Original Research Article

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Urinary tissue factor (uTF), tissue factor pathway inhibitor (TFPI) and plasmin as novel biomarkers in early diagnosis of lupus nephritis

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Abstract

Systemic lupus erythematosus (SLE) is an autoimmune inflammatory disease, with multi systematic affection. Lupus nephritis (LN) is the most frequent cause of renal damage in SLE patients with variable presentations that may progress to end stage renal failure. Coagulation disorders are frequently reported in SLE and LN with higher mortality rates. Renal biopsy is an invasive process, and the existing indicators for LN diagnosis and activity are unreliable. New urinary biomarkers with significant validity, safety, and accuracy are the current focus of most studies. Our study sought to assess the value of urinary tissue factor (uTF), tissue factor pathway inhibitor (TFPI), and plasmin as biomarkers for the early identification and detection of LN and its activity. This was a cross-sectional study, included 100 subjects (80 SLE patients, and 20 healthy controls), they were recruited from the Internal Medicine department, Rheumatology and Nephrology units and outpatient's clinics at Assiut University hospital between the period of 2020 and 2022. All patients underwent full history taking, clinical evaluation, and activity scoring calculation and laboratory investigations. The results showed that the best diagnostic accuracy of LN was observed with TFPI (90% accuracy, sensitivity 80% and specificity 95% with p<0.001 at cutoff point of >193.2 ng/ml), followed by uTF (75.4% overall accuracy at cut off point of >12.6 ng/ml, sensitivity 90% and specificity 68% with p< 0.001) and plasmin (70.3% accuracy at cut off point of >30.5 ng/ml, sensitivity 55% and specificity 78% with *p*< 0.001). Urinary TFPI was the best predictor of LN occurrence with odd ratio of 4.34, (*p*< 0.001). In conclusion urinary TFPI could be used as a diagnostic marker for LN with high accuracy and an early predictor of LN.

Keywords: uTF, TFPI, SLE and LN.

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Introduction

SLE is an autoimmune disease that causes tissue damage through the deposition of immune complexes, microvasculature inflammation, autoantibodies generation, and complement activation. It is a challenging disorder with an

uncertain outcome as it affects different organs, also clinical and serological findings change significantly over time between patients as well as in the same patient.[1] The prevalence, frequency, and severity of clinical and laboratory symptoms of SLE vary according to the ethnic and racial characteristics.[2] It typically affects women of childbearing age due to the effect of estrogen which modulates the lymphocytes activation.[3]

Lupus nephritis (LN); an autoimmune complex glomerulonephritis, one of the most common and severe clinical features of SLE, causing high morbidity and mortality rates.[4] In the United States, approximately 35% of adults with SLE have clinical evidence of nephritis at diagnosis time, with about 50–60% developing nephritis during the first 10 years of disease.[5] Early treatment of acute inflammation, preventing renal scarring, and reaching complete remission of the disease activity are the main aims of LN management.[6] The gold standard for the diagnosis and prognosis monitoring of LN is the renal biopsy; it is an invasive process and cannot be utilized as a regular indicator of nephritis activity. Also, the currently available laboratory investigations, as anti-ds DNA, 24 hours urinary proteins, and complement levels are unreliable.[4,7]

Coagulation disorders are frequently reported in SLE and LN with higher thrombotic events than general population especially in proteinuria or anti phospholipid antibodies detection.[8] Inflammation which occurs in SLE affects all blood coagulation stages, when it occurs in the glomerular stroma, active macrophages promote local production of tissue factor by pro inflammatory cytokines.[9]

Tissue factor, also called platelet tissue factor stimulates fibrin deposition in vivo and produces minute amounts of thrombin,[10] is the primary activator of the extrinsic coagulation cascade. Human crescentic glomerulonephritis greatly increases urinary tissue factor (uTF) glomerular expression.[11]

Tissue factor pathway inhibitor (TFPI) is a matrix-associate serine protease inhibitor, and the basic inhibitor of uTF.[12] TFPI is detected in the kidneys of normal rabbits and in a crescentic model of glomerulonephritis as fibrin deposition might be a main factor of injury.[13] It was also found in cellular crescents, particularly apparent in fibrous or fibro-cellular crescents in glomerulonephritis and glomeruli exhibiting extra capillary proliferation. Therefore, it might indicate how persistent the crescentic lesions are.[13]

Plasmin enhances the recruitment of phagocytic cells, the pro-inflammatory response, and the debris clearance.[14] Additionally, it has a role in dissolving blood clots.[15] The net fibrin accumulation in the glomeruli is generated from pathogenetic pro-coagulant effects by uTF and the protective effects of the plasminogen plasmin system.[16.]

