



Study for the Relationship between Lupus Nephritis and Anti-C1q Antibodies

Ashraf Abdel-Maged Donia^{1*}, Ahmed Ibrahim Amin² and Sameh Soliman Dawoud Mohamed³

¹Urology and Nephrology department, National Institute of Urology and Nephrology, Cairo, Egypt

²Biochemistry department, Faculty of Science, Cairo University

³Desert Research Center, Cairo, Egypt

ABSTRACT

Lupus nephritis (LN) is an inflammation of the kidney caused by systemic lupus erythematosus (SLE), a disease by the immune system. Anti-C1q antibodies have been found in many different systemic autoimmune diseases, they are strongly linked to immune complex disorder most prominently SLE and severe rheumatoid arthritis and have been suggested to be closely associated with lupus nephritis (LN). Generally anti-dsDNA antibodies have been acknowledged as an important tool in the diagnosis of SLE, however their predictive value as to the activity of the disease remains controversial, on the contrary anti-C1q antibodies appear to have a clear-cut relationship with renal complications of SLE not only have they been shown to play a pathogenic role in the development of lupus nephritis but also their serum levels correlate with the presence of active proliferation lupus nephritis. The aim of the study was to further investigate association between serum titer of anti-C1q antibody and disease manifestation of SLE, significant association were found between increased serum titer of anti-C1q antibody and nephritis with subsequent loss of kidney function. The study was carried out in three different groups: healthy group, rheumatoid arthritis group and lupus nephritis group. All groups were subjected to determination of anti-C1q antibody, blood urea nitrogen (BUN) and serum creatinine. The results showed no significant difference in BUN levels between normal and rheumatoid arthritis group in contrast there was a highly significant difference in BUN between normal and lupus group also between rheumatoid arthritis and lupus nephritis group ($p < 0.001$). No significant difference in serum creatinine levels between normal and rheumatoid arthritis group in contrast there was a highly significant difference in serum creatinine between normal and lupus group also between rheumatoid arthritis and lupus nephritis group ($p < 0.001$). No significant difference in serum anti-C1q antibodies levels between normal and rheumatoid arthritis group in contrast there was a highly significant difference in serum anti-C1q antibodies between normal and lupus group also between rheumatoid arthritis and lupus nephritis group ($p < 0.001$). In control group and rheumatoid arthritis groups, only BUN showed a highly significant positive correlation with serum creatinine concentration ($r=0.906$, $r=0.404$) and ($P < 0.001$, $P < 0.05$) respectively while in lupus nephritis group, BUN showed a highly positive correlation with serum creatinine concentration ($r=0.773$, $P < 0.001$) also serum creatinine concentration showed a positive concentration with serum anti-C1q antibody ($r=0.513$, $P < 0.05$). The present study suggests that anti-C1q antibody might be a new parameter for the development of lupus nephritis since the increased of anti-ds DNA antibody and hypocomplementemia (C3 and C4) are serological markers of SLE activity but they are not enough to identify which organ will be affected, while anti-C1q antibody either alone or in combination with other serological markers could give information of the diagnosis of a renal flare with a sensitivity and specificity 100%.

Keywords: Lupus nephritis; Systemic lupus erythematosus; Anti-ds DNA antibody; Anti-C1q antibody; Rheumatoid Arthritis

INTRODUCTION

One of the most complex, beautifully “engineered” organs of the human body is the Kidneys that perform several essential tasks including the excretion of waste products, the maintenance of homeostatic balance in the body and the release of important hormones. To achieve this, human kidneys have a highly developed, superbly refined anatomy and physiology. Some patients with kidney involvement may show rapid progression to renal failure, while others may enter complete and stable remission after adequate therapy. More difficult to manage are the large number of patients who have similar clinical and histological patterns at presentation, but alternate periods of clinical quiescence with renal relapses of different severity. It is still uncertain which, if any, immunologic parameters may help to diagnose a renal flare. The increase in anti-double-stranded DNA (dsDNA) titre or hypocomplementaemia related to classical pathway activation provides no indication as to whether a relapse includes the kidney [1]. Active proliferative glomerulonephritis is a serious manifestation of systemic lupus erythematosus (SLE) that may exist at disease onset or may develop later on during a flare. Clinical nephritis develops in about 50% of patients with SLE. Early diagnosis and rapid treatment of lupus nephritis are crucial to improving survival in SLE patients [2]. The prognostic significance of lupus nephritis indicates a need for identifying early biomarkers that predict nephritis development [3].

A major pathogenic hypothesis is that SLE involves defective renal clearance of immune complexes. Among immunological parameters, consumption of the early components of the classical complement pathway, such as C1q and C4, is strongly associated with the development of active SLE [4]. Low C1q levels, although occasionally caused by a rare genetic abnormality, are usually related to consumption by immune complexes such as dsDNA–anti-dsDNA or nucleosomes–antinucleosomes [1,5]. Another cause of low C1q levels is the presence of anti-C1q antibodies with the formation of C1q/anti-C1q immune complexes [6]. Anti-C1q antibodies have been described in patients with SLE [7] or other autoimmune diseases [8]. Their correlations with hypocomplementemia and glomerulonephritis suggest that anti-C1q may play a pathogenic role [9].

An intact classical pathway of the complement system is essential for protection against immune complex disease; C1q is a central molecule in the first step of the classical complement activation pathway, the globular heads of C1q bind to the Fc regions of immunoglobulins IgM or IgG thus inducing an activation of the other subcomponents of C1, C1r and C1s. Serial measurement of anti-C1q titers will be an effective tool for the guidance of immunosuppressive therapy in SLE patients, anti-C1q autoantibodies may be especially relevant for monitoring of lupus nephritis activity, the highest anti-C1q titers were found in patients with active lupus nephritis [10].

MATERIALS AND METHODS

Patients

The patients included in the current study were admitted and treated at Nephrology department, National Institution of Urology and Nephrology, Egypt, during the period from January 2012 to December 2012. Full clinical data were collected from the clinical sheets of the patients. The study was carried out in three different groups:

First group was control group which was 20 healthy volunteers; second group was 25 patients suffering from rheumatoid arthritis; third group was 20 patients with positive for systemic lupus erythematosus with renal involvement.

All groups were subjected to determination of Anti-C1q Ab by ELISA technique, determination of blood urea nitrogen (BUN) and determination of serum creatinine.

Determination of anti-C1q Ab

Principle:

Anti-C1q is an indirect solid phase enzyme immunoassay (ELISA) for the quantitative measurement of IgG class autoantibodies against anti-C1q in human serum or plasma. Highly purified human C1q is bound to microwells, antibodies against this antigen if present in diluted serum bind to the respective antigen, washing of the microwells removes unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human IgG immunologically detects the bound patient antibodies forming a conjugate / antibody / antigen complex, washing of the microwells removes unbound conjugate, an enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color; the addition of an acid stops the reaction forming a yellow end-product. The intensity of this

yellow color is measured photometrically at 450 nm. The amount of color is directly proportional to the concentration of IgG antibodies present in the original sample.

