Diagnostic and Prognostic Value of Biological Markers in Lupus Nephritis

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Abstract: Lupus nephritis is known as one of the most serious manifestations of systemic lupus erythematosus (SLE). There is increasing interest in identifying biomarkers to predict flare of lupus nephritis (LN). It is hoped that non-invasive markers of renal disease can be used to predict flares and remission of disease. Recently, two promising biological markers, anti C1q autoantibody, and serum prolactin were addressed as diagnostic and prognostic markers for lupus nephritis. Aim of this study wasto determine the diagnostic and prognostic values of anti-C1q antibodies, and prolactin as simple and easy biological markers for lupus nephritis-two years follow up.*Patients and Methods;* 60 active SLE patients divided into two groups, group 1;30 patients with active nephritis, and group 2; 30active SLE patientswithout nephritis. All the patients subjected to complete history andphysical examination. The demographic, clinical and laboratory variables and the disease activity index (SLEDAI) were analyzed. Patients with lupus nephritis WHO class III, IV or V received glucocorticoid and cyclophosphamide for induction of remission. For all the patients, anti C1q, prolactin, anti ds DNA, C3, C4 were measured. Kidney assessment included, urinary sediments, and protein/creatinin ratio, biopsy only for group 1. The length of the follow up was 2 years.*Results;* the age of the patients, and the duration of the disease were significant higher in-group 1than in group2 ($P< 0.001$ for both). No significant difference in two groups considering the sex. Prevalence and level of ant C1q, and prolactin were higher in-group 1 than in-group 2 ($P<0.001$). Both of them were correlated with the renal score activity at the time of sampling, and at the end of the study.Anti ds DNA did not correlated with the renal activity score. Anti C1q had high sensitivity, and accuracy (86.7%, 0.9) than prolactin (50%, 0.7), versus high specificity of prolactin (100 %).Conclusion; anti-C1q antibodies, and prolactin might be valuable serological biomarkers to reflect kidney involvement and to monitor disease activity of LN.
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1.Introduction:

Systemic Lupus (SLE) defined by its clinical picture, together with antibodies directed against one or more nuclear components, particularly anti-double-stranded DNA (dsDNA).Lupus nephritis(LN) is considered to be the major complication or outcome in SLE. (1). Up to 25% of these patients still develop end-stage renal disease (ESRD) 10 years after onset of renal compromise [1]. In addition, if LN develops early in the course of SLE,it becomes a major predictor of poor prognosis [2].

The prognostic significance of lupus nephritis indicates a need for identifying early biomarkers that predict nephritis development. Current laboratory markers for lupus nephritis such as proteinuria, urine protein / creatinine ratio, creatinine clearance, anti-dsDNA, and complement levels are unsatisfactory(3). Future directions in SLE biomarker research should focus on a combination of novel markers with conventional clinical parameters to enhance the sensitivity and specificity for the prediction of renal flares and prognosis in lupus nephritis.Although a large number of novel biomarkers have been studied in lupus nephritis, none of them have been rigorously validated in large-scale longitudinal cohorts of patients with different ethnic background(1).

In 2004, **Trouw*et al***. demonstrated in a mouse model that antibodies against C1q of the complement system (anti-C1q) play a pathogenic role in LN in the presence of immune-complexes (ICs)[4]. C1q is the first component of the classical pathway of complement activation and its main function is to clear immune complexes from tissues and self-antigens generated during apoptosis(5).Anti-C1q could participate in glomerular injury by reducing the clearance of circulating immune complexes(ICs). Monitoring anti-C1q might be valuable for the clinical management of SLE patients as a noninvasive biological marker. Prognosisof lupus nephritis can usually be improved dramatically by treatment.

Prolactin (PRL) is a versatile hormone that is produced by the anterior pituitary gland and various extrapituitary sites including immune cells. Furthermore, PRL has widespread influences on proliferation and differentiation of a variety of cells

in the immune system and is, in effect, a cytokine. PRL-receptors (PRL-R) are distributed throughout the immune system and are included as members of the cytokine receptor superfamily(6). Polymorphisms of the human PRL gene have implications for production of lymphocyte PRL in SLE. Mild and moderate hyperprolactinemia (HPRL) has been demonstrated in 20–30% of SLE patients and is associated with active disease. HPRL may have a role in lupusnephritis and central nervous system involvement of SLE patients (6). However, the clinical significance of PRL has not been investigated in lupus glomerulonephritis (GN).Hyperprolactinemia was prevalent in SLE patients and high levels of PRL in the serum could be related to severe renal disease.Prolactin is related to the activityof the disease.

