

ASSESSMENT THE ROLE OF URINE OSTEOPROTEGERIN AS A BIOMARKER IN LUPUS NEPHRITIS

By

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ABSTRACT

Background: Urinary biomarkers may help in identification and assessment of the activity of lupus nephritis (LN). Urinary osteoprotegerin may reflect renal disease activity better.

Objective: To investigate urinary osteoprotegerin level as a potential marker of lupus nephritis activity.

Patients and Methods: The study included 90 individuals classified into three equal groups: Group (I): clinically and laboratory free, Group (II): patients without renal involvement (non active renal group) and Group (III): patients with active lupus nephritis. Measurement of the level of urinary osteoprotegerin has been done by ELISA from January 2018 till June 2019.

Results: All patients with systemic lupus erythematosus SLE in group II and III had significant higher levels in urinary Osteoprotegerin compared to the control group (I). Moreover, SLE patients with lupus nephritis activity (Group III) had marked increase in urinary osteoprotegerin more than SLE patients without renal involvement (Group II).

The study showed also that, with advance of renal histopathological class in lupus nephritis patients, the urine OPG level showed more increase.

Conclusion: Urinary level of osteoprotegerin may be used as a biomarker for early prediction of lupus nephritis. Also, it may be used for monitoring the progression and follow up of lupus nephritis as well as with shifting from one class to another.

Keywords: Systemic Lupus Erythematosus, Lupus Nephritis, Osteoprotegerin, Urine, Potential Marker.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic inflammatory disease of unknown cause that can affect the skin, joints, kidneys, lungs, nervous system, serous membranes and/or other organs of

the body. Immunologic abnormalities, especially the production of a number of antinuclear antibodies due to the production of autoreactive cells and antibodies (*Massarotti et al., 2020*).

Lupus nephritis (LN) is an important manifestation of systemic lupus erythematosus (SLE) and adversely affects the long term outcome. In spite of aggressive therapy, renal outcome has not improved over the last two decades (*Gupta et al., 2016*).

Lupus nephritis occurs in over 50% of patients with systemic lupus erythematosus (SLE). Lupus nephritis is a major cause of morbidity and mortality in systemic lupus erythematosus (SLE). Reports of 5-year renal survival with treatment ranges from 46 to 95%. Early diagnosis and prompt treatment, however, may significantly improve the long-term prognosis (*Imran et al., 2016*).

Biopsy of an involved organ (eg, skin or kidney) is necessary in some cases. Typical histologic findings in various organs in SLE are discussed in topic reviews devoted to the particular sites of involvement, such as the kidney (*Al-Katheri et al., 2017*).

Renal biopsy is the gold standard for providing information on the histological classes of lupus nephritis and the relative degree of activity and chronicity in the glomeruli. However, it is invasive and serial biopsies that are impractical in the monitoring of lupus nephritis. 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 (*Tony et al., 2016*).

Current laboratory markers for lupus nephritis such as proteinuria, urine protein-to-creatinine ratio, creatinine clearance, anti-dsDNA, and complement levels are unsatisfactory. They lack sensitivity and specificity for

differentiating renal activity and damage in lupus nephritis. Significant kidney damage can occur before renal function is impaired and first detection by laboratory parameters. Persistent proteinuria may not necessarily indicate ongoing inflammation in the kidneys and may be contributed by pre-existing chronic lesions or recent damage in the kidneys during the course of the disease. Flares of nephritis can occur without any observable and recent increase in the degree of proteinuria (*Salem et al., 2018*).

Urine has been in the center of attention among scientists of clinical proteomics in the past decade, because it is valuable source of proteins and peptides with a relative stable composition and easy to collect in large and repeated quantities with a noninvasive procedure. Osteoprotegerin (OPG), a member of the tumor necrosis factor (TNF) receptor family, has been identified as a regulator of bone resorption. It has been demonstrated that OPG is produced by a variety of organs and tissues, including the cardiovascular system (heart, arteries, veins), lung, kidney, and immune tissues, as well as bone (*Jonker et al., 2015*).

The expression and production of the protein is modulated by various cytokines, peptides, hormones, and drugs. Cytokines, including TNF, interleukin (IL)-1, IL-18, transforming growth factor (TGF), bone morphogenetic proteins, and steroid hormones such as 17-estradiol are known to up-regulate OPG mRNA levels. It is hypothesized that kidney excretion plays an important role in the clearance of OPG. Thus OPG concentration in the urine might rise in a lupus nephritis flare, because of the increased production and

excretion from inflamed micro vascular endothelial cells in the kidney (*James et al., 2016*).