Depending on the finding of the coagulation disorders associated with SLE and LN, our study focused on detection of these markers (TFPI, uTF and plasmin) in the urine of those patients for detection their utility in diagnosis of LN and their association with the disease activity scores.

Subjects and Methods

The study was performed in the Department of Internal Medicine, Rheumatology and Nephrology units and outpatient's clinics, Assiut University Hospitals, Egypt, and included 80 SLE patients fulfilled the 2019 ACR criteria for diagnosis of SLE[17] (40 SLE patients without nephritis and 40 patients with biopsy-proven LN). The study also included 20 normal individuals as the control group, they were age and sex matched to study subjects.

All patients were subjected to full history taking, medical history of the current received treatment, history of dialysis and clinical examination, included blood pressure and, temperature (patients considered feverish if temperature above 37.2 °C at the time of examination after exclusion of infections), pulse (rate, rhythm and the peripheral pulsation), with full respiratory, cardiac, articular (arthralgia or arthritis of >2 joints), cutaneous (rash, alopecia and mucosal ulcers), neurological, ophthalmological examination (retinal hemorrhages, serous exudate or hemorrhages in the choroid, optic neuritis, scleritis or episcleritis after exclusion of

hypertension, infection, or drug causes) and serositis (pleurisy or pericarditis detected clinically, radiologically or by electrocardiogram confirmation). All manifestations were presented at the time of visit. eGRF was calculated using the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation by an online calculator.[17]

Assessment of Disease Activity score

All patients underwent assessment of Disease Activity Index-2000 (SLEDAI-2k).[18] For renal involvement, renal SLE Disease Activity Index (rSLEDAI) was used to assess renal disease activity. The score consists of the four kidney-related parameters: pyuria, hematuria, proteinuria, and urinary casts (RBCs or WBCs casts). Score for the renal SLEDAI ranges from 0 to 16. The rSLEDAI score of > 4 was taken as an indicator of active lupus nephritis.[18]

Renal biopsy

Renal biopsy was taken from SLE patients who presented with unexplained impairment in renal function or proteinuria of at least 1 g/24 hours or proteinuria of at least 500 mg/24 hours associated with either microscopic hematuria (5 red blood cells/high-power field on urinalysis), cellular casts, or both according to the American College of Rheumatology (ACR) guidelines for renal biopsy.[19] Ultrasonographic guided renal biopsy was taken by an expert under complete aseptic condition using renal biopsy needle (16G*200mm) for histopathological examination and Immunofluorescence staining for staging.

Exclusion Criteria

All patients presented with renal artery stenosis, congenital renal diseases, renal tumours, other causes of glomerulonephritis, chronic kidney disease due to other causes rather than SLE, pregnancy, coagulation disorders, diabetes mellitus, hypertension, obesity, and other connective tissue diseases were excluded from the study.

Samples collection

All investigations were performed according to the manufacturer's instructions, in Laboratory of Clinical Immunology, Department of Clinical Pathology, Assiut university hospitals, Egypt.

1. Urine Samples were obtained from patients and controls at the time of patient visit or admission. Each sample was divided into 2 parts; the first part was used for complete urine analysis and urinary albumin creatinine ratio. The second part was centrifugated and divided into aliquots and stored at -70 °C until used in the enzyme linked immunoassay (ELISA) for determination of urinary tissue factor, tissue factor pathway inhibitor and plasmin among studied groups.

2. Venous blood samples (about 10 ml) were collected from each study subject under aseptic conditions. Each sample was divided into 2 parts; the first part (4 ml) was collected into 2 tubes containing anticoagulant (K3 EDTA) and sodium citrate. The first tube was used for complete blood count (CBC), erythrocyte sedimentation rate (ESR) and direct Coombs' test. The second tube was used for coagulation profile and D-dimer. The second blood part (6 ml) was allowed to clot in Wassermann tubes. Sera were obtained by centrifugation and divided into aliquots and were used for kidney and liver function tests, serological, CRP and immunological markers.

