Original Article

Study of the Role of Urinary Tweak as a Biomarker of Lupus Nephritis.

Magdy Abd Elaziz Zehairy,[1] Eiman Soliman,[1] Akram Abd El-Moneim Deghady,[2] Doaa Abd Elaziz.[3]

Department of Internal Medicine[1], Unit of Rheumatology & Immunology. Department of Clinical Pathology[2], MBBCh Student[3], Faculty of Medicine, Alexandria University .

Abstract:

Background: Systemic lupus erythromatosis (SLE) is an auto immune disease characterized by overproduction of various auto-antibodies affecting almost all body organs. Renal involvement is common in SLE and often determines the course of the disease. The cytokine TWEAK is one of the novel biomarkers of renal lupus activity. **Objectives:** The aim of the work was to study the role of uTWEAK in lupus patients, its relation to clinical manifestations of the disease especially lupus nephritis. Furthermore, its correlation with the conventional measures of renal lupus activity. **Subjects:** The present study was conducted on 30 patients with biopsy-proven lupus nephritis, 20 SLE patients without nephritis and 20 healthy subjects of matched age and sex as a control. The diagnosis of patients with SLE was based on fulfilling at least four of the American College of Rheumatology Classification Criteria. **Methods:** All patients were subjected to thorough history taking and complete clinical examination to detect various organs involvement by SLE. Laboratory investigations done included: CBC, ESR, FBG, blood urea, serum creatinine, complete urine analysis, 24 hours urinary protein ,urinary protein/creatinine ratio, ANA, Anti-ds DNA, C3, C4, urinary TWEAK by ELISA, SLEDAI for all lupus patients and renal SLEDAI for lupus nephritis**.** **Results:** Among all cases of SLE, the highest mean of uTWEAK was detected in patients with renal manifestations (lupus nephritis) ($p<0.001$). There is a statistically significant increase in uTWEAK level in patients with lupus nephritis than patients with SLE without nephritis ($p<0.001$) as well as than controls ($p<0.001$).In patients with lupus nephritis, there is a statistically significant positive correlation between uTWEAK level and ANA($p=0.034$), Anti-ds DNA($p=0.005$), 24 hours Urinary protein($p=0.001$), urinary protein/creatinine ratio ($p<0.001$), hematuria ($p<0.001$), pyuria ($p=0.016$), proteinuria ($p=0.014$), urinary casts ($p=0.001$), Total SLEDAI ($p=0.018$), Renal SLEDAI($p<0.001$) as well as pathological activity index of renal biopsy($p=0.004$).While there is a significant negative correlation between uTWEAK and C3($p<0.001$) as well as C4($p<0.001$).On the other hand, there is no statistically significant correlation between uTWEAK level and routine lab tests, blood urea, serum creatinine, extra-renal SLEDAI, SLE damage index, histological class of renal biopsy or pathological chronicity index of renal biopsy. **Conclusion:** Our results suggest that uTWEAK can be used as a non-invasive biomarker in adjunction with conventional laboratory measures for detection of LN activity.

Keywords: Systemic Lupus Erythromatosis, Lupus Nephritis, uTWEAK.

Introduction:

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterized by overproduction of various auto-antibodies. The great majority of SLE patients are women in their childbearing years. SLE is a complex disease with variable presentations, course and prognosis that is characterized by remissions and flares. [(1)]Renal involvement is common in SLE and often determines the course of the disease. Nearly 70-80% of all cases of SLE have some clinical manifestations of lupus nephritis, mostly glomerulonephritis. [(1)]

Lupus nephritis (LN), is a common and serious complication, with reports of 5-year renal survival with treatment ranging from 46 to 95%.[(1)] LN is characterized by a relapsing-remitting course, requiring constant follow-up and surveillance and often entailing changing treatments. [(1)]Earlier treatment has a beneficial effect on the prognosis of lupus

nephritis, and it has been shown that late diagnosis of lupus nephritis is correlated with a higher frequency of renal insufficiency. Moreover, delayed diagnosis is associated with an increased incidence of end stage renal disease, underlining the importance of early diagnosis in this disease.[2]

Histological analysis of kidney tissue is a valuable tool for diagnosis, assessment, and prognosis in lupus patients. However, kidney biopsy can be accompanied by significant morbidity and, therefore, is not usually performed serially. Furthermore, there can be a question of how representative are the limited number of glomeruli usually obtained of nephritis activity and chronicity. [3]

A noninvasive, easily obtainable, and accurate marker that can be followed serially may therefore be of great value in monitoring lupus patients. Laboratory markers in current use, which include serological determination of serum anti-double-stranded (ds) DNA antibodies and complement levels, can be helpful clinically, but the correlation between those and lupus renal disease is imperfect and their utility in reflecting disease activity and in predicting outcome remains controversial.[3] Thus, novel biomarkers that are able to discriminate lupus renal activity and its severity, predict renal flares, and monitor treatment response and disease progress are clearly necessary.