This study aimed to assess urine Osteoprotegerin as a potential biomarker for lupus nephritis activity.

PATIENTS AND METHODS

The present study was conducted on 90 individuals. They were selected from outpatient clinic and inpatient of Rheumatology and Internal Medicine Departments of New Damietta Hospital at Al-Azhar University from January 2018 till June 2019.

They were classified into 3 equal groups as follows:

- **Group 1:** Normal subjects, clinically and laboratory free.
- **Group 2:** SLE patients without renal involvement (non-active renal group), they had normal serum creatinine and no proteinuria).
- **Group 3:** SLE patients with active lupus nephritis, based on results of a kidney biopsy demonstrating immune complex-mediated glomerulonephritis, as well as evidence of major renal manifestations attributable to SLE, such as proteinuria and/or elevated serum creatinine.

All subjects submitted to full history taking, general and local examination and laboratory investigations including complete blood count (CBC), erythrocyte

sedimentation rate (ESR), serum creatinine, serum cholesterol, complement C3 and C4, anti-double strand DNA (A-dsDNA), urine protein/creatinine ratio (urine P/Cr ratio), renal biopsy in SLE patients with active renal disease group,

Osteoprotegerin (OPG) concentrations were measured in urine by ELISA. The normal standardized value: UP to 2 ng /l.

Statistical analysis:

Data were collected, coded, revised and entered to the Statistical Package for the Social Science (IBM SPSS) version 20. The data were presented, mean & standard deviations for the quantitative data. The comparison between groups with quantitative data and parametric distribution were done by using One Way Analysis of Variance (ANOVA) test. Spearman correlation coefficients were used to assess the significant relation between two quantitative parameters in the same group. Receiver Operating Characteristic curve (ROC) was used to assess the best cut off point between two groups with its sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and area under the curve (AUC). The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant at $P < 0.05$.

RESULTS

There was a statistically significant increase of hemoglobin in non-active disease group and there was statistically

significant increase of total leukocytic counts and platelets in active renal disease group (Table 1).

Table (1): Comparison between active diseases, non-active disease and control group among CBC

Groups Parameters	Control group (No.=30)		Non active renal disease group (No.=30)		Active renal disease group (No.=30)		P value
	Mean	SD	Mean	SD	Mean	SD	
HB	14.29	0.58	11.88	0.78	9.30	0.80	0.001
TLC	7.09	1.74	11.55	3.03	11.90	4.08	0.001
PLT	321.13	70.12	141.90	30.27	160.07	55.34	0.001
Post hoc test (LSD)							
	Control group VS non active disease group		Control group VS Active disease group		Non active disease group VS active disease		
HB	0.001		0.001		0.001		
TLC	0.001		0.001		0.024		
PLT	0.001		0.001		0.563		

There was a statistically significant increase of erythrocyte sedimentation rate

and creatinine in active renal disease group (Table 2).

Table (2): Comparison between active diseases, non-active disease & control group among ESR and creatinine

Groups Parameters	Control group (No.=30)		Non active renal disease group (No.=30)		Active renal disease group (No.=30)		P value
	Mean	SD	Mean	SD	Mean	SD	
ESR	10.00	2.55	46.67	14.13	72.87	9.95	0.001
CREAT	0.95	0.22	0.99	0.18	2.96	0.87	0.001
Post hoc test							
	Control group VS non active disease group		Control group VS Active disease group		Non active disease group VS active disease		
ESR	0.001		0.001		0.001		
CREAT	0.769		0.001		0.001		

There was a statistically significant difference between control group, non-active renal disease and active renal disease group among HB, TLC and PLT, ESR, Creatinine, Cholesterol, C3, C4, Anti-dsDNA, Urine protein/creatinine ratio, urine level of osteoprotegerin.

There was no statistically significant difference between non active renal group and active renal disease among platelets, C3, C4, and urine protein/creatinine ratio (**Table 3**).

Table (3): Correlation between OPG among all CBC, ESR, Creat, C3, C4, DNA and Urine P/cr ratio in active disease group

Parameters	OPG (up to 2 ng/l)	R	P value
Hb (g/dl)		-0.132	0.487
Tlc /cmm		0.346	0.061
Plt /cmm		-0.155	0.414
Esr (mm/min)		0.008	0.966
Creat (mg/dl)		0.568	0.001
Chol (mg/dl)		0.088	0.642
C3 (mg/dl)		0.053	0.783
C4 (mg/dl)		0.032	0.868
Anti dna ab (Iu/ml)		-0.087	0.648
Urine P / cr ratio (up to 0.3 g/mg)		0.580	0.001

OPG has positive correlation with serum creatinine and urine P/CR ratio in active disease group (**Table 4**).

