Urinary Tumor Necrosis Factor-Like Weak Inducer of Apoptosis (TWEAK) As a Marker for Lupus Nephritis Activity

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Abstract: *Background: Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by auto antibodies directed against nuclear antigens and causing a variety of clinical and laboratory abnormalities and involving multiple organs, causing significant morbidity and mortality. Lupus nephritis (LN) is one of the most serious SLE complications since it is the major predictor of poor prognosis. For LN, the search for an accurate and reliable biomarker is particularly pressing since the only truly reliable method to evaluate LN in current practice is by performing a kidney biopsy, an invasive procedure. Therefore, the search for a lupus biomarker has been especially intense. Objective: The aim of this study is to evaluate the urinary TWEAK (uTWEAK) as a biomarker for lupus nephritis activity. Methods: This study included 40 SLE patients, who were admitted to the inpatient of Nephrology unit of Internal Medicine department and Rheumatology and Rehabilitation department, faculty of medicine, Zagazig university. All individuals in this study were divided into 2 groups: Group I (20 patients): patients with active renal disease and having a renal systemic lupus erythematosus disease activity index(rSLEDAI) score of ≥4 (i.e., at least 1 abnormal result for renal parameters) and Group II (20 patients): patients with inactive renal disease (patients with history of LN whose disease became quiescent for at least 6 months after treatment and having a rSLEDAI score of 0 (i.e. they did not have, at the time of the visit, pyuria, proteinuria, hematuria or urinary casts). All patients included in the study were subjected to proper history taking and thorough medical examination in addition to laboratory investigations like, complete urine analysis, blood urea, serum creatinine, liver function tests, ESR and 24 hours urinary proteins estimation, ANA, AdsDNA antibodies, quantitative determination of serum C3 and C4 and kidney biopsy and uTWEAK estimation, plus abdominal sonography. Results: A statistically significant difference regarding the mean uTWEAK level and the mean rSLEDAI was found between the 2 groups (P<0.001) with significant lower uTWEAK levels in SLE patients without kidney involvement and lower rSLEDAI in patients with non active lupus nephritis. uTWEAK showed sensitivity of 80% and specificity of 75% for the presence of active LN. Conclusion: uTWEAK may play a role in the pathogenesis of LN, and can be used as diagnostic tool to discriminate lupus renal activity and is a sensitive biomarker for early detection of active lupus nephritis.*

Keywords: SLE, lupus nephritis, TWEAK, SLEDAI.

1. Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by autoantibodies directed against nuclear antigens and causing a variety of clinical and laboratory abnormalities. SLE can involve multiple organs, causing significant morbidity and mortality **(1).**

SLE includes a wide spectrum of severity, ranging from relatively mild manifestations (e.g. skin rash or non-erosive arthritis) to seriously disabling or even life threatening complications, such as lupus nephritis (LN) and neuropsychiatric disorders **(2).** LN is one of the most serious SLE complications since it is the major predictor of poor prognosis **(3).**

The search for an accurate and reliable biomarker for LN is particularly pressing since the only truly reliable method to evaluate LN in current practice is by performing a kidney biopsy, an invasive procedure. Therefore, the search for a lupus biomarker has been especially intense **(4).**

The current laboratory markers for LN such as proteinuria, urine protein to creatinine ratio, creatinine clearance, anti-dsDNA, and complement levels are unsatisfactory because they lack sensitivity and specificity for differentiating renal activity and damage in LN **(5)** .

Significant kidney damage can occur before renal function is impaired and first detection by laboratory parameters**.** 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 **(5).**

Urinary biomarkers are easily obtained and probably are best at reflecting the current renal status, as they specifically represent local inflammatory activity **(6).** Tumor necrosis factor–like weak inducer of apoptosis (TWEAK) is a multifunctional cytokine that belongs to the TNF-ligand superfamily**.** These cytokines are involved in multiple biological responses including inflammation, immune responses and tissue repair **(7).**

The major role of TWEAK in LN is centered on its pro-inflammatory and chemokine inducing effects **(8).**

Schwartz and his colleagues supported a role for TWEAK in the pathogenesis of LN, and provided strong evidence for uTWEAK as a candidate clinical biomarker for LN **(9).**

