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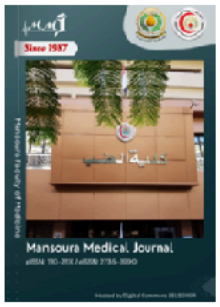

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ORIGINAL STUDY

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Abstract

Background: **Systemic lupus erythematosus (SLE) is an autoimmune inflammatory disease affecting various organs, notably the kidneys, leading to lupus nephritis (LN) in approximately half of patients. Early and accurate diagnosis of LN and initiation of therapy are crucial steps in halting disease progression. Despite its value as a gold standard for diagnosing, classifying, and guiding treatment for LN patients, renal biopsy is expensive, carries risks, and cannot expect the response to immunosuppressive therapy.**

Methods: **The study included 80 SLE patients; 60 patients were biopsy-proven LN (30 patients with active LN, and 30 patients with LN in remission), and 20 without LN. After complete history taking and clinical examination, laboratory investigations were done including measurement of urinary Vitamin D-binding protein (VDBP). The SLE disease activity, and renal disease activity were assessed using SLEDAI and renal SLEDAI score, respectively.**

Results: **Urinary VDBP was significantly higher in LN group with median 3.33 (2.67−4.53) μg/ml when compared with SLE group without LN with median 2.35 (1.40−3.22) μg/ml. However, there were no statistically significant differences in urinary VDBP level between active and remission LN groups, although it was, to some extent, higher in active LN group with median 3.58 (2.73−4.83) μg/ml and 3.23 (1.94−4.25) μg/ml in remission group. Urinary VDBP at cut-off value of 2.12 showed 100% sensitivity and 50% specificity in identifying cases with active LN.**

Conclusion: **Higher urinary VDBP levels may be a potential marker for newly developed LN among SLE patients.**

Keywords: Biomarker, Lupus nephritis, Systemic lupus erythematosus, Systemic lupus erythematosus disease activity index, Vitamin D-binding protein

1. Introduction

Systemic lupus erythematosus (SLE) is a prevalent autoimmune-inflammatory condition that may impact every organ in the body (Luo et al., 2018). About 30−60% of adult SLE patients have renal involvement in the form of lupus nephritis (LN), which increases morbidity and decreases survival in these patients (Davidson, 2016). Prompt and precise diagnosis of LN and treatment start are essential to stop the disease progression (Anders et al., 2020). Routine clinical tests that are commonly used to monitor the activity of LN in everyday medical practice include the quantification of protein in 24-h urine collections, the examination of urinary sediment, and the observation of variations

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in serum anti-double-stranded DNA (anti-dsDNA) antibodies in conjunction with a reduction in complement components C3 and C4 levels. However, for a true representation of the degree of tissue injury and the immunopathological activity occurring in the kidneys in real time, these measurements lack appropriate sensitivity and specificity (Hsieh et al., 2016). A renal biopsy is the gold standard for diagnosing, classifying, and deciding on a LN treatment plan. The biopsy result helps to determine the disease's activity and chronicity, as well as providing prognostic information (Pereira et al., 2017). However, it can not predict which patients will react to immunosuppressive medication, and it is expensive and hazardous (Aragó et al., 2020). Researchers have studied many urinary and serum biomarkers in their search for noninvasive markers that overcome the limitations of the existing ones. Because it is non-invasive and easy to collect, urine is the best biological sample to identify LN biomarkers, and urinary proteins are more selective for renal inflammation than serum proteins (Go et al., 2018).

Vitamin D-binding protein (VDBP) is an albumin-binding protein family member. It is primarily produced by the liver, which binds and transports vitamin D metabolites to target tissues via circulation (Mirkovi et al., 2013a). Normal urine rarely detects VDBP, as the proximal tubular cells typically reabsorb it after the glomerulus filters it. The glomerular filtration and proximal tubular reabsorption of VDBP are important steps in changing 25-hydroxyvitamin D (25 (OH) D) into 1,25-dihydroxyvitamin D (1,25 (OH) 2D) through megalin/cubilin-mediated endocytosis. Simulating glomerular capillary leakage and tubular injury in the urine of SLE patients without proteinuria leads to a decrease in VDBP reabsorption and 1,25 (OH) 2D production. This might make macrophages work harder and boost the production of VDBP, which could improve neutrophil complement-mediated chemotaxis (Go et al., 2018).