Complete blood count (for patients and control subjects) was done on fully automated high-volume hematology analyzer (ADVIA® 2120i System, Germany), according to the manufacturer's instructions. For coagulation profile (prothrombin time and concentration and activated partial thromboplastin time), D-dimer, and lupus anticoagulant (LA) Sysmex CS 2500 instrumentation supplied Siemens Healthineers, Germany), according to the manufacturer's instructions.

Coombs' test was done using anti-IgG ID cards ("LISS/Coombs"-50531-Diamed Gmbh-BIO.RAD, Germany), according to the manufacturer's instructions.

Kidney function tests (serum urea and creatinine), liver function tests (serum total and direct bilirubin, total protein, and albumin) were performed for all study subjects using an automated chemistry analyzer (ADVIA® 1800

high volume chemistry analyzer supplied by Siemens Healthineers, Germany), according to the manufacturer's instructions.

Antinuclear antibody (ANA), anti-double stranded DNA (anti-ds DNA), C-reactive protein (CRP), Complement component 3 and 4 (C3 and C4) were assessed by an automated immunodiagnostic system (Algeria system, Orgentec Diagnostic GmbH, Germany), according to the manufacturer's instructions.

Estimation of urinary tissue factor, tissue factor pathway inhibitor and plasmin

1. The urinary human tissue factor (uTF) was quantitatively estimated using ELISA kits (Cat. no. E1195Hu, Human tissue factor ELISA kit, BT LAB Bioassay Technology Laboratory, Shanghai Crystal Day Biotech CO., LTD. China), according to the manufacturer's instructions. The kit range: 0.05-30 ng/ml with a sensitivity of 0.028 ng/ml.

2. The urinary human tissue factor pathway inhibitor (TFPI) was quantitatively estimated using ELISA kits (Cat. no. E1188Hu, Human tissue factor pathway inhibitor ELISA kit, BT LAB Bioassay Technology Laboratory, Shanghai Crystal Day Biotech CO., LTD. China), according to the manufacturer's instructions. The kit range: 5- 900 ng/ml, with a sensitivity of 2.02 ng/ml.

3-The urinary human plasmin was quantitatively estimated using ELISA kits (Cat. no. E1136Hu, Human plasmin ELISA kit, BT LAB Bioassay Technology Laboratory, Shanghai Crystal Day Biotech CO., LTD. China), according to the manufacturer's instructions. The kit range : 0.5-100 ng/ml, with a sensitivity of0.27 ng/ml.

Statistical Analysis

Data was collected and analyzed by using the Statistical Package for the Social Science (SPSS), version 20, IBM, and Armonk, New York. Quantitative data are expressed as mean ± standard deviation (SD) and were compared with Student t test in case of comparison between two different groups, while ANOVA was used in case of more than two groups followed by post-hoc analysis. Nominal data were given as number (n) and percentage (%). The Chi-squared test was implemented on such data. Pearson correlation was used to determine correlation between uTF, plasmin and TFPI with other variables. Diagnostic accuracy of these new biomarkers was evaluated by the receiver operator characteristics (ROC) curve for diagnosis of SLE and LN. Logistic regression analysis was used to determine possible risk factors for LN in patients with SLE. The level of confidence was kept at 95% and hence, *p* value was considered significant if < 0.05.

Results

The mean age of patients with LN, without LN and the control group was 29.70 ± 10.57, 28 ± 5.46 and 29.70 ± 8.32 years, respectively and the majority of them were females (Table 1). Patients with LN had higher temperature (>37.2 °C) than patients without LN with p=0.05. Patients with LN had significantly higher SLEDAI in comparison to those without LN (14.55 ± 3.44 vs. 6.24±2.33, p < 0.01). rSLEDAI of LN patients was 8.60 ± 3.50.

Table 1. Baseline data and characteristics of studied subjects.

	With LN (n= 40)	Without LN (n=40)	Control group (n= 20)	*p1*	*p2*	*p3*
Age (years)	29.70 ± 10.57	28 ± 5.46	29.70 ±8.32	NS	NS	NS
Sex						
Male	4 (10%)	6 (15%)	4 (20%)	NS	NS	NS
Female	36 (90%)	34 (85%)	16 (80%)			
Duration since SLE diagnosis (years)	4.95 ± 4.42	2.91 ± 2.12	0	NS		
fever	18 (45%)	10 (25%)	0	0.05		
Articular manifestation	11 (27.5%)	12 (30%)	0	NS		

Table 1. Continued.

