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ORIGINAL ARTICLE

The Relation between Plasma Complement 4 d and Lupus Nephritis at Zagazig University Hospitals.

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ABSTRACT

Background: Lupus nephritis is a common and serious manifestation of systemic lupus erythematosus,its diagnosis is established by a combination of clinical ,laboratory, serological data and histology of the kidney. Plasma complement 4d can be used to detect disease activity of lupus nephritis. Aim of the work: The aim of our work was to assess the relation between plasma complement 4 d and lupus nephritis. **Methods:** This study was carried out on 100 Lupus Nephritis patients fulfilled the Systemic Lupus International Collaborating Clinics (SLICC) classification criteria and 100 apparently healthy age & sex matched controls. Plasma complement 4 d was detected by specific sandwich ELISA according to the instruction of the manufacturer**. Results:** Our work showed that 24 hour urine protein,RBCs in urine and pus in urine have significant positive correlation with plasma C4d (unstandardized β=0.001, -0.213, 0.041 and 0.024 respectively) ($p<0.05$). Our results also revealed that there is statistically significant positive relation between plasma c4d and lupus nephritis activity in SLE patients ($p<0.05$). **Conclusions:** The study evaluated the importance of plasma complement 4d in lupus nephritis and confirmed that plasma complement 4d can be used as a very good marker of lupus nephritis activity.

Key words: Systemic Lupus Erythematosis, Lupus nephritis,Complement 4 d.

INTRODUCTION

Systemic Lupus Erythematosus is an inflammatory disease of insidious onset that is predisposed by an auto antibody response to nuclear and cytoplasmic antigens, has a relapsing remitting course and a wide range of prognosis. Women of childbearing period have the highest incidence and prevelance of disease [1]. The etio-pathogensis of SLE involves incorporation of environmental, genetic risk factors and immune system disregulation; including defects of apoptotic cell clearance, disturbance of innate and acquired immunity, production of autoantibodies, complement activation with subsequent organ damage **[2].**Lupus nephritis is a common manifestation of SLE. It is caused by formation of immune complexes which deposit on the mesangium, subendothelial, and/or subepithelial space related to the glomerular basement membrane of the kidney. This leads to an inflammatory process ,in which the complement pathway is activated with the onst of flow of neutrophils and other inflammatory cells as a result [3]. Lupus nephritis is clinically evident in 50-60% of patients with systemic lupus erythematosus (SLE), but is histologically present in most SLE patients, even if there are no clinical manifestations of renal disease [4]. Regarding C4 itself, SLE is related to reduction of total C4 copy number, but increased number of copies is a protective factor .Approximately 50% of SLE-C4-deficient develop glomerulonephritis**[5].**Complement 4d is formed when complement component 4 is splitted into C4a and C4b;C4b is after that cleaved into its inactive products C4c and C4d,the characteristic property of C4d is that it contains a thiol ester site ensuring it remains stably bound to endothelial cell surfaces and extracellular matrix components of vascular basement membranes close to the sites of C4 activation [6]. Glomerular-C4d (G-C4d) deposition

is related to lupus nephritis activity as lupus nephritis pathogenesis involves immune complex deposition which strongly activates complement cascade and gives rise to C4d. So,C4d levels correlate with lupus nephritis activity [7]. As lupus nephritis has poor prognosis regarding both morbidity and mortality and C4d is an end product of C4 after its activation in the pathogenesis of lupus nephritis so they are inversely proportional with each other, C4 consumption in lupus nephritis activity causes decreasing its value but increasing C4d level [8].Thus, the aim of our study was to confirm the role of plasma complement 4 d in the detection of lupus nephritis activity to facilitate and accelerate good management of the patients

