Diagnostic Performance of Urinary Osteoprotegrin as A Novel Biomarker for Early Detection of Lupus Nephritis Activity

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Abstract: Background and aim of study: Renal activity in systemic lupus erythematosus (SLE) is mainly determined by histopathological examination of renal biopsy, but renal biopsy is expensive, invasive, and carries some risk. Osteoprotegerin (OPG) is produced by the heart, lungs, kidney, and bone.The current study was aimed to investigate urinary osteoprotegrin as a biomarker for early detection of lupus nephritis activity and as a potential alternative to kidney biopsy. **Patients and methods**: This study was conducted on 68 patients with systemic lupus erythematosis. Patients were 23 males (33.8%) and 45 females (66.2%) with a mean age of 29.35 ± 12.15 years (range 18–36 years). Patients were divided into two groups, systemic lupus patients with lupus nephritis (group I;43 patients) and systemic lupus patients without lupus nephritis (group II;25 patients).Measurement of urinary OPG (pg/ml) was performed by enzyme linked immunosorbant assay (ELISA) using (Human Osteoprotegerin ELISA Kit). Disease activity was assessed by total systemic lupus erythematosis disease activity index and renal activity by renal systemic lupus erythematosis disease activity index and their correlation with urinary osteoprotegrin was analysed. Kidney biopsy was performed to group1of patients and classified according to International Society of Nephrology/Renal Pathology Society 2003. **Results**: Urinary osteoprotegrin was significantly high in group I compared to group II (*p*value=0.0001). In group I, there was positive correlation between urinary osteoprotegrin and anti-nuclear antibodies, Anti-ds DNA, 24 hrs urinary protein, serum creatinine, hematuria, erythrocyte sedimentation rate, total systemic lupus erythematosis disease activity index and renal activity of systemic lupus erythematosis disease activity index. There was –ve correlation between urinary osteoprotegrin and C3 and C4.The level of urinary osteoprotegrin was higher in classes III and IV of lupus nephritis than classes I,II, and V, but was not statistically significant. The sensitivity and specificity of the urinary osteoprotegrin as a marker of lupus nephritis activity (as determined by the receiver operating curve) were found to be 90.9% and 84.6% respectively with area under curve (0.874), 95% confidence interval 0.771 to 0.942 and *P* value <0.0001. **Conclusion**: Our results suggested that urinary osteoprotegrin can be a useful non invasive biomarker for assessment of lupus nephritis activity in patients with systemic lupus erythematosis and as an early predictor of lupus nephritis flare.Further larger and longitudinal studies are needed to evaluate that urinary OPG is a potential alternative to kidney biopsy

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1.Introduction

Lupus nephritis(LN),one of the most serious manifestations of systemic lupus erythematosus (SLE), usually arises within 5 years of diagnosis and can be seen in up to 60% of all SLE patients [1]. Furthermore, 10–15% of LN patients progress to end-stage renal disease (ESRD) requiring hemodialysis, and the 5-year survival of LN patients is stalled at 82%, whereas 5-year survival for those without LN is 92% [1]. Despite the fact that several efficacious therapies have been used to treat LN, the incidence of ESRD from LN increased during the period of 1982–1985 [2], and from 1996 to 2004 showed no change [3].This may reflect the limitations of our current treatment options, poor access to health care, late diagnosis, or delay in treatment [3].

Earlier treatment has a beneficial effect on the prognosis of LN, and it has been shown that late diagnosis of LN is correlated with a higher frequency of renal insufficiency [4]. Moreover, delayed diagnosis is associated with an increased incidence of ESRD, again underlining the importance of early diagnosis of LN in patients with SLE to control disease [5].

Kidney biopsy and histopathological analysis of kidney tissue is a valuable method for diagnosis, assessment, and prognosis of LN. However, kidney biopsy is expensive,invasive and carry risks and, therefore, is not usually performed serially. Furthermore, with blind needle kidney biopsy, there are the limited number of glomeruli usually obtained that not insufficient for assessment of renal activity and chronicity. Laboratory markers in current use, which include serological determination of serum anti-double-

stranded (ds)DNA antibodies and complement levels, can be helpful clinically, but the correlation between those and lupus renal disease is imperfect. A non-invasive, easily obtainable, and accurate marker that can be followed serially, accurately predict LN activity, pathology and prognosis may therefore be of great value to guide therapeutic decisions and in monitoring LN[6].

