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Assessment of Phosphatidyl Serine-Specific Phospholipase A1, and CH50 in Sera of Systemic Lupus Erythematosus Patients and Its Correlation with Disease Activity, and Severity

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ABSTRACT

Background: Systemic lupus erythematosus (SLE) is a chronic connective tissue disease with multisystem inflammation, complement system aid in deposition of immune complexes. Hydrolyzing phosphatidylserine on the plasma membrane of apoptotic cells and activating neighboring immune cells by producing lysophospholipid, phospholipase A1 promotes the onset of autoimmunity in SLE patients.

Objective: To determine the levels of Phosphatidyl Serine-Specific Phospholipase A1 (PS-PLA1), and the total hemolytic complement in the sera of SLE (CH50) patients and to clarify how these levels correspond to the severity and activity of the disease.

Patients and methods: 80 persons participated in the study (40 SLE patients and 40 healthy as control group). The disease activity level is evaluated as regards the SLE disease activity score, laboratory tests and enzyme linked immune-sorbent test for Phosphatidyl Serine-Specific Phospholipase A1 and the total hemolytic complement.

Results: This study revealed a significant difference in PS-PLA1 and CH50 levels in the patient group when compared to healthy patients. The values of (SLEDAI) and laboratory parameters including antinuclear-antibody, anti-double stranded, erythrocyte sedimentation rate, CBC, and albumin/creatinine ratio levels were significantly associated with PS-PLA1, and CH50 in SLE patients ($P< 0.05$).

Conclusion: Sequential changes of PS-PLA1, and CH50 concentrations in SLE patients and a strong correlation existed between SLEDAI with both markers, suggesting their putative role in the disease pathophysiology.

Keywords: PS-PLA1, CH50, SLE, SLEDAI.

INTRODUCTION

SLE is a chronic autoimmune illness, characterized by B lymphocytes overactivity, immune complexes formation that attack several systems causing tissue damage. SLE patients still have a high risk of morbidity and mortality despite effective treatment [1]. There are 20 to 70 instances of SLE per 100,000 persons, with a female predominance (male- to- female ratio, 1:9) [2].

Novel biomarker, are recently studied promoting early detection of organ outcomes and so management including, lyso phospholipids, which are a group of sphingo-phospholipids [3], that are essential for cell signaling , inflammatory response, endocytosis, and exocytosis [4].

An anionic phospholipid called phosphatidylserine (PS) is typically confined to the inner leaflet of the cell membrane. The externalization of PS during apoptosis acts as a signal for phagocyte identification. The PS-specific phospholipase A_1 (PS-PLA_1) is one of many phospholipases that have been suggested as the culprits [5]. LysoPS is created when PS-PLA_1 is exposed on apoptotic cells [6]. Complement system components have been linked to a higher incidence of SLE. It has emerged as a key player in the development of immune complex deposition associated with SLE [7].

It has been demonstrated that a lack of classical pathway complement component C1 increases the chance of developing the disease in up to 90% of SLE patients [8]. Interestingly, the total hemolytic complement (CH50) has been given varying degrees of clinical relevance in measuring disease activity, a genetic defect in one or more complement proteins might result in a CH50 value of extremely low down to zero, moderate CH50 reductions are commonly seen particularly in cases with immunological complex formation [9].

The study aimed to assess the role of PS- PLA1, and CH50 in SLE disease activity, and severity.

PATIENT AND METHODS

A case-control study was conducted at the Rheumatology, Rehabilitation and Physical Medicine Department, Benha University Hospitals on 40 patients who were diagnosed as systemic lupus erythematous according to the European League against Rheumatism/American College of Rheumatology (ACR/EULAR) 2019 criteria [10]. 40 healthy participants matched for age and sex were selected to be in the control group. Laboratory testing was performed by the Clinical and Chemical Pathology Department of University Hospitals.

Exclusion criteria: Patients with other autoimmune illnesses, cancer, or chronic infection, metabolic disorders and pregnant women.

Patients' group:

40 systemic lupus erythematosus were above the age of 16, their disease duration ranged from three months to six years. 20 patients (50%) were diagnosed with lupus nephritis based on clinical manifestations, laboratory parameters and renal biopsy findings in some patients 15 out of 40 (38%). No patient showed central

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nervous system, cardiovascular and respiratory systems affection. They were taking immunosuppressive medications such as steroids, methotrexate, mycophenolate mofetil, and hydroxychloroquine (HCQ). Disease activity was determined based on the SLEDAI score [11]. They were categorized as mild, moderate, severe, and extremely severe. Few patients actually have scores higher than 45, which implied very severe symptoms, despite the fact that the theoretical maximum score is 105. The level of disease activity is more significant the higher the score.

All eighty subjects underwent a thorough medical history analysis, comprehensive clinical examination, and laboratory investigations, including an analysis of PLA1 (ng/ml), CH50 (pg/ml) (Cat#: E-02704hu, Cloud-Clone Crop., USA), a CBC, ESR mm/h by Westergren method, antinuclear antibodies (ANA IU/ML) by indirect immunofluorescence assays, C-reactive Protein (CRP mg/l) by latex agglutination slide test ,serum creatinine (mg/dl), anti-dsDNA (IU/L), and urinary Albumin/Creatinine Ratio.