Blocking TWEAK/Fn14 interactions may be a promising therapeutic target in immune-mediated renal diseases **(10).** Therefore, the aim of this study is to evaluate the role of urinary TWEAK (uTWEAK) as a biomarker for LN activity

2. Patients and Methods

This study included 40 SLE patients, who were admitted to the inpatient of nephrology unit of Internal Medicine department and Rheumatology and Rehabilitation department, faculty of medicine, Zagazig university. All individuals were divided into two groups:**1.Group I:** 20 patients with active lupus nephritis; 17 females and 3 males with mean age of 27.85±8.34 years, mean SLE duration of 24.55±19.52 months and having a renal systemic lupus erythematosus disease activity index (rSLEDAI) score of ≥4 (i.e., at least 1 abnormal result for renal parameters). **2. Group II:** 20 patients with non active lupus nephritis (patients with history of LN whose disease became quiescent for at least 6 months after treatment and having a renal SLEDAI score of 0 i.e. they did not have, at the time of the visit, pyuria, proteinuria, hematuria or urinary casts). They were 18 females and 2 males with mean age of 28.5±8.48 years and mean SLE duration of 46.70±25.09 months.

SLE patients were selected according to the following inclusion criteria:
1-Age of the patients ranged from (15-40) years old to avoid extremes of age that may affect the results.
2-All SLE patients in this study fulfilled the ACR 1997 revised criteria for the classification of SLE **(11).**

ASSESMENT OF Renal SLEDAI: Kidney disease activity is assessed by rSLEDAI score that consists of the 4 kidney-related items of the SLE Disease activity (hematuria, pyuria, proteinuria and urinary casts). The presence of each one gives a score of 4 points; thus, the score can range from 0 (non-active renal disease) to a maximal score of 16 **(12).**

Exclusion criteria:
1) Patients with diabetes mellitus.
2) Patients with a diagnosis of overlap syndrome (coexistence of lupus with other connective tissue diseases such as rheumatoid arthritis or scleroderma).
3) Patients with urinary tract infections.
4) Patients with End stage renal disease.

3. Methods

All patients of the study were subjected to the followings:
A) Full medical history taking and thorough clinical examination: According to checking patient's records.

B) Laboratory investigations:
All the investigation were done according to the methods applied in the clinical pathology laboratories of Zagazig university hospitals and included:
1) Complete Urine analysis.
2) Estimation of 24 hours urinary proteins.
3) Complete blood count.
4) Random plasma glucose level.
5) Liver function tests.
6) Erythrocyte sedimentation rate (ESR)

7) Serum creatinine and blood urea.
8) Antinuclear antibodies (ANA) and Anti deoxyribonucleic acid (DNA) antibodies by immunoflouresnce technique.
9) Quantitative determination of serum complement levels (C3, C4).
10) 10-Urinary creatinine level: as uTWEAK levels were normalized to urine creatinine concentrations measured in the same spot urine.
11) Estimation of uTWEAK level by Human Tumor necrosis factor related weak inducer of appoptosis(TWEAK), **ELISA KitEIAab® (9,12)**:

The urine samples were aseptically collected (mid-stream, first urine of the day) into a sterile container, centrifuged and stored at ≤ - 20°C until the time of TWEAK estimation. The microtiter plate was pre-coated with an antibody specific to TWEAK. Standards and samples were then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for TWEAK and Avidin conjugated to Horseradish Peroxidase (HRP) was added to each microplate well and incubated. Then a TMB substrate solution was added to each well. Only those wells that contain TWEAK, biotin-conjugated antibody and enzyme-conjugated Avidin exhibited a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change was measured spectrophotometrically at a wavelength of 450 nm. The concentration of TWEAK in the samples is then determined by comparing the optical density of the samples to the standard curve. The lower detection limit was 156pg/ml.

C) Renal biopsy: Renal biopsy was done for patients with active renal disease (group I). The renal pathology was classified according to the revised ISN/RPS system **(Weening et al., 2004).(13).**

Statistical analysis
Data collected throughout history, basic clinical examination, laboratory investigations and outcome measures coded, entered and analyzed using Microsoft Excel software. Data were then imported into Statistical Package for the Social Sciences (SPSS version 20.0) (Statistical Package for the Social Sciences) software for analysis. According to the type of data, the following tests were used to test differences for significance;. Differences between frequencies (qualitative variables) and percentages in groups were compared by Chi-square test. Differences between means (quantitative variables) IN parametric two group by t test in Non parametric by Mann Whitney, correlation by Pearson's correlation scale or qualitative correlation by Spearman. ROC curve was done to determine cutoff. P value was set at <0.05 for significant results & <0.001 for highly significant result.

