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The Relation between Plasma Complemant 4 d and Disease Activity in Systemic Lupus Erythematosus Patients at Zagazig University Hos*pitals.*

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Abstract

Background*: Systemic Lupus Erythematosus is a chronic inflammatory disease that is characterized by an auto antibody response to nuclear and cytoplasmic antigens, has a relapsing remitting course .Plasma complement 4 d can be used to predict activity of systemic lupus erythematosus patients.*

Aim: *The aim of our study was to assess the relation between plasma complement 4 d and disease activity of systemic lupus erythematosus patients.*

Methods:*This study was carried out on 92SLE patients fulfilled the Systemic Lupus International Collaborating Clinics (SLICC) classification criteria and 92 apparently healthy age & sex matched controls, Plasma complement 4 d was detected byspecific sandwich ELISA according to the instruction of the manufacturer* ***(SunRed, Shanghi).***

Results:*Our results revealed that there was statisticallysignificant relations between plasma c4d and skin rashes,malar rash and hair falling($p<0.05$). There washighly significant positive relation between plasma complement 4d and SLEDAI score($p\leq0.001$),but no significant relation between plasma complement 4 d and arthritis ($p=0.456$).*

Conclusion: *Our study evaluated the role of Plasma complement 4d in systemic lupus erythematosus patients and confirmed that plasma complement 4d can be used as a useful marker of the disease activity in SLE patients.*

Key words: *Systemic Lupus Erythematosus, Disease Activity,Complement 4 d.*

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I. Introduction:

Systemic lupus erythematosus (SLE) is an autoimmune disorder leading to inflammation and tissue damage, involving multiple organ systems and is more common in females a 3 : 1 ratio **(1).** SLE is caused by an autoimmune reaction in which the innate and adaptive immune systems direct an inappropriate immune response to nucleic acid-containing cellular particles **(2),**SLE has multiple clinical manifestations that can affect virtually every organ, and can vary dramatically from patient to patient. The most common pattern is a mixture of constitutional complaints with skin, musculoskeletal, renal**,….(3).**Complement 4d is a stable cleavage product of complement 4, accumulating following classical and lectin pathways activation. C4d deposition in a tissue is regarded as an indirect proof of an activation of the complement cascade. It is usually bound stably to the target structure but may eventually enter the circulation **(4),**it can bind with various cells, including reticulocytes , platelets and in the peripheral circulation, but they bind mostly with erythrocytes, erythrocyte binding C4d is present in active SlE**(5).**Autoimmune mechanisms with autoantibodies and a cytotoxic effect of the complement membrane attack complex on epidermal or vascular cells can cause direct skin damage and inflammation of the skin and blood vessel wall, furthermore consumption of complement 4 and increase level of c4d (**6**).Currently used C3 and C4 levels exhibit low sensitivity in follow-up of patients with SLE.The absence of C4d in healthy control subjects makes it perse a better marker.Complement 4d has a much longer half-life than other molecules of complement cascade, allowing it to stay anchored to the cell while other molecules get cleared away by the blood stream; all these properties have established C4d as a stable marker for complement activation and disease activity in auto immune diseases as SLE. **(7).**

II. SUBJECTS & METHODS

Subjects were divided into two groups:

•Group I (SLE group):

It included 92 female patients with systemic lupus erythematosus (SLE),who fulfilled the ACR/ SLICC revised criteria for classification of SLE **(8).**

Their ages ranged from 19-48 years with a mean of 30.35 ± 8.72 years,disease duration ranged from 1 – 15 years.

•Group II (control group):

It included 92 apparently healthy female volunteers. Their ages ranged from 19-48 years with a mean of 30.35 ± 8.72 years .

-Ethical consideration: Approval was taken from the Institusional Review Board (IRB) at zagazig university.

-Type of the study: Case control study.

- Inclusion criteria:

All included patients of SLE fulfilled the Systemic Lupus International Collaborating Clinics (SLICC) classification criteria for SLE **(8**), ourpatients were generally examinedfor presenceof:Skin rashes, oral ulcers, alopecia ,lupus hair,cardiac examination for pericardial rub (effusion) and chest examination for crepitations and pleural rub (effusion). They were also locally examined for:jointswelling,Range of motion,after that assessed for disease activity according to SLEDAI-2K score **(3)** then laboratory investigations were done in the form of

Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP),Creatinine clearance, protein in 24-hour urine collection,complete urine analysis,ANA,Anti-dsDNA antibodies titre andplasma complement 4 d levels in both patients and control.