The aims of the present study were to determine the diagnostic and prognostic values of anti-C1q antibodies, and prolactin as simple and easy biological markers for lupus nephritis-two years follow up.

2.Patients and Methods;

This is a cross-sectionalstudy between September 2010 and September 2012. 60 (55 female, and 5 male patients), from Ain Shams university hospital outpatient clinics, were fulfilling the classification criteria for systemic lupus erythematosus (7), were recruitedin this study.Written informed consent was obtained from all patients.

Two groups were included;

Group 1 *(patients with active lupus nephritis) 30 patients*;

Thirty patients with biopsy-proven renal diseasewere chosen out of 40 SLE patients (10 patients did not complete the study and were excluded. Clinical nephritis was suspected if urinalysis showed protein/creatinine ratio>0.5 g/mmol(7) and/or hematuria or cellular casts with or without increased serum creatinine. These 30 patients werefollowed every 6monthsfor an average of two years.

Group 2 *(patients with active SLE without nephritis)*

Thirty SLE patients had active disease (Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) ≥ 4)at the time of serum sampling, and there was no evidence of lupus nephritis (no proteinuria, hematuria, or cellular casts by routine periodic urinalysis) at any time during follow-up.

Exclusion criteria

Patients with changes in liver function tests (ALT, AST or LDH), end stage renal disease(ESRD defined by a serum creatinine of ≥6 mg/dl or the initiation of renal replacement therapy), changes in thyroid function, or users of drugs that alter the circulating levels of prolactin were excluded Patients below 16 years and pregnant women were excluded from the study.

For all the patients:

Complete history, and physical examination; including time of SLE diagnosis, and its duration from starting renal involvement

S*LE Disease Activity Index Assessment (SLEDAI) was used to assess the disease activity, total points ≥4 considered active (8).*

Evaluation of renal involvement

For patients with renal involvement, renal SLE Disease Activity Index (renal SLEDAI) was used to assess kidney disease activity. The score consists of the four kidney-related parameters including hematuria (>5 red blood cells/high-power field), pyuria (>5 white blood cells/high-power field), proteinuria (>0.5 g/24 hours or urine protein/creatinine ratio >0.5) and urinary casts (heme, granular, or red blood cell). Scores for the renal SLEDAI can range from 0 (inactive renal disease) to a maximum of 16. A SLEDAI score of 4 was taken as an indicator of active lupus nephritis (3).

Renal biopsy specimens from LN patients were classified according to the World Health Organization (WHO) criteria: class I; minimal changes, class II; mesangial alterations, class III; focal proliferative, class IV; diffuse proliferative, and class V; membranous glomerulonephritis, class VI; nephrosclosis(9).

Anti-C1q determination;

IgG anti-C1q antibodies were determined using an ELISA with purified human C1q. Anti-C1q were tested in serum using a commercially available enzyme immunoassay (EIA) kit (Diagenics, Milton Keynes, UK), under 1 M NaCl conditions as described previously.[27] Briefly, standards, controls, and patient sera were incubated with human C1q adsorbed into the microtitre plate. After washing, an IgG horseradish peroxidase labelled conjugate was added in the appropriate dilution. Colour was developed by adding an enzyme substrate (tetramethylbenzidine in citrate buffer with hydrogen peroxidase). The reaction was stopped by adding 0.25 M sulphuric acid and optical densities at 450 nm were measured by a TitertekMultiskan MC apparatus (Flow Laboratories, Herts, UK). Optical densities were then converted to units (U) by being plotted against the autoantibody titre of the standards given by the manufacturer.

