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URINARY VITAMIN D BINDING PROTEIN LEVEL AS A PREDICTOR OF STEROID RESISTANCE IN NEPHROTIC SYNDROME – A CROSS SECTIONAL STUDY FROM SOUTH TAMILNADU

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ABSTRACT

Background: Nephrotic syndrome(NS) is characterised by massive proteinuria, hypoalbuminemia and oedema.Vitamin D binding protein (VDBP) which closely resembles albumin in its molecular weight and isoelectric point is excreted in nephrotic syndrome.At present,there are no non-invasive markers available to screen or diagnose Steroid Resistant Nephrotic Syndrome (SRNS) and biopsy still remains the gold standard method. In this study,we tested the hypothesis that urinary VDBP(uVDBP) levels are increased more in SRNS than Steroid Sensitive Nephrotic Syndrome(SSNS) Objective: To establish the association between urinary VDBP levels and steroid sensitive/resistant nephrotic syndrome. **Methods:** Hospital based Cross sectional study between August 2020 and June 2021.50 children aged 2 to 12 years who were newly diagnosed with nephrotic syndrome or those who presented with relapse were enrolled(study group).47 age and sex matched healthy children with normal urine analysis were also enrolled(control group). Urine was collected under strict asepticmeasures,centrifuged at 5000 rpm for 5 minutes and stored at -80°c until further analysis. uVDBP level was measured using commercially available ELISA kit (R&D Systems). Data were analyzed raw and normalized to urine creatinine. Outcome: Correlation between uVDBP, SSNS and SRNS was measured Results:uVDBP concentration was significantly higher in children with nephrotic syndrome(Mean 848ng/ml,SD 267.67ng/ml,p0.0001) than controls.Among children with nephrotic syndrome,uVDBP was significantly higher in children with Steroid Resistant Nephrotic Syndrome (Mean 1017.46ng/ml, SD 46.94ng/ml,p0.0001) than Steroid Sensitive Nephrotic Syndrome. Conclusion:uVDBP is a potential biomarker discriminating children with Steroid Resistant Nephrotic Syndrome from Steroid Sensitive Nephrotic syndrome.

INTRODUCTION

Nephrotic syndrome (NS) is one of the most common glomerular diseases in children. The disease is characterized by relapsing episodes of oedema, proteinuria, and hypoalbuminemia. Idiopathic nephrotic syndrome in children (0–18 years of age) has a prevalence of 10–50 cases per 100,000 children and an incidence of 1.2 to 16.9 per 100,000 children globally (1).The most common pathological lesion observed is minimal change disease, although in the majority of cases, the pathological lesion pattern is not established with kidney biopsy.Approximately 90% of children are steroid responsive and they have a favourable prognosis while children with Steroid Resistant Nephrotic Syndrome (SRNS) have an unfavourable outcome.

The common histopathological findings on invasive biopsy are minimal change disease (MCD), Focal Segmental Glomerulosclerosis (FSGS) and Mesangioprolifeartive Glomerulonephritis. Children with MCD have a steroid-sensitive course and a good prognosis, whereas those with FSGS and Mesangio-prolifeartive Glomerulonephritis are often resistant to steroid treatment and are associated with poor outcome.Infact, FSGS is the leading cause of End Stage Renal Disease (ESRD) in children and the primary disease often recurs (30 to 40%) in kidney transplant recipients (2) In a child with NS, steroid resistance and hence SRNS can be suspected only if remission is not attained at the end of 6 weeks.[1]Absence of remission at the end

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of 6 weeks is a definite indication for renal biopsy.However, children are not subjected to biopsy at all centres.Biopsy even if performed is not without limitations. The diagnosis of FSGS by renal biopsy in children is limited by the smaller core size of tissue obtained by biopsy and focal nature of the disease. Hence, there is a need for an effective, reliable, non-invasive means of diagnosing SRNS to avoid the unnecessary effects of high-dose corticosteroids and to initiate the alternative treatments for SRNS much earlier.

