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Platelets activation and indices in patients with systemic lupus

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Abstract

Systemic lupus erythematosus (SLE) is an autoimmune disease with higher prevalence in women than men, with a female-to-male ratio ranging from 8:1 to 15:1.SLE is characterized by periods of remission and exacerbation (flares) with prolonged periods of subclinical activity, thus making it a very unpredictable disease needs continuous assessment of disease activity. There is no single biomarker used for that purpose. Platelet activation markers as CD62p and Platelet indices have recently been found to be a simple inflammatory marker used in the assessment of systemic inflammation in many diseases like rheumatoid arthritis, ankylosing spondylitis and, inflammatory bowel diseases. Aim of study, to investigate the platelet indices and their relation to platelet activation and disease activity in SLE patients. A case-control study enrolled forty SLE recruited from AL-Zahraa University Hospital Al-Azhar University. According to Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) score the patients were divided into three groups first one including ten patients without flare (SELEDAI <4), a second group including ten patients moderate flare (SELEDAI 8 – <12) and the third group including twenty patients with severe flare (SELEDAI >or=12). Another forty persons apparently healthy including in the study as a control group. P selectin and Platelet indices (MPV, PDW, PTC) were assessed in all of them and their correlation to the SLEDAI score was analyzed. There was a highly significant increase in P selectin in the active patients more than non-active patients (p-value < 0.05) and a highly significant increase in severe activity compared to moderate activity (p-value < 0.001), and a significant decrease in PCT and MPV in the active patients less than non-active patients (p-value < 0.05) and it is decreased in severe cases compared to moderate activity, non-active and control groups(p-value < 0.05) and it had a significant negative correlation with SELEDAI score. By Using the ROC curve, it was shown that MPV can be used to discriminate between active & non-active SLE patients with a specificity of 66.7% and sensitivity 60%. Also, CD62p can be used to discriminate between active & non-active SLE patients with 66.7% sensitivity & 80% specificity. P selectin, mean platelet volume (MPV), and Plateletcrit (PCT) could be used as a novel marker for SLE activity.

Keywords: SLE – Lupus activity –SLEDAI – p selectin-Platelet indices – Mean platelet volume – Platelet-crit – Platelet distribution width.

1. Introduction

Systemic lupus erythematosus (SLE) is a chronic multi-system autoimmune disease characterized by autoantibodies against nuclear antigens and deposition of immune complexes (IC) in several tissues [1].

Activation of platelets is increased in patients with SLE compared with healthy individuals. Surface expression of P-selectin is a hallmarks of platelet activation. In SLE, platelets have increased surface levels of P-selectin correlates with the SLEDAI score [2].

There is no single biomarker for assessment of disease activity till now but there is a validated measure as SLEDAI is beneficial in day-to-day practice. This activity index uses multiple parameters like clinical and laboratory parameters some of them are simple, easy to use in routine clinical practice and others may not be simple. Platelet indices are a group of platelet parameters determined in automatic CBC profiles; they are related to platelets' morphology and proliferation kinetics and represent platelet activation markers [3].

The present study aimed to investigate the platelet indices and their relation to platelet activation and disease activity in SLE patients.

2. Materials and Methods

2.1 Study design:

A case-control study was conducted on forty control and forty patients. The control group was 7male and 33 female and their ages ranged from 20 to 48 years. Forty patients were fulfilling 2012 SLICC (Systemic Lupus International Collaborating Clinics) criteria for SLE. They were 35 (87%) females and 5 (12.5%) males. Their ages ranged from 18 to 50 years. Their disease duration ranged from 12 to 45 months. According to disease activity the patients were divided into three groups, group I(N=10) patients without flare (SELEDAI <4), group II (N =10) patients with moderate flare (SELEDAI 8 – <12) and group III (20) patients with severe flare (SELEDAI >or=12, the patients were adults' males and females ≥ 18 years old.

2.2 Exclusion criteria:

Patients with a cardiovascular event or venous thrombosis, patient with other autoimmune diseases, patients with known current infection and females were in menstruation or taking oral contraceptive pills were excluded from the study.

