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Research Article

Correlation of red blood cell distribution width in systemic lupus erythematosus patients

Nehal Ibrahim Abbas; Hanan Mostafa Kamel;
Mohammed Abdelrazek Abdelhakeem and Asmaa Raouf Abdelaziz
Department of Clinical Pathology, Faculty of Medicine, Minia University

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Abstract

SLE, or systemic lupus erythematosus, is a chronic, relapsing-remitting autoimmune disease with a wide range of symptoms, from moderate to life-threatening. SLE often affects women of reproductive age because to a combination of genetic susceptibility and environmental, immunological, and hormonal factors. Preventing SLE relapses is an essential part of the disease treatment strategy, and exacerbations are critical events that may have a substantial impact on the prognosis. Anti-alpha enolase antibody and RDW concentrations in SLE patients were evaluated in this research to determine their function in SLE activity. It was conducted in the Clinical Pathology Department, Rheumatology and Rehabilitation Department, Faculty of Medicine, Minia University, Egypt between May 2021 and July 2022. After receiving ethics committee permission, the study recruited 90 volunteers, all of whom provided their written agreement in the form of a signed consent form. I. The Aspects: The participants were categorized as follows: A total of thirty (30) individuals with SLE on exacerbation were enrolled in group I (Active group). Patients were chosen from the Minia University Hospital's Rheumatology Department's in-patient wards. A total of thirty (30) patients in group II (the stable group) were enrolled in the study from Minia University Hospital's outpatient Rheumatology Clinic, the patients were chosen. There were 30 people in group III (the "control group"), and all of them seemed to be in good condition. The ages of all the controls were matched to those of the patients. The following procedures were used on all participants: Consider age, work, place of residence, length of illness, presence of joint and muscle pains, L.L edoema, haemorrhage, fever and dyspnea...etc. and any co-morbidities while obtaining a thorough history of the patient's health. Complete abdominal and local exams are performed. 3) Experiments in the laboratory: Investigations of a routine nature Included CBC, ESR, renal function tests, liver function tests, urine analysis, 24-hour protein in urine, rheumatoid factor and C-reactive protein (CRP). Anti-double-strand DNA, complement C3, and complement C4 antibodies are used to diagnose SLE. (C) Focused studies: Anti-alpha enolase antibody ELISA measurement. 2. Automated blood cell counters discovered RDW. The findings of this investigation were as follows: Additionally, there was a statistically significant rise in haemoglobin levels in group II when compared to the haemoglobin levels of the other two groups. Two of the three groups investigated showed a statistically significant DROP in platelets while the other two groups showed no statistically significant difference in TLC. While group III had a decrease in urea, group I and II saw an increase. When comparing groups, I and II to group III, there was a statistically significant rise in creatinine. • The ALT and AST levels in groups I and II were significantly higher than in group III, although the ALB levels in groups I and II were lower than in group III. There was a statistically significant rise in CRP and ESR in group I, whereas there was a statistically significant increase in RF in group I when compared to group III. There was a statistically significant rise in ANA and Anti-ds DNA in group I, but there was a statistically significant DROP in C3 and C4 when compared to group II. • When comparing groups, I, II, and III, the levels of anti-alpha enolase antibody and RDW were both highly statistically significant. It was shown that anti-alpha enolase Ab had a substantial positive connection with ANA, C3, and C4 on the one hand, and anti-alpha enolase Ab on the other with both TLC and platelets with respect to RDW.

Keywords: Systemic lupus erythematosus; Disease activity; Red cell distribution width; Anti-alpha enolase antibody; Diagnostic performance

Introduction

Lupus, conventionally called systemic lupus erythematosus (SLE) is an autoimmune disorder in which the body immune system erroneously conquers various organs. It is a disease of flares and remissions. Lupus attacks mainly women in childbearing period, although other population get lupus, too.[(1)] Lupus is considered a deadly disease in the 20th century due to deficient diagnostic tools and therapeutic modalities[(2)]. Therefore, identifying markers for early detection of the disease activity and advancement of treatment options become urgent necessity.

The cytoplasmic and glycolytic enzyme families include alpha-enolase. Infections and autoimmune illnesses can manifest with antibodies that target alpha-enolase. The anti-alpha enolase antibodies (AAE-Abs) cause endothelial damage in autoimmune disorders by triggering cell death, disrupting the fibrinolytic system, activating the complement classical cascade, and generating immune-logical complexes.[(3)]

As part of a full blood count, an automated hematology analyzer measures the red cell distribution width (RDW) to determine the variability of red cells. When combined with other red cell indices, RDW helps clinicians distinguish between various forms of anemia.[(4)] Irrespective of infection, anemia, or nutritional deficits, a large body of research suggests that elevated RDW values are associated with several autoimmune disorders.[(5)] As a result, RDW could be useful for estimating the incidence of certain disorders.

