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RESEARCH ARTICLE

The association between urinary angiostatin and urinary angiostatin to urinary creatinine ratio with lupus nephritis and their correlation to disease activity

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Abstract

Background. Currently, renal biopsy is the gold standard tool for diagnosis and prediction of prognosis in lupus nephritis (LN). However it cannot be performed serially and tissue obtained may not represent the renal pathology. Finding a non-invasive, easily obtainable, and accurate marker that performed serially may therefore be of greater value in monitoring LN.

Aim of study. To explore the ability of urinary angiostatin and urinary angiostatin / urinary creatinine (UAng/UCr) ratio to identify SLE with nephritis and their relation with lupus activity.

Subjects and Methods.UAng and UAng/UCr ratio were evaluated in 100 lupus patients and compared to that in the controls. These markers were also compared between patients with and without LN.

Results. UAng and Ln UAng/UCr ratio were significantly higher in SLE patients than controls and also in SLE patients with than without LN.Among SLEpatients,these markers are significantly correlated with SLEDAI score and with serum creatinine while inversely correlated with serum C3 and C4 levels. Among LN patients, UAng and Ln UAng/UCr ratio are significantly correlated with renal SLEDAI score and renal chronicity index but not with renal activity index. A ROC curve analysis revealed the usefulness of UAng and Ln Uang/UCr ratio in discriminating SLE patients with LN from those without LN.

Conclusion. UAng and Ln UAng/UCr ratio are associated with LN class and renal chronicity index .UAng and Ln UAng/UCr ratio are capable of identifying lupus patients with LN from patients without. UAng and Ln UAng/UCr ratio are correlated with lupus activity.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic autoimmune inflammatory disease that can affect any organ in the body***(Tsokos, 2011)***.Renal involvement is common in SLE as estimates had shown that more than half of the patients will develop Lupus nephritis (LN) during the course of the disease***(Cameron, 1999)*** with a considerable frequency of these patients develop LN in the first year after diagnosis ***(SeshanandJennette, 2009)***.LN is a seriouscondition and is associated with significant morbidity and mortality among the SLE patients, however, the extent of renal involvement among these patients varies widely ***(Contreras et al., 2005)***with reports of 5-year renal

survival with treatment ranging from 46 to 95% ***(Korbet et al., 2000; Sidiropoulos et al., 2005)***. Therefore, early identification of SLE patients who had LN can provide a good opportunity to improve the disease outcome and to prevent progression of the disease to end stage renal disease.

Currently, histological examination of renal tissues is the gold standard tool for diagnosis, evaluation, and prediction of prognosis in LN. However, renal biopsy can be associated with significant morbidity and, hence, is not usually performed serially. Besides, it is questionable how representative are the limited number of glomeruli that are obtained of LN activity and chronicity by the use of blinded needle biopsy. A non-invasive, easily obtainable, and accurate marker that performed serially may therefore be of greater value in monitoring LN. Laboratory markers in current use, which include estimation of serum anti-double-stranded (ds)DNA antibodies and complement levels, can be beneficial, but the correlation between those and LN is imperfect***(Reyes-Thomas et al., 2011)***.

Since SLE is a systemic disease, serum biomarkers appearsproper in monitoring for lupus activity. With respect to LN, however, urine is a direct product of the kidney and therefore urinary biomarkers can be more specific for renal damage than serum biomarkers, especiallyin patients with lupus flare.Moreover, urine samples are easily obtained and non-invasive method, making urine samples ideal for a disease that requires repetitive screening.

Several molecules have emerged in recent years as a potential urinary biomarker for LN including interleukin (IL)-6 ***(Iwano et al., 1993)***, vascular cell adhesion molecule (VCAM)-1 ***(Abd-Elkareem et al., 2010)***, Neutrophil gelatinase-associated lipocalin***(Rubinstein et al., 2010),*** TNF-like weak inducer of apoptosis (TWEAK) ***(Liu et al., 2011)***. Nevertheless, the ideal urine biomarker for monitoring LN remains elusive.