Specimen collection:

Whole blood specimens were collected using acceptable medical techniques to avoid hemolysis, then blood was allowed to clot and serum was separated by centrifugation taking into consideration that serum should be clear and non-hemolyzed, contamination by hemolysis or lipemia was best avoided but did not interfere with this assay.

Test procedure:

A sufficient number of microplate modules were prepared to accommodate controls and prediluted patient samples. 100µl of calibrators, controls and prediluted patients samples were pipetted in duplicate into the wells then incubated for 30 minutes at room temperature (20-28°C). The contents of the microwells were discarded and washed 3 times with 300µl of wash solution; 100µl of enzyme conjugate was dispensed into each well, and were incubated for 15 minutes at room temperature. The contents of the microwells were discarded and washed 3 times with 300µl of wash solution. 100µl of TMB substrate solution was dispensed into each well and Incubated for 15 minutes at room temperature. 100µl of stop solution was added to each well of the modules and incubated for 5 minutes at room temperature. The optical density was read at 450 nm and the results were calculated Bi- chromatic measurement with a reference at 600-690 nm.

Calculation of the results:

For Anti-C1q IgG a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice. First the averaged optical densities for each calibrator was calculated well, using lin-log graph paper and the averaged optical density of each calibrator was plotted versus the concentration, the best fitting curve was drawn approximating the path of all calibrator points. The concentration of unknowns may then be estimated from the calibration curve by interpolation [11-15].

Determination of blood urea nitrogen (BUN)

Principle: Berthelot. Enzymatic colorimetric method

Urea in the sample is hydrolyzed enzymatically into ammonia (NH₄⁺) and carbon dioxide (CO₂). Ammonia ions formed reacts with salicylate and hypochlorite (NaClO), in presence of the catalyst nitroprusside to form a green indolphenol. This intensity of the color formed is proportional to the urea concentration in the sample [16].

Procedure:

1.0 ml of buffer was added into blank, standard and samples tubes, buffer solution consisting of Phosphate pH 6.7 50 mmol/L, EDTA 2 mmol/L, Sodium salicylate 400 mmol/L and Sodium nitroprusside 10 mmol/L. One drop of urease enzyme was added into blank, standard and samples tubes then 10µl of standard was added into standard tube and 10µl of each sample into each tube, than mixed and incubated for 3 minutes at 37°C. 200µl of (NaClO) Sodium hypochlorite 140 mmol/L, Sodium Hydroxide 150 mmol/L were added into blank, standard and samples tubes, then components were mixed and incubated for at least 5 minutes at 37°C Absorbance of samples (A_{sample}) and standard (A_{standard}) against reagent blank within 60 minutes at 578nm.

Determination of creatinine

Principle: Jaffè. Colorimetric-kinitic

The assay is based on the reaction of creatinine with sodium picrate as described by Jaffè, creatinine reacts with alkaline picrate forming a red complex, the time interval chosen for measurements avoids interference from other serum constituents, the intensity of the color formed is proportional to the creatinine concentration in the sample [17].

Procedure:

Working reagents (WR) were prepared by mixing equal volumes of picric acid and sodium hydroxide. 1.0ml of working reagent was added into blank, standard and samples tubes, then 100µl of standard was added into standard tube, and 100µl of serum samples were added into samples tubes then mixed well and the absorbance (A₁) was read after 30 seconds against blank at 492nm and after 120 seconds (A₂) of the sample. ΔA was calculated = A₂ – A₁.

Calculation:

Concentration of creatinine mg/dl:

$$\frac{\Delta A_{\text{sample}} - \Delta A_{\text{blank}}}{\Delta A_{\text{standard}} - \Delta A_{\text{blank}}} \times 2 \text{ (standard concentration)}$$

Determination of Anti-ds DNA antibody**Principle:**

Diluted patient serum was added to wells coated with purified dsDNA antigen by ELISA (Enzyme linked immunosorbent assay), specific antibody if present, was bounded to the antigen. All unbounded materials were washed away and the enzyme conjugate was added to bind to antibody-antigen complex, if present excess enzyme conjugate was washed off and substrates was added. The plate was incubated to allow the hydrolysis of the substrate by the enzyme, the intensity of the color generated was proportional to the amount of specific antibody in the sample.

Procedure and calculation: Reagents preparation:

Wash buffer was prepared by adding the contents of buffer bottle (25ml) to 475 ml distilled water. All specimen and kit reagents were brought to room temperature and gently mixed, negative control, positive control and calibrators are ready to use. 1:21 dilutions of the samples were prepared by adding 10 μ l of the sample to 200 μ l of sample diluent and were mixed well. 100 μ l of diluted serum, calibrators and controls were dispensed into appropriate wells and mixed well then incubated for 20 minutes at room temperature. Liquids from all wells were removed and washed well three times by 300 μ l wash buffer then blotted on absorbance paper. 100 μ l of enzyme conjugate was dispensed and incubated for 20 minutes at room temperature then washed three times by 300 μ l wash buffer and blotted on absorbance paper. 100 μ l of TMB substrate was dispensed and incubated for 10 minutes at room temperature then 100 μ l of stop solution (1 M of sulphoric acid) was added. Absorbance (OD) was read at 450 nm using ELISA reader within 15 minutes. Cut-off value was calculated: calibrator OD X calibrator factor CF Antibody (Ab) index of each sample was calculated by dividing the value of each sample by cut-off value (37).

Determination of Sodium (Na) and Potassium (K)**Principle:**

The measurement of sodium and potassium by an ion-selective electrode apparatus (ISE), In an ion-selective electrode, an electrical potential is established across a membrane that is selective to a specific ion, such electric potential of the ion-selective electrode is measured against a reference electrode and it is used to determine the activity or effective concentration of Na and K according to the Nernst equation.

$$E = \bar{E} + S \cdot \log(c)$$

Where E is monitored potential, (\bar{E}) is the standard electrical potential, (S) slope which determined by measuring the electrical potentials of the ion-selective electrode in two calibration solutions that have known concentrations of the measuring ions at different levels and (c) is the effective concentration. Once the \bar{E} and S are determined, the unknown concentration of a sample can be determined by measuring the electric potential of the electrode in a sample.

Procedure and calculation:

Sample was measured by Na and K electrodes containing ISE buffer and ISE standards which was ready to use. Results were obtained by the analyzer which automatically computed the sodium and potassium values of each sample (38).