All patients were subjected to the following: -

Clinical history taking, complete clinical examination, and assessment of SLE disease activity state using SLEDAI score.

Laboratory investigations including: The patients and control groups were subjected to: Complete blood count by (sysmex KX-21N, Japan), erythrocyte sedimentation rate (ESR), kidney function tests,24hour urinary protein, liver function tests, complete urine analysis, anti-nuclear antibody (ANA), anti -double stranded DNA antibody (anti-ds DNA Ab), complement 3 (C3) and complement 4 (C4) level.

Immune phenotyping for detection of CD41a, CD42b, CD62p on platelets by flow cytometry. Platelet indices were obtained from CBC done on an automated cell counter.

2.3 Detection of CD41a, nCD42b, CD62a on platelets by flowcytometry:

Platelet-rich plasma was obtained by centrifugation of citrated blood at 110xg for 10 minutes. 100 ul of platelet-rich plasma of each patient sample was mixed with 10 ul of each one the following monoclonal antibodies: Phycoerythrin (PE) conjugated anti human CD4 (Lot NO.5245924), Fluorescein isothiocyanate

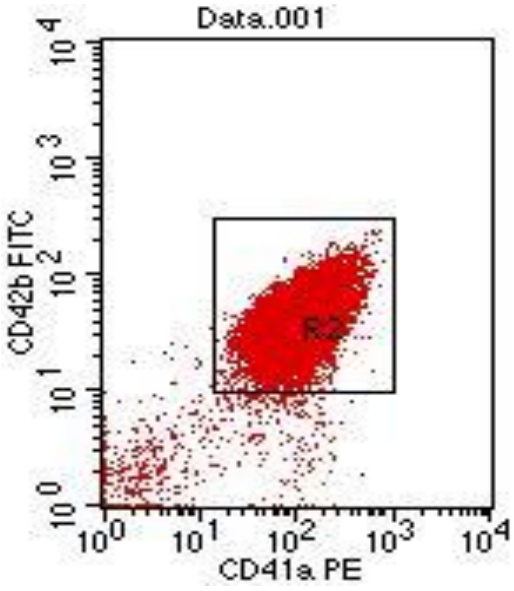
(FITC) conjugated CD42b (Lot NO.6266800), and Allophycocyanin (APC) conjugated CD62P (Lot NO.8137531)**.** All monoclonal antibodies were obtained from BD Biosciences, San Jose, USA. These samples were incubated for 15 to 30 minutes in the dark at room temperature (20ºto 25ºC). After incubation, tubes were washed twice with phosphate buffered saline. Then the pellets were resuspended in sheath fluid. Isotype control (BD Biosciences, San Jose, USA, lot no. 8243903.) was used for detection of nonspecific binding.
The acquisition was done on four colors FACS Caliber (BD, Biosciences, San Jose, USA) using system software Cell Quest pro. Acquisition of at least 100,000 events was done for both test and control tubes. Compensation was established using color calibrate beads (BD Biosciences, San Jose, USA, lot no.8192516).

1- Gating of Platelets:

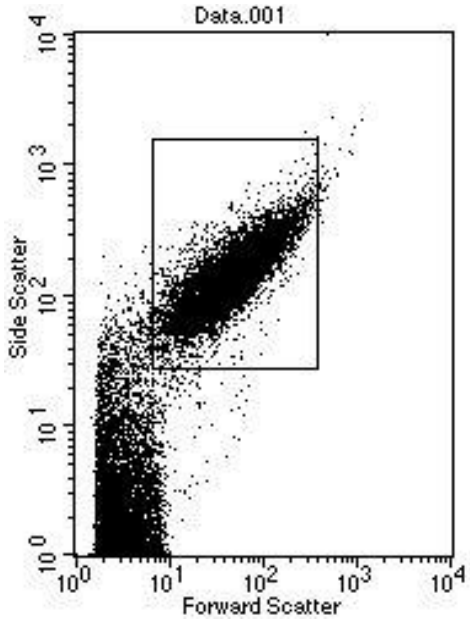
A- With the use of logarithmic amplification, platelets were identified using the forward scatter (FSC) /side scatter (SSC) characteristics as shown in Fig.1. A.