One such biomarker of interest is Vitamin D binding Protein (VDBP) (3). It is produced in liver and it regulates the levels of Vitamin D (VitD) and its metabolites in blood.It resembles albumin in its molecular weight and isoelectric point and is freely filtered. Reabsorption of filtered VDBP requires the normally functioning megalin and cubilin receptors in proximal tubule. The integrity of these tubules is impaired in SRNS which leads to an increased excretion of VDBP in urine (uVDBP).

Many studies have demonstrated the low levels of VitD in both Steroid Sensitive Nephrotic Syndrome (SSNS) and SRNS and the fall is due to loss of VDBP in urine. The deficiency was probably higher in SRNS than SSNSfor the reason already mentioned.Literature search on uVDBP levels in Indian children was sparse.Hence, we proceeded with this study to determine the levels of uVDBP in children with NS and the ability for use as a biomarker in differentiating SRNS from SSNS

METHODS

This cross-sectional study was done at the Paediatric department of a tertiary care centre in South India from August 2020 to June 2021. 50 children in the age group of 2 to 12 years who were diagnosed with nephrotic syndrome, either first episode or relapse of SSNS or SRNS were included in the study.Children were followed up till they attained remission to classify them as SSNS or SRNS.Another 47 children with normal urine analysis served as controls.Written informed consent was obtained from the parents of participants. We excluded children with congenital nephrotic syndrome and severe acute malnutrition.This study was approved by paediatric department and the Institutional ethical committee After cleaning the genitals,10 to 30 ml of early morning urine sample was collected directly to a sterile container.In children below 3 years,urine collection bag was used to collect sample and then transferred to the sterile container.The collected urine was centrifuged at 5000 rpm for 5 min, aliquoted, and stored at –80˚C until analysed.Urine VDBP measured using commercially available ELIZA diagnostic kit. Demographic and clinical data (urine analysis,steroid response history,current relapse status) were recorded at the time of enrolment.Estimated Glomerular filtration rate(eGFR) was calculated using new Schwartz formula.

Remission is defined as urine albumin trace or nil for 3 consecutive days by urine dipstick.First episode was treated with 6 weeks of daily oral prednisolone (2mg/kg) and another 6 weeks of oral prednisolone (1.5mg/kg) on every other day.Relapse is defined as urine albumin 3+ or 4+ for 3 consecutive days after attaining remission. Relapses were treated with oral prednisolone(2mg/kg) till remission followed by oral prednisolone(1.5mg/kg) every other day for 4 weeks. SSNS is defined as the ability to attain complete remission of proteinuria with steroids.SRNS is defined as inability to attain complete remission after 6 weeks of steroid therapy.Two relapses while on alternate day steroid therapy or relapse within 15 days of completion of steroids is termed Steroid Dependent Nephrotic Syndrome(SDNS) (4)

Statistical analysis

The information collected was recorded in a master chart. Data was analysed through using Epi info v7 and SPSS 20 Software. P value < 0.05 was considered as being statistically significant. Quantitative data was expressed as mean +SD.ANOVA one-way statistical was used to compare between the groups. Discrete variables were analysed using t- test. Spearman's correlation analysis was done to find association between study variables.

RESULTS

Fifty children were enrolled over a 11-month period.20 of them were boys and 30 were girls.Table 1 shows the clinical characteristics of cases.There was no significant difference in uVDBP level between males and females(p=0.5).Of the 50 cases,100% had oedema at admission while 90% had oliguria along with oedema. There was no significant difference in uVDBP levels in children presenting with oedema alone and those with oedema and oliguria(p=0.92). SSNS was diagnosed in 68% of children, 16% had SDNS while another16% had SRNS.36% had first episode of NS while 64% were relapsers. Table 2 reveals uVDBP level was significantly higher (p <0.0001) in cases (Mean 848ng/ml, SD 267.67ng/ml) than controls (Mean 611.03ng/ml, SD 250.45ng/ml).Among children with NS (Table 3), SRNS has significantly higher (p<0.0001) excretion of uVDBP (Mean 1017.46ng/ml, SD 46.94ng/ml) than SSNS (Mean 789.83ng/ml, SD 291.04ng/ml)

Table 1. Clinical characteristics of cases.

