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# SERUM PROTEINS, INITIAL AND FOLLOW-UP LIPID PROFILE IN CHILDREN WITH NEPHROTIC SYNDROME

Krishnaswamy D***, Indumati.V**, Satishkumar D*, Vijay V*, Maharudra.Shekanawar*, Amareshwara.Maligi* & Rajeshwari.V*

***Professor & Head, **Associate Professor, *Assistant professors, Department of Biochemistry, VIMS, Bellary, Karnataka, India.

**ABSTRACT: Background & Objectives:** It has been noted that certain factors like diet, malnutrition, genetic traits etc., are known to alter the frequency and severity of lipid pattern. The Indian patient has a different dietary, constitutional and genetic background. Hence, we undertook a study to determine the spectrum of lipid abnormalities in children with nephrotic syndrome. An attempt was also made to correlate the degree of proteinuria and hypoproteinemia, with the rise in serum lipid values in cases of nephrotic syndrome.

**Methods:** Twenty cases of Nephrotic Syndrome, 7 age and sex matched controls were studied. The samples were analysed for Protein profile and Lipid Profile. Lipid profile was measured 8-10 days after treatment of Nephrotic syndrome with initial levels measured within 24 hours of admission to the hospital.

**Results:** There was a significant increase in Total cholesterol, LDLC, VLDL, Non-HDLC, serum phospholipids and triglycerides levels in Nephrotic syndrome patients when compared to normal controls ($P<0.0001$). There was significant decrease in Total protein, serum albumin and HDL-C in Nephrotic patients when compared to Controls. There was a significant difference between the initial and follow-up Lipid profile levels in these patients ($p <0.001$).

**Interpretation & Conclusion:** Our study concludes that, in nephrotic syndrome, there is generalized hyperlipidemia (except HDL) and hypoalbuminemia. The serum cholesterol level in first episode nephrotic syndrome reaches normal at the end of steroid therapy. Hence there is a rationale for treatment.

**Key words:** Lipid profile, Nephrotic syndrome in children, Non-HDL Cholesterol

## INTRODUCTION

Nephrotic syndrome is a kidney disease with proteinuria, hypoalbuminemia, and edema. Nephrotic range proteinuria is 3 grams per day or more. On a single, "spot" urine collection, it is 2 grams of protein per gram of urine creatinine. There are many specific causes of nephrotic syndrome. These include kidney diseases such as minimal-change nephropathy, focal glomerulosclerosis, and membranous nephropathy. Nephrotic syndrome can also result from systemic diseases that affect other organs in addition to the kidneys, such as diabetes, amyloidosis, and lupus erythematosus.

Hyperlipidemia has been recognized as a common finding in nephrotic patients since 1917, when hypercholesterolemia was described as a feature of nephrotic syndrome1. Although pathophysiological aspects of hyperlipidemia have not been completely identified, hypoalbuminemia, increased lipoprotein synthesis and decreased lipoprotein lipase activity are described by various workers2. Some degree of correlation between lipids and serum albumin has been suggested by Thomas et al.3 and between lipidemia and oedema by Peters et al.4. Generally when oedema regresses, lipid level falls but in some case it may continue to persist even after the oedema has disappeared.

Hyperlipidemia is usually observed during the active phase of the disease and disappears with resolution of proteinuria. However, it may persist in some cases, leading to increased risk of atherosclerosis in later life. Hence, close monitoring of lipid levels during remission of nephrotic syndrome is necessary to select high-risk patients[5].

Lipoproteins play an important role in the transport of plasma lipids; their increase or alteration in various fractions may be responsible for hypercholesterolemia, in nephrotic syndrome2. There is increased total cholesterol, LDL cholesterol, VLDL cholesterol and triglycerides and normal or low HDL cholesterol6. However, in Indian children, the degree of hyperlipidemia is not high as in western children5, 7. More recently it has been expressed that hyerlipidemia may contribute to renal injury8. And experimental studies demonstrated that reduction of plasma lipid levels slows progression of glomerular and tubulointerstitial disease9.