A number of these biomarkers has been of recent interest including Neutrophil Gelatinase-Associated Lipocalin (NGAL)[4], Urine Proteomics, Monocyte Chemoattractant protein-1(MCP-1), and TNF-like Weak Inducer of Apoptosis (TWEAK). [5,6]

The cytokine TWEAK was first discovered in 1997[7] and assigned to the TNF superfamily. The TWEAK receptor (TWEAK-R), a TNF receptor superfamily member more commonly known as Fn14. Fn14 is expressed on endothelial cells, vascular smooth muscle cells, kidney, heart, lung, spleen, brain, monocytes/macrophages, and NK cells, but not B or T cells[8], and is upregulated under conditions of tissue stress and inflammation.[9] In kidney cells, TWEAK mediates important biological effects, including modulation of cell survival and upregulation of proinflammatory mediators. In human mesangial cells, podocytes, and tubular cells, TWEAK induces the expression of multiple inflammatory mediators, including Regulated on Activation Normal T-cell Expressed and Secreted(RANTES), (MCP)-1,Interferon gamma-induced Protein-10 (IP-10), Macrophage Inflammatory Protein-1alpha (MIP-1α), Intercellular Adhesion Molecule-1 (ICAM-1), Vascular Cell Adhesion Molecule-1 (VCAM-1), Matrix MetalloProteinase-1 (MMP-1), and Matrix MetalloProteinase-9 (MMP-9). Thereby causing glomerular and tubular injury, which might play an important role in the pathogenesis of lupus nephritis.[10-13]

Aim of the Work:

To study the role of uTWEAK in lupus patients, its relation to clinical manifestations of the disease especially lupus nephritis. Furthermore, its correlation with the conventional measures of renal lupus activity.

Subjects:

The present study was conducted on two groups of SLE patients fulfilling at least four of the American College of Rheumatology classification criteria:[14]

Group A: Thirty SLE patients with biopsy-proven lupus nephritis.

Group B: twenty SLE patients without lupus nephritis.

They were compared with twenty healthy controls of matched age and sex. The setting of the present study was the Alexandria Main University Hospital.

Exclusion Criteria:

1. SLE patients with proteinuria due to other conditions than lupus nephritis as pregnancy and fever or patients with impaired renal functions due to any other cause than lupus nephritis as diabetes mellitus.
2. Patients with lupus nephritis on hemodialysis or with history of renal transplantation.

Methods:

All subjects were subjected to:

- Thorough history taking and complete clinical examination to detect various organs involvement by SLE.
- Laboratory investigations:

1- Routine laboratory tests:
   a. Complete blood Count (CBC).[15]
   b. Erythrocyte sedimentation rate (ESR).[16]
   c. Fasting blood sugar (FBS).[17]
2- Renal function tests:
   a. Blood urea.[18]
   b. Serum creatinine.[19]
   c. Complete urine analysis.[20]
   d. 24 Hs urinary protein.[21]
   e. Urinary protein to creatinine ratio.[21]
3- Immunological profiles:
   a. Anti-nuclear antibodies (ANA).[22]
   b. Anti-ds DNA.[23]
   c. Complement level determination (C3, C4).[24]
4- Urine TWEAK by ELISA.[25,26]
5- SLEDAI[27] for all lupus patients, and renal DAI (rDAI) for lupus nephritis.

Statistical Analysis of the Data:

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. Comparison between different groups regarding categorical variables was tested using Chi-square test. When more than 20% of the cells have expected count less than 5, correction for chi-square was conducted using Fisher's Exact test or Monte Carlo correction. Histogram and QQ plot were used for vision test. If it reveals normal data distribution, parametric tests was applied. If the data were abnormally distributed, non-parametric tests were used. Agreement of the different predictives with the outcome was used and was expressed in sensitivity, specificity, positive predictive value, negative predictive value and accuracy. Receiver operating characteristic curve (ROC) was plotted to analyze a recommended cutoff, the area under the ROC curve denotes the diagnostic performance of the test. Significance of the obtained results was judged at the 5% level.[28,29]

Results:

There was no statistically significant difference between the three studied groups as regard the age and sex (p=1 and 0.444 respectively). (table I)

There was no statistically significant difference between the three studied groups as regard the disease duration (p=0.819).

The level of uTWEAK was significantly higher in LN than non-LNSLE group (p<0.001) and than controls (p<0.001). There was also a significantly increased level of uTWEAK in non-LN SLE group than controls (p<0.001) (table II). The percentage of LN patients with high TWEAK level was significantly higher than that of non-LN SLE patients with high TWEAK level (93.3% versus 10%) (p<0.001). Where as none of the control group had a high TWEAK level (0%).

The highest mean level of uTWEAK was found in SLE patients with renal manifestations (Lupus Nephritis) with (p<0.001) (table II).

Table (III) shows: Regarding immunological profile; in LN group there was a significant positive correlation between uTWEAK and ANA (p=0.034), Anti ds DNA (p=0.005) and a significant negative correlation between uTWEAK and C3 (p<0.001) and C4 (p<0.001). Regarding renal function tests; In the LN group there was a significant positive correlation between uTWEAK and urinary 24 hs. Protein (p=0.001) as well as urinary protein /creatinine ratio (p<0.001).

In LN group, there was also a significant positive correlation between uTWEAK and hematuria (p<0.001), pyuria (p<0.016), proteinuria (p=0.014) and presence of urinary cast (p=0.001) as well as granular type of cast (p=0.025). Whereas, no correlation was found between urinary TWEAK and casts, pyuria and hematuria or proteinuria in non LN SLE group. As for total SLEDAI, a significant positive correlation was detected with uTWEAK in LN group (p=0.018) but there was no correlation in non LN SLE group (p=0.763).