Osteoprotegerin (OPG), a member of the tumor necrosis factor (TNF) receptor family, has been identified as a regulator of bone resorption[7].

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[8].The expression and production of OPG are regulated by various cytokines and hormones[9].

It was hypothesized that kidney excretion plays an important role in the clearance of OPG. Thus, OPG concentration in the urine might rise in a LN flare, because of the increased production and excretion from inflamed microvascular endothelial cells in the kidney, therefore, it was found in a recent study that OPG was associated with measures of lupus renal disease activity, and medium or high levels of OPG were predictive of a urine protein/creatinine ratio of ≥ 0.5[10].

The aim of this study was to evaluate urinary OPG as a biomarker of LN activity and flare and as a potential noninvasive alternative to renal biopsy which is still the "gold standard" to detect LN activity in SLE.

2. Patients and methods

This study was conducted on 68 patients with SLE. Patients were 23 males (33.8%) and 45 females (66.2%) with a mean age of 29.35 ± 12.15 years (range 18–36 years). Patients were divided into two groups, SLE patients with LN (group I;43 patients) and SLE patients without LN (group II;25 patients).LN diagnosed by presence of persistent proteinuria or hematuria[11]. SLE patients with proteinuria other than LN as pregnancy and fever or patients with impaired renal function due to any other cause than LN as diabetic nephropathy or HCV&HBV and other connective tissue diseases were excluded from the study. All these patients were attending the nephrology OPD in Nephrology Department Theodor Bilharz Research institute, Cairo Egypt. Written informed consent was obtained from all patients.

Each patient underwent thorough history taking and complete clinical examination. Routine examinations included urine analysis, renal function tests (serum creatinine, urea, sodium, potassium and uric acid), complete blood count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP). Serum C3 and C4, antinuclear antibodies, anti-dsDNA and anticardiolipin antibodies were also conducted.

Measurement of urinary OPG (pg/ml) was performed by enzyme linked immunosorbant assay technique (ELISA) using (Human Osteoprotegerin ELISA Kit, RayBiotech, USA) and was read by ELISA reader nm filter[12].

Kidney biopsy was performed to group I of patients and classified according to International Society of Nephrology/Renal Pathology Society (ISN/RPS 2003)[13]. Disease activity was assessed by total SLE disease activity index (tSLEDAI), and renal activity by renal SLE disease activity index (rSLEDAI)[14] and their correlations with urinary OPG were analysed.

Statistical analysis

Data were presented as mean ± SD. Or number of cases and percentages. Comparisons between variables in the study groups were performed using unpaired two-tailed Student's *t*-tests (Graphpad Quick Calcs). Mann Whitney U test for independent samples was used to Compare study markers according to disease activity groups. Correlation between various variables was done using Pearson correlation equation for linear relation. Accuracy was represented using the terms sensitivity and specificity. Receiver operator characteristic (ROC) analysis was used to determine the optimum cut off value for the studied diagnostic markers (MedCalc Statistical Software). *P* value less than 0.05 was considered statistically significant

3. Results

Demographic and clinical characteristics of the studied patients are shown in [Table 1]. Laboratory parameters of the studied patients are shown in [Table 2] where,there was statistically significant difference in the level of s.creatinine, bl. urea and 24 hours urinary protein between both groups of patients with *p* value = 0.0034, 0.0018 and 0.0001 respectively[Table 2]. There was no significant difference in the level of hemoglobin level, white blood cells, blood platelets, ESR and CRP between both groups of patients with *P* value = 0.8934, 0.3536, 0.9428, 0.6788 and 5756 respectively[Table 2]. There was no significant difference in the anti-ds DNA titre between both groups (*p* value=0.7147). C3 and C4 were found to be significantly more consumed in group I compared to group II with a *p*-value 0.0023 and 0.0334 respectively[Table 2].

There was statistically significant difference in tSLEDAI and rSLEDAI between both groups (*p* value=0.0007 and 0.0001 respectively[Table 3].

Urinary osteoprotegerin was statistically significant high in group I compared to group II with *p* value 0.0001[Table 4].