Determination of serum Albumin**Principle:**

A coloured complex was formed when bromocresol green was reacted with albumin, the absorbance of albumin-BCG complex was measured bichromatically (600/800) and was proportional to the albumin concentration in the sample.

Procedure and calculation:

Reagents was ready to use which a mixture of 5-chloro-2-methyl-4- isothiazolin-3-one and 2-methyl-4-isothiazoline-3-one in presence of succinate buffer (pH 4.2) 100 mmol/L and Bromocresol green 0.2 mmol/L, results were obtained from calibration curve of the analyzer. (39)

Determination of serum calcium**Principle:**

Total serum calcium is composed of three fractions: free or ionized calcium, protein bound calcium most of which is bound to albumin with only a small portion bound to globulin and complex-bound calcium mainly to phosphate, citrate and bicarbonate. The ionized calcium is physiologically most significant but has proven difficult to assay directly, it may be estimated from total calcium (40).

Procedure and calculation

The calcium procedure is based on calcium ions (Ca^{2+}) reacting with Arsenazo III (2,2-[1,8-Dihydroxy-3,6-disulphonaphthylene-2,7-bisazo]-bisbenzenearsonic acid) to form an intense purple coloured complex, in this method the absorbance of the Ca-Arsenazo III complex was measured bichromatically at 600/ 700 nm. The resulting increase in absorbance of the reaction mixture was directly proportional to the calcium concentration in the sample. (41,42). Reagents were ready to use and results were obtained from calibration curve of the analyzer.

Determination of C3 and C4**Principle:**

Sample was mixed with buffer and anti-serum solution, serum C3 and C4 was reacted with specifically with anti-human C3 antibodies and anti-human C4 antibodies to yield insoluble aggregates, the absorbance of these aggregates is proportional to the C3 and C4 concentration in the serum sample. (43).

Procedure and calculation:

Reagents containing tris buffer (pH 7.2), polyethylene glycol 6000 1.6% w/v, goat anti-C3 antibodies and goat anti-C4 antibodies. Reagents were ready for use and results were obtained from calibration curve of the analyzer.

RESULTS

Serum anti-C1q antibody, blood urea nitrogen (BUN) and serum creatinine have been assessed in all groups.

Table 1: Mean \pm standard error of mean and median of different parameters among groups

Parameter	Group	Normal	Rheumatoid	Lupus nephritis
Blood Hb (g/dl)	Mean \pm SE	11.65 \pm 0.34	12.30 \pm 0.36	8.44 \pm 0.25
	Median	11.25	11.8	8.5
Serum albumin (g/dl)	Mean \pm SE	4.37 \pm 0.11	4.28 \pm 0.11	2.42 \pm 0.20
	Median	4.4	4.2	2.35
BUN (mg/dl)	Mean \pm SE	11.25 \pm 0.93	11.92 \pm 0.76	68.10 \pm 11.74
	Median	11.5	12	54
Serum creatinine (mg/dl)	Mean \pm SE	0.65 \pm 0.04	0.95 \pm 0.34	4.05 \pm 0.73
	Median	0.6	0.6	2.7
Serum sodium (mEq/dl)	Mean \pm SE	140.05 \pm 0.88	140.80 \pm 0.72	127.85 \pm 1.18
	Median	139.5	140	128
Serum potassium (mEq/dl)	Mean \pm SE	4.25 \pm 0.11	4.26 \pm 0.09	4.26 \pm 0.23
	Median	4.25	4.3	4.4
Serum total calcium (mg/dl)	Mean \pm SE	9.06 \pm 0.10	9.04 \pm 0.09	7.40 \pm 0.18
	Median	9.05	9.1	7.6
Serum C3 (mg/dl)	Mean \pm SE	123.75 \pm 5.83	125.52 \pm 6.59	69.60 \pm 3.64
	Median	118.5	127	69.5
Serum C4 (mg/dl)	Mean \pm SE	29.50 \pm 2.41	27.76 \pm 2.37	9.08 \pm 0.83
	Median	27	29	7.95
Serum anti-C1q antibody (U/ml)	Mean \pm SE	42.28 \pm 7.68	54.38 \pm 6.72	1121.75 \pm 184.21
	Median	22.69	60.5	767.5