As regard the correlation between renal SLEDAI and uTWEAK; there was a positive significant correlation between renal SLEDAI and uTWEAK (p<0.001) where as there was no correlation with extra-renal SLEDAI (p=0.622). As for pathological activity index; a positive significant correlation with uTWEAK was found (p=0.004) where as no correlation was found between uTWEAK and pathological chronicity index (p=0.447) or between uTWEAK and SLE Damage index (p=0.251).

Urinary TWEAK level was elevated in all classes of LN. The highest mean level was in class IV+V+III (16.8 pg/ml). This was followed by class IV +V (16.43 pg/ml) then class IV (15.98 pg/ml) then class III (12.63 pg/ml) then class IV+IIIc (11.92 pg/ml) and lastly class V+IIIa (6.9 pg/ml).

No significant statistical difference in mean uTWEAK level was detected among different histological classes of renal biopsy [p=0.539] (table 3).

Relative to renal involvement, uTWEAK showed a sensitivity of 93.33%, specificity of 90%, a positive predictive value of 93.33% and a negative predictive value of 90% with an area under the curve of 0.953 (p<0.001). (Table IV)

Table (I): Comparison between the three studied groups according to demographic data

	Lupus Nephritis (n = 30)		**Lupus only without nephritis (n = 20)**		**Control (n = 20)**		**Test of sig.**	**p**
	No.	**%**	**No.**	**%**	**No.**	**%**		
Sex								
Male	2	6.7	1	5.0	1	5.0	$\chi^2 = 0.346$	$^{MC}p= 1.000$
Female	28	93.3	19	95.0	19	95.0		
Age (years)								
Min. – Max.	13.0 - 44.0		13.0 - 46.0		18.0 - 46.0			
Mean ± SD	28.97 ± 8.19		30.60 ± 11.0		32.30 ± 8.09		F = 0.822	0.444
Median	26.50		30.0		32.0			

χ^2: Chi square test
F: F test (ANOVA)

Table (II): Comparison between the three studied groups according to urinary TWEAK level (pg/ ml)

	Lupus Nephritis (n = 30)	**Lupus only without nephritis (n = 20)**	**Control (n = 20)**	**p_1**	**p_2**	**p_3**
Urinary TWEAK level (pg/ml)						
Min. – Max.	5.50 – 30.10	2.0 – 8.40	1.10 – 6.30			
Mean ± SD	13.86 ± 7.55	4.85 ± 1.86	2.75 ± 1.28	<0.001*	<0.001*	<0.001*
Median	10.10	5.15	2.50			
$^{KW}\chi^2$(p)	50.251* (<0.001*)					

$^{KW}\chi^2$: Chi square for Kruskal Wallis test
Sig. bet. grps was done using Mann Whitney test
p_1: p value for comparing between Lupus Nephritis and Lupus only without nephritis
p_2: p value for comparing between Lupus Nephritis and control
p_3: p value for comparing between Lupus only without nephritis and control
***:** Statistically significant at p ≤ 0.05

Table (III): Correlation between Urinary TWEAK level and different studied parameters

	Lupus Nephritis		Lupus Without Nephritis	
	r_s	p	r_s	p
Personal characteristics				
Age	-0.214	0.256	-0.016	0.947
Sex (male/female)	-0.170	0.369	-0.020	0.934
Disease Duration	-0.199	0.291	0.061	0.798
Immunological profile				
ANA	0.388*	0.034*	0.057	0.810
Anti-ds DNA	0.498*	0.005*	-0.258	0.272
C3	-0.915*	<0.001*	0.048	0.841
C4	-0.729*	<0.001*	0.067	0.778
Routine lab test				
Hb	-0.253	0.178	0.361	0.118
PLT	-0.133	0.482	0.189	0.424
WBcs	-0.129	0.498	-0.088	0.712
ESR	0.074	0.697	0.135	0.569
FBG	-0.142	0.454	0.123	0.606
Renal function test				
Blood urea	0.326	0.078	0.010	0.967
S. creatinine	0.139	0.465	-0.170	0.474
24hr urinary Protein	0.574*	0.001*	-.0038	0.874
Urinary Protein /Creatinine ratio	0.599*	<0.001*	0.062	0.797
Complete urine analysis				
Casts (absent/ present)	0.579*	0.001*	-	-
Granular (absent/ present)	0.409*	0.025*	-	-
Hyaline (absent/ present)	0.268	0.152	-	-
Pyuria (absent/ present)	0.437*	0.016*	-0.191	0.421
Hematuria (absent/ present)	0.704*	<0.001*	0.339	0.144
Proteinuria (absent/ present)	0.442*	0.014*	-	-
Total SLEDAI	0.428*	0.018*	0.072	0.763
Renal SLEDAI	0.900*	<0.001*	-0.019	0.937
Extra Renal SLEDAI	-0.094	0.622	0.144	0.545
SLE Damage Index	0.216	0.251	0.324	0.163
Pathological Activity index	0.512*	0.004*	-	-
Pathological chronicity index	0.144	0.447	-	-

r_s: Spearman coefficient

***:** Statistically significant at $p \leq 0.05$

Table (IV): Agreement (sensitivity, specificity and accuracy) for Urinary TWEAK level (pg/ml) of the whole group of SLE patients (n=50)

		Lupus only without nephritis (n = 20)	Lupus Nephritis (n = 30)	Sensitivity	Specificity	PPV	NPV	Accuracy
Urinary TWEAK level (pg/ml)	**≤6.5**	18	2	93.33	90.0	93.33	90.0	92.0
	>6.5	2	28					

Discussion:

Due to the unpredictable nature of LN, it would be clinically valuable to discover a reliable biomarker for disease activity and progression. TWEAK has been established as a pro-inflammatory cytokine that by binding to its receptor Fn14 induces the secretion of chemokines known to play a major role in the pathogenesis of LN.

