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# Evaluation of Urinary Ceruloplasmin as a Novel Biomarker for Lupus Nephritis

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## Abstract

**Background:** A crucial step in modifying the course of lupus nephritis (LN) is the early identification of kidney disease in systemic lupus patients. LN affects around 30%–60% of systemic lupus erythematosus (SLE) patients and 70% of juvenile SLE. Renal biopsy, which is an invasive procedure with hazards, is considered the gold standard to diagnose LN. Ceruloplasmin (CP) is an acute phase protein that transports most of the circulating copper and functions as an iron oxidase that is associated to iron metabolism, and has been found that urinary exosomal CP increased in kidney diseases due to local tissue pro-inflammatory cytokines such TNF- $\alpha$ , IL-6, and IL-1 $\alpha$ . This study aimed to evaluate the utility of urinary CP as a new biomarker to differentiate LN from nonnephritis (NN), and to evaluate correlation to lupus activity.

**Patients and methods:** Case control study included 60 lupus patients and 30 controls. The case group subdivided into 30 LN and 30 NN patients. The following were measured: complete blood count (CBC), erythrocyte sedimentation rate (ESR) urine analysis, urinary CP, serum creatinine, 24 h protein in urine, eGFR (MDRD Equation), Complement 3 (C3) and Complement 4 (C4), Anti-dsDNA, and anti-nuclear antibody (ANA).

**Results:** Elevated levels of urinary CP were higher in lupus patients than controls and by further stratification; CP found to be greater in nephritis group than NN group, also, CP found to have a significant positive correlation to nephritis activity, and disease duration, and a negative correlation to eGFR, C3 and C4.

**Conclusion:** Urinary CP has a potentiality to differentiate LN from NN.

**Keywords:** Biomarker, Ceruloplasmin, Lupus nephritis, SLE

## 1. Introduction

Lupus nephritis (LN) affects around 30%–60% of adult lupus patients, and up to 70% in juvenile lupus.<sup>1</sup> Despite management recommendations and recognized immune-suppressive medications, 10%–20% of lupus patients may advance to end-stage renal disease (ESRD) within 5 year-diagnosis, and 40% of nephritis class III, IV, and V will develop a form of chronic kidney disease (CKD).<sup>2</sup>

LN is considered a potentially fatal consequence of systemic lupus erythematosus (SLE) and a significant problem due to the difficulties in detecting it before complications arise. Proteinuria, eGFR, urine casts, anti-dsDNA, and complements level are not specific

nor sensitive to assess ongoing nephritis activity from old renal affection. Biomarkers for nephritis activity in Lupus patients have long been considered. Such biomarkers should ideally be able to identify early subclinical flares and might be used to assess therapeutic responses, eliminating the need for repeated renal biopsies and associated potentially dangerous consequences.<sup>3</sup>

Clinical or laboratory signs may not be present when LN first develops, and flares may start without an apparent rise in proteinuria. Also, because proteinuria takes a while to settle, it might be challenging to distinguish between cases of proteinuria caused by irreversible kidney damage and those caused by continued LN activity.<sup>4,5</sup>

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The urinary protein/creatinine ratio, anti-dsDNA titer, and complements levels, which are now the primary laboratory indicators for LN, are insufficient since they are not sensitive enough or specific to detect renal activity or damage.<sup>5,6</sup>

Therefore, renal biopsy continues to be the gold standard for gaining knowledge on the histological class of LN and the degree of disease activity and chronicity. A biopsy is intrusive, not always encouraged, and in some cases, patients may even refuse to have it done. Furthermore, it is impractical to repeat the kidney biopsy when a flare occurs or to track the effectiveness of treatment. Therefore, there is a need for new LN biomarkers that are specific to SLE and renal involvement, easily detectable, do not depend on age, sex, or ethnicity, reflect renal activity, predict flares, and monitor progression of the disease and monitor treatment outcomes.<sup>2,7</sup>

Serum biomarkers have traditionally been the focus of biomarker studies in SLE, although additional samples, including urine and cerebral spinal fluid (CSF), have drawn attention, especially for the monitoring of clinical features. The demand for accurate biomarkers for lupus remains an unfulfilled concern.<sup>8</sup>