Researchers have suggested that urinary VDBP should be used as a noninvasive way to check how much tubulointerstitial inflammation and fibrosis there is because it is known to be higher in people with tubular dysfunction (Lisowska-Myjak et al., 2020). Moreover, urinary VDBP was correlated with the renal SLE disease activity index (SLEDAI) and predicted the development of proteinuria in those patients (Go et al., 2018).

This study aims to assess the urinary VDBP level of Egyptian patients with SLE, as well as its significance as a urinary biomarker for LN and disease activity.

2. Patients and methods

This observational cross-sectional study was conducted at the Internal Medicine Department, Rheumatology and Nephrology Unit of Mansoura University Hospitals in Mansoura, Egypt. It assesses the amounts of VDBP in patients with SLE as a potential urinary biomarker for LN. The study took place over 1 year, from 2022 to 2023.

This study involved 80 patients with SLE who were diagnosed based on the SLE International Collaborating Clinics/American College of Rheumatology Damage Index (SLICC/ACR) (Tedeschi et al., 2018). Among them, 60 patients were confirmed to have LN through renal biopsy (LN group), while the remaining 20 SLE patients did not have LN (SLE without LN group). The LN group patients included two distinct subgroups; the first group (active LN group) comprised 30 patients exhibiting renal flare marked by proteinuria more than 0.5 gm/24 h or other urinary active sediments. The second group (Remission group) comprising 30 patients initially diagnosed with LN via renal biopsy, now in a state of remission devoid of LN flare manifestations characterized by a reduction in proteinuria levels, an improvement or stabilization of kidney function, absence of hematuria and successful tapering of corticosteroids dosages (Malvar et al., 2017). The trial was not eligible to include patients with advanced liver disease, diabetes mellitus, pregnant women, patients with other connective tissue illnesses, or patients with an estimated glomerular filtration rate of less than 60 ml/min.

Every participant underwent a thorough history taking and clinical evaluation. Determination of SLEDAI which is the sum of total score points of 24 descriptors with a minimum score equal to zero and maximum score equal to 105. There are four categories for the SLEDAI score: no, mild, moderate, and severe. The most serious manifestations (renal, neurologic, and vasculitis) are weighted greater than others (such as skin manifestations). Patients are classified as inactive if their score is less than 4, as mildly diseased if it is 4–8, as moderately diseased if it is 9–12, and as severely diseased if it is greater than 12 (Isenberg et al., 2004). Patients with active disease were categorized based on renal involvement as AN (Active LN) if their renal SLEDAI (rSLEDAI) score was greater than or equal to 4 and as ANR (Active non-renal SLE) if their rSLEDAI score was 0. Renal SLEDAI (rSLEDAI) is the amount that represents the total of the four components in SLEDAI connected to urinary examination which include proteinuria, hematuria, pyuria, and urinary casts with score of 4 of each, so it can range between

0 and 16 points (Gupta et al., 2021). The laboratory tests include measuring the levels of serum creatinine, urine analysis along with 24 h urinary protein, tests for C-reactive protein (CRP), erythrocyte sedimentation rate, and full blood count. Additionally, tests for antinuclear antibodies (ANA), anti-double-stranded DNA (dsDNA), serum complement components C3 and C4, and serum albumin are included. Furthermore, the urinary VDBP level was assessed through an ELISA kit using the specified procedure in the provided package insert. Renal samples were evaluated for individuals with LN according to the International Society of Nephrology/Renal Pathology Society (ISN/RPS) categorization system. The classification includes six classes: Class I represents minimal mesangial LN. Class II represents mesangial proliferative LN. Class III represents focal LN (active and chronic; proliferative and sclerosing). Class IV denotes diffuse LN (active and chronic; proliferative and sclerosing; segmental and global). Class V represents membranous LN, whereas Class VI denotes progressive sclerosis LN (Yu et al., 2017).

