

RENAL HISTOPATHOLOGICAL PROFILE IN PATIENTS WITH SILENT LUPUS NEPHRITIS

By

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ABSTRACT

Background: Silent lupus nephritis (SLN) is a life menacing consequence of systemic lupus erythematosus (SLE). This condition is characterized by pathological impairment of the kidney in the obscurity of clinical or laboratory manifestations.

Objective: To reveal the existence of SLN along with the potential differences between overt lupus nephritis (OLN) and SLN among a sample of Egyptian patients based on histopathological assessment.

Patients and Methods: It is a prospective case-control study which was performed at nephrology units, internal medicine department, Elhussein and Sayed Galal university hospitals, faculty of medicine, Al-Azhar University, Cairo, Egypt, throughout the entire period April 2016 to November 2019. Patients aged more than 18 years (216 months) and fulfilled at least 4 of the American College of Rheumatology criteria for the classification of systemic lupus erythematosus (SLE) were enrolled in the current study. Patients were further assorted into two groups; patients with SLN and those with OLN.

Patients were subjected to the following investigations: 1.Complete blood count, using Coulter counter Max-M (Coulter Cooperation, Florida, USA) 2.Erythrocyte sedimentation rate, (Wester green method) (Ref: < 20mm/hr) 3.S. Albumin, S. Creatinine. Albumin/Creatinine ratio. Creatinine clearanc & eGFR using the Modification of Diet in Renal Disease (MDRD) formula. 4. Liver function tests. 5. Coagulation profile. 6. Urine analysis (Fresh morning midstream urine) to exclude infection. 7. Quantitative assessment of proteinuria by Pr/creat. Ratio (Ref. <0.2).

Assessment of auto-antibodies and complement system: Autoantibodies to ds-DNA, RNP. SSA, SSB, Sm and Scl-70, C4 and C3 serum levels were assessed in all of the included patients. In particular, the titer of anti-dsDNA antibodies was evaluated by enzyme-

Radiological evaluation: Patients were subjected to pelvi-abdominal ultrasound in order to obtain valuable data about the morphological appearance of the kidney and to detect any urological abnormality.

Renal biopsy: Percutaneous renal biopsy was carried out under local anesthesia.

Results: An overall 40 patients with SLE who developed lupus nephritis were enrolled in the current study. Among them, 20 patients had OLN, whereas 20 patients were SLN. Based on ISN/RPS Classification, stage II was the predominant stage, 13 patients, among patients with SLN, whilst stage V was the predominant stage among the OLN patients. Additionally, five and three patients were stage III among the SLN and OLN groups, respectively. Furthermore, the presence of RBCs Cast ($r=0.479$, $P=0.032$) in urine and decreased levels of complement ($r=0.676$, $P=0.001$) showed a statistically significant positive correlation with the high grades of lupus nephritis among SLN group.

Conclusion: Patients with SLE should be subjected to close follow up evaluation and renal biopsy for early detection of SLN to determine the activity, severity, and chronicity of LN.

Keywords: Silent, lupus-nephritis, silent lupus erythematosus and overt lupus nephritis.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic multisystem autoimmune disorder that manifested clinically in a relapsing and remitting course. The burden of renal impairment in patients with SLE is considerably high in contrast to other organs affection (*Duli et al., 2017*). The manifestations of Lupus nephritis (LN) could range from asymptomatic microscopic hematuria up to renal failure (*Ishizaki et al., 2015*).

To date, nearly 50% of SLE patients manifested with overt lupus nephritis (OLN) with approximately 55% in Asians and 51% in Africans. LN is characterized by various clinical and pathological manifestations, which ultimately determine the prognosis of SLE (*Davidson et al., 2019*). However, some patients may have a pathological evidence of renal involvement in the absence of the clinical or laboratory manifestations of renal impairment. Thereafter, the actual prevalence of LN among SLN is estimated to be higher than reported (*Hoover and Costenbader, 2016*).

Silent lupus nephritis (SLN) is a life menacing consequence of SLE. This condition is characterized by pathological impairment of the kidney in the obscurity of clinical or laboratory abnormalities (*Moroni et al., 2016*). Pathological lesions in patients with SLN are usually mild. On the contrary, some patients might be presented with diffuse proliferative glomerulonephritis which unfortunately accompanied by a mortality rate of 60% (*Xu et al., 2014*).