B- Platelets were further identified by expression of PE CD41a and FITCE CD42b as shown in Fig.1. B.

2. Platelets were evaluated for the expression of the activation marker CD62P(APC) using a single histogram where CD62P(APC) was represented on the X-axis.



(A)



(B)

Figure (1): Representative dot plot showing platelet distribution and gating by: (A) Forward scatter (FSC) /side scatter (SSC) characteristics. (B) Expression of PE CD41a and FITCE CD42b.

2.4 Statistical analysis:

Recorded data were analyzed using the statistical package for social sciences, version 20.0 (SPSS Inc., Chicago, Illinois, USA). Quantitative data were expressed

as mean± standard deviation (SD). Qualitative data were expressed as frequency and percentage. The following tests were done:
Independent-samples t-test of significance was used when comparing between two means. Chi-square (x2) test of significance was used to compare proportions between qualitative parameters. Pearson's correlation coefficient (r) test was used to assess the degree of association between two sets of variables laboratory findings were compared among the groups using one-way ANOVA for continuous variables. The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant as the following:
Probability (P-value). P-value <0.05 was considered significant. P-value <0.001 was considered highly significant. P-value >0.05 was considered insignificant. ROC curve was performed to determine the value of the p selectin and MPV Cutoff as disease activity biomarkers. The sensitivity, specificity, positive predictive value, and negative predictive value, and p-value were also calculated.

3. Results

In the present study as regard to CBC parameters it shows highly statistically significant decrease in RBCs, Hb, HCT, MCH& PLTs in our patients compared with control (p-value < 0.001) and statistically significant decrease in MCV in patients compared with control (p-value < 0.05) and highly statistically significant increase in ESR in our patients compared with control (p-value < 0.001) as shown in Table 1. Regarding p selectin and platelet indices in our study, there was a significant increase in CD62p MFI in the active patients more than non-active patients (p-value < 0.05) and a highly significant increase in severe activity compared to moderate activity (p-value < 0.001) and a significant decrease in PCT and MPV in the active patients more than non-active patients (p-value < 0.05) also there is a statistically decreased in PCT and MPV in severe cases compared to moderate activity, inactive and control groups (p-value < 0.05) as shown in Table 2 and Table 3. Results of MFI of CD62 using single histogram in studied groups are shown in Fig.2.

In our study, we found a statistically significant increase inCD62P in thrombocytopenic patients compared to patients with normal platelets(p-value<0.05). Also, there was a statistical and highly statistically significant decrease in MPV &PCT (p-value<0.05) and (p-value<0.001) respectively in thrombocytopenic patients compared to patients with normal platelets but no differences between patients with thrombocytopenia & patients with normal PLT count as regard PDW as shown in Table 4. Our result showed a highly significant negative correlation between CD62 and MPV in all patients and non-active patients and a significant Negative correlation between CD62 and MPV in the active patients as shown in Fig.3. Also, there was a highly significant Negative correlation between CD62 and PCT in all patients and this correlation is significantly negative in the active patients. Also, there was a highly significant Negative correlation between MPV & SLEDI in all patients and a significant Negative correlation between MPV & SLEDI in non-active patients as shown in Table 5. The receiver operating characteristic curve (ROC) showed that MPV can be used to discriminate between active & non-active SLE patients at a cutoff level of < 9.35, with 66.7% sensitivity, 60% specificity, 62.5% PPV and 64.3% NPV (AUC = 0.75 & p-value = 0.015).Also, CD62 can be used to discriminate between active & non-active SLE patients at a cutoff level of > 55.5, with 66.7% sensitivity, 80% specificity, 76.9% PPV and 70.6% NPV (AUC = 0.76 & p-value = 0.016) as show in Table 6.

Table (1): Comparisons between studied groups as regard to CBC and routine parameters.