	With LN (n= 40)	Without LN (n=40)	Control group (n= 20)	*p1*	*p2*	*p3*
Cutaneous manifestation	12 (30%)	19(47.5%)	0	NS		
Neurological manifestation	5 (12.5%)	4 (10%)	0	NS		
Eye manifestation	3 (7.5%)	2 (5%)	0	NS		
Serositis	8 (20%)	7 (17.5%)	0	NS		
Dialysis	8 (20%)	0	0	<0.001		
Blood Pressure						
SBP (mmHg)	121.50 ± 11.36	119 ± 12.52	117.11±4.87	NS	NS	NS
DBP (mmHg)	77.50 ± 8.60	75.50 ± 10	76.76 ± 8.81	NS	NS	NS
SLEDAI	14.55 ± 5.44	6.24±2.33	----------	< 0.01		
rSLEDAI	8.60 ± 3.50	----------	----------			
Current medications						
steroid	30 (75%)	32 (80%)	----------			
Azathioprine with steroid	10 (25%)	8 (20%)	----------			
Hydroxy-chloroquine with steroids	30(75%)	39(97.5%)	----------			
Anti-platelets/ anticoagulants	3(7.5%)	0	----------			

Data are expressed as mean (±SD), frequency (percentage). *P* > 0.05 is not significant (NS). LN: lupus nephritis; DBP: diastolic blood pressure; SBP: systolic blood pressure. SLEDAI: SLE Disease Activity Index, rSLEDAI: renal SLE Disease Activity Index, *p*1 compares between patients with nephritis and those without nephritis; *p*2 compares between patients with nephritis and the control group; *p*3 compares between those without nephritis and control group.

Baseline laboratory data of studied groups

Coomb's test and different autoantibodies were not different between patients with LN and those without LN (p> 0.05). Patients with LN had significantly lower levels of Complement 3 and 4, and positive lupus anticoagulant was detected only in 2 subjects with LN. All studied autoantibodies were negative in the control group and also, they had normal level of C3 and C4. Other laboratory data are presented in (Table 2).

Table 2. Baseline laboratory data of studied groups.

	With LN (n= 40)	Without LN (n=40)	Control group (n= 20)	*p1* value	*p2* value	*p3* value
Hemoglobin (mg/dl)	9.97 ± 1.75	11.37 ± 1.35	12.16 ± 1.58	<0.001	<0.001	NS
WBCs (10^3/μl)	7.51 ± 5.75	5.41 ± 3.24	6.36 ± 1.76	NS	NS	NS
Platelets (10^3/μl)	303.85±148.3	217.25±165.52	289.20±101.52	NS	NS	NS
Reticulocytes (%)	1.46 ± 0.60	1.12 ± 0.88	0.38 ± 0.22	NS	<0.001	NS
Albumin (mg/dl)	29 ± 6.31	32 ± 3.33	45.45 ± 3.66	<0.001	<0.001	0.02
aPTT (second)	31.03 ± 5.58	28.27 ± 5.06	27.30 ± 7.95	NS	NS	NS
D-dimer (μg/ml)	4.26 ± 2.45	1.66 ± 0.91	0.09 ± 0.07	<0.001	<0.001	<0.001
ESR (ml/hour)	91.40 ± 38.60	59.75 ± 22.77	11.35 ± 4.52	<0.001	<0.001	NS
CRP (mg/dl)	13.82 ± 12.99	12.08 ± 11.92	3.36 ± 3.04	<0.001	<0.001	0.03
Urea (mmol/L)	16.10 ± 11.47	6.83 ± 3.78	5.29 ± 1.97	<0.001	<0.001	NS
Creatinine (μmol/L)	231 ± 189	80.36 ± 21.28	61.85 ± 21.87	<0.001	<0.001	NS

Table 2. Continued.