Physical proximity to the kidney makes urine an intriguing possibility for LN diagnosis and monitoring. An acute-phase protein with renal origins 'ceruloplasmin (CP)' can increase under stressful or inflammatory conditions and may serve as an early indicator of a number of different kidney illnesses.<sup>9</sup>

The study of urinary CP as a biomarker is made possible by the fact that collecting a urine sample is not a difficult or intrusive process. CP is an acute phase protein that transports most of the circulating copper and functions as an iron oxidase that is associated to iron metabolism. As a result, CP may rise because of proinflammatory cytokines such IL-6, TNF- $\alpha$ , and IL-1 $\alpha$ .<sup>7</sup>

This work aimed to evaluate the utility of urinary CP as a new biomarker to differentiate LN, and to evaluate the correlation to activity.

## 2. Patients and methods

The current case-control study got conducted after obtaining the Approval certificate under registration code (Rheu-med.9 Med.Research.Urinary.CP.biomarker.LN\_000007) from Al-Azhar Faculty of Medicine's Ethics Committee. Patients were recruited from the rheumatology outpatient clinic and inpatient departments of Al-Azhar University Hospitals, after obtaining an informed consent prior to enrolment, from November 2020 to June 2021.

This study included: 60 adult SLE patients (Case Group) fulfilled 4 or more of revised ACR lupus criteria.<sup>10</sup> Then subdivided into two subgroups:

Lupus Nephritis (LN) group: 30 LN patients had their renal biopsy within last 6 months before enrollment.

Non-Nephritis (NN) group: 30 SLE patients without renal involvement.

With exclusion criteria of concurrent autoimmune illness, active UTI or systemic infection, end stage renal disease (ESRD), or dialysis, pregnancy, malignancy, or cognitive impairment.

(Control group): 30 healthy individuals matched for same age and sex of the case group, not complaining from rheumatic disorders, or chronic medical condition.

All participants were subjected to demographic data, clinical features, and the following investigations: Urinary CP level, CBC, ESR, CRP, Complete Urine Analysis: Proteinuria, RBCs, WBCs counts, Casts. S.creatinine, eGFR (MDRD) equation, anti-nuclear antibody (ANA), and Anti-dsDNA, Complement 3 (C3) and Complement 4 (C4), and 24 h Protein in Urine.

### 2.1. Outcome measures

All patients were diagnosed following ACR criteria. SLEDAI was used to evaluate disease activity, and renal SLEDAI (rSLEDAI) also utilized to evaluate the kidney activity in (LN group). Hematuria, pyuria, proteinuria, and urine casts constitute the four kidney-related criteria that compose the score. The rSLEDAI scores can vary from zero (inactive renal disease) to as high as 16. A rSLEDAI score of equal to 4 or greater considered to represent an active LN. A score of less than 4 on the rSLEDAI was also used to indicate inactive LN. NIH activity and chronicity indices scores, as well as the (ISN/RPS) histopathological classification of nephritis were used to assess renal biopsies of LN group.

Urinary CP level estimated using (ELISA noncompetitive sandwich method). Samples collected and processed as following: Morning urine sample collected by sterile tube. Centrifuged at 2000–3000 RPM for ~20 min; after which it was filtered through 0.25  $\mu$ m membranes to extract cell debris, then urinary CP was measured with commercial ELISA kits.

### 2.2. Statistical analysis

The statistical program for social sciences, version 23.0, was used to evaluate the recorded data (SPSS

Table 1. SLE patients vs control group regarding demographic data.

Demographic data	Patients (n = 60)	Control (n = 30)	Test value 't'/ $\chi^2$	P-value (Significance)
Sex				
Female	56 (93.3%)	28 (93.3%)	$\chi^2:0.00$	1.000 (NS)
Male	4 (6.7%)	2 (6.7%)		
Age (years)				
Mean $\pm$ SD	31.93 $\pm$ 10.36	34.17 $\pm$ 10.31	t:0.97	0.340 (NS)
Range	18–54	22–59		

Inc., Chicago, Illinois, USA). The numerical inputs were displayed as mean, standard deviation, and ranges. Also, qualitative variables were presented as number and percentages. *Shapiro-Wilk and Kolmogorov-Smirnov tests* were used to examine the data for normality.