PLA1's principal: The enzyme-linked Immunosorbent assay (ELISA) kit employed a double-antibody sandwich method to measure the amount of PLA1 in samples. Test the sample well using 10 ul of the testing sample and 40 ul of the sample diluent. PLA1 antibodies were mixed with and added to.

CH50's principal: The enzyme-linked Immunosorbent assay (ELISA) kit employed a double-antibody sandwich method to measure the amount of CH50 in samples. Test the sample well using 10 ul of the testing sample and 40 ul of the sample diluent. CH50 antibodies were mixed with and added to.

Ethical approval: This study was governed by the Helsinki Declaration on the Ethical Principles in Human Research. The study's procedure was approved by the University Faculty of Medicine's Human Subject Research Ethics Committee (code RC-3-2023). Before taking part in the study, all participants had to complete an informed written consent forms.

Statistical analysis

The acquired data were tabulated and analyzed using SPSS version 16 Categorical data (X2) was analyzed using a test. The Shapiro-Wilks test, which assumes normality at $P > 0.05$, was used to determine whether quantitative data were normal. The means and standard deviations (SD) were used for variables with normally distributed distributions.

The Student "t" test was used to analyze the distribution of data between two groups that were unrelated to one another. The Man Whitney U test was used to assess non-parametric variables. An ANOVA was used to compare the variances between the means of the three separate groups. There were several Bonferroni comparisons utilized. 95% CI.

RESULTS

Patients' age ranged from 20 to 53 years old (36.5 ± 10.7) with more than 90% of them, being females (36 females, 4 males) [F: M 9:1]. The control group consisted of a total of 30 women and 10 men [F: M 3:1] with ages ranging from 19 to 57 years old (39.7 ± 10.8). The disease duration ranged from 3 months to 6 years (Median disease duration was 1y) (Table 1).

Table (1): Demographic, clinical, and laboratory characteristics of the SLE group (no =40)

Variable	**SLE patients (no.=40)**
Demographic	
Age (years)	Range: 20 - 53 Mean ±SD; 36.5 ± 10.7
Duration of illness (years)	Range: 3M-6Y Median: 1y
Clinical	**NO (%)**
Photosensitivity	22 (55%)
Mala rash	28 (70%)
Hematological	4 (10%)
Arthritis	20 (50%)
Artharlagia	31 (78%)
Lupus nephritis	20 (50%)
SLEDAI IQR (6.25-20)	
Mild	27 (67%)
Moderate	7 (18%)
Severe	6 (15%)
Laboratory	**Mean ±SD**
Hemoglobin (gm/dl)	9.2 ± 2.2
TLC ($x10^9$/L)	7.08± 1.71
PLTS ($x10^9$/L)	210.5 ± 51.31
ESR (mm/h)	73.1± 17.8
Creatinine (mg/dl)	1.5± 1.0
CRP (mg/L)	12.0±2.8
ACR (mg/g)	2.0±0.4

SLEDAI: Systemic lupus disease activity index, d Hb: hemoglobin, TLC: Total leucocytic count, ESR: Erythrocyte sedimentation rate, CRP: C-Reactive protein AlB/CR: Albumin creatinine ratio.

PS-PLA1 and CH50 levels showed significant difference in cases (7.57 ± 1.88) and (12.28 ± 2.65) compared to the control group (5.24 ± 1.30) and (9.51 ± 2.36) respectively, ($p < 0.001$) for both (Table 2).

Table (2): Comparing the studied groups according to the studied markers.

Variable	SLE patients (n=40)		Controls (n=40)		St."t" Test	P
	Mean	±SD	Mean	±SD		
Serum PS-PLA1 (ng/ml)	7.57	1.88	5.24	1.30	4.76	**<0.001**
Serum CH50 (pg/ml)	12.28	2.65	9.51	2.36	4.22	**<0.001**

PS- PLA1: Phosphatidyl Serine-Specific Phospholipase A1, CH50: Total hemolytic Complement, Bold values are significant at p<0.05.

Clinical manifestations (arthritis, malar rash, photosensitivity) and serum PS-PLA1 did not substantially, associate with one another (p > 0.05) except for lupus nephritis (p < 0.05) (Table 3).

Table (3): Serum PS-PLA1 according to clinical manifestations

Variable	Serum PS-PLA1 (ng/ml)		St."t"	P
	Mean	± SD		
Lupus nephritis	6.24	1.54	0.16	**0.05**
	7.81	1.92		
Arthritis	6.69	1.65	1.27	0.21
	7.83	1.91		
Malar rash	7.83	1.51	0.62	0.53
	7.36	1.80		
Photosensitivity	7.47	1.83	0.43	0.67
	7.82	1.94		

PS- PLA1: Phosphatidyl Serine-Specific Phospholipase A1 **St."t"**: Student test, .Bold values are significant at p<0.05.

