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Association of anti C1q and ds-DNA levels with the pathogenesis of Lupus Nephritis among SLE patients

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Abstract

Background: Lupus nephritis (LN) is the most common and serious complication associated with SLE and it results in significant morbidity and mortality. It is known by several studies that patients of LN have higher levels of anti-dsDNA and anti-C1q compared with SLE patients without renal involvement. The current study was designed to determine and compare the level of anti-dsDNA and anti-C1q in patients of SLE with and without lupus nephritis in the Pakistani population. This current study was also aimed at providing proof that anti-C1q levels are more prominent in LN/non-LN SLE as compared to anti-dsDNA. This project may help in the determination of results in Pakistan and contribute to the further confirmation of the sensitivity of anti-C1q.

Method: The patient samples were collected from Sheikh Zayed hospital, Lahore. These patients were clinically diagnosed by the Rheumatologists as SLE and LN positive on the basis of ACR and SLEDAI scoring criteria. This study was performed and samples were analyzed in the Department of Medical and Laboratory Sciences, Imperial College of Business Study, Lahore on the patient's serum by ELISA technique.

Result: About 38% (12) patients with LN were positive for anti-dsDNA and 31% (9) SLE patients without LN were positive whereas about 38.7% (12) were anti-dsDNA negative in LN cases and 58.6% (17) in SLE without LN. In case of anti-C1q 100% (31) of these LN patients were positive and 93.1% (27) patients SLE without LN showed positive anti C1q results. Only 6.9% (2) patients showed negative results for anti-C1q in LN negative patients

Conclusion: The higher levels of anti-C1q suggest that it may be a better diagnostic marker for LN than that of anti-dsDNA and that it can be helpful in the prognosis of SLE patients.

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Systemic Lupus Erythematosus, Lupus Nephritis, ELISA



Introduction

Autoimmunity is the failure of an organism in recognizing its own constituent parts as self, thus leading to an immune response against its own cells and tissues [1]. The loss of self-tolerance due to inadequate deletion of central and peripheral auto-reactive lymphocytes lead to various autoantibody formations is the cause of autoimmunity [2, 3].

Systemic lupus erythematosus (SLE) is an autoimmune disease that affects multiple organs of the body. It exhibits low grade inflammation and may lead to fatal damage to different organs [4]. The incidence of SLE among women is greater than males. The overall worldwide prevalence of SLE ranges from 7.4 in males to 159.4 in females per 100,000 people respectively [5]. The pathogenesis of SLE involves a multitude of cells and molecules that participate in apoptosis, innate and adaptive immune responses [6]. SLE patients exhibiting abnormal renal function are indicative of lupus nephritis. The changes in the renal morphology of SLE patient's biopsy show a wide range of vascular, glomerular and tubulointerstitial lesions. It is the most common cause of death among the SLE patients around the world [7]. Renal disease may also be manifested by an increased serum creatinine level, or by the presence of hematuria, pyuria, or both. The urine protein-to-creatinine ratio has been found to be a reliable measure of proteinuria in LN [8].

The early part of the classical pathway of complement system plays an important role in the protection of humans against SLE [9]. Studies have suggested that C1q plays an important role in the clearance of apoptotic cells, processing of immune complexes from tissues and host defence against infections [10]. IgG mainly binds to the globular portion of C1q whereas anti-C1q antibodies bind to the collagenous portion of C1q and this collagenous part is considered as the immunogenic part of the molecule [11]. Hereditary deficiency of C1q is a high risk for the development of SLE, but on contrary, SLE itself causes consumption of C1q in the majority of patients who are not C1q deficient by birth [12].

A significant association has been established between the low levels of C1q presence of anti-C1q antibodies. But unlike C1q other complements such as C3 and C4 have varying levels in different SLE patients. Most of the observations suggest that the levels of C3 and C4 decrease quite significantly in SLE patients. It can be

concluded that in SLE, these autoantibodies are produced in response to activation of classical pathway and against the collagenous part of C1q complement. This autoantibody is also found in patients with hypocomplementemic urticarial vasculitis, and mesangiocapillary glomerulonephritis [13]. Antibodies to double-stranded DNA (Anti-dsDNA) are a group of anti-nuclear antibodies and their target antigen is double stranded DNA. Anti-dsDNA is found in 40-60% of SLE patients, they are associated with renal involvement but do not correlate well with disease activity [14]. One possible mechanism for anti-dsDNA and their role in nephritis is the formation of immune complexes that arise by indirect binding to DNA or nucleosomes that are adhered to glomerular basement membrane (GBM) [15, 16].

Different studies have presented contrasting results, which make the role of these antibodies controversial in the onset of LN [18]. Sinico *et al* demonstrated high levels of anti-C1q and anti-dsDNA in LN [19]. Whereas Yang *et al* reported around 39.7% of LN patients had both anti-C1q and anti-dsDNA simultaneously whereas 14.7% patients had none of them [20].

A mounting body of evidence suggests that these autoantibodies play pivotal role in the pathogenesis of SLE and LN, yet their role is defined poorly. Moreover, it is hypothesized that increased levels of anti-dsDNA and anti-C1q are associated with prognosis of LN. Hence, the current study is designed to determine the level of these antibodies and establish its possible correlation with disease severity in patients of SLE with and without LN.