The further tests were conducted: When comparing two means, the independent-samples *t*-test of significance was utilized, and the *Mann Whitney U test* was used for comparisons between two groups in nonparametric data. Using the  $\chi^2$  test, groups with qualitative data were compared.

If one or both sets of variables were skewed, the degree of correlation between them evaluated using *Spearman's rank correlation coefficient (rs)*.

Positive: Raising the independent variable raises the dependent variable. Negative: Raising the independent variable decreases the dependent variable. The pattern of the resultant dots on a scatter plot, a graph where the values of two variables are shown along two axes, reveals the presence of correlation.

To determine the overall predictability of the parameter and the appropriate cut-off value with detection of sensitivity and specificity at this cut-off value, receiver operating characteristic (ROC curve) analysis was employed.

### 3. Results

Our study comprised 60 SLE participants (30 Nephritis patients and 30 NN patients) matched for 30 healthy controls. When compared with the healthy control group, there was no difference between the two groups regarding demographic data. The characteristics of the research subjects were shown in Table 1.

The overall level of CP was considerably greater in SLE patients (Mean: 329.60 ng/ml) than control group (Mean: 183.75 ng/ml) as shown in Table 2.

In addition, LN group had significant higher levels of CP than NN group as shown in Table 3.

Additionally, our study revealed that patients with higher SLEDAI and rSLEDAI had greater levels of CP than inactive nephritis group.

Also, our study found that in LN group CP exhibited a negative highly significant association with eGFR [r: 0.687 – (0.001)] and C3 [r: –0.371– (0.001)], as well as a significant positive correlation regarding renal biopsy class, activity and chronicity indices, disease duration, pyuria, and ESR.

There was also a substantial positive significant correlation between CP and collection of 24 h urinary protein (r: 0.752–0.001), s creatinine (r: 0.776–0.001), and CRP (r: 0.653–0.001).

Using the ROC curve diagnostic performance; urinary CP cut-off value has been found at levels greater than 276.77 ng/ml among LN subgroup with 82% sensitivity and 80.9% specificity, 81.7% positive predictive value and 82.1% negative predictive value as shown in Table 4.

### 4. Discussion

Since up to 75% of lupus patients will eventually have LN, it is crucial to identify LN as early as possible. S. albumin, C3 and C4, anti-dsDNA are traditional LN biomarkers, as well as urinalysis, and serum creatinine, and eGFR Tsokos.<sup>11</sup>

Unfortunately, the sensitivity and specificity for identifying LN flares in levels of C3 and C4, and anti-dsDNA are poor. Since, only 25% of lupus patients who have low C3 or low C4 or high anti-dsDNA experience an nephritis flare Lindblom and colleagues.<sup>8</sup>

Before kidney function becomes reduced and initially detected by lab results, considerable kidney damage may have developed Aljaberi and colleagues.<sup>12</sup>

Although renal biopsy is the preferred method, it is an invasive technique and prone to sampling error. And so, to enhance LN identification and management, we need credible, noninvasive biomarkers Carmona-Fernandes and colleagues, Hanly and colleagues.<sup>13,14</sup>

Table 2. SLE patients vs control group regarding ceruloplasmin level.

Urinary ceruloplasmin Level (ng/ml)	Patients (n = 60)	Control (n = 30)	U test	P-value (Significance)
Mean $\pm$ SD	329.60 $\pm$ 99.96	183.75 $\pm$ 32.57	–7.704	<0.001**
Range	230.17–570	106.44–227.84		

Table 3. LN group vs NN group regarding urinary ceruloplasmin level.

Urinary Ceruloplasmin level (ng/ml)	LN (n = 30)	NN (n = 30)	t-test value	P-value (Sign.)
Mean ± SD	407.00 ± 87.66	252.20 ± 15.78	-6.656	<0.001**
Range	278–570	230.17–276.77		

Table 4. Ceruloplasmin Cut-off value in discrimination LN vs NN.