S.No	Variables	n	%
1	Male Female	20 30	40 60
2	Oedema Oedema with oliguria	5 45	10 90
3	Hypertension Haematuria	16 1	32 2
4	First episode Relapse	18 32	36 64
5	Normal eGFR Reduced eGFR	37 13	74 26
6	Steroid responsive Steroid dependent Steroid resistant	34 8 8	68 16 16

Table 2. Comparison of urinary VDBP between cases and controls.

Group	n	Mean	SD	T	P value
Case	50	848	267.67	4.23	0.0001
Control	47	611.03	250.45		

Tab 3. Mean comparison of Urinary Vitamin D binding protein between each type of NS

S.no	Type	n	uVDBP	P value
1	SSNS	34	789+-291.04	0.0001
2	SDNS	8	925+-209.37	
3	SRNS	8	1017+-46.94	
4	Control	47	611+-250.45	

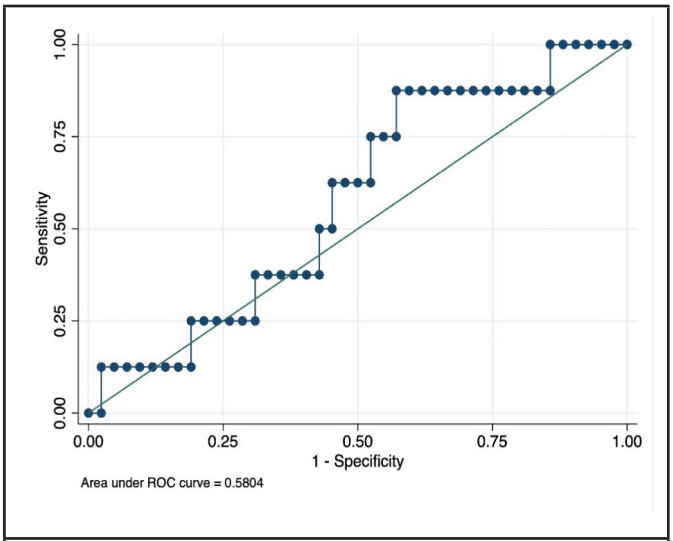

Figure 1. ROC curve for Steroid Dependent Nephrotic Syndrome

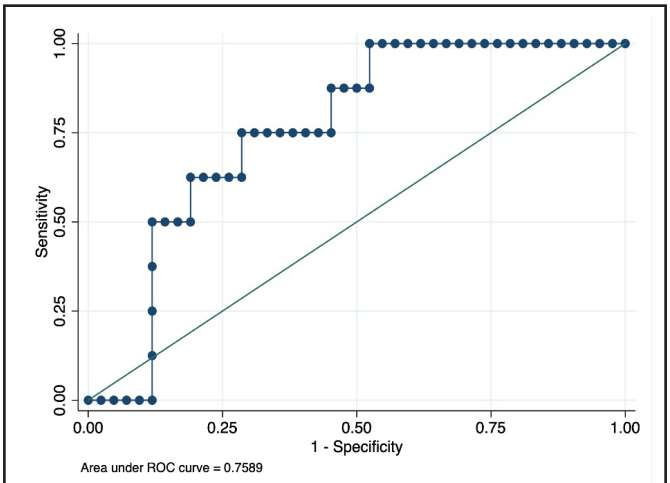

Figure 2. ROC curve for Steroid Resistant Nephrotic Syndrome

DISCUSSION

SSNS has a favourable outcome while Steroid resistant nephrotic syndrome is associated with poor outcome and can result in progression to End stage renal disease. Currently there are no diagnostic markers for differentiating SRNS from SSNS.Steroid responsiveness is usually diagnosed by observing the response after treatment with steroids.Alternatively,diagnosis can be made by invasive renal biopsy to study the histopathological type but this is usually avoided in children. In our study,main objective was to study whether urinary vitamin d binding protein can be used as a non-invasive biomarker to differentiate SRNS from SSNS.