Among the investigated molecules angiostatin emerged as a promising marker of nephritis. Angiostatin is a bioactive fragment of plasminogen, and has been known to have modulatory function in angiogenesis and inflammation ***(Wu et al., 2010)***. The goal of this study is to explore the ability of urinary angiostatin and of the urinary angiostatin / urinary creatinine (UAng/UCr) ratio to identify SLE with nephritis and the relation of these markers with lupus activity.

Subjects and Methods

This study was conducted on 100 consecutive patients with SLE who were attending the outpatient clinic of nephrology and Rheumatology & Rehabilitation in different areas in saudiaarabia, between june 2014 and July 2015. All patients met the American College of Rheumatology (ACR) revised criteria for the classification of SLE ***(Hochberg, 1997)***. All SLE patients were females, their age ranged from 20 to 44 years and their disease duration ranged from 1 to 14 years. The study included also 50 age-matched apparently healthy females who served as a control group.

For inclusion, patients had to have adequate renal biopsy samples for histological diagnosis. Based on the renal pathological findings at renal biopsy the SLE patients were dichotomized into two groups: (a) SLE patients with biopsy proven LN (BPLN) and (b) patients who had no LN at renal biopsy examination. Patients with any renal disease due to causes other than SLE, patients with disease that lead to renal impairment (diabetes and hypertension), patients with concurrent infection or tumor, patients with rheumatic diseases other than LSE were not allowed to participate in the study. All patients and controls provided a written consent prior to the participation in the study. The research followed the tenets of the Declaration of Helsinki.

Clinical Assessment of the Patients

The clinical assessment of the patients included interview for history taking to report demographic and clinical data regarding age, sex, and duration of disease. The medical history and drugs used for the treatment were obtained during the interview and from the medical files of the patients.

Assessment of SLE Activity

Lupus disease activity was calculated using the SLE Disease Activity Index (SLEDAI). LN was assessed clinically with the renal SLE disease activity index (renal SLEDAI) consisted of 4 kidney related items of the total SLEDAI:

hematuria, pyuria, proteinuria and urinary casts/HPF. The presence of each one of the 4 parameters takes a score of 4 points, thus the renal SLEDAI score ranges from 0 (inactive renal disease) to a maximum score of 16 ***(Bombardier et al., 1992).***

Collection and preparation of Serum Samples

After an overnight fasting, venous blood samples were collected from every subject by sterile venipuncture on the same day of history taking and clinical examination. Two millilitres of blood was delivered into citrated tube for ESR determination. The separated serum was kept frozen at -20^{o} C till the time of analysis. Estimation of CRP (quantitative) was done using *Turbox CRP kit* (for protein analyzer Turbox plus) by *turbidimetrymethod****(Fischer et al., 1976)***. Erythrocyte sedimentation rate (ESR) was also measured using *Westergren method*. Antinuclear antibody (ANA), anti-dsDNA, serum creatinine, serum C3 and C4 levelswere also assessed. Angiostatin was assessed using the human angiostatinkit which is an in vitro enzyme-linked immunosorbent assay (ELISA) for the quantitative measurement of human Angiostatin in serum and in urine.

Urine Sample Collection

Midstream clean-catch urine samples were collected. Patients are requested to first cleanse the urethral area and the midstreamurine is then collected into a clean specimen . A urinalysis was performed on the urine samples, for estimation of UAng and UCr.

Renal biopsy

Renal biopsy-confirmed LN cases were classified according to the 2003 ISN/RPS classification ***(Weening et al., 2004)***. Data regarding immunofluorescence findings were available for 100% of the patients. Activity indices (AIs) and chronicity indices (CIs) were calculated (maximum scores, 24 for AI and 12 for CI) and interstitial fibrosis was evaluated for each biopsy specimen and was graded semiquantitatively using a scoring system from 0 to 3 (0 = no changes, 1 = mild, 2 = moderate, and 3 = severe) ***(Austin et al., 1983)***.

