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Urinary Epidermal Growth factor as a marker for Lupus Nephritis: Clinical, Laboratory and Histopathological Study

--Manuscript Draft--

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Is this study a clinical trial? *A clinical trial is defined by the World Health Organisation as 'any research study that prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects on health outcomes'.*	No

Title page

Cover Letter:

Dear Editor of the journal of the Egyptian Rheumatology and Rehabilitation; we have the honor to submit our manuscript (**Urinary Epidermal Growth factor as a marker for Lupus Nephritis: Clinical, Laboratory and Histopathological Study)** in your valuable journal, we have conducted this research for the purpose of testing the value of using Human Urinary Epidermal Growth Factor as a non-invasive, easy and cheap test for detecting and following up the progression of lupus nephritis.

The article has not been published and is not under consideration for publication elsewhere.

Key words; SLE, Nephritis, Renal biomarkers, Urinary Epidermal Growth factor, renal biopsy.

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Urinary Epidermal Growth factor as a marker for Lupus Nephritis: Clinical, Laboratory and Histopathological Study

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Declarations:

- Ethics approval and consent to participate: The protocol of the study was approved by the local Ethics Committee with number IBR≠S20-135 and conforms to the guidelines of the Declaration of Helsinki
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Urinary Epidermal Growth factor as a marker for Lupus Nephritis: Clinical, Laboratory and Histopathological Study

Running title: Urinary Epidermal Growth Factor in SLE Nephritis

Abstract

Background: Lupus nephritis can be seen in up to 60% of all SLE patients with 10–15% of nephritis patients progress to end-stage renal disease, late diagnosis of lupus nephritis is correlated with a higher frequency of renal insufficiency. The study aim is determination of the value of Urinary Human Epidermal Growth Factor (urinary EGF) as an early biomarker of lupus nephritis in SLE patients and its relevance to disease activity and renal histopathology.

Results: the study included 58 SLE patients and 30 healthy control, a significant difference was noticed between SLE and controls in urinary protein, creatinine, protein/creatinine ratio, and urinary EGF. The mean level of urinary EGF was less in class IV and V renal nephritis than in class I, II and III.

A significant difference in urinary EGF (33±29, 27±16, P=0.04) between class II and class III Lupus nephritis, with no significant differences in Urinary protein, creatinine, Protein/creatinine ratio, SLEDAI. On the other hand, the comparison between class II and IV showed no only significant difference in urinary EGF (33±29, 11.7±4.9m P=0,003), but also in SLEDAI (37.4±8, 70.5±27, P= 0.007), and Protein/creatinine ratio (0.98±0.62, 3±1.8, P=0.006).

Conclusion: This study raises the attention to test the sensitivity of urinary EGF in detecting the early and the subsequent changes in renal pathology of SLE patients as an easy, non-invasive, accurate, cheap marker that could help in following up the nephritis progression and adjusting the plan of treatment, also it can be used to guide the time of biopsy or as an alternative in cases where renal biopsy is contraindicated.

Background:

One of the potentially life-threatening diseases is the Systemic Lupus Erythematosus (SLE). SLE owns a broad range of clinical manifestations with often unpredictable temporal sequence of organ involvement, and disease flares that may cause permanent injury,[1]. Lupus nephritis (LN) can be seen in up to 60% of all SLE patients with 10–15% of nephritis patients progress to end-stage renal disease (ESRD) requiring hemodialysis [2].

late diagnosis of lupus nephritis is correlated with a higher frequency of renal insufficiency [3]. The increased incidence of ESRD underlines the importance of early diagnosis in this difficult to control disease with unpredictable course [4].

The ideal biomarker in SLE patients with suspicion or confirmation of LN should have the following properties: 1) be specific for renal involvement, 2) have a good correlation with kidney activity or damage, 3) be useful for serial monitoring, 4) be superior to conventional clinical or laboratory parameters, 5) possess the ability to assess the severity of renal involvement, 6) be cost-effective, and 7) easy to perform and available in most clinical laboratories [1].

In a longitudinal study by Moroni et al [5] anti-dsDNA, anti-C1q, C3, and C4 all had poor positive predictive values (ranging from 28% to 38%). Although the best multivariate analysis model for renal flare prediction was obtained by combining anti-C1q with C3 and C4, their data clearly showed that anti-C1q antibodies were less reliable in predicting flares in non-proliferative nephritis and flares in the presence of anti-phospholipid antibodies. Furthermore, none of these traditional markers has been shown to possess the ability to predict histology. Clearly, the lack of specificity of our current markers for lupus nephritis and inability to predict histology highlight the pressing need for a true biomarker for lupus nephritis.

