The Potential of Vitamin-D-Binding Protein as a Urinary Biomarker to Distinguish Steroid-Resistant from Steroid-Sensitive Idiopathic Nephrotic Syndrome in Iraqi Children

Ali Mohammed Abd Alridha*, Dheyaa Jabbar Kadhim, Ayad Hussein Ali Alkhazrajy*****

**Department of Clinical Pharmacy and Therapeutics, Faculty of Pharmacy, University of Kufa, Najaf, Iraq, **Department of Clinical Pharmacy, College of Pharmacy, University of Baghdad, Baghdad, Iraq, ***Babylon Hospital for Maternity and pediatrics, Babylon, Iraq.*

ABSTRACT

Objective: To determine the ability of uVDBP to differentiate SRNS from steroid-sensitive nephrotic syndrome (SSNS) in Iraqi children.

Materials and Methods: This cross-sectional study enrolled children with SRNS (n=31) and SSNS (n=32) from the pediatric nephrology clinic of Babylon Hospital for Maternity and Pediatrics over three months. Patients' characteristics in terms of demographics, clinical data, and urinary investigations were collected. Quantitative analysis of uVDBP levels was undertaken via a commercially available ELISA kit.

Results: The median uVDBP values were significantly higher (p-value<0.001) in the SRNS group (median=10.26, IQR=5.91 µg/mL) than in the SSNS group (median=0.953, IQR=4.12 µg/mL). A negative correlation was noted between uVDBP levels and estimated glomerular filtration rate (eGFR) (Spearman's rho coefficient = −0.494, p=0.001). Nevertheless, the rise in uVDBP concentrations was still considerable in children with SRNS whose eGFR measurements were above 60 mL/min/1.73 m^2. The study revealed a good discriminatory power for uVDBP as a predicting parameter to distinguish SRNS from SSNS (AUC= 0.909, p<0.0001. The optimal uVDBP cut-off value of 5.781 µg/mL was associated with a sensitivity of 83.9% and specificity of 84.4% to differentiate SRNS from SSNS.

Conclusion: Considering its significant discriminatory strength, uVDBP can be considered as a potential marker to noninvasively distinguish children with SRNS from those with SSNS.

Keywords: Nephrotic syndrome; biomarker; vitamin D binding protein; steroid-sensitive nephrotic syndrome; steroid-resistant nephrotic syndrome (Siriraj Med J 2023; 75: 248-258)

INTRODUCTION

Nephrotic syndrome (NS) is a common glomerulopathy that occurs in children. The disorder is distinguished by episodic events of relapses that involve edema, proteinuria, and hypoalbuminemia.[1–3] The two prevalent forms of the disease often found in the histopathological study of an invasive renal biopsy, are minimal-change disease (MCD) and focal segmental glomerulo-sclerosis (FSGS).[4–7] Children with steroid-resistant nephrotic syndrome (SRNS) are at higher risk of the condition worsening and development of complications in comparison to children with steroid-sensitive nephrotic syndrome (SSNS).[8–12] Furthermore, reports indicated that the number of cases with SRNS is escalating which is likely attributed to the growing number of cases diagnosed with FSGS worldwide (including Iraq).[13–16] FSGS is the second leading cause of the end-stage renal disease (ESRD) and chronic renal failure in childhood.[17,18]

Corresponding author: Ali Mohammed Abd Alridha
E-mail: alim.abdalridha@uokufa.edu.iq
Received 11 January 2023 Revised 23 February 2023 Accepted 27 February 2023
ORCID ID:http://orcid.org/0000-0002-5580-9849
https://doi.org/10.33192/smj.v75i4.260831

Responsiveness to steroid therapy has been reported to provide a better prediction of prognosis as compared to a renal biopsy study.[1] Hence, children with idiopathic nephrotic syndrome (INS) complete an imperative trial of high-dose steroid therapy (for a variable duration of up to three months) which can be considered as both therapeutic and diagnostic intervention. If successful remission is not attained, the patient diagnosis is presumed SRNS and a biopsy study is warranted to identify the histopathological type.[19–22] On the other hand, identification of SRNS (specifically FSGS) is commonly missed with a single kidney biopsy because of the focal nature of the glomerular lesions, which mandate performing several biopsies for accurate diagnosis of FSGS.[23]

Vitamin D deficiency is a common complication in NS, which was reasoned to develop predominantly due to urinary losses of vitamin D binding protein (uVDBP).[24,25] In children with NS, a greater extent of the decline in serum vitamin D level was noted in SRNS as compared to SSNS suggesting that the severity of uVDBP losses is more prominent in SRNS than in SSNS.[26–28] In that regard, the uVDBP levels were assessed in SRNS and SSNS patients from India and the United States (US) and significantly higher concentrations were reported in SRNS as compared to SSNS.[29,30] However, SSNS subjects with proteinuria on urinalysis showed a higher trend of uVDBP levels than that in SSNS cases without proteinuria. It was questioned whether the increased levels of uVDBP were a reflection of more pronounced proteinuria rather than the disparity in steroid responsiveness in INS patients.[31]

Additionally, when the findings of the SRNS groups were examined separately, the reported uVDBP levels in the studied populations from the US and India were far from equivalent. The uVDBP levels in the SRNS groups from both studies (the American and the Indian, respectively) were 13659 (median; IQR 477–22,979) and 701.12 (mean; SD ± 371.64) ng/mL.