On personal computers, the Statistical Package for Social Science (SPSS) for Windows version 29 (IBM Corp., Armonk, NY) was used to code, process, and analyze the gathered data. Quantitative data were represented as means [±standard deviation (SD)] for parametric variables or as appropriate medians (interquartile range; (IQR)) for nonparametric variables. Qualitative data were described as percentages and numbers. Utilizing the Kolmogorov–Smirnov test, the normality of the variable distribution was evaluated. When comparing two groups, normally distributed variables were compared using the *t*-test, while non-normally distributed variables were compared using the Mann–Whitney test. Utilizing the χ^2 test allowed for the comparison of qualitative variables. Multiple variable correlation with urine VDBP was examined using Spearman's rank correlation. Utilizing binary logistic regression analysis, significant LN predictors and associations were identified. To predict the diagnosis of LN and the activity of LN in the patients under study, a receiver operating characteristic (ROC) curve was used to determine a cut-off point of urinary VDBP. The cut-off point was selected based on the best possible specificity without sacrificing the sensitivity of choice. A significance threshold of 5% ($P \leq 0.05$) was applied.

Before being included, every participant gave their written, informed consent. The consent process entailed a clear explanation of the study's significance and the assurance of data confidentiality. Prior to commencement, the study design received approval from the Institutional Review Board (IRB) at the Faculty of Medicine, Mansoura University, under the code number MS.21.05.1497.

3. Results

This study includes 80 patients (60 cases SLE with LN and 20 cases without LN). The LN group in this study then classified into Active group and group in remission (each 30 patients). Table 1 shows the Anthropometric and clinical data of the studied groups, there were no statistically significant differences in SLEDI score between LN group and SLE group without LN. However, renal SLEDI score was significantly greater in LN group than SLE group without LN (*P* value < 0.001). on the other side, Both SLEDI and renal SLEDI scores were statistically significantly higher in the active LN group when compared with the LN group with remission (*P* value < 0.001 for each score).

Table 2 shows a comparison of laboratory data including the studied groups. Urinary VDBP was statistically significantly higher in LN group compared with SLE patients with no LN (*P* value < 0.002). Mean hemoglobin concentration in active LN patients (9.7 gm/dl) was significantly lower when compared with patients in remission (11.6 gm/dl) with *P* value less than 0.001. Also, Serum albumin was significantly lower in the active LN group with *P* value less than 0.001. Both erythrocyte sedimentation rate and CRP were statistically significantly higher in the active LN group (*P* value < 0.001 for each). Also, urinary protein, red blood cells and white blood cells were significantly higher in active LN group (*P* value < 0.001,<0.001, and 0.013, respectively). Quantitative proteinuria was significantly higher in LN group (*P* value < 0.001). Both ANA titer and Anti-ds DNA titer were significantly greater in LN group (*P* value < 0.001 for each titer). There were no statistically significant differences in urinary VDBP level between active LN and LN with remission groups although it was slightly higher in active LN group.

Fig. 1 shows VDBP among the study groups.

Based on renal biopsy, the distribution of LN classes in the study included Class IV LN being the most common with 44 patients, followed by Class III LN with eight patients, Class VI LN with five patients, and Class II and Class I LN with two and one patient, respectively.

Table 3 shows correlation of urinary VDBP with different variables in LN patients showing statistically significant negative correlation between VDBP and serum C3 level.

Table 4 shows Analysis of ROC curves for urine VDBP cutoff levels. The urine VDBP was tested for

Table 1. Anthropometric and Clinical data of the studied groups.

	All patients ($n = 80$)	LN ($n = 60$)	SLE without nephritis ($n = 20$)	*P* value	Active LN ($n = 30$)	LN in remission ($n = 30$)	*P* value
Age, years (mean ± SD)	32.5 ± 9.3	31.18 ± 8.97	36.25 ± 9.39	**0.034**	27.8 ± 7.7	34.6 ± 8.9	**0.003**
Sex: N (%)							
Male	14 (17.5)	13 (21.7)	1 (5)	0.089	6 (20)	7 (23.3)	0.089
Female	66 (82.5)	47 (78.3)	19 (95)		24 (80)	23 (76.7)	
Body weight, kg (mean ± SD)	77.1 ± 14.3	77.03 ± 11.83	77.15 ± 20.35	0.973	75.9 ± 12.3	78.1 ± 11.4	0.500
BMI, kg/m^2 Median (range)	27.9 (25.3–30)	28.20 (25.43–29.70)	27.59 (23.65–32.55)	0.820	27.8 (25.5–29)	28.6 (25.2–30.9)	0.408
Duration of disease, months Median (range)	42 (12–84)	42 (12–84)	42 (9.75–93)	0.916	24 (1.75–75)	48 (24–84)	0.078
SBP (mmHg) Median (range)	130 (110–140)	130 (120–140)	110 (100–120)	**<0.001**	130 (120–140)	130 (117.5–140)	0.213
DBP (mmHg) Median (range)	80 (70–80)	80 (70–90)	70 (60–77.5)	**<0.001**	80 (80–90)	80 (70–80)	0.068
SLEDI Median (min–max)	6.5 (4–20)	12 (6–16)	7.5 (4–12.75)	0.110	16 (13.75–20)	6 (4–10)	**<0.001**
Renal SLEDI Median (min–max)	4 (0–8)	8 (4–8)	0 (0–4)	**<0.001**	8 (8–12)	4 (0–4)	**<0.001**