In group I of patients,there was positive correlation between urinary OPG and ANA (r=0.53; p=0.04), Anti-ds DNA (r=0.74; p=0.01), 24 hrs urinary protein (0.86; *p*=0.01), s. creatnine(r=0.57; *p*=0.04), hematuria(r=0.61; *p*=0.02), ESR(r=0.83; *p*=0.01), tSLEDAI(r=0.62; *p*=0.01) and rSLEDAIi (r=81; *p*=0.01). There was –ve correlation between urinary OPG and C3 (r=0.82; *p*=0.01) and C4(r=0.52; *p*=0.03)[Table 5].

The level of urinary OPG was higher in classes III and IV of LN than class I,II and V(10.2± 5.5 and 10.7±3.4 vs. 6.4±7.4, 6.6 ±3.2 and 7.5±5.1),but was not statistically significant(*p* value>0.05)[Table 6].

The sensitivity and specificity of the urinary OPG as a marker of LN activity (as determined by the ROC Curve) were found to be 90.9% and 84.6% respectively with area under curve (0.874), 95% confidence interval 0.771 to 0.942 and *p* value <0.0001[Fig.1].

Table 1:Demographic and clinical characteristics of the studied two groups

Variables	Group I(n=43)	Group II(n=25)	*p* value
Age (years)	27.4 ± 5.96	27.9 ± 6.84	0.7531(NS)
Gender(F/M)	28/15(65.1%/34.9%)	17/8(68%/32%)	
Disease duration(years)	5.3 ± 3.15	4.1 ± 2.4	0.1046(NS)
Systolic Bl. Pressure (mmgh)	125.11 ± 19.3	110.5 ± 10.13	0.0008*
Diastolic Bl. Pressure (mmgh)	79.4 ± 12.6	69.8 ± 5.12	0.0006*
Artheralgia/arthritis	31(72%)	14(56%)	
Skin rash	23(53.5%)	12(48%)	

NS=non significant

Table 2:Laboratory parameters in the studied patients

Variables	Group I(n=43)	Group II(n=25)	*p* value
S.creatinine (mg/dl)	0.96 ± 0.35	0.72 ± 0.24	0.0034*
Blood urea	72.23±48.47	39.21±18.92	0.0018*
24 hours Ur. Proteins (gm)	2.34 ± 1.9	0.15 ± 0.13	0.0001*
ESR (mm/min)	75.3 ± 38.9	71.2 ± 39.7	0.6788(NS)
CRP(IU/ml)	10.41±7.63	9.37±6.83	0.5756(NS)
Hemoglobin (gm/dl)	12.8 ± 2.80	12.9 ± 3.21	0.8934(NS)
White Bl. Cells (x1000/ml)	8.3 ± 4.13	7.4 ± 3.24	0.3536(NS)
Platelets (x1000/ml)	292.5 ± 101.1	294.3 ± 96.5	0.9428(NS)
ANA	1/930	1/86	
Anti-ds DNA	187.32±86.43	179.57±79.42	0.7147(NS)
C3 (g/l)	0.63 ± 0.51	1.17 ± 0.9	0.0023*
C4 (g/l)	0.18 ± 0.15	0.26 ± 0.14	0.0334*
Urine analysis Hematuria Cast	 37(86%) 3376.7%)	 0(0%) 0(0%)	

ESR=erythrocyte sedimentation rate.CRP=C-reactive protein.ANA=antinuclear antibodies.NS=non significant

Table 3:Comparison between tSLEDAI and rSLEDAI in the studied patients

Variables	Group I(n=43)	Group II(n=25)	*p* value
tSLEDAI	12.9 ± 6.8	7.4 ± 4.9	0.0007*
Rsledai	8.10 ± 5.4	1.1 ± 2.3	0.0001*

tSLEDAI=total systemic lupus erythematosis disease activity index
rSLEDAI=renal systemic lupus erythematosis disease activity index

Table 4: Comparison between OPG in group I and group II

	Group I(n=43)	Group II(n=25)	*p*- value
OPG(pg/ml)	8.71±3.52(1.3-17.9)	4.10±2.21(0.53-8.42)	0.0001*

OPG = osteoprotegrin

Table 5: Correlations between OPG with clinical and laboratory variables in the studied patients in both groups.

