Spot Urine Albumin Creatinine Ratio- A Reliable And More Convenient Test For Diagnosis Of Nephrotic Syndrome In Children

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Abstract: Aim and objective - *Nephrotic syndrome is a nonspecific disorder in which the kidneys are damaged, causing them to leak large amounts of protein (proteinuria at least 3.5 grams per day per $1.73m^2$ body surface area) from the blood into the urine. It is common among 2 – 6 years old boys. To diagnose childhood nephrotic syndrome precise urine protein excretion has to be measured for which 24 hrs urine sample needs to be collected. But the procedure 24 hrs urine sample collection of a child becomes very tedious and cumbersome and the exact volume may not be correctly collected leading in inaccurate estimation of protein excretion. So in our study we have done a comparative analysis of 24 hr urine protein levels and spot urine albumin creatinine ratio in patients and normal healthy controls and evaluated the efficacy of both methods.*
Material and methods *– Patients attending the department of pediatrics of HMCH were assessed and healthy age matched children were used as control. Urine protein was measured using dye based method, urine Creatinine was measured by Jaffe's method and urine albumin was measured using immunoturbidemetric method.*
Result *– 24 hrs urine protein values measured in patients with nephritic matched well with the urine albumin creatinine ratio.*
Conclusion *– The result showed the spot urine albumin creatinine ratio is as accurate as 24 hrs urine protein estimation and can be used in pediatric age group for diagnostic parameter in nephrotic syndrome.*

I. Introduction:

Nephrotic syndrome in children is a very common kidney disorder. Generalized edema in an otherwise healthy or well looking child is the typical clinical picture. It is characterized by laboratory derangements like massive (nephrotic range) proteinuria, hypoalbuminemia, hyper cholesterolemia without derangements of RFT[1]. Estimation of 24 hour UP is the gold standard for diagnosis of NS in children. Collection of 24 UP however is a much difficult task in children. Can albumin/creatinine ratio in random spot urine replace it as a diagnostic tool? The answer to this question can be very helpful in deceasing mortality and morbitity in children due to nephritic syndrome.

II. Aims and objective:

The study is designed to correlate "spot urine albumin- creatinine ratio (ACR)" with "24hrs urinary protein excretion" so that it can be a more reliable and convenient test for diagnosis of nephrotic syndrome (NS) in children.

Scope of the study:
1. To ascertain whether albumin/creatinine ratio in a randomly collected spot urine sample can replace 24 hour urinary proteinuria as a diagnostic tool for childhood NS?
2. Nephrotic range proteinuria can be picked up from spot urine sample instead of 24 hour urine collection.
3. Prediction of a formula to find out 24 hr urinary protein from spot urine sample in children.

III. Material & Methods:

•   Patients :
– Prospective observational study was conducted in the department of Biochemistry at Hi-tech Medical College, Rourkela from July 2017 to June 2018 after clearance from Ethical Committee, HMCH.
– Total no. of 50 patients clinically diagnosed and subsequently proved biochemically to be Nephrotic syndrome was recruited from indoor Pediatrics ward of HMCH.

– Control group of 20 patients in the study was also included. Those patients admitted to pediatric ward for other illnesses without proteinuria or with mild non nephrotic range proteinuria.

Inclusion criteria:
Case:
- Age ≤ 14years.
- Heavy proteinuria 3+/4+, hypoalbuminemia (≤2.5 gm/dl), hypercholesterolemia (≥200 mg/dl).
    Control:
- No proteinuria at all.
- Mild (1+/2+ proteinuria) where clinical as well as biochemical diagnosis of NS is ruled out.

Exclusion criteria:
Case:
- Severe complicated cases of NS especially associated renal failure (ARF).
- Associated with gross hematuria or RBC cast on urine examination.
Control:
- Significant renal disease/massive proteinuria(3+/4+)

Laboratory methods:
❖ In all pts. 24hrs.urine was collected to estimate 24hrs urinary protein & randomly chosen spot urine was collected to measure Alb/Cr ratio(ACR).
❖ Serum albumin, cholesterol, creatinine done in all patients.
❖ Serum albumin: by BCG method (Accurex).
❖ Creatinine (urine/serum): Jaffe's method.
❖ Urinary protein: Pyrogallol red method, End Point
❖ Urinary albumin: turbidimetric method using 3% sulphosalicylic acid.
❖ Serum cholesterol: Enzymatic, colorimetric method (CHOD-PAP).

