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

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ABSTRACT

Background: 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.

Aim of the study: this study aimed to investigate association between serum titer of anti-C1q antibody and disease manifestation of SLE. **Methodology**: 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.

Results: there was no significant difference in BUN levels between the normal and rheumatoid arthritis groups in contrast there was a highly significant difference in BUN between the normal and lupus groups also, between the rheumatoid arthritis and lupus nephritis groups ($p<0.001$). No significant difference was detected in serum creatinine levels between the normal and rheumatoid arthritis groups in there was a highly significant difference in serum creatinine between the normal and lupus groups and also between the rheumatoid arthritis and lupus nephritis groups ($p<0.001$). No significant difference was realized in serum anti-C1q antibodies levels between the normal and rheumatoid arthritis groups in contrast there was a highly significant difference in serum anti-C1q antibodies between the normal and lupus groups and also between rheumatoid arthritis and lupus nephritis groups ($p<0.001$). In the 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 ($r=0.513$, $P<0.05$).

Conclusion: the present study suggested 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 may 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 100% sensitivity and specificity.

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

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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 involved 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 suggested 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 may 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].

PATIENTS AND METHODS

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.

This study was carried out in three different groups: the first group was the control group which was 20 healthy volunteers; the second group was 25 patients , they were suffering from rheumatoid arthritis; the third group was 20 patients with positive systemic lupus erythematosus and 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[11-14].

Determination of blood urea nitrogen (BUN):

Principle: Berthelot. Enzymatic colorimetric method

Urea in the sample is hydrolyzed enzymatically into ammonia (NH_4^+) and carbon dioxide (CO_2). 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 [15].

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 [16].

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[17].

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.

$$E = É + S \cdot \log(c)$$

Where E is monitored potential , (É) 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 É and S are determined, the unknown concentration of a sample can be determined by measuring the electric potential of the electrode in a sample[18].

Determination of serum Albumin:

Principle:

A colored 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[19]

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 estimated from total calcium [20].

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 [23].

The study was approved by the Ethics Board of Cairo University.

RESULTS

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

Table 1: Mean ± standard error of mean and median of the different parameters among groups

Parameter / Group		Normal	umatoid patients	Lupus nephritis patients
Blood Hb (g/dl)	Mean±SE Median	11.65±0.34 11.25	12.30±0.36 11.80	8.44±0.25 8.50
Serum albumin (g/dl)	Mean±SE Median	4.37±0.11 4.40	4.28±0.11 4.2	2.42±0.20 2.35
BUN (mg/dl)	Mean±SE Median	11.25±0.93 11.50	11.92±0.76 12.00	68.10±11.74 54.00
Serum creatinine (mg/dl)	Mean±SE Median	0.65±0.04 0.60	0.95±0.34 0.60	4.05±0.73 2.70
Serum sodium (mEq/dl)	Mean±SE Median	140.05±0.88 139.50	140.80±0.72 140	127.85±1.18 128.00
Serum potassium (mEq/dl)	Mean±SE Median	4.25±0.11 4.25	4.26±0.09 4.30	4.26±0.23 4.40
Serum total calcium (mg/dl)	Mean±SE Median	9.06±0.10 9.05	9.04±0.09 9.10	7.40±0.18 7.60
Serum C3 (mg/dl)	Mean±SE Median	123.75±5.83 118.5	125.52±6.59 127.00	69.60±3.64 69.50
Serum C4 (mg/dl)	Mean±SE Median	29.50±2.41 27.00	27.76±2.37 29.00	9.08±0.83 7.95
Serum anti-C1q antibody (U/ml)	Mean±SE Median	42.28±7.68 22.69	54.38±6.72 60.50	1121.75±184.21 767.50