A great deal of evidence is now available to show that the incidence of Nephrotic Syndrome varies from place to place due to changes in food habits, climate, type of work and ethnic origin. It has also been noted that certain factors like diet, malnutrition, genetic traits etc., are known to alter the frequency and severity of lipid pattern. The Indian patient has a different dietary, constitutional and genetic background. Hence, we undertook a study to determine the spectrum of lipid abnormalities in children with nephrotic syndrome. An attempt was also made to correlate the degree of proteinuria and hypoproteinemia, with the rise in serum lipid values in cases of nephrotic syndrome.

## MATERIALS AND METHODS

Twenty cases of Nephrotic Syndrome admitted in paediatric department of Chaluvamba hospital, Mysore, Karnataka, 7 age and sex matched controls were studied. The Nephrotic cases were selected according to the criteria proposed by International study of kidney diseases in children(ISKDC) , that is children with oedema, Proteinuria(more than 3 gm in 24 hrs/1.73 m2 ) , hypoproteinemia(serum albumin less than 2.5 gm/dl), hyperlipidemia[10](M.Phadke, Oct 1990).

Exclusion Criteria: Children with liver disorders, oedema due to Kwashiorkor, oedema due to CCF and Children suffering from kidney diseases other than nephrotic syndrome.

The samples were analysed for Protein profile (Serum Total protein, serum albumin, serum globulin, A:G ratio, urinary proteins, Blood urea & serum creatinine), Lipid Profile(Total cholesterol, HDL-C, LDLC, VLDL, Non-HDLC, serum phospholipids and triglycerides). Lipid profile was measured 8-10 days after treatment of Nephrotic syndrome with initial levels measured within 24 hours of admission to the hospital.

Serum Protein was estimated by modified Lowry's method[11], Serum Albumin was estimated by Biuret method [12], Urinary Proteins were estimated by Esbach's albuminometer.

Serum Urea was estimated by Diacetly Monoxime method[13], Serum Creatinine was estimated by Jaffe's Method[13] , Serum Phospholipids were estimated by Ammonium Ferrothocyanate Method[14] , Serum Triglycerides were estimated by Acetyl Acetone Method [15] , Serum Total Cholesterol was estimated by $FeSO_4$ –acetic acid method[16] , Serum HDL Cholesterol was estimated by Heparin/$Mn^{++}$ precipitation method[13] , VLDL Cholesterol was calculated using Friedewald formula: VLDL Chol(mg/dl)= Triglyceride X 0.2., Non-HDL Cholesterol was calculated as Total Cholesterol- HDL Cholesterol.

Informed consent was taken from patients and healthy individuals. The study was approved by the Ethical & Research Committee of the institution on the use of human subjects in the Research. 2 ml fasting venous blood was collected in a plain bulb under aseptic precautions from patients with Nephrotic Syndrome and healthy controls. Serum was separated after half an hour and protein profile was carried out immediately and Lipid assay within 12 hours.

One way ANOVA followed by Bonferroni multiple comparison test, paired and unpaired students's 't', correlation coefficient was employed for the statistical analysis of the data to compare the groups.

## RESULTS

A total number of twenty patients suffering from nephrotic syndrome were studied. Age ranged from one and half years to twelve years. Mean age being 5.85 years. Twelve patients were between 1-4 yrs, 5 between 5-9 yrs and 3 patients between 10-12 years. Thirteen were male and 7 were female children. All 20 patients were fresh cases of nephrotic syndrome with no history of any relapse. Four patients were from rural areas and sixteen from urban area. Only one patient was of lower middle class and remaining 19 were from lower income group. The results of various parameters at the time of admission and at the time of discharge are shown in Table 1 and Table 2.

**Table 1: Protein Profile in controls and in nephrotic syndrome patients at the time of admission and at the time of discharge.**

| Protein Profile | Controls | Nephrotic syndrome (At Admission) | Nephrotic syndrome (At Discharge) |
|---|---|---|---|
| **Serum Total Protein(g/dl)** | 7.41 ± 0.35 | 4.08 ± 0.63* | 6.74 ± 0.57# |
| **Serum Albumin(g/dl)** | 4.17 ± 0.23 | 1.17 ± 0.29* | 4.13 ± 0.83# |
| **Serum globulin(g/dl)** | 3.20 ± 0.22 | 2.37 ± 0.54 | 2.67 ± 0.46# |
| **Urinary Protein(gm/24 hrs)** | Nil | 3.28 ± 0.71* | Nil |
| **Blood Urea(mg/dl)** | 26.71 ± 2.36 | 34.85 ± 11.38 | 27.00 ± 4.11# |
| **Serum Creatinine(mg/dl)** | 0.62 ± 0.149 | 1.08 ± 0.56 | 0.74 ± 0.16# |

All values are expressed as Mean ± SD; * P <0.01 when compared to controls; # P<0.01 when compared to the values at the time of admission.