More studies in different populations are still required to provide further information about the capability of uVDBP in predicting steroid-responsiveness in children with INS. In Iraq, no research, to our knowledge, was undertaken to investigate the ability of uVDBP as a biomarker to differentiate children with SRNS from SSNS. Thus, we conducted this study to evaluate the potential of uVDBP as a noninvasive biomarker to predict steroid responsiveness in Iraqi children with INS.

MATERIALS AND METHODS

Setting and study design

This cross-sectional study was conducted in the department of pediatrics at Babylon Hospital for Maternity and Pediatrics from March to June 2022. The Human Research Committee of Babylon Directorate of Health (Decision number: 44 on 28/3/2022) and Research Ethics Committee of the University of Baghdad – College of Pharmacy (Approval number: RECAUBCP17102021A on 17/10/2021) approved the study protocol. Informed consent was acquired from all study participants (or parents/legal caretakers) before their enrollment in the study.

Participants

Patients aged 1-14 years, who were already diagnosed with steroid-sensitive or steroid-resistant INS, were recruited from the pediatric nephrology consultation clinic. Steroid-sensitive nephrotic syndrome (SSNS) was identified as succeeding in acquiring successful remission (<1+ proteinuria on early morning urine dipstick) after 4 weeks of daily prednisolone [2 mg/kg/d (maximum 60 mg/d)].

Steroid-resistant nephrotic syndrome (SRNS) was identified as failing to acquire successful remission after 8 weeks of daily prednisolone [2 mg/kg/d (maximum 60 mg/d)] or 4 weeks of daily prednisolone [2 mg/kg/d (maximum 60 mg/d)] then another 4 weeks of alternate day prednisolone [1.5 mg/kg/d (maximum 50 mg/d)] or 6 weeks of daily prednisolone [2 mg/kg/d (maximum 60 mg/d)] then another 6 weeks of alternate day prednisolone [1.5 mg/kg/d (maximum 50 mg/d)].

The exclusion criteria included patients with fever, gross hematuria, acute kidney injury, active or recurrent urinary tract infection, and nephrotic syndrome secondary to systemic diseases such as lupus nephritis, viral infections, or diabetes. Serum fasting blood glucose was documented to exclude diabetes mellitus. Additionally, patients were screened for viral antibodies (HIV, HBV, and HCV), autoantibodies (anti-double stranded DNA antibodies and anti-nucleic acid antibodies), and low serum complement C3 levels to exclude NS secondary to viral infections and autoimmune diseases such as systemic lupus erythematosus. The patients were approached during their routine follow-up visit to the clinic and were recruited consecutively after their consent and satisfaction with the study's inclusion and exclusion criteria.

Sample size estimation

An online calculator (https://sample-size.net/sample-size-ci-for-auroc/) was used to calculate the sample size.[32] The expected area under the ROC curve and the width of the confidence interval (0.90 and 0.16, respectively) was estimated based on the results of previous studies.[29,30]

A sample size of 63 was calculated when the proportion of the sample having the positive studied outcome (steroid resistance) is 50% of the sample size. Thus, 31 patients were included in the positive outcome group (SRNS) and 32 patients were recruited for the negative outcome group (SSNS).

Data collection

Data about the clinical and demographical characteristics of all the study participants were collected in a predetermined sheet at the time of enrollment. The glomerular filtration rate (eGFR) was calculated based on the participant's height and serum creatinine using the updated Schwartz equation.[33]

A commercially available ELISA kit (Bioassay Technology Laboratories, Zhejiang, China) was utilized to measure the VDBP levels in urine. Urine collection was performed as part of a routine clinic visit. After collection, the urine sample (early morning void) was subjected to centrifugation at 3000 RPM for 20 min, aliquoted, and stored at -80 °C. Repeated freeze-thaw cycles of more than two times were not allowed.

Statistical analysis

The Statistical Package for Social Sciences (SPSS) statistics software (version 22) was used to execute the statistical analysis. The categorical data of demographical and clinical characteristics were described using frequencies and percentages and tested the differences using the Chi-square analysis. The normal data of both study groups (height, serum albumin, and eGFR) were compared using the unpaired t-test while the non-normal data were compared using the Mann-Whitney U test. The receiver operator characteristics (ROC) curve was also analyzed to determine the discriminatory power of uVDBP level to distinguish SRNS patients from SSNS patients. Spearman rank correlation analysis was performed to evaluate the association of the uVDBP levels with the renal function of the studied nephrotic syndrome patients, which was represented by eGFR. A finding was considered statistically significant if the p-value was lower than 0.05.

RESULTS

The patients' characteristics in both groups (SSNS and SRNS) were comparable except for serum albumin, serum creatinine, and blood urea (p-value <0.05; Table 1). The number of participants receiving concomitant treatment with ACEI and diuretics was higher in the SRNS group (n= 8 and 14, respectively) than in the SSNS group (n= 2 and 5, respectively). The mean values of the eGFR based on the Schwartz equation were significantly lower in the SRNS group (63.61 ± 14.31mL/min/1.73 m^2, p-value<0.05) in comparison to those in the SSNS group (72.89 ± 14.71mL/min/1.73 m^2).