	With LN (n= 40)	Without LN (n=40)	Control group (n= 20)	*p1* value	*p2* value	*p3* value
eGFR (ml/min/1.73m)	58.47 ± 8.34	96.11 ± 3.56	101.34 ± 5.55	<0.001	<0.001	NS
uACR (mg/g)	500.60±376.27	16.45 ± 7.58	10.20 ± 5.59	<0.001	<0.001	NS
Immunological markers (percentage %)						
Positive ANA	39 (95%)	38 (95%)	0	NS		
Positive anti-dsDNA	36 (90%)	34 (85%)	0	NS		
Consumed C3	30 (75%)	10 (25%)	0	<0.001		
Consumed C4	22 (55%)	4 (10%)	0	<0.001		
Positive LA	2 (5%)	0 (0%)	0	NS		
Positive Coomb's test	10 (25%)	4 (10%)		NS		

Data expressed as mean (±SD), frequency (percentage). *P* > 0.05 is not significant (NS). p1 compares between patients with nephritis and those without nephritis; p2 compares between patients with nephritis and the control group; p3 compares between those without nephritis and the control group. WBC: white blood cells; uACR: urinary albumin creatinine ratio; ANA: Antinuclear Antibody Test; CRP: C- reactive protein; ESR: erythrocyte sedimentation rate; Anti-dsDNA; Anti-double stranded DNA antibodies; LA: Lupus Anticoagulant.

Urinary tissue factor, tissue factor pathway inhibitor and plasmin among studied groups

Patients with LN had significantly higher level of TFPI and uTF in comparison to those without LN (*p*<0.001 for both) (Table 3). However, plasmin level was not different in patients with LN and patients without LN (*p* =0.18). But plasmin was significantly different between patients with nephritis and without nephritis when compared to the control group (*p* = 0.001 and *p*=0.02, respectively).

Table 3. Urinary tissue factor, tissue factor pathway inhibitor and plasmin among studied groups.

Marker	LN (n= 40)	Without LN (n=40)	Control group (n= 20)	*p1*	*p2*	*p3*
TFPI (ng/ml)	337.30 ± 164.41	119.35 ±56.97	42.68 ± 12.81	<0.001	<0.001	0.01
uTissue factor (ng/ml)	19.96 ± 6.22	15.42 ± 3.95	3.67 ± 2.32	<0.001	<0.001	<0.001
Plasmin(ng/ml)	32.83 ± 11.98	28.09 ± 11.96	20.02 ± 9.06	NS	<0.001	0.02

*p*1 compares between patients with nephritis and those without nephritis; *p2* compares between patients with nephritis and the control group; *p3* compares between those without nephritis and the control group. *p* > 0.05 is not significant (NS). uTF: urinary tissue factor; TFPI: tissue factor pathway inhibitor.

Correlations of urinary tissue factor, tissue factor pathway inhibitor and plasmin with different variables

It was found that uTF had positive correlations with the duration of SLE, d-dimer, ESR, CRP, urea, creatinine, uACR, SLEDAI and rSLEDAI. It had negative correlations with hemoglobin, eGFR, and albumin. TFPI had positive correlations with d-dimer, ESR, CRP, creatinine, ACR and SLEDAI. In addition, TFPI had negative correlations with hemoglobin. Plasmin had positive correlations with duration of SLE, d-dimer, ESR, CRP, urea, creatinine, uACR, SLEDAI and rSLEDAI, with negative correlations with hemoglobin and eGFR.

Table 4. Correlations of urinary tissue factor (uTF), tissue factor pathway inhibitor (TFPI), and plasmin with different variables.

	uTF		TFPI		plasmin	
	r	*p*	r	*p*	r	*p*
Age (year)	0.08	NS	-0.11	NS	-0.01	NS
Duration since SLE diagnosis (yr)	0.29	0.02	0.15	NS	0.52	< 0.001
SBP (mmHg)	0.11	NS	0.11	NS	0.13	NS
DBP (mmHg)	0.02	NS	0.02	NS	0.14	NS
Hemoglobin (mg/dl)	-0.51	< 0.001	-0.26	0.04	-0.46	< 0.001
Leucocytes (10^3/μl)	0.12	NS	-0.10	NS	0.13	NS
Platelets (10^3/μl)	0.19	NS	-0.10	NS	0.40	NS
Reticulocytes (%)	0.11	NS	-0.07	NS	0.40	0.05
Albumin (mg/dl)	-0.29	0.02	-0.11	NS	-0.04	NS
aPTT (second)	0.08	NS	-0.07	NS	-0.15	NS
D-dimer (μg/ml)	0.43	< 0.001	0.36	< 0.001	0.57	< 0.001
ESR (ml/hour)	0.62	< 0.001	0.50	< 0.001	0.66	< 0.001
CRP (mg/dl)	0.40	< 0.001	0.29	0.02	0.31	0.01
Urea (mmol/l)	0.40	< 0.001	0.20	NS	0.52	< 0.001
Creatinine (μmol/l)	0.55	< 0.001	0.25	0.05	0.53	< 0.001
eGFR (ml/min/1.73m2)	-0.39	< 0.001	-0.11	NS	-0.47	< 0.001
uACR (mg/g)	0.52	< 0.001	0.32	0.01	0.35	< 0.001
rSLEDAI	0.67	< 0.001	0.04	NS	0.33	< 0.001
SLEDAI	0.62	< 0.001	0.37	< 0.001	0.67	< 0.001