Prolactin measurement;

Prolactin serum levels were measured in all patients by electrochemiluminescence (Elecsys, Roche Diagnostics, Mannheim, Germany), with analytical sensitivity of 5.0 pg/mL and 0.047 ng/mL,

respectively. 29.9 ng/mL for prolactin. Blood collection for such measurements was performed simultaneously with those for the tests used for determining SLEDAI.

Other laboratory measurements;

Anti -dsDNA antibodies were determined using an ELISA with enzyme-linked immunoassay technology.

Complete blood picture, kidney function tests, liver function tests, erythrocyte sedimentation rate (ESR) and urine analysis were done for all the studied patients. Complement levels (C3, C4) by Nephelometry.

Follow up regimen;

Patients had been followed up as outpatients in Ain Shams outpatients' clinics for two years since nephritis was diagnosed and therapy started, based on clinic-pathological correlation or on renal biopsy data. Our therapeutic approach included high doses of corticosteroids (prednisone 1-2 mg/kg/day orally) in every patient or 1g/day doses of methylprednisolone intravenously, during 3-5 consecutive days, in more severe cases. Intravenous (IV) monthly cyclophosphamide was used in 0.5 to 1 g/m^2 doses of body surface area as a first-choice of immunosuppressive agent for induction of class III, IV or V nephritis remission. Azathioprine was used for nephritis maintenance treatment after 6-9 months of cyclophosphamide IV (7).

Renal function tests, albuminuria, C3, C4, Serum C1q, and prolactin were determined at baseline and at 6-month intervals for two years. The remission of LN includes complete remission and partial remission. Complete remission was defined as urinary protein excretion <0.3 g/day, normal urinary sediment (RBC < 5/HP, WBC < 5/HP), normal serum albumin and normal renal function. Partial remission was defined as having any one of the following items: decrease of serum creatinine to <1.4mg/dl for patients with a baseline serum creatinine level ≥1.4 μmol/L but <2.9 mg/dl; decrease of serum creatinine by >50% for patients with a baseline serum creatininelevel >2.9 mg/dl; decrease of urinary protein excretion by >50% and <3.0 g/day, with a serum albumin level ≥30 g/L and stable renal function(10).

Statistical analysis

All statistical analysis was carried out using the SPSS 11.0 program (Microsoft software). Demographic,and clinical were reported. Results are given as the median, range, and (inter- quartile range (IQR). Nonparametric tests (Mann-Whitney *U* test), and chi-square test were used to compare the groups with and without lupus nephritis. $P < 0.05$ was considered significant. Receiver Operating Characteristic (ROC) curve was used to asses sensitivity and specificity.

3.Results:

The study sample comprised 30 patients diagnosed to have SLE with active lupus nephritis (group 1), and 30 patients with active SLE without nephritis (group 2). Out of the 30 patientsof group 1, 10 patients (30%) presented with renal impairment and 6 patients (20%) with nephrotic syndrome. The main extra-renal SLE manifestations were joints, mucous-cutaneous, hematologic and serositis, found in 96%, 63%, 67% and 5% of the patients. All our patients had highanti ds DNA. The median SLEDAI value at diagnosis was 12 expressing disease in considerable activity, that is, SLEDAI $\geq$ 4.

Renal biopsy shows 9 patients with grade III, 11 patients grade IV, 10 patients with grade V.non- of the patients had glomerulosclerosis (class VI).

Demographic data of the patients showed in table 1.Table 1 showed that group 1 had elder age, with long duration, and lower disease activity score than group 2 (P<0.001, P<0.001, P<0.023 respectively). No significant differences were found between the groups for the sex ratio.

Table1; Demographic data of the patients;

	Group 1	Group 2	Z	*P*-value
Age (years) Median Range	 37 26-49	 25 19-32	23.184	<0.001*
Sex (F/M)	27/3	28/2		1.00
Duration of the disease (years) at sampling time Median Range	 18 8-22	 3 0. 50-6	15.11	<0.001*
SLEDAI at first serum sample (points) Median Range	 12 4-28	 16 4-30	4.155	0.023*

Comparing the laboratory data of the patients, there were significant higher level of anti C1q, PRL, and PCR, and significant lower level of Hb, in group 1than group 2 (P< 0.001, 0.007, 0.001- P< 0.001, 0.001). No significant difference was found between the two groups as regarding anti ds-DNA.

