AIM OF THE WORK

To investigate the specificity and sensitivity of anti-beta$_2$ glycoprotein I antibodies (anti-β_2 GPI) by ELISA in Egyptian Systemic Lupus Erythematosus (SLE) patients with features of Antiphospholipid Syndrome (APS) compared to IgG/IgM anticardiolipin antibodies (IgG/IgM aCL) and lupus anticoagulant (LAC), and to examine the possible correlations between clinical manifestations and anti- β_2 GPI levels.

SUBJECTS

Fifty young patients with systemic lupus erythematosus (SLE) according to the American college of Rheumatology (ACR) were recruited:

Group "I": Thirty systemic lupus erythematosus patients having secondary antiphospholipid syndrome. (28 females, 2 males aged from 21-54 years old)

Group "II": Twenty systemic lupus erythematosus patients without antiphospholipid syndrome. (19 females, one male aged from 18-46 years old)

Group "III": Twenty young healthy subjects served the purpose of control. (18 females, 2 males aged from 20-44 years old)

Informed concent was obtained before the study.

Inclusion criteria:

* Age between 16-55 years old.
* Systemic lupus erythematosus according to (ACR) [37] is diagnosed when the patient present with at least four criteria of the following:
  - Malar rash
  - Discoid rash
  - Photosensitivity
  - Oral ulcers
  - Arthritis
  - Serositis
  - Renal disorder
  - Neurologic disorder
  - Haematologic disorder
  - Immunologic disorder
  - Positive ANA antibody

* Antiphopholipid syndrome is diagnosed when at least one of the clinical and one of the lab criteria are met [83]

  - Clinical criteria:

    - Vascular thrombosis

    - Pregnancy morbidity: one or more unexplained fetal deaths or, one or more premature births or, three or more unexplained spontaneous abortions.

  - Lab criteria:

    - Present lupus anticoagulant or,

    - Positive anticardiolipin antibodies IgG or IgM or,

    - Positive antiβ2 glycoprotein antibodies IgG or IgM.

Positivity should be present on two or more occasions at least 12 months apart for any test.

Exclusion criteria:

- Age above 55 years old.
- Overlap syndrome, or those who meet the criteria of other autoimmune disease
- Family history of thrombosis due to other cause than antiphospholipid syndrome.

METHODS

The following data were registered for each of the studied subjects:

I- Demographic data:

***Personal history:** e.g.: name, age, sex, marital state, habits, occupation, residence

II) Disease related data:

1- **Present history:** (onset, duration, presentation and progression)

Stroke, deep vein thrombosis (DVT), pulmonary embolism, myocardial infarction, pregnancy morbidity

2- **Past history:** (including risk factors for thrombosis)

Diabetes mellitus (DM), metabolic diseases, hyperlipidemia, rheumatic and congenital heart disease, hypertension, connective tissue diseases, blood and coagulation disorders, malignancy, ischemic heart disease and arrythmias (including any previously done investigations either laboratorial or radiological)

3- **Family history:** (including connective tissue diseases, any thrombosis causing disease and pregnancy morbidity)

4- **Drug history:** (including Procainamide, quinidine, propranolol, hydralazine, Phenytoin, chlorpromazine, Interferon alpha, quinine, amoxicillin and oral contraceptives). [14,80]

5- **Obstetrical history:** The obstetrical clinical criteria of APS include:

- One unexplained death of a morphologically normal foetus at or beyond the tenth week of gestation OR,
- ≥ One premature births of a morphologically normal neonate at or beyond the 34^{th} week of gestation, due to severe preeclampsia, eclampsia, or placental insufficiency OR,
- ≥ Three unexplained consecutive spontaneous abortions before the tenth week of gestation (maternal anatomic or hormonal abnormalities and chromosomal causes excluded). [43,89]

6- **Menstrual history**: Frequency, amount, associated symptoms.