BMI, body mass index; DBP, diastolic blood pressure; LN, lupus nephritis; SBP, systolic blood pressure; SD, slandered deviation; SLEDAI, systemic lupus erythematosus disease activity index.

Table 2. Comparison of laboratory data among the studied groups.

	All patients ($n = 80$)	LN ($n = 60$)	SLE without nephritis ($n = 20$)	*P* value	Active LN ($n = 30$)	LN with remission ($n = 30$)	*P* value
Serum creatinine (mg/dl) Median (range)	0.9 (0.7–1.2)	0.9 (0.7–1.2)	0.9 (0.7–1)	0.663	0.9 (0.6–1.2)	0.9 (0.8–1.2)	0.349
eGFR (ml/min/1.73 m^2) Median (range)	101.6 (86.3–135.2)	102.85 (88.83–135.58)	94.3 (84.7–135.2)	0.756	110.3 (87.9–153.3)	98.2 (87.9–117.5)	0.217
WBCs ($X10^9$/L) Median (range)	5.9 (4.5–8.2)	5.95 (4.83–7.75)	5.8 (3.9–8.8)	0.777	6.3 (4.9–7.9)	5.8 (4.8–7.8)	0.723
Hemoglobin (gm/dl) (mean ± SD)	10.7 ± 2.1	10.7 ± 2.3	10.8 ± 1.6	0.796	9.7 ± 2	11.6 ± 2.2	**<0.001**
Platelets ($X10^9$/L) (mean ± SD)	235 ± 66	238.6 ± 64.6	225 ± 72	0.439	242 ± 72	235 ± 56	0.660
Serum albumin (g/dl) Median (range)	3.2 (2.6–3.9)	3.15 (2.6–3.8)	3.5 (3–3.9)	0.098	2.6 (2.3–3)	3.8 (3.4–4)	**<0.001**
ESR (mm/h) Median(range)	35.5 (20–80)	36.5 (22–83.8)	33 (12.75–70)	0.605	70 (33–100.75)	25 (17.25–37.75)	**<0.001**
CRP (mg/l) Median(range)	0 (0–12)	0 (0–15)	0	**0.005**	12 (0–24.75)	0	**<0.001**
Urinary protein (number of +) Median (range)	1 (0–2)	1 (0–2)	0	**<0.001**	2 (2–3)	0 (0–1)	**<0.001**
Urinary RBCs/HPF Median (range)	4 (2–7)	4 (2.25–10)	2 (2–3.75)	**<0.001**	7.5 (4–14.25)	3 (2–4.75)	**<0.001**
Urinary WBCs/HPF Median (range)	8 (3–12)	9.5 (4–19.5)	3 (2–4)1	**<0.001**	11.5 (5–22)1	8 (3–10)	**0.013**
Quantitative proteinuria Median (range)	350 (135–2062)	515 (224.75–2388)	109.5 (77.25–154.25)	**<0.001**	2376 (1821–3350)	239.5 (135.75–427.5)	**<0.001**
ANA titer (number of folds) Median (range)	2 (0–5.75)	3 (0–7)	0 (0–1.75)	**0.008**	5.5 (3–9)	0 (0–2.25)	**<0.001**
Anti-ds DNA titer Median (range)	1 (0–4)	2 (0–5)	0.5 (0–2)	**0.005**	4 (2–6.25)	0 (0–2.25)	**<0.001**
Serum C3 (mg/dl) Median (range)	97.5 (83.25–104.5)	97 (74–102)	100 (86.5–106.5)	0.325	95 (66.5–102)	98 (93.5–105.5)	0.155
Serum C4 (mg/dl) (mean ± SD)	19.6 ± 7.4	18.5 ± 9.4	22.7 ± 8.9	0.080	16.9 ± 10.9	20 ± 7.3	0.215
Urinary VDBP (μg/ml) Median (range)	2.99 (2.31–4.21)	3.33 (2.67–4.53)	2.35 (1.40–3.22)	**0.002**	3.58 (2.73–4.83)	3.23 (1.94–4.25)	0.337

