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Evaluating The Role of Serum Circulating Immune Complex Level in Lupus Nephritis Patients

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ABSTRACT

Background: Systemic lupus erythematosus (SLE) is associated with significant multisystem complications. Lupus nephritis (LN) is one of the major complications. Circulating immune complexes (CIC) may represent a promising diagnostic biomarkers in LN. This study aimed to evaluate the role of serum circulating immune complexes in Lupus Nephritis and it's relation to the renal biopsy.

Patients and Methods: Ninety subjects were included, 30 with systemic lupus with nephritis (group 1A), 30 with lupus without nephritis (Group 1B) and 30 healthy subjects. All were clinically evaluated and the value of laboratory measurements were recorded. The serum circulating of CIC were determined and correlated with other clinical and laboratory data as well as disease activity index. The assay of serum circulating immune complex level by quantitative Sandwich enzyme linked immunosorbent assay (ELIZA) technique. The assay of CIC was performed using a commercially available kits. The detection range was 2 to 600 ng/ml. The kit minimum detectable level of CIC was 1.765ng/ml.

Results: In group A, the results of the renal biopsy was class III LN among 13 patients and Class IV LN among 17 patients. The results of renal biopsy was significantly associated with anti-dsDNA, ESR, Activity index and urine total protein (TP), where anti-ds-DNA was significantly reduced and each of ESR, activity index and urine TP were significantly increased in class IV than class III (the mean values were 71.4 (IU/ml), 86.8 (mm/hour), 18.5, and 3.6 g/dl in class IV compared to 155.8, 69.4, 16.6 and 2.17 in class III, respectively). CIC was significantly correlated with activity index score in both 1A and 1B groups. CIC in discrimination SLE groups showed that, at value ≤ 84 (ng/ml) had a sensitivity, specificity, PPV, NPV of 100%, 100.0%, 100.0% and 100.0% respectively. Similar values were recorded for discrimination between 1B and control groups at a cutoff value of ≤ 189.

Conclusion: The CIC can be used as diagnostic and follow up biomarkers in the patients with systemic lupus wither with or without nephritis. It is specifically correlated with disease activity index.

Keywords: Immune complexes; Systemic Lupus; Nephritis; Disease Severity Index; Renal Biopsy.

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INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic multisystem autoimmune diseases. It frequently involves the kidneys. The development of the disease is usually due to exposure to environmental factors (triggers) in individuals with genetic predisposition. It is characterized by multi-system inflammations, lost tolerance against nuclear autoantigens, lymphoproliferation, production of polyclonal autoantibody and immune complex disease. The commonly involved organs include skin, joints, kidneys, heart, lungs, central nervous system (CNS) and serous membranes [1,2]. Lupus nephritis (LN) usually developed early in the clinical course of SLE in up to 50% of patients. The survival of patients with SLE was improved after the introduction of new effective diagnostic tests and new therapies. However, the risk of death is still higher among SLE than the general populations. Highly active lupus nephritis requires greater immunosuppressive treatment. However, the risk of death is still higher due to opportunistic infection. Additionally, the long term treatment by a high dose corticosteroids represents a risk factor for the development of coronary atherosclerosis and cardiovascular diseases. In lupus nephritis, glomerular immune complexes activates the complement system and engages leukocyte Fc receptors to initiate the process of inflammation and renal injury. In addition, LN has vary pleomorphic clinical and morphologic expressions. Clinically, it may be presented by asymptomatic hematuria, proteinuria, nephrotic syndrome or rapidly progressive renal failure as all compartments of the kidney can be affected (e.g., glomeruli, tubules, interstitial tissue and blood vessels) [3-5].