Statistical Analysis

All statistical analyses were performed using SPSS for windows version 20.0 (SPSS, Chicago, IL). Continuous data were expressed as mean ±standard deviation (SD), while categorical data were expressed in number and percentage. Continuous data were checked for normality and equality of distribution, prior to any analysis being performed. The UA/UG ratio variable was skewed and was logarithmically transformed to attain a normal distribution. The differences among SLE cases and controls and differences between the BPLN patients and SLE patients without LN were determined by independent samples t test for continuous data or chi-square test for categorical data. Receiver operating characteristic (ROC) curve for the UAng/UCr ratio test was drawn and the area under the curves (AUC) for was calculated to assess the ability of this marker to distinguish the SLE patients who had LN from SLE patients without nephritis.95% confidence interval for differences between means of the UAng/UCr ratio between SLE patients and controls was also calculated. All analyses were 2-tailed. Statistical significance was set at $p<0.05$.

Results

In the current study we have enrolled 100 consecutive SLE patients and 50 apparently healthy controls. All subjects (patients and controls) were females. The ages of the SLE patients ranged from 20 to 44 years with an average of 30.2 ±5.6 years while the ages of the controls ranged from 20 to 41 years with an average of 29.5 ±4.6 years. Despite that the patients and controls were matched for age and sex, the SLE patients had significantly higher Ln UAng/UCr ratio than controls (4.7 ±1.7 and 2.4 ±0.3 respectively, 95% CI, 1.82; 2.78, $p<0.001$). Also, the Uang was significantly higher in the SLE patients than in the controls (47.9 ±65.6 versus 2.9 ±1.1 respectively, $p<0.001$) whereas serumangiostatin did not differ significantly between the SLE patients and the controls. Serum creatinine was significantly higher in the SLE patients compared to the control (Table 1).

Table 2 compares the clinical and laboratory findings between the BPLN patients and the SLE patients without LN. BPLN patients had significantly longer disease duration and SLEDAI score than SLE patients without LN. BPLN patients had significantly lower C3 and C4 serum levels than SLE patients without LN. Moreover, BPLN patients

had significantly higher serum creatinine, UAng and higher Ln UAng/UCr ratio than SLE patients without LN whereas serum angiostatin did not differ significantly between the two groups.

Among the SLE patients, UAng and Ln UAng/UCr ratio aresignificantly correlated with SLEDIA score and with serum creatinine while inversely correlated with serum C3 and C4 levels. Also, among the BPLN patients, UAng and Ln UAng/UCr ratio aresignificantly correlated with the renal SLDAI scoreand renal chronicity index but not with renal activity index (Table 3).

As shown in Figure 1, among the patients with BPLN, the Ln UAng/UCr in patients with Class 2 LN was 5 ±0.3, in patients with class 3 LN was 7 ±0.8 and in patients with class 4 LN was 7.4 ±0.2. These differences were significant (F= 81.924, p<0.001).Also, among the patients with BPLN, the UAng in patients with Class 2 LN was 37.3 ±8.9, in patients with class 3 LN was 116.7 ±53.2 and in patients with class 4 LN was 164.3 ±83.8 ($x10^3$pg/ml). These differences were significant (F=19.939, p<0.001).

We have conducted ROC analysis to estimate the diagnostic ability of the Ln UAng/UCr ratio in the discrimination between the BPLN patients from patients with SLE but without LN. As shown in Figure 3, the AUC was found to be 0.877 with 95% CI = 0.805 – 0.949. The AUC for the UAng was 0.819 for the discrimination between the BPLN patients from patients with SLE but without LN (95% CI = 0.742 – 0.895) (Figure 4).