The concentration of PS-PLA1 in the serum was directly related to how serious was the condition? (P= 0.002). Moreover, there was a significant increase in serum PS-PLA1 levels when comparing mild to severe disease activity and moderate to severe disease activity (P = 0.001 and = 0.049 respectively). Serum CH50 levels were significantly correlated with the SLEDAI score (P=0.009), but still steady in different SLE patients disease activity grade (p > 0.05) (Table 4 & Figures 1 & 2)**.**

Table (4): Serum PS-PLA1 and Serum CH50 in regard to SLEDAI score

Activity grade	N	Serum PS-PLA1 (ng/ml)		F	P	Multiple Comparisons	P
		Mean	± SD				
Mild	27	6.85	1.70	7.76	**0.002**	Mild≠Moderate Mild≠Severe Moderate≠Severe	P1=1.0 P2=**0.001** P3=**0.049**
Moderate	7	7.53	1.87				
Severe	6	10.3	0.94				
Activity grade	N	Serum CH50 (pg/ml)		F	P	Multiple Comparisons	P
		Mean	± SD				
Mild	27	3.91	0.69	5.31	**0.009**	Mild≠Moderate Mild≠Severe Moderate≠Severe	P1=01.39 P2=01.21 P3=0.79
Moderate	7	3.14	0.66				
Severe	6	2.9	0.55				

PS- PLA1: Phosphatidyl Serine-Specific Phospholipase A1, CH50: Total hemolytic Complement. Bold values are significant at p<0.05

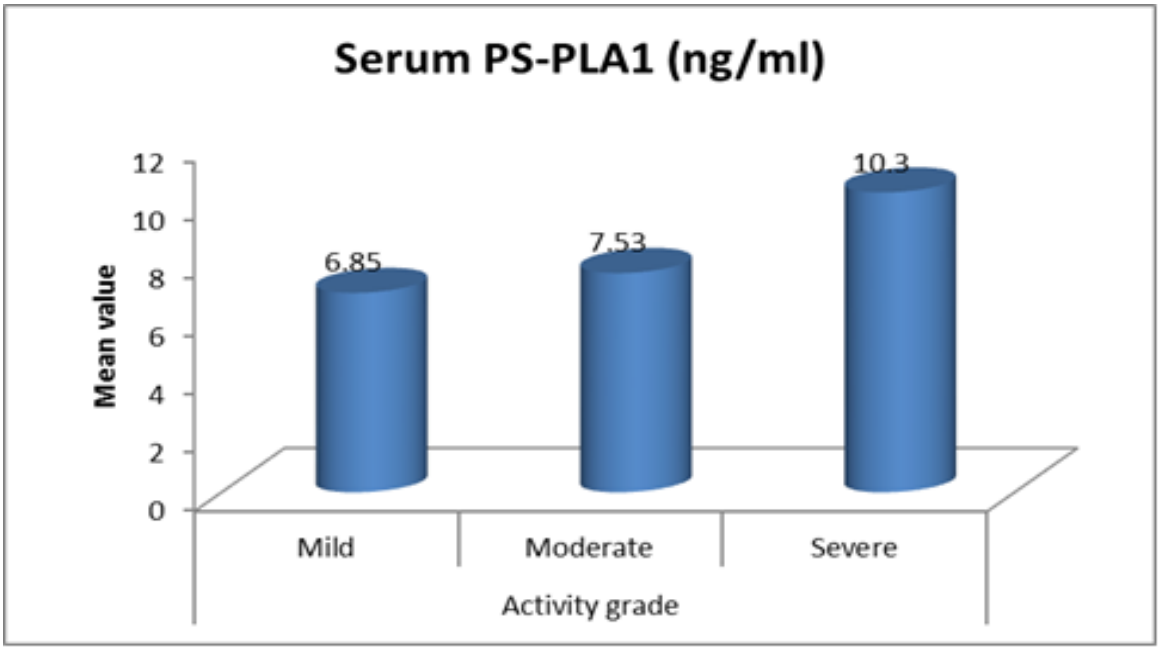

Fig. (1): Serum PS-PLA1 according to SLE activity grade.