On studying the different SLE manifestations, there was no significant difference between LN and non-LN SLE patients in the occurrence of constitutional, mucocutaneous, joint, occular, cardiac, pulmonary, neurological or hematological manifestations.

Regarding the immunological tests, ANA was positive in all (100%) SLE patients. The mean ANA titre in LN group was higher than in the non-LN SLE group yet with no significant statistical difference between the two groups (p=0.377). Anti-ds DNA was also positive in 100% of SLE patients, with higher mean anti-ds DNA level in LN than in non LN SLE patients but again without reaching statistical significance (p=0.063). It has been shown that patients with active lupus nephritis often have raised levels of anti-ds DNA antibodies with evidence supporting their pathogenic role in LN.[30,31]

This comes in accordance with Farid et al[32] who studied 88 SLE patients divided as 44 patients with biopsy-proven LN and 44 patients without LN and they found that all patients of SLE with and without nephritis had positive ANA (100%). He also found that Anti-ds-DNA was higher in the LN group (84.09%) compared with the non-LN group (70.45%), but the difference was not statistically significant (P = 0.082).

In our study, the mean value of C3 in LN patients was lower than that in non-LN SLE patients (0.52 ± 0.42 versus 1.19 ± 0.41g/L) with a statistically significant difference between the two groups (p< 0.001). Similarly, the mean value of C4 was lower in LN patients than that in non-LN SLE patients (0.18 ± 0.15 versus 0.29 ± 0.16g/L) with a significant statistical difference between the two groups (p=0.014). Low levels of C3 and C4 in LN usually indicate disease activity or flare, this can be explained by consumption of the complement system to clear immune complexes from the blood.[33-35] In contrast to our results, Farid et al found no statistically significant difference between LN and non-LN SLE patients regarding C3 and C4.

As regard the renal functions, LN patients had significantly higher levels of serum creatinine (p=0.001), blood urea (p<0.001), 24 hs. urinary proteins(p<0.001) and PCR (p<0.001) than non-LN SLE patients. These results come in agreement with El-shehabi et al. who found a statistically significant difference between the LN and non-LN SLE patients regarding serum creatinine(p=0.001) as well as 24 hs. urinary proteins (p<0.001). Similarly, Pitashny et al found a statistically significant difference between the two groups regarding urinary PCR (p<0.0001).

In the current study, LN patients had more prevalent urinary changes than SLE patients without LN, which is an expected finding. Pyuria was detected in 76.7% of LN patients and 25% of non-LN SLE patients. Hematuria was present in 43.3% of LN cases and 5% in non-LN SLE patients. Urinary casts were present in 26.7% of LN cases while no casts were detected in non LN SLE patients.

Regarding the type of urinary cast, 20% of LN cases had granular cast while 3.3% only of them had hyaline cast. Similarly, Proteinuria was found in 86.7% of LN patients while no proteinuria was found in non LN SLE group. This comes in agreement with Pitashny et al who found a statistically significant difference between the two groups regarding proteinuria (p<0.0001).

In our study, assessment of the disease activity in SLE patients was done by applying SLEDAI. Every patient had got a score which is the sum of scores applied to certain symptoms and some selected laboratory parameters. We found that the mean total SLEDAI in LN group was significantly higher than non LN SLE group (24.53 ± 8.24 versus 16.2 ± 7.25) [p=0.001]. As regards the renal activity, it was assessed by the renal SLEDAI (rSLEDAI) score that consists of the 4 kidney-related items of the SLE Disease Activity Index 2000 (SLEDAI-2K) (hematuria, pyuria, proteinuria and urinary casts).[36] The presence of each one of the 4 parameters gives a score of 4 points thus, the rSLEDAI score can range from 0 (non-active renal disease) to a maximal score of 16. The mean valueof rSLEDAI was found to be also significantly elevated in the LN than the non-LN SLE group (9.33 ± 4.25 versus 1.2 ± 1.88) [p < 0.001]. On the other hand, extra-renal SLEDAI which is calculated by substracting the renal SLEDAI score from the total SLEDAI had no significant statistical difference between the two groups [p=0.818]. This can be explained by the fact that almost all patients of LN group were in activity raising the score of rSLEDAI hence, raising the score of total SLEDAI.

El-shehaby et al.[37] who studied 73 SLE patients divided as 50 patients with active LN and 23 patients with inactive LN or non-renal patients similarly found a statistically significant difference between LN and non-LN SLE patients regarding total SLEDAI (p=0.0002) as well as rSLEDAI (p=0.0002). Also, similar to our results, Pitashny et al. found a statistically significant difference between the two groups regarding total SLEDAI (p<0.0001) as well as rSLEDAI.

Meanwhile, there was no statistically significant difference between the two groups regarding SLE Damage index in the current study (p=0.155).