Sedky et al [6] assessed the levels of urinary LXA4 in SLE patients, and showed that the urinary LXA4/creatinine ratio levels were significantly lower in cardiovascular and neuropsychiatric manifestations and non-significantly lower in patients with nephritis.

Studies which demonstrated the potential use of urinary biomarkers of LN activity, showed correlation with disease activity, renal flare, histological damage and that may help in monitoring the response to immunosuppressive treatment, however, studies about biomarkers in LN still involve relatively few cohorts. The urinary biomarkers are still not superior to renal biopsy, which remains the gold standard to determine LN activity and chronicity [7].

Human epidermal growth factor (urinary EGF), a 6.000 molecular weight polypeptide, was first isolated by Cohen and Carpenter in 1975 [8]. EGF is a growth factor that stimulates cell growth, proliferation and differentiation by binding to its receptor EGFR, some studies found that urinary EGF has a role in the development of body organs such as brain, lungs, blood vessels and kidneys [9].

Urinary EGF is locally produced in several tissues, such as Henle's loop and the distal convoluted tubule in the kidney, salivary glands and duodenum [10]. In the kidney, urinary EGF is involved in the repairing process of renal tissues [11].

High concentrations of urinary EGF can be found in the urine. Based on in vitro experiments, it has been previously suggested that urinary urinary EGF originates from the ultrafiltrate. However, in vivo, it was shown in rats and in humans that the urinary urinary EGF is mainly produced in the kidney itself. Therefore, it is generally accepted that the urinary urinary EGF excretion reflects the renal EGF production [11-13]. Reduced concentrations of urinary EGF in the urine have been previously observed in diabetes nephropathy, IgA nephropathy, adult polycystic kidney disease, and children with chronic renal failure [14, 15]. Also, the possibility that urinary EGF might serve as a surrogate marker for functional regeneration of the renal tubules, reflecting their ability to respond to future acute or chronic injury was recently put forward [16].

This study is conducted to determine the value of Urinary Human Epidermal Growth Factor (urinary EGF) as an early biomarker of lupus nephritis in SLE patients and its relevance to disease activity and renal histopathology progression.

Methods:

A cross sectional observational study included 58 patients diagnosed with SLE, fulfilling the 2012 Systemic Lupus International Collaborating Clinics (SLICC) classification criteria of SLE [17] and have signs of renal involvement (hematuria, urinary cast, proteinuria or histopathologic picture of nephritis, these patients have been admitted to the Department of Rheumatology and Rehabilitation, that was during the period between August 2017 and December 2019, The protocol of the study was approved by the local Ethics Committee with number IBR≠S20-135 and conforms to the guidelines of the Declaration of Helsinki.

Patient and Public Involvement: the researchers explained the study protocol and aim of the work to all the participants, and patients' consent were signed.

Drug induced lupus, discoid lupus without systemic manifestations and diabetic patients were excluded. The following data were collected: full medical history, general examination, cardiovascular, chest, abdominal, neurological and locomotor system examination, age at disease onset (defined at the time of onset of symptoms attributed to SLE), the duration of the disease (defined as the time from disease onset until the date of visit), the clinical features of SLE, routine laboratory and autoimmune tests for each patient had been done.

For disease activity assessment we used the SLEDAI index [18].

-Thirty healthy control matched for age and sex were recruited from the officers in the hospital and volunteers.

- Laboratory Renal investigations: The patients and healthy volunteers were instructed to collect a spot midstream urine sample in a clean sterile container. For SLE patients the sample was collected at the day of renal biopsy.

1. Spot urine protein/creatinine ratio (u-P/C ratio): Both urinary protein and creatinine concentrations were measured by turbidimetric assay and Kinetic colorimetric Jaffé method, respectively, using Cobas c311 Chemistry Analyzer System (Roche Diagnostics, GmbH, Mannheim, Germany). u-P/C ratio in spot urine samples was calculated by dividing the urinary protein concentration in mg/dL by urine creatinine concentration in mg/dL. u-P/C ratio of less than 0.2 mg/mg was considered within normal limits, whereas a ratio in excess of 3.5 was considered as "nephrotic-range" proteinuria,[19]
2. Detection of Urinary Human Epidermal Growth Factor (EGF): After collection of urine sample in a sterile container, it was centrifuged at the speed of 2000-3000 rpm for 20 min. Supernatant was removed, if precipitation was appeared, the

