

Paraoxonase Activity and Lipid Profile in Paediatric Nephrotic Syndrome: A Cross-sectional Study

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ABSTRACT

Introduction: Dyslipidaemia of Nephrotic Syndrome (NS) is known to be linked to oxidative reactions and atherosclerosis. Paraoxonase (PON1) has been implicated in the prevention of Low Density Lipoprotein (LDL) lipid peroxidation and also degrades biologically active oxidised lipids in lipoprotein.

Aim: The present study was taken up to assess PON1 levels in paediatric nephrotic syndrome and also to see if any correlation exists between lipid parameters and PON1.

Materials and Methods: This study consists of Group 1 with 40 cases of NS in the age group of 2-14 years and Group 2 with 40 age and sex matched healthy controls. Lipid profile and paraoxonase activity was measured in serum samples of both the groups.

Results: Statistical analysis by student's t-test showed that the mean levels of Total Cholesterol, Triglycerides, LDL, and VLDL were significantly increased in Group 1 when compared to Group 2 ($p < 0.001$). The mean levels of HDL were similar in both groups. The levels of PON1 were significantly lowered in Group 1 when compared to Group 2. Correlation studies showed no significant correlation between lipid profile and PON1.

Conclusion: Cases have atherosclerotic dyslipidaemia and significantly decreased PON1 activity. Decreased PON1 may lead to increased oxidation of LDL accelerating the process of atherosclerosis.

Keywords: Atheroprotective enzyme, Atherosclerosis, Dyslipidemia, Hyperlipidemia, Nephrotic syndrome

INTRODUCTION

Hyperlipidaemia has been recognized as a common finding in nephrotic patients since 1917. The lipid profile seen in Nephrotic Syndrome (NS) is atherogenic and it predisposes to premature Coronary Artery Disease (CAD) [1,2]. Hyperlipidaemia is not always connected with nephrotic disease activity and may sometimes persist for long time during remission, especially in frequently relapsing NS which worsens further prognosis [3].

Human Serum Paraoxonase (PON1) is tightly associated with apolipoprotein A1 in High Density Lipoprotein (HDL) and has highest activity in the liver and blood. PON1 has been implicated in the prevention of Low Density Lipoprotein (LDL) lipid peroxidation and also degrades biologically active oxidized lipids in lipoprotein [4,5]. Oxidized LDL formation in the sub endothelial space of arterial wall is a key initial step in atherosclerosis. Moreover, there is an inverse relationship between the level of oxidized lipid products and PON1 activity. There is reduced activity of PON1 in subjects who are at high risk of developing atherosclerosis [6,7]. NS in adulthood is associated with an increased risk of atherosclerosis and coronary heart disease [8]. HDL from PON1 knockout mice cannot prevent oxidation of LDL in a co-culture model (in mice liver cells and HepG2 Human Hepatoma cell lines) simulating the artery wall and their macrophages contain more oxidized lipid. The same is true in human population studies [9]. One prospective study has shown that reduced serum PON1 activity was an independent predictor of coronary disease [10]. Dyslipidaemia of NS is known to be linked to oxidative reactions. The oxidative reactions cause endothelial dysfunction, increased lipid oxidation. There is impaired activity of some antioxidant enzymes in the acute phase of idiopathic NS i.e., PON1, that may increase the risk of development of atherosclerosis [1].

It is important to know the antioxidant defense status i.e., PON1 activity in NS patients. Very few studies have investigated the levels PON1 activity and its relation to dyslipidaemia in paediatric NS in Indian population. There is dramatic improvement in the life

expectancy of children afflicted with NS over the last 15 years, so, the occurrence of dyslipidaemia with its associated morbidity is of particular concern.

AIM

Hence, the present study was taken up with the aim to study the derangement of serum lipids and PON1 activity in nephrotic syndrome and also to know if any correlation exists between the lipid parameters and PON1 activity.

MATERIALS AND METHODS

It was a hospital based cross-sectional comparative study. The study was conducted in Department of Biochemistry, Jawaharlal Nehru Medical College, Belgaum, over a period of twelve months from February 2010 to February 2011.

Study Participants and Groups

The study participants included two groups, Group 1 consisted of 40 cases of NS patients in the age group of 2 to 14 years of both sex and Group 2 consisted of 40 age and sex matched healthy controls.

Inclusion Criteria

Group 1 comprised of clinically, diagnostically confirmed cases of NS in active phase (both newly diagnosed and old cases with acute relapse), age 2 to 14 years of either sex admitted or attending the paediatric unit of KLES Dr Prabhakar Kore Hospital and Medical Research Centre, Belgaum. Group 2 consisted of age and sex matched healthy children who were selected from among those who came for routine immunization and follow up in the paediatric Out Patient Department (OPD).