In the current study, the level of uTWEAK was significantly higher in LN group (ranging from 5.5 to 30.1 pg/mL with a mean of 13.86 ± 7.55 pg/mL) than non-LN SLE group (ranging from 2 to 8.4 pg/mL with a mean of 4.85 ± 1.86 pg/mL) [p<0.001]. uTWEAK was also significantly higher in LN than the control group (ranging from 1.1 to 6.3pg/mL with a mean of 2.75 ± 1.28 pg/mL) [p<0.001]. On comparing uTWEAK levels between non LN SLE patients and controls, there was also a significant statistical difference between the two groups [p<0.001]

This runs in accordance with Schwartz N. et al.[38] Who performed a cross-sectional study of a large, multi-center cohort of 66 SLE patients (divided into 23 LN patients and 43 non-LN SLE patients). He reported that the levels of uTWEAK were significantly higher in the LN group when compared to those with inactive or no nephritis (ranging from 9.9 to 23 with a mean of 16.3 versus a range from 2.3 to 16.8 with a mean of 5.5) pg/mg creatinine (p=0.001). Similarly, In a further multicenter longitudinal study done later by the same author[39] involving 30 biopsy-proven LN patients and five control groups (normal, nonrenal SLE, rheumatoid arthritis, osteoarthritis and non-SLE renal disease) he found that uTWEAK levels were significantly higher in LN patients than non LN SLE patients (p=0.005) and were also higher in LN patients than normal controls (p=0.003). On the other hand, El-shehaby et al. found that significantly higher levels of uTWEAK were observed in LN compared with non-LN SLE patients (p<0.001) and in LN patients compared with control subjects (p<0.001) while there was no statistically significant difference between non-LN SLE and control group.

On comparing the prevalence of high versus low uTWEAK levels in the studied groups, we found that 93.3% of LN group had high uTWEAK levels where as only 10% of non- LN SLE group had high uTWEAK levels with a statistically significant difference between the two groups (p<0.001). None of the control group had a high uTWEAK level. The cut off value applied was suggested by the statistical package for social sciences (SPSS) programme.[29] This suggests that uTWEAK is related to the renal involvement

rather than other system involvement in SLE patients.

On studying the correlation between uTWEAK and SLE manifestations, a significant positive correlation was found between patients with renal involvement and the level of uTWEAK ($p<0.001$) while no significant correlation was found between any of the extra-renal manifestations and uTWEAK. This implies that uTWEAK was primarily dependent on the renal component of the disease and it is unrelated to the systemic (non-renal) SLE manifestations. Yet, this assumption still needs to be confirmed by further larger studies.

No correlation was found between uTWEAK and the different demographic data; age, sex or disease duration. This comes in agreement with El-shehaby et al.

Regarding the correlation between uTWEAK and conventional markers of lupus activity, a significant positive correlation was found between uTWEAK and anti-ds DNA ($r=0.498$, $p=0.005$). There was also a significant negative correlation between uTWEAK and C3($r=-0.915$, $p<0.001$) as well as between uTWEAK and C4 ($r=-0.729$, $p<0.001$). This runs in agreement with Schwartz N. et al who found that uTWEAK was correlated with common serologic indicators of SLE renal activity; positively with anti-ds DNA antibodies ($r=0.459$, $p=0.008$) and negatively with C3 ($r=-0.262$, $p=0.019$) as well as C4 ($r=-0.269$, $p=0.016$). Similarly, El-shehaby et al who found a significant negative correlation between uTWEAK and C3 ($P<0.001$) as well as C4($P<0.001$).

Concerning the laboratory measures, no correlation was found between uTWEAK and Hb level, WBC count, platelet count or ESR.

Regarding renal function tests; no correlation was found between uTWEAK and blood urea or between uTWEAK and serum creatinine. This runs in accordance with Schwartz N. et al who also did not find a correlation between uTWEAK and BUN or serum creatinine. The authors suggested that uTWEAK levels did not correlate well with serum BUN and creatinine as this cytokine is situated very proximally in the pathogenesis of LN. If this is true, uTWEAK may turn out to be helpful as a forecaster of flares, with higher diagnostic accuracy in the pre-flare period rather than following flare onset. Longitudinal studies with more patients are needed to study this in detail.[39]Similarly, El-shehaby et al found no correlation between uTWEAK and serum creatinine($p=0.217$).

In the current study, there was a significant positive correlation between uTWEAK and 24 hs. urinary proteins ($r=0.574$, $p=0.001$) as well as urinary PCR ($r= 0.599$, $p<0.001$)), the latter being a simple, reliable and valuable tool to monitor LN progression.[40] In agreement with this result, El-shehaby et al found also a significant correlation between uTWEAK and 24 hs. Urinary protein ($p=0.03$).

There was a significant positive correlation between uTWEAK and hematuria ($r=0.704$, $p<0.001$), pyuria ($r=0.437$, $p=0.016$), presence of urinary casts ($r=0.579$, $p=0.001$) as well as granular type of cast ($r=0.409$, $p=0.025$). This runs in agreement with El-shehaby et al as they found a significant correlation between uTWEAK and hematuria ($p=0.03$), pyuria ($p=0.01$) as well as urinary casts($p<0.001$).

As regard the correlation between uTWEAK and proteinuria, we found a significant positive correlation ($r=0.442$, $p=0.014$).This was in contrast to Schwartz N. et al who did not find a correlation between uTWEAK and proteinuria ($r=0.073$, $p=0.562$). He suggested that This finding indicated that high urinary TWEAK levels are not a result of damage to the glomerular filtration barrier and non-specific protein loss into the urine, but rather the source of uTWEAK is the kidneys themselves, reflecting local inflammatory activity.