**Table 2: Lipid Profile in controls and in nephrotic syndrome patients at the time of admission and at the time of discharge.**

| Lipid Profile | Controls | Nephrotic syndrome (At Admission) | Nephrotic syndrome (At Discharge) |
|---|---|---|---|
| **Serum Total Cholesterol(mg/dl)** | 150 ± 9.86 | 377 ± 91* | 151 ± 24# |
| **Serum Triglycerides(mg/dl)** | 112.7 ± 17.2 | 424.0 ± 14.3* | 127.0 ± 39.0# |
| **Serum Phospholipids(mg/dl)** | 203 ± 12.46 | 368 ± 101* | 205 ± 28# |
| **LDL Cholesterol(mg/dl)** | 48.3 ± 4.96 | 243 ± 2.00* | 78.8 ± 26.5# |
| **VLDL Cholesterol(mg/dl)** | 51.85 ± 5.18 | 85.5 ± 29.7* | 25.3 ± 7.90# |
| **HDL Cholesterol(mg/dl)** | 50.57 ± 3.99 | 48.1 ± 8.5 | 47.6 ± 8.8 |
| **Non-HDL Cholesterol(mg/dl)** | 99.4 ±5.87 | 328.47 ± 89.6* | 103±16.38# |

All values are expressed as Mean ± SD; * P <0.01 when compared to controls; # P<0.01 when compared to the values at the time of admission.

On admission serum protein levels were significantly low (P < 0.02) compared with normal controls. After treatment the values are significantly increased (P<0.001) and the percentage increase was 65.2%. Compared to controls the serum albumin levels were significantly low (p<0.001) in Nephrotic syndrome patients at the time of admission. The percentage decrease was 58.9%. At the time of discharge the serum albumin increased significantly (P < 0.001). The percentage increase was 141.5%. On admission there was no significant difference in the serum globulin levels between controls and nephrotic patients. But on the verge of discharge values showed slight significant increase (P< 0.05) with a 12.6% increase. In 17 out of 20 cases the A:G ratio is reversed. In 3 patients ratio is less than 1:1. After treatment 19 out of 20 patients showed an A: G ratio which is nearly normal. Only in one case the ratio remained at 1:1. There is no significant elevation of blood urea and serum creatinine levels although there is an apparent increase at the time of admission, which significantly decreased at the time of discharge ( P< 0.001).

On admission Serum Phospholipids, Serum Triglycerides, serum Total Cholesterol, LDL Cholesterol, VLDL Cholesterol and Non-HDL Cholesterol levels were significantly high ($P < 0.001$) compared with normal controls with a percentage increase of 81.3%, 277.6%, 150%, 403%, 64.9% and 330.45% respectively. After treatment the values are significantly decreased ($P<0.001$) and the percentage decrease was 44.3%, 70.05%, 59.9%, 67.6%, 70.4% and 31.4% respectively.

## DISCUSSION

The studies on hyperlipidemia in nephrotic syndrome emphasize the temporal and causal relationship between hypoalbuminemia and hyperlipidemia. Gheradi et al have shown in their study that VLDL and IDL increased exponentially as the serum albumin concentration fell, where as LDL increased more slowly but linearly[17]. It has been proposed that the altered protein binding of free fatty acids in nephrotic plasma facilitated uptake of free fatty acids by the liver, thus leading to an increased hepatic triglyceride synthetic rate [18]. When the concentration of albumin falls, an increased proportion of free fatty acids and other amphipathic lipids normally bound to albumin may be bound instead to VLDL[19] This would have at least two effects on lipoprotein metabolism. First these compounds may affect the interaction of triglyceride rich lipoproteins with lipoprotein lipase. And second, the concentration of unbound free fatty acids may increase. These unbound free fatty acids may inhibit lipoprotein lipase, thus further reducing the rate of VLDL triglyceride clearance.