It is suggested that, several phenotypic renal diseases of SLE are due to the formation of immune complexes (ICs) either formed by foreign or self-antigens and antibodies in biological fluids in the kidney tissues. The clinical and biological properties, abnormal amounts or deposition of IC are suggested to have a relationship with the pathogenesis process of the disease and it was widely studied. However, the specific relationship between each phenotype and ICs is not well understood. Little is known about factors what governs the ICs production and deposition in specific renal tissues and why different organs are exposed to ICs deposition in certain phenotype. Recently, the introduction of new technologies enables the recognition of ICs and its antigens in affected tissues and body fluids. This may provide a key information to discover an important trigger for the autoimmunity and immunological abnormality process in SLE [6-10].

THE AIM OF THE WORK

The aim of the this work was to evaluate the role of serum circulating immune complexes level in Lupus Nephritis patients and it's relation to the findings of the renal biopsy.

PATIENTS AND METHODS

This was a case control study that was conducted at Al-Azhar University hospitals (Al-Hussin and Damietta Hospitals) during the duration from July 2023 to March 2024. A total of 90 subjects (A convenient sample) were included. They were 60 adult patients with SLE, aged 18 50 years. They were selected from the outpatient clinic of the internal medicine departments (Nephrology units). The diagnosis of SLE was constructed according to EULAR/ACR 2019 Criteria [11]. The disease activity and severity was estimated according to SLEDAI-2K descriptors and scores [12].

Patients with SLE were divided into two equal groups (each 30 patients). The first group (group 1A) included patients with renal involvement. Their Diagnosis was based on the international society of nephrology and renal society classification of LN 2003) [13]. The second group (Group 1B) included those without renal involvement. The control group composed of 30 healthy subjects matched for age and sex.

We **excluded** patients with other systemic immunological diseases known to affect the kidney (e.g., diabetes mellitus, hypertension, heart disease, liver disease and malignancies).

Ethical aspects: the study protocol was reviewed and approved by the Research Ethics Committee, Faculty of Medicine Al-Azhar University before recruiting the first patient. In addition, an informed consent was signed by each patient confirming the acceptance to participate in the study. The collected data was only used for the purpose of this study and patient anonymity was assured by coding of data. The study was completed in line with the ethical codes for research conduction and reporting developed by the world health organization.

Methods

Initially, each patient was evaluated from the clinical point of view by the information about medical history and standard clinical examination. The duration SLE, clinical presentation and treatment received special attention. In addition, venous blood samples were collected and treated to be used in the determination of different laboratory tests. These included the scoring indices of SLEDAI-2K and EULAR/ACR 2019 Criteria, Erythrocyte sedimentation rate (ESR), antinuclear antibodies (ANA), anti-double stranded DNA (Anti-dsDNA) antibody, C3 and C4 levels. In addition, the urine was tested for hematuria, proteinuria, albumin /creatinine ratio, total protein in urine of 24 hours. Furthermore, the estimated glomerular filtration rate (e-GFR) was calculated and results of renal biopsy were documented and correlated with other results. All the laboratory assays were conducted at Clinical Pathology Department of Al-Azhar university hospitals (El-Hussein and Damietta hospitals).

The assay of serum circulating immune complex level by quantitative Sandwich enzyme linked immunosorbent assay (ELIZA) technique. Firstly, 5 ml of venous blood were withdrawn from each patient and control subjects under complete aseptic conditions, into a plain tube. Serum was separated by centrifugation of the plain tube at 3000 revolution per minute (RPM) for 20 min at room temperature after complete blood clotting and stored at -20^{o}C till the time of analysis. Repeated

freezing/thawing of samples were avoided.

