The Value of Estimating Paraoxonase Activity in Nephrotic Children

Saad Ahmed Mansour[1], Mayy Abd Alfattah Neemat-Allah[1], Amal Saeed El Shal[2], Shaymaa Sherif Abd El Aziz Ibrahim*[1]

[1]Pediatrics Department and [2]Biochemistry Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt

*Corresponding author: Shaymaa Sherif Abd El Aziz Ibrahim, Mobile: (+20)01014901316, Email: drshaymaasherif@gmail.com

ABSTRACT

Background: Nephrotic syndrome (NS) is a series of symptoms caused by kidney damage. This involves protein in the urine, low levels of blood albumin, elevated blood lipids, and high levels of swelling.

Objective: This study aimed to evaluate paraoxonase1 (PON1) activities in children with nephrotic syndrome before and after treatment and compare it with healthy control individuals.

Patients and Methods: A case control study was carried out at Pediatric Nephrology Unit and Outpatient Nephrology Clinics of Children Hospital at Zagazig University over a period of fifteen months from August 2017 to November 2018, included 40 children classified into three groups; group1: included 20 child of newly diagnosed nephrotic syndrome children; Group 2: comprised same nephrotic patients of group 1 in remission stage after one month of daily divided dose of steroid; group 3 included 20 children apparently healthy and matched with the previous groups in age and sex (control group).

Results: There was a high significant difference in the lipid profile among the three studied groups. There was a significant difference in serum PNO1 levels among three studied groups. There was a high significant decrease in serum PNO1 in group1 than control group. There was no significant difference in PNO1 serum levels between group 2 (remission) and control group.

Conclusion: PON1 activity of newly diagnosed cases is significantly decreased antioxidant ability to prevent lipid oxidation. Hyperlipidemia with diminished antioxidant potential due to reduced PON1 collectively predisposes NS patients to the possibility of atherosclerosis.

Keywords: Paraoxonase, Antioxidants, lipid profile; Nephrotic Syndrome.

INTRODUCTION

Abnormal lipid metabolism is typical in patients with kidney disease[(1)]. This effect is most pronounced in nephrotic syndrome (NS) where there is a marked rise in plasma lipid levels[(2)].The lipid profile seen in the NS is atherogenic and predisposes to premature coronary artery disease (CAD) [(3)]. Paraoxonase1 (PON1) is mainly synthesized in the liver[(4)]. Human serum PON1 is closely associated with apolipoprotein A1 in high density lipoprotein (HDL) and has the highest expression in the liver and blood. PON1 has been involved in the prevention of low density lipoprotein (LDL) lipid peroxidation and also degrades biologically active oxidized lipids in lipoprotein[(5)]. Oxidized LDL formation in the sub-endothelial space of the arterial wall is a crucial initial step in atherosclerosis. There is also an inverse relationship between the amount of oxidized lipid products and the activity of PON1. PON1 activity is decreased in subjects at high risk of developing atherosclerosis [(6)].

PON1 plays a vital function in the antioxidant system. Decreased paraoxonase levels reduces the antioxidant activity of high-density lipoprotein and induce glomerulosclerosis. Few studies have investigated paraoxonase activity and antioxidant status in NS[(4)].

AIM OF THE WORK

The present study aims to evaluate PON1 activities in children with nephrotic syndrome before and after treatment and comparing it with healthy control individuals. Also to study any correlation exists between PON1 activity and lipid profile.

PATIENTS AND METHODS

A case control study was carried out at Pediatric Nephrology Unit and Outpatient Nephrology Clinics of Children Hospital at Zagazig University over a period of fifteen months from August 2017 to November 2018. Forty children were included in this report. They were randomly divided into three groups**.**

Group 1: This group comprised 20 children of newly diagnosed nephrotic syndrome (Acute). The cases were assessed for the clinical, laboratory parameters at baseline. Each of our nephrotic patients had NS characters in the form of generalized edema, proteinuria > 40 mg/m^2/h, hypoalbuminemia (serum albumin < 2.5 g/dL) and hypercholesterolemia (serum cholesterol > 200 mg/dL).The age of patients ranged from 2-10 years (median, 6 years), both sexes were presented (65% male and 35% female). They were treated at Nephrology Unit of Pediatric Department of Children Hospital. All patients were steroid sensitive nephrotic syndrome

Received:10 /5 /2020
Accepted:19 /6 /2020

(SSNS) who enter remission in response to steroids therapy alone within 4 weeks.