Aim of the Work:

This research set out to determine if serum AAE-Abs and RDW may be useful indicators for SLE disease activity prediction.

Subjects and Methods

The present investigation was place between March 2021 and April 2022 at the Clinical Pathology Department and the Rheumatology and Rehabilitation Department at the Faculty of Medicine, Minia University, Minia, Egypt.

Previous research indicated that in order to achieve 80% power at the 0.05 significance level, this study included 60 individuals with SLE. Based on the criteria outlined by Petri et al. (6), patients were diagnosed with SLE. Two groups were formed from the subclassification.

Twenty-five patients with active lupus erythematosus disease (SLEDAI) and a score more than 4 were included, whereas thirty patients with inactive lupus erythematosus (SLEDAI) and a score less than 4 were also included. For this research, we enlisted the help of 30 healthy individuals to act as a control group.

Patients with other autoimmune disorders, cardiovascular, kidney and liver diseases, acute infection, chronic inflammatory diseases, benign or malignant hematological diseases, as well as those with a history of blood transfusion 3 months prior participation in this study, were excluded.

Each participant gave his/her written, informed consent, and the Local Ethical Committee (No.817/08/2021) gave the approval to the study.

A comprehensive medical history, physical examination, and battery of laboratory testing were administered to both healthy controls and patients with SLE in this research. All of the following tests were performed: complete blood count (CBC), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), urine analysis, 24-hour protein in urine test, kidney and liver function tests, rheumatoid factor (RF), antinuclear antibody (ANA), anti-dsDNA, complement (C)3 and C4, evaluation of serum levels of anti-alpha enolase antibody (AAE-Abs), and RDW.

Blood sampling protocol:

About 8 ml of venous blood was withdrawn from each subject by using a disposable plastic

syringe after disinfection of skin with isopropyl alcohol (70%) swaps. This sample was divided as follow:

- 1.8 ml of blood on a tube containing 0.2 ml trisodium citrate for detection of ESR (Dilution 1:4).
- 1.8 ml in ethylenediaminetetraacetic acid (EDTA) containing tube for CBC.
- 4 ml on plain tube. Prior to centrifugation, the blood was allowed to coagulate in the incubator for 30 minutes. Assays for renal and liver function tests, CRP, RF, and AAE_Abs were performed using the expressed serum.

Methods

A) Routine investigations:

1- Complete blood count (CBC): It was performed using **Sysmex XN-1000TM AUTOMATED HEMATOLOGY ANALYSER, France.**

- **Principles and Technology:** Dedicated channels for fluorescent flow cytometry using a semi-conducting laser for hydrodynamic focusing.
- **RDW** stemmed from the constraints of the CBC. Microscopical analysis of a blood film stained with Leishman's solution validated the differential leucocytic count.

2- Renal function tests (blood urea & serum creatinine) **and Liver function tests** (ALT, AST and serum Albumin): following the instructions provided by the manufacturer, we used the **auto-analyzer SELECTRA PRO XL from ELIT ech** Group, clinical chemistry automation systems, Netherlands, in conjunction with commercially available kits.

3- CRP: using **GENRUI, Biotech Inc, kinetic assay, China** immunofluorescence method.

4-RF: using latex agglutination method**.**

5- ESR: Determined by **conventional Westergren method.**

6-Urine analysis:

- Random mid-stream sample was taken from the patient to examine visually the color, aspect, odor, volume and foam. Scientifically, by means of the dipstick method for urine we examined pH, sugar, proteins, ketones, bilirubin, White blood cells, nitrites, leukocyte esterase.
- Urinary protein: The protein-error-of-indicators principle is the foundation of this test. The presence of protein causes any color to appear at a constant pH. For "negative" responses, the color spectrum goes from yellow to green to green-blue, and vice versa for "positive" reactions.
- Microscopically for white blood cells, red blood cells**,** bacteria, yeast, parasites, casts and crystals

7- 24 hr. protein in urine:

Collection of urine started at 8:00 am by discarding the first urine specimen at 8 am, all then after quantity of urine throughout the following 24 hours, up to the sample at the following 8:00 am which was collected. Used a large container with label including patient identification, date and time of collection. Using **auto-analyzer SELECTRA PRO XL, ELITech Group, clinical chemistry automation systems, Netherlands**, for doing the test by using this equation:

Total protein * total urine volume / 100.000

B) Diagnosis of SLE:

1. ANA.

1. It is essential that all reagents be allowed to cool to room temperature (20-26°c) before the experiment may begin. Fill the holder with the necessary amount of microwells or strips. To prevent unnecessary exposure to water vapor, promptly place unused strips back into the desiccants' bag and tightly close.