		Groups				F	P-value
		Non active (n = 10)	Moderate active (n = 10)	Severe active (n = 20)	Control (n = 40)		
WBCs $x10^3$/ul	**Mean**	5.8	7.5	5.8	7.1	**1.7**	**0.161 NS**
	±SD	2.4	4.9	2.8	1.7		
RBCs ($x10^{12}$/ul)	**Mean**	4.1	3.8	4.3	4.7	**12.8**	**< 0.001 HS**
	±SD	0.7	0.5	0.5	0.4		
Hb (g/dl)	**Mean**	10.6	9.2	11.0	12.9	**31.7**	**< 0.001 HS**
	±SD	1.6	1.4	1.3	0.9		
Hct (%)	**Mean**	33.2	30.3	36.3	39.4	**18.7**	**< 0.001 HS**
	±SD	5.0	5.1	3.6	3.3		
MCV (fl)	**Mean**	78.2	82.3	81.6	84.2	**3.07**	**0.033 S**
	±SD	11.2	7.5	5.0	3.5		
MCH (pg)	**Mean**	25.2	25.5	26.2	28.1	**10.6**	**< 0.001 HS**
	±SD	4.3	1.4	1.7	1.1		
MCHC (g/d)	**Mean**	32.0	31.2	31.3	32.6	**4.9**	**0.004 S**
	±SD	1.7	1.9	1.7	1.2		
PLTs ($x10^3$/ul)	**Mean**	279.1	202.0	161.1	277.4	**11.5**	**< 0.001 HS**
	±SD	52.5	107.9	92.8	65.6		
ESR (mm/h)	**Mean**	12.5	69.8	69.7	10.0	**38.2**	**< 0.001 HS**
	±SD	3.3	38.7	39.3	4.3		
Urea (mg\dl)	**Mean**	34.8	64.0	52.6	26.8	**19.4**	**< 0.001 HS**
	±SD	13.3	31.0	20.1	9.0		
Creat (mg\dl)	**Mean**	1.2	2.2	1.4	0.9	**15.1**	**< 0.001 HS**
	±SD	0.5	1.2	0.7	0.3		
U.protein (mg\dl)	**Mean**	0.3	1.7	1.2	0.2	**16.9**	**< 0.001 HS**
	±SD	0.1	1.8	0.9	0.1		

Table (1): Comparisons between studied groups as regard to CBC and routine parameters.