urine sample was centrifuged again. The supernatants were collected and were divided into aliquots and stored at -80˚C. Urinary EGF was measured in urine samples using an enzyme-linked immunosorbent assay (ELISA) kit (SinoGeneClon Biotech Co., Ltd, No.28 Cangxin Road,YuHang District 311112, HangZhou, China, CATALOG #: SG-10583), according to the manufacturer' instructions. A standard curve was prepared by serial dilution of the standard supplied with the kit. Standards and diluted urine samples were added to a 96-well plate pre-coated with purified Human EGF antibody. The plate was incubated for 30 min. at 37 °C. Following complete plate washing, combined EGF which with HRP labeled conjugate, became antibody-antigen-enzyme-antibody complex. The detection antibody was incubated for 30 min. at 37 °C, and after complete plate washing, tetramethylbenzidine (TMB) substrate was added and incubated for 15 min. at 37 °C with avoidance of light. TMB substrate became blue color as HRP enzyme-catalyzed. The enzyme reaction was terminated by the addition of a stop solution with change of color from blue to yellow. The absorbance of the color change was measured at 450 nm using the Thermo Fisher Scientific Multiskan EX Microplate Reader (Thermo Fisher Scientific Oy, FI-01621 Vantaa, Finland). The concentration of human EGF in samples, was determined by comparing the O.D. of the samples to the standard curve.

Statistical analysis: Data analyzed by SPSS version 20.0 statistical package, data were presented as number and percent, mean ±SD, or median and range as appropriate. Student's *t*-test, and multivariate analysis were used for comparing means between different groups, Pearson correlation coefficient (*r*) was used to test the association between quantitative variables. A *p*-value less than 0.05 was considered statistically significant.

Results:

The patients' mean age is 47.2±14.6 years, with 53 females (91.4%) and the mean disease duration in years is 5.4±4.2, SLEDAI mean ±SD was 12.3±9.4, with minimum 2 and maximum 22, medications were (100%

Hydroxychloroquine, 69% Mycophenolate mofetil, and 17.2% Azathioprine, 86% corticosteroid), the main data of the patients is displayed in (table 1).

The comparison between the patients and the control group was significant in urine protein, urine creatinine, protein/creatinine ratio and in urine EGF level (table 2).

We found no significant differences in urinary EGF level between; speckled and homogenous ANA pattern, positive and negative anti-dsDNA.

No significant correlation of urinary EGF level with urinary P/C ratio, or with any SLE disease parameters and renal laboratory tests except for ESR there was a negative significant correlation (r= -0.72, P=0.002).

With classification of renal biopsy according to the International society of nephrology/renal pathology society 2003 classification of lupus nephritis [20] we noticed that the mean level of urinary EGF was less in class IV and V than in class I, II and III. Table (3), and histopathological picture for sample of patients in figure (1).
Histological renal biopsy showed significant negative correlation with urinary EGF (r= -0.55 p= 0.008) figure (2), and ESR (r= -0.56 p=0.03).

Due to the small number of patients in class I and V nephritis, we started the comparison with class II.
By independent T test between class II and class III Lupus nephritis, there was no significant differences in Urine protein, Urine creatinine, Protein/creatinine ratio, SLEDAI, but there was significant differences in urinary EGF (33±29, 27±16, P=0.04) , while comparison between class II and IV showed significant differences in SLEDAI (37.4±8, 70.5±27, P= 0.007), in Protein/creatinine ratio (0.98±0.62, 3±1.8, P=0.006), and urinary EGF (33±29, 11.7±4.9m P=0,003).

we further tested the relation of renal biopsy with urinary EGF, protein/creatinine ratio and SLEDAI through the linear regression analysis

which showed only significance with the urinary EGF level. Table 4 and supplementary tables (4a-4b-4c).

ROC curve has been created to test urinary EGF level as a predictor of Lupus Nephritis and showed cut off value at <40.6, figure (3).

Discussion:

Renal biopsy with histological study of kidney tissue is an esteemed tool for diagnostic classification and prognostication in Lupus nephritis patients, but we can't deny the accompanied significant morbidity with the procedure of renal biopsy, that why it is not usually performed serially. Furthermore, with an essentially "blind" needle biopsy, there can be a question of how representative are the limited number of glomeruli usually obtained of kidney activity and chronicity [1].