4. Results

The results showed a statistically significant difference regarding the mean value ±SD of hemoglobin(HB) , serum albumin , 24 hours urinary proteins , serum creatinine, blood urea and erythrocyte sedimentation rate (ESR) between both groups as shown in **table (1).**

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Moreover, A statistically significant difference regarding anti-double stranded deoxyribonucleic acid (Anti-dsDNA) between groups was found. It was positive in 85% of patients with active lupus nephritis compared to 45% positive in patients with non active lupus nephritis. Also, **table (1)** showed a statistically significant difference regarding the mean value±SD of uTWEAK level and rSLEDAI between both groups with significant lower uTWEAK levels in SLE patients without kidney involvement and lower rSLEDAI in patients with non active lupus nephritis.

Positive correlations were observed between uTWEAK (pg/mg Cr) and 24 hours urinary proteins, serum creatinine, blood urea and ESR in SLE patients, while negative correlations were observed between uTWEAK and C3 , C4 and serum albumin as shown in **table (2).**

In active lupus nephritis there was significant positive correlation between uTWEAK levels and rSLEDAI , while in non active lupus nephritis, there was no significant correlation between uTWEAK levels and age, disease duration, 24 hours urinary proteins, C3 and C4 as shown in **table (3).**

There was direct correlations between uTWEAK and rSLEDAI and Activity index of renal biopsy. However, there was no significant correlation between uTWEAK levels and chronicity index of renal biopsy as shown in **table (4).**

A nonparametric receiver operator characteristics (ROC) curve was constructed to quantify how well uTWEAK distinguishes between SLE patients with or without nephritis, the sensitivity and specificity of the uTWEAK as a marker of nephritis in SLE patients were found to be 80% sensitivity and 75% specificity and area under the curve (AUC)is0.872(**figure1).**

Table 1: Comparison between active and non active nephritis regarding the mean values ±SD of different variables

variable	Active N(20)	Non active N(20)	P
Age(years) Mean ±SD	27.85±8.34	28.5±8.48	0.829
Gender: male female	No % 3 15 17 85	No % 2 10 18 90	0.9
SLE duration (months)	24.55±19.52	46.70±25.09	0.152
Hemoglobin (mg/dl)	9.40	11.37	.006*
Serum albumin (gm/dl)	2.38	3.59	.000**
24 hours urinary	3.27	0.3256	.000**
Serum creatinine (mg/dl)	2.68	0.91	0.001*
Blood urea (mg/dl)	74.45	33.60	0.001*
ESR (mm/h)	97.25±32.55	34.90±11.89	0.000**
C3 (mg/dl)	96.60 ±29.39	104.85±28.05	0.370
C4 (mg/dl)	25.95 ± 8.34	30.55 ±8.85	0.161
Anti nuclear Antibodies (ANA)	100% (+ve)	100% (+ve)	1
Anti-dsDNA	85% (+ve)	45% (+ve)	0.008*
uTWEAK (pg/mg Cr.)	15.28 ±4.90	8.08 ±3.95	0.000**
rSLEDAI	8.60 ±2.50	0.0000	0.000**

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).

Table 2: Correlation between uTWEAK and demographic and laboratory data of SLE patients.

Variable	Pearson	(2-tailed)
Age(years)	0.014	0.930
Disease duration (months)	-0.123	0.451
HB (gm/dl)	-0.276	0.085
Serum albumin (gm/dl)	-0.651 **	0.000**
24 hours urinary proteins (gm)	0.678**	0.000**
Serum creatinine (mg/dl)	0.374*	0.017*
Blood urea (mg/dl)	481**	0.002
ESR	0.528**	0.000**
C3 (mg/dl)	-0.314 *	0.049
C4 (mg/dl)	-0.505 **	0.001
rSLEDAI	0.652**	0.000**

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed)

Table 3: Correlation between uTWEAK and demographic and laboratory data of active and non active lupus nephritis.