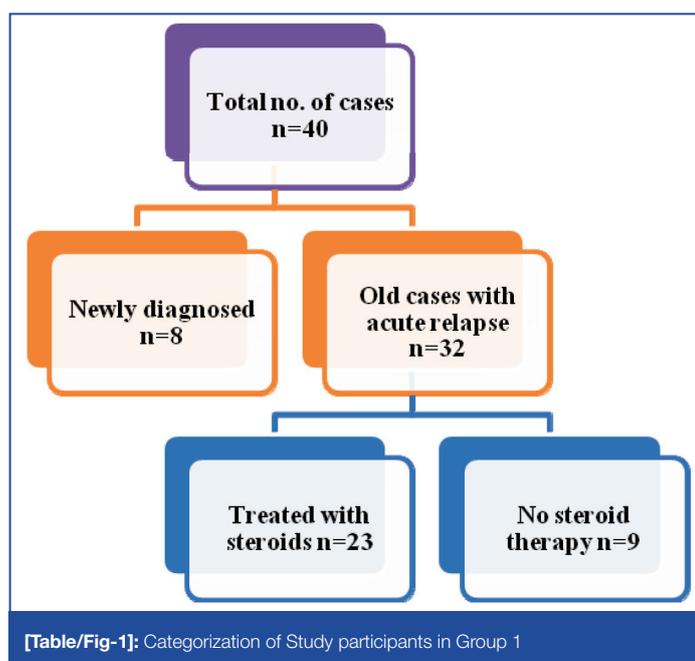
Exclusion Criteria

Familial hyperlipidaemia, patients with other renal pathology, patients with liver disease, and malnourished children were excluded from the study.

Ethical Approval

Ethical clearance has been obtained from the Institutional Ethical Clearance Committee, J. N. Medical College, Belgaum. The study was conducted in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. An informed consent was obtained from parents of patients and control group before collecting the blood sample.

Patient details, clinical details and lab results were noted in the proforma. We noted down the duration of illness, the number of relapses the child had in the past, treatment history - whether the child was given steroid therapy, compliance for the same was noted for each patient to look for its correlation with PON1 [Table/Fig-1].



Sample collection

5ml of venous blood was collected from the patients and controls under aseptic precautionary measures using disposable syringe in plain tubes. The blood was allowed to clot for 30 minutes. Serum was then separated by centrifugation for 10 minutes at 2000 rpm within one hour of collection and analysed within 24 hours or kept at -20°C if analysis was delayed.

METHOD OF ASSAY

The following parameters were analysed from the serum sample: Total cholesterol (TC) by CHOD/PAP Enzymatic method [11]; HDL-C by Polyethylene glycol precipitation method [12]; Triglycerides (TG) by GPO-PAP enzymatic method [13]. LDL, VLDL – calculated by Friedewald's formula [14].

The analysis was done spectrophotometrically using coral reagent kits from CREST BIOSYSTEMS, INDIA.

PON1 activity was tested by Spectrophotometric method using P-nitrophenyl acetate (from ACROS ORGANICS) as a substrate. The increase in the absorbance at 402 nm due to formation of p-nitrophenol was measured. 50µL serum was added to 2ml of Tris-HCl buffer (25mM, pH 7.4, 25°C) containing 1.0mM CaCl₂, and 5.5 mM/L p-nitrophenyl acetate. For non-enzymatic hydrolysis of p-nitrophenyl acetate, 2 ml of buffer was taken in spectrophotometric cuvette and 50 µl of substrate was added to it. The rate of change in absorbance (A) was monitored at 30, 60, 90, 120 and 150 seconds at 402 nm. The ΔA (rate of change in absorbance)/min was calculated. The difference in absorbance was calculated by deducting the ΔA obtained by non enzymatic hydrolysis of substrate from ΔA obtained in the presence of serum. The calculation was done omitting the first 30 seconds in order to

allow the reaction to reach a steady state. The molar absorptivity of 14000 M⁻¹cm⁻¹ at pH 7.4 for the substrate p-nitrophenyl acetate was used to calculate the PON1 activity in U/L [15].

STATISTICAL ANALYSIS

The data was analysed by SPSS software. Results were expressed as Mean ± SD. Student's t-test and chi square test were used for group wise comparisons. Relationship between variables was measured by Karl Pearson's correlation coefficient. A statistical significance was set at 5% level of significance (p<0.05).

RESULTS

Demographic parameters of both Group1 and Group 2 are shown in [Table/Fig-2]. There was no statistical difference in the sex distribution (p-value 0.178) and mean age between the two groups both the groups were comparable.