The median uVDBP values were significantly higher (p-value<0.001) in the SRNS group (median=10.26, IQR=5.91 µg/mL) than in the SSNS group (median=0.953, IQR=4.12 µg/mL). The difference between the two groups was statistically significant (Fig 1).

A subgroup analysis was performed to examine the difference in the uVDBP level between the SSNS patients with proteinuria and those without proteinuria. The uVDBP median in SSNS patients with proteinuria was 2.63 (IQR=5.72) while the median in those without proteinuria was 0.85 (IQR=3.91). No statistically significant difference was found (p-value>0.05, Fig 2A).

Another subgroup analysis was conducted in the SRNS to determine if there is a difference in the uVDBP levels among children with calcineurin inhibitor (CNI) therapy and those without CNI therapy (Fig 2B). The difference was insignificant between the two subgroups (median [IQR] =10.26 [5.32] in CNI-treated children compared to 9.13 [6.53] in non-CNI treated children; p-value>0.05).

The median uVDBP values were negatively correlated with eGFR (the Spearman's rho coefficient = – 0.494, p<0.01), as illustrated by the increase of uVDBP levels in INS patients that was accompanied by a corresponding decline of renal function based on eGFR measurements. However, high levels of uVDBP were still present in SRNS subjects despite preserved renal function (eGFR>60 mL/min/1.73 m^2; Fig 3).

The receiver operator characteristic (ROC) curve analysis was conducted to assess the uVDBP capability as a predicting indicator of steroid responsiveness in INS. The levels of the uVDBP parameter showed a high discriminatory power (Fig 4) to characterize SRNS patients from SSNS patients with an AUC of 0.909 (p<0.0001). The analysis also revealed that the optimal cutoff value of 5.781 µg/mL yielded a sensitivity of 83.9% and a specificity of 84.4% to distinguish SRNS patients from SSNS patients (Fig 4 and Table 2).

DISCUSSION

Children with INS who are resistant to steroids are at a high risk of sustaining complications and progressing to end-stage kidney failure. The routine approach to diagnosing SRNS is to observe the outcome of a trial of a long-term prednisolone course, which is typically followed by an invasive kidney biopsy for the prediction of treatment responsiveness and disease progression. This approach entails that individuals with SRNS are being

TABLE 1. Demographic characteristics and clinical data of the study participants.

Characteristics	The study participants (n=63)		P-value
	SSNS (n=32)	SRNS (n=31)	
Age at enrollment [years; median (IQR)]	6.48 (3.4)	8.5 (6)	0.104§
Gender [male; frequency (%)]	20 (62.5)	21 (67.7)	0.432
Age at onset of disease [years; median (IQR)]	4 (2)	3 (5.5)	0.19§
Weight [Kg; median (IQR)]	21 (10.6)	25 (25)	0.132§
Height (cm; mean ± SD)	111.81 ± 17.14	118.77 ± 26.09	0.218‡
Serum albumin (gm/L; mean **± SD**)	37.92 ± 7.15	32.36 ± 11.29	**0.024‡**
Serum creatinine [umol/L; median (IQR)]	54 (22.8)	65 (32)	**0.007§**
Blood urea [mmol/L; median (IQR)]	2.8 (1.45)	4.1 (2.8)	**0.003§**
eGFR (mL/min/1.73 m²; mean ± SD)	72.89 ± 14.71	63.61 ± 14.31	**0.014‡**
Presence of hypertension (frequency, %)			
Systolic blood pressure >95 percentile	4 (12.5)	5 (16.1)	0.479*
Diastolic blood pressure >95 percentile	3 (9.4)	7 (22.6)	0.138*
Pathology upon biopsy (frequency, %)			
Focal segmental glomerular sclerosis	-	1 (3.2)	NA
Membranoproliferative glomerulonephritis	-	1 (3.2)	NA
Minimal change disease	-	3 (9.7)	NA
No biopsy	32 (100)	26 (83.9)	NA
Immunosuppressant regimen (frequency, %)			
Prednisolone	32 (100)	3 (9.7)	NA
Prednisolone and cyclosporine	0	18 (58.1)	NA
Prednisolone and tacrolimus	0	3 (9.7)	NA
Prednisolone and chlorambucil	0	1 (3.2)	NA
Prednisolone and mycophenolate mofetil	0	6 (19.4)	NA
Concomitant medications (frequency, %)			
ACEI	2 (6.3)	8 (25.8)	**0.036***
Statin	2 (6.3)	6 (19.4)	0.118*
Diuretic	5 (15.6)	14 (45.2)	**0.011**

* Significance value for Fisher's Exact Test. § Significance value for Mann-Whitney U test.
‡ Significance value for Independent samples t-test. Statistically significant p-values are in bold.
Abbreviations: SSNS: steroid-sensitive nephrotic syndrome; SRNS: steroid-resistant nephrotic syndrome; SD: standard deviation; IQR: interquartile range; eGFR: estimated glomerular filtration rate; ACEI: angiotensin-converting enzyme inhibitor.