We seriously need a noninvasive, easily obtainable, and accurate marker that can be followed serially in monitoring lupus patients. pathologic studies provide limited information because patients are not biopsied frequently and clinical measures provide limited information since they do not reflect intrarenal injury very well. In the previous studies many laboratory markers have been used, which include serological determination of serum anti-double-stranded (ds)DNA antibodies and complement levels, and those can be helpful clinically, but the correlation between them and lupus renal disease is lacking. Sensitivity and specificity for active lupus nephritis among all SLE patients different with different studies and tests used [5, 21, 22].

Our study included 58 SLE adult patients, 53 (91%) of them are females, with the mean disease duration in years is 5.4±4.2, the patients showed significant differences with the healthy control in the renal lab tests (urine protein- protein/creatinine ratio and urinary EGF level) which matches with the results of many other studies [11, 13, 18, 23].

Interestingly, we did not find significant correlations between urinary EGF level and urinary P/C ratio, despite that urinary protein is known to be generally a simple marker for detecting renal glomerular disease activity, and that could be explained by the earliest change in the level of urinary

EGF than p/c ratio, similar observations were present in studies tested the urinary chemokines correlation with the u-P/C ratio [24, 25].

Other clinical parameters such as u-P/C ratio, urinary EGF level did not show any significant correlation with the SLE disease parameters and renal laboratory tests but the ESR showed negative significant correlation (r = -0.72 P=0.002).

In the study of Worawichawong, et al 2016 [25] they tested the combined use of urinary biomarkers with opposing actions such as EGF and MCP-1 (Monocyte Chemoattractant Protien-1) to offer additional information compared to either cytokine alone. Previously, the ratio of urinary biomarkers, they found urinary EGF/MCP-1 ratio is independently associated with tubulointerstitial severity in primary glomerulonephritis. However, they did not address the benefit of EGF/MCP-1 ratio over EGF alone at discriminating renal histological grade when the additional costs of the MCP-1 assay is considered. By contrast, Neutrophil gelatinase-associated lipocalin (NGAL) appeared to be strongly associated with proteinuria, and less useful as a biomarker of tubulointerstitial disease severity compared to EGF. They recommended for further prospective studies to support and evaluate role of EGF or EGF/MCP-1 as candidate biomarkers to guide to therapy in various types of GN.

It was essential in this study to use the most approved tool for detecting the renal affection in SLE nephritis patients, it is the histological study of renal biopsy, and that work showed significant negative correlation with only urinary EGF level (r= -0.55 p= 0.008) and ESR (r= -0.56 p=0.03). This significant negative correlation with urinary EGF has been translated by the noticeable differences in its level among the renal histology classes, not only this, but it gave significant differences between two close classes (II and III), while with widening the interclasses comparison (class II and IV) the significant differences between the urinary EGF levels increased, and the other parameters appeared to give significant differences (SLEDAI, u-P/C ratio) this observation raise the attention to test the sensitivity of urinary EGF level for detecting the subsequent changes in renal pathology in SLE patients and so on, can be an excellent, non-invasive, accurate, cheap marker for following up the nephritis progression and adjusting the

plan of treatment, and to support our finding Linear regression analysis of renal histological biopsy with the urinary EGF, protein/creatinine ratio and SLEDAI was done, and again has supported the urinary EGF importance by being significant only with it, and ROC curve has shown a cut off value for urinary EGF <40.6 with a sensitivity 90.4% and specificity 83.3%.

According to our knowledge this study is one of the few studies which tested the level of urinary EGF in adult systemic Lupus nephritis and discussed its relationship with diseases activity index and histological study in Arab SLE patients with determining cut off value.

Conclusion: SLE renal biopsy histopathological results were parallel to the decrease in urinary EGF level. Urinary EGF is a simple urine test showed significant reduction at the very early stage of Lupus nephritis (class I) and showed a significant correlation as well with renal nephritis changes among classes.

Urinary EGF is a noninvasive, cheap and easy way to follow the progression of Lupus nephritis and could help in the early management and monitoring of progression.

The urinary EGF owns the advantage as a LN biomarker by having a good correlation with kidney activity and damage, useful for serial monitoring, can be superior to conventional clinical or laboratory parameters, possess the ability to assess the severity of renal involvement, little cost-effective, and easy to perform and available in most clinical laboratories

Limitation of the study:

Our study is a cross sectional, while we recommend a future longitudinal study in order to provide more information and accuracy for its course and use as a biomarker for follow up of SLE nephritis.

The study did not include SLE patients without nephritis as a comparative group, which is a step forward will be done in an extension study of this one.