Table 1. Characteristics of the SLE patients and controls

	SLE patients		Controls		P
	Range	Mean ±SD	Range	Mean ±SD	
Age (years)	20 – 44	30.2 ±5.6	20 – 41	29.5 ±4.6	0.446
SLE duration (years)	1 – 14	6.4 ±3.1			
SLEDAI score	2 – 19	9.4 ±5.3			
Renal SLEDAI score	0 – 13	4.3 ±4.2			
ANA positivity (n, %)	96, 96%				
Anti-dsDNA positivity (n, %)	78, 78%				
ESR 1st hour (mm)	9 – 40	17.4 ±8.3			
CRP (mg/dl)	0.6 – 7	3.5 ±1.9			
C3 (mg/dl)	33.3 – 187	110.2 ±42.2			
C4 (mg/dl)	8.1 – 60	25.4 ±13.9			
Serum creatinine (mg/dl)	0.8 – 3.1	1.2 ±0.4	0 – 1.1	0.8 ±0.3	<0.001
Serum angiostatin ($x10^3$pg/ml)	1 – 15.6	7.8 ±4.6	1.8 – 13.3	7.4 ±3.8	0.596
UAng($x10^3$pg/ml)	5.4 – 273	47.9 ±65.6	1.4 – 4.5	2.9 ±1.1	<0.001
Ln UAng/UCr ratio	3 – 7.6	4.7 ±1.7	1.96 – 2.81	2.4 ±0.3	<0.001
Azathioprine (n, %)	78, 78%				
Hydroxychloroquine (n, %)	34, 34%				
Cyclophosphamide (n, %)	12, 12%				
Steroids (n, %)	100, 100%				
Biopsy proven LN (n, %)	44, 44%				

Table 2. Characteristics of the SLE patients and controls

	SLE patients with BPLN	SLE patients without LN	p
	Mean ±SD	Mean ±SD	
Age (years)	29.3 ±5.5	30.9 ±5.7	0.168
SLE duration (years)	7.2 ±3.6	5.7 ±2.5	0.019
SLEDAI score	13.5 ±3.5	6.5 ±4.3	<0.001
ANA positivity (n, %)	44, 100%	52, 92.9%	0.070
Anti-dsDNA positivity (n, %)	36, 81.8%	42, 75%	0.414
ESR 1st hour (mm)	17.37 ±8.6	17.36 ±8.1	0.996
CRP (mg/dl)	3.4 ±1.9	3.8 ±1.9	0.323
C3 (mg/dl)	87.5 ±42.7	128 ±32.4	<0.001
C4 (mg/dl)	19.8 ±11.9	29.7 ±13.8	<0.001
Serum creatinine (mg/dl)	1.5 ±0.5	1 ±0.1	<0.001
Serum angiostatin ($x10^3$pg/ml)	8.7 ±4.4	7.2 ±4.6	0.102
UAng($x10^3$pg/ml)	98.6 ±71.8	8 ±2.4	<0.001
Ln UAng/UCr ratio	6.3 ±1.2	3.4 ±0.3	<0.001
Renal pathological findings at renal biopsy			
LN class (n, %)			
Class 2	16, 36.4%		
Class 3	18, 40.9%		
Class 4	10, 22.7%		
Activity index	10.1 ±4.7		
Chronicity index	4.3 ±2.1		
Current drugs used			
Azathioprine (n, %)	31, 70.5%	47, 83.9%	0.106
Hydroxychloroquine (n, %)	11, 25%	23, 41.1%	0.092
Cyclophosphamide (n, %)	8, 18.2%	4, 7.1%	0.092
Steroids (n, %)	44, 100%	56, 100%	1.000

Table 3. Correlation of the UAngandLnUAng/UCr ratio with SLE duration, SLEDAI score, Renal SLEDAI score, C3 serum level, C4 serum level and Serum creatinine