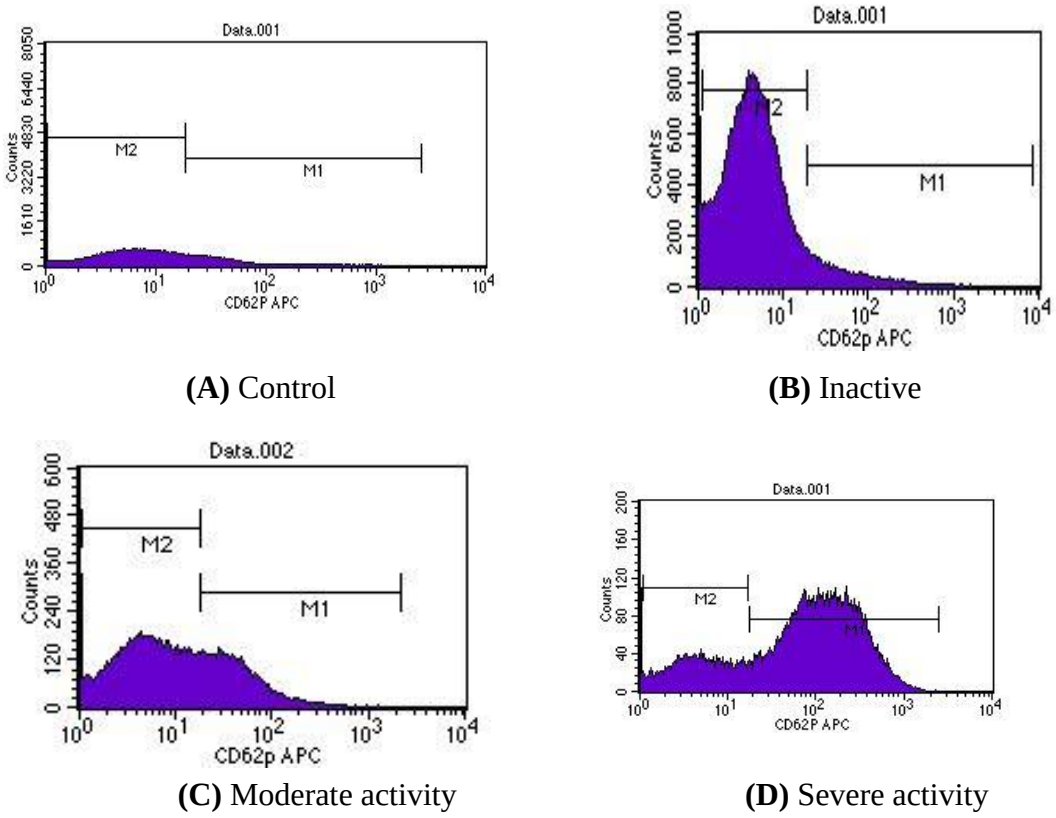
		Groups				F	P-value
		Non active (n = 10)	Moderate active (n = 10)	Severe active (n = 20)	Control (n = 40)		
ALT (iu\L)	**Mean**	25.4	19.0	23.6	21.0	**0.5**	**0.682 NS**
	±SD	33.4	15.3	7.7	6.9		
AST (iu\L)	**Mean**	50.6	32.2	43.4	18.3	**3.4**	**0.022 S**
	±SD	80.6	31.0	40.4	5.8		
T. Bili (mg\dl)	**Mean**	0.5	0.3	1.0	0.8	**11.5**	**< 0.001 HS**
	±SD	0.7	0.1	0.3	0.2		
D. Bili (mg\dl)	**Mean**	0.3	0.1	0.4	0.4	**2.2**	**0.099 NS**
	±SD	0.7	0.1	0.2	0.2		
ALB (g\l)	**Mean**	3.9	3.0	3.9	4.0	**8.07**	**< 0.001 HS**
	±SD	0.3	0.7	0.9	0.5		
C3 (mg\dl)	**Mean**	105.3	62.9	59.6	142.1	**56.01**	**< 0.001 HS**
	±SD	37.5	17.7	19.1	27.3		
C4 (mg\dl)	**Mean**	21.0	14.0	16.6	26.9	**8.4**	**< 0.001 HS**
	±SD	13.7	8.3	10.3	7.7		

Table (2): Comparisons between non active& active patients as regard p selectin and PLT indices.

		Activity		Test	P-value
		Non active (n = 10)	Active (n = 30)		
CD62 MFI	Median	42.4	69.9	MW = 72.5	0.014 S
	IQR	19.1 – 56.6	43.9 – 124.8		
MPV (fl)	Median	10.3	8.8	MW = 72.5	0.014 S
	IQR	8.85 – 11.2	7.9 – 10.1		
PDW (%)	Median	11.25	11	MW = 147.5	0.939 NS
	IQR	9 – 11.9	9 – 12.9		
PCT (%)	Median	0.27	0.12	MW = 51	0.001 S
	IQR	0.25 – 0.29	0.08 – 0.23		

Table (3): Comparisons between studied groups as regard p selectin and PLT indices.

		Groups				F	P-value
		Non active (n = 10)	Moderate active (n = 10)	Severe active (n = 20)	Control (n = 40)		
CD62 MFI	Mean	41.8	71.6	90.3	41.7	8.2	< 0.001 HS
	±SD	24.6	44.4	60.0	22.3		
MPV (fl)	Mean	10.01	9.35	8.67	9.93	5.6	0.002 S
	±SD	1.21	1.29	1.10	1.20		
PDW (%)	Mean	10.90	11.42	10.95	12.38	1.64	0.187 NS
	±SD	1.56	2.51	2.14	1.92		
PCT (%)	Mean	0.27	0.18	0.14	0.27	19.9	< 0.001 HS
	±SD	24.6	44.4	60.0	22.3		



(A) Control **(B)** Inactive

(C) Moderate activity **(D)** Severe activity

Figure (2): Mean fluorescence intensity (MFI) of CD62p +ve platelet (measured in area under M1 marker) using single histogram in (A)- Control (B) non active (c) -Moderate activity (D) –Severe activity.