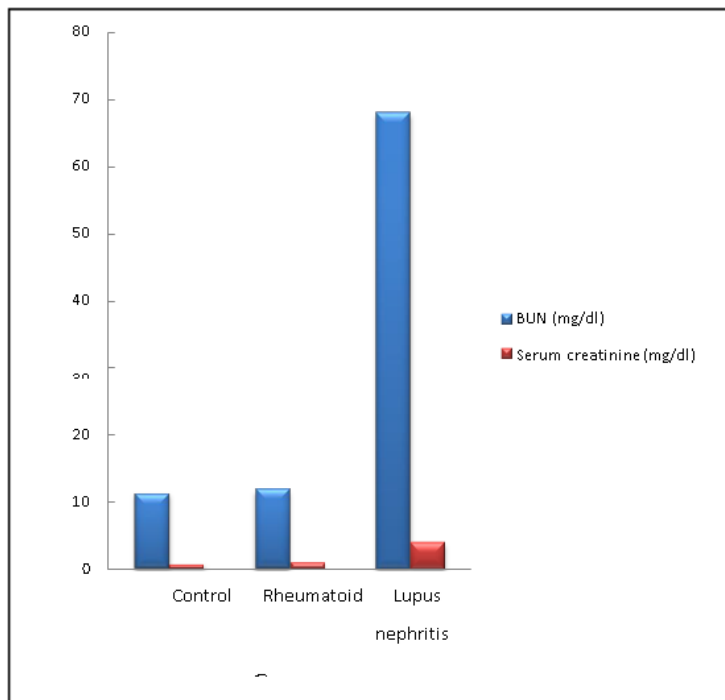


Figure 1: Mean of BUN and serum creatinine among groups

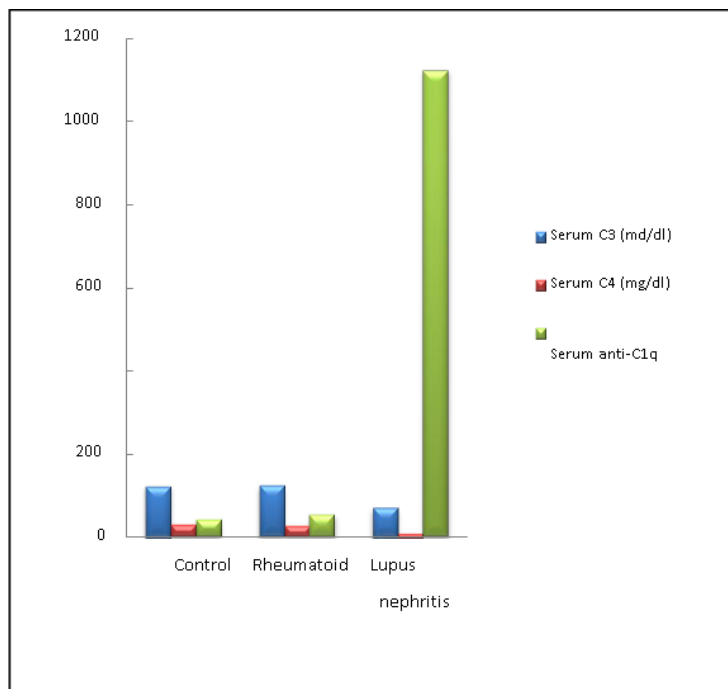


Figure 2: Mean of serum C3, C4 and anti-C1q antibody among groups

Table (2) shows no significant difference in blood urea nitrogen levels between normal and rheumatoid groups. In contrast, there is a high significant difference in blood urea nitrogen levels between normal and lupus nephritis group and between rheumatoid and lupus nephritis group ($p < 0.001$).

Table 2: Comparison of BUN among different groups using T-test

	Normal	Rheumatoid	Lupus
Normal	-	N.S	p<0.001
Rheumatoid	N.S	-	p<0.001

*p<0.05 is significant
* p<0.01 or p<0.001 is highly significant

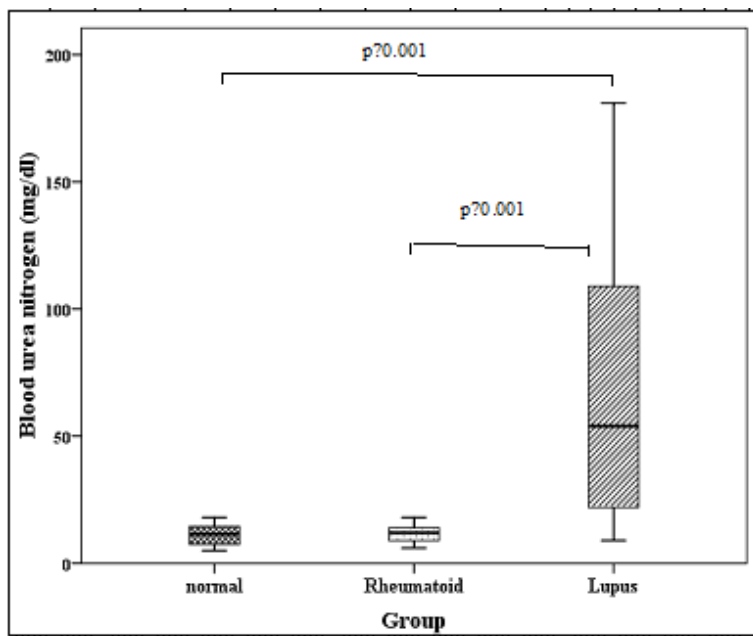


Figure 3: Box plot shows significant difference in BUN among different groups

Table (3) shows no significance difference in serum creatinine levels between normal and rheumatoid groups. In contrast, there is a high significant difference in serum creatinine levels between normal and lupus nephritis group and between rheumatoid and lupus nephritis group (p<0.001).

Table 3: Comparison of serum creatinine among different groups using T-test

	Normal	Rheumatoid	Lupus
Normal	-	N.S	p<0.001
Rheumatoid	N.S	-	p<0.001

*p<0.05 is significant
* p<0.01 or p<0.001 is highly significant

Table (4) shows no significance difference in serum C3 levels between normal and rheumatoid groups. In contrast, there is a high significant difference in serum C3 levels between normal and lupus nephritis group and between rheumatoid and lupus nephritis group (p<0.001).

Table 4: Comparison of serum C3 among different groups using T-test

	Normal	Rheumatoid	Lupus
Normal	-	N.S	p<0.001
Rheumatoid	N.S	-	p<0.001

*p<0.05 is significant
* p<0.01 or p<0.001 is highly significant

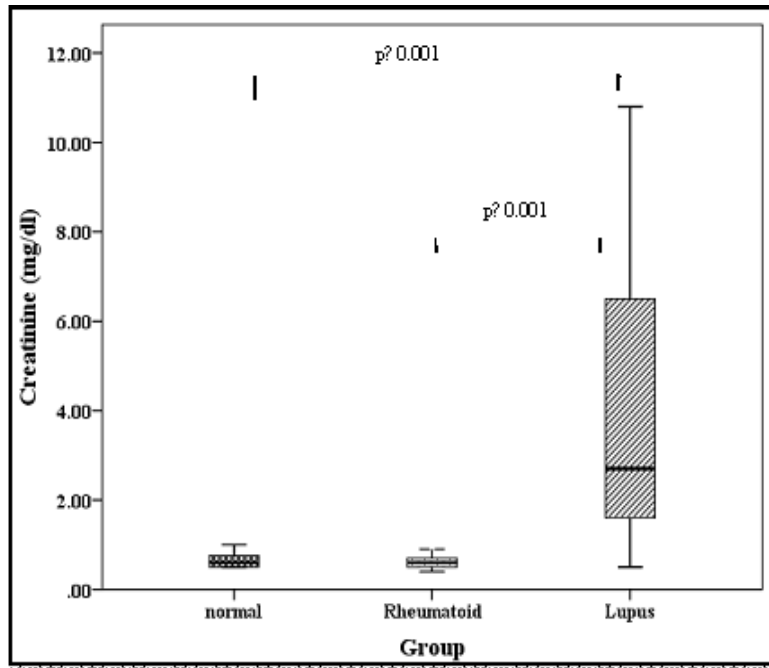


Figure 4: Box plot shows significant difference in serum creatinine levels among different groups

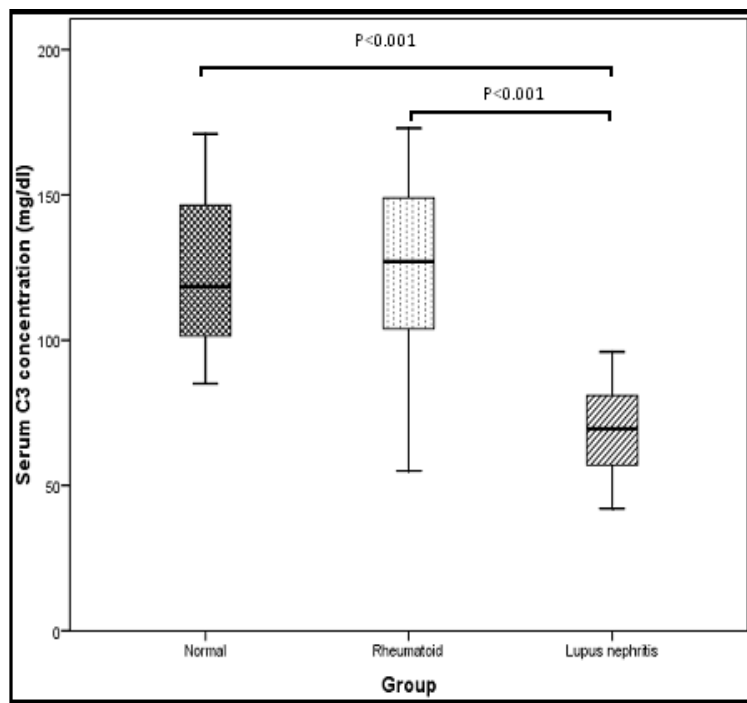


Figure 5: Box plot shows significant difference in serum C3 levels among different groups