Table2; Comparisons between patients' laboratory values of different parameters at the sampling time;

						Mann-Whitney Test	
		Range (unit/ml)	Median	IQR	Mean rank	Z	*P*-value
Anti C1q	Group 1	11.100 - 190.000	34.850	93.950	43.117	-5.601	<0.001*
	Group 2	3.000 - 23.000	10.500	6.000	17.883		
PRL	Group 1	5.000 - 250.000	17.500	27.500	36.583	-2.705	0.007*
	Group 2	2.000 - 20.000	11.500	6.250	24.417		
PCR	Group 1	0.200 - 6.400	2.1	3.41	45.3	-6.819	<0.001*
	Group 2	0.100 - 0.300	0.1	0.300	15.7		
Hb	Group 1	4.000 - 15.200	12.000	3.050	40.417	-4.427	<0.001*
	Group 2	8.000 - 11.000	9.500	1.000	20.583		
Anti ds-DNA	Group 1	5.000 - 150.000	39.000	41.000	28.567	-0.858	0.391
	Group 2	12.000 - 100.000	40.000	30.000	32.433		

PRL; prolactin, PCR; protein/ creatinine ratio, Hb; hemoglobin .

As regarding the patients parameters using chi-square, more patients in group 1 had Pus cell and RBC in the urine(P<0.001), low C3 and low C4 (P<0.001) than in group 2, while more patients with abnormal Platelets in group 2 than in group 1,but it did not reach a significant value (P<0.073), and no significant results between TLC between both groups.

Table 3;Comparisons between patients'laboratory parameters at the sampling time;

	Group 1		Group 2		Total		Chi-square	
	N	%	N	%	N	%	X^2	*P*-value
PUS*	10	33.33	0	0.00	10	16.67	15.876	<0.001*
RBC*	8	26.67	0	0.00	8	13.33	12.326	<0.001*
PLT	1	3.33	5	16.67	6	10.00	3.208	0.073
TLC	4	13.33	9	30.00	13	21.67	2.506	0.113
C3	7	23.33	0	0.00	7	11.67	10.631	<0.001*
C4	8	26.67	0	0.00	8	13.33	12.326	<0.001*

RBC; red blood cell, PLT; platelet, TLC; total leucocytic count, * Pus and RBC in urine

The incidence of Anti C1q in all our active SLE patients with or without nephritis was elevated in 75% of our patients The incidence of anti C1q in patients with LN (group 1) was 100% , while the incidence in active lupus without nephritis (group2) was 50%. Anti C1q was significantly high in the first group (P< 0.001).

Table 4; Prevalence of anti C1q in all the patients

Anti C1q	Groups						Chi-Square	
	Group 1		Group 2		Total			
	N	%	N	%	N	%	X^2	*P*-value
Normal			15	50	15	25	25.891	<0.001*
Abnormal	30	100	15	50	45	75		
Total	30	100.00	30	100.00	60	100.00		

The incidence of high PRL level in all our active SLE patients was 75%, the incidence in group1 was 50% and none of our patients in group 2 had high PRL level the difference was statistically significant (*P*<0.001).

Table 5; Prevalence of Prolactin in all the patients;

PRL	Groups						Chi-Square	
	Group 1		Group 2		Total			
	N	%	N	%	N	%	X^2	*P*-value
Normal	15	50.00	30	100.00	45	75.00	20.000	<0.001*
High >20	15	50.00	0	0.00	15	25.00		
Total	30	100.00	30	100.00	60	100.00		

Anti-C1q was positively correlated with C3, and C4 (*P*<0.001, and 0.009) (Table 6). PRL was correlated positively with C3, C4, and RBC in urine (*P*< 0.024, 0.001, 0.007) (Table 7).