Cut-off	Sen.	Spec.	PPV	NPV	AUC [95% C.I.]	P-value
>276.77	82.9%	80.9%	81.7%	82.1%	0.82 (0.74–0.95)	<0.001

Serum biomarkers known to be more likely a reflection of systemic response than organ response, even though they are more stable. However, a more direct evaluation of kidney disease activity may be better with urine biomarkers Soliman and Mohan.<sup>3</sup>

Since urinary biomarkers may be easily gained and reflect the current kidney condition, urine biomarkers seem to be more promising than serum biomarkers since they directly represent the direct local inflammatory products of activity Salem and colleagues.<sup>15</sup>

Besides, urinary exosomes include proteins and indicators unique to the kidney, they represent a distinct area of research. As Examining urine exosomes and extracellular vesicles of renal diseases may deliver on the long-desired promise of replacing invasive tissue biopsies with noninvasive 'liquid biopsies Pisitkun and colleagues, Gudehithlu and colleagues.<sup>16–18</sup>

CP is an acute-phase protein derived from the kidney; it may be an early indicator of different kidney disorders Gudehithlu and colleagues.<sup>9</sup>

Our study aimed to evaluate the utility of urinary CP as a potential surrogate biomarker to differentiate LN from NN and to investigate the correlation to disease activity.

In our study, we found a highly statistically significant difference ( $P < 0.001$ ) regarding urinary CP level between LN group (mean: 407 ng/mL) and NN group (mean: 252.20 ng/mL). These results were in line with Suzuki and colleagues,<sup>19</sup> and Urrego and colleagues,<sup>7</sup> where both found that CP would be a promising biomarker for LN than traditional measures.

Our study revealed that in LN; CP level shows a significant positive correlation regarding renal biopsy class, activity and chronicity indices, disease duration, 24 h protein in urine (0.752–0.001), pyuria, s. creatinine (0.776–0.001), ESR and CRP (0.653–0.001).

Also, Urinary CP level shows a highly significant negative correlation regarding eGFR (–0.687

–0.001), C3 (–0.371–0.001). which came in concordance with Brunner and colleagues<sup>20</sup>

Besides, we found no correlation between CP to hematuria, CBC, or Complement 4.

Our study also found a positive correlation between concentrations of CP and renal biopsy class, as well as a positive correlation to activity and chronicity indices, disease duration, pyuria, and ESR.

Also, we found an excellent cut-off value for CP to differentiate LN; as urinary CP level has been found at levels greater than 276.77 ng/mL among LN subgroup with 82% sensitivity and 80.9% specificity, 81.7% positive predictive value and 82.1% negative predictive value and this was in agreement with Urrego and colleagues, Urrego-Callejas and colleagues studies<sup>7,21</sup> as they observed that TF and CP were promising biomarkers for nephritis and could differentiate active LN from inactive LN rather than discrimination from NN.

Up till now, there is not a single biomarker that can replace an established clinical criterion (renal biopsy) on its own to diagnose nephritis and to assess the progression of the disease and forecast renal flares. Instead, a constellation of biomarkers found to be required to develop a useful index for managing LN patients. Hence, Brunner and colleagues<sup>20</sup> developed a biomarker-based Renal Activity Index for Lupus (RAIL) after demonstrating that CP, MCP-1, adiponectin, neutrophil gelatinase-associated lipocalin (NGAL), hematopexin, and KIM-1 were the leading predictors of LN activity Brunner and colleagues.<sup>20</sup>

#### 4.1. Limitations

Our study's first drawback was the study design for a predictor biomarker, due to feasibility of the study it would be better if a prospective cohort study follows SLE patients longitudinally with multiple measures of CP at baseline diagnosis then at timed intervals till progression into LN and along treatment course. We were unable to assess the behavior of biomarkers over time or their relevance to therapy using the case-control research design.

Another drawback of our study was the inability to compare CP level to renal biopsy concurrently at

the same time with considerate adjustment of possible urinary confounders.

#### 4.2. Conclusion

Urinary CP can be considered as a promising biomarker that might be used to characterize renal affection in LN and to monitor nephritis activity. Subsequently, it can be used to evaluate response to ongoing treatments.

#### Consent for publication

Non applicable.

#### Availability of data and material

On reasonable request, information will be given.

#### Authors contributions

M.M.H and H.M.A contributed to manuscript writing, and S.A.F and H.M.A supervised the work.

#### Disclosure

The authors have no financial interest to declare in relation to the content of this article.

#### Authorship

All authors have a substantial contribution to the article.

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#### Conflicts of interest

The authors declared that there were NO conflicts of Interest.

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