Complement 4 d:

Plasma complement 4 d was measured by specific sandwich ELISA according to the instruction of the manufacturer **(SunRed, Shanghi)** with test principles were**:**The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of Human Complement Fragment 4d (C4D) in samples.Add Complement Fragment 4d (C4D) to monoclonal antibody Enzyme well which is pre-coated with Human Complement Fragment 4d (C4D) monoclonal antibody,incubation; then, add Complement Fragment 4d (C4D) antibodies labeled with biotin, and combined with Streptavidin-HRP to form immune complex; then carry out incubation and washing again to remove the uncombined enzyme.Then add Chromogen Solution A, B, the color of the liquid changes into the blue, And at the effect of acid, the color finally becomes yellow. The chroma of color and the concentration of the Human Substance Complement Fragment 4d (C4D) of sample were positively correlated.Plasma Collected from blood by venipuncture, sample coagulation at 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 Method

Data analysis was performed using the software SPSS (Statistical Package for the Social Sciences) version 20. Quantitative variables were described using their means and standard deviations.Categorical variables were described 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. To compare means of two groups,Mannwhitney 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. Pearson correlation and Spearman rank correlation coefficients were used to assess strength and direction of a linear relationship between two variables.ROC curve was used to determine the best cutoff of a certain marker for diagnosis of a certain health problem. Linear regression analysis was used to determine the extent to which there is a linear relationship between a dependent variable and one or more independent variables.The level statistical significance was set at 5% ($P<0.05$). Highly significant difference was present if $p\leq0.001$.

III. Results:

Our study showedthat there is statistically highly significant difference between the studied groups regarding plasma C4d**(higher among lupus group).**(**Table 1**)**.** Our study alsorevealed thatthere is statistically non-significant relation between plasma C4d and presence of arthritis.(**Figure 1**).

Table (1) Comparison between the studied groups regarding plasma C4d:

Plasma C4d	**Groups**		**Test**	
	Lupus group	**Control group**	**Z**	**P**
	N=92 (%)	**N=92 (%)**		
Median **Range**	0.348 0.01 – 4.883	0.127 0.009 – 2.736	-3.549	<0.001**

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

Figure (1) Boxplot showing relation between presence of arthritis and plasma C4d

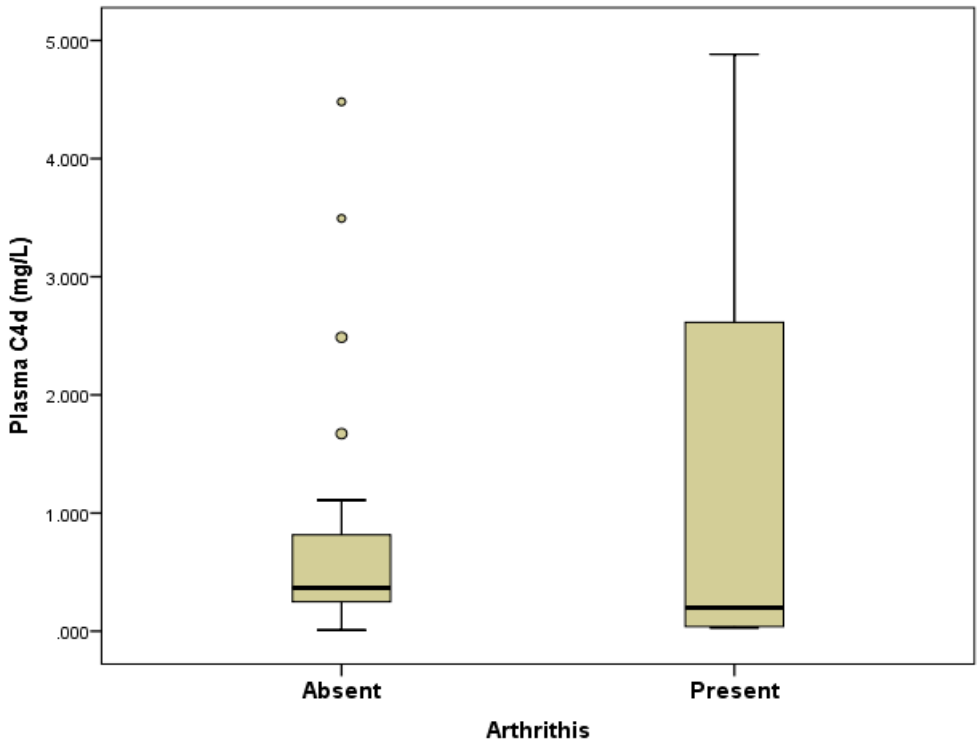


Table (2) Relation between plasma C4d and skin,mucous membrane and hair affection among patients with lupus:

	Plasma C4d		Test	
	N	**Median (range)**	**Z**	**p**
Skin rash:				
Absent	68	0.281(0.01 – 4.883)	-4.414	<0.001**
Present	24	1.063 (0.345 – 4.883)		
Hair loss:				
Absent	64	0.266 (0.01 – 4.483)	-5.843	<0.001**
Present	28	1.672 (0.345 – 4.883)		
Ulcer:				
Absent	84	0.348 (0.01 – 4.883)	-0.666	0.506
Present	8	0.405 (0.345 – 0.456)		
Malar rash:				
Absent	72	0.31 (0.01 – 4.88)	-4.396	<0.001**
Present	20	1.672 (0.345 – 4.883)		

(Z Mann whitney test *p<0.05 is statistically highly significant)

Figure (2) Scatter dot graph showing significant positive correlation between plasma C4d and 24 hour urine protein among the studied patients.

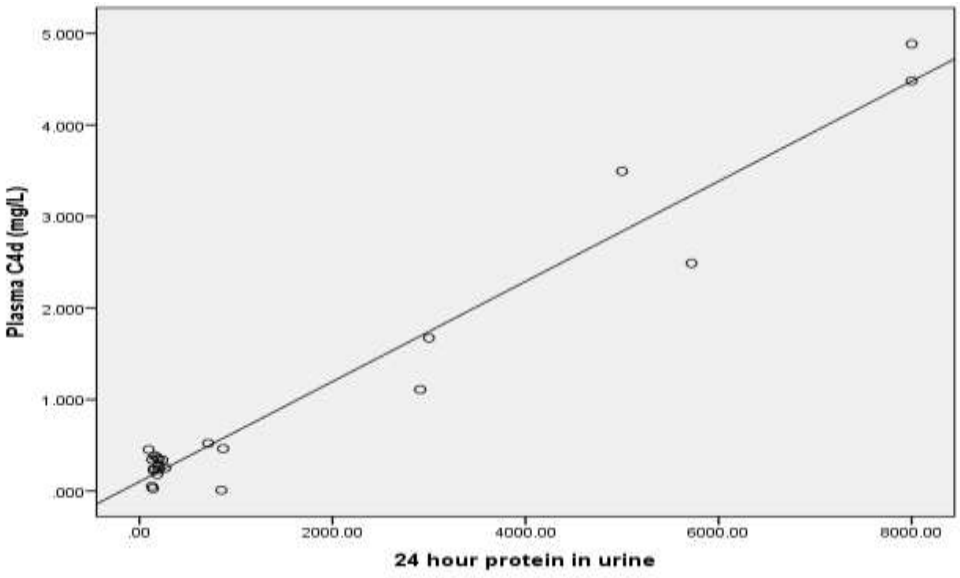


Table (3): Correlation between plasma C4d and SLEDAI of the studied patients:

Parameters	Plasma C4d	
	r	P
SLEDAISCO (SLE disease activity score)	0.63	0.001**

(r Spearman rank correlation coefficient *p<0.05 is statistically significant **p≤0.001 is statistically highly significant)