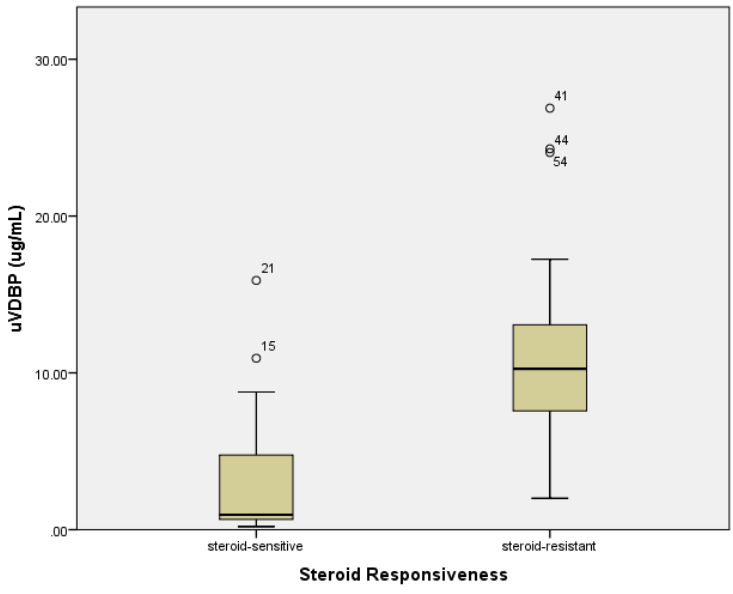
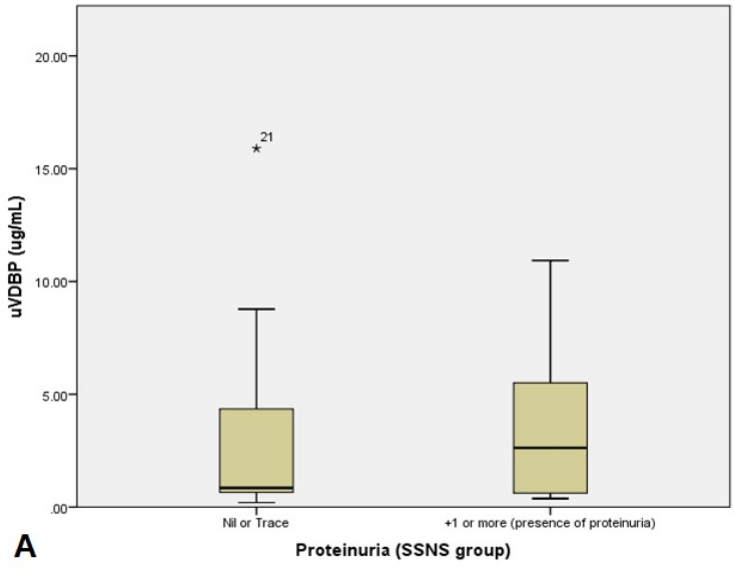


Fig 1. Urine vitamin D-binding protein (uVDBP) levels in the studied children with idiopathic nephrotic syndrome. Median uVDBP was significantly higher in the steroid-resistant group (median [IQR]= 10.26 [5.91]) than in the steroid-sensitive group (median [IQR]= 0.953 [4.12]; p-value<0.001).



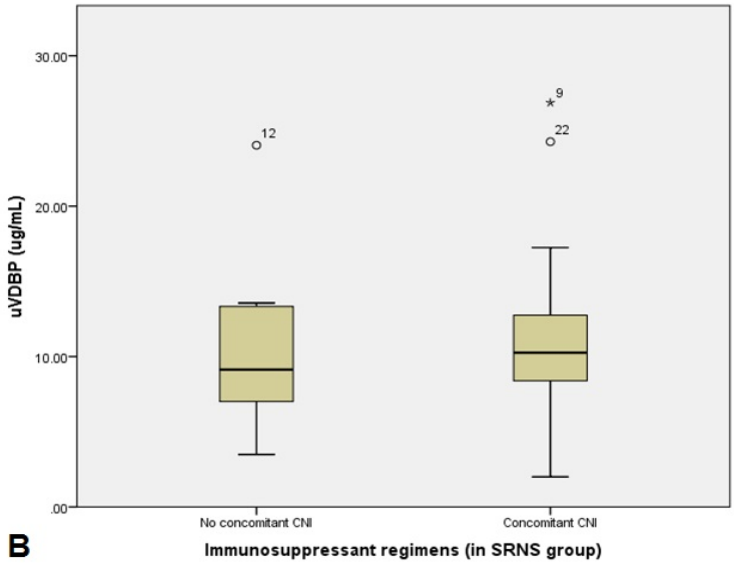


Fig 2. Subgroup analysis of uVDBP levels in children with SSNS and SRNS. A is a comparison of uVDBP levels in SSNS subgroups (SSNS patients with proteinuria and SSNS patients without proteinuria). B is a comparison of uVDBP levels in SRNS subgroups [SRNS patients with calcineurin inhibitor (CNI) use and SRNS patients without CNI use].