	UAng		Ln UAng/UCr ratio	
	r	p	r	p

In the SLE patients

Correlation with SLE duration	0.071	0.485	0.218	0.029
Correlation with SLEDAI score	0.469	<0.001	0.640	<0.001
Correlation with C3 serum level	-0.725	<0.001	-0.737	<0.001
Correlation with C4 serum level	-0.566	<0.001	-0.596	<0.001
Correlation with Serum creatinine	0.840	<0.001	0.730	<0.001
In BPLN patients				
Correlation with renal SLEDAI score	0.981	<0.001	0.883	<0.001
Correlation with renal biopsy activity index	0.237	0.122	0.194	0.208
Correlation with renal biopsy chronicity index	0.607	<0.001	0.494	<0.001

Figure 1. Comparison between the Ln UAng/UCrratio among Class2, 3 and 4 LN in the patients with BPLN.

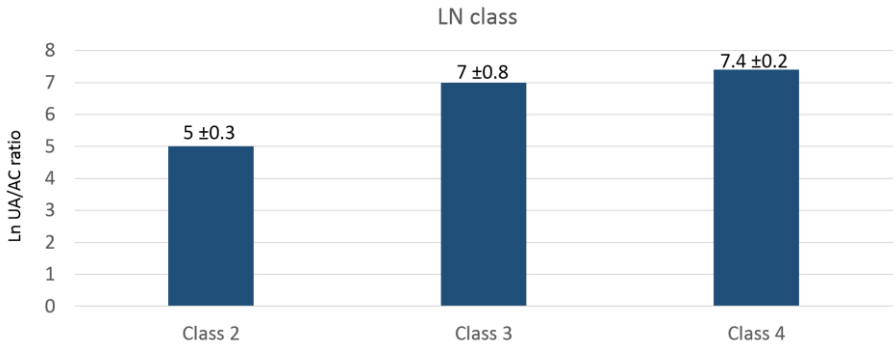
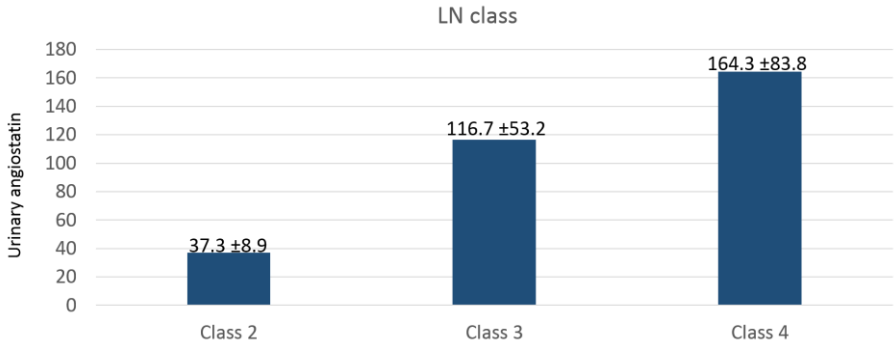


Figure 2. Comparison between the UAngamong Class2, 3 and 4 LN in the patients with BPLN.



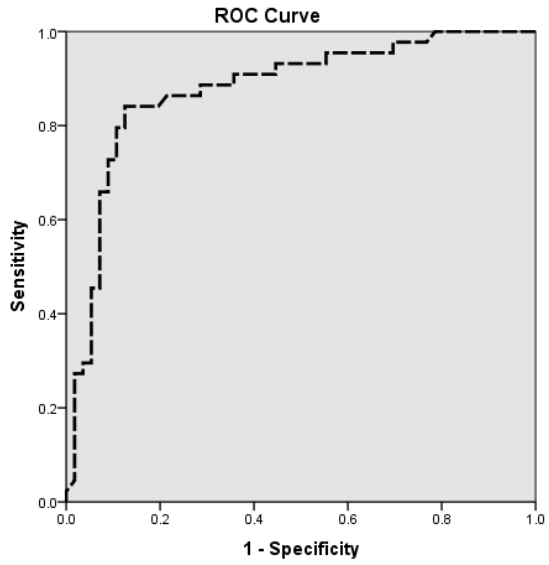


Figure 3. The Receiver operating characteristics curve evaluation of Ln UAng/UCr ratio in the diagnosis of presence of LN among the SLE patients (AUC=0.877).