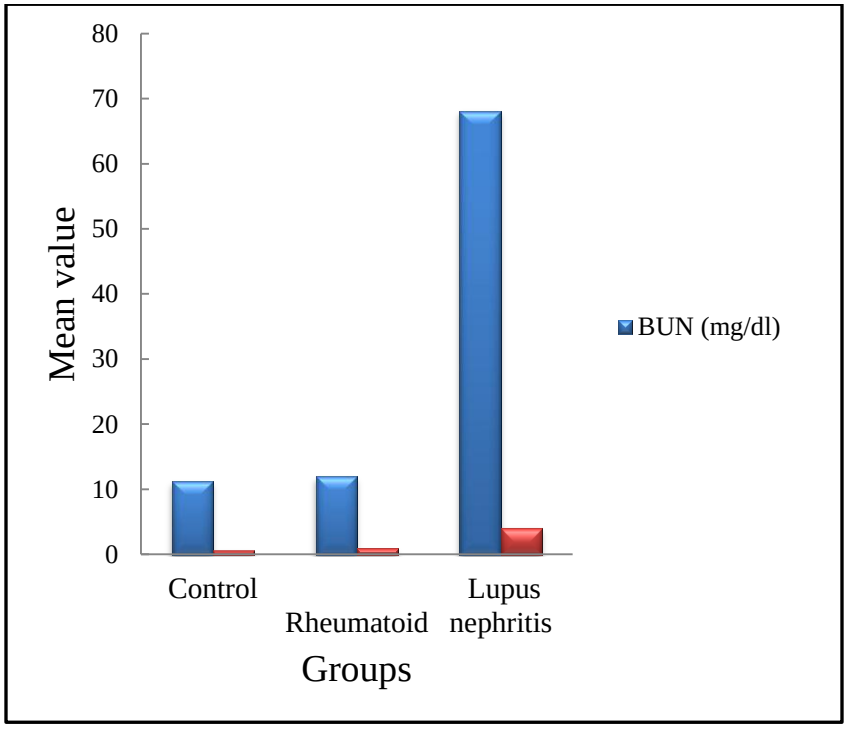

Fig. 1: mean of BUN and serum creatinine among the groups

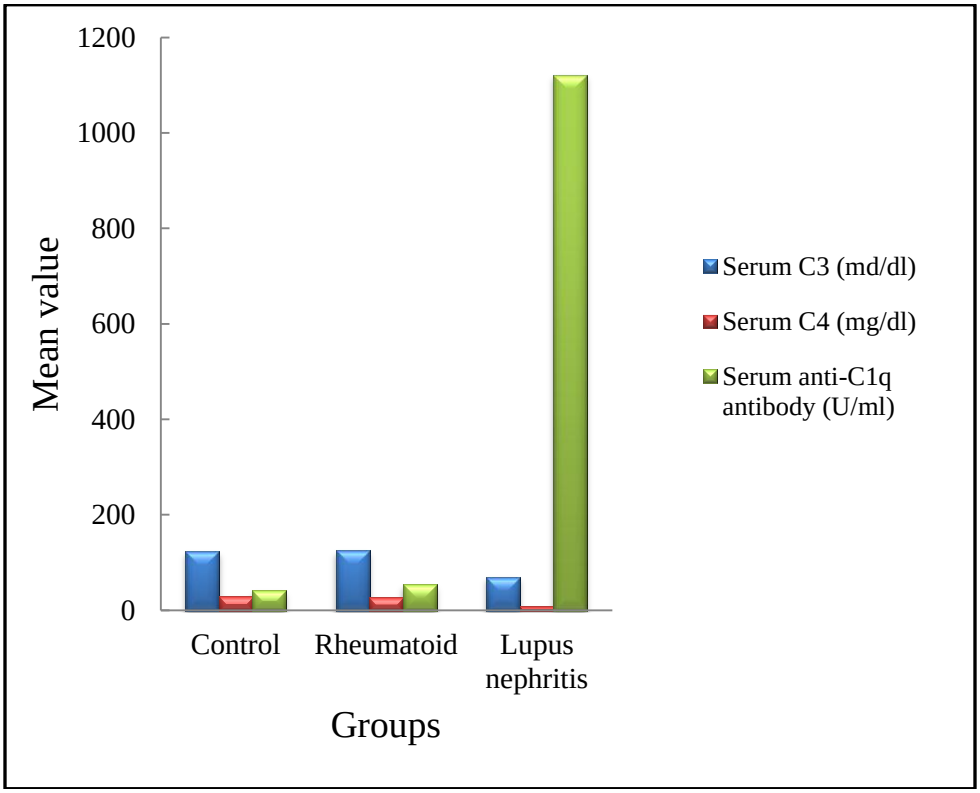

Fig.2: mean of serum C3, C4 and anti-C1q antibody among the groups

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

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