Abbreviations:

Antinuclear Antibodies	ANA
Anti-phospholipid	APL
Complement 3	C3
Complement 4	C4
Discoid lupus	DLE
Double strands DNA	ds-DNA
End Stage Renal Disease	ESRD
Epidermal Growth Factor Receptor	EGFR
Erythrocyte sedimentation Rate	ESR
Glomerulonephritis	GN
Lupus Nephritis	LN
Systemic Lupus Erythematosus	SLE
Systemic Lupus Erythematosus Disease Activity Index	SLEDAI
Systemic Lupus International Collaborating Clinics	SLICC
Epidermal Growth Factor	EGF
Urine Protein/Creatinine ratio	u-P/C ratio

References:

1. Reyes-Thomas, J., I. Blanco, and C. Putterman, Urinary biomarkers in lupus nephritis. Clin Rev Allergy Immunol 2011;40: 138-150.
2. Weening, J.J., D'Agati, V.D., Schwartz, M.M., Seshan, S.V., Alpers, C.E., Appel, G.B., et al., The classification of glomerulonephritis in systemic lupus erythematosus revisited. J Am Soc Nephrol 2004;15.2: 241-250.
3. Esdaile, J. M., L. Joseph, T. MacKenzie, M. Kashgarian, and J. P. Hayslett. The benefit of early treatment with immunosuppressive agents in lupus nephritis. J Rheumatol 1994;21: 2046-2051.
4. Faurschou, M., Starklint, H., Halberg, P. and Jacobsen, S., Prognostic factors in lupus nephritis: diagnostic and therapeutic delay increases the risk of terminal renal failure. J Rheumatol 2006; 33:1563-1569.
5. Moroni, G., Radice, A., Giammarresi, G., Quaglini, S., Gallelli, B., Leoni, A., et al., Are laboratory tests useful for monitoring the activity of lupus nephritis? A 6-year prospective study in a cohort of 228 patients with lupus nephritis. Ann Rheum Dis 2009;68: 234-237.
6. Abdou, M. M. S., Effat, D. A., Mansour, L. A., Abd El Baky, N. M., & Salam, M. M. A, Urinary lipoxin A4 as a biomarker for systemic lupus erythematosus. Egypt Rheumatol Rehabil, 2015. **42**(2): p. 55-61.
7. Gasparin, A.A., Pamplona Bueno de Andrade, N., Hax, V., Tres, G.L., Veronese, F.V. and Monticielo, "Urinary biomarkers for lupus nephritis: the role of the vascular cell adhesion molecule-1." Lupus 28, no. 3 (2019): 265-272
8. Cohen, S. and G. Carpenter, Human epidermal growth factor: isolation and chemical and biological properties. Proc Natl Acad Sci 1975; 72: 1317-1321.
9. Betsholtz, C., Insight into the physiological functions of PDGF through genetic studies in mice. Cytokine Growth Factor Rev 2004;15: 215-228.
10. Zeng, F. and R.C. Harris. Epidermal growth factor, from gene organization to bedside. Semin Cell Dev Biol 2014;22:2-11.
11. Chou, J.S., I.W. Reiser, and J.G. Porush, Aging and urinary excretion of epidermal growth factor. Ann Clin Lab Sci 1997;27:116-122.
12. Li, Z., Zhao, Z., Liu, X., Su, Z., Shang, X. and Wen, J. Prediction of the outcome of antenatal hydronephrosis: significance of urinary EGF. Pediatr Nephrol 2012;27:2251-2259.
13. Grandaliano, G., Gesualdo, L., Bartoli, F., Ranieri, E., Monno, R., Leggio, A., et al., MCP-1 and EGF renal expression and urine excretion in human congenital obstructive nephropathy. Kidney Int 2000;58:182-192.
14. Dincer, Y., C. Akkaya, and S.J.U.N.O.A.J. Alagöz, Assessment of urinary epidermal growth factor level in patients with chronic kidney disease. Urol Nephrol Open Access J 2018; 6:131-134.
15. Meybosch, S., De Monie, A., Anné, C., Bruyndonckx, L., Jürgens, A., De Winter, B.Y., et al., Epidermal growth factor and its influencing variables in healthy children and adults. PloS one 2019; 14: e0211212 .
16. Ju, W., Nair, V., Smith, S., Zhu, L., Shedden, K., Song, P.X., et al., Tissue transcriptome-driven identification of epidermal growth factor as a chronic

kidney disease biomarker. Science translational medicine, 2015;7: 316ra193-316ra193.