2. Pour 100µL each of the following into the wells: the diluted patient samples, the ELISA Negative Control, the ANA ELISA Low Positive, and the ANA ELISA High Positive, all of which have been prediluted. After 30 minutes of incubation on a flat surface at room temperature, cover the wells. Once the final sample has been added, the incubation period may start.

3. The next step is to wash the wells by aspirating their contents carefully. After that, aspirate after adding 200-300µL of the diluted HRP Wash buffer to each well. For a total of three washes, repeat this cycle twice more. After each wash, turn the plate upside down and tap it on absorbent material to soak up any remaining liquid. After each washing cycle, be sure to drain the wells entirely. Keep the aspiration procedure in the same order as the sample addition.

4. Pour 100 microliters of the HS HRP IgG Conjugate into every well. Standard aseptic

conditions and competent laboratory procedures should be used to extract the conjugate from the bottles. Take out of the container only the quantity of conjugate that is needed for the test. Never put unused conjugate back into the bottle to prevent possible contamination from microbes and chemicals.
incubate the wells for 30 minutes as in step 2.

5. Wash step: Repeat step 3.

6. Put 100µL of TMB Chromogen into each well and let it incubate at room temperature for 30 minutes in the dark.

7. To every well, add 100µL of HRP Stop Solution. Add the HRP Stop Solution in the same order and at the same time as the TMB Chromogen. Mix the wells by gently tapping the plate with a finger.

8. Within an hour of stopping the reaction, read the absorbance (OD) of each well at 450 nm.

For use requiring bichromatic measurements, 620nm can be used as a reference wavelength Quality Control

1. Each batch of samples should be tested with the ANA ELISA Low Positive, the ANA ELISA High Positive, and the ELISA Negative Control to guarantee that all reagents and processes are functioning correctly.

It is essential that the prediluted ANA ELISA High Positive absorbance be higher than that of the low positive absorbance, which in turn must be higher than that of the negative control prediluted ELISA control.

3. The absorbance for the prediluted ANA ELISA High Positive must be more than 1.0, whereas the absorbance for the prediluted ELISA Negative Control must not exceed 0.15.

4. The absorbance value for the ANA ELISA Low Positive must be more than 0.15 or twice the ELISA Negative Control.

Calculation of Results

Sample OD = Sample Value / ANA ELISA Low Positive OD (units) x ANA ELISA Low Positive(units) (units)

Interpretation of Results

Negative <20 units	
Moderate Positive 20-60units	- 20 60
Strong Positive 60<	60<

2. Anti-double strand DNA.

1. Bring all the ingredients to room temperature (between 20 and 26 degrees Celsius) before starting the experiment. Fill the holder with the necessary amount of microwells or strips. To prevent unnecessary exposure to water vapor, promptly place unused strips back into the desiccants' bag and tightly close.

2. For each well, add 100µL of the following: diluted patient samples, ELISA Negative Control, dsDNA ELISA Positive, dsDNA ELISA calibrator, and ELISA Prediluted. Place a cover over each well and let them sit on a flat surface at room temperature for 30 minutes. Once the final sample has been added, the incubation period may start.

3. The next step is to wash the wells by aspirating their contents carefully. After that, aspirate after adding 200-300µL of the diluted HRP Wash buffer to each well. For a total of three washes, repeat this cycle twice more. After each wash, turn the plate upside down and tap it on absorbent material to soak up any remaining liquid. After each washing cycle, be sure to drain the wells entirely. Keep the aspiration procedure in the same order as the sample addition.

4. Add 100µL of the HS HRP IgG Conjugate to each well. Conjugate should be removed from the bottles using standard aseptic conditions and good laboratory techniques. Remove only the amount of conjugate from the bottle necessary for the assay. to avoid potential microbial and/or chemical contamination, never return unused conjugate to the bottle.

incubate the wells for 30 minutes as in step 2.

5. Wash step: Repeat step 3.

6. Put 100µL of TMB Chromogen into each well and let it incubate at room temperature for 30 minutes in the dark.

7. To every well, add 100µL of HRP Stop Solution. Add the HRP Stop Solution in the same order and at the same time as the TMB Chromogen. Mix the wells by gently tapping the plate with a finger.

8. Within an hour of stopping the reaction, read the absorbance (OD) of each well at 450 nm. The reference wavelength for bichromatic measurements is 620 nm.

Quality Control

1. To guarantee that all reagents and methods work as intended, it is recommended to run the ELISA negative control, the ELISA calibrator, and the dsDNA ELISA positive with each batch of samples.

2. As a general rule, the prediluted dsDNA ELISA Positive must have a higher absorbance than both the dsDNA ELISA calibrator and the prediluted ELISA Negative Control.

3. A positive prediluted dsDNA ELISA result must be more than 1.0, but a negative prediluted ELISA result must not be more than 0.2.