Table (4): Relation between OPG among biopsy in active disease group

Biopsy	OPG	Mean	SD	P value
Class II		3.74	1.61	0.001
Class III		6.84	1.71	
Class IV		11.75	1.70	

There was a statistically significant increase urine level of osteoprotegerin and

renal biopsy classes in a direct proportional manner (**Table 5**).

Table (5): Cut of point, sensitivity and specificity of OPG between active disease group and control group

Cut off point	AUC	Sensitivity	Specificity	-PV	+PV
>1.8	0.993	93.33	100	93.7	100.0

Figure (1) showed high sensitivity and specificity of OPG:

- The cut of point of High sensitive OPG >1.8.

- Its sensitivity is 92.33%.
- Its specificity is 100%.
- The positive predictive value is 100%.
- The negative predictive value is 93.7%.

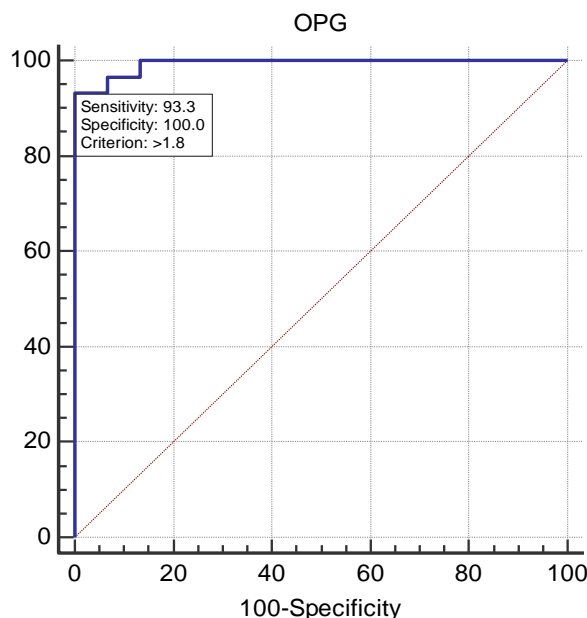


Figure (1): Cut of point of urine level of osteoprotegerin in active disease group

DISCUSSION

Our study has conducted on 90 individuals, divided into two groups as follow: Group (I) 30 normal subjects, clinically and laboratory free. Group (II): 30 SLE patients without renal involvement (non-active renal group), Group (III): 30 SLE patients with active lupus nephritis.

Regarding to hematological findings, our study found that there was statistically significant difference between control group and active renal disease among HB, TLC and PLT but there was statistically significant difference between non active renal group and active renal disease among HB, TLC only. Our results were supported by *Newman et al. (2013)* who

reported that many patients have a mild anemia, which is most often due to the anemia of chronic diseases and thrombocytopenia is also frequently seen.

There was a statistically significant difference between control group and non-active renal disease as regards to ESR and creatinine. A similar finding was reported by *Ferguson and Waikar (2012)* who stated that most renal abnormalities emerge soon after diagnosis as an elevated serum creatinine concentration in patients with SLE.

There was a statistically significant difference between control group and non-active renal disease as regards to cholesterol. *Abdalla et al. (2017)* mentioned that the lipid profile of the SLE patients showed hypercholesterolemia.

As regards to immunological finding, there was a statistically significant difference between control group and non-active renal disease among C3 only and statistically significant differences between control group and active renal disease among C3 and C4. *Raymond et al. (2018)* reported that measurement of serum complement levels C3 and C4 may be helpful, since hypocomplementemia is a frequent finding in active SLE.

There was a statistically significant difference between control group and the studied groups as regards to anti-dsDNA. *Carubbi et al. (2019)* reported that anti-dsDNA antibodies were evaluated and the following results were obtained.

As regards to urine protein/creatinine ratio, there was no statistically significant difference between control group and non-active renal disease, there were statistically significant differences between control group and active renal disease P/CR ratio. *Yu et al. (2014)* mentioned that persistent proteinuria greater than 0.5 grams per day which is one of the American colleges of rheumatology (ACR) diagnostic criteria of SLE.