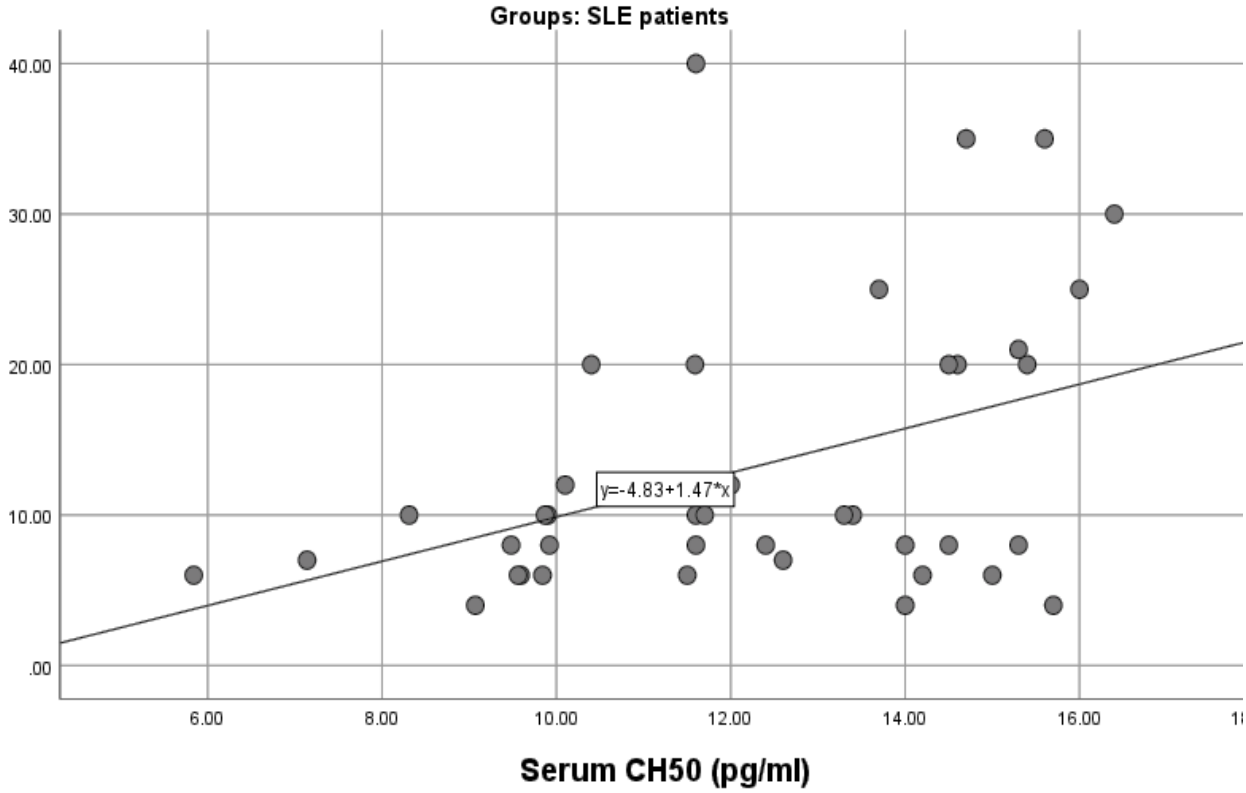

Fig. (2): Scatter graph showed significant positive correlation between CH50 and SLEDAI

Our research demonstrated a strong statistically significant positive association between serum PLA1 levels and laboratory variables (ANA, Anti dsDNA, ESR, creatinine and Albumin/creatinine ratio), (r=.539, p=<0.001), (r = 0.339, p = 0.011), (r =.285, p = 0.075), (r =.340, p = 0.032) and (r =.375, p = 0.017) consecutively. Also, these parameters were positively correlated with CH50 (r=0.407, p=0.009), (r=.355, p=0.025), (r=.429, p=0.006), (r=.432, p=0.005) and (r= .479, p=0.002) respectively. On the contrary, a strong negative association between serum PLA1, CH50 concentrations with hemoglobin, total leukocyte count, and the platelets count has been established (r=-.323, p=0.042), (r=-.403, p= 0.01), (r=-.318, p=0.046), (r=-.527, p=0.001), (r=-.406, p= 0.009) ,(r=-.432, p=0.005) respectively. Demographic data showed insignificant association with the study markers (p<0.05) (Table 5 and Figures 3 & 4).

Table (5): Correlation between Serum PS-PLA1 level, Serum CH50, and the demographic, clinical, laboratory variables in SLE group

Variable	PS-PLA1		CH50	
	R	P	R	P
Age	0.123	0.87	.222	.23
Disease duration	0.102	0.53	0.013	0.93
Antinuclear antibody (iu/ml)	0.539	**<0.001**	0.407	**0.009**
SLEDAI	0.452	**0.003**	0.381	**0.015**
Anti ds DNA(iu/l)	0.339	**0.011**	0.355	**0.025**
Hb (gm/dl)	-0.323	**0.042**	-0.527	**<0.001**
TLC ($x10^9$/L)	-0.403	**0.01**	-0.406	**0.009**
PLTs ($x10^9$/L)	-0.318	**0.046**	-0.432	**0.005**
ESR (mm/h)	0.285	**0.075**	0.429	**0.006**
Creatinine (mg/dl)	0.340	**0.032**	0.432	**0.005**
CRP(mg/l)	0.204	0.21	0..278	0.16
ALB/CR (g/mg)*	0.375	**0.017**	0.479	**0.002**
Serum CH50 (pg/ml)	0.603	**<0.001**		

ANA: Antinuclear antibody, SLEDAI: Systemic lupus disease activity index, Anti ds DNA: Anti double stranded Hb: hemoglobin, TLC: Total leucocytic count, ESR: Erythrocyte sedimentation rate, CRP: C-Reactive protein AlB/CR: Albumin creatinine ratio, .PS- PLA1: Phosphatidyl Serine-Specific Phospholipase A1, CH50: Total hemolytic Complement .Bold values are significant at p<0.05 .*Spearman' correlation coefficient (rho).