Table (5) shows no significance difference in serum C4 levels between normal and rheumatoid groups. In contrast, there is a high significant difference in serum C4 levels between normal and lupus nephritis group and between rheumatoid and lupus nephritis group ($p < 0.001$).

Table 5: Comparison of serum C4 among different groups using T-test

	Normal	Rheumatoid	Lupus
Normal	-	N.S	p<0.001
Rheumatoid	N.S	-	p<0.001

*p<0.05 is significant
* p<0.01 Or p<0.001 is highly significant

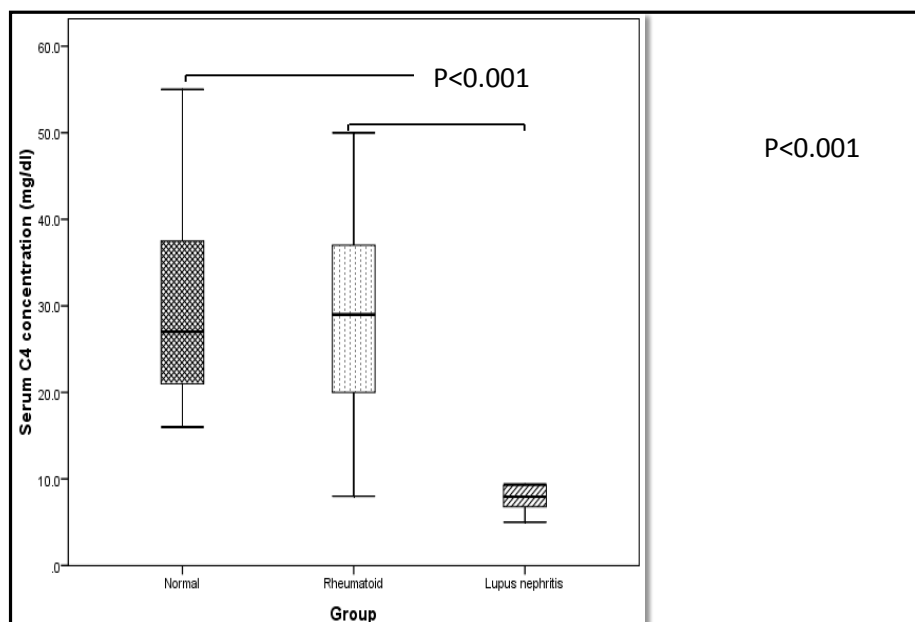
**Figure 6: Box plot shows significant difference in serum C4 levels among different groups**

Table (6) shows no significant difference in serum anti C1q antibody levels between normal and rheumatoid groups. In contrast, there is a high significant difference in serum anti-C1q antibody levels between normal and lupus nephritis group and between rheumatoid and lupus nephritis group(p<0.001).

Table 6: Comparison of serum anti-C1q antibody among different groups using T-test

	Normal	Rheumatoid	Lupus
Normal	-	N.S	p<0.001
Rheumatoid	N.S	-	p<0.001

*p<0.05 is significant
* p<0.01 or p<0.001 is highly significant

In lupus nephritis group, BUN showed a highly significant positive correlation with serum creatinine concentration ($r = 0.773$, $p < 0.001$). Also, serum creatinine concentration showed a significant positive correlation with serum anti-C1q antibody ($r = 0.513$, $p < 0.05$).

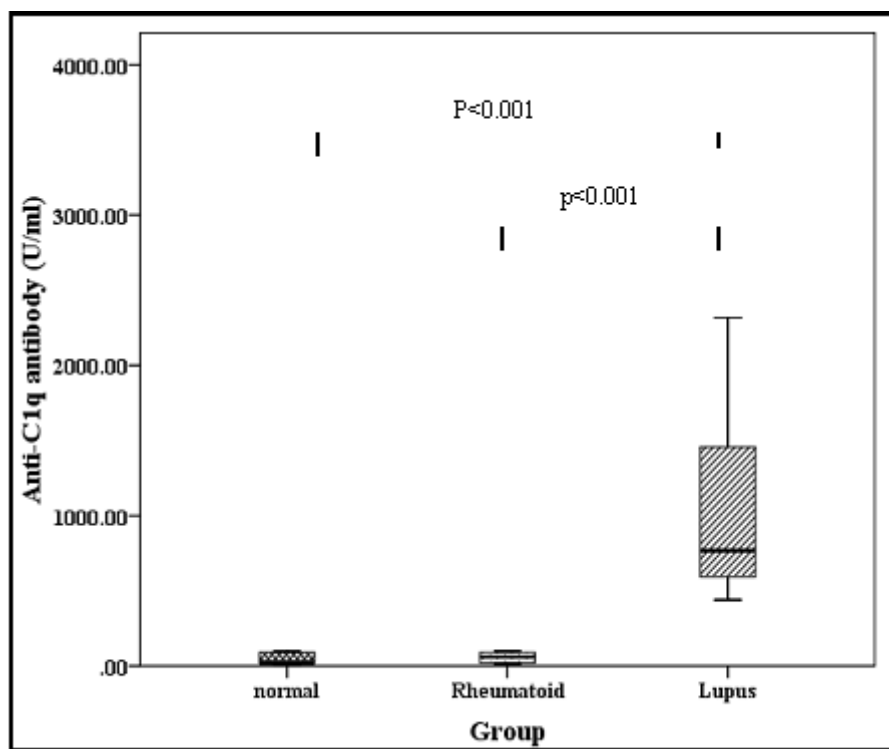


Figure 7: Box plot shows significant difference in anti C1q antibody levels among different groups

Table 7: Correlation between different parameters within lupus nephritis patients group