Percutaneous renal biopsy is the gold standard tool for the diagnosis and classification of renal impairment coupled with assessment of the disease activity (*Wen, 2011* and *Hsieh et al., 2012*). For patients with SLN, renal biopsy is necessary for precise diagnosis and timely detection of renal involvement (*Haladyj and Cervera, 2016*).

The current study was executed to reveal the existence of SLN along with the potential differences between OLN and SLN among a sample of Egyptian patients based on histopathological assessment.

SUBJECTS AND METHODS

The present investigation was conducted in consideration of the Declaration of Helsinki, and ethical research board confirmation was obtained from the ethics unit of the Faculty of Medicine, Al-Azhar University. All participants assigned informed consents after clear explanation of the study process and possible side effects.

This was a prospective case-control study which was performed at Nephrology Unit, Internal Medicine Department, Al-Hussein and Sayed Galal University Hospitals, Faculty of Medicine, Al-Azhar University, Cairo, Egypt, throughout the entire period April 2016 to November 2019.

Eligibility criteria:

Patients aged more than 18 years and fulfilled at least 4 of the American College of Rheumatology criteria for the classification of SLE were enrolled in the current study. Patients were further

assorted based on the presence of clinical and laboratory evidence of LN into two groups; patients with SLN and those with OLN.

The condition of SLN was clarified among patients with the absence of clinical renal manifestations, normal creatinine levels (0.6-1.4 mg/dl), normal creatinine clearance (70-120 ml/minute/1.73 m² body surface), normal urinary sediment, and absence of clinical proteinuria (\leq 300 mg/day in 24 hours urine collection).

Patients were included in the OLN group if they had one or more of the following characteristics; clinical evidence of LN such as hypertension ($>140/90$) or edema, high creatinine levels (>1.4 mg/dl), low creatinine clearance (<70 ml/minute/1.73 m² body surface), abnormal urinary sediment (> 5 leucocytes and/or >5 red cells 40x power field), and presence of clinical proteinuria (> 300 mg/day in 24 hours urine collection).

Exclusion criteria:

Patients refused to be subordinated to renal biopsy, and those with life menacing disorders such as severe hypertension, diabetes mellitus, advanced renal failure, or those with abnormal coagulation profile were excluded from the study. Similar to that, patients with drug-induced nephritis, congenital renal or urological diseases were ousted from the study.

Patient's evaluation:

1. Clinical evaluation:

Detailed history taking and clinical evaluation were implemented for all patients to reveal patient's age, sex, weight,

onset of SLE, duration of the disease, clinical manifestations of SLE, co-morbidities, along with generalized examination.

2. Laboratory assessment:

Patients were submitted to laboratory evaluation which included the following tests; blood profile, liver functions, renal functions apart from serum creatinine, urea, and creatinine clearance coupled the albumin/creatinine ratio and estimation of glomerular filtration rate.

3. Radiological evaluation:

Patients were subjected to pelvi-abdominal ultrasound in order to obtain valuable data about the morphological appearance of the kidney and to detect any urological abnormality.

Renal biopsy:

Percutaneous renal biopsy was carried out under local anesthesia and after ultrasonography localization of the left renal pole. The obtained tissues were stained for immunofluorescent and optical microscopy. In detail, paraffin sections were stained by Hematoxylin-Eosin, PAS, Gomori trichrome and silvermethenamine-hematoxylin stains. Immunofluorescent microscopy was used to assess human IgG, IgA, IgM, C3 and C4 after treatment of the obtained renal sections by fluoresceinated antiserums. The obtained renal biopsies were classified based on ISN/RPS classification. Besides that, the activity and chronicity indexes were calculated (*Touma et al., 2011* and *Mubarak & Nasri, 2014*).

Statistical analysis:

Continuous normally distributed data were reported in the form of mean, and standard deviation (SD). On the other hand, continuous non-normally distributed data were illustrated using median and range and were compared using Mann-Whitney U test. Subsequently, categorical variables were expressed using number and percentage and its particular groups were compared using Pearson's chi-square

test. Correlation analysis was conducted using Spearman's rank correlation coefficient for categorical variables. The overall statistically significant difference was established at $p < 0.05$. Statistical analysis was performed using SPSS software version 23 for Windows (SPSS Inc., Chicago, IL, USA). The figures were renovated using GraphPad Prism (GraphPad Software, Inc, San Diego) software version 7.