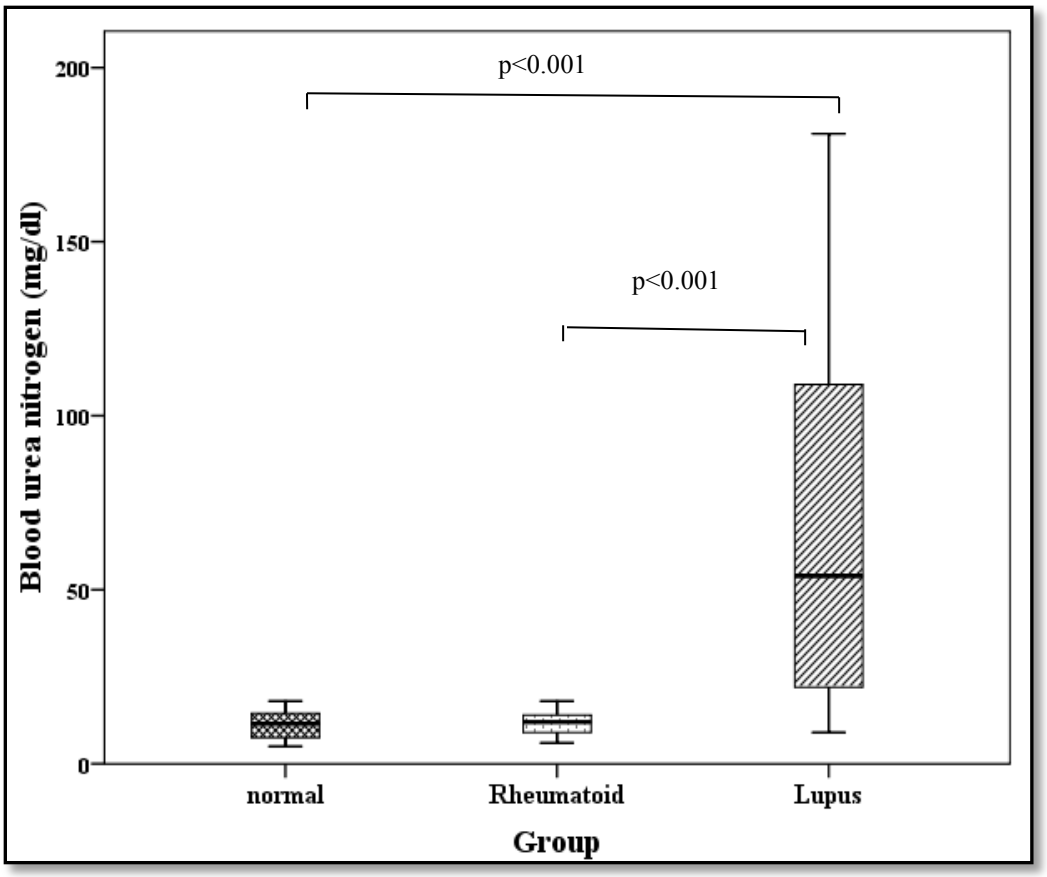

Fig. 3: box plot shows significant difference in BUN among the different groups

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

Table3: comparison of serum creatinine among the 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