Methods

The samples for this study were obtained from Shaikh Zayed Hospital Lahore and they were processed in the lab of Imperial Business College. It was a Cross-Sectional Comparative study and convenient sampling technique was used. A total of sample size was 60 patients in two groups. The first group had SLE patients and the second group had LN patients, both type of patients were clinically diagnosed by the clinician. Both groups included diagnosed case of SLE according to American College of Rheumatology criteria (ACR criteria) and Systemic lupus erythematosus disease activity index (SLEDAI) and excluded pregnant and other autoimmune disorder patients.

Three (3) ml blood was drawn in gel vacutainer (without anticoagulant) from each patient and after centrifugation serum was separated and stored at -20°C until the tests were performed. The samples were then processed and analysed in the lab and levels of anti-C1q and anti-dsDNA (positive = $\geq 30\text{U/ml}$) A (positive = $\geq 75\text{U/ml}$) were estimated via ELISA technique) kits for anti-C1q (Cat.no.E0344Hu, BIOASAY technology) and anti-dsDNA (Quanta lite 704650, INOVA diagnostics). These kits were based on the indirect ELISA method; Diluted patient serum was added to wells and incubated. All unbound materials were washed away. After that an enzyme conjugate was added which bonded to the antibody antigen complex. Excess enzyme conjugate was washed off and TMB Chromogenic substrate was added. The enzyme conjugate catalytic reaction was stopped at a specific time. The intensity of the color generated was thus proportional to the amount of bound target antibody. The results were read by a micro-well reader on BIO-RAD microplate reader Model 550.

Statistical Analysis

Mean \pm SD was given for quantitative variables e.g. age. Qualitative variables such as sex and age, they were expressed as frequencies and percentages. Two independent 't' test was applied to compare serum anti-C1q and anti-dsDNA levels with LN. Mann-Whitney U test was applied for comparison between anti-C1q and anti-dsDNA. A p-value of ≤ 0.05 was considered as statistically significant. The level of significance in this study was 5%.

Results

The frequency and percentage of males and female patients having LN are shown in table 1. The results show that 97% (3) males were positive for LN out of total 4 male patients whereas 90% (28) of females out of 56 patients who showed positive LN. The remaining 96.6% (28) showed LN negative.

Frequency and P- value of Anti-C1q

Frequency and percentage of anti-dsDNA and anti -C1q in both groups of SLE with and without lupus nephritis were seen. Whereas 100%(31) of these LN positive patients showed positive result for anti-C1q and 93.1% (27) patients without LN showed positive anti -C1q results. Only 6.9% (2) patients showed negative results

for anti c1q in LN negative patients, these results are shown in table 1. Table displays the values of anti-C1q antibody in LN. Table shows the median value of 108.653 (0.11-424) and a p-value of 0.013 for anti-C1q which showed significance in LN patients as compared to anti-dsDNA.

Variables	LN(n=30)	SLE (n=30)	P-value
Age (Mean \pm SD)	27.95 \pm 9.78	26.24 \pm 7.53	0.458
Gender			
Male	3 (97%)	1 (3.4%)	--
Female	28 (90%)	28 (96.6%)	--
Autoantibody			
Anti-c1q	31(100%)	27 (93%)	0.013*
Anti-dsDNA	12 (38%)	9 (31%)	0.314
Total (N=60)	43 (71.6%)	36 (60%)	--

Table 1: Diagrammatic distribution of anti-c1q and anti-dsDNA with SLE and LN

Parameter	Median	IQR
Anti-C1q	108.653	0.11-424
Anti-dsDNA	39.85	0.73-2091

Table 2: Median, IQR and P-value of Anti C1q and Anti -dsDNA antibody in SLE patient with LN

Frequency and P-value of Anti-dsDNA

The table 1 showed that 38% (12) patients with LN were positive for anti-dsDNA and about 38.7% (12) were anti-dsDNA negative, The percentage for SLE patients with positive anti-dsDNA were 31% (9) and 58.6% (17) of these patients showed negative anti-dsDNA results. Table 2 shows median values and p value of anti-dsDNA antibody in LN patients .This table shows the median value of 39.85 (0.73-2091) and a p value of 0.314 which was insignificant for anti-dsDNA levels.

Table 3 shows the frequency and percentage of complement component C3 and C4 levels in LN positive patients and LN negative patients. LN positive patients with lower of C3 were found to be 25.8% (8) with the majority of patients with having 64% (20) having normal C3 levels. Similarly patients belonging to LN negative group showed that 34% (10) had low levels of C3 and the rest of 55% (16) showed normal C3 levels. Patients without LN showed 41.4% (12) low results for C4 and 48.3% (14) showed normal results, whereas patients of LN positive patients showed 34.3% (10) patient to have low C4 levels and remaining 58% (18) showed normal C4 levels.