Table 6; Correlation between serum level of anti Cq1 and laboratory parameters at the sampling time;

		Anti C1Q				Mann-Whitney Test	
		Range	Median	IQR	Mean rank	Z	*P*-value
PUS	**Normal**	12.000 - 190.000	31.250	81.300	14.850	-0.572	0.567
	Abnormal	11.100 - 122.000	77.500	99.650	16.800		
RBC	**Normal**	11.100 - 190.000	31.250	69.400	13.932	-1.619	0.106
	Abnormal	21.200 - 122.000	112.400	95.400	19.813		
TLC	**Normal**	11.100 - 190.000	34.850	93.900	15.808	-0.488	0.625
	Abnormal	12.000 - 117.000	64.500	101.500	13.500		
C3	**Normal**	11.100 - 119.000	28.000	56.000	12.348	-3.557	<0.001*
	low	44.000 - 190.000	122.000	41.200	25.857		
C4	**Normal**	11.100 - 190.000	25.450	58.150	12.955	-2.627	0.009*
	low	30.000 - 122.000	115.400	60.500	22.500		

RBC; red blood cell, ,TLC; total leucocytic count, * Pus and RBC in urine

Table 7; Correlation betweenprolactinserum level, and laboratory parameters at the sampling time.

		PRL				Mann-Whitney Test	
		Range	Median	IQR	Mean rank	Z	*P*-value
PUS	**Normal**	9.000 - 200.000	12.500	24.000	14.125	-1.213	0.225
	Abnormal	5.000 - 250.000	34.000	28.875	18.250		
RBC	**Normal**	5.000 - 47.000	12.500	19.000	12.886	-2.703	0.007*
	Abnormal	11.000 - 250.000	40.000	142.250	22.688		
TLC	**Normal**	5.000 - 250.000	18.000	27.750	15.462	-0.061	0.951
	Abnormal	11.000 - 40.000	17.500	24.000	15.750		
C3	**Normal**	5.000 - 250.000	13.000	16.000	13.500	-2.261	0.024*
	Abnormal	12.000 - 200.000	38.000	38.000	22.071		
C4	**Normal**	5.000 - 40.000	12.000	13.500	11.750	-3.879	<0.001*
	Abnormal	30.000 - 250.000	45.500	128.500	25.813		

RBC; red blood cell, TLC; total leucocytic count, * Pus and RBC in urine

At the end of the study (after two years); serum of anti C1q level, and serum PRL level, were positively correlated with PCR, the activity score of the patients at the end of the study (score 2) (*p*<0.004, *P*<0.001 respectively). Also there was positive correlation with the activity score at the serum sampling (score 1 *P*<0.001).

Table 8; Correlation betweenserum antiC1q level**,** and prolactin level at the end of the study, and laboratory data and the patients' scores (at the sampling time, and at the end of the study) in lupus nephritis patients.

	Anti C1Q		PRL	
	r	***P*-value**	**r**	***P*-value**
PRL	0.390	0.033		
Hb	-0.180	0.340	-0.193	0.306
ANTIDNA	0.150	0.429	0.086	0.650
PCR	0.574	0.001*	0.510	0.004*
SCORE1	0.565	0.001	0.589	0.001*
SCORE2	0.704	<0.001*	0.562	0.001*

PRL; prolactin, PCR; protein/ creatinine ratio, Hb; hemoglobin, score 1; activity score at the sampling time.Score 2; activity score at the end of the study.

In this study 36.67% of our patients improved, while 63.33% either became worse or no response to the treatment.

Table 9; Follow up end point in lupus nephritis patients after two years.

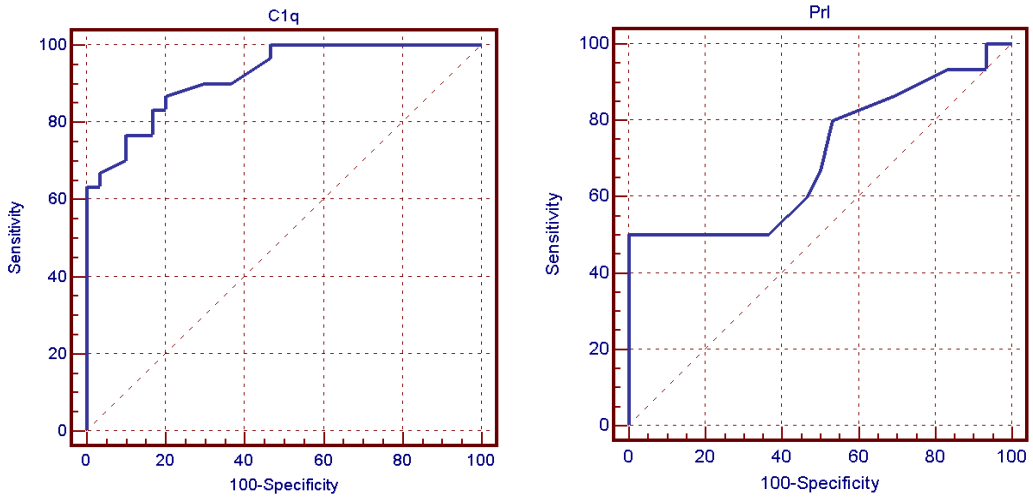
Monitoring of Lupus Nephritis (2 years follow up)		
	N	**%**
No change	9	30.00
Worse	10	33.33
Remission	11	36.67
Total	30	100.00