The plasma enzyme, lecithin cholesterol Acyl transferase (LCAT) is a likely modulator of plasma triglyceride pathway. Cohen et al[20] examined patients, with proteinuria for serum LCAT activity and has shown that there is diminished LCAT activity in patients with proteinuria, which appeared to be caused by substrate or acceptor limitation. Compensatory synthesis of hepatic protein leading to an increased VLDL secretion rate and diminished VLDL clearance due to diminished availability of fatty acids acceptors, have both been suggested as the role of albumin in the dyslipoproteinemia of nephrotic syndrome.

In nephrotic syndrome, elevation of VLDL and IDL is paralleled by a fall of HDL.[6] HDL is important in the catabolism of both chylomicrons and VLDL. So a deficiency of HDL may play a role in accumulation of VLDL and IDL. HDL Cholesterol in nephrotic syndrome may be normal or low. But the ratio of LDL cholesterol to HDL cholesterol is generally increased.[6,]

More recent data suggests that measurement of Non -HDL Cholesterol level (Calculated as Total Cholesterol minus HDL Cholesterol) could be more representative of all atherogenic, apolipoprotein (apo) B containing lipoproteins –LDL,VLDL,IDL and Lipoprotein(a). Although apolipoprotein B can be assessed directly, measurement of Non-HDL Cholesterol is more practical, reliable, inexpensive and can be considered as a surrogate marker for apolipoprotein B in routine clinical practice[21]. Hence Non-HDL cholesterol can be included in Lipid Profile. The normal Non-HDL Cholesterol level is < 130mg/dl.

### Relation between Albumin and Serum Lipids in Nephrotic Syndrome

In our study, we observed an inverse correlation between albumin and cholesterol. When albumin was too low (range 1.0 – 1.5 gm%), the serum cholesterol was very high (mean = 417.33mg%). Whereas when albumin was between 1.5 – 2.0 gm%, the mean cholesterol was 364.46 mg%. However the correlation is not statistically significant ($P> 0.01$). We observed an inverse relation between serum albumin(range 1.0 – 1.5 gm%) and Triglycerides and it was statistically significant ($P< 0.005$). There was an inverse relation between serum albumin and VLDL cholesterol and it was statistically significant ($P<0.005$). We also observed an inverse relation between serum albumin(range 1.0 – 1.5 gm%) and Phospholipids but it was not statistically significant ($P > 0.1$). Heymann et al[22], found no correlation between the developmental of hyperlipidemia and hypoalbuminemia and postulated that the severity of hyperlipidemia is related to the amount of nephrotic kidney tissue present.

In the present study, we observed a direct relation between serum albumin and HDL Cholesterol. When serum albumin was too low (1.0-1.5gm%),the HDL Cholesterol was also low(mean=42.13mg%)where as when albumin was between 1.5-2.0gm%,the mean HDL Cholesterol was 45.27mg% but the correlation was not statistically significant(P>0.01).Mallik et al[23] also had similar observations.. Falaschi F et al[24] observed patients with nephrotic range proteinuria (> or=3.5 gm/24 hrs) had a significantly higher carotid initima media wall thickness than did those without (p<0.02) patients with nephrotic range proteinuria. In patients of nephrotic syndrome the severity of proteinuria correlated positively with serum cholesterol (r = + 0.60) and with serum triglyceride (r = + 0.58).

We noticed that the degree of lipid increase was not that high as reported by Western workers. Milne[25] reported that the total cholesterol in nephrotic syndrome may be higher than 1000 mg%. In our study the mean total cholesterol was 377 ± 91mg% and highest value was 662 mg%. Dyaneshwar. D.K, in his dissertation submitted to Rajiv Gandhi University of Health sciences, Bangalore in 2006 observed that the mean total cholesterol was 422.61mg% and highest value was 676 mg%. Thus we observed low serum lipids in Indian children.

Querfeld[27], suggested that, there is rationale for treatment, since dyslipidemia may contribute to the development of atherosclerosis and the progression of chronic renal failure. However, the benefits of treatment with lipid lowering drugs have not been proven. Short term studies in adults with nephrotic syndrome have documented safety and efficacy of lipid-lowering drugs, including 'Statins', Fabric acids, fish oil and probucol. Statins are the most effective, resulting in a decrease of total cholesterol levels by about 30-40%. Prospective controlled studies in children evaluating efficacy and safety and lipid lowering drugs are needed.

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