ANA, antinuclear antibody; Anti-ds DNA, anti-double-stranded DNA; C3,4, complement 3,4 and VDBP, vitamin D binding protein; CRP, C reactive protein; eGFR, estimated glomerular filtration rate; ESR, erythrocyte sedimentation rate; RBC, red blood cells; WBC, white blood cells.

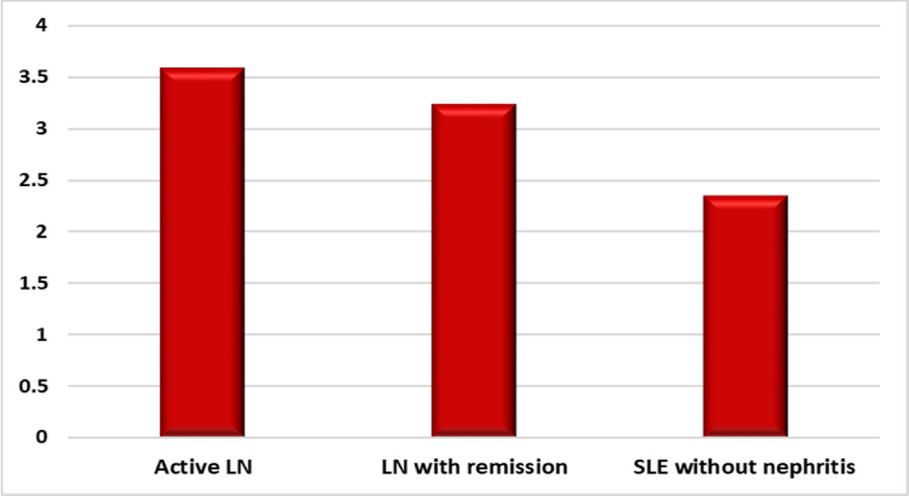

Fig. 1. Vitamin D binding protein among the three groups of the study.

Table 3. Correlation of urinary vitamin D binding protein with different variables in LN patients.

SLEDAI score	Rho	0.179
	P value	0.172
Renal SLEDAI	Rho	0.023
	P value	0.863
eGFR	Rho	0.092
	P value	0.483
Anti-ds DNA titer	Rho	0.168
	P value	0.199
Serum C3	Rho	−0.271*
	P value	0.036
Serum C4	Rho	−0.120
	P value	0.362
24-h urinary protein	Rho	0.077
	P value	0.557
Urine RBC	Rho	0.136
	P value	0.299
Urine WBC	Rho	0.120
	P value	0.361

eGFR, estimated glomerular filtration rate; SLEDAI, systemic lupus erythematosus disease activity index; ANA, antinuclear antibody; Anti-ds DNA, anti-double-stranded DNA; C3,4, complement 3,4 and WBC, white blood cells; RBC, red blood cells.

LN activity detection using ROC analysis, and the results showed that the area under the curve (AUC) was 0.572. Fig. 2 illustrates the equivalent sensitivity and specificity for the detection of LN activity using the urinary VDBP ideal cut-off value of 2.08, as determined by the Youden index.

The AUC was found to be 0.782 when ROC analysis was performed to evaluate urinary VDBP for the identification of active LN vs SLE patients without LN. As shown in Fig. 3, the equivalent sensitivity and specificity for identifying SLE patients with active LN vs those without LN were 100% and 50%, respectively, using the urinary VDBP optimum cut-off value of 2.12, as established by the Youden index.

The area AUC was found to be 0.685 when ROC analysis was performed to assess urinary VDBP for identification of LN with remission vs SLE patients without LN. As shown in Fig. 4, the equivalent sensitivity and specificity for identifying LN with remission vs. SLE patients without LN were 66.7% and 65%, respectively, using the urinary VDBP ideal cut-off value of 2.82, as determined by the Youden index.