In our study, we observed that NS was more common in girls (n-30, 60%) than boys (n-20, 40%). This is in contrast to previous studies by Sahana et al, who found that males are three times more likely than females to get nephrotic syndrome[5]. Six males and two females are members of SRNS. This is consistent with prior research by Pawan Mutalik et al, who found that males are more affectedthan girls.[6]

The most common age group for presentation was 2-6 years (n-34, 68%),with an average age of 5.3 years. This is similar to prior research by Pandya and Mehta et al, who found a mean age group of 4.08 years. However, no age group was found to have a higher risk of developing SRNS in this study.[7]

In this study, 100% of the cases had oedema as a presenting complaint (n-50, 100%) and 90% of the cases had oliguria (n-45, 90%). We conclude tha to edema is the most prevalent clinical symptom of NS, which can be caused by hypoalbuminemia and oliguria due to a decrease in intravascular fluid volume as a result of fluid migrating from intravascular to extravascular space. This is comparable to previous study by Agarwal and Singh et al, in which 100% of the participants had oedema and 26% had oliguria. Atypical features such as hypertension were observed in 32% and haematuria in 2%, which is consistent with prior studies that found hypertension in 27.1 percent and haematuria in 5.6 percent. A total of 13 cases (n -13, 26%) had a decreased eGFR as determined by the Schwartz formula. Three of the SRNS had a lowered eGFR, while the other five had a normal eGFR. As a result,we infer that eGFR in SRNS can be normal or low.

The primary goal of this study was to determine urinary vitamin D binding protein levels in patients and controls in order to determine a cut-off point for distinguishing SRNS from SSNS. From our study, we observed that among 50 children, 68% had SSNS,16% SDNS and another 16% had SRNS.uVDBPconcentrations was higher in patients (n-50, 848.00± 267.67 ng/ml) than in healthycontrols (n-47, 611.03±250.45 ng/ml), and this difference was statistically significant (p <0.0001).By using ANOVA test, it was found that children with SRNS (n-8, 1017.46± 46.94ng/ml) had substantially greater uVDBP levels than patients with SDNS (n-8,925.79±209.37ng/ml) and SSNS (n-34,789.83± 291.04ng/ml) which was also statistically significant(p<0.0001). This was similar to prior research by Benet etal, who observed higher uVDBP levels in SRNS than SSNS (2).

Urinary levels of vitamin D binding protein has good diagnostic validity in identifying those with SRNS (Figure 1&2). It has an Area Under the Curve (AUC) of 0.76 with 95% CI of 0.61-0.91. At a cut off value of 936ng/ml, it has a sensitivity of 100% and a specificity of 56%. This cut off can be used, when uVDBP is used a screening test along with other parameters. Running multiple tests in series would improve the specificity of uVDBP.If it is used as a standalone test for screening SRNS, a higher cut off should be chosen. Cut off value of 1000 would have a sensitivity and specificity close to 70 to 75% (For 1012, sensitivity is 75% and specificity is 71%).

Our study is not without limitations.This was a single-centre cross-sectional study involving children who were already receiving treatment when the study began.All the children with SRNS were diagnosed by steroid responsiveness only and biopsy was not done. The number of children with SSNS and SRNS were not equal. To confirm our preliminary findings, a large multi-centre prospective study determining urine VDBP levels at baseline before treatment and following up on the uVDBP levels with illness progression would berequired.

We therefore conclude that uVDBP can be an upcoming urinary biomarker to distinguish SRNS from SSNS.

What is already known – Proteinuria is universal in NephroticSyndrome.

What this study adds – Measuring uVDBP may help to predict Steroid resistance very early.

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Conflict of interest: None

Ethical approval:

The study was approved by the Institutional Ethics Committee of Madurai Medical College, Madurai and performed as per the standards laid down by the Declaration of Helsinki for medical research involving human subjects.

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