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Urinary lipid quantitation in nephrotic syndrome

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ABSTRACT

Nephrotic syndrome is characterized by proteinuria, lipiduria, hypoalbuminemia, and edema. Studies determining the levels of urinary cholesterol and urinary triglycerides are very few. Study was carried out in 44 primary (group I), 54 secondary (groupII) cases of nephrotic syndrome and 40 healthy controls for group I cases and 30 for group II cases. Urinary cholesterol, urinary triglycerides, serum albumin, creatinine and fasting lipid profiles were estimated. There was significant increase in urinary cholesterol and urinary triglycerides excretion in group I cases compared to group II ($p<0.001$). There was significant rise in lipid parameters in cases compared to controls ($p<0.05$). Correlation was observed between urinary total protein and urinary total cholesterol ($r=0.936$ $p<0.001$, $r=0.599$ $p< 0.001$), urinary total triglycerides ($r=0.456$ $p<0.001$, $r =0.440$ $p<0.001$). Negative correlation was observed between serum albumin and urinary total cholesterol in group I cases ($r=-0.474$ $p<0.001$). In conclusion we found significantly increased lipiduria in primary nephrotic syndrome compared to secondary nephrotic syndrome.

KEYWORDS

Nephrotic syndrome;
Lipiduria; proteinuria;
Lipoproteins;
Hypercholesterolemia;
Chloroform: methanol.

INTRODUCTION

The association between lipemic serum and coagulable urine has been recognized for over 150 years and by the early part of century the occurrence of hypercholesterolemia, lipidiuria and hyperlipidemia in nephrotic syndrome was established. Later, serum lipoproteins were studied in nephrotic syndrome patients[1]. Nephrotic hyperlipidemia is characterized by elevated serum cholesterol and triglyceride levels, which are almost entirely due to increase in very low density lipoproteins and low density lipoprotein fractions[2]. Lipiduria in nephrotic syndrome mainly consists of cholesterol, triglycerides and high density cholesterol. High density lipoprotien particles which are slightly larger than albumin are excreted in urine, where as low density lipoproteins, which are much larger, are not excreted[3].

According to previous study mean cholesterol ex-

cretion in nephrotic urine is 0.8mg/dl[4], but finding was based on small sample size (n=5).

There are very few reports regarding the quantity of cholesterol and triglyceride excretion in nephrotic urine, this is due to fact that the methods available for estimation of urinary lipids were very laborious, time consuming and sparse. Most of the methods require whole 24 hour sample of urine in large aliquots, and needs processing like dialysis, concentration and lipid extraction steps. None of previous studies reported regarding the quantiation of urinary cholesterol and triglycerides in primary and secondary nephrotic subjects. In the present study we have quantitated urinary cholesterol and triglycerides in both primary and secondary nephrotic syndrome patients and compared among them.

MATERIALS & METHODS

Samples

The study was carried out on 44 primary (group I) and 54 secondary nephrotic syndrome patients (group II) aged between 3-75 years. Fourty age and sex matched healthy controls both for primary and secondary nephrotic syndromes were used. In both groups the patients were with proteinuria>100mg/dl and creatinine clearance of >35ml/min/1.73m^2 were selected for the study. All the patients were recruited from Kasturba Medical College Hospital, Manipal, India, who came with symptoms and signs of nephrotic syndrome. Diagnosis of underlying renal lesion was made on clinical grounds and confirmed by appropriate laboratory tests and renal biopsy. Ethical clearance was obtained from institutional review committee and informed consent was obtained from the subjects involved in the study.

Twenty-four hour urine was collected from these patients with hydrochloric acid as preservative. The urinary samples were stored at 4 degree centigrade until analysis and after centrifugation the clear urine samples that are free of any sediment were processed for the determination of protein, cholesterol, triglycerides. Fasting blood samples were also collected from these patients and serum was separated subsequently and processed for determination of fasting lipid profile, serum albumin, total protein, serum creatinine.