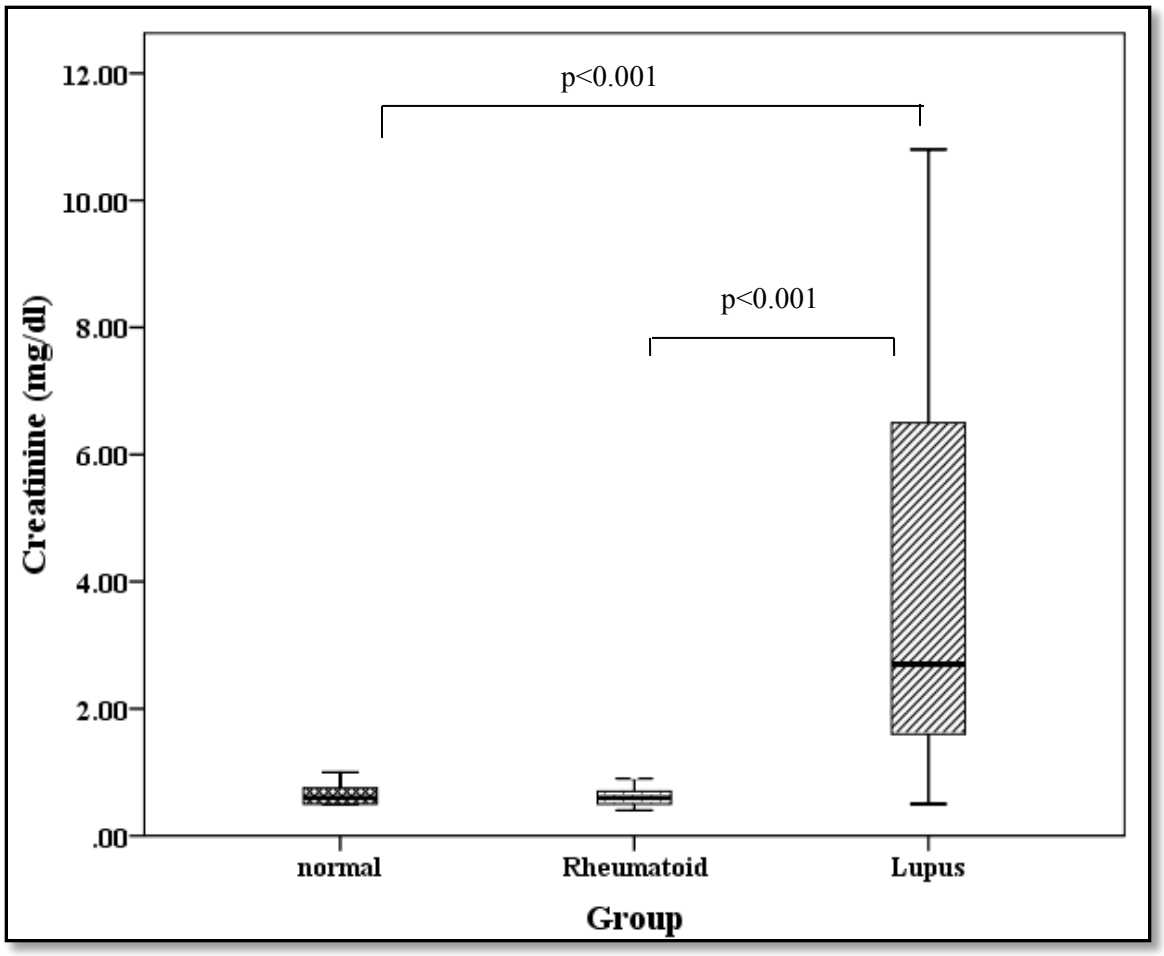

Fig. 4: box plot shows significant difference in serum creatinine levels among the different groups

Table 4 showed no significance difference in serum C3 levels between the normal and rheumatoid groups. In contrast, there was 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