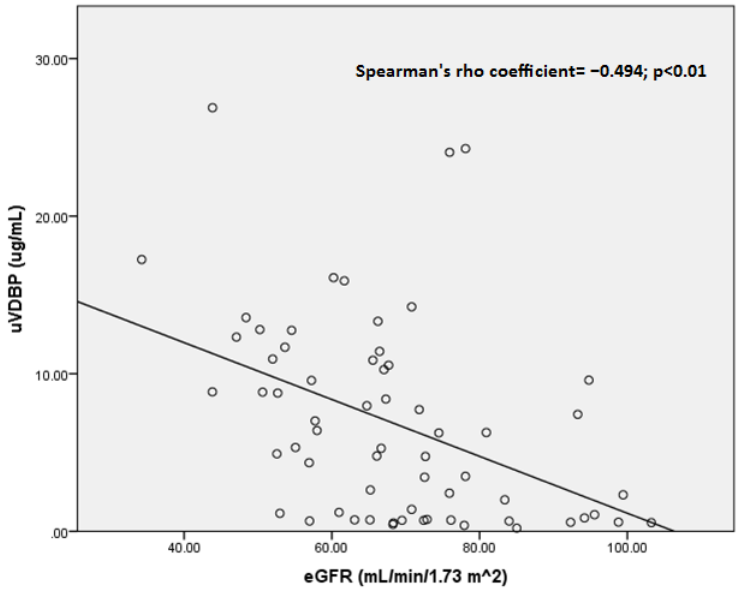


Fig 3. Correlation of uVDBP with renal function in children with idiopathic nephrotic syndrome (n=63). Median uVDBP is negatively correlated with estimated glomerular filtration rate (eGFR), as illustrated by the increase in uVDBP levels in nephrotic syndrome patients that is accompanied by a corresponding decline in renal function based on eGFR measurements.

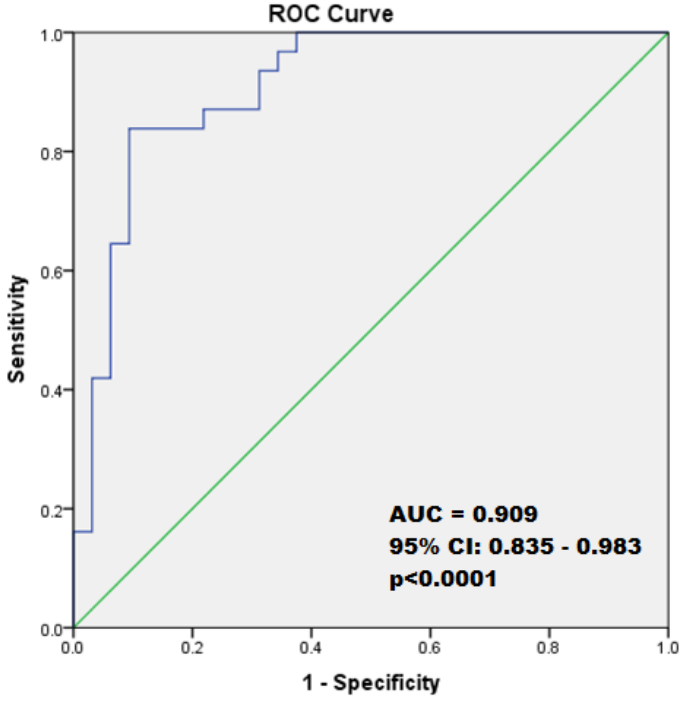


Fig 4. Receiver operator characteristic (ROC) curve analysis of uVDBP. The uVDBP parameter has good discriminatory power to distinguish SRNS from SSNS. ROC analysis revealed an AUC of 0.909 (95% CI: 0.835-0.983; p<0.0001) for the detection of SRNS. The optimal cut-off value was 5.781 µg/mL uVDBP, with a sensitivity of 83.9% and specificity of 84.4%.

exposed unnecessarily to high-dose steroid regimens, as well as prompting the postponement of administering alternative and potentially more effective regimens. Presently, there are no validated markers available for diagnostic purposes for SRNS. This study attempted to provide information to fill this gap by assessing the capability of uVDBP as a non-invasive biomarker to discriminate a priori between children with SRNS and those with SSNS.

In this study, the ROC curve analysis of uVDBP found a significantly reliable discriminatory power to discern patients with SRNS from patients with SSNS (AUC= 0.909, p<0.0001). This result was consistent with the results from similar studies in the United States and India (AUC= 0.87 and 0.897, respectively; p<0.0001).[29,30] We also investigated the impact of potentially interfering factors on the study findings, such as proteinuria status and CNI use. Although children with SRNS showed significantly higher uVDBP levels compared to those with SSNS, there were more patients with active proteinuria in the SRNS group (n=17) than in the SSNS group (n=7). Moreover, most SRNS patients (n=21) used a concomitant CNI, which is potentially nephrotoxic. Such interfering factors could further expand the difference in the uVDBP levels between the two groups. However, the subgroup analyses revealed no statistically significant differences in

TABLE 2. Results of the ROC curve analysis for uVDBP levels and the coordinates of the curve