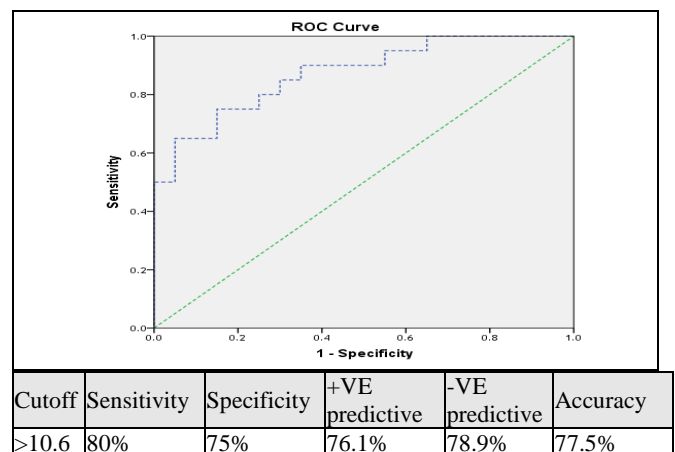| Variable	Pearson Correlation (Active nephritis)	(2-tailed) p	Pearson Correlation (Non active nephritis)	(2-tailed) p
Age	-0.063	0.792	0.159	0.503
Disease duration (months)	-0.209	0.376	0.250	0.288
24 hours urinary proteins (gm)	0.477*	0.033	-0.212	0.369
C3 (mg/dl)	-0.540 *	0.014	0.032	0.892
C4 (mg/dl)	-0.643-**	0.002	-0.225	0.340
rSLEDAI	0.652**	0.000**	B	

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).
B. Cannot be computed because at least one of the variables is constant.

Table 4: Correlation between uTWEAK and activity and chronicity index of renal biopsy

Variable	Correlation Coefficient	Sig. (2-tailed)
Activity index	0.675**	0.001
Chronicity index	0.255	0.278

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed)

Cutoff	Sensitivity	Specificity	+VE predictive	-VE predictive	Accuracy
>10.6	80%	75%	76.1%	78.9%	77.5%

Figure 1: Roc curve of TWEAK of SLE patients with active nephritis versus SLE patients without active nephritis with AUC is 0.872.

5. Discussion

Assessment of renal functional activity (eg , markers of GFR), such as serum creatinine and BUN, has limitations. Because, the GFR may be still preserved while there is severe inflammation thus making it difficult to assess its true changes **(14).**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 **(5).**

With respect to LN, urinary biomarkers may be more specific for kidney damage than serum biomarkers, particularly in SLE patients with active systemic disease. **Schwartz and colleagues** reported that cytokines and chemokines secreted locally within the kidney are instrumental in the pathogenesis of LN. Furthermore, cytokines and chemokines excreted in the urine are an excellent indicator of their local production and secretion, and thus may have more potential than a serum-based marker to reflect inflammatory activity in the kidney**(15).** Obtaining urine for laboratory testing is much easier and less invasive, making urine a more ideal biological sample for a disease that requires repetitive screening **(16).**

Among the more promising suggested biomarkers are the chemokines inducible protein 10 (IP-10) and monocyte chemoattractant protein 1 (MCP-1).It is very interesting that both IP-10 and MCP-1 are among the proinflammatory chemokines induced by TWEAK in mesangial **(10)** and tubular cells **(17).** Therefore, TWEAK may eventually turn out to be a more accurate biomarker than MCP-1, as it is more proximally situated in the inflammatory cascade, and it induces multiple cytokines that are relevant to the pathogenesis of LN **(4).**

Researches also reported that TWEAK/human fibroblast growth factor-inducible14(Fn14) induced apoptosis of glomerular mesangial cells and tubular epithelial cells with induction of pro- inflammatory cytokines, thereby causing glomerular and tubular injury, which might play an important role in the pathogenesis of LN **(10).**

Similar to TNF, TWEAK is processed into a soluble form that circulates as a trimer, believed to be the primary mediator of its biological effects **(4).**