The assay of CIC was performed using a commercially available kit supplied by Antibodies online (Catalog No. ABIN626335) through SunRed Biotechnology Laboratory (Shanghai, China). The kit used a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of Human circulating immune complexes (CIC) in samples. At first, the circulating immune complex (CIC) were added to monoclonal antibody samples which was pre-coated with Human circulating immune complex (CIC) monoclonal antibody. The wells were incubated, then, circulating immune complex (CIC) antibodies labeled with biotin, and combined with Streptavidin-HRP were added to form immune complex. After that, the sample was re-incubated and washed to remove the uncombined enzyme. Chromogenic Solution A, B, were added and the color of the liquid changes into the blue and then turned yellow under the effect of acid. The concentration of the human circulating immune complexes (CIC) of sample were determined according to the chroma of color (optical density). To interpret results, a standard curve was constructed by plotting the optical density (OD) for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis. The concentration of human CIC in each sample was then determined by comparing the OD of each sample to the standard curve. The concentration was measured by ng/ml. The detection range was 2 to 600 ng/ml. The kit minimum detectable level of CIC was 1.765ng/ml.

In addition, Anti-nuclear Antibody was assayed by ANA, ELISA Kit (Catalogue no #MBS702970), Anti-double stranded DNA (Catalogue no #MBS6507401), Polyclonal Antibody to Complement Component 4 (C4) (Catalogue no #MBS2028022) and Polyclonal Antibody to Complement Component 3 (C3) (Catalogue no #MBS2001664) supplied by MyBioSource Inc (Southern California, San Diego (USA)) through local distributors.

Statistical analysis: Data were analyzed using Statistical Program for Social Science (SPSS) version 24 (IBM Inc., USA). Qualitative data were presented by their relative frequencies and percentage. Quantitative data were expressed by the arithmetic mean and standard deviations (SD). Then, the groups were compared by one way analysis of variance (ANOVA) test (for more than two means) with calculation of post-hoc least significant differences, independent samples student test (for two means). The categorical data associations were measured by Chi square test or Fisher exact test. The Pearson's correlation coefficient was calculated and receiver operation characteristic (ROC) curve was built to detect the diagnostic value and the best cutoff for a specific laboratory test. The p value < 0.05 was set as the margin of significance to interpret results.

RESULTS

In the current work, no significant differences were reported between study groups as regards the patient age and gender. The majority of patients were in their fourth decade of life with female sex predilection (the female gender represented 86.7%, 100.0% and 73.3% of groups 1A, 1B and control groups, respectively). In addition, the duration of SLE, alopecia, cerebritis, bleeding, thrombosis, serositis, oral ulcer, arthritis, and medical treatment, (except MMF, azathioprine and hydroxychloroquine). Clinically, renal manifestations were significantly increased in group 1A than 1B (100.0% vs 0.0% respectively), while pulmonary, cardiac, photosensitivity and malar rash were significantly increased in 1B than 1A groups (53.3%, 46.7%, 80.0% and 100.0% vs 6.7%, 6.7%, 33.3%, 80.0% successively). All patients in groups 1A and 1B received steroids, calcium and vitamin D, while MMF was significantly increased in 1A than 1 B group (63.3% vs 33.3%, respectively), while azathioprine and hydroxychloroquine were significantly increased in 1B than 1A groups (43.3%, 100.0% vs 0.0% and 0.00%, respectively) (Table 1).

Results of the laboratory data showed a statistically significant variance between study groups. Firstly, data in control group is significantly different than that of groups 1A and/or 1 B groups. In addition, ANA, anti-ds DNA, C3, eGFR, and CIC were significantly reduced in 1A than 1B groups, while C4, ESR, ACR, urine TP, and activity index score were significantly increased in the 1A than 1 B groups (Table 2).

The abnormalities in laboratory data showed significant increase of positive ANA, anti-ds DNA, low C3, low C4, and higher ESR in both SLE groups than the control group. However, hematuria, proteinuria, higher ACR, higher urine TP (24 h) and low eGFR were restricted to the 1A group, with significant differences between groups (Table 3).

In group A, the results of the renal biopsy was class III LN among 13 patients (43.3%) and Class IV LN among 17 patients (56.7%). In the 1A group, the results of renal biopsy was significantly associated with anti-dsDNA, ESR, Activity index and urine TP, where anti-ds-DNA was significantly reduced and each of ESR, activity index and urine TP were significantly increased in class IV than class III (the mean values were 71.4m 86.8, 18.5, and 3.6 in class IV compared to 155.8, 69.4, 16.6 and 2.17 in class III, respectively). Otherwise, no significant association was recorded for ANA, C3, C4, ACR, eGFR, and CIC (Table 4).