RESULTS**Patient's demographic characteristics:**

An overall 40 patients with SLE who developed lupus nephritis were enrolled in the current study. Among them, 20 patients had overt lupus nephritis (OLN), whereas 20 patients were silent lupus nephritis (SLN). There was no statistically significant difference between both groups regarding the age of the patients ($p=0.583$), whereby, the mean age of the patients was 29.1 ± 8.43 and 27.65 ± 8.15 in

the SLN and OLN groups, respectively. There was a statistically significant difference ($P < 0.001$) between SLN and OLN groups regarding the frequency of hypertensive patients. In particular, 17 patients had hypertension in OLN group, whereby only one patient had hypertension among SLN group. On the contrary, neither did any patient experienced diabetes in our study (**Table 1**).

Table.(1): Demographic characteristics of the included patients

Groups Variables	SLN (20)	OLN (20)	P-Value
	Mean +SD/ Number (%)	Mean +SD/ Number (%)	
Age	29.1±8.43	27.65±8.15	0.583
Sex			
Female	16(80%)	12 (60%)	0.168
Male	4 (20%)	8 (40%)	
Co-Morbidities			
Hypertension	1 (5%)	17 (85%)	P<0.001
Diabetes	0	0	
Creatinine	1.01±0.23	3,24±0.97	P>0.001
Urea	27.65±9.04	105,75±27.31	P>0.001
24H urine collection			
24H urine collection	72.5(22-500)*	3000(700-4000)*	P>0.001
Proteinuria	85(0-900)*	2200(1500-4200)	P>0.001
Urine sediment			
RBCs cast	2(10%)	8(40%)	P>0.001
Granular cast	0	8(40%)	P>0.001
C3	17(85%)	3(15%)	P>0.001
C4	17(85%)	3(15%)	P>0.001
<p>SLN=Silent lupus nephritis, OLN=Overt lupus nephritis, * the results of t-test. *data represented in the form of median and range.</p>			

Having the laboratory assessment, the mean values of creatinine and urea increased considerably in the OLN groups (P<0.001) relative to SLN patients. In details, the mean value of creatinine was 1.01±0.23 mg/dl and 3.24±0.97 mg/dl among the SLN and OLN groups, respectively. Subsequent to that, the mean value of urea was 27.65±9.04 mg/dl in the SLN group, whereby it was 105.75±27.31 mg/dl among the OLN group. On the same hand, the median values of 24H urine collection and proteinuria were statistically (p>0.001) high in the OLN group when compared with SLN group. In this respect, the median value of 24H urine collection was 72.5 ml and 3000 ml in the SLN and OLN groups, respectively. Similar to that, the mean values of proteinuria were 85 mg in the SLN group, whereas it was 200 mg among the OLN group.

The pattern of urine sediments showed a statistically significant difference between both groups apart from an equal proportion of patients (8) had RBCs Cast and Granular cast among the OLN group. Additionally, two patients had RBCs Cast among the SLN group. Patients among SLN group experienced a noticeable decline in the levels of C3 and C4 (P<0.001), whereas 17 patients had decreased values of both C3 and C4.

There was no statistically significant difference between SLN and OLN patients (p=0.202) regarding the activity index with a means of 14.5±4.09 and 12.8±4.17 in the SLN and OLN groups, respectively. Conversely, both groups experienced statistically significant difference regarding the chronicity index, whereas OLN patients encountered significant (P<0.001) higher points [7 (4-

12)] of the chronicity index in contrast with SLN patients [4 (0-8)].

Based on ISN/RPS Classification, stage II was the predominant stage, 13 patients, among patients with SLN, whilst stage V was the predominant stage among

the OLN patients. Additionally, five and three patients were stage III among the SLN and OLN groups, respectively. Eventually, no patients encountered stage VI among the SLN group (**Table2**).