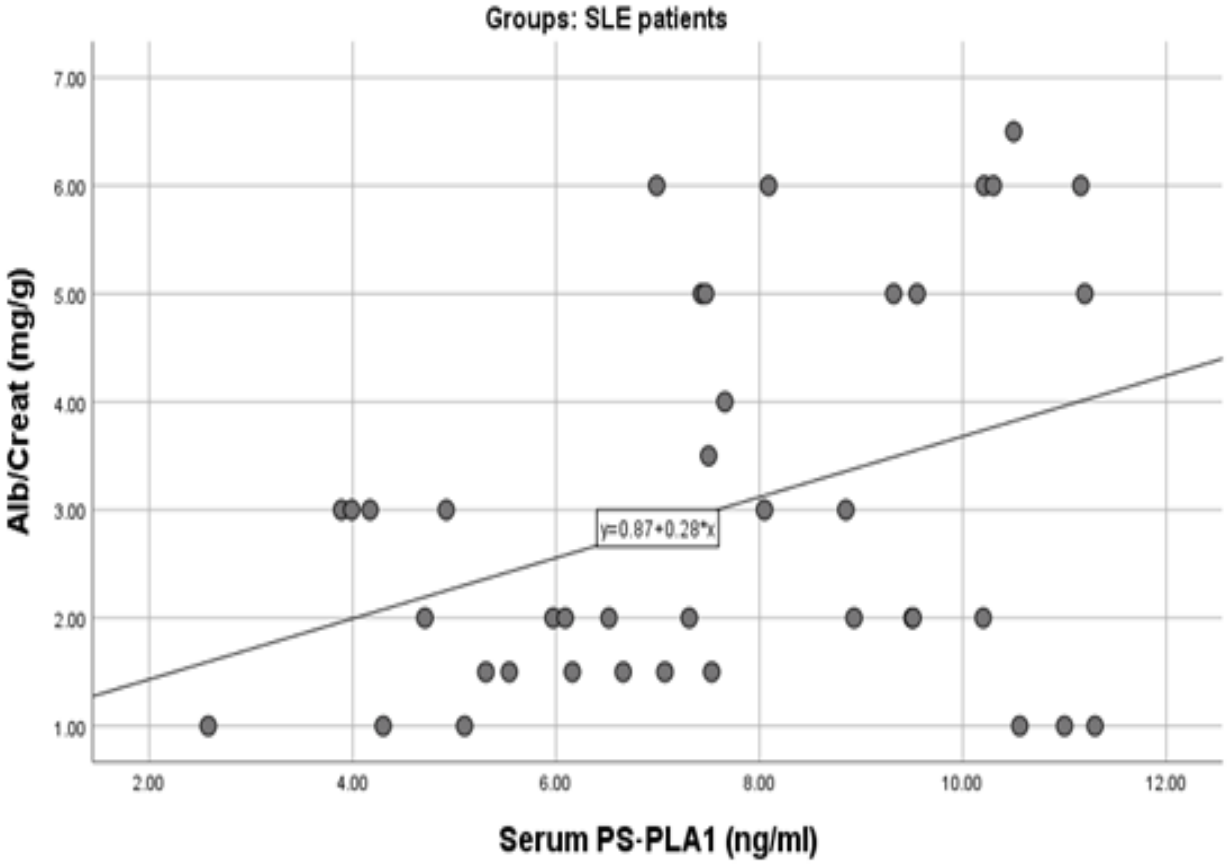

Fig. (3): Scatter graph showed significant positive correlation between serum PS-PLA1 and ACR**.**