METHODS

Study design and subjects: This is a case-control study performed on two groups: **Group1** (Lupus Nephritis group), It included 100 female patients with systemic lupus erythematosus (SLE),who fulfilled the ACR/ SLICC revised criteria for classification of SLE and have lupus nephritis activity [9]. Their ages ranged from 19-48 years with a mean of 30.56 ± 8.37 years,disease duration ranged from 1 – 15 years. Group2 (control group), It included 100 apparently healthy female volunteers. Their ages ranged from 19-48 years with a mean of 30.56 ± 8.3. Approval of Institutional Review Board (IRB) was taken and all participants signed an informed written consent before enrolment in the study and the study design was approved by the Ethical Committee of Faculty of Medicine, Zagazig University.The work has been carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Clinical examination and disease activity: Our patients were generally examined for presence of: Loin Pain, Skin rashes, oral ulcers, alopecia ,lupus hair, cardiac examination for pericardial rub (effusion) and chest examination for crepitation and pleural rub (effusion), after that assessed for disease activity using SLEDAI-2K score [10]. Activity categories defined on the basis of SLEDAI scores [11]

- No activity (SLEDAI; 0),
- Mild activity (SLEDAI; 1–5),
- Moderate activity (SLEDAI; 6–10),
- High activity (SLEDAI; 11–19),
- Very high activity (SLEDAI ≥ 20)

Laboratory investigations: In the form of Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), Creatinine clearance, protein in 24-hour urine collection, urine analysis, ANA, Anti-dsDNA antibodies titre and plasma complement 4 d levels in both patients and control.

Plasma complement 4 d measurement: Plasma complement 4 d was measured by specific sandwich ELISA according to the instruction of the manufacturer with test principles were**:** The kit uses a double-antibody sandwich (ELISA) to assay the level of Complement 4d (C4D) in samples. Add (C4D) to monoclonal antibody Enzyme well which is pre-coated with Human (C4D) monoclonal antibody, incubation; then, add Complement Fragment 4d (C4D) antibodies labeled with biotin, and combined with Streptavidin-HRP in order to form immune complex; then carry out incubation and washing again to remove the uncombined enzyme. After that add Chromogen Solution A, B, the color of the liquid becomes blue, and by acid, the color changes to yellow at the end. The chroma of color and the concentration of the Human (C4D) of sample were positively correlated. Plasma Collected from blood by venipuncture, sample is coagulated at usual room temperature 10-20 minutes, centrifugation 20-min at the speed of 2000-3000 r.p.m. & supernatant stored at -20° C (avoid repeated freeze-thaw cycles).

STATISTICAL ANALYSIS

Analysis of data was done using the software SPSS (Statistical Package for the Social Sciences) version 20. Describing of quantitative variables using their means and standard deviations. Describing of categorical variables using their absolute frequencies and were compared using Chi square test and Fisher exact test when appropriate. Kolmogorov-Smirnov (distribution-type) and Levene (homogeneity of variances) tests were used to verify assumptions for use in parametric tests. Mann whitney test (used with non-normally distributed data) was used to compare medians of two groups and independent sample t test (used with normally distributed data) were used to compare means of two groups. To compare medians of more than two groups over time, Kruskal Wallis test was used. Spearman rank correlation coefficients was used to assess strength and direction of a linear relationship between two variables. Using ROC curve to determine the best cutoff of a certain marker for diagnosis of a certain health problem. Using linear regression analysis to show the extent to which there is a linear relationship between a dependent variable and one or more independent

variables. The level of statistical significance was set at 5% (P<0.05). Highly significant difference was present if p≤0.001.