III- Clinical examination:

1- **General appearance** (e.g. development, nutrition, deformities, attention to grooming)

2- **Vital signs**
   - Blood pressure (mmHg)
   - Heart rate (beat/minute)
   - Temperature (˚ C)
   - Respiratory rate (Cycle / minute)

3- **Head & neck examination**

4- **Chest examination**

5- **Cardiac examination**

6- **Abdominal examination**

7- **Neurological examination**

8- **Musculo-skeletal examination**

IV- Laboratory assessment:

A- Routine investigations:

1- Immunological investigations including: ANA[92], antidsDNA antibodies by ELISA[93].

2- Coagulation investations including: aPTT. [94]

3- Haematological investigations including: Complete Blood Count using cell counter analyzer (SYSMEX-KX21)[95].

4- Blood chemistry including:

   a- Serum urea [96],b- serum creatinine [97],c-complete urine analysis[98],

   d- Albumin/creatinine ratio in urine or 24 hrs urinary proteins. [99]

B- The specific investigations:

The specific investigations performed to the subjects were:

1- Anti β_2 glycoprotien 1 IgM & IgG: [100-102]

a 4-mL blood serum sample in a red-topped tube ; serum is preferred

PRINCIPLE OF THE TEST:

Highly purified beta-2-glycoprotein I is bound to microwells. Antibodies against the coated antigen, if present in diluted patient sample, bind to the respective antigen. Washing of the microwells removes unbound unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human antibodies immunologically detect the bound patient antibodies forming a conjugate/antibody/antigen complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound

conjugate hydrolyzes to form a blue colour. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow colour is measured photometrically at 450 nm. The amount of colour is directly proportional to the concentration of antibodies present in the original sample.

CONTENTS OF THE KIT:

- CALIBRATOR (A): 1×1.5 Calibrator A 0 U/ml, containing serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
- CALIBRATOR (B): 1x 1.5 ml Calibrator B 6.3 U/ml, containing beta-2-glycoprotein I antibodies in a serum/buffer matrix.
- CALIBRATOR (C): 1x 1.5 ml Calibrator C 12.5 U/ml, containing beta-2-glycoprotein I antibodies in a serum/buffer matrix.
- CALIBRATOR (D): 1x 1.5 ml Calibrator D 25 U/ml, containing beta-2-glycoprotein I antibodies in a serum/buffer matrix.
- CALIBRATOR (E): 1x 1.5 ml Calibrator E 50 U/ml, containing beta-2-glycoprotein I antibodies in a serum/buffer matrix.
- CALIBRATOR (F): 1x 1.5 ml Calibrator F 100 U/ml, containing beta-2-glycoprotein I antibodies in a serum/buffer matrix.
- CONTROL (+VE): 1x 1.5 ml Control positive, containing beta-2-glycoprotein I antibodies in a serum/buffer matrix.
- CONTROL (-VE): 1x 1.5 ml Control negative, containing beta-2-glycoprotein I antibodies in a serum/buffer matrix.
- DILUENT: 15 ml Sample Buffer P, containing PBS, BSA, detergent, concentrate (5 x).
- CONJUGATE (G): 15 ml Enzyme Conjugate; containing anti-human IgG antibodies, HRP labelled; PBS, BSA. Ready to use.
- CONJUGATE (M): 15 ml Enzyme Conjugate; containing anti-human IgM antibodies, HRP labelled; PBS, BSA, detergent. Ready to use.
- TMB: 15 ml TMB Substrate; containing 3,3', 5,5'- Tetramethylbenzidin, colorless. Ready to use.
- STOP: 15 ml Stop solution; contains acid. Ready to use.
- WASH: 20 ml Wash Buffer, containing Tris, detergent, preservative sodium azide 0.09%; 50 x conc.

TEST PROCEDURE:

Sampling: blood samples were taken from patients and control subjects, and serum was separated, left at -20°C until processed.