The biochemical parameters i.e., lipid profile and PON1 of both the groups are shown in [Table/Fig-3]. Statistical analysis by student's t-test showed that the mean levels of TC, TG, LDL, and VLDL were significantly increased in Group1 when compared to Group 2 (p <0.001). The mean levels of HDL were similar in both groups as shown in [Table/Fig-3]. The levels of PON1 are significantly lowered in Group 1 when compared to Group 2 (p<0.001).

We also looked for any effect of steroid therapy in the past on PON1 activity in the acute phase of NS. Comparative analysis of PON1 in old cases of NS with acute relapse among the subjects from group1, who were treated with steroids (n=23) and cases who did not receive steroid therapy in the past (n=09) showed that the levels of PON1 were significantly higher in cases who have not received steroid therapy in comparison to patients who received steroid therapy with a p-value of 0.033 as shown in [Table/Fig-4].

We found no significant correlation between lipid profile parameters- TC, TG, HDL, LDL, VLDL and PON1 (p>0.05) within the groups. But the relation was different in both the groups. Group 1 showed a negative correlation between non HDL lipids and PON1, whereas Group 2 showed a positive relation. HDL correlated positively with PON1 in Group 1, whereas the relation was negative in case of Group 2 as shown in [Table/Fig-5]. Additionally, when we looked for correlation between duration of illness and PON1 in Group 1, we found no significant correlation (p>0.05). [Table/Fig-6] shows the correlation based on the duration of illness.

Parameters	Group 1	Group 2
Age (in years ± SD)	6.2 ± 3.2	7.1 ± 2.4 [#]
Males (n)	25	19 [#]
Females(n)	15	21 [#]

[Table/Fig-2]: Comparison of demographic parameters in Group 1 and Group 2. [#]p>0.05 (not significant)

Parameters	Group 1 (n=40)	Group 2 (n=40)	p
Total cholesterol (mg/dL)	364 ± 94.6	168.0 ± 24.5**	<0.001
Triglycerides (mg/dL)	285.8 ± 88.2	129.4 ± 29.6**	<0.001
HDL (mg/dL)	50.7 ± 15.5	49.3 ± 6.0 [#]	>0.05
LDL (mg/dL)	256.7 ± 83.3	92.4 ± 24.1**	<0.001
VLDL (mg/dL)	57.8 ± 18.4	26.0 ± 5.8**	<0.001
PON1 (IU/L)	218.7 ± 35.3	302.3 ± 43.4**	<0.001

[Table/Fig-3]: Comparison of lipid profile and PON1 in Group1 and Group 2 (values expressed as mean ± SD). [#]p>0.05 (not significant), *p<0.05 (significant), **p<0.001 (highly significant)

Steroid therapy in the past	Received (n=23)	Not received (n=09)	P
PON1 (IU/L)± SD	212.0 ± 32.2	238.0 ± 20.5*	<0.05

[Table/Fig-4]: Comparison of PON1 with respect to steroid therapy received in the past among old cases in Group1. *p<0.05 (significant)

Parameters	PON1 in Group 1 (n=40) r	p	PON1 in Group 2 (n=40) r	p
Total cholesterol (mg/dL)	-0.22	0.16 [#]	0.08	0.62 [#]
Triglycerides (mg/dL)	-0.25	0.12 [#]	0.25	0.13 [#]
HDL (mg/dL)	0.10	0.53 [#]	-0.09	0.60 [#]
LDL (mg/dL)	-0.21	0.18 [#]	0.06	0.72 [#]
VLDL (mg/dL)	-0.26	0.10 [#]	0.26	0.11 [#]

[Table/Fig-5]: Correlation between lipid profile and PON1 in Group 1 and Group 2. [#]p>0.05 (not significant)

Variables	r	p
Duration of illness vs POM	-0.1802	0.2659 [#]

[Table/Fig-6]: Correlation between duration of illness (in years) and POM and in Group 1. [#]p>0.05 (not significant)

DISCUSSION

In agreement with previous studies [1,16-19] our subjects in Group 1 had significantly increased levels of TC, TG, LDL and VLDL in comparison to Group 2 ($p < 0.001$). In contrast to the study done by El-Melegy et al., who found lower levels of HDL in cases, we found no significant differences in the mean levels of HDL between Group 1 and Group 2 [1]. This is in accordance to study done by Muls et al., who found normal levels of HDL in NS [20]. They also reported abnormal distribution of HDL subtypes. Also, Hu P et al., observed higher levels of HDL in nephrotic cases compared to controls [19]. There is increased levels of HDL₃ and decreased HDL₂ [20-22] which measured together give normal values for total HDL, but result in defective reverse cholesterol transport pathway.