The level of uTWEAK Was found to be strongly correlated with total SLEDAI ($r=0.428$, $p=0.018$) as well as renal SLEDAI ($r=0.900$, $p<0.001$). However, this correlation was no longer significant when only the extra-renal component of the index was correlated with uTWEAK in LN ($r=-0.094$, $p=0.622$). These results are consistent with the findings by Schwartz N. et al. who proved also that uTWEAK was significantly correlated with the total SLEDAI as well as renal SLEDAI but not with the extra-renal SLEDAI. These results were confirmed in an other study by the same author[39,40] in which they found a significant correlation between uTWEAK and total SLEDAI ($p<0.001$) as well as between

uTWEAK and rSLEDAI (r=0.388, p=0.047) while no correlation was found with extra-renal SLEDAI (p=0.426). This comes also in accordance with El-shehaby et al. who found a significant correlation between uTWEAK and tSLEDAI (p<0.001) as well as rSLEDAI (p<0.001). The finding of significant correlation of uTWEAK with total SLEDAI and rSLEDAI but not extra-renal SLEDAI suggests that elevation in uTWEAK is an indicator of renal activity, not extra-renal activity.

Moreover,we compared the mean level of uTWEAK of LN patients with rSLEDAI ≤ 8 (8.24 ± 1.71) with that of LN patients with rSLEDAI > 8 (21.21± 5.52) and we found a significant statistical difference between these two sub groups (p<0.001). This furtherly indicates that higher levels of uTWEAK are present in patients with higher grades of renal activity.

Regarding the disease damage, no correlation was found between uTWEAK and SLEDDI (p=0.251). This indicates that the elevation of uTWEAK level may be related to disease activity rather than damage.

In this study, renal biopsy was done in all the 30 patients of LN group. Biopsies were classified according to International Society of Nephrology/Renal Pathology Society (ISN/RPS) 2003 Classification of Lupus Nephritis[41]. We found that the highest percentage of patients had class III LN (36.7%) followed by class IV (26.7%) then class IV+IIIc (16.7%) then class IV+V (13.3%) then class V+IIIa and class IV+V+III (3.3% each), while no patients were found in classI, II or VI.

On studying the correlation of uTWEAK and the different histological classes of LN, we found that there was no significant difference in the levels of uTWEAK among the different classes of renal biopsy. Accordingly, uTWEAK could not discriminate between the histological classes of renal biopsy in the current study. This was confirmed by the results of other studies such as that done by Xuejing Z. et al. (who studied 46 patients with LN divided as 12 of nonactive LN and 34 of active LN)[42] as well as studies done by Schwartz {38,39} as they also did not find a significant difference in uTWEAK levels among different classes of LN. This indicates that uTWEAK can not replace renal biopsy for detection of renal involvement in SLE patients. The authors suggested that This may be due to the fact that TWEAK is predominantly a pro-inflammatory cytokine that contributes to active renal inflammation, while additional factors and mediators are necessary to induce progression into chronicity. Genetic background and/or environmental triggers may influence disease progression and perhaps even contribute to the evolution of the different WHO classes. Yet, they also suggested that this finding still remained to be confirmed in larger numbers of patients displaying each of these histological subtypes. This also applies for other studied biomarkers in LN as none of them up till now proved to replace renal biopsy.[37,43]

Regarding the correlation between uTWEAK and renal biopsy indices; in the current study, a significant positive correlation was found between uTWEAK and pathological activity index (r=0.512, p=0.004) while no correlation was found between uTWEAK and pathological chronicity index (r=0.144, p=0.447). This runs in agreement with Xuejing Z. et al who found that uTWEAK levels of patients with LN had significantly positive correlation with activity index (r=0.825, p<0.01) but had no significant correlation with chronicity index (p>0.05). They concluded that uTWEAK levels could reflect the level of histological activity in LNpatients so, the expression of TWEAK may be relevantto glomerular and tubulointerstitial lesions. They suggested that the elevation of uTWEAK level may be related to the increased expression of TWEAK in kidney. This was shown in a cross sectional cohort study by Albert Einestein College of Medicine of 66 SLE patients divided as 23 LN patients and 43 non-LN SLE patients compared with 19 healthy controls, they found that there was a statistically significant difference in serum TWEAK between SLE patients and controls[p=0.034] where as there was no statistically significant difference in sTWEAK between LN and non-LN SLE patients [p=0.747]).[39] However, it is still uncertain whether the kidney is the only organ expressing TWEAK, which needs to be confirmed by further studies in the future.[42]

It is well known that the relapsing-remitting course of LN, among the most serious complications of SLE thus it requires close monitoring and often frequent treatment

adjustments throughout patients' lives. Trend towards re biopsy upon renal flares is increasing.[44] as some authors in their studies found that histological transformations were common, and they occurred when the previous biopsy had non-proliferative lesions as well as when lesions were proliferative. Treatments were modified after repeat renal biopsy in the majority of patients. In this experience, kidney repeat biopsies were useful in guiding treatment of LN flares.[44] However, is impractical as a clinical tool to be repeatedly used upon frequent relapses as it is considered as an invasive maneuver with possible complications. Moreover, contra indications for this procedure may be encountered in some patients such as thrombocytopenia, bleeding tendency and severe hypertension.[45] A dependable biomarker that can reflect the patient's renal disease activity is therefore highly desirable. This may eventually enable clinicians to institute treatment of flares earlier, and hopefully improve the significant short- and long-term morbidity associated with lupus renal disease.