RESULTS

Our work showed that there is non-significant difference among the studied groups regarding age or gender **Table (1),** it showed that there is highly significant difference between the studied groups regarding plasma C4d (higher among lupus nephritis group) **Table (2).** Our work concluded that 24 hour urine protein,RBCs in urine, pus in urine were (significantly positively associated) with plasma C4d (unstandardized β=0.001, -0.213, 0.041 respectively) ,but serum albumin is negatively associated with it **Table (3)** The study revealed that there is non-significant relation between plasma complment 4 d and ESR in studied group **Table (4)** ,on the other hand there is significant positive relation between disease activity and C4d among the studied patients,(c4d value increases with increasing the disease activity)**Table (5).**Our study also showed that there is non-significant relation between disease activity and anti-dsDNA among the studied patients,(p>0.05).**Table (6)** The work showed that the best cutoff of plasma C4d in diagnosis of lupus nephritis activity among the studied patients is ≥0.342 with area under curve 0.77, sensitivity 84%, specificity 76%, positive predictive value 77.8%, negative predictive value 82.6%, , accuracy 80%(p<0.05) **(Figure 1).**

Table (1) Comparison between the studied groups regarding demographic characteristics:

demographic characteristics	Groups		Test	
	Lupus Nephritis group N=100(%)	Control group N=100 (%)	t	P
Gender: Female	100 (100)	100 (100)		
Age (years): Mean ± SD	30.56 ± 8.37	30.56 ± 8.37	-0.375	0.708
Range	19 - 48	19 – 48		

t Independent sample t test

Table (2) Comparison between the studied groups regarding plasma C4d:

Plasma C4d	Groups		Test	
	Lupus nephritis group N=100 (%)	Control group N=100 (%)	Z	p
Median	0.347	0.131		
Range	0.01 – 4.883	0.009 – 2.736	-4.69	<0.001**

Z Mann Whitney test **p≤0.001 is statistically highly significant

Table (3) linear stepwise regression analysis of factors significantly associated with plasma C4d among patients with lupus nephritis:

Model	Unstandardized Coefficients		Standardized Coefficients	t	P
	β	SEM	β		
24 hour protein in urine	**0.001**	**0.000**	**1.154**	**28.263**	**<0.001****
RBCs in urine	**-0.213**	**0.046**	**-0.352**	**-4.646**	**<0.001****
Pus in urine	**0.041**	**0.014**	**0.241**	**2.921**	**0.01***
Serum albumin (g/dL)	**0.441**	**0.076**	**0.289**	**5.779**	**<0.01****

(**p≤0.001 is statistically highly significant *p<0.05 is statistically significant)

Table (4) correlation between plasma C4d and ESR:

Parameters	Plasma C4d	
	r	P
ESR	0.137	0.173

*p value more than .05 is non significant

Table (5) Relation between C4d and disease activity among the studied patients:

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Flare	C4d	
	Median (range)	P
No (n=34)	0.237 (0.01 – 4.883)	
Mild (n=18)	0.052 (0.028 -0.523)	<0.001**
Moderate (n=14)	0.464 (0.348 – 4.48)	
High (n=24)	0.411 (0.251–2.488)	
Very high (n=10)	1.672 (0.01 – 4.883)	

KW Kruskal Wallis test **p≤0.001 is statistically highly significant

Table (6) Relation between anti dsDNA and disease activity among the studied patients:

Disease activity	Anti dsDNA		Test	
	Negative	Positive	χ^2	p
	N=45(%)	N=55(%)		
No	16 (35.6)	18 (32.7)		
Mild	8 (17.8)	10 (18.2)		
Moderate	8 (17.8)	6 (10.9)	1.99	0.15
High	10 (22.2)	14(25.5)		
Very high	3 (6.7)	7 (12.7)		

χ2 Chi square for trend

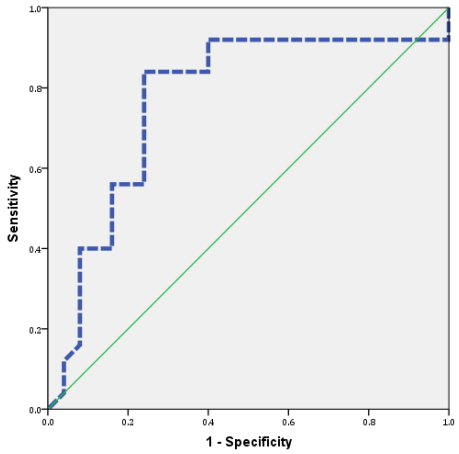

Figure (1) ROC curve showing performance of C4d in detection of Lupus Nephritis activity among the studied patients