The potential sources of TWEAK in the kidney include infiltrating monocytes and T lymphocytes, tubular epithelial cells and mesangial cells **(18).** The major role of TWEAK in LN is centered on its pro-inflammatory and chemokine inducing effects **(8).** Since TWEAK/Fn14 axis may play an important role in the induction and development of SLE, blockade of the axis might represent a novel therapeutic strategy for the treatment of SLE **(19).**

Reyes-Thomas and colleagues reported that urinary biomarkers may be more specific for kidney damage than serum biomarkers, particularly in SLE patients with active systemic disease **(16)**. This was supported by **Schwartz and colleagues** who found that urine but not serum levels of TWEAK are elevated in patients with active LN **(9).**

Therefore, in our study we preferred to measure uTWEAK levels rather than its serum levels in SLE patients.

To determine whether uTWEAK correlates with LN activity, we compared uTWEAK levels of patients with active LN (rSLEDAI score $\geq$ 4) with uTWEAK levels of patients with non active lupus nephritis (with a rSLEDAI score of 0). In our study, The findings clearly showed that uTWEAK levels were significantly higher (P<0.001) in SLE patients with active LN (15.28±4.90 pg/mg Cr) compared to SLE patients with non active lupus nephritis(8.08±3.95 pg/mg Cr). Similar results were found by **Schwartz and colleagues,** In their study, high uTWEAK levels were found to reflect the presence of LN in SLE patients even better than clinical markers in widespread use, such as anti-dsDNA Ab and C component levels **(9).**The same data were also reported by **El-shehaby and colleagues (20) and Zhu and colleagues (12).**

We found that uTWEAK levels were significantly positively correlated with rSLEDAI (p<0.001). Similar results were found by **Schwartz and colleagues (4,9)** and **El-shehaby and colleagues (20).**

In our study, uTWEAK levels significantly positively correlated with ESR (p<0.001). This is in agreement with **Dooley** who reported that elevated ESR is associated with active L.N**(21).** On the contrary, these results were different from what was found by **El- shehaby et al**; who found that there is no correlation between uTWEAK levels and ESR**(20)**

Regarding 24 urinary proteins, uTWEAK levels significantly positively correlated with it(p<0.001). Similar results were found by **El-shehaby and colleagues (20).** On the contrary, **Schwartz and colleagues** did not find a significant correlation between uTWEAK and the degree of proteinuria**(4)**

On the other hand, significant negative correlations were observed between uTWEAK (pg/mg Cr.) and both C3 (p=0.049) and C4 (p=0.001), respectively. Similar results were found by **Schwartz and colleagues (4)** and **El-shehaby and colleagues (20).** This is expected as depressed C3 and C4 levels are associated with active LN **(21)**. In our opinion, the later findings have important clinical implications, although C components (C3 and C4), known to be consumed in SLE patients with LN, yet in SLE C components can be consumed in other immune-complex-mediated lesions, such as vasculitis with a stable renal function

Given that, uTWEAK could be the novel and more specific biomarker of potential diagnostic and prognostic roles to assess LN. Moreover, we found that uTWEAK levels significantly positively correlated with serum creatinine (p=0.017) and significant negative correlations with serum albumin (p<0.001).Similar results were found by **El-shehaby and colleagues (20).**

In an effort to explore the relation between uTWEAK levels and the renal histology, renal biopsy was done for patients with active nephritis uTWEAK levels showed significantly

positive correlation with the histological renal activity index (p=0.001), but not renal chronicity index (p=278). This confirms that the elevation of uTWEAK level shows a stronger association with the renal disease activity rather than with the degree of renal insufficiency.

So, uTWEAK levels may be used to monitor the renal disease activity without the need of repeating renal biopsy which is an invasive procedure to monitor the renal disease activity. Similar results were found by **Zhu and colleagues** who also found that the uMCP-1 levels of patients with LN had significantly positive correlation with activity index, but had no significant correlation with chronicity index (12).

In Conclusion , uTWEAK can be a useful biomarker for LN activity in different situations in which it is difficult to ensure the presence of renal disease activity as during pregnancy, especially if the patient is suspected to have preeclampsia, or in the postpartum period. Moreover it can be used to identify potential candidates for therapies intended to block the TWEAK signaling pathways.