Enough microplate modules were prepared for all calibrators / controls and patient samples.

1. 100 µl of calibrators, controls were pipetted and prediluted patient samples into the wells. Incubated for 30 minutes at room temperature (20-28 °C). The contents of the microwells were discarded and washed 3 times with 300 µl of wash solution.
2. 100 µl of enzyme conjugate into each well were dispensed. Then incubated for 15 minutes at room temperature. The contents of the microwells were discarded and washed 3 times with 300 µl of wash solution.
3. 100 µl of TMB substrate solution were dispensed into each well. Then incubated for 15 minutes at room temperature.
4. 100 µl of stop solution to each well of the modules were added . Then incubated for 5 minutes at room temperature.

The optical density at 450 nm (reference 600-690nm) was read and the results were calculated.

The developed colour was stable for at least 30 minutes and was read during this time.

CALCULATION OF RESULTS:

For quantitative results the optical density of each calibrator versus the calibrator concentration were plotted to create a calibration curve. The concentration of patient samples were interpolated from the calibration curve.

Interpretation of results:

IgG & IgM:

- Negative:< 5 U/ml,
- Borderline:5 - 8 U/ml,
- Positive:> 8 U/ml.[100-102]

2- Anticardiolipin antibodies (IgM, IgG): [101,103]

Obtain a 7-mL blood serum sample in a red-topped tube. Serum is preferred over plasma for testing.

PRINCIPLE OF THE TEST:

Highly purified cardiolipin is coated on microwells saturated with beta-2-glycoprotein I. Antibodies against the coated antigen, if present in diluted patient sample, bind to the respective antigen. Washing of the microwells removes unbound unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human antibodies immunologically detect the bound patient antibodies forming a conjugate/antibody/antigen complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue colour. The addition of an acid stops the reactionforming a yellow end-product. The intensity of this yellow colour is measured photometrically at 450 nm. The amount of colour is directly proportional to the concentration of antibodies present in the original sample.

CONTENTS OF THE KIT:

- CALIBRATOR (A): 1x 1.5 ml Calibrator A 0 GPL-U/ml / 0 MPL-U/ml, containing serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
- CALIBRATOR (B): 1x 1.5 ml Calibrator B 7.5 GPL-U/ml / 5 MPL-U/ml, containing Cardiolipin antibodies in a serum/buffer matrix.
- CALIBRATOR (C): 1x 1.5 ml Calibrator C 15 GPL-U/ml / 10 MPL-U/ml, containing Cardiolipin antibodies in a serum/buffer matrix.
- CALIBRATOR (D): 1x 1.5 ml Calibrator D 30 GPL-U/ml / 20 MPL-U/ml, containing Cardiolipin antibodies in a serum/buffer matrix.
- CALIBRATOR (E): 1x 1.5 ml Calibrator E 60 GPL-U/ml / 40 MPL-U/ml, containing Cardiolipin antibodies in a serum/buffer matrix.
- CALIBRATOR (F): 1x 1.5 ml Calibrator F 120 GPL-U/ml / 80 MPL-U/ml, containing Cardiolipin antibodies in a serum/buffer matrix.
- CONTROL (+VE): 1x 1.5 ml Control positive, containing cardiolipin antibodies in a serum/buffer matrix.
- CONTROL (–VE): 1x 1.5 ml Control negative, containing cardiolipin antibodies in a serum/buffer matrix.
- DILUENT: 15 ml Sample Buffer; containing PBS, BSA, detergent, , concentrate (5 x).
- CONJUGATE (G): 15 ml Enzyme Conjugate IgG; containing anti-human IgG antibodies, HRP labelled; PBS, BSA. Ready to use.

- CONJUGATE (M): 15 ml Enzyme Conjugate IgM; containing anti-human IgM antibodies, HRP labelled; PBS,BSA, detergent. Ready to use.
- TMB: 15 ml TMB Substrate, containing 3,3', 5,5'- Tetramethylbenzidin. Ready to use.
- STOP: 15 ml Stop solution; contains acid. Ready to use.
- WASH: 20 ml Wash Buffer, containing Tris, detergent, preservative sodium azide 0.09%; 50 x conc.