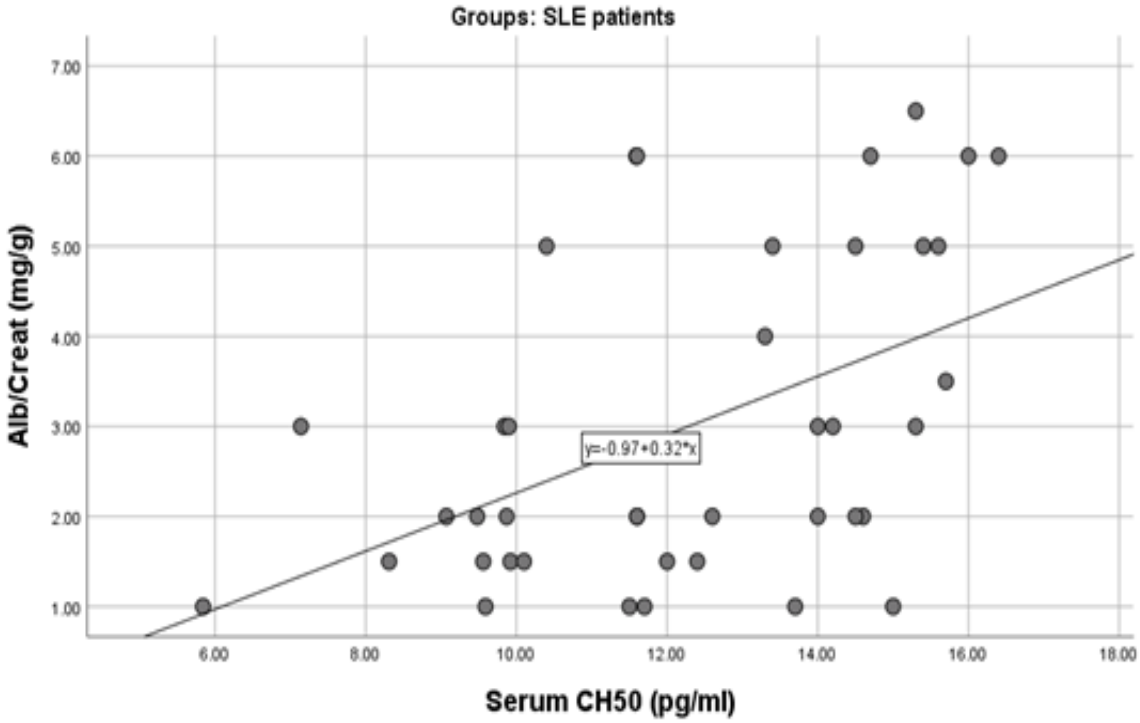

Fig. (4): Scatter graph showed significant positive correlation between serum CH50 and ACR

Regarding the major predictors of the SLEDAI, factors that were shown to be significantly connected with SLEDAI were entered into a multiple linear regression analysis (activity). Serum PS-PLA1, CH50, and albu/creat ratio were discovered to be the most reliable indicators of SLE activity (Table 6).

Table (6): Multiple linear regression analysis for the predictors of activity in SLE

Model summary	**R^2**		**Adjusted R^2**	**SEE**	**F**	**P-value**
	0.638		0.542	1.86	10.49	**<0.001**
Variable	**Unstandardized Coefficients**		**Standardized Coefficients**	**95% CI of B**	**T**	**P**
	B	**Std. Error**	**Beta**			
(Constant)	-4.81	1.43	------	-9.02-(-0.02)	3.03	**0.006**
Age	0.13	0.12	0.15	-0.12-0.38	1.06	0.3
Anti dsDNA(iu/l) titre	0.018	0.034	0.095	-0.09-0.052	0.54	0.59
ANA(iu/ml)	0.012	0.069	0.029	-0.15-0.13	0.18	0.85
Serum PS-PLA1 (ng/ml)	2.13	0.54	0.33	0.87-6.9	2.41	**0.026**
Serum CH50 (pg/ml)	2.34	0.44	0.39	0.72-5.3	2.58	**0.016**
ESR(mm/h)	0.009	0.076	0.025	-0.15-0.17	0.12	0.91
CRP(mg/l)	0.074	0.061	0.27	-0.05-0.2	1.21	0.23
Creat (mg/dl)	1.12	1.03	0.62	-1.96-7.9	1.33	0.19
Alb/creat	1.98	0.77	0.28	1.05-9.9	2.09	**0.043**

AlB/CR: Albumin creatinine ratio. PS- PLA1: Phosphatidyl Serine-Specific Phospholipase A1, CH50: Total hemolytic Complement .Bold values are significant at $p<0.05$.

The ROC curve study produced an excellent AUC of 0.900 for a PS-PLA1 cut-off value of ≥ 9.12 ng/ml, a sensitivity of 85.7%, a specificity of 78.8%, positive predictive value of 46.23% and negative predictive value of 96.34%. According to the study's findings, the CH50 cut-off value was ≥13. 5 pg/ml, with very good AUC of 0.855, a sensitivity of 85.7%, specificity of 69.7%, positive predictive value of 37.5%, and a negative predictive value of 95.8% (Fig. 5).