4. A minimum absorbance of 0.25 or twice that of the ELISA Negative Control is required for the ANA ELISA calibrator.

3. Assessment of C3 and C4:

Pre-Assay Preparation

1. Reagents: C3/C4-specific antibodies, enzyme-conjugated secondary antibodies, substrate, standards, and controls.

2. Sample collection: Serum or plasma samples.

3. Sample preparation: Dilute samples according to manufacturer's instructions.

ELISA Procedure

1. Coating: Coat microtiter plates with C3/C4-specific capture antibodies.

2. Blocking: Block non-specific binding sites.

3. Sample addition: Add diluted samples to wells.

4. Incubation: Incubate for 1-2 hours at room temperature.

5. Washing: Wash plates to remove unbound antibodies.

6. Detection antibody: Add enzyme-conjugated secondary antibodies.

7. Incubation: Incubate for 30 minutes to 1 hour.

8. Substrate addition: Add substrate (e.g., TMB or pNPP).

9. Color development: Measure absorbance at 450 nm (C3) or 405 nm (C4).

Calculation and Interpretation

1. Standard curve: Plot standard concentrations vs. absorbance.

2. Sample calculation: Calculate C3/C4 concentrations using the standard curve.

3. Reference ranges: Compare results to established reference ranges.

C) Special investigations:

•RDW and Anti-alpha enolase antibody:

1. RDW

Parameters of RDW taken from CBC from **Sysmex XN-1000TM AUTOMATED HEMATOLOGY ANALYSER, France**.

By using the cell counter, Currently, there are two RDW measurements that are used: RDW-CV (red cell distribution width - coefficient of variation) and RDW-SD (red cell distribution width - standard deviation).

Using the distribution curve width and the mean cell size, the RDW-CV was computed. The mean cell size standard deviation was divided

by the red blood cell MCV and then multiplied by 100 to get the percentage.
In femtoliters (fL), the RDW-SD measured the true width of the red cell distribution curve. Twenty percent above the baseline is where the distribution curve was found to be widest. The RDW-SD more faithfully represents the variation in red cell size since it is an actual measurement and is thus unaffected by the MCV. Adults typically have an RDW-SD between 40.0 and 55.0 fl.

2. Anti-alpha enolase antibody:
Test principle
The quantity of anti-alpha enolase antibody (anti-αENOL Ab) in samples was assessed using a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) using the kit. A micro elisa well was prepared by adding an anti-alpha enolase antibody (anti-αENOL Ab), followed by incubation and washing. The anti-alpha enolase antibody (anti-αENOL Ab) was tagged with HRP before being added. The unbound enzyme was removed after further incubation and washing. Then, Chromogen Solution A and B were added, causing the liquid's color to shift to blue. The acid eventually caused the color to become yellow. The sample's anti-alpha enolase antibody (anti-αENOL Ab) presence was confirmed by measuring the absorbency (OD value) using a micro plate reader with a 450nm wavelength and comparing the result to the critical value.

Assay Procedure:
1. Control wells, testing sample wells, and blank wells were all set up independently. The blank wells did not contain any sample or HRP-conjugate reagent, but all other operations were identical.
The standard well was supplemented with 50 µl of diluted standard, while the testing sample well on the assay plate received 40 µl of sample dilution. Following this, 10 µl of testing sample (with a final dilution degree of 5 times) was added, and the mixture was gently shaken before being incubated at 37 oC for 30 minutes.
2. Toss out Fill up each well with diluted washing liquid 30 times, then spin the container for 30 seconds. After that, pat the liquid dry with absorbent paper. After each of the five iterations, pat the area dry.
Except for the blank well, 50µl of HRP-conjugate reagent was applied to every well. Shaken gently, the mixture was then incubated at 37 ℃ for 30 minutes.

Statistical analysis:
Research was conducted using SPSS, version 20, for statistical analysis. The Kolmogorov Smirnov test was used to check whether the quantitative data was normally distributed. We used Student's t-test to compare two groups, analysis of variance (ANOVA) to compare all three groups, and a Bonferroni post hoc test to find the least significant statistical difference between each pair of groups for normally distributed variables that were reported as mean + standard deviation (SD). For variables that did not follow a normal distribution, we used the median (interquartile range, or IQR) and compared the two sets of data using the Kruskal-Wallas and Mann-Whitney U tests. When applicable, we used a Chi-square test or Fisher's exact test to compare qualitative values, which were presented as numbers (%). We used the Spearman correlation test for non-parametric data and Pearson's correlation test for non-parametric variables to investigate relationships between them. On a scale from 0 to 1, values of the correlation coefficient (r) were as follows: 0.024 = weak, 0.25-0.49 = fair, 0.5-0.74 = moderate, and > 0.75 = high. We used receiver operating characteristic (ROC) curves to determine the best serum AAE-Abs and RDW cutoff values for SLE disease activity prediction. It was considered significant when the p-value was less than 0.05.