CIC was proportionately and significantly correlated with activity index score in both 1A and 1B groups. However, the correlation in group 1A was mild ($r = 0.21$), while in group 1B, it was moderate ($r = 0.51$). No other significant correlations were recorded between CIC and other variables or between activity index score and other variables (Tables 5, 6).

Regarding the diagnostic performance of CIC in discrimination between SLE groups, the cutoff value ≤ 84 had a sensitivity, specificity, PPV, NPV of 100%, 100.0%, 100.0% and 100.0% respectively. Similar values were recorded for discrimination between 1B and control groups at a cutoff value of ≤ 189 (Table 7).

Table (1): Comparison between study groups regarding patient demographics, clinical data and received treatments

Variable		Group 1A(n=30)	Group 1B(n=30)	Controls (n=30)	Test	P value
Age (years)	Mean±SD	30.6 ± 8.9	31.7 ± 7.7	30.4 ± 7	0.21	0.804
Gender (n,%)	Male	4(13.3%)	0 (0.0%)	8 (26.7%)	0.31	0.732
	Female	26(86.7%)	30 (100.0%)	22 (73.3%)		
Duration of SLE (years)	Mean ±SD	6.1 ± 3.9	6.9 ± 4.5	-	0.73	0.467
	Range	0.25 - 17	1 – 14	-		
Clinical Presentation (n,%)	Alopecia	11(36.7%)	16 (53.5%)	-	1.68	0.194
	Renal	30 (100.0%)	0 (0.0%)	-	60.0	<0.001*
	Pulmonary	2(6.7%)	16(53.3%)	-	15.5	<0.001*
	Cardiac	2(6.7%)	14 (46.7%)	-	12.2	<0.001*
	Cerebritis	6(20.0%)	10 (33.3%)	-	1.36	0.243
	Bleeding	4(13.3%)	4(13.3%)	-	0.001	1.00
	Thrombosis	4(13.3%)	3 (10.0%)	-	0.16	0.688
	Serositis	11(36.7%)	8(26.7%)	-	0.69	0.405
	Photosensitivity	10 (33.3%)	24 (80.0%)	-	13.3	<0.001*
	Malar rash	24 (80.0%)	30(100.0%)	-	6.66	0.01*
	Oral ulcer	22(73.3%)	24 (80.0%)	-	0.37	0.542
	Arthritis	30 (100.0%)	30 (100.0%)	-	0.001	1.00
Treatment (n,%)	Steroids	30(100.0%)	30(100.0%)	-	0.001	1.00
	Calcium	30(100.0%)	30(100.0%)	-	0.001	1.00
	Vitamin D	30(100.0%)	30(100.0%)	-	0.001	1.00
	Cyclophosphamide	11(36.7%)	7 (23.3%)	-	1.27	0.260
	MMF	19(63.3%)	10 (33.3%)	-	5.4	0.02*
	Azathioprine	0(0.0%)	13 (43.3%)	-	16.5	<0.001*
	Hydroxychloroquine	0(0.0%)	30(100.0%)	-	60.0	<0.001*

Table (2): Laboratory data and activity index among study groups.