DISCUSSION

Lupus nephritis is clinically present in 60% of SLE patients and is histologically present in most SLE patients even if no clinical manifestations of renal affection, so evaluating renal function in SLE is very important as early diagnosis and management can improve the outcome **[4].** Glomerular-C4d (G-C4d) deposition is related to lupus nephritis activity as lupus nephritis pathogenesis involves immune complex deposition which strongly activates complement cascade and gives rise to C4d. So, C4d levels correlate with lupus nephritis activity **[7].** The study compares patients with lupus nephritis (group A) versus healthy control patients (group B) with no significant difference regarding age and sex (p>0.05) **(Table1),** This is compatible with that of **Martin et al** [12] who studied two groups: one lupus nephritis group and other healthy control group and stated that comparison regarding age and sex was insignificant.

Our study revealed statistically highly significant difference between the studied groups regarding plasma c4d level (it is higher among lupus nephritis group). (p<0.001) **(Table 2),**this is compatible with **Abd El Halim et al study [7]** who performed a similar study and concluded a similar difference between the two groups and illustrated that by the role of immune complex deposition that strongly activates complement cascade and gives rise to C4d in the pathogenesis of lupus nephritis

Our study concluded statistically significant negative correlation between plasma complement 4 d and serum albumin, in the same context plasma c4d has a positive correlation with 24 hour urine protein, urine RBCS and pus cells in urine **(Table 3);** and this is compatible with **Martin et al** [12] study which concluded that plasma complement 4d level though increases in SLE activity, it reaches its highest level of increasing in lupus nephritis activity and so can be used to detect lupus nephritis activity which is so important to start aggressive treatment as soon as possible. This result is also in harmony with **Chen et al** [13] who suggested the same opinion.

On the other hand our work studied the relation between the plasma complement 4d level and the SLE Disease Activity Index (SLEDAI) score **(table 5)** and showed a positive significant correlation which is matched with **Merrill et al** [14] study, who concluded that plasma complement 4 d increases with the increasing SLEDAI score and illustrated that by the role of plasma complement 4 in the pathogenesis of SLE specially lupus glomeruniphritis. All these studies give support to the role of plasma complement 4 d as a noninvasive biomarker in detection of lupus nephritis activity.

The study revealed non significant relation between SLEDAI Score and presence of anti-ds-DNA **(p=0.82) (table 6)**. This suggested that complement 4d level is better than anti ds-DNA as a marker for SLE and lupus nephritis activity. The previous conclusion is compatible with **Arriens et al** [15], who illustrated that elevated autoantibodies against dsDNA (anti-dsDNA) was reported to correlate with disease activity in SLE,however, anti-dsDNA was also found in clinically inactive SLE patients with a relatively high percentage. Also this is in harmony with **Putterman et al** [16] who concluded that complement 4 d has higher sensitivity than anti ds-DNA. **Mora et al** [17] have the same opinion in their study which showed a higher correlation between lupus nephritis activity and complement 4d than that with anti ds-DNA.

This work showed statistically non-significant correlation between plasma C4d and ESR**. (Table 4).**These last two results regarding the relation between Anti ds-DNA and SLE disease activity &ESR and complement 4 d confirmed the purpose of our research regarding the plasma complement 4 d to be a better marker for disease activity detection in lupus nephritis.

CONCLUSIONS

Our study evaluated the role of Plasma complement 4d in lupus nephritis and showed that almost our patients of lupus nephritis activity at Zagazig University Hospitals had higher level of plasma complement 4d than normal control involved people, so confirmed that plasma complement 4d can be used as a very good marker of Lupus nephritis activity

Conflict of interest: None
Financial Closure: None

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