	Gender	Blood Hb	Serum albumin	BUN	Serum creatinine	Serum sodium	Serum total calcium	Serum C3	Serum C4	Anti-C1q antibody	Anti-dsDNA
Gender	-	r = 0.59 p<0.01	r = 0.37 N.S	r = -0.199 N.S	r = -0.087 N.S	r = 0.163 N.S	r = 0.471 p<0.05	r = -0.349 N.S	r = 0.508 p<0.05	r = -0.292 N.S	r = 0.187 N.S
Blood Hb	r = 0.59 p<0.01	-	r = 0.535 p<0.05	r = -0.546 p<0.05	r = -0.409 N.S	r = 0.367 N.S	r = 0.576 p<0.01	r = 0.335 N.S	r = 0.574 p<0.01	r = -0.326 N.S	r = 0.076 N.S
Serum Albumin	r = 0.37 N.S	r = 0.535 p<0.05	-	r = -0.379 N.S	r = -0.177 N.S	r = 0.717 p<0.01	r = 0.54 p<0.05	r = 0.411 N.S	r = 0.467 p<0.05	r = -0.224 N.S	r = 0.422 N.S
BUN	r = -0.199 N.S	r = -0.546 p<0.05	r = -0.379 N.S	-	r = 0.773 p<0.001	r = -0.185 N.S	r = -0.658 p<0.01	r = -0.415 N.S	r = -0.457 p<0.05	r = 0.417 N.S	r = 0.054 N.S
Serum creatinine	r = -0.087 N.S	r = -0.409 N.S	r = -0.177 N.S	r = 0.773 p<0.001	-	r = -0.261 N.S	r = -0.514 p<0.05	r = -0.282 N.S	r = -0.361 N.S	r = 0.513 p<0.05	r = 0.235 N.S
Serum sodium	r = 0.163 N.S	r = 0.367 N.S	r = 0.717 p<0.01	r = -0.185 N.S	r = -0.261 N.S	-	r = 0.51 p<0.05	r = 0.319 N.S	r = 0.307 N.S	r = -0.093 N.S	r = 0.62 p<0.01
Serum total calcium	r = 0.471 p<0.05	r = 0.576 p<0.01	r = 0.54 p<0.05	r = -0.658 p<0.01	r = -0.514 p<0.05	r = 0.51 p<0.05	-	r = 0.381 N.S	r = 0.337 N.S	r = -0.45 p<0.05	r = 0.314 N.S
Serum C4	r = 0.508 p<0.05	r = 0.574 p<0.01	r = 0.467 p<0.05	r = -0.457 p<0.05	r = -0.361 N.S	r = 0.307 N.S	r = 0.337 N.S	r = 0.796 p<0.01	-	r = -0.310 N.S	r = 0.196 N.S

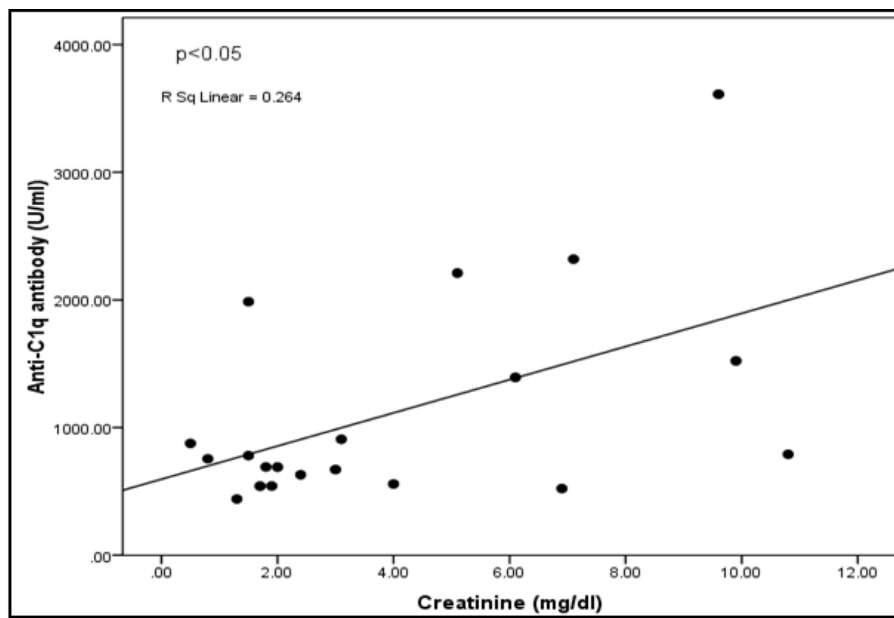


Figure 8: Correlation between serum creatinine and serum anti-C1q antibody within lupus nephritis patients

Group

ROC curve

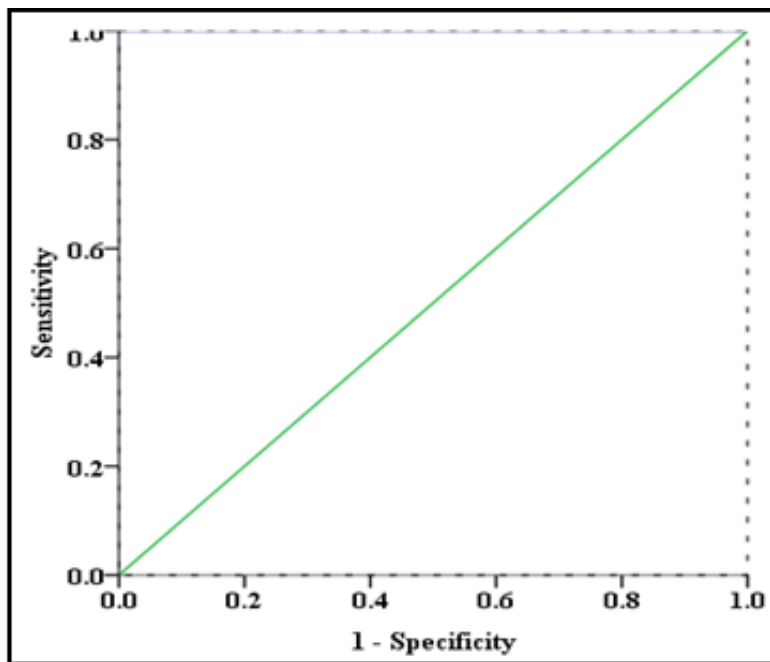


Figure 9: ROC curve of anti C1q antibody between lupus and non-lupus patients

	AUC	p	Cut-off value	Sensitivity	Specificity	PPV	NPV	Accuracy
Anti C1q antibody	1.0	<0.001	269.25 U/ml	100%	100%	100%	100%	100%

ROC curve for anti C1q antibody was significant between patients with and without lupus nephritis ($p < 0.001$).

Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy were 100%.

DISCUSSION

The increased levels of anti-double strand DNA antibodies (anti-dsDNA) and hypocomplementemia are serological markers of SLE activity, but they are not enough to identify which organ will be affected (18). Several studies have described that anti-C1q antibody (anti-C1q), antibodies against collagen-like region of first component of the classical complement pathway (19) might be regarded as immunological markers of SLE with renal involvement in particular, (20) detection of anti-C1q either alone or in combination with other serological markers of disease activity could give complementary information to the diagnosis of a renal flare (21).