Methods

All the glassware, apparatus used for the study were washed initially with detergent solution and rinsed with distilled water and dried in hot air oven. Cholesterol and triglyceride enzymatic kits were obtained from Pointe Scientific Inc. USA. Special reagents were obtained from Sigma Chemicals. All other chemicals used for the study were of analytical grade. Fasting lipid profile was estimated by enzymatic method using automated analyzer, Hitachi model 912. Total cholesterol estimation was done by cholesterol oxidase method; high density lipoprotein was estimated by same method after precipitating the low density lipoproteins, very low density lipoprotein and chylomicrons [5]. Triglycerides were estimated by enzymatic mixture containing lipoprotein lipase, glycerol kinase and glycerol-3-phosphate oxidase and peroxidase[6]. The levels of serum total protein and albumin were measured using automated analyzer, the albumin concentration was determined by its ability to bind with bromocresol green (BCG), an anionic dye to form blue green colored complex[7,8]. Biuret method was done for estimation of serum total protein using bovine serum albumin as standard[9]. Jaffe's method was used for serum creatinine estimation by automated analyzer[10]. Urinary total protein was measured by Biuret method using bovine serum albumin as standard[11].

Determination of urinary cholesterol and triglycerides were done by precipitating the 24 hour clear urine sample in aliquots and precipitated lipids were extracted into chloroform using (1:1vol/vol) chloroform: methanol mixture. Chloroform layer containing urinary lipids were evaporated under gentle stream of nitrogen gas. Reconstituted cholesterol oxidase and triglyceride reagent were added to separate tube containing precipitate of urinary lipids obtained by similar and simultaneous processing as mentioned above. After 10 mins of incubation the color developed was read at 540 nm (12). For all the estimations, calibration curve was prepared using appropriate standards and using the calibration curve values were calculated for deciliter of serum and urine. All the above estimations are carried out with appropriate blank solutions. All the spectral analysis was made at 25 degree C using Genesys 10 UV single beam spectrophotometer equipped with one cm quartz cuvette.

Full Paper

Statistical analysis

Statistical analysis was done using statistical package for social sciences (SPSS) version 10. The results were expressed as mean ± SD. A p value <0.05 was considered statistically significant. Unpaired student t test was used to compare mean values. Pearson correlation was applied to correlate between the parameters.

RESULTS

There was significant increase in urinary total cholesterol and urinary total triglyceride excretion in group I cases compared to group II ($p<0.001$). Significant increase in serum total cholesterol and triglycerides ($p<0.001$, $p<0.001$) was observed in group I cases and also in group II cases ($p<0.001$, $p<0.05$) compared to their respective controls. Correlation was observed between urinary total protein and urinary total cholesterol in group I and group II cases ($r=0.936$ $p<0.001$, $r=0.599$ $p<0.001$) respectively. Urinary total protein was also correlated with urinary total triglyceride in group I and group II cases ($r=0.456$ $p<0.001$, $r=0.440$ $p<0.001$) respectively. A significant negative correlation was observed between serum albumin and urinary total cholesterol ($r=-0.474$ $p<0.001$) in group I cases (Figure 1). Mean ±SD for all the parameters and patient characteristics were depicted in TABLE 1.

TABLE 1: Patient characterstics and Mean ± SD of all serum and urinary parameters

	Group I (Primary)		Group II (Secondary)	
	Controls (10)	Cases(44)	Controls(30)	Cases (54)
AGE	7 ± 4	10 ± 7	52 ± 17	58 ± 19
SEX(M+F)	6 + 4	25 + 19	20 + 10	34 + 20
TC(mg/dl)	155 ± 25	338±184**	175 ± 29	206 ± 84*
TG(mg/dl)	110 ± 15	301± 185**	136 ± 34	182 ± 83*
HDL (mg/dl)	45 ± 8	42 ± 5	40 ± 6	35 ± 5
LDL (mg/dl)	88±14	226± 105**	118 ±16	145 ± 62*
TP(g/dl)	6.8 ± 0.5	5.3 ± 1.6**	7.3 ± 0.3	6.1± 0.6**
ALB(g/dl)	4.3 ± 0.4	2.1 ± 0.6**	4.3 ± 0.3	2.9±0.6**
CREAT(mg/dl)	0.7 ± 0.3	0.8 ± 0.3	0.9 ± 0.3	1.2 ± 0.3
UTP(g/day)	-	4.6 ± 0.6*	-	3.1 ± 0.1
UCH(mg/day)	-	12 ± 5.2**	-	3.3 ± 1.6
UTG (mg/day)	-	7.5 ± 3.4*	-	3.4 ± 1.6

**** p< 0.001, *p< 0.05; TC-Total Cholesterol; TG- Triglycerides; HDL-High Density Lipoprotein; LDL-Low Density Lipoprotein; TP- Total Serum Protein; ALB-Serum Albumin; CREAT-Serum Creatinine; UCH- Urinary Cholesterol; UTG- Urinary Triglycerides; M+F- Male+Female**