Statistical analysis:
- The data were entered in Microsoft excel sheet and statistical analysis were performed.
- Linear correlation between 24hrs urine protein (x- axis) and spot urine ACR (y- axis) was found out.
- Sensitivity and specificity at various cut off points of ACR to detect Nephrotic syndrome in children were plotted in both tabular and graphical ways.
- Diagnostic accuracy (validity) of spot urine ACR was evaluated by receiver operating characteristic (ROC) curve which was plotted by using SPSS software.
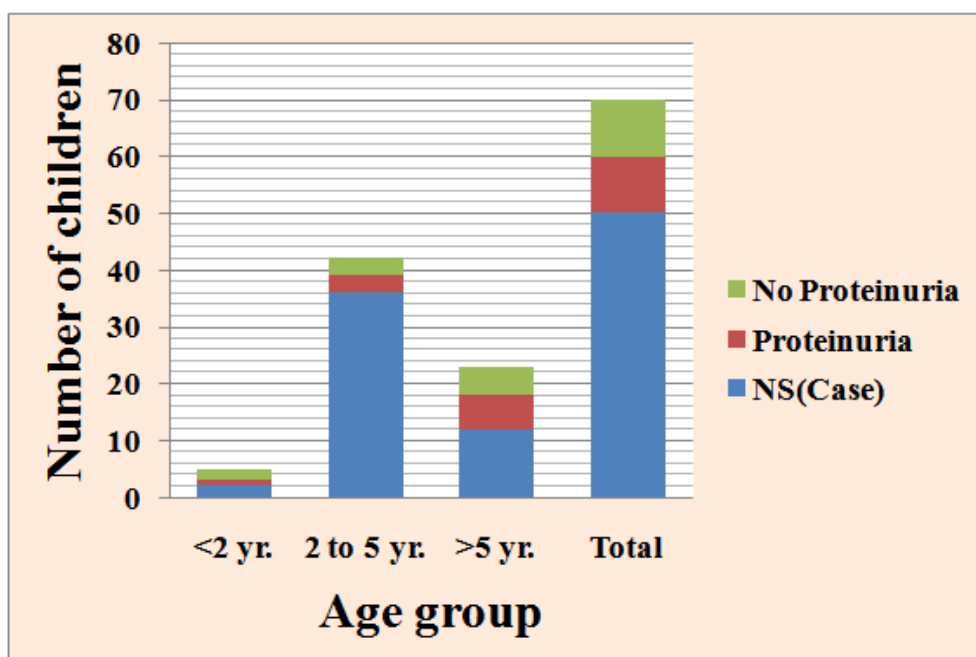

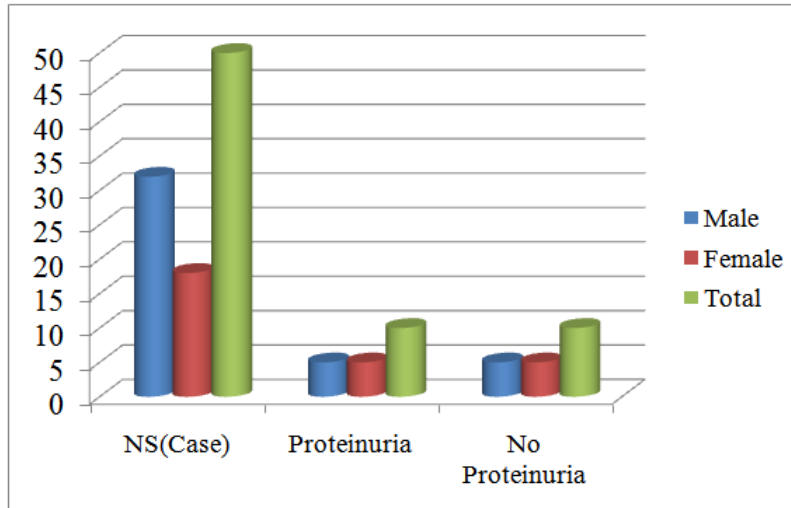**Fig.1- Distribution of study population as per age**