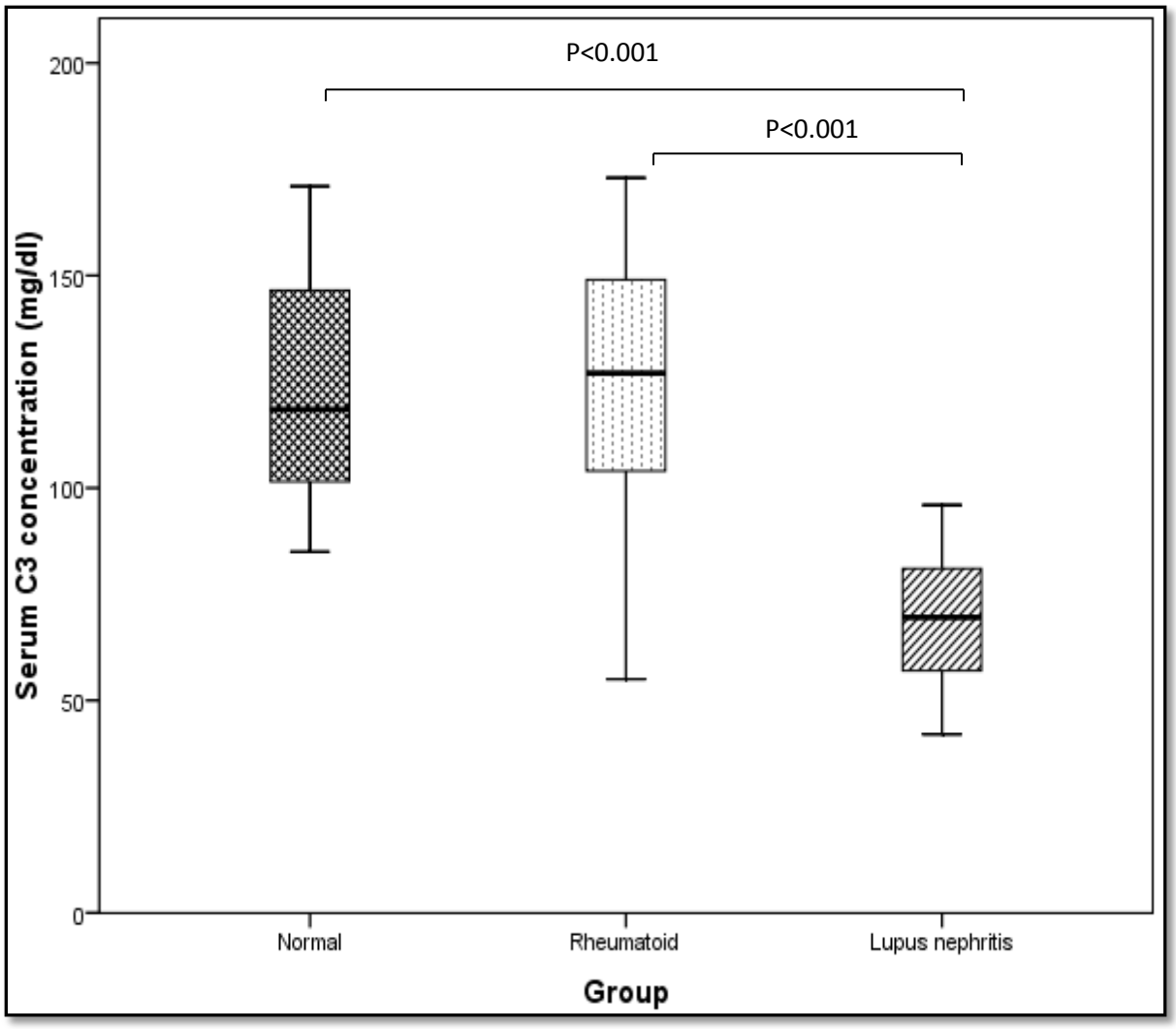

Fig. 5: box plot shows significant difference in serum C3 levels among the different groups

Table 5 showed no significance difference in serum C4 levels between normal and rheumatoid groups. In contrast, there was 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 the 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 0r p<0.001 is highly significant