Variables	Group I		Group II	
	r	*p* value	r	*p* value
OPG				
ANA	0.53	0.04*	0.48	0.04*
Anti-ds DNA	0.74	0.01*	0.45	0.03*
C3	0.82	0.01*	-0.74	0.01*
C4	0.52	0.03*	-0.13	0.12(NS)
24 hrs urinary protein	0.86	0.01*	0.42	0.03*
S.creatnine	0.57	0.04*	0.17	0.08(NS)
Bl.urea	0.24	0.07(NS)	0.26	0.06(NS)
Hematuria	0.61	0.02*	0.45	0.03*
Cast	0.47	0.03*	0.46	0.04*
ESR	0.83	0.01*	0.43	0.03*
Duration of disease	0.26	0.07(NS)	0.18	0.07(NS)
Systolic blood pressure	0.17	0.12(NS)	0.27	0.06(NS)
Diastolic blood pressure	0.28	0.07(NS)	0.19	0.07(NS)
tSLEDAI	0.62	0.01*	0.47	0.04*
Rsledai	0.81	0.01*	0.06	0.13(NS)

Table 6: Comparison between OPG of SLE patients with lupus nephritis in relation to renal biopsy.

Variables	Class I LN(n=2)	Class II LN(n=4)	Class III LN(n=14)	Class IV LN(n=19)	Class V LN(n=4)	*p* value
OPG(pg/ml)	6.4±7.4	6.6 ±3.2	10.2± 5.5	10.7±3.4	7.5±5.1	>0.05(NS)

OPG = osteoprotegrin.NS = non significant

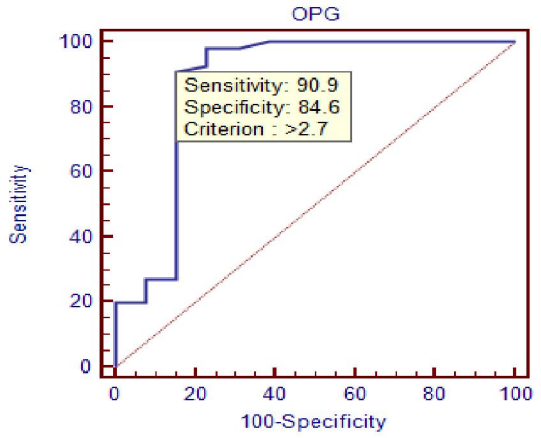

Fig.1: The sensitivity and specificity of the osteoprotegrin (OPG) level as a marker of LN activity as determined by the receiver operating characteristic curve (ROC curve).

4. Discussion

Lupus nephritis (LN) is a major cause of morbidity and mortality in SLE. As the course of LN is often unpredictable, it is important to identify reliable, noninvasive methods to repeatedly assess the condition of the kidneys in these patients. Urinary biomarkers are easily obtained and probably are best at reflecting the current renal status, as they specifically represent local inflammatory activity[15].

Laboratory markers in current use, which include serological determination of serum anti-double-stranded (ds)DNA antibodies and complement levels, can be helpful clinically, but the correlation between those and lupus renal disease is unsatisfactory. Sensitivity and specificity for active lupus nephritis among all SLE patients varied according to different studies and tests used (enzyme immunoassay vs. immunofluorescence)[16–18].

Esdaile *et al.* [18], evaluated these markers as predictors 3, 6, and 9 months prior to a renal flare as determined by the rSLEDAI, while Moroni *et al.*[17], showed more sensitivity and specificity of detecting nephritic and proteinuric flares of patients who already carried the diagnosis of LN. In the prospective longitudinal study by Moroni *et al.*[18], 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 histopathology [18]. Clearly, the lack of specificity of the current markers for LN and inability to predict histology highlight the importance of the need for a true biomarker for LN.

Serum biomarkers may indeed be appropriate monitoring and diagnostic tools for systemic disease activity, as SLE is a systemic disease. However, urinary biomarkers may be more specific for kidney damage than serum biomarkers in patients with LN, particularly in SLE patients with active systemic disease. Furthermore, obtaining urine for laboratory testing is much easier and less invasive, making urine a more ideal biological sample for a disease that requires serial screening.