aPTT: activated partial thromboplastin time, uACR: urinary albumin creatinine ratio. Data expressed as r value (strength of correlation) and *P* > 0.05 of correlation is not significant (NS).

Diagnostic accuracy of uTF, TFPI and plasmin in diagnosis of SLE

For diagnosis of SLE, it was found that the best diagnostic accuracy was observed with uTF with cutoff point of > 8.8 ng/ml and 100% accuracy with AUC was 1.0. It was found that TFPI had overall accuracy of 95.4% with AUC of 0.99 at cut off point of > 67.9 ng/ml. While plasmin had the least accuracy for diagnosis of SLE of 65.1% with AUC of 0.74 at cut off point of >27.5 ng/ml (Table 5).

Table 5. Accuracy of urinary tissue factor (uTF), tissue factor pathway inhibitor (TFPI) and Plasmin in diagnosis of SLE.

Indices	uTF	TFPI	Plasmin
Sensitivity	100%	93%	60%
Specificity	100%	100%	75%
PPV	100%	100%	83.5
NPV	100%	87%	48.4%
Accuracy	100%	95.4%	65.1%
Cutoff point	> 8.8	> 67.9	> 27.5
Area under curve	1	0.99	0.74
p value	< 0.001	< 0.001	< 0.001

p value was significant if < 0.05. uTF: urinary tissue factor; TFPI: tissue factor pathway inhibitor; PPV: positive predictive value; NPV: negative predictive value.

Diagnostic accuracy of uTF, TFPI and plasmin in diagnosis of LN

For diagnosis of LN, it was found that the best diagnostic accuracy was observed with TFPI at cutoff point of > 193.2 ng/ml, 90% accuracy with AUC of 0.94. Followed by uTF, had 75.4% overall accuracy with AUC of 0.86 at cut off point of > 12.6 ng/ml. Plasmin had 70.3% accuracy, AUC of 0.71 at cut off point of >30.5 ng/ml (Figure 1 and Table 6).

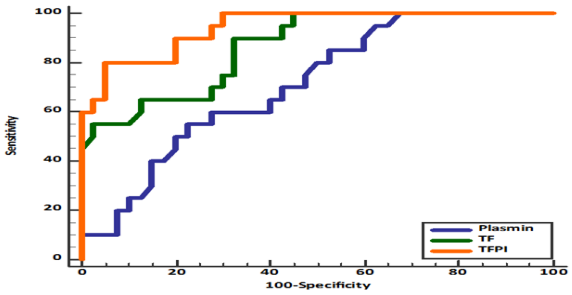

Figure 1. Receiver operator characteristics (ROC) curve for accuracy of urinary tissue factor (uTF), tissue factor pathway inhibitor (TFPI) and Plasmin in diagnosis of LN.

Table 6. Accuracy of urinary tissue factor (uTF), tissue factor pathway inhibitor (TFPI) and Plasmin in diagnosis of LN.

Indices	uTF	TFPI	Plasmin
Sensitivity	90%	80%	55%
Specificity	68%	95%	78%
PPV	58%	89%	55%
NPV	93%	91%	78%
Accuracy	75.4%	90%	70.3%
Cutoff point	> 12.6	> 193.2	> 30.5
Area under curve	0.86	0.94	0.71
p value	< 0.001	< 0.001	< 0.001

p value was significant if < 0.05. uTF: urinary tissue factor; TFPI: tissue factor pathway inhibitor; LN: lupus nephritis; PPV: positive predictive value; NPV: negative predictive value.

Predictors of lupus nephritis in patients with systemic lupus erythematosus

Based on the current study, predictors for lupus nephritis among patients with SLE were SLEDAI (odd's ratio= 1.26) and urinary level of TFPI (odd's ratio= 4.34) (Table 7).

Table 7. Predictors of lupus nephritis in patients with SLE.