TEST PROCEDURE:

Enough microplate modules for all calibrators / controls and patient samples were prepared.

1. 100 µl of calibrators, controls were pippetted and prediluted patient samples into the wells. Incubated for 30 minutes at room temperature (20-28 °C). The contents of the microwells were discarded and washed 3 times with 300 µl of wash solution
2. 100 µl of enzyme conjugate into each well were dispensed. Incubated for 15 minutes at room temperature. The contents of the microwells were discarded and washed 3 times with 300 µl of wash solution.
3. 100 µl of TMB substrate solution into each well were dispensed. Incubated for 15 minutes at room temperature.
4. 100 µl of stop solution were added to each well of the modules. Incubated for 5 minutes at room temperature.

The optical density at 450 nm (reference 600-690nm) was read and the results were calculated.

The developed colour was stable for at least 30 minutes and was read during this time.

CALCULATION OF RESULTS

For quantitative results the optical density of each calibrator versus the calibrator concentration were plotted to create a calibration curve. The concentration of patient samples were interpolated from the calibration curve.

Expected values:

In a normal range study with samples from healthy blood donors the following ranges have been established with this ELISA assay: Cut-off (IgG: 10 GPL-U/ml, IgM: 7 MPL-U/ml).

Interpretation of results:

IgG: Negative:< 10 GPL-U/ml, Positive:≥ 10 GPL-U/ml

IgM: Negative:< 7 MPL-U/ml, Positive:≥ 7MPL-U/ml.[101,103]

3- Lupus anticoagulant: [101,104]

LAC is a misnomer, since it is clinically associated with clotting tendency rather than anticoagulation activity, and only 50% of people with LAC meet the criteria for SLE.

The International Society of Thrombosis and Haemostasis (ISTH) criteria for lupus anticoagulant detection include 4 mandatory steps, in the following sequence:[100,103]

1. Prolongation of a phospholipid-dependent clotting assay (activated partial thromboplastin time [aPTT], kaolin clotting time [KCT], diluted Russell's viper venom test [dRVVT], diluted prothrombin time [dPT]); two tests that have different assay principles should be used, usually the dRVVT and aPTT; this is the screening step

2. Mixing study with 1:1 proportion of patient's plasma and a normal pooled plasma without preincubation and reassessment of the clotting assay used in step one; if it remains prolonged, an inhibitor is present, either LAC or a specific factor inhibitor; if it corrects, then LAC is excluded and the cause is likely a specific factor deficiency

3. Confirmation that the inhibitory activity is phospholipid-dependent by relative correction of the abnormal clotting time when the concentration of phospholipid is increased in the screening test(s) that yielded abnormal results; this step confirms that the cause of abnormal mixing study is LAC (phospholipid-dependent inhibitor), not a specific factor inhibitor; knowing the clinical history helps in diagnosis—thrombosis in case of LAC or hemorrhage in the case of factor deficiency

4. Exclusion of other coagulopathies that may yield similar results or accompany the LAC presence; specific factor assay might be necessary

The results from the above tests are considered positive when they are above the local cutoff value. For tests in steps 1 and 2, the cutoff value is the 99th percentile of the distribution of the tests performed on plasmas. In the confirmatory tests in step 3, the cutoff value is equal to the mean of the individual percentage of corrections, calculated with the following equation:

[(Screen - confirm)/screen] × 100† A blood serum sample is collected in 0.109 M sodium citrate (in a blue topped tube). Blood should be collected before the start of anticoagulation therapy or after a sufficient period following discontinuation.

Platelet-free (< 10,000/μL) plasma preparation via centrifugation must be done with special care, as it may cause platelet fragmentation and the release of phospholipids, which may significantly affect the results.