Entering significant variables in a binary logistic regression equation analysis ($P > 0.001$) resulted in exclusion of age ($P = 0.662$), SBP ($P = 0.707$), CRP ($P = 0.362$), antidsDNA ($P = 0.058$), and urinary VDBP ($P = 0.162$) from being predictive variables/associates for diagnosis of LN. Conversely, renal SLEDI (OR = 2.228, $P = 0.015$) and quantitative proteinuria (OR = 1.020, $P = 0.006$) were significant predictors/associates for LN as shown in Table 5.

Table 4. Analysis of receiver operating characteristic curves for cutoff values for urinary Vitamin D binding protein.

	Cutoff	AUC (95%CI)	*P* value	Sensitivity	Specificity
Active LN versus LN with remission	2.08	0.572 (0.424–0.720)	0.337	100%	26.7%
Active LN versus SLE without LN	2.12	0.782 (0.651–0.913)	**0.001**	100%	50%
LN with remission versus SLE without LN	2.82	0.685 (0.536–0.834)	**0.028**	66.7%	65%

AUC, area under curve; LN, nephritis; SLE, systemic lupus erythematosus.

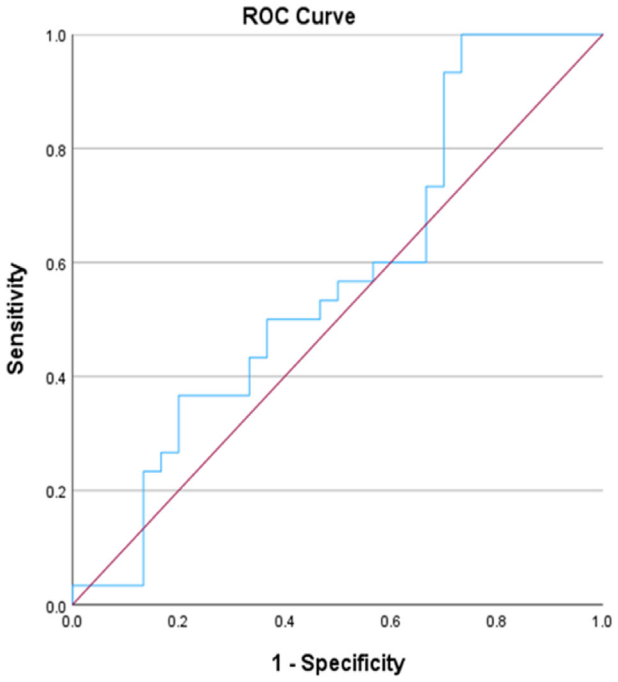

Fig. 2. Active lupus nephritis versus lupus nephritis with remission.

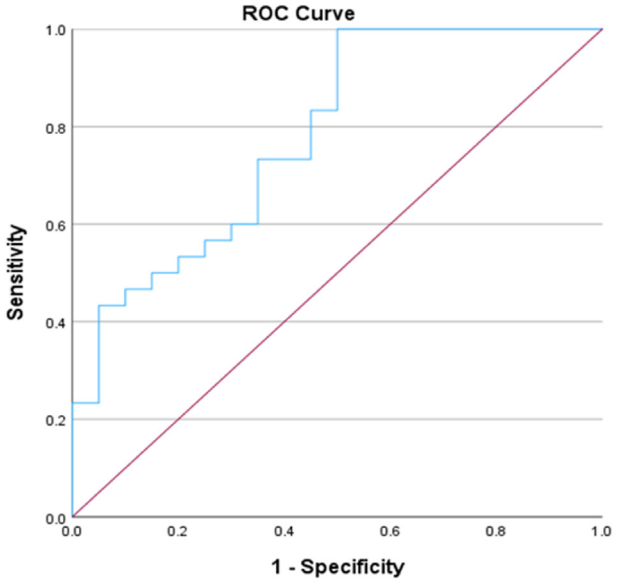

Fig. 3. Active lupus nephritis versus systemic lupus erythematosus without lupus nephritis.

4. Discussion

The purpose of this cross-sectional study was to assess the urinary VDBP levels in Egyptian SLE patients and its significance as a urinary biomarker for LN and disease activity.

The current study included 80 SLE patients (30 cases with active LN, 30 cases LN with remission in addition to 20 cases SLE without LN as a control group).