	Groups			F	P-value
	Group IA (n = 30)	Group IB (n = 30)	Control (n = 30)		
ANA (IU/ml)	128.7 ± 103.7$^{\&}$	239.2 ± 92.2	9.1 ± 4.5$^{\#}$	61.8	< 0.001 *
Anti-ds-DNA (IU/ml)	108 ± 94.9$^{\&}$	178.2 ± 152.2	6.7 ± 3.9$^{\#}$	20.7	< 0.001 *
C3 (mg/dl)	35 ± 22.7$^{\&}$	40.8 ± 30.5	152 ± 32.2$^{\#}$	157.4	< 0.001 *
C4 (mg/dl)	8.2 ± 5.4$^{\&}$	7.1 ± 2.4	25.7 ± 7.4$^{\#}$	108	< 0.001 *
ESR (mm/hour)	79.2 ± 23.5$^{\&}$	65.9 ± 25.4	7.7 ± 3$^{\#}$	108.1	< 0.001 *
ACR (mg/g Creat)	1331.6 ± 873.5$^{\$}$	22.6 ± 6.4	22.1 ± 5.3	67.3	< 0.001 *
Urine TP (g/24 h)	3 ± 28$^{\$}$	0.1 ± 001	0.1 ± 0002	64.9	< 0.001 *
eGFR (ml/min)	28 ± 15.8$^{\$}$	122.9 ± 6.7	89.8 ± 45.2$^{\#}$	820.8	< 0.001 *
Activity index score	17.7 ± 2.2	9.8 ± 1.96	-	14.8	< 0.001 *
CIC (ng/ml)	57.6 ± 14.9 $^{\&}$	135.5 ± 26.7	421.2 ± 81.7 $^{\#}$	61.8	< 0.001 *

# indicates significant difference between control group and each of groups IA or IB; $ indicates significant differences between group 1A and each of group Ib or Control group; & indicates significant differences between groups IA and IB. NB; Data are presented as (mean±SD, Min. – Max.).

Table (3): Comparison of study groups regarding abnormal laboratory data

Variables	Abnormality	Groups			F	P-value
		Group IA (n = 30)	Group IB (n = 30)	Control (n = 30)		
ANA (IU/ml)	Positive	28 (93.3%)	30(100.0%)	0 (0.0%)$^{\#}$	81.8	<0.001*
Anti-ds-DNA(IU/ml)	Positive	26 (86.7%)	29(96.7%)	0(0.0%)$^{\#}$	71.3	<0.001*
C3 (mg/dl)	Low	30(100.0%)	27 (90.0%)	0(0.0%)$^{\#}$	78.3	<0.001*
C4 (mg/dl)	Low	23 (76.7%)	28 (93.3%)	0(0.0%)$^{\#}$	60.5	<0.001*
ESR (mm/hour)	High	30(100.0%)	30(100.0%)	0(0.0%)$^{\#}$	90.0	<0.001*
Hematuria	Positive	25 (83.3%)	0(0.0%)	0(0.0%)$^{\#}$	69.2	<0.001*
Proteinuria	Nil	0(0.0%) $^{\$}$	30(100.0%)	30(100.0%)	90.0	<0.001*
	+	4 (13.3%)	0(0.0%)	0(0.0%)		
	++	14 (46.7%)	0(0.0%)	0(0.0%)		
	+++	12(40.0%)	0(0.0%)	0(0.0%)		
ACR (mg/g Creat)	High	30(100.0%)$^{\$}$	0(0.0%)	0(0.0%)	90.0	<0.001*
Urine TP (g/24 h)	High	30(100.0%)$^{\$}$	0(0.0%)	0(0.0%)	90.0	<0.001*
eGFR (ml/min)	Low	30(100.0%)$^{\$}$	0(0.0%)	0(0.0%)	90.0	<0.001*

# indicates significant difference between control group and each of groups IA or IB; $ indicates significant differences between group 1A and each of group Ib or Control group; NB; Data are presented as (number and percentages).

Table (4): Association between renal biopsy and other studied data in IA group.