Several autoantibodies, especially those against double stranded DNA (anti- dsDNA) are believed to play a major role in the induction of glomerular inflammation, (22) raised titers of anti-dsDNA and hypocomplementemia are reported to be associated with the activity of the disease, (23) however the lack of specificity of these biological markers for renal exacerbations has led to the search for other autoantibodies that might contribute to nephritis and help diagnose a renal flare (24).

In this study the mean and standard error of mean of serum Anti-C1q antibody was 42.28 ± 7.68 U/ ml with median 22.69 U/ ml in normal group , 54.38 ± 6.72 U/ ml with median 60.50 U/ ml in rheumatoid group while 1121.75 ± 184.21 U/ ml with median 767.5 U/ ml in lupus nephritis group. There was no significant difference in serum anti-C1q antibody levels between normal and rheumatoid group in contrast there was a high significant difference in serum anti-C1q antibody levels between normal and lupus nephritis group and between rheumatoid and lupus nephritis group ($p < 0.001$).

Serum C3 and C4 within normal range in normal and control groups while decreased in lupus nephritis group due to consumption of the early components of the classical complement pathway C3 and C4 which is strongly associated with increase in anti-C1q antibody.

This was in agreement with three other studies found significantly higher titers of anti-C1q antibodies in patients with active disease compared with those with inactive SLE (25-27).

Moroni et al. detected a significant association and high titer of anti-C1q antibody and anti-ds DNA antibody in active SLE patients with nephritis, (28) and *Matrat et al.* confirmed that: the presence of anti-C1q and anti-dsDNA Abs was associated with a high risk of renal flare, whereas the absence of both Abs excluded such an event (29).

Anti-C1q might be of important help in the diagnosis of suspected proliferative lupus nephritis, particularly in situations when standard parameters such as urinalysis, creatinine, serum complement levels and anti-dsDNA antibodies do not allow a clear-cut decision about treatment modifications and/or the necessity of a renal biopsy, very high titers of anti-C1q strongly increase the likelihood of the presence of severe lupus nephritis. Vice versa, and maybe more importantly, a negative test result almost excludes the presence of an active glomerulonephritis and therefore might help avoid unnecessary renal biopsies and/or treatment modifications (30).

Hewala et al. found that presence of anti-C1q antibody and anti-ds DNA antibody in lupus nephritis patients and both of them were significantly associated with lupus nephritis in active patients, none of patients with active lupus nephritis had anti-C1q antibody only and none was negative for both anti- ds DNA antibody and anti-C1q antibody (31).

In this study; only blood urea nitrogen (BUN) showed a highly significant positive correlation with serum creatinine concentration in normal group ($r=0.906$, $p < 0.001$).

In rheumatoid group only blood urea nitrogen (BUN) showed a significant positive correlation with serum creatinine concentration ($r=0.404$, $p < 0.05$).

In lupus nephritis group blood urea nitrogen (BUN) showed a highly significant positive correlation with serum creatinine concentration ($r=0.773$, $p < 0.001$) also serum creatinine concentration showed a significant positive correlation with serum anti-C1q antibody ($r=0.513$, $p < 0.05$).

In the same context; *Trendelenburg et al.* found strong positive correlation between anti-C1q and the occurrence of active proliferative lupus nephritis corresponding to a prevalence of $>97\%$, in comparison anti-C1q were found in only about one-third of SLE patients having either inactive lupus nephritis or no lupus nephritis at all. In addition to the high prevalence of anti-C1q in patients with biopsy-proven active lupus nephritis, these patients had the highest

titers observed in this study, furthermore anti-C1q titers strongly decreased during successful treatment [32]. In this study, for anti-C1q antibody was significant between patients with and without lupus nephritis ($p < 0.001$). Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy were 100%. This was agreement with *Trendelenburg et al.* reported that, for the detection of an active glomerulonephritis in SLE patients, the anti-C1q antibody assay showed a particularly high sensitivity (97.2%) while specificity was 70.3% [30-32]. In the same context, the sensitivity of anti-C1q antibody was 15/15 (100%) for subsequent severe lupus nephritis, the specificity of anti-C1q antibody assay was 95.7%, the positive predictive value (PPV) for subsequent severe lupus nephritis 15/30 (50%) and the negative predictive value (NPV) was 18/18 (100%) [33]. This finding was similar to the studies reported by *Zang et al.* and *Katsumata et al.* who suggested that the circulating anti-C1q antibodies may bind to the C1q deposits in the kidneys of LN patients and this consumption of serum anti-C1q antibodies by binding to C1q-containing immune complexes could be responsible for the lack of significant difference among LN and non-LN patients [34].

The present result supports the study done by *Moroni et al.* [35] which showed 87% sensitivity and 92% specificity for anti-C1q in predicting SLE nephritis activity, it also agrees with the study by *Sinico et al.* which showed a strong association of anti-C1q with active SLE nephritis, anti-C1q in the latter study had a better predictive value for active nephritis than other parameters such as C3/C4 consumption and anti-ds DNA [36].

SUMMARY AND CONCLUSION

The aim of the study was to further investigate association between serum titer of anti-C1q antibody and disease manifestation of systemic lupus erythematosus (SLE), significant association were found between increased serum titer of anti-C1q antibody and nephritis with subsequent loss of kidney function, in addition the development of nephritis was preceded by a significant increase in serum titer of anti-C1q antibody. The present study suggests that anti-C1q antibody might be seen as new parameter for the development of lupus nephritis since the increased levels of anti-ds DNA antibody and hypocomplementemia (C3 and C4) are serological markers of systemic lupus erythematosus activity but they are not enough to identify which organ will be affected.

In conclusion, anti-C1q antibody might be regarded as immunological markers of systemic lupus erythematosus with renal involvement in particular; detection of anti-C1q antibody either alone or in combination with other serological markers could give information of the diagnosis of a renal flare with a sensitivity and specificity 100%.