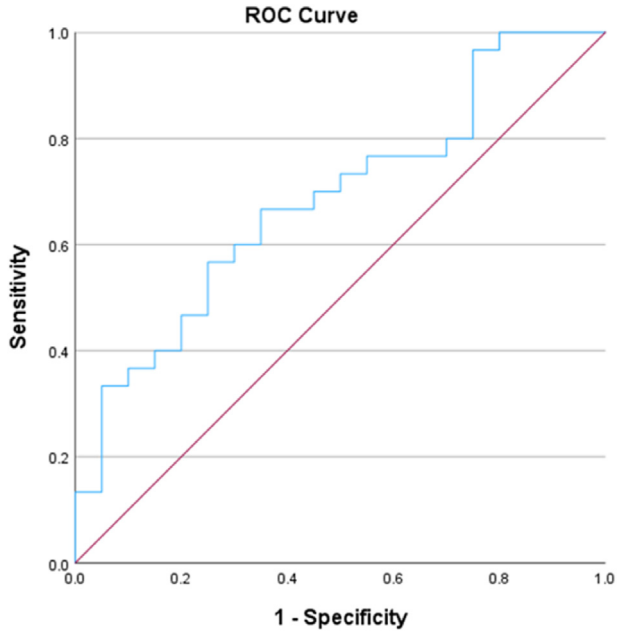

Fig. 4. Lupus Nephritis with remission versus systemic lupus erythematosus without lupus Nephritis.

Table 5. Binary logistic regression of lupus nephritis.

	Beta	OR	*P* value	95% CI	
				Lower	Upper
Age	0.027	1.028	0.662	0.909	1.161
SBP	0.015	1.015	0.707	0.937	1.100
Renal SLEDI	0.801	2.228	**0.015**	1.172	4.235
CRP	0.106	1.111	0.362	0.886	1.395
Quantitative proteinuria	0.019	1.020	**0.006**	1.006	1.034
Anti-ds DNA titer	0.558	1.747	0.058	0.980	3.115
Urinary VDBP	0.382	1.466	0.162	0.857	2.507

Anti-ds DNA, anti-double-stranded DNA; CRP, C reactive protein; OR; odds ratio; SBP, systolic blood pressure; SLEDAI, systemic lupus erythematosus disease activity index; VDBP, vitamin D binding protein.

According to the current study, the LN group's systolic and diastolic blood pressure was statistically substantially higher than the SLE groups without LN, while there were no statistically significant differences between active and remission LN patients as the median systolic and diastolic BP in remission group are comparable to those in active LN group. These findings are supported by Shaharir et al. results which showed high incidence of persistent hypertension between inactive LN patients with conserved renal function of 53.1% (Shaharir et al., 2015). This can be explained by renal glomerular damage and renal vascular endothelial dysfunction that occurs in LN patients either with flare or in remission (Munguia-Realpozo et al., 2019).

The LN group and the SLE group without LN did not differ in SLEDAI scores in a way that was

statistically significant. because SLE patients may have any other disease activity rather than LN which, sequentially, leads to high SLEDAI score. However, SLEDAI score was statistically significantly higher in the active LN group when compared with LN group with remission. These results were in agreement with Emam et al. and Go et al. results, which found that SLEDAI score was significantly higher in the active LN group than remission group (Go et al., 2018; Munguia-Realpozo et al., 2019). Compared with the SLE group without LN, the renal SLEDAI score was statistically substantially higher in the LN group. Additionally, the active LN group's rSLEDAI score was statistically substantially greater than that of the remission-only LN group. The rSLEDAI score was statistically substantially higher in active LN patients compared with LN patients in remission in the Go et al. and Liu et al. studies (Go et al., 2018; Liu et al., 2020).

The present study found that both ANA titer and Anti-ds DNA titer were statistically significantly higher either in LN patients when compared with patients with no LN, or in the active LN group when compared with remission LN group. This agreed with study of Samir et al. and Emam et al. who found that patients with LN either active or inactive had a statistically significantly higher level of ANA in comparison to those with no LN (Emam et al., 2014; Samir et al., 2018). In contrast, the results of Go et al. study showed no statistically significant difference in Anti-ds DNA level between all previous groups (Go et al., 2018).