	Renal biopsy		Test	P-value
	Class III (n = 13)	Class IV(n = 17)		
ANA (IU/ml)	133.3 ± 102.4	125.3 ± 107.7	0.2	0.838
Anti-ds-DNA (IU/ml)	155.8 ± 107	71.4 ± 66.8	2.65	0.013*
C3 (mg/dl)	31.4 ± 25.5	37.8 ± 20.7	0.75	0.457
C4 (mg/dl)	8 ± 3.9	8.3 ± 6.5	0.1	0.914
ESR (mm/hour)	69.4 ± 17.4	86.6 ± 25.2	2.11	0.044 *
Activity index	16.6 ± 1.4	18.5 ± 2.3	2.5	0.017 *
ACR	998.3 ± 475.5	1586.4 ± 1026.6	1.9	0.067
Urine TP	2.17 ± 0.86	3.6 ± 2.3	2.11	0.044*
e-GFR	26.5 ± 13.4	29.1 ± 17.6	0.44	0.661
CIC (ng/ml)	60.2 ± 10.1	55.5 ± 17.8	0.83	0.408

Table (5): Correlation between CIC and other variables in SLE groups.

CIC	Group IA		Group IB	
	r	p-value	r	p-value
Age	-0.05	0.778	-0.05	0.81
SLE duration	0.16	0.468	0.01	0.977
ANA	-0.21	0.277	-0.16	0.414
Anti-ds-DNA	0.02	0.929	-0.16	0.397
C3	-0.18	0.343	0.18	0.35
C4	-0.25	0.184	-0.24	0.207
ESR	-0.05	0.776	-0.33	0.075
ACR	-0.15	0.44	0.18	0.33
Urine TP	-0.18	0.345	-0.25	0.186
e-GFR	0.05	0.783	-0.02	0.938
Activity index score	0.21	0.022*	0.51	0.004*

Table (6): Correlation study between activity index score and other studied data in all studied groups.

Activity index score	Group IA		Group IB	
	r	p-value	r	p-value
Age	-0.09	0.637	-0.18	0.349
SLE duration	-0.22	0.3	-0.02	0.933
ANA	-0.18	0.351	0.00	0.996
Anti ds-DNA	-0.14	0.455	-0.15	0.436
C3	-0.12	0.514	0.26	0.163
C4	-0.12	0.528	-0.21	0.273
ESR	-0.25	0.192	0.08	0.633
ACR	-0.07	0.722	0.1	0.552
Urine TP	-0.03	0.889	-0.06	0.745
eGFR	0.09	0.648	-0.18	0.329
CIC	0.21	0.022*	0.51	0.004*

Table (7): Diagnostic performance of CIC in discrimination of studied groups.

CIC	Cut off	AUC	Sensitivity	Specificity	PPV	NPV	p-value
IA vs IB	≤ 84	1.0	100%	100%	100%	100%	< 0.001
IB vs Control	≤ 189	1.0	100%	100%	100%	100%	< 0.001

DISCUSSION

Renal biopsy is the gold standard for diagnosis of lupus nephritis (LN) allowing for classification measurement of activity and chronicity of LN, which guide current treatment lines. Unfortunately, frequent renal biopsies are not practical due to invasive nature and associated morbidity of this procedure [14]. The aim of the study was to evaluate the role of serum circulating immune complexes level in lupus nephritis patients and it's relation to renal impairment parameters including renal biopsy findings. It was significantly reduced in patients with renal impairment than those with preserved renal function and control groups. But, it is also reduced in SLE with preserved renal function than the control groups. It is specifically able to differentiate between healthy and SLE subjects with preserved renal function and between those SLE with nephritis than those

with preserved renal function. However, its correlation with the results of renal biopsy was not statistically significant.

It is well known that, Immune complexes are involved in SLE pathogenesis, either by in situ formation in affected organs or in the form of circulating immune complexes. **Jha A *et al.*[15]** showed that a commercial C1Q-based CIC ELISA differentiated active from inactive disease and correlated well with disease activity scores. This is in line with the current work. Immune complexes are made from many antigens (not simply from DNA antigens). Thus, it is logical to estimate the overall circulating immune complexes. This is what we performed in the current work and this agrees with previous studies in literature [16]. In addition, an interesting study by **Elbagir *et al.*** [17] reported an increased levels of circulating immune complexes in Sudanese SLE than SLE patients from Sweden. They used their studies to explain the higher activity of SLE in black than white populations. The previous studies concentrate on the role of CIC in diseases activity. The positive correlation between diseases activity scores and CIC in both SLE with nephritis and those without in the current work are in line with these studies.