Results
The participants of this study are allocated into 3 groups: **group I:** it included 30 patients with active SLE, they were 23 females and 7 males with age ranging from 18 to 43 with mean $\pm$SD of 39.2$\pm$10.1 years; **group II**: it comprised 30 patients with inactive SLE, they were 24 females and 6 males ranging in age from 20 to 45 with mean $\pm$ SD of 42$\pm$11 years, and **group III**: it consisted of 30 healthy controls, they were 22 females and 8 males, their ages ranged from 19 to 44 with mean $\pm$SD of 41$\pm$12.1 years.

Table 1 shows no statistically significant differences between the studying groups as

regards age, gender, occupation, residence and duration of illness, however the SLEDAI score was significantly higher in group I versus group II (12.3±6.46 vs.1.47±0.99,p< 0.00).

Table (1): Demographic and clinical characteristics of the study groups.

Variable	Group (I) Active SLE n=30	Group (II) Inactive SLE n=30	Group (III) Controls n=30	P value		
				I vs II	*I vs III*	*II vs III*
[1]Age (years)				*P=0.53*		
	39.2±10.1	42±11	41 ± 12.1	0.08	0.20	0.18
[2]Gender [n (%)]				P=0.921		
Male	7(23.3)	6(20)	8(26.7)	0.79	0.75	0.53
Female	23(76.7)	24(80)	22(73.3)			
[3]Duration of illness (months)	36.2± 11	34.7 ± 10.07	----------	------		
				0.1		
[2]Occupation [n (%)]				P=0.69		
Worker	15(50)	17(56.7)	15(50)	0.69	0.59	0.94
Non-worker	15(50)	13(43.3)	15(50)			
[2]Residence [n (%)]				P=0.351		
Rural	15(167)	7(23.3)	6(20)	0.30	0.63	0.51
Urban	25(83.3)	23(76.7)	24(80)			
[3]SLEDAI score	12.3±6.46	1.47±0.99	----------	----------		
				<0.001		

SLE= systemic lupus erythematosus; n= number; SLEDAI= Systemic lupus erythematosus disease activity index score

[1]: Data are expressed as mean ± SD and compared by ANOVA followed by Bonferroni post Hoc test

[2]: Data are expressed as number (percentage) and compared by Chi-Square test

[3]: Data are expressed as mean ± SD and compared by Student's t-test

Blood value donate significant results

Table (2) showed that:

When SLE patients with active disease were compared with those without, there was no statistical significant difference as regard TLC, blood urea, ALT and RF titre. They displayed statistically significant lower levels of hemoglobin (9.8 ± 0.9 g/dl vs. 10.8 ± 1.0 g/dl, p< 0,001), serum albumin(3.4±0.5 g/dl vs.4.0 ± 0.4 g/dl , p< 0,001), C3 **(** 67(38.7 – 105.2) mg/ dl vs. 98 (86 – 110.5) mg/ dl, p< 0,001**)** and C4 **(** 12.5 (8-28) mg/ dl vs. 22.5 (19.7 – 28) mg/ dl , p=0,034**)** o contrary , they showed statistically significant higher values of serum creatinine (1.1 ± 0.5 mg/ dl vs. 0.9 ± 0.3 mg/ dl, p = 0.04), AST (32.4 ± 6.6 U / L vs. 28.6 ± 7.2 U / L, p= 0.038), ESR 1st hour (67.5 (45-85) mmHg vs. 10 (6-12) mmHg , P< 0.001) CRP (2(1-3.25) mg / L vs . 1 (0-2) mg / L, P= 0.012), AAE. Abs (11.5 ± 0.9 ng / ml vs. 6.8 ± 0.6 ng / ml, p< 0.001) RDW (15.04 ± 0.41 % vs. 13.3 ± 0.5%, p = 0.005), ANA (41.5±15.6 **U**vs. 33.4±10.8 **U**, p= 0.017) and anti–ds DNA (311.5(30.2–546) IU/ml vs. 50 (31.5 – 72.5) IU / ml, p < 0.001) only TLC was matched in SLE patient with active disease with healthy controls, but they showed significantly lower levels of hemoglobin and serum albumin (9.8 ± 0.9 g / dl vs. 12.8 ± 0.8 g/dl, p< 0.001 and 3.4 ± 0.5 g/dl vs. 4.4 ± 0.4 g/dl , p < 0.001 , respectively) and statistically higher levels of urea , creatine , AST,ALT ,ESR,RF titre, AAE-Abs and RDW (0.001, 0.001, 0.001, 0.002, 0.001, 0.017, 0.015, 0.001 respectively) (**table 2)**