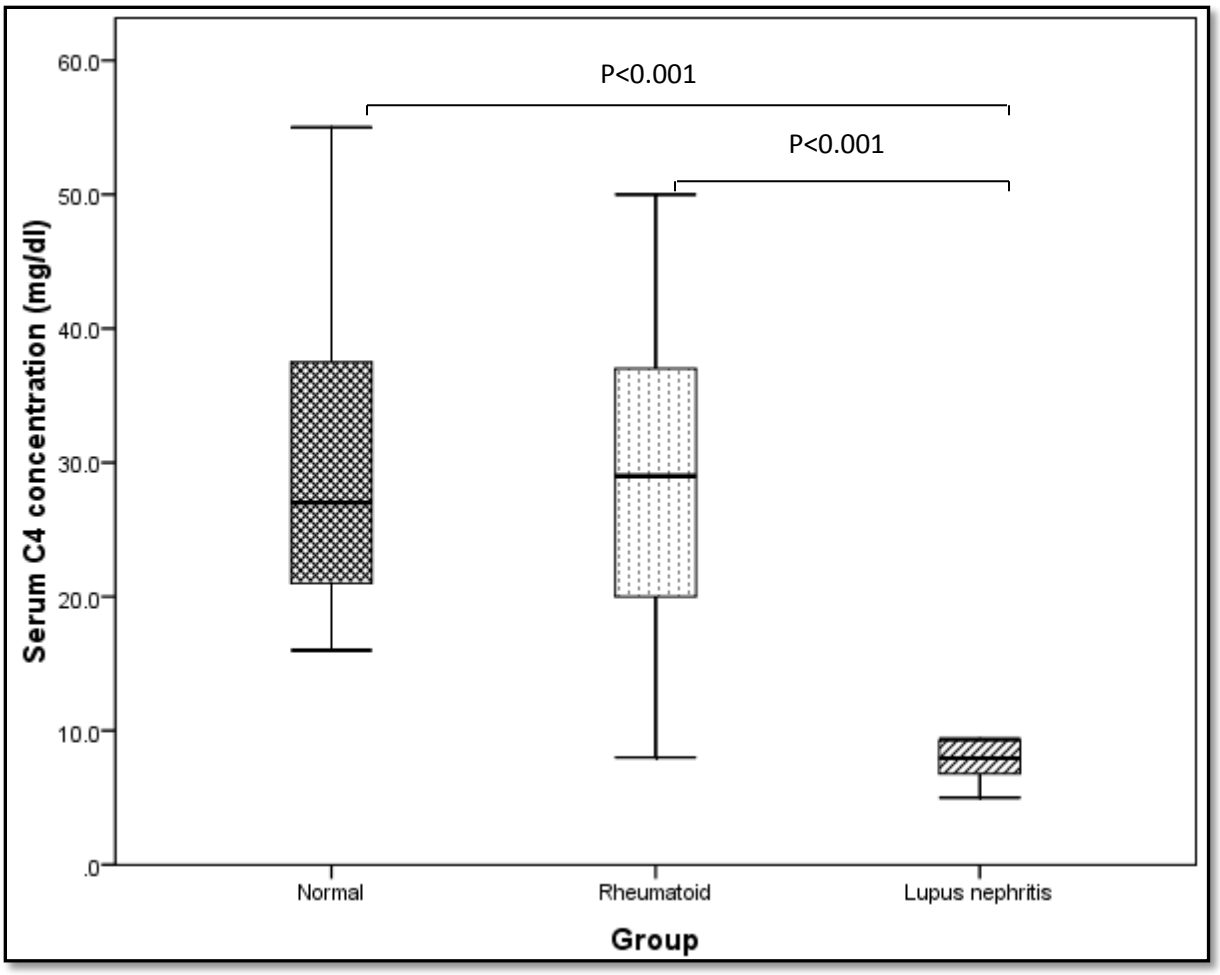

Fig. 6: box plot shows significant difference in serum C4 levels among the different groups

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

Table 6: comparison of serum anti-C1q antibody among the 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