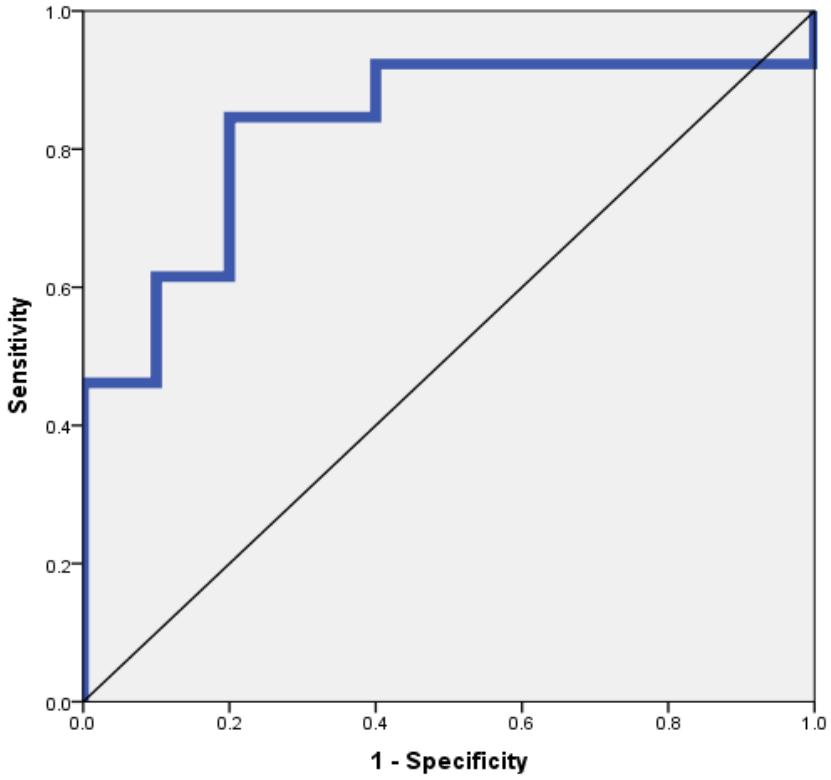
Table (4) Relation between anti dsDNA and disease activity among the studied patients:

Disease activity	Anti dsDNA		Test	
	Negative	Positive	χ^2	p
	N=20 (%)	N=72 (%)		
No	4 (20)	20 (27.8)		
Mild	0 (0)	16 (22.2)		
Moderate	8 (40)	4 (5.6)	0.212	0.645
High	8 (40)	20 (27.8)		
Very high	0 (0)	12 (16.7)		

χ2 Chi square for trend

The best cutoff of plasma C4d in diagnosis of higher SLE activity among the studied patients ≥0.342 with area under curve(AUC) 0.831, sensitivity 84.6%, specificity 80%, positive predictive value(PPV) 84.6% , negative predictive value (NPV) 80% , positive likelihood ratio (PLR) 4.23, negative likelihood ratio(NLR) 0.19, accuracy 82.6% (p<0.05)

Figure (20) ROC curve showing performance of C4d in prediction of higher disease activity among the studied patients



The study showed that there is statistically significant relation between plasma C4d and presence of skin rash, hair loss or malar rash (C4d is higher in patients with positive lesion),there is statistically non-significant relation between plasma C4d and presence of ulcer**(Table 2),** also there issignificant positive correlation between plasma C4d and 24 hour urine protein among the studied patients **(Figure 2).**

Our study revealed that there is statistically significant positive correlation between plasma C4d and SLEDAISCO.(**Table 3)**and showeda non-significant relation between disease activity and anti-dsDNA among the studied patients**.(Table 4)**

The study revealedthat the best cutoff of plasma C4d in diagnosis of higher SLE activity among the studied patients ≥0.342 with area under curve(AUC) 0.831, sensitivity 84.6%, specificity 80%, positive predictive value(PPV) 84.6% , negative predictive value (NPV) 80% , positive likelihood ratio (PLR) 4.23, negative likelihood ratio(NLR) 0.19, accuracy 82.6% ($p<0.05$).**(Figure 3).**

IV. Discussion:

Systemic Lupus Erythematosus is a chronic inflammatory disease that is characterized by an auto antibody response to nuclear and cytoplasmic antigens, has a highly variable prognosis and damage to different organs or systems, including skin, musculoskeletal, renal and the central nervous system **(2)**

Our study revealed statistically highly significant difference between the studied groups regarding plasma c4d level (it is higher among lupus group).($p<0.001$) **(Table 1),**this is compatible with that of **Abd El Halim et al., 2018 (9) ,**the study revealed statistically non significant relation between plasma complement 4 d and arthritis. **(Figure 1)**,this is the same opinion as **Martin et al; 2017 (7)** .**Leffler et al.,2012 (10) andNzeusseu et al.,2007 (11)**were agree with the same result and illustrated that by finding that arthritis in SLE patients has a different pathogenesis in regard to complement activation,so complement 4 d level can not be a marker for presence of arthritis.