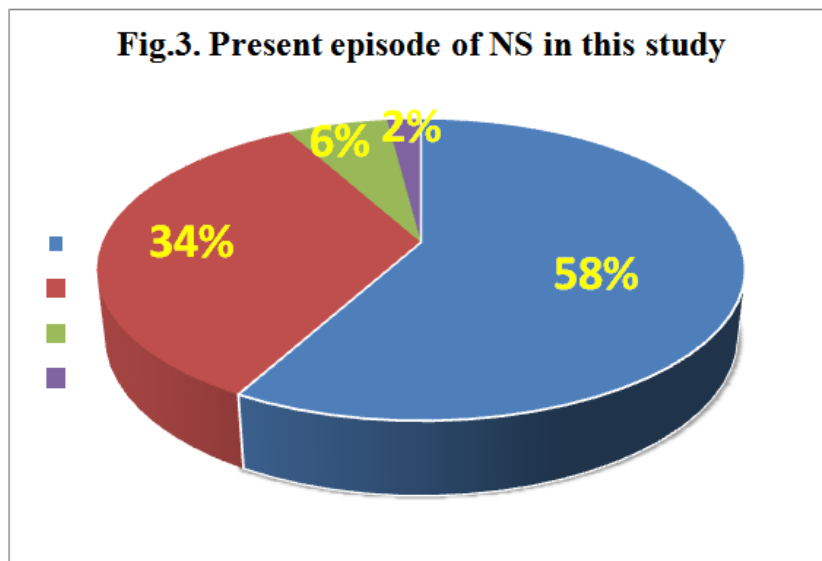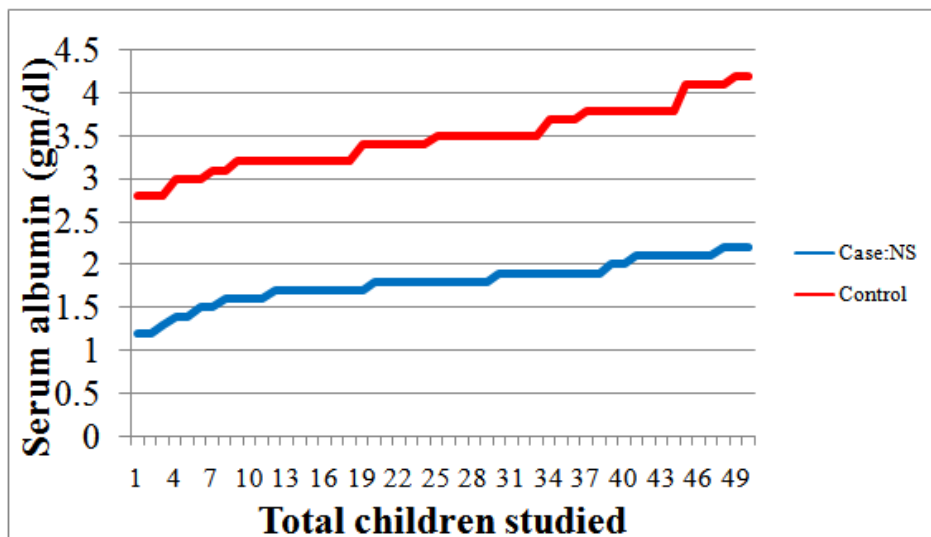**Fig.2- Distribution of study population as per sex**

Fig.4- Serum albumin in NS versus control.