REFERENCES

- [1] MJ Walport. *N Engl J Med*, **2001**, 344, 1058-1066.
- [2] FA Houssiau; D D'Cruz D; J Vianna; GR Hughes. *Clin Exp Rheumatol*, **1991**, 9, 345-349.
- [3] FA Houssiau; C Vasconcelos; D D'Cruz; GD Sebastiani; E de Ramon Garrido; MG Danieli; D Abramovicz; D Blockmans; A Mathieu; H Direskeneli; M Galeazzi; A Gul; Y Levy; P Petera; R Popovic; R Petrovic; RA Sinico; R Cattaneo; J Font; G Depresseux; JP Cosyns; R Cervera. *Arthritis Rheum.*, **2004**, 50, 3934-3940.
- [4] MJ Walport. *Arthritis Res.*, **2002**, 4 (Suppl 3), S279-293.
- [5] MJ Walport. *N Engl J Med.*, **2001**, 344, 1140-1144.
- [6] AG Sjöholm; U Martensson; G Sturfelt. *J Rheumatol.*, **1997**, 24, 871-878.
- [7] M Mosca; D Chimenti; F Pratesi; C Baldini; C Anzilotti; S Bombardieri; P Migliorini. *J Rheumatol.*, **2006**, 33, 695-697.
- [8] A Braun; J Sis; R Max; K Mueller; C Fiehn; M Zeier; K Andrassy. *Scand J Rheumatol.*, **2007**, 36, 291-298.
- [9] R Flierman; MR Daha. *Mol Immunol.*, **2007**, 44, 133-138.
- [10] CE Siegert; MR Daha. *Immunobiology.*, **1998**, 199, 295-302.
- [11] MJ Walport. *Arthritis Res.*, **2002**, 4 (Suppl 3), S279-293.
- [12] M Botto; C Dell'Agnola; AE Bygrave; EM Thompson; HT Cook; F Petry; M Loos; PP Pandolfi; WJ Walport. *Nat Genet.*, **1998**, 19, 56-59.
- [13] MJ Walport; KA Davies; M Botto. *Immunobiology.*, **1998**, 199, 265-285.
- [14] M Trendelenburg; JA Schifferli. *Z Rheumatol.*, **2000**, 59, 172-175.
- [15] K Conrad; W Schöblier; F Hiepe. *Pabst Science Publishers*, **2001**, 38.
- [16] A Tabacco; F Meiattini; E Moda; P Tarli. *Clin Chem.*, **1979**, 25, 336-337.
- [17] Murray R.L. Creatinine. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 1261-1266 and 418.

- [18] JC Wasmuth; B Grun; B Terjung. *Clin Chem.*, **2004**, 50, 2169-2171.
- [19] V Agnello; D Koffer; JW Eisenberg; RJ Winchester; HG Kundel. *J Exp Med.*, **1971**, 134, 228-241.
- [20] CE Siegert; MR Daha; ML Westedt; E van der Voort; F Breedveld. *J Rheumatol.*, **1991**, 18, 230-234.
- [21] Y Yin; X Wu; G Shan; X Zhang. *Lupus*, **2012**, 21, 1088-1097.
- [22] BH Hahn. *N Engl J Med.*, **1998**, 338, 1359-1368.
- [23] DA Isenberg; CT Ravirajan; A Rahman; J Kalsi. *Lupus*, **1997**, 6, 290-304.
- [24] Z Amoura; S Koutouzov; JC Piette. *Curr Opin Rheumatol.*, **2000**, 12, 369-373.
- [25] Y Katsumata; K Miyake; Y Kawaguchi; Y Okamoto; M Kawamoto. T Gono; S Baba; M Hara; H Yamanaka. *Arthritis Rheum.* **2011**, 63(8), 2436-2444.
- [26] P Horák; Z Hermanová; J Zadrazil; H Ciferská; M Ordeltová; L Kusá; M Zurek; T Tichý. *Clin Rheumatol*, **2006**, 25: 532-536.
- [27] Wu FQ; Zhao Q; Cui XD; Zhang W. *Rheumatol Int*, **2011**, 31(4), 501-505.
- [28] G Moroni; M Trendelenburg; N Del Papa; S Quaglini; E Raschi; P Panzeri; C Testoni; A Tincani; G Banfi; G Balestrieri; JA Schifferli; PL Meroni; C Ponticelli. *Am J Kidney Dis*, **2001**, 37, 490-498.
- [29] A Matrat; C Veysseyre-Balter; P Trolliet; E Villar; F Dijoud. J Bienvenu; Fabien N. *Lupus*, **2011**, 20(1), 28-34.
- [30] M Trendelenburg; M Lopez-Trascasa; E Potlukova; S Moll; S Regenass; V Frémeaux-Bacchi; J Martinez-Ara; E Jancova; ML Picazo; E Honsova; V Tesar; S Sadallah; Schifferli. *J Nephrol Dial Transplant*, **2006**, 21, 3115-3121.
- [30] A El-Hewala; GS Nageeb; EE El-shahawy; DM Sharaf; AA Omran; FA El-Messallamy; S Eassa. *Egypt Rheumatol*, **2011**, 33(4), 203-208.
- [31] M Trendelenburg; M Lopez-Trascasa; E Potlukova; S Moll; S Regenass; V Frémeaux-Bacchi; J Martinez-Ara; E Jancova; M Luz Picazo; E Honsova; V Tesar; S Sadallah; J Schifferli. *Nephrol Dial Transpl.*, **2006**, 21, 3115-3121.
- [32] OC Meyer; P Nicaise-Roland; N Cadoudal; S Grootenboer- Mignot; E Palazzo; G Hayem; P Dieudé; S Chollet-Martin. *Arthritis Res Ther.*, **2009**, 11, R87.
- [33] PC Chen; CR Wang; MF Liu; FF Chen; CC Liang. *Asian Pac J Allergy Immunol.*, **2002**, 20, 223-227.
- [34] G Moroni; A Radice; G Giammarresi; S Quaglini; B Gallelli; A Leoni; ML Vecchi; P Messa; RA Sinico. *Ann Rheum Dis.*, **2009**, 68, 234-237.
- [35] RA Sinico; L Rimoldi; A Radice; L Bianchi; B Gallelli; G Moroni. *Ann N Y Acad Sci*, **2009**, 1173, 47-51.
- [36] KH Wong; JW Lawton; SK Cheng; SS Lee; CS Lau. *Pathology*, **1998**, 30, 57-61.
- [37] NW Tietz, El Pruden, O Siggaard-Anderson. Electrolytes. In: Tietz NW, ed. *Fundamentals of clinical chemistry*. Philadelphia: WB Saunders Company, **1987**, 614-624.
- [38] DS Young. Effects of preanalytical variables on clinical laboratory tests, 2nd ed. Washington: AACC Press, **1997**, 15-16.
- [39] L Thomas. Calcium (Ca). In: Thomas L, ed. *Clinical Laboratory diagnostics. Use and assessment of clinical laboratory results*. Frankfurt / Main: TH-Books Verlagsgesellschaft, **1998**, 231-241.
- [40] HJ Gitelman. *Anal Biochem.*, **1967**, 18, 521-531.
- [41] FH Pollard; JV Martin., **1956**, 81, 348-353.
- [42] AA Ismail; N Snowden. *Ann Clin Biochem.*, **1999**, 36, 565-578.