In the current study, we concluded that uTWEAK excretion correlated strongly with renal disease progression and activity and not with extra renal disease activity score in LN. More over TWEAK was also correlated with pathological activity index of renal biopsy.

In addition, uTWEAK served as a biomarker of LN activity and was correlated with conventional markers of lupus renal activity (anti-dsDNA Abs, C3 and C4).

Moreover, In the current study, uTWEAK showed a sensitivity of 93.33%, specificity of 90%, a positive predictive value of 93.33% and a negative predictive value of 90% with an area under the curve of 0.953 ($p<0.001$). This comes in agreement with El-shehaby et al. who found that uTWEAK showed a sensitivity of 89%, specificity of 56%, a positive predictive value of 93% and a negative predictive value of 66.7% with an area under the curve of 0.816 .

But as none of the biomarkers studied till now proved to replace the valuable role of renal biopsy,[37,43] future directions in SLE biomarker research should focus on combination of novel markers with conventional clinical and laboratory parameters to enhance the sensitivity and specificity for the prediction of renal flares and to improve prognosis in LN.

Conclusions:

We found that, the mean level of uTWEAK was strongly correlated with renal SLEDAI as well as the pathological activity index of renal biopsy. In addition, it showed a high sensitivity, specificity and positive predictive value for renal involvement and activity so, it can be used as a non invasive biomarker for early detection of LN activity yet it can not replace the need for renal biopsy. Accordingly, uTWEAK can be used in association with other conventional markers to detect renal flare early in SLE patients.

Recommendations:

Further longitudinal studies on larger sample size are promptly needed to test the value of urinary TWEAK in early detection of renal involvement in lupus patients as well as its prognostic value in follow up of SLE patients.

References:

1- **Korbet SM, Lewis EJ, Schwartz MM.** Factors predictive of outcome in severe lupus nephritis. Lupus Nephritis Collaborative Study Group. Am J Kidney Dis 2000; 35:904-14.

2- **Esdaile JM, Joseph L, MacKenzie T, et al.** The benefit of early treatment with immunosuppressive agents in lupus nephritis. J Rheumatol 1994; 21:2046-51.

3- **Illei GG, Tackey E, Lapteva L, et al.** Biomarkers in systemic lupus erythematosus: II. Markers of disease activity. Arthritis Rheum 2004; 50:2048-65.

4- **Devarajan P.** Neutrophil gelatinase- associated lipocalin (NGAL): a new marker of kidney disease. Scand J Clin Lab Invest 2008; 241(Suppl):84-9.

5- **Rovin BH, Birmingham DJ, Nagaraja HN, et al.** Biomarker discovery in human SLE nephritis. Bull NYU Hosp Jt Dis 2007; 65:187-93.

6- **Rovin BH, Song H, Birmingham DJ, et al.** Urine chemokines as biomarkers of human systemic lupus erythematosus activity. J Am Soc Nephrol 2005; 16:467-73.

7- **Chicheportiche Y, Bourdon PR, Xu H, et al.** TWEAK, a new secreted ligand in the tumor necrosis factor family that weakly induces apoptosis. J Biol Chem 1997; 272:32401-10.

8- **Maecker H, Varfolomeev E, Kischkel F, et al.** TWEAK attenuates the transition from innate to adaptive immunity. Cell 2005; 123:931-44.

9- **Wiley SR, Cassiano L, Lofton T, et al.** A novel TNF receptor family member binds TWEAK and is implicated in angiogenesis. Immunity 2001; 15:837-46.

10- **Campbell S, Burkly LC, Gao H, et al.** Proinflammatory effects of TWEAK/Fn14 interactions in glomerular mesangial cells. J Immunol 2006; 176:1889-98.

11- **Schwartz N, Michaelson JS, Putterman C.** Lipocalin-2, TWEAK, and other cytokines as urinary biomarkers for lupus nephritis. Ann NY Acad Sci 2007; 1109:265-74.

12- **Gao HX, Campbell SR, Burkly LC, et al.** TNF-like weak inducer of apoptosis (TWEAK) induces inflammatory and proliferative effects in human kidney cells. Cytokine 2009; 46:24-35.

13- **Sanz AB, Justo P, Sanchez-Nino MD, et al.** The cytokine TWEAK modulates renal tubulointerstitial inflammation. J Am Soc Nephrol 2008; 19:695-703.

14- **PubMed Health.** American College of Rheumatology Criteria for Classification of Systemic Lupus Erythematosus. 1997. Available at: http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0041704/.

15- **Mayo Clinic Staff.** Tests and diagnosis. 2011. Available at: file:///F:/Lupus%20Tests%20 and %20 diagnosis%20-%20MayoClinic_ com.htm.

16- **Vilá LM, Alarcón GS, McGwin G Jr, et al.** Systemic lupus erythematosus in a multiethnic cohort (LUMINA): XXIX. Elevation of erythrocyte sedimentation rate is associated with disease activity and damage accrual. J Rheumatol 2005; 32: 2150-5.

17- **Koca SS, Karaca I, Yavuzkir MF, et al.** Insulin resistance is related with oxidative stress in systemic lupus erythematosus. Anadolu Kardiyol Derg 2009; 9: 23-8.