Group 2: This group comprised same nephrotic patients of group (1) on remission (3 consecutive days of trace or negative protein on urinalysis testing**).** All the cases received oral prednisolone in a dose of 2 mg/kg/d (60 mg/m^2/d) for 4 wk and tapered to the same dose but single daily dose every other day at morning for 2-3 wk. In addition to steroid other drugs as antacid, ezapril, aspocid, calcium and one alpha were used. All cases were reassessed after 4 wk of starting the steroid for the clinical, laboratory parameters (after remission).

Group 3: Twenty healthy children of comparable age and gender were enrolled in study.

Ethical Clearance: Authorized informal consent was obtained from the patient parents to participate in the study. **The approval for the study was obtained from the Pediatrics and Biochemistry Departments of Zagazig University Hospitals after the approval of the Institutional Review Board (IRB).** The work was carried out for human studies in accordance with the World Medical Association's Code of Ethics (Helsinki Declaration).

Inclusion criteria: Cases of newly diagnosed NS. Age: 2 to 12 years. Sex: Both sexes were included. All patients were SSNS.

Exclusion criteria: Patients received intravenous methyl prednisolone. Patients received medication before admission. Familial hyperlipidemia. Children had microscopic hematuria. Children obtained some type of antioxidant medication. Patients with chronic renal failure (CRF) or other renal illness. Patients suffering from liver disease. The malnourished children. Patients who had severe infection.

All participants were subjected to complete history taking regarding***:*** Age and sex. Onset of disease, course and response to therapy. Initial symptoms of edema, ascites, effusion, scrotal edema, hypertension, respiratory distress, skin infection and gastroenteritis and medications.

Also they were subjected to clinical examination including***:*** Weight and height and body mass index, Site of edema and its severity which varies from patient to patient.

Regarding laboratory investigation, routine laboratory investigations of NS including: CBC, total protein, serum, albumin, ALT, ESR, C3, renal function tests including serum creatinine and urea and urine samples were collected for detection of proteinuria.

Principle of the method:

My BioSource Human PON1 activity assay kit is a solid phase ELISA (Enzyme-Linked Immunosorbent Assay) sandwich in vitro for the quantitative measurement of human PON1. This procedure uses a quantitative immunoassay enzyme sandwich technique. Antibody unique to PON was pre-coated on a microplate. Standards and samples were piped into tubes, and any PON present was bound by an immobilized antibody. After elimination of any unbound substances, a PON-specific biotin-conjugated antibody was applied to the wells. After washing, enthusiastic in conjugated horseradish peroxidase (HRP) was applied to the well. After washing to eliminate any unbound avidin-enzyme reagent, a substrate solution was applied to the well and the color was developed in proportion to the amount of PON bound in the initial stage. The color production was stopped and the color intensity was measured.

Standard: was prepared according to the instructions of the manufacturer.

Statistical analysis

Recorded data were analyzed using the statistical package for the social sciences, version 20.0 (SPSS Inc., Chicago, Illinois, USA). Quantitative data were expressed as mean± standard deviation (SD), median, and range. Qualitative data were expressed as frequency and percentage.

The following tests were done:

- Mann-Whitney test of significance was used when comparing between two means.
- Chi-square (x^2) test of significance was used in order to compare proportions between two qualitative parameters.
- The confidence interval was set to 95% and the margin of error accepted was set to 5%. The p-value was considered significant as the following:

- P-value <0.05 was considered significant.
- P-value <0.001 was considered as highly significant.
- P-value >0.05 was considered insignificant.

RESULTS

Table (1), showed Non-significant difference among the three studied groups as regard demographic data.

Table (1): Demographic data of nephrotic syndrome patients and healthy children:

	Nephrotic syndrome cases (N=20)		Healthy children (N=20)		P-value
	Mean ± SD		Mean ± SD		
Age (years)	6.35 ± 2.51		5.75 ± 2.936		>0.05
	N	**%**	**N**	**%**	
Sex:					
Male	13	65	10	50	>0.05
Female	7	35	10	50	

Table (2), showed that there was a significant difference in the lipid profile among the three studied groups.