Bengtsson *et al.* [18] **and Nezlin** [19] evaluated CIC in SLE disease by serial measurement in inactive, active and after remission in addition to control group. They reported that the concentration of DNA in plasma of healthy donors is about 266 ng/ml compared to 2-4 µg/ml in SLE disease and the level of anti-DNA is significantly low compared to SLE disease. In healthy persons nuclear antigens are cleared by natural antibodies these natural IC probably play a role in suppressing B cells upon interaction with their surface receptors. The level of CIC in active stage is significantly low compared to inactive SLE group. But this fact is constant if associated with deficient C1q and inconstant if not associated with C1q deficiency. They reported that these findings are due to several abnormalities present in SLE disease. Among of which the increase in apoptosis without adequate clearance of apoptotic material especially if associated with C1q deficiency as C1q and classical complement pathway components are essential for development of tolerance against nuclear antigens. The second is the increased production of autoantibodies against autoantigens in SLE disease, and several abnormalities of CIC kinetics in SLE disease have been identified. Increased clearance of IC from blood due to increased hepatic uptake. Impaired retention of IC within liver with release of IC back to circulation and impaired IC clearance by spleen (i.e defect in macrophage function]. They reported that the decreased level of CIC in active stage of SLE reflect deposition of CIC in tissues causing and contribute to development of clinically detectable flare. So evaluating of CIC longitudinally is of prognostic value.

In our study the level of CIC was significantly low in SLE with nephritis compared to SLE without nephritis. This finding going with **Wener** [20] who reported that glomeruli of kidney is the preferable site of CIC deposition due the following: 1) the large volume of plasma are filtrated through the capillary wall with transient retention of macromolecules including immune complexes, 2) the glomerular capillary wall contains a fenestrated endothelium, allowing large molecules to traverse the glomerulus than other capillary beds, 3) fixed negative changes are present in glomerular basement membrane allowing cationic autoantigens as DNA and histone to firm binding, 4) the observation that glomerular epithelial cells have a receptors for C3b which may play a role in deposition of CIC in the kidney.

In the present study the level of CIC correlates with disease activity index in both SLE groups (with and without nephritis). However, there was no correlation between CIC and anti-ds DNA. This going with **Bengtsson *et al.*** [18] and **Nezlin** [19]**.** The fact that high anti-ds DNA level do not necessarily associated with high level of CIC must be taken into consideration and it depends on kinetics of CIC. CIC did not correlate with ESR, immuno-logical parameters and renal parameters. There was no permeability to perform correlation between CIC and clinical presentation and line of therapy as clinical presentation and line of therapy were not subclassified as comparable subgroups.

In the present study, no correlation was found between CIC and renal biopsy finding which could be due to low number of cases and the restricted stages to stage III and IV in our study. On the other hand renal biopsy correlated with disease activity index, degree of inflammation (ESR), anti- ds DNA levels which is of great value to predict renal involvement in SLE disease and total urinary proteins of 24 hours which is one of the main renal parameters that reflects degree of kidney involvement in LN. So there is no correlation between circulating IC in blood and degree of IC deposited in kidney evaluated by renal biopsy. These findings go partially with study done by **Chen *et al.*** [21]**.**

Regarding clinical presentations, our results are in line with **Wu *et al.*** [22] who stated that musculoskeletal symptoms were the most frequent clinical manifestation in SLE patients. On the other hand, **El Garf *et al.*** [23] disagreed with these findings and found that mucocutaneous manifestations are the commonest (90%), while **Ibrahem *et al.*** [24] reported that the most frequent clinical manifestations in Egyptians with SLE were constitutional symptoms (87%), followed by mucocutaneous (76%), musculo-skeletal (64%) and hematological manifestations (64%). These discrepancies may be due to different sample size and the nature of SLE disease that can affect many body organs and give a wide spectrum of clinical manifestations. In addition, ethnic factors may be responsible as reported by **Elbagir *et al.*** [17]. Furthermore, the specific nature of the disease with remission-recovery cycles may be responsible, and the time of recruitment in relation to disease stage or activity is a crucial factor.