Receiver operating characteristic curve (ROC) curve for detecting sensitivity and specificity of C1q, and PRL showed, Sensitivity to detect the disease was 86.7% for C1q and 50% for PRL. As regard specificity, it was 80% for C1q, and 100% for PRL.

Table 10; Sensitivity and Specificity of anti C1q and prolactin.

ROC curve between positive and negative outcome					
Cutoff	**Sens.**	**Spec.**	**PPV**	**NPV**	**Accuracy**
C1q> 10 *	86.7	80.0	81.2	85.7	0.921
PRL> 20 *	50.0	100.0	100.0	66.7	0.703

Roc; Receiver operating characteristic curve



Receiver operating characteristic curve (**ROC**) showed sensitivity and specificity of anti C1q, and prolactin for detection of Lupus nephritis.

4.Discussion

Clinical nephritis develops in about 50% of patients with SLE, and is an important risk factor for death (7). Therefore, markers for the diagnosis and follow-up of nephritis are very important. However, the lack of specificity of (anti ds DNA) (biological) markers for renal exacerbations has led to the search for other autoantibodies that might contribute to nephritis and help diagnose a renal flare (11)Recently, it has been clarified the role of anti C1q as a marker for flare up of lupus nephritis (12), and the role of prolactin in active lupus nephritis (13). In this research, we try to evaluate both of them as simple serum biological markers for flare up and follow up of the patients with lupus nephritis.

Anti C1q considered as nephritogenic autoantibody, play a central role in the pathogenesis of lupus nephritis. In our study, anti C1q was elevated in all our patients with active nephritis (100%).In comparisons with anti C1q in active SEL patients without nephritis was 50%. Our results were the same postulated by **Meyer *et al.,***2009 that no lupus nephritis can occur in the absence of anti-C1q antibodies, and recorded 100% of active lupus nephritis and 45% of lupus without nephritis (14). Similar data reportedby **Horak and his colleagues** in a series of 33 patients [15], and **Fang** and his colleagues in a series of 150 patients [16], all with lupus nephritis.Up to now, the prevalence of anti-C1q in active lupus nephritis has not been uniformly established (17)Although many studies were agreed with our result, demonstrated a high prevalence of anti-C1q in active lupus nephritis, the data presented in other reports suggested lower negative predictive values for anti-C1q (18).Differences might be attributed totheses points; first, differences in the cut off point of anti C1q,the cut-off used in our study was lower than that low prevalence studies. In our study the cut off was 10 unit/ml, while in low prevalence studiesthe cut off pointranged from 40-55 unit/ml. Moreover, most of our patients were grade VI, and V renal histology.

