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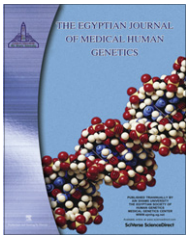

ORIGINAL ARTICLE

Prevalence and clinical significance of anti-C1q antibodies in cutaneous and systemic lupus erythematosus

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Abstract Autoantibodies against C1q are strongly linked to immune-complex disorders like systemic lupus erythematosus (SLE). Although anti-C1q antibodies have received much interest in the recent years, their biological functions remain unclear. Anti-C1q antibodies are strongly associated with lupus nephritis. The aim of this study was to determine the prevalence of anti-C1q antibodies in Egyptian lupus patients as well as to evaluate the associations between anti C1q antibodies and clinical and serologic parameters of patients with cutaneous and systemic lupus erythematosus.

Fifty-eight patients of lupus erythematosus were recruited in the study, and they were divided into 3 groups according to their clinical presentations and laboratory investigations; group (1) consists of 20 patients with musculoskeletal manifestations, mainly arthritis (34.5%), group (2) consists of 12 patients with lupus nephritis (20%), and group (3) consists of 26 patients with cutaneous lupus (44.8%). Fourteen age and sex matched healthy subjects served as controls. Complete blood picture, kidney function tests, liver function tests and anti-double stranded DNA were done for all the studied patients. Anti-C1q antibodies were determined by immunometric enzyme immunoassay for all the studied subjects.

Anti-C1q antibodies were positive in (63.8%) of lupus erythematosus (LE) patients and (0%) of controls. Moreover, the serum anti-C1q antibodies titers were significantly higher ($P < 0.001$) in all lupus erythematosus patients (both systemic and cutaneous) when compared to healthy controls. Surprisingly, serum anti-C1q antibodies were significantly higher in patients with cutaneous lupus

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than those with lupus nephritis ($P < 0.001$). Anti-C1q titers were significantly correlated with levels of anti–double stranded DNA ($P < 0.001$), as well as with proteinuria (< 0.05) in lupus nephritis patients.

It was concluded that anti C1q antibodies might play a pathogenic role in the pathogenesis of cutaneous lupus and could positively be associated with evolution to SLE. Moreover, it could predict patients who subsequently develop nephritis, thus early use of immune modulators in cutaneous lupus could improve patients' prognosis by decreasing the possibility of evolution to systemic lupus complications, mainly nephritis.

1. Introduction

Systemic Lupus erythematosus (SLE) is an autoimmune disease characterized by a broad clinical spectrum from cutaneous lesions to severe systemic manifestation. SLE is characterized by acute and chronic inflammatory lesions located either in skin (cutaneous lupus erythematosus – CLE) or in any tissue and organ such as kidney and central nervous system [1].

The complement system is one of the major effector mechanisms of the innate immune system and it plays an important role in immunity [2]. 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 [3,4]. A prolonged exposure of C1q epitopes to the immune system could eventually lead to an autoimmune response against itself [4]. Autoantibodies against C1q have been found in many different autoimmune diseases, and in 3–5% of normal individuals. Initially, anti-C1q antibodies were observed in 100% of patients with hypocomplementemic urticarial vasculitis and in 30–48% of patients with SLE [5,6]. Subsequently they were strongly linked to other immune-complex disorders such as rheumatoid vasculitis and rheumatoid arthritis [1]. Over a third of SLE patients have a high level autoantibodies–antigen complex that contains some complement proteins, especially C1q as the trigger protein in the classical complement activation pathway. So, the SLE, as an autoimmune disease, is certainly related to disorders caused by the activation of a complement system that finally leads to tissue damage [7]. The hereditary deficiency (complement genes mutations) of this component; C1q; is a known risk factor for the development of SLE [3]. In such a case, some components of the complement system might be inactivated. Anti-C1q antibodies were first identified as low-molecular weight C1q precipitins in the sera of patients with systemic lupus erythematosus over 30 years ago. Anti-C1q antibodies are strongly associated with the development of proliferative lupus nephritis, so much so that active renal inflammation in SLE patients is very unlikely if these antibodies are not present [8]. Although anti-C1q antibodies have received much interest in the recent years, their biological functions remain unclear. However, their high negative predictive value for active lupus nephritis suggests a pathogenic role in SLE patients. In addition, clearance of anti C1q antibodies by repeated plasmapheresis or C1q immunoabsorption improved the clinical status of SLE patients [9,10].