Hyperlipidaemia and dyslipidaemia in NS occurs due to abnormalities in lipoprotein metabolism i.e. increased synthesis of VLDL, LDL, decreased catabolism of VLDL and LDL and altered reverse cholesterol transport pathways. The exact cause for these disturbances is not yet clear [8].

Further, we also found in this study, the levels of enzyme PON1 that hydrolyses oxidized lipids, is decreased significantly in Group 1 when compared to Group 2 as shown in [Table/Fig-3]. This is in accordance to studies done by researchers Kniażewska MH and Ece et al., [16,23]. The phenotypic changes induced by hyperlipidaemia in microcirculation leads to platelet activation, lipid peroxidation and generation of radicals; the mediator being oxidative and nitrosative stresses which along with impaired activity of some antioxidant enzymes-PON1 in the acute phase of NS increase the risk of early atherosclerosis development [1,16,23-26]. Pathology studies have established that atherosclerotic disease begins in childhood and will progress faster if risk factors are present [27]. With hyperlipidaemia in NS, there is increased accumulation of lipoproteins in intima of blood vessels which due to changes induced by free radicals get oxidized to ox LDL and ingested by macrophages via scavenger receptors forming foam cells. Once ox LDL saturates macrophages and smooth muscle cells start accumulating lipids, the process of atherosclerosis becomes progressive and cannot be reversed back [27].

The formation of biologically active oxidized lipids can be prevented by the antioxidants present in LDL and the microenvironment where LDL is trapped. The decrease in PON1 levels leads to elevated levels of ox LDL, as the antioxidant activity of the HDL is reduced. This results in increased lipid peroxidation of lipoproteins and elevated levels of lipid hydroperoxides with decreased hydrolysis of ox LDL predisposing to the risk of atherosclerosis [25,28-30]. This relationship between PON1 activity and lipid peroxidation of lipoproteins is also confirmed by another study which established a negative correlation between HDL-PON1 activity and the levels of lipid hydroperoxides associated with HDL and LDL [30].

Additionally, in our study we found the levels of PON1 are significantly higher in active cases who have not received steroid therapy in the past in comparison to patients who received steroid therapy (among the old cases of Group 1) as seen in [Table/Fig-4]. This suggests that steroids will neither suppress the oxidative stress nor correct the lipid abnormalities in NS [23] and some other modality of treatment is required to prevent formation of oxidized lipids. The results of study done by researchers suggest that nephrotic children may have prolonged periods of hyperlipidaemia even after clinical remission [31]. The abnormal lipid profile is more likely seen in children with frequent relapses even during their remission [32]. Lipid abnormalities seen in NS constitutes atherogenic dyslipidaemia and can predispose to subclinical atherosclerosis in adulthood. This necessitates the coronary artery disease risk assessment and intervention in paediatric population [33].

We found no significant correlation between TC, TG, LDL, VLDL and PON1 in both Group 1 and Group 2. This is in accordance to a study done in healthy children in Slovakia who found no correlation between PON1 and lipid parameters [7]. We found no significant correlation between HDL and PON1 in Group 1 and Group 2. Some studies have shown a positive correlation between HDL and PON1 [4,23,34,35]. In a study in adult population there was a positive correlation between PON1 and LDL, VLDL in cases with vascular diseases and in controls PON1 correlated with HDL [36]. The relationship of PON1 and lipid profile is different in Group 1 versus Group 2 in our study though not statistically significant [Table/Fig-5], suggests other factors influenced PON1 activity more in cases than in controls. One of these factors may be the co-regulation of the PON1 activity with lipoprotein levels. The joint regulation of PON1 with lipoproteins differs in these two groups, suggesting PON1 activity predicts risk of atherosclerosis.

LIMITATIONS

HDL subtypes were not estimated separately. Ox LDL which would be a better marker to estimate the risk of atherosclerosis and correlate with PON1 activity was not measured due to cost reasons.

CONCLUSION

In total the cases of NS had significant increased levels of all lipids except for HDL cholesterol. Also PON1 activity is significantly decreased in these cases indicating decreased antioxidant capacity to prevent lipid oxidation. Steroid therapy in the past for relapsers did not improve lipid profile or PON1 levels in acute relapse. Hyperlipidaemia with decreased antioxidant capacity due to reduced PON1 collectively predisposes the NS patients to the risk of atherosclerosis. PON1 could be used as a marker along with Lipid profile to assess risk for atherosclerosis in NS.

Thus, it can be stated that Nephrotic syndrome is associated with dyslipidaemia and decreased levels of PON1, which may accelerate the development of atherosclerosis and thus requires monitoring of these parameters and finding new approaches towards treating them.

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