Table (2): Lipid profile of studied groups.

	Group 1 (N=20)	Group 2 (N=20)	Control group (N=20)	P-value
	Mean ± SD	Mean ± SD	Mean ± SD	
Triglyceride (Normal 60-150 mg/dl)	246.8 ± 54.1	137.9 ± 8.8	60.5 ± 3.73	<0.001
Total cholesterol (Normal 125-250mg/dl)	318.2 ± 41.41	182.2 ± 9.3	153.75 ± 11.11	<0.001
HDL (Normal <40mg/dl)	32.45 ± 1.08	51 ± 3.77	70.65 ± 12.44	<0.001
LDL (Normal <170mg/dl)	196.05 ± 23.19	121.6 ± 8	92.5 ± 6.22	<0.001
VLDL (2-30 mg/dl)	48.6 ± 10.83	27.59 ± 6.76	12.13 ± 0.759	<0.001

Table (3), showed that there was a significant difference in serum PON1 levels among studied groups. A significant decrease of serum PON1 in acute and remission phases of NS. A highly significant decrease of serum PON1 in acute phase of NS than healthy children (group 3). No significant difference in serum PON1 between remission and control groups.

Table (3): Serum paraoxonase1 level in studied groups.

	Group 1 (N=20)	Group 2 (N=20)	Control (N=20)	-value
	Mean ± SD	Mean ± SD	Mean ± SD	
Serum PON1 levels (Normal 31.25-2000 mIU/ml)	8.76 ± 1.06	54.4 ± 1.4	62.9 ± 8	0.002
				0.004
				<0.001
				>0.05

Table (4), showed that there was a negative significant correlation between LDL and serum PON1 levels in acute patients of NS (Group 1). A negative non-significant correlation between LDL and serum PON1 levels in remission stage of NS (Group 2). A positive significant correlation between HDL and serum PON1 levels in group1. A positive non-significant correlation between HDL and serum PON1 levels in group 2.

Table (4): Association between PON1 levels and selected study parameters in acute and remission groups of NS.

	Group 1 (N=20)		Group 2 (N=20)	
	r*	P-value	r*	P-value
MI (kg/m^2)	-0.026	>0.05	0.065	>0.05
rotein/Creatinine ratio	-0.04	>0.05	-0.274	>0.05
erum urea (mg/dl)	0.05	>0.05	0.003	>0.05
erum creatinine (mg/dl)	-0.289	>0.05	-0.195	>0.05
erum albumin (mg/dl)	0.035	>0.05	-0.036	>0.05
rotein in 24 hours (Mg)	-0.127	>0.05	-0.247	>0.05
emoglobin level (mg/dl)	-0.011	>0.05	0.06	>0.05
3 (mg/dl)	0.252	>0.05	0.216	>0.05
SR (mm/hr)	0.057	>0.05	-0.355	>0.05
riglyceride (mg/dl)	-0.04	>0.05	-0.102	>0.05
otal cholesterol (mg/dl)	-0.143	>0.05	-0.004	>0.05
DL (mg/dl)	**-0.24**	**0.014**	-0.511	>0.05
LDL (mg/dl)	-0.311	>0.05	-0.102	>0.05
DL (mg/dl)	**0.361**	**0.048**	0.046	>0.05

r* Pearson correlation coefficient

DISCUSSION

Persistent long-term hyperlipidemia is nephrotoxic and leads to chronic progressive glomerular and tubulointerstitial injuries. LDL apheresis has been increasingly used in clinical practice for the treatment of kidney disease with focal segmental glomerulosclerosis (FSGS) nephrotic syndrome. Effective management of hyperlipidemia with HMG-CoA reductase inhibitors or LDL apheresis in drug-resistant NS patients can prevent the progression of renal disease and the resolution of NS symptoms in some patients[7].