In many previously reported cases, the levels of anti-C1q antibodies increased prior to the exacerbation of lupus nephritis (14). In our study, none of our active SLE patients without nephritis developed lupus nephritis along the time of follow up. This may be because none of these patients had high anti C1q initially. Furthermore, we did not take renal biopsy from none-nephritis patients, although, some of SLE patients who did not fulfill the criteria for active lupus nephritis, may revealed class II, III, IV, or V lupus nephritis on renal biopsy (19). So, some of our patients who did not have renal overt nephritis may have developed over a period of time.Taking in consideration, duration of follow up two years in our study, versus 10 years in other (10). Like others: (19, 20)we found a good correlation between anti-C1q and other recognized markers of renal disease activity in SLE namely, urine sediment, renal activity score, PCR, low C3, and low C4. Curiously, although high anti-dsDNA values,it did not associated with activity of nephritis, there was no difference in prevalence of anti-dsDNA in patients with and without nephritis (all our patients has high anti ds DNA), supporting previous evidence of the superior specificity of anti-C1q over anti-dsDNA for renal flares (20). It is clear that these findings could have been influenced by the fact that some of the serum samples were taken in the first four months after beginning immunosuppressive treatment, as anti-dsDNA values fall very rapidly after treatment. (20).

Level of Anti-C1q titers did not correlate with anti-dsDNAtiters (P = 0.4). But, it was positively correlated with the SLE renal activity score at the time of sampling and at the end of the study (P<0.001). Thisresult was in accordance with **Mayer**2009(14). But in contrast with **Fang *et al.,***who recorded positive correlation between anti ds DNA and anti C1q.(16). This can be explained by; a large number in their study, and they used IgG subclass of anti C1q. Also, taking in consideration that the study was applied on chenese patients and our study on Egyptian patients. This antibody should not be taken as a general marker of disease activity, in contrast to anti-ds-DNA antibodies. This was agreed with**Olivier** and hiscollogues (19).

This significant inverse correlation between anti-C1q and C3 and C4 in our study confirmed the role of anti C1q in the pathogenesis of LN. As in other studies this decrease in the complement classical pathway was associated with active renal disease.In addition, anti-C1q had been recovered at necropsy from the glomeruli of patients with proliferative lupus nephritis(21). Two possible mechanisms may explainhow this antibody exerts their nephritogenic effect. Anti-C1q may either contribute to the formation of circulating immune complexes that are deposited in the kidneys or contribute to local formation of immune complexes on the glomerular basement membrane. By interfering with activation of the complement system through the classical pathway, anti-C1q may hamper immune complex solubilisation, further contributing to immune complex deposition in the kidney.Animal studies indicated that IgG was a focus for C1q, and anti-C1q antibodies could only be pathogenic to the kidney in the premise of combination with glomerular C1q-containing immune complexes (22) This may explain why glomerulonephritis does not

develop in the presence of anti-C1q antibodies alone (in patients with rheumatoid arthritis and hypocomplementaemic urticarial vasculitis syndrome).

A successful treatment of active lupus nephritis typically decreases the titer of anti-C1q autoantibodies. The anti-C1q test remained high, even after 2 years of immunosuppressive therapy in 19 out of 30 (63.3%) nephritis patients in our series, this was agreed with **Meyer *et al***.,2009It was 62%. Fang and col- leagues only 47% of their patients were positive at the end of the study (14) Where, 30% in the study by **Moroni** and colleagues [22], it was lower than our results but in this study they considered only the complete remission patients, in our study eleven of fifteen patients achieved only partial responses. Furthermore, most of our patients were grade V, and VI glomerulonephritis comparing with grades III, IV, and V glomerulonephritis in other studies.

In our study,the sensitivity of anti C1q for diagnosis of lupus nephritis was 86.7%, specificity was 80%, with positive predictive value 81.2, and negative predictive value 85.7. Accuracy was 0.921. These results were in accordance with **Moroni*et al.*** [22], they had almost the same result that, in proliferative lupus nephritis, elevation of anti-C1q level predicted renal flares with a sensitivity and specificity of 81% and 71%, respectively.

Anti-C1q titers correlated with active renal disease in both basic data ($P< 0.0001$) and at the end of the study ($P< 0.0001$). In patients with biopsy-proven lupus nephritis, anti-C1q titers appear to be strongly related to renal disease activity. Their measurement may be useful for confirming the diagnosis of renal flares of lupus nephritis. This results support the results of **Moroni** and his colleagues (22).