Table 2: Baseline laboratory findings in the study groups

Variable	Group (I) Active SLE n=30	Group (II) Inactive SLE n=30	Group (III) Controls n=30	P value		
				I vs II	*I vs III*	*II vs III*
1- Hemoglobin (g/dl)	(8.2-12.5)	(8.8-12.7)	(11.8-14.6)		**<0.001**	
Mean ± SD	9.8 ± 0.9	10.8 ± 1.0	12.8 ± 0.8	**<0.001**	**<0.001**	**<0.001**
1- Total leucocytic count(x10^3/µl)	(2.9-17.8)	(4.0-11.0)	(4.0-9.8)		0.736	
Mean ± SD	6.4 ± 4.3	6.8 ± 1.9	6.3 ± 1.5	0.533	0.922	0.471
1- Platelets (x10^3/µl)	(165-550)	(165-390)	(175-477)		**<0.001**	
Mean ± SD	235.6 ±113.5	213.1 ±49.6	315.8 ± 88.9	0.324	**0.001**	**<0.001**
					0.324	
1- Urea (mg/dl)	(20-100)	(15-65)	(15-38)		**0.001**	
Mean ± SD	38.7±17.0	33.2±12.1	26.2±6.2	0.152	**0.001**	**<0.001**
1- Creatinine (mg/dl)	(0.7-3.0)	(0.5-1.2)	(0.5-1.1)		**0.008**	
Mean ± SD	1.1±0.5	0.9±0.3	0.79 ± 0.18	0.046	**<0.001**	**0.035**
1- AST:(u/L)	(19-41)	(15-40)	(10-30)		**<0.001**	
Mean ± SD	32.4±6.6	28.6±7.2	18.9±4.7	**0.038**	**<0.001**	**<0.001**
1- ALT(u/L)	(20-41)	(10-43)	(10-32)		**<0.001**	
Mean ± SD	31.6±6.2	28.4±11.0	19.1±6.0	0.183	**0.002**	0.261
1- ALB: (g/dl)	(2.5-4.6)	(3.3-4.9)	(3.7-5.1)		**<0.001**	
Mean ± SD	3.4±0.5	4.0±0.4	4.4±0.4	**<0.001**	**<0.001**	**<0.001**
2-ESR 1st hour (mmHg)	23-150	4-15	3-9		**<0.001**	
Mean ± SD	66.7±27.6	9.13±3.1	5.3±1.6	**<0.001**	**<0.001**	**0.003**
Median, (IQR)	67.5(45-85)	10(6-12)	5(4-6)			
2-CRP: (mg/l)	0-24	0-12	0-5		**0.019**	
Mean ± SD	3.93±6.18	1.37±2.2	1.5±1.3	0.012	**<0.001**	0.359
Median, (IQR)	2(1-3.25)	1(0-2)	1(0-3)			
2-RF:(IU) ml	0-64	0-32	0-7		0.041	
Mean ± SD	8.4±13.7	4.2±6.7	2.7±2.4	0.068	0.017	0.895
Median, (IQR)	4(2-7)	2(.75-5.25)	2(.75-5)			
1-Anti-alpha enolase Ab: ng/ml	(10.4-13.7)	(5.9-7.9)	(3-5.4)		**<0.001**	
Mean ± SD	11.5±0.9	6.8±0.6	4.5±0.5	**<0.001**	**0.015**	0.527
1-RDW: %	(14.5-16)	(12.5-14.2)	(11-12.5)		**<0.001**	
Mean ± SD	15.04±0.41	13.3±0.5	11.9±0.3	**0.005**	**<0.001**	**<0.001**
1-ANA: units	(21-67)	(20-60)	--------		***I vs II***	
Mean ± SD	41.5±15.6	33.4±10.8			**0.017**	
2-Anti-ds DNA: IU/ml	6-801	12-101	--------		***I vs II***	
Mean ± SD	316.4±283.5	52.0±27.2			**<0.001**	
Median, (IQR)	311.5(30.2-546)	50(31.5-72.5)				
2-C3:mg/dl	25-137	29-120	--------		***I vs II***	
Mean ± SD	72.5±36.9	96.3±22.1			**<0.001**	
Median, (IQR)	67(38.7-105.2)	98(86-110.5)				
2-C4:mg/dl	7-34	5-31	--------		***I vs II***	
Range Mean ± SD	17.5±10.3	22.4±6.5			**0.034**	
Median, (IQR)	12.5(8-28)	22.5(19.7-28)				

SLE: Systemic Lupus Erythematosus, n: range AST: aspartate transaminase, ALT: alanine transaminase, ALB: albumin ESR Erythrocyte Sedimentation Rate, CRP C-Reactive Protein, RF:Rheumatoid Factor, RDW Red Cell Distribution Width, ANA Anti-Nuclear Antibody , Anti-ds DNA :Anti Double Strand DNA,C3 Complement 3, C4 Complement 4

