parameter	Group 1	Group 2	Difference	% change	p-Value
Albumin(gms/dl)	2.47±0.57	3.44±0.48	0.97	39.27	0.000***
MDA (nmol/L)	8.40±2.18	5.12±1.98	3.28	39.04	0.000***
TAC (mmol/L)	1.30±0.20	1.67±0.24	0.37	28.46	0.000***
Zinc (µg/dl)	92.75±20.14	117.25±20.94	24.5	26.41	0.000***
Copper (µg/dl)	88.01±20.92	101.37±17.43	13.36	15.18	0.000***
TC (mg/dl)	305.90±63.00	270.37±44.26	35.53	11.61	0.000***
TG (mg/dl)	267.68±69.63	142.80±23.26	124.88	46.65	0.000***
HDL-C (mg/dl)	44.00±07.00	46.61±6.56	2.61	5.93	0.006***
LDL-C (mg/dl)	208.36±61.42	195.19±44.41	13.17	6.32	.077NS

*** Extremely significant, ** highly significant, NS- Insignificant

4. Discussion

Dyslipidemia is a contributory factor in the progression of initial glomerular injury in NS [10] .In the present study significantly increased mean serum level of TC , LDL-C , TG and significantly decreased level of HDL-C in group 1 was observed when compared to healthy control, although dyslipidemia is a common complication of NS. The increased level of serum TC could be attributed to impaired metabolism of mevalonate by the nephrotic kidney. This allows a greater cholesterol availability that coupled with an enhanced Hydroxy Methyl Glutaryl-CoA (HMG-CoA) reductase activity leads to increased hepatic cholesterol synthesis and unbalanced lipid homeostasis [10]. 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 development of atherosclerosis [11].

In our study we also found that serum MDA level was increased in group 1 as compared to healthy control and serum TAC, Copper and Zinc was significantly decreased in group1 as compared to healthy control. These findings are corroborating with the finding of previous studies [12, 13, and 10]. Elevated plasma MDA level in nephrotic syndrome is strongly associated with the severity of nephrotic syndrome and renal injury. [14] Zinc deficiency was probably a consequence of reduced absorption of Zinc in conjugation with excessive urinary loss [15]. Nephrotic Syndrome 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 [16]. Both ceruloplasmin and copper levels decrease in nephrotic syndrome patients [17]. Hypocupremia associated to Nephrotic syndrome is secondary to renal loss of copper proteins. Per-oxidation of lipid membrane raises the concentration of the MDA that results in lowering of the concentration of antioxidants because of consumption (4). Albumin is a leading preventive but not a chain breaking antioxidant of serum. In the present study, significantly lower level of mean serum albumin was observed in group 1 in comparison with those of the healthy control. It is reported that even at very low concentration, albumin has a high antioxidant activity [12]. Mean serum levels of TAC significantly decreased in SSNS adults. These decreased levels suggest depletion, possibly because of consumption for neutralizing excessive circulating oxidants [13].

In Present study we observed normalization of serum Zinc, Copper and HDL-C level in group 2 after the use of corticosteroid induction therapy. There was no normalization in serum level of TC, TG, LDL-C, MDA, and TAC in group 2 however, despite the use of corticosteroids. These findings are in agreement with previous study [18]. From our study and considering the results of some other experimental studies it is believed that steroids directly or indirectly impair the antioxidant reactions and lead to over production of reactive oxygen species (ROS).The use of antioxidant therapy in NS opens a promising field in prevention of oxidative stress related pathologies in renal patients. Vitamin C, and E and also combination magnesium, Zinc, Vitamin C and E supplements was effective in improvement of glomerular but not tubular renal function in type 2 diabetes patients [19].The beneficial effects of antioxidants, minerals and B-complex vitamins on oxidative stress in Nephrotic Syndrome patients was also reported by dwivedi *et al.* (7).

5. Conclusion

The findings of the present study display an increased oxidative stress and decreased antioxidant response in SSNS adults. Additionally, although there is clinical remission no normalization of the biochemical indices was observed despite the use of corticosteroids. Therefore, there might be a potential role for regular lipid monitoring during the follow-up of Nephrotic patients to identify high-risk patients, who should be evaluated as candidates for a lipid lowering therapy. Therefore, the present study recommends the combined use of steroid, antioxidanttherapy, and lipid lowering therapy in such adults to prevent development of NS related complications with more frequently and long term follow up in large number of patients would be necessary.

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