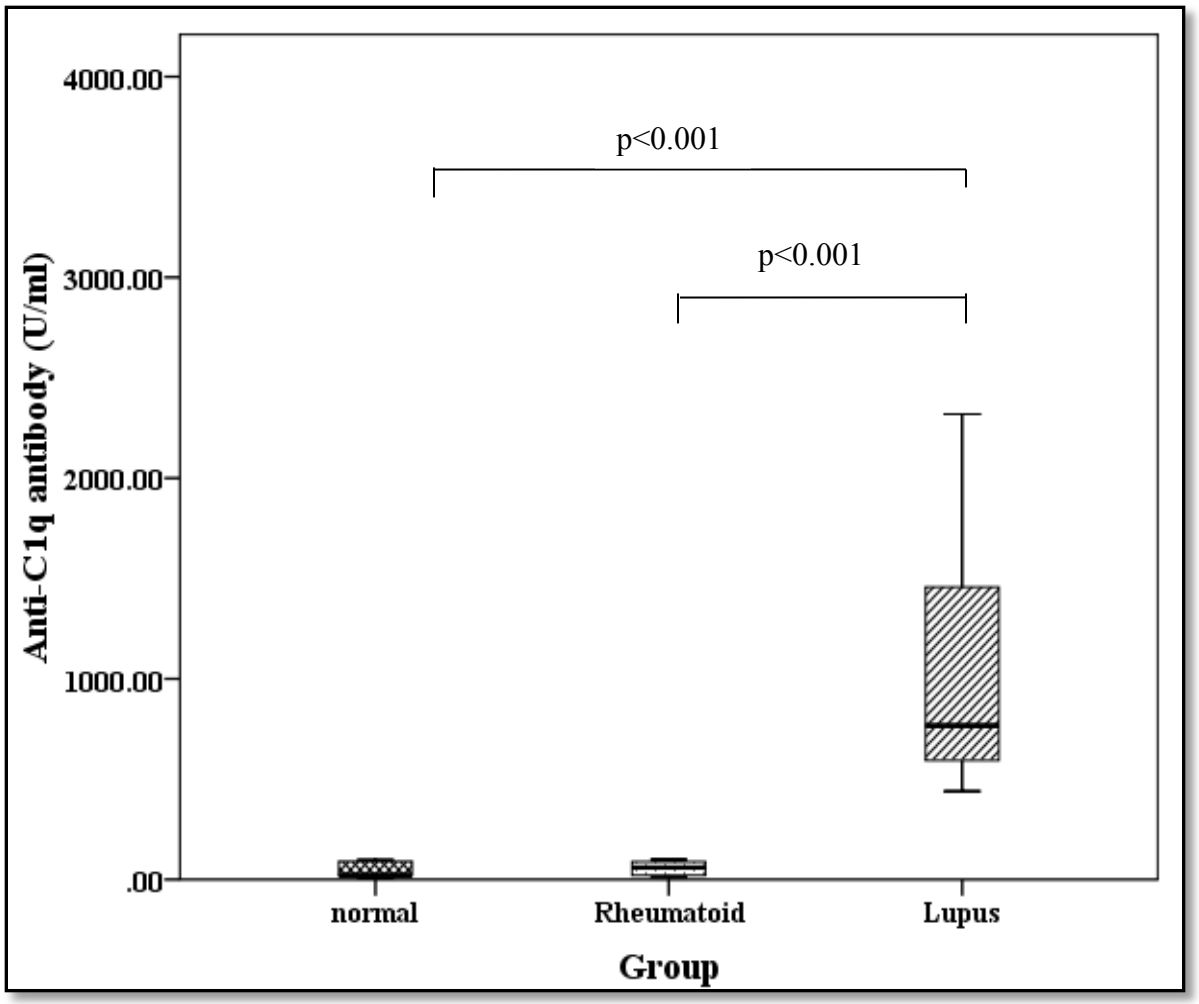

Fig. 7: box plot shows significant difference in anti C1q antibody levels among the different groups

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).