Fig.5. Serum cholesterol in NS vs controls
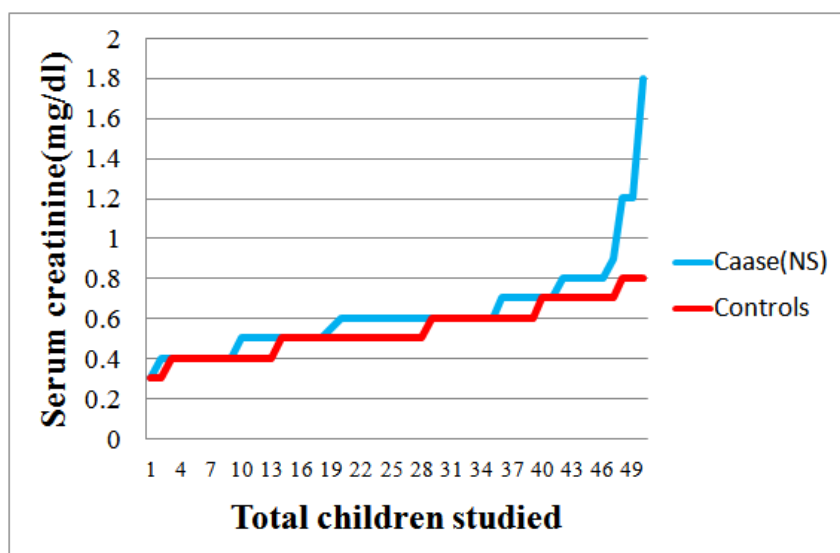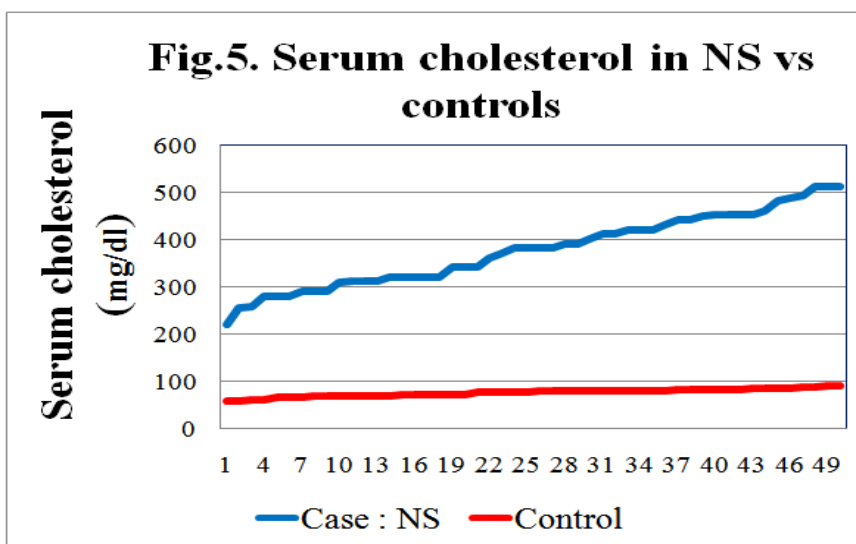
Fig.6- Serum creatinine in NS versus controls
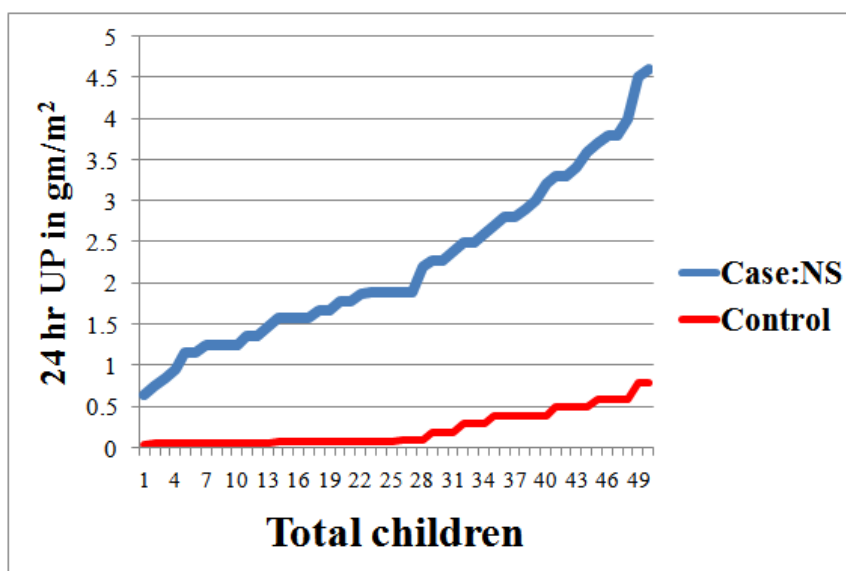
Fig.7- 24 hr UP in case versus control

Parameter	Mean	SD
24UP : case	1.992	0.794584
24UP : control 1	0.468	0.173897
24UP:control 2	0.063	0.01567
Alb/Cr :Case	5.472	3.345972
Alb/Cr :Control 1	1.2	0.648074
Alb/Cr :Control 2	0.39	0.128668
Serum albumin:case	1.798	0.247015
Serum albumin:control 1	3.38	0.335989
Serum albumin:control 2	3.62	0.407704
Serum Cholesterol:case	376.44	76.22233
Serum Cholesterol:control 1	75.9	5.3427
Serum Cholesterol:control 2	75.8	11.48719
Serum Creatinine:case	0.629	0.246214
Serum creatinine:control 1	0.59	0.119722
Serum creatinine:control 2	0.47	0.11595