Table (4): Comparisons of CD62P & PLT indices in thrombocytopenic patients compared with patients with normal PLTs.

		PLTs		T	P-value
		Thrombocytopenia (n = 15)	Normal PLTs (n = 25)		
CD62 (MFI)	Mean	103.8	54.1	3.2	0.003 S
	±SD	64.9	34.7		
MPV (fl)	Mean	8.4	9.4	2.9	0.006 S
	±SD	1.0	1.2		
PCT (%)	Mean	0.1	0.2	7.3	< 0.001 HS
	±SD	0.0	0.1		
PDW (%)	Mean	10.5	11.4	1.2	0.234 NS
	±SD	1.9	2.1		

Table (5): Comparisons of CD62P & PLT indices in thrombocytopenic patients compared with patients with normal PLTs. (r): Pearson correlation coefficient. S: p-value < 0.05 is considered significant. HS: p-value < 0.001 is considered highly significant. NS: p-value > 0.05 is considered non-significant.

Variables	All patients		Active patients		Non-active pat.	
	R	p-value	R	p-value	R	p-value
CD62 vs age	-0.02	0.904 NS	0.06	0.771 NS	-0.29	0.425 NS
CD62 vs MPV	-0.58	< 0.001 HS	- 0.47	0.008 S	-0.93	< 0.001 HS
CD62 vs PDW	0.03	0.836 NS	-0.02	0.928 NS	0.40	0.25 NS
CD62 vs PCT	-0.53	< 0.001 HS	- 0.43	0.018 S	-0.43	0.214 NS
PDW vs SLEDI	-.219	.174 NS	.393	.723 NS	.100	.783 NS
MPV vs SLEDAI	-0.571	0.000 HS	-0.160	0.658NS	-0.52	0.003 S