Table (2): The pattern of chronicity and activity indices among the studied groups

Variables	Groups	SLN	OLN	P-Value
		Mean +SD	Mean +SD	
Activity index		14.5±4.09	12.8±4.17	0.202
Chronicity index		4 (0-8)*	7 (4-12)*	P<0.001
ISN/RPS Classification among the studied groups				
Variables		SLN	OLN	P-Value
		Number (%)	Number (%)	
Biopsy staging				
II		13 (65%)	1 (5%)	P<0.001
III		5 (25%)	3 (15%)	
IV		1 (5%)	5 (25%)	
V		1 (5%)	10 (50%)	
VI		0	1 (5%)	
SLN=Silent lupus nephritis, OLN=Overt lupus nephritis, * data represented in the form of Median (Range), ** the results of Mann-Whitney U test Abbreviations; SLN=Silent lupus nephritis, OLN=Overt lupus nephritis				

The results of the current study revealed that; there was no statistically significant correlation between age ($r=0.047$, $P=0.84$), sex ($r=-0.35$, $P=0.11$) proteinuria ($r=0.33$, $P=0.15$) urea ($r=0.3$, $p=0.1$), and 24H urine collection ($r=0.073$, $p=0.75$) and the staging grade of lupus nephritis among patient with silent lupus

nephritis. On the other hand, the presence of RBCs Cast ($r=0.479$, $P=0.032$) in urine and decreased levels of C3, and C4 ($r=0.676$, $P=0.001$) showed a statistically significant positive correlation with the high grades of lupus nephritis among SLN group (**Table 3**).

Table(3): Correlations between the staging and demographic characteristics

Parameter	Correlation	r	P-Value
Age		0.047	0.84
Sex		-0.35	0.11
Proteinuria		0.33	0.15
Creatinine		0.017	0.94
Urea		0.3	0.1
24H urine collection		0.073	0.75
RBCs Cast		0.479	0.032
C3		0.676	0.001
C4		0.676	0.001
SLN=Silent lupus nephritis, OLN=Overt lupus nephritis, r= correlation coefficient			

DISCUSSION

Lupus nephritis is one of the most frequent and serious complications of SLE, although long-term prognosis may be dramatically improved by early detection of nephritis coupled with the employment of the current therapeutic protocols. Therefore, a precise histologic diagnosis is required for rational management and follow-up of the glomerular lesion in SLN (*Fu et al., 2019*).

In the present work, there was a patient developed hypertension among SLN group, whereby 17 patients had hypertension among OLN group. Additionally, all paraclinical parameters counterpart S. creatinine, S. urea, and 24H urine collection were at their normal range among the SLN group. It is also important to stress that renal lesions were found in SLN group without clinical renal manifestations, regardless of the time of evolution from the apparent onset, age of the patients, gender or degree of extra-renal clinical activity of the disease, in contrast to OLN group.

These findings were compatible with the definition of SLN which stated that; silent nephritis, is a serious pathological impairment of the kidney which presents in some patients with SLE, in the obscurity of abnormal urinalysis results or other clinical manifestations such as elevated serum creatinine and hypertension (*Wang et al., 2018*).

As the onset of overt nephritis is thought to be exacerbated in silent lupus nephritis, our findings suggest that silent lupus nephritis represents an early phase or mild form of lupus nephritis. In our study, there was no significant difference between SLN and OLN group regarding

the activity index despite being relatively high among OLN group. These results suggest that at the initial diagnosis of SLE it is quite difficult to distinguish patients who will develop overt nephritis from those whose renal disease will remain silent. Having histopathological findings, the results of the current study revealed that the majority of cases among SLN group had mild histopathological staging (stages II and III) relative to OLN group (stages IV and V). Owing to the additional significant difference between SLN and OLN groups regarding the chronicity index, these findings bring to light that patients with lupus nephritis may experience a course of SLN prior to the development of clinical and paraclinical manifestations. However, this finding should be confirmed in prospective studies with adequate follow-up periods.

In accordance with our results, some studies displayed that the majority of patients (63.3%) among SLN were II stage, whereby 37.5% and 25% staged IV and V among OLN group, respectively (*Houssiau and Lauwerys, 2013*). The pattern of glomerular injury seen in SLE is primarily related to the site of formation of the immune deposits, which are located in the mesangium, subendothelial and/or sub-epithelial compartments of the glomerulus (*Fibbe and Rabelink, 2017*). Renal histopathological findings have been investigated as important predictors of renal and patient survival in lupus nephritis, and patients with proliferative changes are known to have worse renal survival than those with mesangial lesions (*Tang et al., 2018*).