Table 1- Mean value of different biochemical parameter in case & control
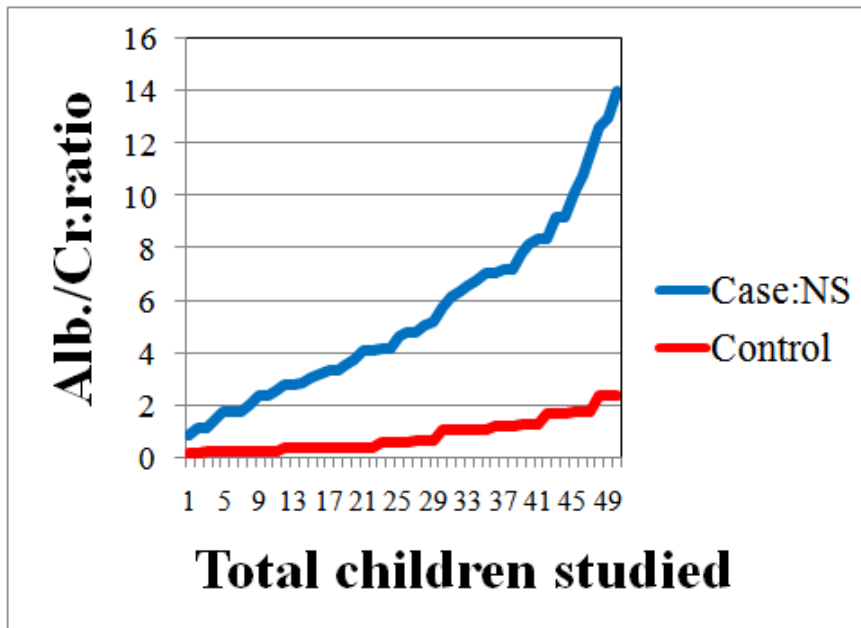

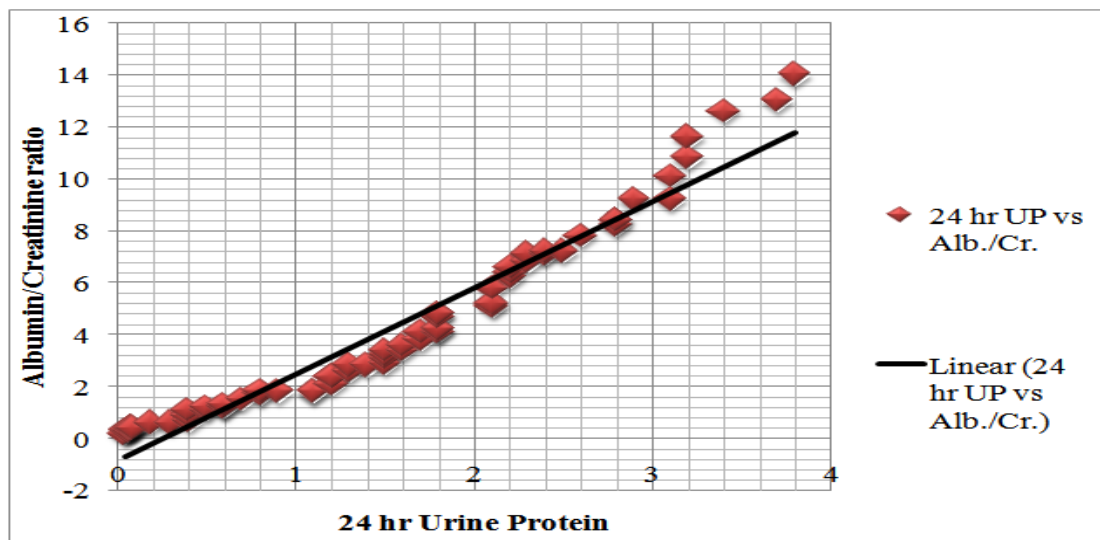**Fig.8- Albumin/Creatinine ratio case (NS) versus control**