Specific events that occur during the development of systemic lupus erythematosus (SLE) can be quite variable among individual patients. In 88% of SLE patients, autoantibodies are present in an average of 3.3 years before diagnosis [11]. Identifying patterns that distinguish early clinical events in SLE and the presence of associated autoantibodies which precedes the fulfillment of clinical criteria of SLE could help in predicting the evolution of these patients to SLE [1].

Renal involvement is a serious clinical feature of systemic lupus erythematosus and can present at any stage of the disease. Although its treatment and outcome have improved, lupus nephritis is still a major contributor to morbidity [12], an increase in anti-C1q antibody titer has been suggested to be one of these autoantibodies which is able to predict renal flares in lupus nephritis so that monitoring anti-C1q might be valuable for the clinical management of SLE patients as a noninvasive biological marker. Therefore, it can influence therapeutic decisions and reduce the number of invasive procedures such as renal biopsies in patients with SLE [13].

Notably, there is increasing evidence that early diagnosis and treatment could increase SLE remission rate and improve patient prognosis. Although it has been shown that autoantibodies appear before clinical manifestations in SLE patients, currently we cannot predict which autoantibody positive subjects will eventually develop the disease. Thus, great effort should be taken in order to identify new biomarkers able to improve our diagnostic potential, from these, anti-C1q antibodies are among the most promising [14]. Although anti-C1q antibodies are expressed mainly in SLE patients with lupus nephritis [15–17], some cutaneous lupus patients expressed anti-C1q antibodies [18].

The aim of this study was to determine the prevalence of anti-C1q antibodies in Egyptian lupus erythematosus patients as well as to evaluate the associations between anti-C1q antibodies and clinical and serologic parameters of patients with cutaneous and systemic lupus erythematosus.

2. Subjects and methods

Fifty-eight lupus erythematosus patients (M/F: 3/55; mean age ± SD: 41 ± 10.3 years) were recruited in the study from those attending Rheumatology and Immunology Unit of Internal Medicine Department and Dermatology Department of Mansoura University Hospital from the period of March, 2010 to June, 2011. Patients were divided into 3 groups according to their clinical presentations and laboratory investigations; group (1) comprised of 20 SLE patients with musculoskeletal manifestations, mainly arthritis (34.5%), group (2) comprised of 12 patients with lupus nephritis (20%), both groups fulfilling at least 4 of 11 American College of Rheumatology (ACR) criteria for classification of SLE [19] but had neither skin lesions nor photosensitivity, and group (3) consists of 26 patients with cutaneous lupus (photosensitivity, malar rash and/or discoid lupus) (44.8%) who did not fulfill a

maximum of 4 out of 11 ACR criteria for SLE classification, and had neither proteinuria nor elevated serum creatinine. In addition 14 age and sex matched healthy subjects served as the control group. Subjects with positive hepatitis C antibodies, serum hepatitis B surface antigen expression and signs of acute microbial inflammation were excluded.

Complete history, physical examination and complete blood picture, kidney function tests, liver function tests, erythrocyte sedimentation rate (ESR) and urine analysis were done for all the studied patients. Renal biopsies were performed to confirm lupus nephritis by histopathology in most of the lupus nephritis patients.

All participants underwent an immunological study including anti-double stranded (anti-ds) DNA (IU/ml) and anti-C1q antibodies serum level. Anti-c1q was assayed by immunometric enzyme immunoassay supplied by Orgentec Diagnostika GmbH (Germany) [20]. Results were expressed as unit/ml (U/ml) and positive anti-C1q was considered if the serum level was more than 10 U/ml, as recommended by the manufacturer as the cutoff value. Informed consent was obtained from all participants.