1: Data are expressed as mean ± SD and compared by ANOVA followed by Bonferroni post Hac test
2: Data are expressed as median (IQR) and compared by Kruskal wallas test and followed by Mann Whitney test between each tow groups, Bold values denote significant results

Table (3): Shows that in SLE patients, a significant positive correlation was found between values of AAE-Abs and ANA levels (r= 0.581, P= 0.001). whereas values of AAE-Abs were correlated negatively with C3 (r=0.493, P= 0.006) and C4 (r=0.437, P=0.016). concerning RDW choose significant negative correlation between it and TLC, Platelets, C3 and C4 (r=0.473, P= 0.008) (r=0.472, P= 0.008) (r=0.209, P = 0.267) (r=0.144, P= 0.447) respectively .

Table (3): Correlations between blood levels of each anti-alpha enolase anti bodies and red cell distribution width and some of the studied laboratory parameters

Variables	Anti-alpha enolase anti bodies		Red Cell Distribution Width	
	r	P-value	r	P-value
HB (g/dl)	-0.063	0.740	-0.209	0.267
TLC (x10^3 /µl)	0.010	0.957	**- 0.473**	**0.008**
PLT (x10^3 /µl)	-0.108	0.596	**-0.472**	**0.008**
CRP (mg / dl)	0.065	0.734	0.241	0.199
RF (IU / ml)	0.002	0.990	0.343	0.063
ESR 1st hour (mmHg)	0.088	0.642	0.084	0.660
UREA (mg / dl)	0.111	0.561	0.150	0.429
Creatinine (mg / dl)	0.049	0.796	0.009	0.963
ALT (u / L)	0.103	0.587	0.186	0.325
AST (u / L)	0.074	0.699	0.088	0.644
ALB (g / dl)	-0.303	0.103	-0.353	0.056
ANA (units)	**0.581**	**0.001**	0.004	0.985
Anti-dsDNA (IU/ml)	0.355	0.054	0.009	0.962
C3 (mg/dl)	**-0.493**	**0.006**	**-0.209**	0.267
C4 (mg/dl)	**-0.437**	**0.016**	**-0.144**	0.447
RDW %	0.099	0.602	0.099	0.602
24hr protein urine (N=17)	0.270	0.295	0.318	0.213
Total SLEDAI score 2K	0.276	0.140	0.307	0.099

HB: Hemoglobin, TLC: Total leucocytic count, PLT: Platelets, CRP: C-Reactive Protein, RF: Rheumatoid, Factor, ESR: Erythrocyte Sedimentation Rate, ALT: alanine transaminase, AST: aspartate transaminase, ALB: albumin, ANA: Anti-Nuclear Antibody, Anti-dsDNA: Anti Double Strand DNA, C3: Complement 3, C4: Complement 4, RDW: Red Cell Distribution Width, N: number of cases, r: correlation coefficient
Grades of r :(r) ranged from 0-1: 0.024= weak; 0.25-0.49= fair; 0.5-0.74= moderate, and > 0.75= strong. Bold values denote significant results .

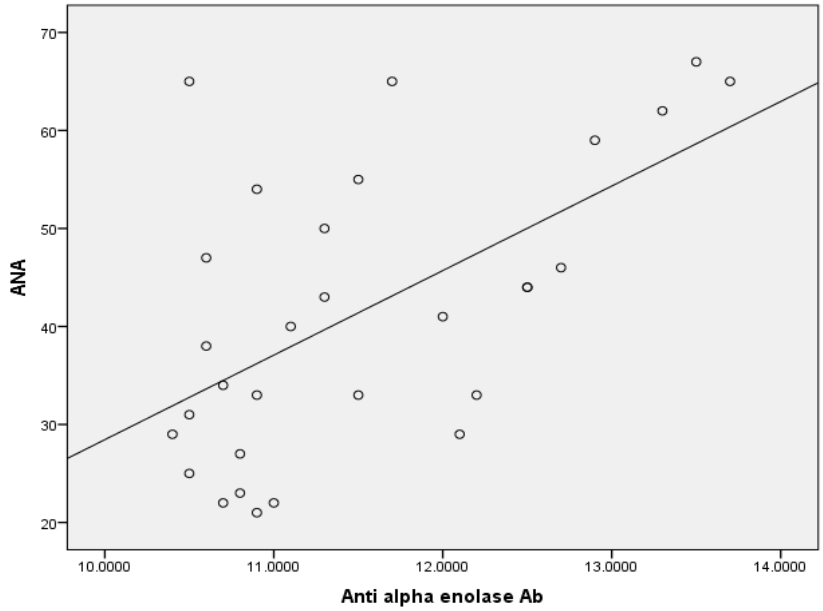

Figure (1): Correlation between Antialpha enolase Ab and ANA in active group

ROC curve showed that the area under the curve of AAE-Abs was 1(95% CI=0.0960-1, p<0.001) at a cut off value of >5.4 ng/ml that yield a sensitivity, specificity, PPV and NPV of 100%, 100%, 100%, 100% respectively.
As regard RDW, the area under ROC curve was 0.982 (100% CI=0.928-0.998, P<0.001) at a cut off value of of >12.5% with a sensitivity of 95%, robust NPV of 90.9%, specificity of 95% and PPV of 100% (**table 4)**