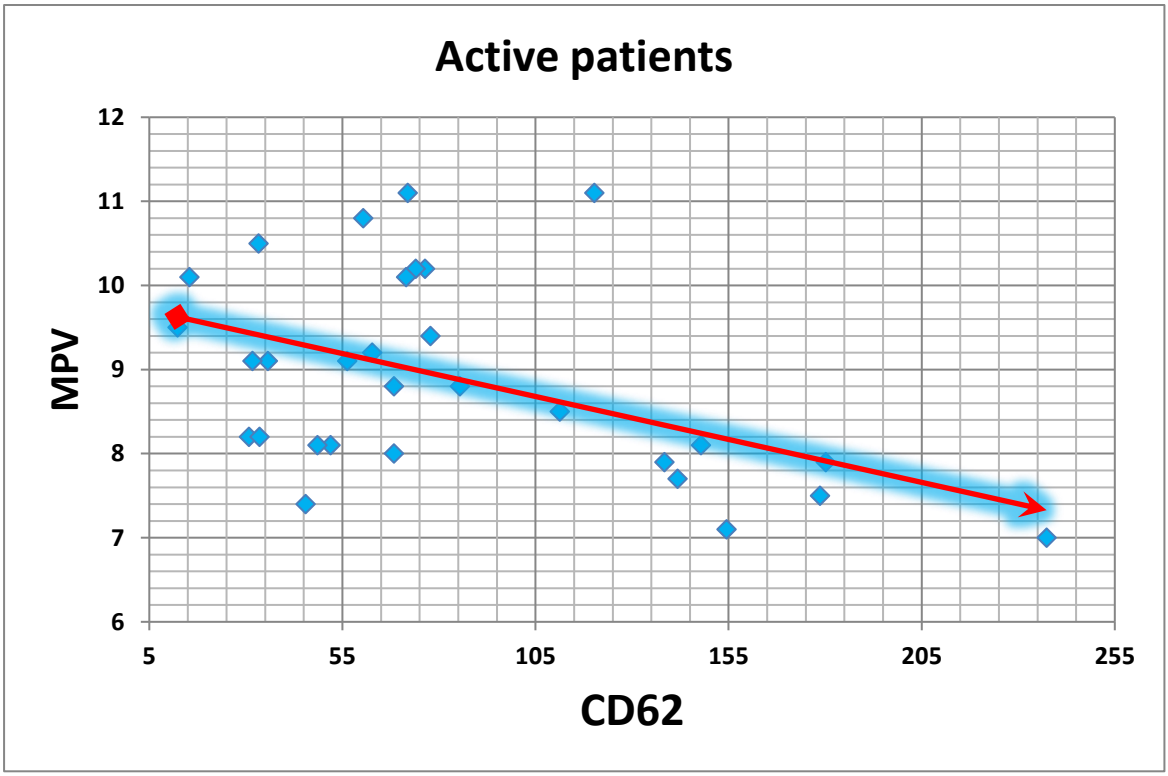


Figure (3): Negative correlation between CD62 & MPV in the active patients. The receiver operating characteristic curve (ROC) showed that MPV can be used to discriminate between active & non-active SLE patients at a cutoff level of < 9.35, with 66.7% sensitivity, 60% specificity, 62.5% PPV and 64.3% NPV (AUC = 0.75 & p-value = 0.015).Also, CD62 can be used to discriminate between active & non-active SLE patients at a cutoff level of > 55.5, with 66.7% sensitivity, 80% specificity, 76.9% PPV and 70.6% NPV (AUC = 0.76 & p-value = 0.016) as show in table (6).

Table (5): Diagnostic performance of MPV & CD62 in discrimination of active and non-active groups. PPV: positive predictive value. AUC: Area under curve. NPV: negative predictive value.

	Cut off	AUC	Sensitivity	Specificity	PPV	NPV	p-value
MPV (fl)	**< 9.35**	**0.75**	**66.7%**	**60%**	**62.5%**	**64.3%**	**0.015**
CD62p (MFI)	**> 55.5**	**0.76**	**66.7%**	**80%**	**76.9%**	**70.6%**	**0.016**

4. Discussion

Systemic lupus erythematosus is the multisystemic, autoimmune disease characterized by heterogenous organ involvement and production of an array of autoantibodies [4].

SLE can be associated with thrombotic complications and increased cardiovascular morbidity. A wide range of research on the pathogenesis of SLE focus on the formation of autoantibodies and autoantibody induced immune complex as well as dysregulation of lymphocyte function and activation of the complement system. However, platelets also play an important role in the inflammatory activity and immune response. Growing evidence indicates that platelets are activated in SLE patients and contribute to the pathogenesis of SLE [5].

In our study there was a highly significant decrease in RBCs, Hb, HCT, MCH, & PLTs (p-value < 0.001) and significant decrease in MCV in patients compared with control (p-value < 0.05). Also, there was a highly significant increase in ESR in our patients compared to the control (p-value < 0.001).

In parallel to our study Manzano et al., [6] found that RBCs, Hb, HCT, MCV, MCH, and platelet counts were reduced in SLE patients compared to controls.

In the current study, there was a significant increase in CD62 p MFI in the active patients more than in non-active patients. Also, there was a highly significant increase in CD62p expression in the severe activity group compared to the moderate activity group.

Our study was supported by Stadtlober et al., [7] and Manzano et al., [6] found that increased level of P-selectin, in SLE patients compared to controls.

Guillotin et al., [8] measured the levels of soluble selectins, they found that patients with active SLE had increased levels of both soluble and microparticulate selectins compared to control and inactive SLE.

Mean platelet volume is a measure of the average size of platelets in a blood sample. A high MPV indicates the presence of more young platelets circulating in the blood [9].

The current study revealed that a significant decrease in MPV in the active patients compared to non-active patients (p-value < 0.05) and a statistically decreased in severe cases compared to

moderate activity group, non-active, and control groups (p-value < 0.05).

In agreement with the present study, Saragih et al., [10] Hartmann et al., [11], and Khan et al., [12] found that adult patients with active SLE had decreased MPV when compared to the non-active disease group.