17. Petri, M., Orbai, A.M., Alarcón, G.S., Gordon, C., Merrill, J.T., Fortin, P.R., et al., Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. Arthritis Rheum 2012;64:2677-2686.
18. Gladman, D.D., D. Ibanez, and M.B. Urowitz, Systemic lupus erythematosus disease activity index 2000. J Rheumatol, 2002;29:288-291.
19. Ginsberg, J.M., Chang, B.S., Matarese, R.A. and Garella, S., Use of single voided urine samples to estimate quantitative proteinuria. N Engl J Med 1983;309:1543-1546.
20. Park, M.H., International Society of Nephrology/Renal Pathology Society 2003 Classification of Lupus Nephritis. Korean J Pathol 2006; 40:165-175.
21. Oelzner, P., Deliyska, B., Fünfstück, R., Hein, G., Herrmann, D. and Stein, G., Anti-C1q antibodies and antiendothelial cell antibodies in systemic lupus erythematosus–relationship with disease activity and renal involvement. Clin Rheumatol 2003; 22:271-278.
22. Abd Elazeem, M.I., Mohammed, R.A. and Abdallah, N.H., "Correlation of serum interleukin-10 level with disease activity and severity in systemic lupus erythematosus." Egypt Rheumatol Rehabil 45, no. 1 (2018): 25.
23. Birmingham, D.J., Merchant, M., Waikar, S.S., Nagaraja, H., Klein, J.B. and Rovin, B.H., Biomarkers of lupus nephritis histology and flare: deciphering the relevant amidst the noise. Nephrol Dial Transplant, 2017; 32(suppl_1): i71-i79.
24. Stangou, M., Alexopoulos, E., Papagianni, A., Pantzaki, A., Bantis, C., Dovas, S., et al., Urinary levels of epidermal growth factor, interleukin- 6 and monocyte chemoattractant protein- 1 may act as predictor markers of renal function outcome in immunoglobulin A nephropathy. Nephrol, 2009; 14:613-620.
25. Worawichawong, S., Worawichawong, S., Radinahamed, P., Muntham, D., Sathirapongsasuti, N., Nongnuch, A., Urine epidermal growth factor, monocyte chemoattractant protein-1 or their ratio as biomarkers for interstitial fibrosis and tubular atrophy in primary glomerulonephritis. Kidney Blood Press Res, 2016; 41:997-1007

Figure titles/legends:

Figure 1: Different stages of LN in investigated cases including *A*- mesangial proliferative LN (stage II), *B*- focal active / chronic LN (stage III A/C), *C*- diffuse segmental LN (Stage IV-S) and *D*- diffuse global LN (IV-D). Hematoxylin and Eosin stain; magnification is X400 for all.

Figure 2: Correlation between the histological renal biopsy and urinary EGF

Figure 3: ROC curve analysis of urinary EGF relation with the Histological Renal Biopsy

Table 1: The main demographic data for the patients;

Variable	Mean± SD	**Variable**	Percent %	**Variable**	Percent %
Disease duration yr	5.4±4.2	Females	91.4	ANA	100
SLEDAI	12.3±9.4	Alopecia	45.5	dsDNA	60
WBC (x 10^9 / l)	7.2±3.4	Malar rash	54.5	Speckled ANA	60.5
PLT (x 10^9 / l)	272±135	photosensitivity	77.3	Homogenous ANA	33.5
Hgb (g/dl)	9.9±2.4	DLE rash	31.8	Others ANA patterns	6
U-P/C (mg/mg)	1.2 ±1.2	Oral/nasal ulcers	54.5	Anti La	25
ESR (mm.)	71±49.8	Pericarditis	13.6	Anti Ro	45
C3 (mg/dl)	62±35.4	Pleurisy	9.6	Ribosomal P0	20
C4 (mg/dl)	23±12.6	Anti Sm	10	APL	5.3

WBC; white blood cells, PLT platelet count, Hgb Hemoglobin, ESR; erythrocytes sedimentation rate, C3; complement 3, C4; complement 4, DLE; discoid lupus, Both; both speckled and homogenous ANA, APL; Anti-phospholipid Abs, U P/C; urinary protein/creatinine ratio.

Table 2: Comparison between the patients and the controls

Variable	Patients (58)	Control (30)	P-value
Age, year	31.6±9.4	32.5±6.4	0.7
Urine protein	106.6±63.8	5.7±0.95	0.002
Urine creatinine	128.4±31.3	136±145	0.42
Protein/creatinine	1.02±0.57	0.06±0.036	0.03
Urinary EGF	30.2±16.7	50.7±0.9	0.001

P value less than 0.05 is significant.
Urinary EGF; Urinary Epidermal Growth Factor.