Predictors	Odd's ratio	95% CI	*p* value
Age	1.09	0.90-2.10	NS
Male sex	1.02	0.56-3.44	NS
Duration of SLE	1.07	0.22-3.01	NS
SLEDAI	1.26	1.11-2.34	0.01
uTF	0.70	0.34-1.09	NS
TFPI	4.34	2.01-8.87	< 0.001
Plasmin	0.87	0.22-1.11	NS

p > 0.05 is not significant (NS). uTF: urinary tissue factor; TFPI: tissue factor pathway inhibitor; SLE: systemic lupus erythematosus; SLEDAI: SLE-disease activity index; CI: confidence interval.

Discussion

SLE has a variety of clinical features affecting the skin, joints, and other organs. LN is a primary cause of morbidity and mortality, altering the course and prognosis of SLE.[4] It has greater risk for thrombosis occurrence mainly if associated with heavy proteinuria.[20] Many studies illustrated the poor reliability in LN diagnosis depending on the clinical features alone or the current laboratory markers.[21,22] Consequently, we focused on screening and identifying non-invasive biomarkers for the early diagnosis and monitoring of SLE and LN. We performed a cross-sectional study, included 80 SLE patients and 20 normal controls.

We found that patients with LN had significantly lower hemoglobin levels. Anemia in LN could be explained by different and complex causes. In our study autoimmune hemolytic anemia was detected in 25% of LN patients' positive Coombs test. D-dimer is a fibrin degradation product that plasmin releases from cross-linked fibrin. Higher D-dimer level does not always signify thrombosis, as many conditions can lead to initiation of intravascular fibrin production without overt thrombus formation.[23] In our study D-dimer was significantly higher among patients with LN (p < 0.001), and also significantly elevated in the group of lupus without nephritis than the control group with (p<0.001). This could be explained by its plasma clearance via renal excretion. It was reported that serum fibrinogen levels were raised in the nephrotic syndrome which accompanied by an increased activity of the fibrinolytic system.[24]

Also, patients with LN had significantly higher CRP and ESR, this agreed with Lee et al., 2008,[25] who reported significantly higher CRP levels in SLE patients with organ damage than in those without such damage. Serum CRP levels rise equivalent to disease activity in inflammatory cases. However, its behavior in SLE has been surprising and subject to controversy.[26] It was suggested that an elevated CRP could occur in SLE patients presented with serositis,[25] polyarthritis,[27] and nephritis as well as chronic renal failure,[28] therefore, this could explain our findings.

Patients with LN nephritis had significantly lower level of complement 3 (75% vs. 25%, p< 0.001) and C4 (55% vs. 10%; p< 0.001) compared to patients without nephritis and this agreed with the other studies, which detected the association of low serum C3 with LN,[29] and the complex association between renal flares and consumption of C3 and/or C4.[30] Rossi et al., 2022[31] hypothesized that low C3 in LN cases is due to the expression of chronic acyl carrier domain (ACP) over activation with unclear mechanisms.

We studied the level of the three urinary markers in SLE patients and results showed that urinary TFPI, uTF and plasmin were significantly higher in both groups of SLE (with nephritis (p<0.001, p<0.001and p<0.001) and without nephritis (p =0.01, p<0.001 and p=0.02, respectively) compared to the controls. The highest accuracy, sensitivity and specificity in lupus diagnosis (100%) detected with uTF, followed by TFPI accuracy (95.4%), sensitivity (93%) and specificity (100%), the least accuracy 65.1% was detected with plasmin, this agreed with Salah Eldeein et al., 2021[32] who studied only the urinary plasmin levels in SLE and control groups and found that there was significant difference in the mean plasmin between the two groups (p< 0.001), being higher in SLE cases.

On studying these markers in LN patients, we found that patients with LN had significantly the highest level of urinary uTF, TFPI and plasmin compared to the other groups. uTF had the highest sensitivity (90%) for diagnosis of LN, while the highest accuracy and specificity observed with TFPI (90% and 95%, respectively), plasmin presented with the lowest sensitivity and specificity (55% and 78%, respectively, p < 0.001 for all). The elevation of these factors in cases of LN can referred to the concept that inadequate stimulation of the coagulation system and the fibrinolysis system has been associated with the pathophysiology of glomerular injury and renal active macrophage cells produced and encourage regional uTF synthesis as renal stromal inflammation takes places[33]. The permeability of renal glomeruli, which depends on the fibrinolysis counter system, is compromised because this activates

the extrinsic coagulation pathway, which causes thrombin to be activated and then a fibrin clot to form renal glomeruli, which relies on the fibrinolysis counter system [10]. According to experimental data, dysregulation of the human TFPI/uTF balance early in the glomerulonephritis is likely to play a significant role in the onset of pathological glomerular fibrin deposition and the exacerbation of the disease.[34]