Table (4): validation of cut off values of anti-Alpha enolase antibodies and red cell distribution width in the prediction of disease activity in systemic lupus erythematosus patients.

	AUC-ROC curve	95%C.I.	p- value	Cut- off point	Sensitivity	specificity	PPV	NPV	Cut – off Point
Anti alpha enolase anti bodies (ng/ml)	1	0.960-1	**<0.001**	>5.4	100	100	*100*	*100*	*> 5.4*
RDW %	0.982	0.928-0.998	**<0.001**	>12.5	100	95	100	90.9	> 12.5

AUC: area under the curve, C.I: confidence interval, PPV: positive predictive value and NPV: negative predictive value, RDW: Red Cell Distribution Width
Bold values denote significant results.

Breaking of tolerance to nuclear self-antigens and the generation of pathogenic autoantibodies define SLE, an autoimmune illness. The skin, eyes, kidneys, heart, muscles, and joints are just some of the organs that may be affected by this difficult and complicated condition.[7]

SLE's aetiology is still a mystery, although environmental, hormonal, and genetic variables, as well as an unbalanced immune system, all play a part.[8]

A higher rate of SLE in females has long been documented (49.6 per 100,000), and it is more common in women of childbearing age (one in 500 cases). Biphasic patterns were discovered in males between 30 and 39 years old and 60 and 69 years old, with a prevalence rate of 6 per 100,000.[9]

These results showed that there were highly statistically significant increased levels of anti-alpha enolase ab in group I when compared to group II and group III also between group II with group III (P=<0.001).

Consistently, [10][11] reported that In a study that examined the serum levels of anti-alpha-enolase Ab in SLE patients, the active group had significantly higher levels than the stable and control groups. α-enolase is a part of the complex extracellular trappings (NETs), which are released by neutrophils through an active process called NETosis. Lupus flares cause the production of circulating α-enolase antibodies because NETosis is a key factor in lupus autoimmunity.[12]

Reported[13][14] that there was a positive correlation between anti-alpha enolase antibody, Anti ds DNA antibody and Complements (C3 and C4) in SLE patient groups it was due to increasing production of antibodies like anti-alpha enolase antibody and Anti-dsDNA antibody during flares of SLE that helped in consumption of complements in glomeruli of kidney leading to renal affection.

Reported[10][11] that there was strong degree of specificity and sensitivity (100%, 100%) respectively of anti- alpha enolase antibody in active group than stable and control groups.

In the current work there were highly statistically significant increasedlevels of RDW in group I when compared with group II (p=0.005), or withgroup III (P= <0.001) and group II with group III (P= 0.001).

In agreement with these results [15] [16] [17] [18]reported that the levels of RDW inpatients with SLE in active group were significantly higher than those in stableand control groups through a study that investigating RDW in SLE patients,sustained autoantibodies that were formed in SLE caused activation of naturalkiller (NK) cells. NK cells caused lysis of the target cells. The hematopoieticsystem was very much vulnerable to these effects. Thus, autoantibodies caused**.**

Our analysis of the area under the ROC curve revealed that the cut off value of AAE- Abs was > 5.4 ng/ml that was the optimal value for accurate prediction of disease activity in SLE patients with an area under curve 1.0 that corresponded to PPV and NPV of 100% for each.it has been reported that NPV of 100% is desirable to minimize the oversight of patients who are at risk. Similarly, the area under ROC curve for RDW was 0.982 at a cut off value > 12.5% that yielded a sensitivity,specificity ,PPV,NPV of 100%,95%,100%,and 90.9% respectively. These results indicated that each AAE-Abs and RDW ca be used as a sole predictors for disease activity I SLE patients.

Undoubtedly, the current study has some limitations. first, the relatively small number of sample size. Second, the study was case-control and hospital based, therefore, the possibility of over estimating the studied markers could not be excluded. Third, complete autoimmune work-up was not done. Finally, the study data were obtained from a single center.

Conclusions

Overall, the findings of this study informed that anti-alpha enolase antibody and RDW levels could be considered an appropriate diagnostic marker in patients with active SLE and could differentiate them from patients with stable conditions.

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