In fact, patients with diffuse proliferative lupus nephritis may have a

mortality rate of 60% with renal failure being a prominent factor leading to death despite therapy with corticosteroids and cytotoxic drugs. Thereafter, it has been suggested that renal biopsies may be necessary at the time of diagnosis in all patients with lupus to define prognosis and possibly therapeutic strategy. This is might be attributed to the evidence that clinical findings of kidney involvement in LN are nonspecific and may be seen in other forms of renal injury (*Almaani et al., 2017*).

Clinical manifestations underestimate the severity of renal involvement in the SLE patient. In our investigation, patients with stage III (25%) among SLN had low levels of creatinine, urea, and proteinuria relative to those with stage II. According to the pathophysiology of the disease, the presence of hematuria and proteinuria in the urine test may be a bookmark of renal impairment due to SLE. Although not very sensitive according to the scientific evidence; therefore its absence underestimates in some chances the degree of renal compromise by autoimmunity (*Stillman, 2016*).

Despite the evidence obtained in the current study, there were some limitations. The limited sample size, which represents only a small proportion of the Egyptian patients with relatively similar environmental and demographic factors, may restrict the capability to generalize our results. Additionally, the lack of adequate follow up period which limits the ability to detect the progression of SLN in a short term and long-term period to reveal patients who will develop OLN among SLN group.

CONCLUSION

Patient is subjected to close follow up evaluation and renal biopsy for early detection of SLN in order to determine the activity, severity, and chronicity of LN.

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تقييم الأنسجة الكلوية فى مرضى الذئبة الحمراء الغير مصاحب بمشاكل كلوية ظاهرة

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خلفية البحث: إتهاب الكلية الصامت فى مرضى الذئبة الحمراء يعد خطراً مهدداً للحياة خاصة فى غياب الأعراض الإكلينيكية والمعملية الظاهرة، والذي ينعكس بشكل ملحوظ على معدل تدهور المرض ونتيجة على المدى القصير والبعيد.

الهدف من البحث: الكشف عن وجود التهاب الكلية الصامت جنباً إلى جنب مع الاختلافات المحتملة بين التهاب الكلية العنفي والصامت بين عينة من المرضى المصريين على أساس تقييم التشريح الباثولوجي.

المرضى وطرق البحث: انها دراسة حالة وضوابط مرتقبة تم إجراؤها في وحدات أمراض الكلية، قسم الطب الباطني، مستشفيات جامعة الحسين وسيد جلال، كلية الطب، جامعة الأزهر، القاهرة، مصر، طوال الفترة من أبريل 2016 إلى نوفمبر 2019. تم تسجيل المرضى الذين تزيد أعمارهم عن 18 عامًا وتتفق حالتهم مع أربعة أو أكثر من معايير الكلية الأمريكية لأمراض الروماتيزم لتصنيف الذئبة الحمراء في الدراسة الحالية. تم تنويع المرضى إلى مجموعتين؛ المرضى الذين يعانون من التهاب الكلية العنفي والمرضى الذين يعانون من التهاب الكلية الصامت.

نتائج البحث: تم تسجيل ما مجموعه 40 مريضاً من مرض الذئبة الحمراء الذين أصيبوا بالتهاب الكلية في الدراسة الحالية. من بينهما،

كان 20 مريضاً يعانون من إتهاب الكلية العنقي، في حين أن 20 مريضاً كانوا يعانون من التهاب الكلية الصامت بناءً على تصنيف ISN/RPS، كانت المرحلة الثانية هي المرحلة السائدة، 13 مريضاً، بين مرضى التهاب الكلية الصامت، بينما كانت المرحلة الخامسة هي المرحلة السائدة بين مرضى التهاب الكلية العنقي بالإضافة إلى ذلك، خمسة وثلاثة مرضى صنفوا في المرحلة الثالثة بين مجموعات مرضى التهاب الكلية العنقي والصامت. على التوالي. علاوة على ذلك، أظهر وجود كرات الدم الحمراء المصبوب في البول وانخفاض مستويات عوامل التكملة وجود علاقة إيجابية ذات دلالة إحصائية مع درجات عالية من التهاب الكلية الصامت في مرضى الذئبة الحمراء.

الاستنتاج: يجب إخضاع المرضى الذين يعانون من مرض الذئبة الحمراء للتقييم عن كثب مع أخذ عينة من الكلى للكشف المبكر عن التهاب الكلية الصامت لتحديد نشاط، وشدة المرض.