It is widely recognized that the presence of anti-anti-dsDNA antibodies and ANA is correlated with LN (Bagavant et al., 2004). Furthermore, the involvement of the kidneys has been linked to deposition of anti-dsDNA antibodies in various renal structures such as the glomerulus, basement membrane, and mesangium among patients with SLE exhibiting active nephritis. This correlation has emerged as a valuable indicator for anticipating the development of LN, with anti-dsDNA antibody levels demonstrating a strong relationship with disease activity (Andrejevic et al., 2013).

In the present study, it was noted that urinary VDBP levels were statistically significantly higher in the LN group, with median value of 3.33 (IQR2.67–4.53), in contrast to the SLE group without LN, where the median was 2.35 (IQR 1.40–3.22). This observation aligns with the research of Go et al. which revealed a marked increase of urinary VDBP in SLE patients with LN compared with those devoid of LN in a urinary proteomic assessment (Go et al., 2018). Moreover, Morell et al. documented a significant increase in VDBP levels in the context of active LN when compared with a control cohort of healthy individuals (Morell et al., 2021). Similarly, Liu et al. found in their study that urinary VDBP levels were significantly higher in active LN patients in comparison to those in SLE patients without renal involvement (Liu et al., 2020).

Elevated urinary levels of VDBP in LN patients are associated with tubulointerstitial inflammation, independently of albuminuria. This suggests that the excretion of VDBP in SLE patients with no proteinuria may indicate glomerular capillary leak and subclinical tubular injury, leading to diminished reabsorption of VDBP (Mirkovi et al., 2013b).

In this work, there were no significant statistical differences observed in urinary levels of VDBP between the active and remission groups of LN. The median VDBP levels were marginally higher in the active LN group (3.58 μg/ml with a range of 2.73–4.83 μg/ml) compared with the remission group (3.23 μg/ml with a range of 1.94–4.25 μg/ml). Conversely, a study conducted by Go et al. in 2017 noted a significant elevation in VDBP levels among SLE patients with LN, particularly in those presenting with active LN. Similarly, a subsequent study by Go et al. in 2018 demonstrated markedly higher levels of VDBP in SLE patients with active LN when compared with individuals with inactive LN or SLE patients without LN (Go et al., 2017, 2018).

The reason for these different results may be because both active LN patients and those in remission have comparable degrees of tubulointerstitial inflammation, which is considered the primary determinant of urinary VDBP, even though they may have different levels proteinuria. This reason also explains our results that there were no significant correlations between urinary VDBP with 24 h urinary protein in LN patients.

As regard the utility of urinary VDBP for the detection of active LN versus SLE patients without LN, our results considered it a fair test as shown by ROC curve analysis with the area under curve (AUC) equal 0.782. Using urinary VDBP optimum cut-off value of 2.12. The matching sensitivity and specificity for identifying SLE patients with active LN versus those without LN were 100% and 50%, respectively, as indicated by the Youden index. In the previous study by Go et al., the cutoff value obtained by ROC analysis was much lower than the median VDBP value in LN patients with active proteinuric flare with high sensitivity (80.0%) and good negative predictive value (94.4%) (Go et al., 2018).

Finally, compared with individuals without LN, patients with SLE and LN may have higher urine

levels of VDBP. Furthermore, our findings suggest that elevated VDBP levels in the urine may function as a prognostic indicator for the onset of LN in patients with SLE.

4.1. Limitations of the study

Firstly, the study's sample size was restricted to 80 individuals restricting the generalizability of its findings. Additionally, there is a notable lack of follow-up on the progress of LN patients and their responses to induction therapy over time. So, to improve the reliability of results, it is recommended that additional studies be done with bigger sample sizes. Moreover, there is a need for additional research to validate the utility of VDBP as a diagnostic tool in active LN cases. Expanding the scope of future studies to include measuring serum vitamin D and serum VDBP levels, and examining their correlation with urinary VDBP, will provide a more comprehensive understanding of these associations.

Ethics approval & consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

The study was approved by the Institutional Review Board of Mansoura University (Approval Code No: MS.21.05.1497).

Consent for publication

Written informed consent was waived by the Institutional Review Board.

Availability of data and material

All data generated or analyzed during this study are included in this published article.

Conflict of interest

The authors declare that they have no conflict of interest.

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Authors contributions

All authors have read & approved the manuscript.

Study concept and design was proposed by OME and EAY

Database search by OME, MS, and EN.

Laboratory analysis and interpretation of data: MSE.

Clinical assessment: OME, MS, EAY.

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