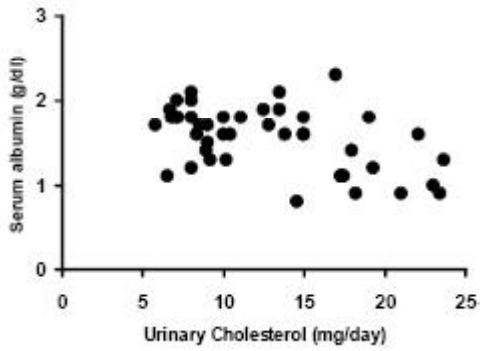

Figure 1: Correlation graph between Urinary cholesterol and Serum albumin in primary nephrotic syndrome patients

DISCUSSION AND CONCLUSION

Association of heavy proteinuria along with hyperlipidemia is regarded as integral feature of nephrotic syndrome. Characteristically, total plasma cholesterol and triglycerides levels are elevated. In agreement with previous studies, we observed elevated levels of serum cholesterol and triglycerides in both primary and secondary nephrotic subjects compared to controls. The mechanisms underlying these abnormalities are multifactorial, involving both increased rates of hepatic synthesis and defective clearance and catabolism of circulating particles[13]. Though the hyperlipidemia reverts to normal once the proteinuria is normalized, but considered as most common cause of cardiovascular risk factor in children and adults. In adults nephrotic hyperlipidemia has got 11 fold increases in total death rate due to coronary event[14].

The hypoalbuminemia in the nephrotic syndrome is contributed by increased loss of albumin in the urine. In agreement with previous studies, serum concentrations of cholesterol correlated with renal clearance of albumin. Serum albumin negatively correlated with urinary cholesterol in primary nephrotic patients (Figure 1). It is due to fact that hypoalbuminemia stimulates albumin synthesis and also other hepatic proteins like very low density lipoprotein which are rich in cholesterol which are filtered through the glomerulus[14]. In our study 80% primary nephrotics were of minimal change disease which is characterized by altered membrane permeability and loss of negatively charged albumin and very low density lipoproteins which are rich in cholesterol. This finding is in accordance with Klahr *et .al*, who

demonstrated that the amount of glomerular permeability correlated with the urinary total cholesterol excretion[15]. Several studies shown that hyperlipidemia in the nephrotic syndrome can attribute to progression of renal damage in children as well as adults and also the effectiveness of lipid lowering drugs in preventing the progression[16].

Human urine normally contains very small amounts of lipids. However in nephrotic syndrome urinary excretion of cholesterol, cholesterol esters, triglycerides, free fatty acids and phospholipids is considerably increased. In our study we observed increased excretion of urinary lipids in both type nephrotic subjects but degree of lipiduria is more marked in primary compared to secondary nephrotic subjects and showed good correlation with degree of proteinuria. This may be due to fact that many of urinary lipids originate from plasma and also the hyperlipidemia is more severe in primary nephrotics [16]. The finding of positive correlation between urinary total cholesterol and urinary total protein would be compatible with enhanced glomerular filtration of plasma lipoproteins as the cause of lipiduria in the nephrotic syndrome[17]. Urinary total triglyceride was well correlated with urinary total protein only in both primary and secondary nephrotic syndrome, it may due to high triglyceridemia has rapid triglyceride turnover which is not seen in mild to moderate triglyceride elevation[14].

According to previous studies the values of mean urinary cholesterol excretion and the values varies from one study to other. Klahr et al showed mean concentrations of urinary cholesterol in 17 nephrotic patients was 34mg/L and Martin et al have shown 35 mg/L with anisotropic droplets and 8.7mg/dl in urine without anisotropic droplets[15]. In our study the mean cholesterol excretion in primary was 12.8 mg/day and 3.3 mg/day in secondary nephrotics. Our results were very much in concordance with that of Martin et al and we used the clear urine sample devoid of cellular debris[4]. We obtained mean urinary total triglycerides values of 7.5mg/day and 3.5mg/day in primary and secondary nephrotics respectively.

In conclusion, hyperlipidemia and lipidiuria of primary is more marked compared to secondary nephrotic syndrome and correlated well with proteinuria. The mean urinary total cholesterol and urinary total triglyceride excretion in large study group which constitutes both primary and secondary nephrotic syndrome.

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