Table 3: Histological Classification of Renal biopsy

	Percent % of patients	Urinary EGF (Mean±SD)
Class I	3.4	32±17.8
Class II	60.3	33±29
Class III	22.4	27.3±16
Class IV	10.3	11.7±4.9
Class V	3.4	13.4±5.7

Measurement of the level of urinary epidermal growth factor in each class of histological Lupus nephritis.

Table 4: Linear Regression for Renal biopsy with each of urinary EGF, Protein/ Creatinine Ratio and SELDAI

Linear Regression	Urinary EGF	Protein/creatinine Ratio	SLEDAI
Renal Biopsy	Sig 0.036	Sig 0.46	Sig 0.98

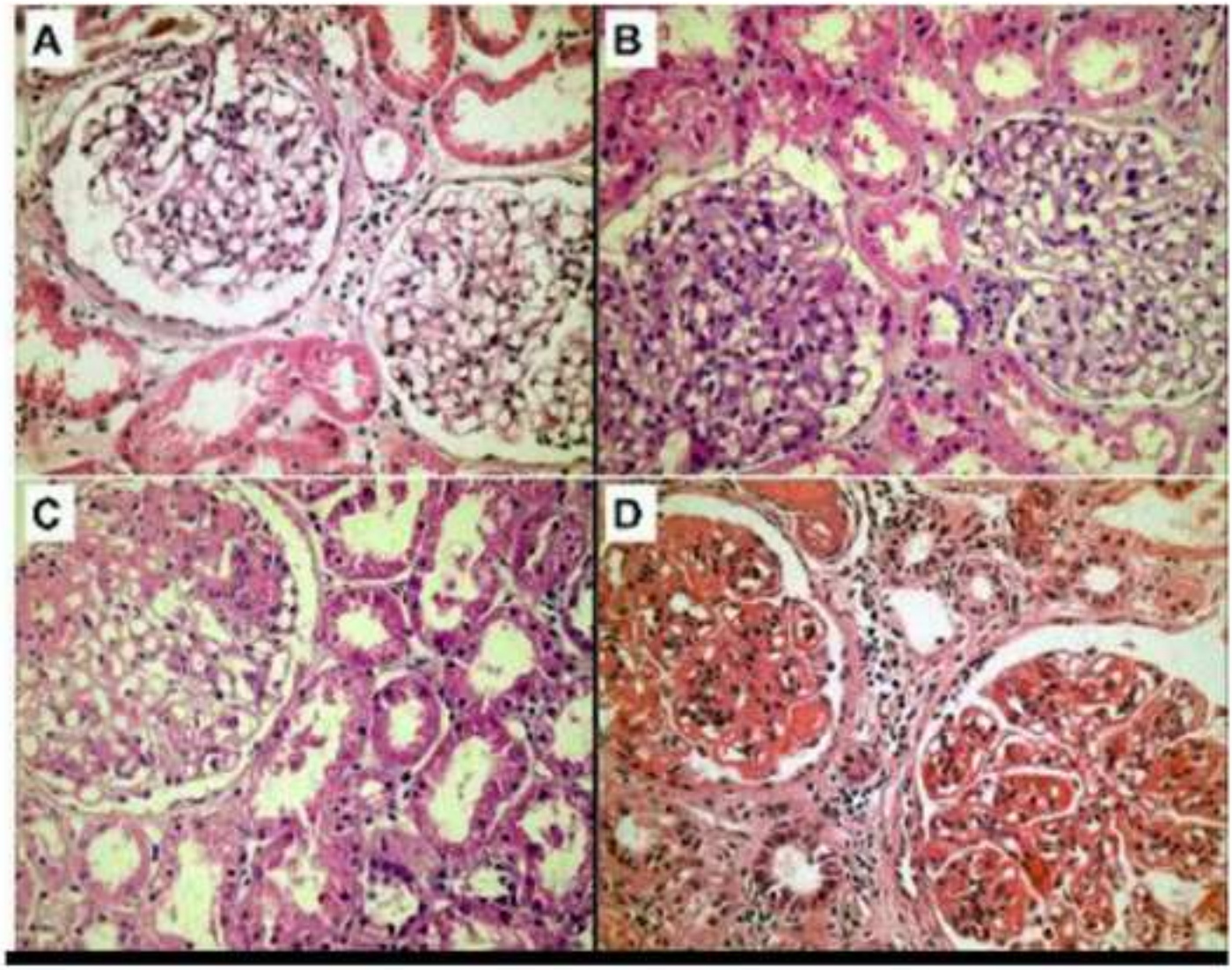
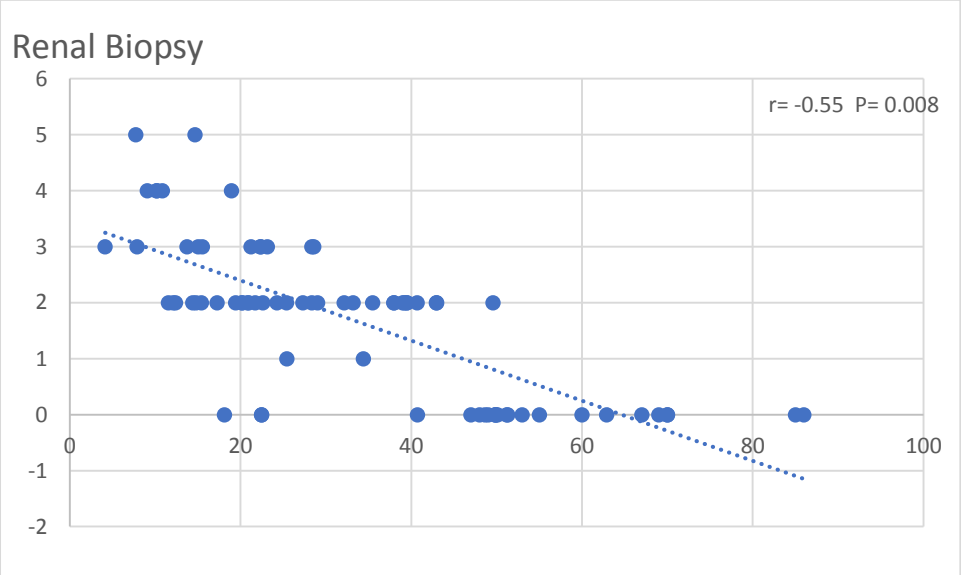