Regarding the relation between plasma complement 4d and skin manifestations, our study revealed statistically significant relations between plasma c4d and skin rashes,malar rash and hair falling **($p<0.05$) (Table 2)** and this is matched with **Panelius and Merri.,2015 (6)**who recommended that autoimmune mechanisms with autoantibodies and a cytotoxic effect of the complement membrane attack complex on epidermal cells can cause direct skin damage and inflammation of the skin, furthermore consumption of complement 4 and increase level of c4d.

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**(figure 2)** ; and this is in agreement with **(Martin et al; 2017)** studywhich concluded that plasma complement 4d level though increases in SLE activity,it reaches its highest level of increasing in lupus nephritis and so can predict lupus nephritis flare which is so important to start aggressive treatment as soon as possible.(7).

On the other hand our work studied the relation between the plasma complement 4d level and the activity of SLE in means of SLE Diease Activity Index (SLEDAI) score **(table 3)** ,this work showed positive significant correlation which is matched with **(Merrill et al., 2018)** study (12).

The study revealed non significant relation between SLE disease activity and presence of anti ds-DNA **($p=0.82$)(table 3)**. This suggested that complement 4d level is better than anti ds-DNA as a marker for SLE activity,this previous conclusion is compatible with **(Arriens et al.,2017)** 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. (13).

V. CONCLUSION:

Our study evaluates the role of Plasma complement 4d in SLE and confirms that plasma complement 4d can be used as a useful marker of the disease activity in SLE patients.

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Conflict of interest: no Conflict of interest.

References:

1-Alzughayyar T, Abukhalaf S and Abuniejma F, et al .,(2020): Systemic Lupus Erythematosus with Multiple Autoimmune Disease Presented with Extensive Peripheral Gangrene.Hindawi.Volume 2020 |Article ID 8278275 | https://doi.org/10.1155/2020/8278275.

2-Bartels C and Muller D.,(2019): Systemic Lupus Erythematosus (SLE) Practice Essentials. Lupus Sci Med. 7:1-15.

3-Gladman D.D. Ibanez D and Urowitz M.B., (2002): Systemic lupus erythematosus disease activity index 2000. J Rheumatol; 2:288–291.

4-Klikovits T. Stockhammer P and HegedusB.,(2017): Circulating complement component 4d (C4d) correlates with tumor volume, chemotherapeutic response and survival in patients with malignant pleural mesothelioma. Sci Rep.3:78-89.

5-Chen C. Tai S and Chen H, et al., (2015): Analysis of Erythrocyte C4d to Complement Receptor 1 Ratio: Use in Distinguishing between Infection and Flare –Up in Febrile Patients with Systemic Lupus Erythematosus. Bio Med Research International(BMRI) Journal.2:330-347.

6-Panelius J and Merri S., (2015): Complement system in dermatological diseases – fire under the skin. Frontiers in immunology. 2:110-122.

7-Martin M. Karolina I and Björk A, etal., (2017): Plasma C4d as a marker for lupus nephritis in SLE. Arthritis Research and Therapy. 19: 1- 266.

8-Petri M. Orbai A.M. and Alarcon GS, et al., (2012): Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus.Arthritis Rheum. 8:267-286.

9-Abd El Halim H . Salah M and Ismail W, et al., (2018): Erythrocyte and glomerular C4d deposits as a biomarker for active lupus nephritis. The Egyptian Rheumatologist Journal.3:169-171.

10-Leffler J.Martin M and Gullstrand B, et al.,(2012): Neutrophil extracellular traps that are not degraded in systemic lupus erythematosus activate complement exacerbating the disease. J Immunol.7:3522-3531.

11-Nzeusseu A. Galant C and Theate I ,et al .,(2007): Identification of distinct gene expression profiles in synovium of patients with systemic lupus erythematosus. Arthritis Rheum.5:1579-1588.

12-Merrill J.Petri M and BuyonJ,et al.,(2018): Erythrocyte-bound C4d in combination with complement and autoantibody status for the monitoring of SLE. Lupus Sci Med.5:212-218.

13-Arriens C. Jonathan D and Melissa E, et al.,(2017): Systemic lupus erythematosus biomarkers: the challenging quest. Rheumatology (Oxford) journal. 8:133-150