The significant increase of ESR in SLE groups than controls and in nephritis than non-nephritis SLE agree with the studies of **Stojan *et al.*** [25] **and Wang *et al.*** [26] who reported that the higher ESR value is a key sign of inflammation in SLE and can be used to monitor SLE disease activity. In addition, the low values of complements 3 and 4 in SLE than the control groups, with no difference between nephritis and non-nephritis subgroups is in line with **Truszewska *et al.*** [27] and **Giaglis *et al.*** [28] who reported decreased C3 and C4 in SLE, which could be due to the increased apoptosis and overproduction of nucleic acid fragments during SLE activity, forming antigen-antibody complexes leading to C3

and C4 consumption with SLE activity. In addition the complement system may become a target of adaptive immune response as autoantibodies against several complement components are often found in SLE patients. On the other hand, these findings were contradictory to **Walport** [29] and **Tug *et al.*** [30] who did not find any change in the level of C3 and C4 in patients in SLE than the control groups. They explained these findings by the compensatory effect of the liver. In addition, these are acute phase reactant that could also obscure a complement consumption. Authors also declared that complement split products could be of significant value in evaluating of C3 and C4 and inherited complement deficiency due to genetic polymorphism already found in a lot of lupus patients not due to activity.

In line with the current work, **Abdelazeem *et al.*** [31] and **Abd-El hamed *et al.*** [32] stated that the percentage of positive ANA and anti-ds-DNA cases in SLE with nephritis was significantly higher compared to SLE without nephritis. This finding supported by the fact that anti-ds DNA is a diagnostic indicator for disease activity and especially for renal involvement.

In the present study, renal parameters (hematuria, proteinuria, urinary total protein of 24 hours, Albumin/ creatinine ratio and eGFR were significantly differ in the SLE with nephritis than the SLE without nephritis. These results agree with **Kamel *et al.*** [33] **and Abd-El hamed *et al.*** [32] who stated that the increased total protein level of 24 hours, pus cells and RBCs in SLE with nephritis that the SLE without nephritis can be attributed to the glomerular injury which was evidenced by renal biopsy in those patients and 24 hours urinary protein is important in diagnosis of lupus nephritis. **Cojocaru *et al.*** [34] stated that although only approximately 50% of patients with SLE develop clinically evident renal disease, urine analysis of asymptomatic patients often show hematuria and proteinuria and 24 hours urinary proteins is a conventional biomarker for lupus nephritis. Studies done by **Wu *et al.*** [22] and **Zeitoun *et al.*** [35] stated that eGFR does not differ between active and inactive SLE patients. In our study all cases of SLE were active but activity index of SLE with nephritis was significantly higher compared to SLE without nephritis. They were in group III and IV in histopathology of renal biopsy. These discrepancy could be attributed to changes in phenotypic presentation, renal biopsy results and lines of therapy.

In conclusion, the CIC could be used as diagnostic and follow up biomarkers in the patients with systemic lupus with or without nephritis. It is specifically correlated with disease activity index. The availability and simplicity of ELISA determination of CIC is a better and reasonable diagnostic method than the renal biopsy. At least it could be used as a rapid screening tool and the renal biopsy confined to confirmation of diagnosis. However, the small number of subjects included in the current work represented a limiting step against globalization of the results. Future large-scale longitudinal studies are recommended.

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