This may be attributed to the consumption of the large and activated platelets at the site of inflammation, leaving small platelets behind. Also, overproduction of pro-inflammatory cytokines can suppress bone marrow following the production of small-size platelets [12].

In agreement with our results, the study of Pryzwara-Chowaniec et al., [13] found that a significant decrease in MPV in SLE patients compared to the control group.

In disagreement with the present study, SHERIF et al., [3] showed no statistical significance among studied groups between non active and active patients as regards MPV. Also, Talat et al., [1] showed that the MPV was significantly increased in patients with SLE as compared to the control group and in active stage of SLE than in remission stage. However, their study was done on children under 17 years old.

Platelet-crit (PCT) is the volume occupied by platelets in the blood as a percentage and is calculated according to the formula PCT= Platelet count x MPV/10,000. The normal range for PCT is 0.22- 0.24%. [9].

As regards PCT we found a statistically significant decrease of PCT in the active patients compared to non-active patients (p-value < 0.05) and a highly significant decrease of PCT in the severe activity group compared to moderate activity, non-active patients, and control group (p-value < 0.001).

In agreement with the present study results obtained by SHERIF et al., (3) found that PCT showed lower values in lupus patients with severe flare than other groups.

Platelet distribution width is an indicator of volume variability in platelet size and is increased in the presence of platelet anisocytosis [9].

In the present study, there were no statistically significant differences in PDW in all studied groups (p-value > 0.05).

our result was supported by SHERIF et al., [3] and Islamouglu and Demirbas,[14] studies, they revealed that no statistical significance among his SLE patients and control groups. Also, Talat et al., [1] found that no significant difference in children between the active stage and remission stage regarding the PDW value. He also found that PDW in SLE patients is more than control.

In disagreement with the current study Saragih et al., [10] found that PDW was higher in the flare group compared with non-flare.

Thrombocytopenia reflects platelet activation and can be considered as a marker of disease activity and as a marker for the long-term appearance of comorbidities [15].

In our study we found a statistically significant increase in CD62P in thrombocytopenic patients compared to patients with normal platelets (p-value < 0.05). Also, there was a statistical and highly statistically significant decrease in MPV and PCT (p-value < 0.05) and (p-value < 0.001 respectively in thrombocytopenic patients compared to patients with normal platelets but no statistically significant differences between them as regard PDW (p-value > 0.05).

Philo et al., [16] found that 13.3% of his SLE patient were thrombocytopenic, 8%

of them had normal MPV while 5.3% had increased MPV.

In the present study, there was a highly significant negative correlation between CD62and (MPV& PCT) with a p-value (< 0.001) While there is no significant correlation between CD62 and PDW. Also, there was a highly significant negative correlation between MPV & SLEDI score and a significant positive correlation between MPV &Platelet count in all patient groups, also the same correlation was found between non active and control group.

In disagreement with our study, Talat et al., [1] found that the MPV was positively correlated with SLEDAI in the active stage of the disease, while SHERIF et al., (2019) found that there is no correlation between MPV and SELEDAI, but they supported our results about the correlation between SELDAI score and PDW.

This In disagreement with the current study Chen et al., [17] in his study that showed a positive correlation between PDW and SELEDAI in all patients.

In the present study by using ROC curve, it was shown that MPV can be used to discriminate between active & non-active SLE patients at a cutoff level of < 9.35, with 66.7% sensitivity, 60% specificity, 62.5% PPV and 64.3% NPV (AUC = 0.75 & p-value = 0.015).Also, CD62 can be used to discriminate between active & non-active SLE patients at a cutoff level of > 55.5, with 66.7% sensitivity, 80% specificity, 76.9% PPV and 70.6% NPV (AUC = 0.76 & p-value = 0.016).

5. Conclusion:

Mean platelet volume, p selectin and PCT could be used as a marker of disease activity. MPV could be used to discriminate between active & non-active SLE patients at a cutoff level of < 9.35, with 66.7% sensitivity. P selectins and MPV may be a promising new direction **for the prognosis of SLE.**

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