As regards to urine level of osteoprotegerin, there were statistically significant differences between control group and non-active renal disease, with high sensitivity (98.5%) and specificity (92.5%), Also, there were statistically significant difference between control group and active renal disease among urine level of osteoprotegerin with high sensitivity (92.33%) and specificity (100%), and there were statistically significant differences between non active renal group and active renal disease.

The study showed also that the patient with active lupus nephritis have higher urine OPG level (mean: 7.11ng/l) than lupus patients without active nephritis (mean: 4.41 ng/l).

Statistically, there was no significant correlation between urine OPG level in lupus patients in non-active group and all laboratory parameters tested in this study, and when we made a correlation between urine OPG in lupus patients with active nephritis in relation to other parameters, we found that: there was a significant correlation between urine OPG level among serum creatinine level and urine protein creatinine ratio, but there was no statistically significance among the other laboratory parameters. *El-Shehaby et al. (2011)* reported that urinary levels of OPG positively correlated with renal involvement in lupus patients with reasonable sensitivity, specificity, and predictive values to detect lupus nephritis.

As regards to renal biopsy, there was a statistically significant increase in urine level of osteoprotegerin and renal biopsy classes in a direct proportional manner. When we make correlation between renal biopsy results in lupus patients with active nephritis in relation to osteoprotegerin, we found that there was statistically significant relation between the advance of renal pathology of renal biopsy in active lupus patients and urine OPG level.

We noticed that with the advance of renal class in lupus nephritis patient, the urine OPG level tends to increase, while patients with class IV have mean urine OPG level (11.75ng/l) higher than whom with class III (6.84ng/l), higher than whom in class II (3.74ng/l). *Gupta et al. (2016)*, reported that u OPG is derived

from kidneys and helps differentiate active SLE patients with and without LN. It shows modest correlation with disease activity and has a potential to predict poor response to therapy and relapse of LN.

In our study, urinary levels of OPG positively correlate with lupus nephritis patients.

CONCLUSION

Patients with lupus nephritis have higher urine OPG level than lupus patients without nephritis, with advance of renal class in lupus nephritis patient, the urine OPG level tends to increase. Urinary level of osteoprotegerin may be used as a biomarker for early prediction of lupus nephritis and for monitoring and follow up the progression of nephritis as well as with shifting from one class to another.

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None declared by the authors.

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تقييم دور أوستيوبروتيجرين البولي كعلامة بيولوجية لالتهابات الكلي في مرضي الذئبة الحمراء

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خلفية البحث: العلامات البيولوجيه البوليه ربما تساعد قي تحديد وتقييم نشاط التهاب الكلي في مرضي الذئبه الحمراء. اوستيوبروتيجيرين البولي ربما يعكس النشاط المرضي للكلي بطريقه افضل.

الهدف من البحث: تقصينا مستوي اوستيوبروتيجيرين البولي كعلامه بيولوجيه لنشاط التهابات الكلي في مرضي الذئبه الحمراء.

المرضى وطرق البحث: إشملت الدراسة على 90 شخص تم تصنيفهم لثلاثة مجموعات متساوية. الأولى: تشمل أشخاص طبيعيين إكلينيكيا ومعمليا، والثانية: مرضي مصابون بالذئبة الحمراء مع عدم وجود إعتلال بالكلي، والثالثة: مرضي مصابون بالذئبة الحمراء مع وجود إعتلال بالكلي بسبب الذئبة الحمراء. وقد تم قياس مستوى أوستيوبروتيجيرين البولي بطريقة الاليزا.

نتائج البحث: جميع مرضي الذئبة الحمراء في المجموعتين الثانيه والثالثه لديهم إرتفاع كبير في مستوي أوستيوبروتيجيرين البولي بالمقارنه بالمجموعة الضابطة. وعلاوه علي ذلك، فان مرضي الذئبة الحمراء والذين لديهم التهابات نشطة بالكلي (المجموعه الثالثه) لديهم

زيادة في مستوي اوستيوبوتيجيرين البولي أكبر من مرضي الذئبة الحمراء الذين ليس لديهم إتهاب بالكلي. والدراسة أوضحت أيضا أنه مع تقدم درجة التشريح المرضي للكلي فإن أوستيوبروتيجيرين البولي أظهر زيادة أكبر.

الاستنتاج: يمكن استخدام أوستيوبروتيجيرين البولي كعلامة بيولوجية للتنبؤ المبكر ومتابعة تطور التهاب الكلي في مرضي الذئبة الحمراء.

الكلمات الدالة: الذئبة الحمراء، إتهاب الكلي الذئبي، أوستيوبروتيجيرين البولي.