Test result variable: urinary vitamin D binding protein level (μg/mL)				
Area Under the Curve				
Area	Standard Error[a]	Asymptotic Significance[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.909	0.038	<0.0001 (2.381E-8)	0.835	0.983
Coordinates of the Curve				
Positive outcome when the result is more than or equal to[c]			Sensitivity	1 – Specificity
-.8000			1.000	1.000
0.2890			1.000	0.969
0.4080			1.000	0.938
0.4865			1.000	0.906
0.5425			1.000	0.875
0.5615			1.000	0.844
0.5785			1.000	0.813
0.6160			1.000	0.781
0.6505			1.000	0.750
0.6745			1.000	0.719
0.6965			1.000	0.688
0.7040			1.000	0.656
0.7180			1.000	0.625
0.7260			1.000	0.594
0.7420			1.000	0.563
0.8035			1.000	0.531
0.9525			1.000	0.500
1.0980			1.000	0.469
1.1715			1.000	0.438
1.2960			1.000	0.406
1.6945			1.000	0.375
2.1565			0.968	0.375
2.3670			0.968	0.344
2.5225			0.935	0.344
3.0290			0.935	0.313
3.4605			0.903	0.313
3.9195			0.871	0.313
4.5500			0.871	0.281
4.7630			0.871	0.250
4.8465			0.871	0.219
5.0910			0.839	0.219
5.2920			0.839	0.188
5.7810			0.839	0.156
6.2560			0.839	0.125
6.3330			0.839	0.094
6.7035			0.806	0.094

TABLE 2. Results of the ROC curve analysis for uVDBP levels and the coordinates of the curve (Continued)

Test result variable: urinary vitamin D binding protein level (μg/mL)		
Coordinates of the Curve		
Positive outcome when the result is more than or equal to[c]	Sensitivity	1 – Specificity
7.2150	0.774	0.094
7.5725	0.742	0.094
7.8450	0.710	0.094
8.1780	0.677	0.094
8.5830	0.645	0.094
8.8045	0.645	0.063
8.8400	0.613	0.063
9.2105	0.581	0.063
9.5835	0.548	0.063
9.9270	0.516	0.063
10.3995	0.484	0.063
10.6980	0.452	0.063
10.8960	0.419	0.063
11.1770	0.419	0.031
11.5500	0.387	0.031
12.0025	0.355	0.031
12.5395	0.323	0.031
12.7775	0.290	0.031
13.0645	0.258	0.031
13.4440	0.226	0.031
13.9035	0.194	0.031
15.0710	0.161	0.031
15.9955	0.161	0.000
16.6720	0.129	0.000
20.6490	0.097	0.000
24.1675	0.065	0.000
25.5825	0.032	0.000
27.8800	0.000	0.000

[a] Under the non-parametric assumption

[b] Null hypothesis: true area = 0.5

[c] There is at least one tie between the negative actual state group and the positive actual state group for the test result variable [uVDBP (μg/mL)]. The smallest observed test value minus 1 is the lowest cutoff value. The highest observed test value plus 1 is the greatest cutoff value. The averages of two consecutively ordered observed test values are the remaining cutoff values.

uVDBP concentrations among SSNS patients with active proteinuria compared to those without active proteinuria, as well as among SRNS patients with concomitant CNI compared to those without concomitant CNI. Thus, the higher concentration of uVDBP in the SRNS group is not fully attributed to the patient's level of proteinuria or use of concomitant CNI. Valles et al. observed elevated uVDBP concentrations in patients with micro-albuminuria and chronic kidney disease (CKD) patients with macro-albuminuria. The uVDBP levels were still significantly higher even after maximal anti-proteinuric therapy and resolution of proteinuria. Proteinuria-independent levels of uVDBP are consistent with the findings of this study.[34]

Interestingly, the SRNS patients in this study had significantly lower eGFR compared to the SSNS group. Moreover, this study highlighted the presence of a negative correlation between uVDBP level and renal function. These findings may likely justify the higher uVDBP in the SRNS group. A plausible interpretation would be the fact that uVDBP is a low-molecular-weight protein that undergoes free filtration through the glomerulus, but the proximal convoluted tubules govern its reabsorption (via cubulin and megalin receptor-mediated transport).[24,35] Thus, a chronic process of tubular damage, such as that anticipated to occur in SRNS, most likely compromises the reabsorption and markedly increases the excretion of uVDBP.

Other markers have been investigated in an attempt to evaluate their ability to predict steroid responsiveness in NS patients. Woroniecki et al. assessed the potential of a urinary cytokine panel to distinguish steroid-resistant from steroid-responsive patients. The study demonstrated the ability of urinary TGF-beta[1] to discriminate FSGS from MCD but disclosed that a statistically significant difference in the marker expression was not found between SRNS and SSNS patients (p-value=0.21).[36] An earlier study discovered a distinct urinary proteome capable of predicting steroid responsiveness using mass spectrometry (surface-enhanced laser desorption/ionization). In the latter study, the proteomic profiling identified a 4.144 KD protein that identified all SRNS and SSNS patients with a positive predictive value of 96% and a negative predictive value of 88.2%. However, the significance of this discovery for clinical practice is hindered by the unavailability of such a mass spectrometry technique in most laboratories and the anonymity of the identifier protein sequence.[37] To our knowledge, this is the first study to determine the discriminatory ability of uVDBP as a non-invasive marker for the differentiation of SRNS from SSNS in Iraqi children with INS.