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

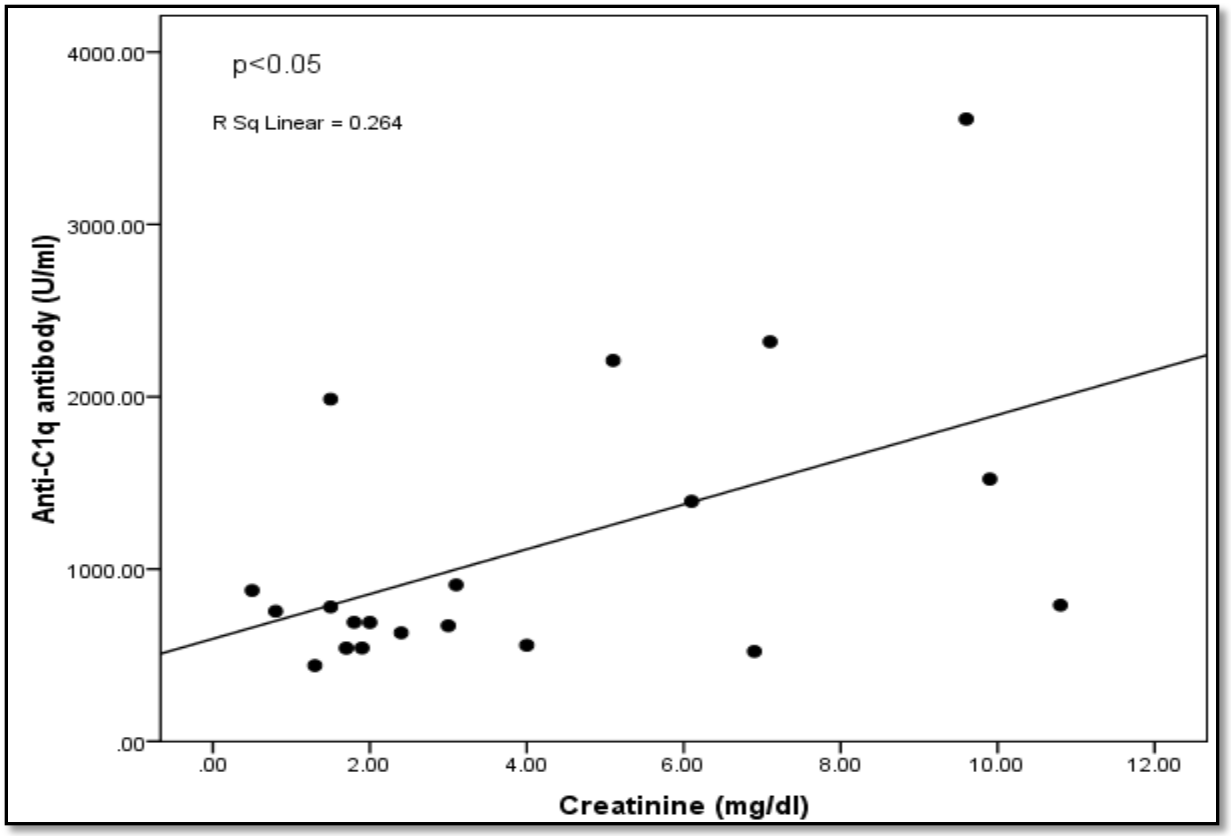

Fig. 8: correlation between serum creatinine and serum anti-C1q antibody within lupus nephritis patients group

ROC curve

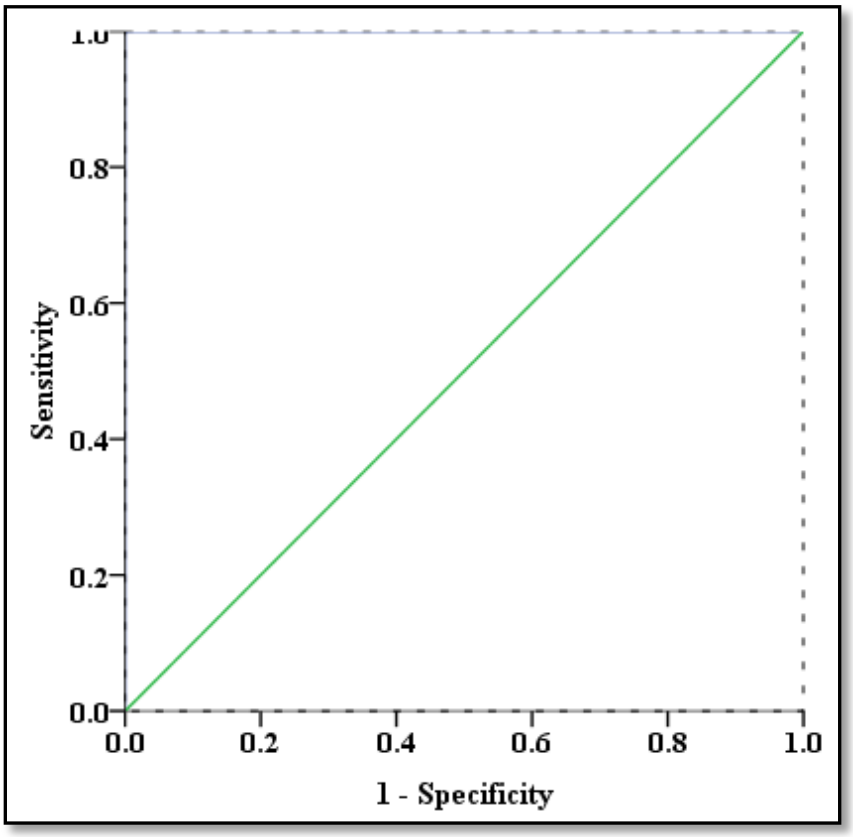

Fig. 9: ROC curve of anti C1q antibody between lupus and non-lupus patients

	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 [24].Several studies have described that anti-C1q antibody (anti-C1q), antibodies against collagen-like region of first component of the classical complement pathway [25].might be regarded as immunological markers of SLE with renal involvement in particular[26]., 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 [27].Several autoantibodies, especially those against double stranded DNA (anti-dsDNA) are believed to play a major role in the induction of glomerular inflammation [28] raised titers of anti-dsDNA and hypocomplementemia are reported to be associated with the activity of the disease [29], 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 [30].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 [31,33]. **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[34] 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 [35]. 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 [36]. 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 [36].

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 [37].

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 is in 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% (36). 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%) [38].

The present result supports the study of **Sinico *et al.*** who 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 [39].

SUMMARY AND CONCLUSION

This study aimed to investigate the 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 increased 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. 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%.

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