References

[1] **Salah S., Lotfy H., Mokbcl et al.:** Damage index in childhood-onset systemic lupus erythematosus in Egypt. Pediatr. Rheumatol .Online J 2011; 9:36.

[2] **Gatto M., Zen M., Ghirardello A. et aL :** Emerging and critical issues in the pathogenesis of lupus. Autoimmun. Rev.2013; 12:523-36.

[3] **De Zubiria S. and Herrcra-Diaz C:** Lupus nephritis: an overview of recent findings. Autoimmune Dis. 2012;1-21.

[4] **Schwartz N, Su L, Burkly LC. et al.,:** Urinary TWEAK and the activity of lupus nephritis. J Autoimmun.2006; 27(4):242-50.

[5] **Mok CC:** Biomarkers for Lupus Nephritis: A Critical Appraisal. Journal of Biomedicine and Biotechnology; 2010:638413.

[6] **Schwartz N, Michaelson J and Putterman C:** Lipocalin-2, TWEAK, and other cytokines as urinary biomarkers for lupus nephritis. Annals of the New York Academy of Sciences.2007; 1109: 265–274.

[7] **Foster D, Parrish-Novak J, Fox B. et al.,:** Cytokine-receptor pairing: accelerating discovery of cytokine function. Nature Rev Drug Discov 2004; 3(2):160–70.

[8] **Campbell S, Burkly LC, Gao HX. et al.,:** Proinflammatory effects of TWEAK/Fn14 interactions in glomerular mesangial cells. J Immunol 2006; 176(3):1889-1898.

[9] **Schwartz N, Rubinstein T, Burkly LC. et al.,:** Urinary TWEAK as a biomarker of lupus nephritis: a multicenter cohort study. Arthritis Res Ther. 2009 ; 11(5): 143.

[10] **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(1):24-35.

[11] **Hochberg MC :** Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum. 1997; 40(9): 1725.

[12] **Zhu X, Tan J, Li J. et al.,:** Urinary TWEAK Level as a marker of Lupus Nephritis activity in 46 cases. Journal of biomedicine and biotechnology. Volume 2012, Article ID 359647, 7 pages.

[13] **Weening J., D'Agati V., Schwartz M. et al. :** The classification of glomerulonephritis in systemic lupus erythematosus revisited. J. Am. Soc. Nephrol.2004; 15:241-250.

[14] **Manoharan A and Michael P :** Biomarkers in Lupus Nephritis. Rheum Dis Clin N Am. 2010; 36: 131–143.

[15] **Schwartz N, Michaelson J and Putterman C:** Lipocalin-2, TWEAK, and other cytokines as urinary biomarkers for lupus nephritis. Annals of the New York Academy of Sciences.2007; 1109: 265–274.

[16] **Reyes-Thomas J, Blanco I. et al.,** : Urinary Biomarkers in Lupus Nephritis. Clin Rev Allergy Immunol. 2011; 40(3):138-50.

[17] **Sanz AB, Justo P, Sanchez-Niño MD. et al., :** The cytokine TWEAK modulates renal tubulointerstitial inflammation. J Am Soc Nephrol.2008; 19(4):695–703.

[18] **Muñoz-García B, Martín-Ventura JL, Martínez E. et al., :** Fn14 is upregulated in cytokine-stimulated vascular smooth muscle cells and is expressed in human carotid atherosclerotic plaques modulation by atorvastatin. Stroke 2006; 37(8):2044–53.

[19] **Leng R, Pan H, Qin W. et al., :** TWEAK as a target for therapy in systemic lupus erythematosus. Mol Biol Rep 2011; 38(1):587–592.

[20] **El-shehaby A, Darweesh H, El-Khatib M. et al., :** Correlations of Urinary Biomarkers, TNF- LikeWeak Inducer of Apoptosis (TWEAK), Osteoprotegerin (OPG), Monocyte Chemoattractant Protein-1 (MCP-1), and IL-8 with Lupus Nephritis. J Clin Immunol 2011; 31(5):848-856.

[21] **Dooley MA :** Clinical and laboratory features of lupus nephritis. In: Dubois' Lupus Erythematosus. 7th ed. Wallace DJ, Hahn BH, eds. Philadelphia, PA: Lippincott Williams & Wilkins 2007: 1112-1130.