This study has several limitations that should be considered before drawing any conclusions. This pilot investigation was conducted in a single center and with a cross-sectional design. The sample in each group was small and included patients with ongoing steroid therapy at the time of recruitment. There was also a wide disparity among the uVDBP values of the SRNS patients. Variable data might be expected when treatment response is investigated in children with INS. Studying groups with small sample sizes could augment such variability. However, the normality of the uVDBP data distribution was analyzed, and non-parametric statistical tests were performed because the uVDBP data were not normally distributed. Moreover, we were led to believe that the analysis outcomes are indeed powerful because of the strength of the significant difference between the two groups (p-value<0.001).

To validate the results of this study, an investigation with a larger sample size and a prospective cohort design that is carried out in multiple centers is necessary. Furthermore, it is important to consider the possibility that the increase in uVDBP concentration is likely attributed to renal tubular damage, which may develop in various types of chronic kidney disease rather than being an underlying process that is distinctive to patients with SRNS. Nevertheless, in the context of INS, the findings of this study revealed that uVDBP has a promising potential to merit clinical applicability to identify patients with SRNS. The availability of a urinary marker with the capability of predicting steroid responsiveness in children with SRNS may help clinicians to personalize the management and improve the chances of controlling this serious and progressive form of the disease. Additionally, uVDBP may also be beneficial in preventing children from being exposed to unnecessary trials of high-dose steroids and other potent immunosuppressive regimens to which they are likely non-responsive.

CONCLUSION

The present study revealed that uVDBP can discriminate SRNS from SSNS in Iraqi children with significant reliability. The availability of a urinary marker with the capability of predicting steroid responsiveness can provide valuable information to develop more personalized therapy for children with INS.

ACKNOWLEDGMENTS

The authors would like to extend their thanks and appreciation to all the patients and caregivers for their participation in the study and to all the clinic staff for their cooperation in facilitating the conduct of this study.