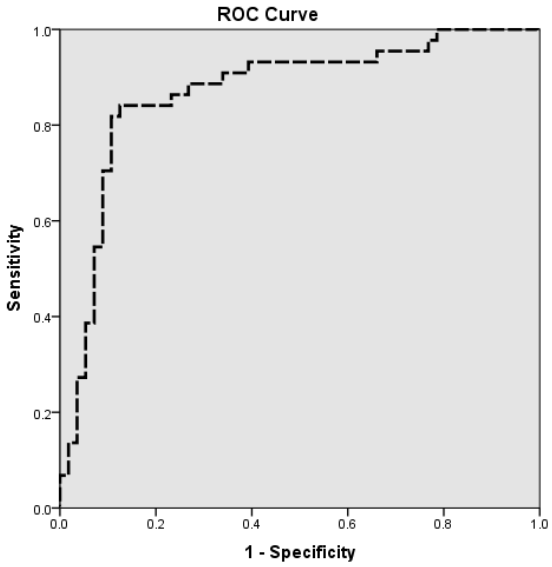


Figure 4. The Receiver operating characteristics curve evaluation of UAng in the diagnosis of presence of LN among the SLE patients (AUC=0.819).

Discussion

A major finding of this study is that the average UAng level of the SLE patients were significantly higher than the controls and were significantly higher in lupus patients with BPLN than those without LN. ***Wu et al. (2003)*** performed a study in which they scanned 274 protein molecules of interest simultaneously in urine samples from patients with LN and healthy controls to identify novel urinary biomarkers of LN. Among the molecules screened, the levels of UAng were increased by almost two orders of magnitude in LN samples compared with healthy controls.In contrast to the urine analysis, our results have shown that there was no significant increase of angiostatin in the serum of SLE patients compared to healthy controls. Serum angiostatin also did not differ significantly between lupus patients with BPLN than patients without lupus nephritis. This finding is in agreement with the results of ***Wu and co-workers (2013)***.

The finding that UAng varied significantly between the SLE patients with BPLNcompared to lupus patients without nephritis and in SLE patients compared to the healthy controls while the serum angiostatindid not show any significant increase of this biomarker between the SLE patients with BPLNcompared to lupus patients without nephritis and in SLE patients compared to the healthy controls may be attributed to the lack of homeostasis mechanisms in the urine. Unlike urine, changes of the levels of most biomarkers in the blood cannot be tolerated for long before they induce homeostasis mechanisms of the body to remove or correct the serum level of the biomarkers. On the contrary, urineaccumulates all kinds of changes of biomarkers as they produced by the kidney and therefore can be a better source for biomarker detection***(Huang et al., 2012 and Gao, 2014)***.

Another major finding of this study is that the Ln UAng/UCr ratio of the SLE patients were significantly higher than the controls and were significantly higher in lupus patients with BPLN than those without LN.In the study of ***Wu et al (2013)***, the UAng expressed as the natural logarithm of the absolute values of UAng normalized against urine creatinine levels (i.e. natural logarithm of UAng/UCr) was significantly higher in the SLE patients with LN as compared to the healthy controls, in agreement to our findings.

Our results had shown that UAng andLn UAng/UCr ratio are significantly correlatedwith SLEDAI in the SLE patients. UAng, Ln UAng/UCr ratio are significantly correlated with renal SLEDAI in the SLE patients with BPLN. ***Wu et al, (2013)*** classified the SLE patients into two groups; SLE patients with inactive disease (SLEDAI $\leq$2) and SLE patients with active disease (SLEDAI >2) and found that the natural logarithm UA/UCrratiowas significantly

higher in the patients with active lupus diseases than those with inactive disease, in agreement with our findings. Moreover,they found that SLE patients with renal SLEDAI = 0 had a significantly lower natural logarithm UAng/UCr ratio than SLE patients with renal SLDAI >0.