The current study showed age of onset for SSNS cases ranged 2-10 years. This is in agreement with**, Rhuma *et al.* [8] who** reported that INS is a disease of pre-school aged children with peak age incidence of 2-3 years. Also**, Saraswathi *et al.* [9]** who reported that childhood NS can occur at any age but is most common between the ages of 1½ and 5 years. The initial presentation indicated that the age had a substantial effect on the incidence of disease distribution and that 70 per cent of MCNS patients were younger than 5 years of age; 20–30 per cent of adolescent nephrotic patients had MCNS[9].

Our study showed obvious male predominance (65% male and 35% female) which supported by **Rhuma *et al.*[8]** and **Ephraim *et al.*[10]** who reported INS affected males more than females. Current study showed no significant difference among studied groups as regard age and sex.

Current study showed a highly significant difference in the lipid profile among the three studied groups. Our subject in group 1 had a very significant increase in TG, TC, LDL, a very small decrease in HDL and no substantial difference in VLDL relative to group 2. Group 2 had a very substantial drop in HDL and a non-significant difference between TC and LDL relative to the control group. **El-Melegy *et al.* [3]** were in agreement with our study for TG, TC, LDL and HDL. Disagree with our findings; **Hu *et al.* [11]** who observed higher rates of HDL in nephrotic cases compared to controls, but did not agree with our findings; **Patil *et al.* [12]** who found no substantial differences in mean rates of HDL between group 1 and group 2, which was in accordance with the analysis by **Muls *et al.* [13]** who found normal levels of HDL in NS and also reported irregular distribution of HDL subtypes. There is an increase in HDL3 levels and a decrease in HDL2 as found by **Muls *et al.* [13].** Calculated together, they have normal values for total HDL which result in a faulty reverse cholesterol transport pathway. Hyperlipidemia and dyslipidemia in NS occur due to defects in lipoprotein metabolism, i.e. increased synthesis of VLDL, LDL, reduced catabolism of VLDL and LDL, and altered transport pathways of reverse cholesterol. The precise cause of these disruptions is not yet clear [14].

As for the key parameter in our PNO1 serum sample that hydrolyses oxidized lipids, there was a substantial difference in PON1 serum levels between the three classes. A very large drop in PON1 serum levels was observed in children with (Group1) acute nephrotic syndrome relative to the control group and a substantial difference was observed in children in group 1 relative to group 2 (NS recovery cases). We also observed that at remission (Group2), the PON1 level of serum increased and exceeded the normal control group level with no noticeable difference. Our findings were in line and endorsed by the previous study[4].

Our analysis found a significant negative correlation between LDL and PON1 and a significant positive correlation between HDL and PON1 in group 1. According to our research, previous studies showed a strong association between HDL and PON1 [4, 5].

Nonetheless, **Patil *et al.*** [12] disagreed with our findings, as they found no substantial association between HDL and PON1 in acute and remission cases of NS. The function of the PON1 serum is highly variable and its regulation is complex. Decreased PON1 may lead to a reduction in HDL antioxidant potential and may contribute to the accelerated development of glomerulosclerosis in patients with long-lasting NS. Reduced PON1 activity may also be the product of increased oxidative stress and/or lower HDL levels [12].

In the adult population sample, there was a negative association between PON1 and LDL, VLDL in vascular disease and control cases. PON1 was positively associated with HDL. Joint control of PON1 with lipoproteins varies in these two classes, indicating that PON1 activity predicts risk of atherosclerosis [15]. PON1 and lipid profile was different in acute cases relative to recovery cases in our research. However, the association was not statistically important, suggesting that other factors affected PON1 behavior more in cases than in controls. One such factor may be the co-regulation of PON1 activity with lipoprotein levels.

CONCLUSION

Newly diagnosed cases of NS showed a large rise in all lipids except HDL cholesterol. PON1 activity in newly diagnosed patients is substantially decreased suggesting a reduced antioxidant capacity to prevent lipid oxidation. Hyperlipidemia with diminished antioxidant potential due to reduced PON1 collectively predisposes NS patients to the possibility of atherosclerosis.

RECOMMENDATION

Large prospective epidemiological studies will be required for steroid-resistant nephrotic syndrome (SRNS) and frequent relapse and steroid dependent NS to determine PON1 level and effect of steroid on PON1 and lipid profile and probability of PON1 as a potential diagnostic tool.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Funding information: None declared.

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