Our data agreed with those of a study by Fawzy et al., 2022[35] who showed that the mean urinary plasmin was significantly higher in SLE cases with LN than non-LN patients and healthy controls (p<0.001). However, our results disagreed with those of Qui et al., 2019[4] who found that active LN patients showed higher urinary levels of plasmin (p<0.0001), uTF (p<0.01) and TFPI (p<0.001) with highest sensitivity appeared with plasmin reaching 100% compared to the inactive LN patient. Angiostatin, the autocatalytic product of plasmin, was hypothesized by some authors to be highly expressed inside the kidneys of LN patients.[36] The origin of plasmin in the murine model was investigated by Svenningsen et al., 2009,[37] who proposed that urinary plasmin in patients with LN primarily originates from the kidneys and that tubular urokinase-type plasminogen activator could activate plasminogen and convert it to plasmin in nephrotic urine. Although other studies as those of Chu et al., 1988[38] and Argüelles et al., 1991[39] studied the level of plasmin but in serum and reported no change in SLE patients' serum levels. These different results may be related to variable disease activity status at the time of testing, as plasminogen is acute phase response,[40] or this difference could result from the change in detection of the plasmin in serum and urine, and the timing difference of the sampling may have an effect.

In the present work, we studied the correlations of urinary TFPI, uTF and plasmin with different clinical and laboratory variables. uTF and plasmin had positive correlations with duration of SLE, d-dimer, SLEDAI and rSLEDAI and negative correlations with hemoglobin, eGFR and albumin. However, TFPI was positively correlated with d-dimer, ESR, CRP, creatinine, ACR and SLEDAI and negatively correlated with hemoglobin. This was nearly in agreement with findings of a study by Qin et al., 2019[4] who found that plasmin, uTF and TFPI correlated positively with rSLEDAI (r = 0.26 p < 0.01, r = 0.50 p < 0.0001, r = 0.33 p < 0.0001, r = 0.40 p < 0.0001, respectively). Plasmin also showed a weak but statistically significant negative correlation with eGFR (r = - 0.23, p < 0.05). Also, the study by Fawzy et al. 2022[35] showed that urine plasmin levels were significantly correlated with the SLEDAI score (r=0.63, p=0.001), rSLEDAI score (r=0.34, p=0.015), disease duration, ESR, CRP, 24-h urinary proteins, serum creatinine and BUN with significant negative correlations with C3 and C4.

We studied the predictors of LN occurrence in SLE patients, and found that the SLEDAI and TFPI are the predominant predictors of LN. The study by Qin et al, 2019 [4] found that urine plasmin emerged as the strongest independent predictor of eGFR, after adjusting for age, gender, and ethnicity. According to their study, next to plasmin, urine TFPI emerged as the only other independent predictor of eGFR and renal disease status. Indeed, it was the only urine marker that could have potential discrimination when added to urine plasmin, in distinguishing active LN. Studies examining the circulating levels of TFPI in SLE patients have yielded contradicting conclusions. Some studies showed that plasma TFPI concentration and activity were lower in SLE patients compared to healthy controls,[41] while others found elevated free TFPI levels that correlated with lupus disease activity and endothelial damage.[42] In conclusion, based on our study findings, urinary TFPI could be proposed as a diagnostic marker for LN with high accuracy and an early predictor of LN.

Author Contributions

MHM, EAT, SSE, ASAM, ASAB and RAM; conceptualization, data collection, formal analysis, methodology, visualization, writing original draft, writing review and editing. ASAM; conceptualization, methodology and performed the laboratory investigations. EAT, SSE, ASAM supervision; read and agreed to the published version of the manuscript. The authors read and approved the final manuscript.

Declaration of Conflicting Interests

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Ethical approval

The study protocol was reviewed and approved by the Institutional Review Board of Faculty of Medicine, Assiut University, Egypt (approval dated May 2020). The study clinical trial approval is NCT04218890.

Informed consent

An informed consent was obtained for each study participant before included in the study.

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