Fig 9- 24 hr Urinary Protein versus Albumin/Creatinine

Alb/Creat	TP	FN	FP	TN	Sensitivity	Specificity
≥0.5	50	0	10	10	1	0.5
≥1	49	1	7	13	0.98	0.65
≥2	43	7	1	19	0.86	0.95
≥ 3	36	14	0	20	0.72	1
≥4	30	20	0	20	0.6	1
≥ 5	23	27	0	20	0.46	1
≥ 6	20	30	0	20	0.4	1
≥7	16	34	0	20	0.32	1
≥8	11	39	0	20	0.22	1
≥9	8	42	0	20	0.16	1
≥10	6	44	0	20	0.12	1

Tab.2- SENSITIVITY AND SPECIFICITY AT VARIOUS CUT-OFF POINTS OF "ALBUMIN/CREATININE RATIO" TO DETECT NEPHROTIC SYNDROME

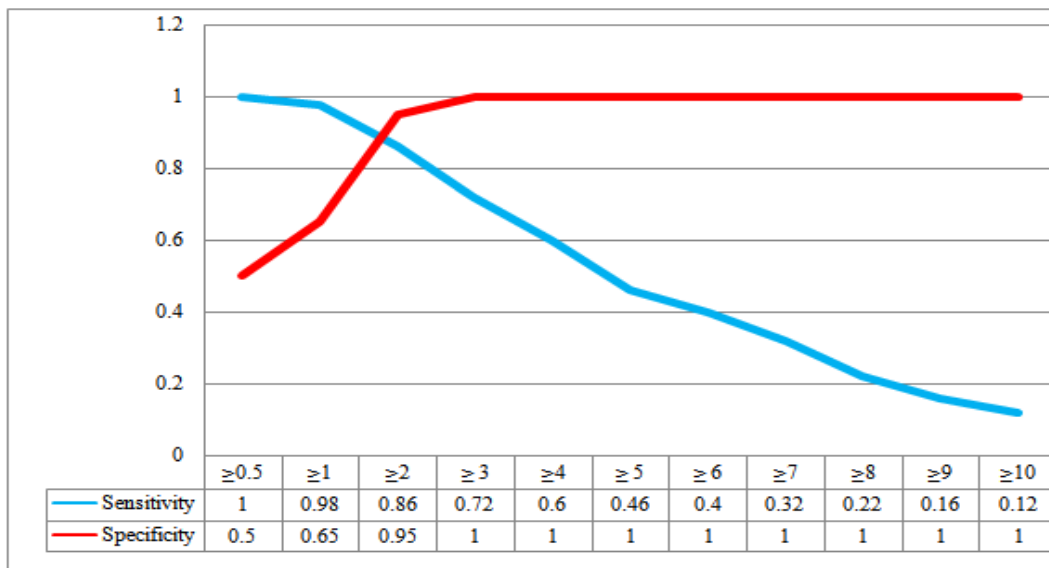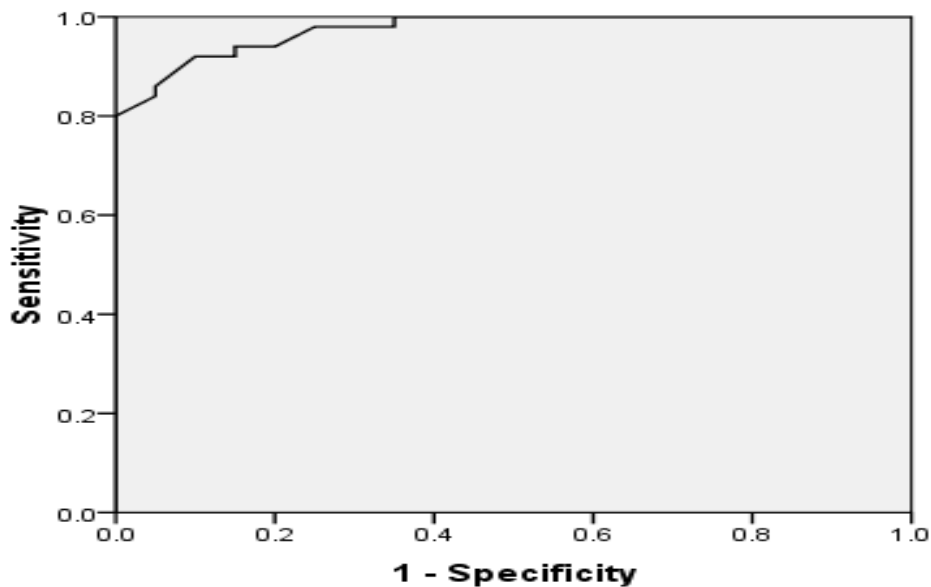|	≥0.5	≥1	≥2	≥ 3	≥4	≥ 5	≥ 6	≥7	≥8	≥9	≥10
Sensitivity	1	0.98	0.86	0.72	0.6	0.46	0.4	0.32	0.22	0.16	0.12
Specificity	0.5	0.65	0.95	1	1	1	1	1	1	1	1