2.1. Statistical analysis

The statistical analysis of data was done using *SPSS* (SPSS, Inc. Chicago, IL), program statistical package for Social Science (version 16). To test the normality of data distribution K-S (Kolmogorov–Smirnov) test was done and only significant data were revealed to be nonparametric. The description of the data was done in form of mean ± standard deviation (mean ± SD for quantitative data. Nonparametric data were expresses as median and range. For quantitative data student *t*-test was used to compare between two groups. Mann–Whitney test and Kruskal Wallis were used for non parametric data. To test the association between variables Pearson correlation co-efficient test was used. P is considered significant if $\leqslant 0.05$ at confidence interval 95% [21].

3. Results

The clinical parameters of the studied lupus erythematosus groups are described in Table 1.

The prevalence and titers of anti-C1q Antibodies were significantly ($P < 0.0001$) higher in systemic lupus erythematosus (SLE) patients than in controls (Table 2).

Anti-C1q antibodies were significantly correlated with proteinuria in lupus nephritis patients, but not with serum creatinine, serum albumin, ESR, hemoglobin, leucocytes or platelets (Table 3).

Table 1 Clinical parameters of groups of lupus erythematosus patients.

Group	Number ($n = 58$) (%)	
Group 1		
Musculoskeletal manifestations (Arthritis)	20	34.48
Group 2		
Lupus nephritis	12	20.68
Group 3		
Cutaneous lupus	26	44.82

Table 2 Comparison between prevalence and serum titers of anti-C1q antibody in systemic lupus erythematosus patients versus control.

Anti-C1q antibodies	Systemic lupus erythematosus ($n = 58$) (%)	Controls ($n = 14$) (%)	P
Prevalence	37/58 (63.8%)	0/14 (0%)	< 0.0001
Serum titers			
Median (range):U/ml	17.6 (1.5–341)	2.5 (0.9–4.2)	< 0.0001

Table 3 Association between the anti-C1q antibodies level and the laboratory parameters of systemic lupus erythematosus patients.

Laboratory parameter	Anti-C1q antibodies	
	r	P value
Serum creatinine	−0.152	NS
Proteinuria	0.504	< 0.001
Serum albumin	−0.022	NS
Erythrocyte sedimentation rate	0.951	NS
Hemoglobin	−0.191	NS
Leucocytes	0.271	NS
Platelets	−0.254	NS
Anti-ds DNA titer	0.459	< 0.001

Table 4 Relation between anti-C1q and anti-double stranded DNA in lupus erythematosus patients.

Anti-ds DNA	Systemic lupus erythematosus number/total (%)	Anti-C1q antibodies median (range)	P
Positive	36/58 (62.1%)	34.0 (2.7–341)	
Negative	22/58 (37.9%)	6.7 (1.5–38)	< 0.0001

Table 5 Relation between anti-C1q antibodies and clinical parameters in LE patients.

Parameters	Anti C1q (U/ml)		P
	Median	Range	
Arthritis (20/58)	4.65	1.5–22	
Nephritis (12/58)	31.05	11–46.8	< 0.0001
Skin (26/58)	36.0	12.5–341	

Moreover, there was statistically significant positive correlation ($P < 0.001$) between titers of anti-C1q antibodies and anti-double stranded-DNA titers (Table 3). Furthermore, the prevalence of anti-C1q antibodies was significantly higher in lupus erythematosus patients who were positive for anti-double stranded DNA ($P < 0.0001$) in comparison to negative anti-ds DNA patients (Table 4).

Surprisingly, anti-C1q antibodies titers were significantly higher in patients with cutaneous lupus when compared to lupus nephritis patients and systemic lupus erythematosus with musculoskeletal manifestations, (the median values were 36.0, 31.05, and 4.65 U/ml respectively, $P < 0.0001$ for all). Data are shown in Table 5 and Fig. 1.

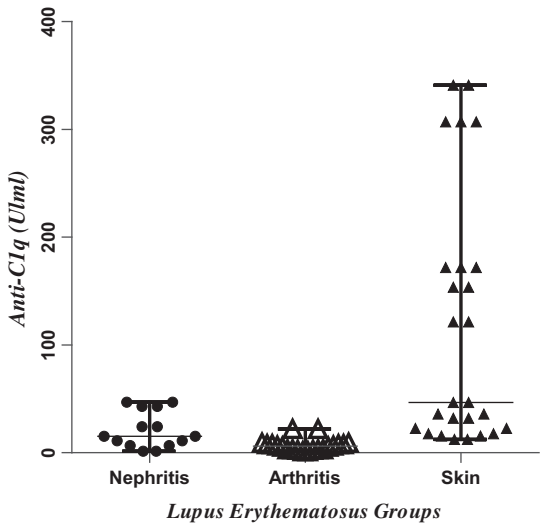

Figure 1 Anti-C1q antibodies in different groups of lupus erythematosus.