In line with these findingsthe result of the current study revealed that SLE patients with LN had a significantly lower C3 and C4 serum levels than those without nephritis. These findings confirm the previous findings of many studies ***(Ho et al., 2001; Linnik et al., 2005; Narayanan et al., 2010).*** In addition, our results have shown that C3 and C4 are negatively correlated with UAng and with Ln UA/UCrratio. Since C3 and C4 are associated with LN it seems reasonable that these markers are negatively correlated with UAng biomarker.

In the current study, A ROC curve was constructed to assess the usefulness of UAng and Ln UAng/UCrratio in discriminating SLE patients with LN from those without LN. ROC curve analysis, revealed that UAng has the capacity to discriminate SLE patients with LN from those without LN. Our results have shown that UAng has a high level of sensitivity and specificity to discriminating SLE from those without LN with an area under curve (AUC) = 0.819. Likewise, A ROC curve results showed that the Ln UAng/UCr ratiohad a high level of sensitivity and specificity of in discriminating SLE patients with LN from SLE without LN an AUC = 0.877.

These AUC values appear promising (AUC values were all above 0.75) compared with previous biomarker candidates assessed similarly. Various protein biomarkers in the urine have been examined for their potential ability to distinguish LN SLE patients from non-LN SLE. Urinary TNF-like weak inducer of apoptosis distinguishes LN SLE patients from non-LN SLE with an AUC of 0.724, sensitivity of 0.50 and specificity of 0.90 ***(Schwartz et al., 2009)***. Lipocalin-2 or neutrophil gelatinase-associated lipocalin, differentiated LN patients from non-LN patients yielding a sensitivity of 0.50, specificity of 0.91, and AUC of 0.71 ***(Pitashny et al., 2007)*** with similar findings noted in pediatric SLE ***(Brunner et al., 2006)***. Another study reported that, in SLE patients, the two markers; the urinary monocyte chemoattractant protein-1 and urinary osteoprotegerin, differentiated those with high renal activity (renal activity score <4) from SLE patients with low renal activity (renal activity score <4) with AUCs of 0.66 and 0.73, respectively ***(Kiani et al., 2009)***. Similar findings have been reported for two adhesion molecules, urinary VCAM-1 and urinary ICAM-1 ***(Abd-Elkareem et al., 2010)***.

Conclusion

UAng and Ln UAng/UCr ratio are associated with LN class and renal chronicity index as identified by renal biopsy. UAng and Ln UAng/UCr ratio are capable of identifying lupus patients with LN from patients without. UAng and Ln UAng/UCr ratio are correlated with lupus activity.

References

Abd-Elkareem MI, Al Tamimy HM, Khamis OA, et al.(2010):Increased urinary levels of the leukocyte adhesion molecules ICAM-1 and VCAM-1 in human lupus nephritis with advanced renal histological changes: preliminary findings. *ClinExpNephrol.*,14(6):548–557.

Austin HA 3rd, Muenz LR, Joyce KM, et al.(1983**):**Prognostic factors in lupus nephritis. Contribution of renal histologic data. Am J Med. , 75(3):382-91.

Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang CH.(1992):Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. Arthritis Rheum. ,35(6):630-40.

Brunner HI, Mueller M, Rutherford C et al.(2006): Urinary neutrophil gelatinase-associated lipocalin as a biomarker of nephritis in childhood-onset systemic lupus erythematosus. *Arthritis Rheum.*, 54(8):2577–2584

Cameron JS. (1999): Lupus nephritis. J Am SocNephrol. , 10(2):413-24.

Contreras G, Pardo V, Cely C, et al.(2005):Factors associated with poor outcomes in patients with lupus nephritis. Lupus., 14(11):890-5.