Fig.10- Specificity& Sensitivity of Albumin/Creatinine ratio in detecting NS

Area under the Curve (AUC) = 0.975 Fig.11- ROC Curve

IV.   Results:

- Fig. 1: Shows distribution of study population as per age where 60 % of children belongs to 2-5yrs, 33% belongs to > 5yrs and only 7% belongs to < 2yrs of age.
- Male: Female ratio was 3.5:2 in our study which is similar to the ratio shown by ISKDC[1] group of 3:2.
- Fig.3 shows different episodes of Nephrotic syndrome included in our study 1st episode- 58%,2nd- 34%,3rd - 6% and 4th -2%
- Fig.4: Shows serum albumin levels of all cases as compared with control. Mean serum albumin level in cases is 1.8 ± 0.25gm/dl as compared to control value of 3.38 ± 0.34gm/dl & 3.62 ± 0.4gm/dl in patients with proteinuria and without proteinuria respectively.
- Fig. 5 shows graphical comparison of serum cholesterol levels in cases & control with significant difference. Mean cholesterol level was 376.4 ± 76.2mg/dl in NS as compared to 75.9 ± 5mg/dl in proteinuric control and 75.8 ± 11.5mg/dl in non proteinuric control.
- Fig. 6 shows serum creatinine levels almost similar in cases and controls indicating that complicated renal failure patients are not included as cases.
- Fig.7 shows the significant difference in 24hr urine protein excretion in cases (mean 1.992 ± 0.795gm) as compared to proteinuric control mean $(0.468\pm0.174$gm) and non proteinuric control (mean 0.063 ± 0.016gm).
- Fig.8 :Shows   significant differences in albumin creatinine ratio(ACR) in cases with mean 5.47 ± 3.35 as compared to proteinuric control (mean-1.2 ± 0.6) and non-proteinuric control (mean-$0.39+0.13$).
- Table-1 shows the mean values of different biochemical parameters in cases and control.
- Fig.9 shows graphically the linear correlation between ACR & 24hrs urinary protein excretion. The value of 24hr urine protein excretion can be calculated from spot urine ACR using the   formula derived from the linear relationship between them. (Alb /Cr ratio = 2.938 x 24hrs urinary protein)
- Table-2 summarizes the sensitivity and specificity at various cut off point of ACR to detect NS in children which has been shown graphically in Fig.10. At cut off point of ACR 2 the sensitivity is 86% and specificity 95%.
- Fig.11 represents the ROC curve showing diagnostic accuracy of ACR at 2 as evidenced by the area under curve (AUC) of 0.975 which is statistically highly significant.

V.   Summary and conclusion:

Montero, et al. [2] found out there was no relevant correlation between spot urine protein/creatinine ratio and 24-h urine total protein excretion in nephrotic-range proteinuria. According to study by Abitbol, et al.[3]the random urinary protein/creatinine ratio appeared to offer the most sensitive test for classification of proteinuria in children with nephrosis, with the advantages of ease and expediency not afforded by the 24-hour urine quantitation. Ginsberg, et al.[4] in their study concluded that the determination of the protein/creatinine ratio in single urine samples obtained during normal daylight activity, when properly interpreted by taking into consideration the effect of different rates of creatinine excretion, can replace the 24-hour urine collection in the clinical quantitation of proteinuria. Antunes, et al. [5] supported the use of the P/C ratio as an accurate test to define critical levels of proteinuria. Stoycheff, et al. [6] concluded that the historical definition of nephrotic-range proteinuria appears reasonable in patients with diabetic kidney disease. The linear correlation between the 24 hr urinary protein excretion and spot ACR is well established in our study. As 24 hrs urine collection is a tedious procedure in children, so ACR can be a good alternative diagnostic tool for childhood NS. ACR cut-off value of more than 2 is a highly sensitive and specific tool for diagnosis of childhood NS.2h hrs urinary protein excretion can be estimated from the value of spot urine ACR as both have linear correlation in nephrotic range proteinuria of childhood.

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