PRL is structurally similar to members of cytochine/hemopoietic family and plays an important role in modulating the immune response. PRL is secreted from the pituitary gland as well as other organs and cells particularly lymphocytes. PRL has an immune stimulatory effect and promotes autoimmunity. PRL interferes specifically with B cell tolerance induction, enhances proliferative response to antigens and mitogens and increases the production of immune globulins, cytokines and autoantibodies. New reports suggest that HPRL is implicated in lupus nephritis. The association between HPRL and high levels of IL-6 in lupus nephritis suggest a bidirectional communication between immune and neuroendocrine system in SLE (13).

The incidence of PRL in all our patients was 25%.Same result by**Dostal*et al.*,** summed up this information hyperprolactin,in 20–30% ofpatients with SLE investigated; nevertheless, opinion continues to vary about its connection with greatly increased clinicalactivity of the disease(23). 50% of our patients with lupus nephritis had high level of PRL than patients without nephritis($P< 0.007$).Our results support the supposition that high serum PRL act as stimuli for T and B-lymphocytes to infiltrate renal tissue.These findings support those previously obtained by researchers (24, 25)

In our study, PRL level was elevated only in patients with nephritis (group1), (mean rank was 36.583,versus 24.417 $P<0.007$). Bromocriptine, a dopaminergic agonist, lowers serum PRL levels by inhibiting PRL secretion from the anterior pituitary. This mechanism may explain the beneficial effects of bromocriptine on disease activity in SLE(26)Additionally, in a recent study, **Peeva*et al.*** demonstrated in mice that treatment with PRL, which causes mild to moderate HPRL, similar to that present in SLE patients, breaks tolerance and induces a lupus-like disease in non-spontaneously autoimmune mice with a susceptible genetic background, suggesting that only a subset of SLE patients are likely to have PRL-responsive disease(27). Further studies on the role of PRL in SLE are needed, even if its importance is limited to a minority subgroup of SLE with PRL-modulated disease that can benefit from drugs that decrease PRL secretion or block its biological action at PRL receptor level.

Hyperprolactinemia (HPRL) is associated with several autoantibodies(28). In human and experimental models of SLE, there is evidence of a relationship between HPRL and hypocomplementemia in our study high level of PRL was correlated negatively with C3, and C4, ($P< 0.001$, and $P<0.007$ respectively).This confirm the hypothesis that PRL may therefore participate in the pathogenesis of lupus nephritis, and the presence of PRL may reflect an abnormal communication between the immune system and the neuroendocrine system in active SLE. Lymphocytes from patients with active SLE produce increased amounts of PRL, and this extrapituitary PRL may participate in aberrant immune processes in SLE(24).

In this study, follow up of SLE patients with active renal nephritis. Level of PRL was successively reduced in 36.6% in the patients with remission either complete or partial after successful treatment. Level of PRL was correlated with the renal activity score at the time of sampling and the end of the study.

Prolactin considered an important link between the immune and endocrine systems. Prolactin stimulated disease in autoimmune NZB/NZW mice. Treatment of the mice with the prolactin-lowering

dopamine agonist, bromocriptine, suppressed anti-DNA and prolonged life spans. These findings have been applied to humans with SLE (29).In support of this theory that bromocriptine, which selectively inhibits secretion of PRL from the anterior pituitary, decreased glomerular deposition in kidneys of lupus nephritis mice (30). So, Selective inhibition of PRL secretion or action may be a promising adjuvant therapy in selective patients of active SLE with or without LN.

PRL level was correlated positively with renal involvement in SLE with low sensitivity and high specificity, and fair accuracy than anti C1q (50%, 100%, 0.7 respectively). It did not increase the sensitivity of anti C1q in diagnosis of lupus nephritis.

Summary

In SLE, anti-C1q washigh in 75% of active SLE patients (with or without nephritis), 66.7% of themhad active lupus nephritis (high sensitivity and specificity). While prolactin was high in 25% of active SLE patients (with or without nephritis), all of them had active lupus nephritis (High specificity and low sensitivity). A successful treatment of active lupus nephritis typically decreases the titer of anti-C1q and prolactin.Patients withhigh anti-C1q had high change of no remission disease with treatment. PRL, as immunomodulation hormone may have a role in the pathogenesis of lupus nephritis.

Conclusions

Anti-C1q antibodies, and prolactin might be a valuable serological biomarker to reflect kidney involvement and to monitor disease activity of lupus nephritis.

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