REFERENCES

1. Eddy AA, Symons JM. Nephrotic syndrome in childhood. Lancet Lond Engl. 2003;362(9384):629–39.
2. Ehren R, Benz MR, Brinkkötter PT, Dötsch J, Eberl WR, Gellermann J, et al. Pediatric idiopathic steroid-sensitive nephrotic syndrome: diagnosis and therapy -short version of the updated German best practice guideline (S2e) - AWMF register no. 166-001, 6/2020. Pediatr Nephrol Berl Ger. 2021;36(10):2971–85.
3. Noone DG, Iijima K, Parekh R. Idiopathic nephrotic syndrome in children. Lancet Lond Engl. 2018;392(10141):61–74.
4. Arif MK, Arif M, Amjad N. A histopathological outlook on nephrotic syndrome: A pediatric perspective. Indian J Nephrol. 2016;26(3):188–91.
5. Ahmed NF, Ibrahim R. Childhood nephritic syndrome Clinical manifestations and histopathlogical spectrum. J Fac Med Baghdad. 2007;49(3):304–6.
6. Azat NFA, hameed NN, Sahib OA. Pediatric Glomerular Diseases (Review of histopathological subtypes). J Fac Med Baghdad. 2010;52(1):1–2.
7. Wine R, Vasilevska-Ristovska J, Banh T, Knott J, Noone D, Gbadegesin R, et al. Trends in the epidemiology of childhood nephrotic syndrome in Africa: A systematic review. Glob Epidemiol. 2021;3:100061.
8. Gipson DS, Chin H, Presler TP, Jennette C, Ferris ME, Massengill S, et al. Differential risk of remission and ESRD in childhood FSGS. Pediatr Nephrol Berl Ger. 2006;21(3):344–9.
9. Roberti I, Vyas S. Long-term outcome of children with steroid-resistant nephrotic syndrome treated with tacrolimus. Pediatr Nephrol Berl Ger. 2010;25(6):1117–24.
10. Hari P, Bagga A, Jindal N, Srivastava RN. Treatment of focal glomerulosclerosis with pulse steroids and oral cyclophosphamide. Pediatr Nephrol Berl Ger. 2001;16(11):901–5.
11. FRANKUL FarM, Fahmi N, Ahmed L. Hypertension in children with nephrotic syndrome. J Fac Med Baghdad. 2005;47(1):5–8.
12. Hilmanto D, Mawardi F, Lestari AS, Widiasta A. Disease-Associated Systemic Complications in Childhood Nephrotic Syndrome: A Systematic Review. Int J Nephrol Renov Dis. 2022;15:53–62.
13. Bonilla-Felix M, Parra C, Dajani T, Ferris M, Swinford RD, Portman RJ, et al. Changing patterns in the histopathology of idiopathic nephrotic syndrome in children. Kidney Int. 1999;55(5):1885–90.
14. Kari JA. Changing trends of histopathology in childhood nephrotic syndrome in western Saudi Arabia. Saudi Med J. 2002;23(3):317–21.
15. Mohammed TF, Al-Badri AA, Abd Al-Latteef A, Abdulhussain RMH. Trends of Histopathology in Childhood Nephrotic Syndrom. Iraqi Postgrad Med J. 2009;1(1):47–55.
16. Asinobi AO, Ademola AD, Okolo CA, Yaria JO. Trends in the histopathology of childhood nephrotic syndrome in Ibadan Nigeria: preponderance of idiopathic focal segmental glomerulosclerosis. BMC Nephrol. 2015;16(1):213.
17. Gipson PE, Gipson DS. CHAPTER 27 - Focal segmental glomerulosclerosis. In: LERMA EV, SPARKS MA, M. TOPF J, editors. Nephrology Secrets (Fourth Edition) [Internet]. Elsevier; 2019. p. 186–91. Available from: https://www.sciencedirect.com/science/article/pii/B9780323478717000368
18. Ahmed NF, Hussain HH. Chronic Renal Failure in Children Admitted to Children Welfare Teaching Hospital. Iraqi Postgrad Med J. 2008;7(1):12–7.
19. Trautmann A, Vivarelli M, Samuel S, Gipson D, Sinha A, Schaefer F, et al. IPNA clinical practice recommendations for the diagnosis and management of children with steroid-resistant nephrotic syndrome. Pediatr Nephrol Berl Ger. 2020;35(8):1529–61.
20. Tullus K, Webb H, Bagga A. Management of steroid-resistant nephrotic syndrome in children and adolescents. Lancet Child Adolesc Health. 2018;2(12):880–90.
21. Schijvens AM, Teeninga N, Dorresteijn EM, Teerenstra S, Webb NJ, Schreuder MF. Steroid treatment for the first episode of childhood nephrotic syndrome: comparison of the 8 and 12 weeks regimen using an individual patient data meta-analysis. Eur J Pediatr. 2021;180(9):2849–59.
22. Rovin BH, Adler SG, Barratt J, Bridoux F, Burdge KA, Chan TM, et al. KDIGO 2021 Clinical Practice Guideline for the Management of Glomerular Diseases. Kidney Int. 2021;100(4):S1–276.
23. Glassock RJ. Con: kidney biopsy: an irreplaceable tool for patient management in nephrology. Nephrol Dial Transplant. 2015;30(4):528–31.
24. Jassil NK, Sharma A, Bikle D, Wang X. VITAMIN D BINDING PROTEIN AND 25-HYDROXYVITAMIN D LEVELS: EMERGING CLINICAL APPLICATIONS. Endocr Pract. 2017;23(5):605–13.
25. Yousefzadeh P, Shapses SA, Wang X. Vitamin D Binding Protein Impact on 25-Hydroxyvitamin D Levels under Different Physiologic and Pathologic Conditions. Int J Endocrinol. 2014;2014:981581.
26. Weng FL, Shults J, Herskovitz RM, Zemel BS, Leonard MB. Vitamin D insufficiency in steroid-sensitive nephrotic syndrome in remission. Pediatr Nephrol Berl Ger. 2005;20(1):56–63.
27. Solarin AU, Adekunle MO, Oladimeji AB, Olutekunbi OA, Lamina MO, Oshomah-Bello OE, et al. Vitamin D status and serum vitamin D binding protein levels in Nigerian children with nephrotic syndrome. Afr J Nephrol. 2022;25(1):149–54.
28. Marzouk H, Ghobrial E, Khorshied M, Mohammed M. Vitamin D level in nephrotic syndrome, Factors of impact? GEGET. 2019;14(2):53–61.
29. Bennett MR, Pordal A, Haffner C, Pleasant L, Ma Q, Devarajan P. Urinary Vitamin D-Binding Protein as a Biomarker of Steroid-Resistant Nephrotic Syndrome. Biomark Insights. 2016;11:1–6.
30. Choudhary A, Mohanraj PS, Krishnamurthy S, Rajappa M. Association of Urinary Vitamin D Binding Protein and Neutrophil Gelatinase-Associated Lipocalin with Steroid Responsiveness in Idiopathic Nephrotic Syndrome of Childhood. Saudi J Kidney Dis Transplant. 2020;31(5):946–56.
31. Aoun AH. Query About Validity of uVDBP as a Biomarker of Steroid-Resistant Nephrotic Syndrome. Biomark Insights. 2022;17:11772719221078372.
32. Hanley JA, McNeil BJ. The meaning and use of the area under a receiver operating characteristic (ROC) curve. Radiology. 1982;143(1):29–36.
33. Schwartz GJ, Muñoz A, Schneider MF, Mak RH, Kaskel F, Warady BA, et al. New equations to estimate GFR in children with CKD. J Am Soc Nephrol. 2009;20(3):629–37.
34. Vallés P, Peralta M, Carrizo L, Martin L, Principi I, Gonzalez A, et al. Follow-up of steroid-resistant nephrotic syndrome:

tubular proteinuria and enzymuria. Pediatr Nephrol Berl Ger. 2000;15(3–4):252–8.
35. Bouillon R, Schuit F, Antonio L, Rastinejad F. Vitamin D Binding Protein: A Historic Overview. Front Endocrinol. 2019;10:910.
36. Woroniecki RP, Shatat IF, Supe K, Du Z, Kaskel FJ. Urinary cytokines and steroid responsiveness in idiopathic nephrotic syndrome of childhood. Am J Nephrol. 2008;28(1):83–90.
37. Woroniecki RP, Orlova TN, Mendelev N, Shatat IF, Hailpern SM, Kaskel FJ, et al. Urinary proteome of steroid-sensitive and steroid-resistant idiopathic nephrotic syndrome of childhood. Am J Nephrol. 2006;26(3):258–67.