Fischer CL, Gill C, Forrester MG, Nakamura R.(1976**)**:Quantitation of "acute-phase proteins" postoperatively. Value in detection and monitoring of complications. Am J ClinPathol., 66(5):840–846.

Gao Y.(2014): Opportunities you do not want to miss and risks you cannot afford to take in urine biomarker era. MOJ Proteomics Bioinform., 1(1): 3.

Ho A, Magder LS, Barr SG, Petri M.(2001): Decreases in anti-dsDNA levels are associated with concurrent flares in patients with systemic lupus erythematosus. Arthritis Rheum .,44: 2342-9.

Hochberg MC.(1997):Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. ArthritisRheum.,40(9):1725.

Huang JT, Chaudhuri R, Albarbarawi O, Barton A, Grierson C, et al.(2012): Clinical validity of plasma and urinary desmosine as biomarkers for chronic obstructive pulmonary disease. Thorax.67(6): 502-508.

Iwano M, Dohi K, Hirata E, et al.(1993): Urinary levels of IL-6 in patients with active lupus nephritis. ClinNephrol.,40: 16-21.

Kiani AN, Johnson K, Chen C et al.(2009): Urine osteoprotegerin and monocyte chemoattractant protein-1 in lupus nephritis. *J. Rheumatol.*, 36(10):2224–2230

Korbet SM, Lewis EJ, Schwartz MM.(2000): Factors predictive of outcome in severe lupus nephritis. Lupus Nephritis Collaborative Study Group. Am J Kidney Dis.,35:904–914.

Linnik MD, Hu JZ, Heilbrunn KR.(2005): Relationship between anti-dsDNA antibodies and exacerbation of renal disease in patients with systemic lupus erythematosus. Arthritis Rheum .,52: 1129-37.

Liu ZC, Zhou QL, Li XZ, et al.9(2011): Elevation of human tumor necrosis factor-like weak inducer of apoptosis in peripheral blood mononuclear cells is correlated with disease activity and lupus nephritis in patients with systemic lupus erythematosus. Cytokine. 53: 295-300.

Narayanan K, Marwaha V, Shanmuganandan K, Shankar S.(2010): Correlation between Systemic Lupus Erythematosus Disease Activity Index, C3, C4 and Anti-dsDNA Antibodies. MJAFI ., 66 : 102-107.

Pitashny M, Schwartz N, Qing X et al.(2007): Urinary lipocalin-2 is associated with renal disease activity in human lupus nephritis. *Arthritis Rheum.*, 56(6):1894–1903

Reyes-Thomas J, Blanco I, Putterman C. (2011): Urinary biomarkers in lupus nephritis. Clin Rev Allergy Immunol. ,40(3):138-50.

Rubinstein T, Pitashny M, Levine B, et al.(2010):Urinary neutrophil gelatinase-associated lipocalin as a novel biomarker for disease activity in lupus nephritis. Rheumatology (Oxford). 49: 960-971.

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

Seshan SV, Jennette JC.(2009): Renal disease in systemic lupus erythematosus with emphasis on classification of lupus glomerulonephritis: advances and implications. Arch Pathol Lab Med.,133:233–248.

Sidiropoulos PI, Kritikos HD, Boumpas DT.(2005):Lupus nephritis flares. Lupus. 14:49–52.

Tsokos GC.(2011): Systemic lupus erythematosus. N Engl J Med.,365(22):2110-21.

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

Wu T, Du Y, Han J, et al. (2013):Urinary angiostatin--a novel putative marker of renal pathology chronicity in lupus nephritis.Mol Cell Proteomics. 12(5):1170-9.

Wu T, Fu Y, Brekken D, et al. (2010): Urine proteome scans uncover total urinary protease, prostaglandin D synthase, serum amyloid P, and superoxide dismutase as potential markers of lupus nephritis. J Immunol., 184: 2183-2193.