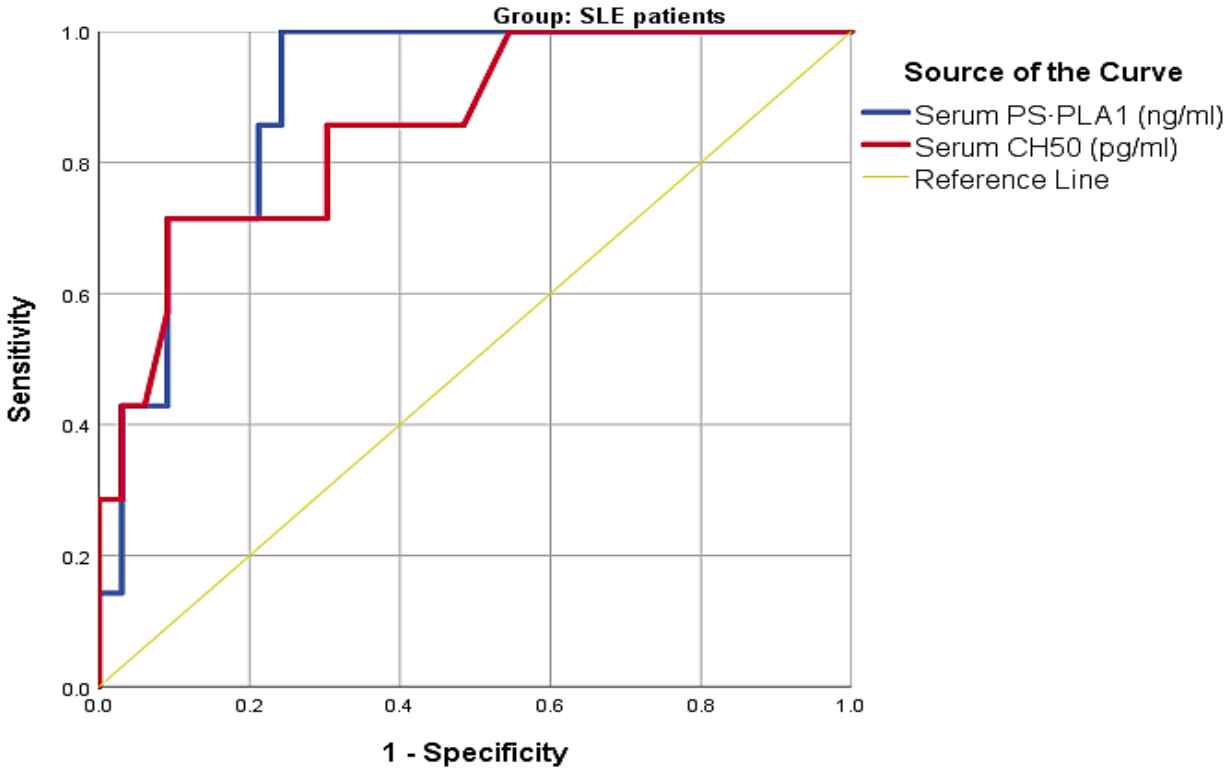

Fig. (5): ROC curve for the performance of the studied markers in Prediction of severe grade SLE.

DISCUSSION

Systemic lupus erythematosus has a variety of phenotypes with multisystem involvement and is associated with significant morbidity and mortality. The precise pathophysiology of the disease is still not well understood, despite recent breakthroughs in our understanding of its pathological underpinnings. SLE diagnosis can be difficult as a result [12].

The aim of this work was to assess the serum levels of PS-PLA$_1$ and CH50 and explore whether these molecules could impact systemic lupus erythematosus activity, and severity.

In this study, participants in both research groups had significantly different serum PS-PLA1 and CH50 levels **(**P <0.001). This was confirmed in 2013 by **Sawada *et al.*** [13] study in which, the blood levels of PS-PLA1 were assessed, when patients with SLE had considerably greater blood PS-PLA1 levels than healthy controls. Furthermore, PS-PLA1 serum levels decreased after immunosuppressive treatment in SLE patient. In addition, a second study performed by **Sawda *et al.*** [14] as extended research concerning this topic, carried on a large number (161 SLE patients and 237 healthy controls), where those with SLE had significantly higher blood levels of PS-PLA1 than did controls (p<0.001).

SLE, Systemic Sclerosis, and Sjogren's syndrome were associated with greater PS-PLA1 levels. Abnormal gene expression has been reported to impact PS-PLA1 expression and is upregulated in SLE, SSc, and SS [15]. An essential part of the pathophysiology of SLE is played by PSPLA1. By effectively hydrolyzing PS on the plasma membrane of apoptotic cells, and immune cells (macrophages and T cells) via the production of LysoPS, PSPLA1 demonstrates tight substrate specificity for PS, which results in the development of autoimmunity in SLE patients. Additionally, PSPLA1 may hydrolyze PS on apoptotic cells, masking its presence and reducing the ability of macrophages and dendritic cells to recognize apoptotic cells. A PSPLA1 antagonist may be a contender for the treatment of SLE patients given the elevated expression of PSPLA1 in SLE [16]. On the contrary, the study of **Han *et al.*** [17] conducted on patients with SLE showed lower CH50 levels than healthy individuals (p= 0.05). In addition, this was against the **Zhao *et al.*** [18] study where, they discovered that 37% of SLE patients and all controls had normal complement levels. Patients with inactive SLE also had normal complement and lower CH50 levels in 21% of cases.

Some of the variables that may affect plasma complement levels include the acute phase response, patient's variation in complement gene copy number, expression and protein synthesis and catabolism. Low complement levels do not work well as SLE diagnostic indicators as a result of these variables [19]. Though, decreased complement levels are not always linked to disease flare-ups, and measuring complement levels has drawbacks due to variations in genetic polymorphisms, variability in synthesis, and the possibility of autoantibodies activating complement in vivo regardless of disease activity [20].