4. Discussion

In the present study it was observed that there was significant relation between serum anti-C1q antibodies and proteinuria in lupus nephritis patients, on the other hand there was no correlation between anti-C1q antibodies and serum creatinine, serum albumin, complete blood picture parameters as well as ESR. These results were in accordance with previous studies that confirmed correlation between anti-C1q antibodies and lupus nephritis [15–17,22] and in parallel with a recent report by Fang et al. [23]. However, this previous study demonstrated significant correlations with creatinine and leucopenia [23]. This discrepancy in results may be ascribable to differences in patients' population and anti-C1q antibodies assays.

Moreover, a significant positive relation was found between anti-C1q antibodies and positivity as well as titers of anti-ds DNA. These results were consistent with those from a study by Mok et al., who demonstrated that the anti-C1q antibodies were correlated to positive anti-ds DNA and also confirmed that anti-C1q antibodies were more specific than anti-ds DNA for concurrent both active renal and extra renal lupus, and that the absence of both anti-ds DNA and anti-C1q antibodies had a high negative predictive value for renal activity [24].

Interestingly, in the present study it was found that in cutaneous lupus patients, serum levels of anti-C1q antibodies were statistically significantly higher when compared to other lupus groups as well as to controls, although some previous studies detected no difference between serum anti-C1q antibody in systemic lupus and cutaneous lupus manifestations [12,13,25], in which the number of patients recruited with cutaneous manifestations was relatively small. A recent study by Eugenia and colleagues stated that anti-C1q antibodies might play a pathogenic role in subcutaneous lupus erythematosus (SCLE) pathogenesis and being positively associated with cutaneous apoptosis markers and might be associated with a negative prognosis and secondary SLE development [18]. Thus, the present study suggests that anti-C1q antibodies besides their good negative predictive value they have for lupus nephritis in SLE patients, might have a positive predictive value for cutaneous lupus evolution to SLE, especially lupus nephritis. This suggestion was supported also by a previous study by Heilen et al. who found that the clinical features that were observed earliest in lupus patients were discoid rash and seizures, and that anti-double-stranded DNA antibodies were associated with renal disease and appeared before evidence of nephritis in most patients. This means that the development of organ-associated autoantibodies generally precedes the appearance of their associated clinical features [1].

In recent years, some therapeutic options have emerged as appropriate interventions for early SLE treatment such as antimalarials, vitamin D, statins, and vaccination with self derived peptides. All these immune modulators seem to be particularly useful when introduced in an early stage of the disease [14]. We suggest that these immune modulators could be used also early in cutaneous lupus to decrease the possibility of evolution to SLE.

In conclusion, our results confirmed that anti-C1q antibodies are present in a significant percentage of cutaneous lupus and in SLE patients with active renal involvement, suggesting that these antibodies might play a pathogenic role in the pathogenesis of cutaneous lupus and that it could be a useful additional marker for early diagnosis. Moreover, it could be of interest for monitoring and follow-up of cutaneous lupus erythematosus (CLE) patients and in predicting those at risk of subsequent renal involvement or flare. Thus, besides the good negative predictive value that these antibodies have for lupus nephritis in SLE they may have a positive predictive value for cutaneous LE evolution to SLE. So, determination of the level of anti-C1q antibodies in serum specimens could have not only a great diagnostic importance but also have a good therapeutic challenge if used early in the disease. Further large-scale multicentre prospective studies are needed to elucidate the clinical significance of anti-C1q antibodies in the prognosis of cutaneous lupus and to explore the possibility of its role in evolution to systemic lupus erythematosus and validate the importance of early immune modulators treatment in cutaneous lupus to improve patients' prognosis and decrease subsequent development of systemic lupus erythematosus complications, mainly lupus nephritis.

Conflict of interest

The authors declare that there is no conflict of interest.

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