Figure 1: Different stages of LN in investigated cases including A-mesangial proliferative LN(stage II), B-focal active/chronic LN (stage III A/C), C-diffuse segmental LN (stage IV) and D- diffuse global LN (IV-D).

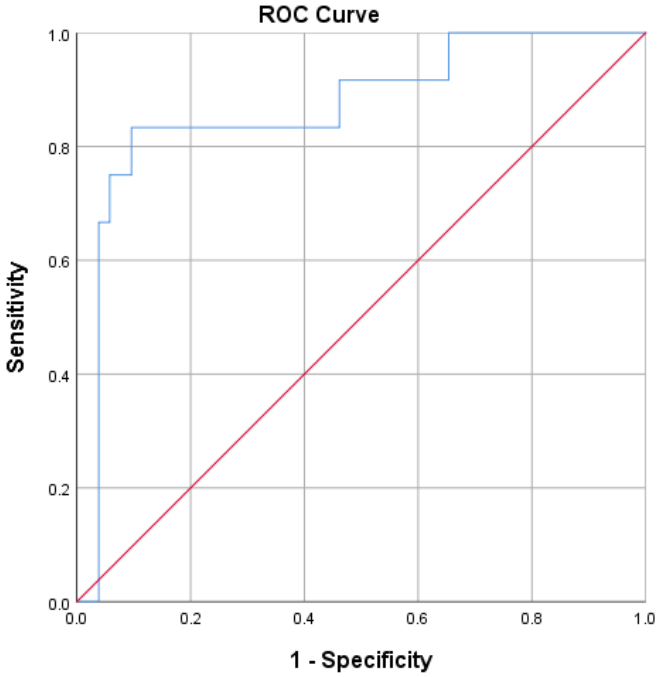
Figures

Figure 2: Correlation between the histological renal biopsy and urinary EGF



Negative significant correlation with r= -0.55 and P= 0.008

Figure 3: ROC curve analysis of urinary EGF relation with the Histological Renal Biopsy



ROC curve analysis of urinary EGF as a predictor of Lupus Nephritis

Variable	Best cut off point	AUC (95 % CI)	Sensitivity (%)	Specificity (%)	PPV (%)	NPP (%)	P value
Urinary EGF	≤40.6	0.869 (0.745:0.992)	90.4	83.3	95.9	66.7	<0.0001