According to the present work, clinical symptoms like photosensitivity, malar rash, and arthritis appeared not to be connected with PS-PLA1 level. However, lupus nephritis was impacted by variations in PS-PLA1 serum levels. The same results were detected by **Iwata *et al.*** [21] who stated that PS-PLA$_1$ has markedly increased in sera of lupus nephritis patients. But, neither pathological findings nor clinical traits like proteinuria or a deterioration in renal function in LN are correlated with the PS-PLA1 level ($p>0.05$). This is consistent with **Sawda *et al.*** [14] who described the association between the level of PS-PLA1 and kidney pathology, although the serum levels of PS-PLA1 were greater in patients with renal manifestation than in those without. Unfortunately, there were just few previous researches that have dealt with this topic, which made the comparison to be difficult.

Our study demonstrated that the mean serum PS-PLA1 concentration was 6.852.23 ng/ml, 7.532.11 ng/ml, and 10.30.94 ng/ml for mild, moderate, and severe disease activity respectively. It was well noted that PS-PLA1 showed a significant increase with different disease activity classes ($P =0.002$). Moreover, a significant fluctuation in blood levels were observed when comparing mild to severe disease activity and moderate to severe disease activity ($P= 0.001$, $p=0.049$ respectively). This is similar to **Kurano *et al.*** [22] results, which elucidated that serum PS-PLA1 levels were shown to be substantially related to clinical stage progression. Also similar results were reported by **Sawda *et al.*** [14] where there was a significant positive correlation between the SLEDAI and serum PS-PLA1 ($r=.549$, $P < .0001$) for all SLE patients. This is against **Iwata *et al.*** [21] study, which revealed that, PS-PLA1 levels did not correlate with the SLE Disease Activity Index (SLEDAI) score, clinical, and laboratory results (such as as anti-double-stranded DNA antibody and complements).

Our research demonstrated a strong statistically significant positive association between serum PLA1, CH50 levels and laboratory variables (ANA, Anti dsDNA, ESR, creatinine, Albumin/creatinine ratio), ($p<0.05$). On the contrary, a strong negative association between serum PLA1, CH50 concentrations with hemoglobin, total leukocyte count, and the platelets count had been established ($p<0.05$). According to **Sawda *et al.*** [13] study, there is a substantial positive association between serum PLA1 levels and ANA. Currently, highly statistically significant negative association had been found between serum PLA1 levels, and haemoglobin, total leukocyte count, and platelet count. Also, another study by **Sawda *et al.*** [14] in 2019 discovered that PS-PLA1 was statistically significant ($P =0.0001$) in relation to anti-dsDNA antibodies ($P= 0.0001$), however statistically insignificant ($P = 0.67$) in relation to WBC, lymphocytes. This is against findings of **Rahman and Isenberg** [23] who noticed that PS-PLA1 and anti-dsDNA antibody titers did not correlate. On contrary, **Sanduh *et al.*** [20] demonstrated that there was no correlation between hemolytic tests and clinical activity except for CH50- and the physician's judgement ($r=0.62$, $P=0.028$).

Swollen joint counts, proteinuria, leucocyte counts, and platelet counts are also included in the recently suggested SLE disease activity score, but quantitative serological immunological indicators such as serum complement levels or anti-dsDNA antibody titers are excluded [24].

In the current study, utilizing a cut-off value of PS-PLA1 $\geq$ 9.12 ng/ml, an excellent AUC (9.00) was reached using the analysis of PS-PLA1 ROC curves to predict SLE severity, it denoted high sensitivity (85.7%), and specificity (78.8%) values. CH50 at a cut-off $\geq$13.5 pg/ml showed a sensitivity of 85.7% and specificity of 69.7%. The best cut-off values for separating untreated SLE patients from healthy controls, as determined by ROC analysis were 18.4 and 18.2 ng/ml, respectively in a research by **Sawada *et al.*** [14]. The latter cut-off value was utilized to assess the diagnostic performance of serum PS-PLA1, which was shown to have 71.4% sensitivity in untreated SLE patients and 57.5% specificity versus disease controls.

LIMITATIONS

The significantly small sample size is the study's main drawback. Increasing the sample size will help clarify the relationship between PS-PLA1 levels and clinical symptoms. Another drawback was the absence of ongoing assessments of blood PS-PLA1 levels in SLE patients receiving treatment. Self-funded research, limit the studying of the correlation of these biomarkers with other assessment tools for systemic lupus erythematosus, including other autoantibodies, radiological, and pathological evaluation tools.

CONCLUSION

Significant high serum levels of PS-PLA1 in SLE patients, denotes its strong potential role in the pathogenesis of SLE. Significant link with disease activity, severity and outcomes were explained by significant correlation of PLA1 with the SLEDAI score points to the urgency of the conduction of new researches for investigation of its association with certain organ damage and so can be used as an accessible tool for disease activity monitoring and treatment follow up.

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