18- **Satirapoj B, Wongchinsri J, Youngprang N, et al.** Predictors of renal involvement in patients with systemic lupus erythematosus. Asian Pac J Allergy Immunol 2007; 25: 17-25.

19- **Couchoud C, Pozet N, Labeeuw M, et al.** Screening early renal failure: cut-off values for serum creatinine as an indicator of renal impairment. Kidney Int 1999; 55: 1878- 84.

20- **Simeville JA, Maxted WC, Pahira JJ.** Urine analysis: a Comprehensive review. Am Fam Physican 2005; 71: 1153-63.

21- **Christopher-Stine L, Petri M, Astor BC, et al.** Urine protein-to-creatinine ratio is a reliable measure of proteinuria in lupus nephritis. The Journal of Rheumatology 2013; 31: 1557-9.

22- **Keren DF.** Antinuclear antibody testing. Clin Lab Med 2002; 22:447-74.

23- **Ippolito A, Wallace DJ, Gladman D, et al.** Autoantibodies in systemic lupus erythematosus: comparison of historical and current assessment of seropositivity. Lupus 2011; 20: 250-5.

24- **Ho A, Barr SG, Magder LS, et al.** A decrease in complement is associated with increased renal and hematologic activity in patients with systemic lupus erythematosus. Arthritis Rheum 2001; 44: 2350-7.

25- **Xuejing Z, Jiazhen T, Jun L, et al.** Urinary TWEAK level as a marker of lupus nephritis activity in 46 cases. J Biomed & Biotechnol 2012; 2012:1-7.

26- **Schwartz N, Su L, Burkly LC, et al.** Urinary TWEAK and the activity of lupus nephritis. J Autoimmun 2006; 27:242-50.

27- **Gladman DD, Ibanez D, Urowitz MB.** Systemic lupus erythematosus disease activity index 2000. J Rheumatol 2002; 29:288-91.

28- **Kotz S, Balakrishnan N, Read CB, et al.** Encyclopedia of statistical sciences. 2nd ed. Hoboken, N.J.: Wiley-Interscience; 2006.

29- **Kirkpatrick LA, Feeney BC.** A simple guide to IBM SPSS statistics for version 20.0. Student ed. Belmont, Calif.: Wadsworth, Cengage Learning; 2013.

30- **Dang H, Harbeck RJ.** The in vivo and in vitro glomerular deposition of isolated anti-double-stranded-DNA antibodies in NZB/W mice. Clin Immunol Immunopathol1984; 30:265–78.

31- **Koffler D, Schur PH, Kunkel HG.** Immunological studies concerning the nephritis of systemic lupus erythematosus. J Exp Med1967; 126:607–23.

32- **Farid EM, Hassan AB, Abalkhail AA, et al.** Immunological aspects of biopsy-proven lupus nephritis in Bahraini patients with systemic lupus erythematosus 2013;24(6):1271-9.

33- **Walport MJ, Davies KA.** Complement and immune complexes. Res Immunol 1996; 147:103.

34- **Morgan BP, Walport MJ.** Complement deficiency and disease. Immunol Today 1991; 12:301.

35- Rabbani MA, Tahir MH, Siddiqui BK, et al. Renal involvement in systemic lupus erythematosus in Pakistan. J Pak Med Assoc 2005; 55:328-32.

36- Gladman DD, Ibanez D, Urowitz MB. Systemic lupus erythematosus disease activity index 2000. J Rheumatol 2002;29:288-91.

37- El-shehaby A, Darweesh H, El-Khatib M, et al. Correlations of Urinary Biomarkers, TNF-Like Weak Inducer of Apoptosis (TWEAK), Osteoprotegerin (OPG), Monocyte Chemoattractant Protein-1 (MCP-1), and IL-8 with Lupus Nephritis2011.

38- Schwartz N, Lihe Su, Burkly LC, et al. Urinary TWEAK and the activity of lupus nephritis Journal of Autoimmunity 2006; 242-50.

39- SchwartzN, Rubinstein T, Burkly LC, et al. Urinary TWEAK as a biomarker of lupus nephritis: a multicenter cohort study. Arthritis Res Ther 2009; 11(5): R143.

40- Matar HE, Peterson P, Sangle S, et al. Correlation of 24-hour urinary protein quantification with spot urine protein: creatinine ratio in lupus nephritis 2012;21(8):836-9.

41- Weening JJ, D'Agati VD, Schwartz MM, et al. The classification of glomerulonephritis in systemic lupus erythematosus revisited. J Am Soc Nephrol 2004; 15(2):241-50.

42- Xuejing Zhu, Jiazhen Tan, Jun Li, et al. Urinary TWEAK Level as a Marker of Lupus Nephritis Activity in 46 Cases. Journal of Biomedicine and Biotechnology 2012; 7:359-66.

43- Pitashny M, Schwartz N. Urinary lipocalin-2 is associated with renal disease activity in human lupus nephritis. Arthritis & Rheumatism 2007; 56(6):1894-903.

44- Greloni G, Scolnik M, Marin J, et al. Value of repeat biopsy in lupus nephritis flares. Lupus Sci Med 2014;1:4.

45- Lefaucheur C, Nochy D, Bariety J. Renal biopsy: procedures, contraindications, complications. 2009 Jul;5(4):331-9.