Variable	C3	C4
LN	8 (25.8%)	10 (34.3%)
SLE	10 (34 %)	12 (41.4%)

Table 3: Variables frequency of C3 and C4 with LN and SLE

Discussion

This cross sectional study was performed to determine the levels of anti-C1q and anti-dsDNA in SLE patients with (and/or without) referral to lupus nephritis. The targeted aim of this study was to understand the relevance of anti-C1q as a better predictive and diagnostic marker for SLE/LN patients in comparison to anti-dsDNA. Many studies in the past decade showed that anti-C1q has a better diagnostic value as compared to anti-dsDNA, which has been used as a gold standard for SLE diagnosis for several years. This current study comes in agreement with numerous studies but simultaneously shows disagreement with a few too with respect to the percentages of results in SLE patients. This may be due to ethnicity, region and gender differences, which can be further studied in the future.

In the current study, there were 56 (93.3%) females and 4(6.6%) males. This number is in agreement with Leung *et al.*, and Rasheed *et al.*, who have documented 92.7% females and 7.3% males, and 95.7% and 7% males respectively [21, 22]. The number of males was higher in group-II patients with LN 3 (75%) as compared to group-I, 1(25%) whereas the frequency of females was equal in both groups 28 (50%), thus it is evident that number of female SLE patients is more than males. This may be due to the hormonal differences between males and female that makes female more prone to SLE. Similarly in a study conducted by Ding *et al.*, it is suggested that out of the total number of SLE patients enrolled in their study 458 were females and only 58 were male patients; therefore we suggest that this female to male ratio of affected patients is in accordance to this present study [23].

In the current study it was seen that anti-C1q levels showed significant results in both types of SLE patients. All LN patients were positive for anti-C1q [with p value (0.013)], whereas in patients without Nephritis 93.1% showed positive anti-C1q, and 6.9% showed negative anti C1q. Picard *et al.*, agrees with these results suggesting that anti-C1q levels remain high in patients of SLE with and without LN [24].

In case of anti-dsDNA, 31% patients showed positive results whereas 58.6% showed negative results.

Furthermore, 38.7% LN patients showed anti-dsDNA positivity {with p value (0.314)} and equal percentage of 38.7% to show negative anti-dsDNA levels in LN patients. Therefore with regards to anti-C1q levels, this study is in agreement with Omrana *et al.*, which showed that anti-C1q has a 92.7% sensitivity in SLE patients and a specificity of 94.12% respectively [25]. Similarly in another study conducted by Birmingham *et al.*, he results showed that anti-C1q were significantly higher in patients with lupus nephritis in the state of flare with 63% positive results. Hence the study of Birmingham *et al.* is not in agreement with this study as difference in the percentage of positivity are far apart [26]. This varying difference between the percentages depends on the consideration of active flared LN in Birmingham's study and general LN selection in the present study.

The current study further suggests that the levels of anti-C1q are significantly higher compared to anti-dsDNA in SLE patients having renal discrepancy as well as without renal involvement. Therefore this study can be backed by Picard *et al* findings, which suggest a 73% to 19% ratio between anti-C1q and anti-dsDNA [24]. However with respect to the levels of anti-dsDNA patients with SLE and nephritis, Radanova *et al* study in 2015 does not come in agreement with the this study. Radanova's study claims a 56.6% level of anti-dsDNA in LN patients whereas we suggest the levels to be only 38.8% [27]. This difference between both the studies may be due to the reason that Radanova's study is based on three stages of lupus nephritis flare in patients where as our study had no such criteria.

The patients C3 and C4 levels in present study were 64% and 58% respectively falling in the normal range, whereas 25.8% and 34.3% respectively were below normal range. So this way our study shows no agreement with Li *et al* which showed that the C3 levels were lowered in LN patients whereas C4 remained normal. This difference in results between the present study and the study by Li *et al* may be due to difference in patient number and the ethnicity differences [28].

Levels of creatinine and 24 hour urinary proteins are indicative of renal insufficiency and thereof we determined these levels in patients with SLE. These patients at some stage of disease do develop lupus nephritis and increase in the levels of creatinine and proteins in urine. This is due to inefficient clearing, therefore in the current study all the enrolled patients of

lupus nephritis showed elevated levels of creatinine and 24 hour urinary proteins. Hence these high levels of creatinine and 24 hour urinary are in agreement with Rasheed *et al* and Mok *et al* who also described that these high levels are indicative of lupus nephritis in SLE patients [22, 29].

Therefore based on our findings we conclude that anti-C1q levels are higher in SLE patients as well as in lupus nephritis patients in comparison with anti-dsDNA, hence it is suggestive that anti-dsDNA can be replaced by anti-C1q as a predictive marker for SLE with and without lupus nephritis. It is also suggested that anti-C1q is a better and more effective tool for the control of SLE as treatments can be initiated before the disease enters into a phase of flare. The indication of anti-C1q in patient's serum may be suggestive of SLE prevalence and may also pin down the disease progressing towards lupus nephritis. Even though anti-dsDNA was found in SLE patient but its lower levels in SLE is suggestive that it may be of least importance with regard to application as a diagnostic marker. Instead we conclude that anti-C1q could be a more reliable and accurate diagnostic marker for lupus nephritis.

Conflict of Interest Statement

The authors declare that there is no conflict of interest regarding the publication of this paper.

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