Osteoprotegerin (OPG), a member of the tumor necrosis factor receptor superfamily, is a soluble decoy receptor for the osteoclast differentiation factor receptor-activator of nuclear factor κB ligand (RANKL) that inhibits interaction between RANKL and its membrane-bound receptor RANK[19]. The RANKL/OPG/RANK axis has been shown to regulate bone remodeling[20,21] and was more recently found to be involved in carcinogenesis as well as central thermoregulation[22,23].This system has also been linked to the development of atherosclerosis and plaque destabilization[24,25]. RANKL exhibits several properties with relevance to atherogenesis, such as promotion of inflammatory responses in T cells and dendritic cells, induction of chemotactic properties in monocytes, induction of matrix metalloproteinase (MMP) activity in vascular smooth muscle cells (SMC), and RANKL has also been found to have prothrombotic properties[20,26]. In observational studies, elevated circulating OPG levels have been associated with prevalence and severity of coronary artery disease, cerebrovascular disease, and peripheral vascular disease[20,26]. Circulating OPG levels are increased in patients with acute coronary syndrome[27] and enhanced expression has been found within symptomatic carotid plaques[28]. Elevated OPG levels have also been associated with the degree of coronary calcification in the general population as a marker of coronary atherosclerosis[29]. OPG has been reported to predict survival in patients with heart failure after acute myocardial infarction,[30] to predict heart failure hospitalization and mortality in patients with acute coronary syndrome,[27] and to be associated with long-term mortality in patients with ischemic stroke[31]. There are also a few studies that show a relationship between OPG and cardiovascular disease (CVD) and related mortality in the general population[24,32].

Many LN biomarker studies have used cross-sectional cohorts to identify novel markers of disease activity. However,several recent studies examined lupus biomarker expression prospectively, to identify biomarkers that can predict the future course of LN [33].

The present study showed that the novel urinary OPG biomarker was found to be significantly higher in patients with active LN than those without active LN and this may support the role of urinary OPG as a potential noninvasive marker of LN activity and going with what was reported by Adnan *et al.*[10], who tested the urinary OPG as potential biomarker for LN in 87 lupus patients and he found the urinary OPG to be significantly higher in patients with active LN than those without active LN.

Our study showed that urinary OPG was significantly higher in patients with active LN than those without active LN and also urinary OPG was found to be strongly correlated with SLEDAI which was first developed by Claire *et al.*[34], as a reliable sensitive index for lupus activity, and was proved later by Goulet *et al.*[35], to be directly correlated with the prognosis and long term mortality in patients with active LN, and that raise the importance of OPG to be not only a marker of lupus activity but also a proposed predictor of prognosis in patients with active LN.

In our study, urinary OPG levels were strongly negatively correlated with C3 and C4 components consumption in SLE patients with LN and that support also the proposal that OPG may be considered as a useful practical biomarker of LN activity.

We noticed a strong positive correlation between urinary OPG and 24 hours urinary proteins which is an essential diagnostic and prognostic marker of LN and that also support other findings in this study regarding correlation of urinary OPG and LN activity.

The present study showed that, The level of urinary OPG was higher in classes III and IV of LN than classes I,II, and V, but was not statistically significant(p value>0.05), this may be due to a few number of patients in classes I,II and V compared to classes III and IV. So we need more larger and longitudinal studies to evaluate that urinary OPG is a potential alternative to renal biopsy.

As regards the sensitivity and specificity of the commonly used markers of LN activity; Moroni *et al.*[36], reported that the traditional clinical biomarkers for SLE, including complement components 3 and 4(C3, C4) and anti-double-stranded DNA antibodies have low sensitivity (49 to 79%) and specificity (51 to 74%) for concurrent renal flare, however in our study the sensitivity and specificity of the urinary OPG level as a marker of LN were found to be 90.9% and 84.6% respectively, that means urinary OPG level has highly comparable sensitivity and specificity to the traditional clinical biomarkers for LN activity.

In conclusion; the urinary OPG suggested to be a useful non invasive biomarker for assessment of LN activity in SLE patients and as an early predictor of LN flare. This may enable physicians to initiate treatment earlier, and improve the outcomes of patients with LN. Further larger study, including longitudinal evaluation and correlation with concurrent renal biopsies, is

needed before this biomarker can be used for practical purpose.

Conflicts of interest

The authors declare that they have no conflict of interest.

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