Frozen plasma is required if testing is delayed, and frozen plasma must be thawed at 37°C.[100,103]

STATISTICAL ANALYSIS

Statistical analysis of the data[104]

Data were fed to the computer and analyzed using IBM ***SPSS software package version 20.0.*** [105] Qualitative data were described using number and percent. Quantitative data were described using mean and standard deviation for normally distributed data while abnormally distributed data was expressed using median, minimum and maximum. Comparison between different groups regarding categorical variables was tested using Chi-square test. When more than 20% of the cells have expected count less than 5, correction for chi-square was conducted using Fisher's Exact test or Monte Carlo correction. The distributions of quantitative variables were tested for normality using ***Kolmogorov-Smirnov test, Shapiro-Wilk test and D'Agstino test, also Histogram and QQ plot were used for vision test.*** If it reveals normal data distribution, parametric tests was applied. If the data were abnormally distributed, non-parametric tests were used. For normally distributed data, comparison between different groups were analyzed using F-test (ANOVA) and Post Hoc test (Scheffe) for pair wise comparison, while for abnormally distributed data, Kruskal Wallis test was used to compare between different groups and Post Hoc test was assessed using Mann-Whitney Test. Significance test results are quoted as two-tailed probabilities. Significance of the obtained results was judged at the 5% level.

Agreement of the different predictives with the outcome was used and was expressed in sensitivity, specificity, positive predictive value, negative predictive value and accuracy. Significance test results are quoted as two-tailed probabilities. Significance of the obtained results was judged at the 5% level.

RESULTS

The present study was carried out on three groups of subjects:

Group "I": Thirty systemic lupus erythematosus patients having secondary antiphospholipid syndrome. (28 females, 2 males aged from 21-54 years old)

Group "II": Twenty systemic lupus erythematosus patients without antiphospholipid syndrome. (19 females, one male aged from 18-46 years old)

Group "III": Twenty young healthy subjects served the purpose of control. (18 females, 2 males aged from 20-44 years old)

1- Statistical analysis of the demographic data:

Statistical analysis of the demographic data of the studied groups is shown in Table (7).

- **The age:** in group (I) ranged between 21-54 years with median = 28 years old. In group (II) the age ranged between 18-46 years old with median =28.5 years old , whereas the age of the control group (III) ranged between 20-44 years old with median = 32years old. There is no statistically significant differences between groups ($^{KW}\chi^2$= 4.662, p= 0.097) (Figure 13) (Table 8).
- **As regarding sex:** in group (I) female number is 28 which is considered as 93.3%, while the male number is 2 which is considered as 6.7%. in group (II) female number is 19 which is considered as 95%, while the male number is only one which is considered as 5%. In group (III) female number is 18 which is considered as 90%, while the male number is 2 which is considered as 10%. (χ^2=0.395, P= 1.000) (Figure 14) (Table 8).

Table (7): Comparison between the studied groups according to demographic data

	Group I (n=30)		Group II (n=20)		Group III (n=20)		Test of Sig.	p
	No.	%	No.	%	No.	%		
Sex								
Male	2	6.7	1	5.0	2	10.0	χ^2=	^{MC}p=
Female	28	93.3	19	95.0	18	90.0	0.395	1.000
Age								
Min. – Max.	21.0 – 54.0		18.0 - 46.0		20.0 – 44.0		$^{KW}\chi^2$=	
Mean ± SD.	29.77 ± 7.62		28.05 ± 7.86		33.0 ± 8.0		4.662	0.097
Median	28.0		28.50		32.0			

Group I: SLE + antiphospolipid
Group II: SLE
Group III: Control

χ^2: Value for Chi square test
MC: Monte Carlo test
$^{KW}\chi^2$: Chi square test for Kruskal Wallis test
Sig. bet. grps was done using Mann Whitney test
*: Statistically significant at $p \leq 0.05$

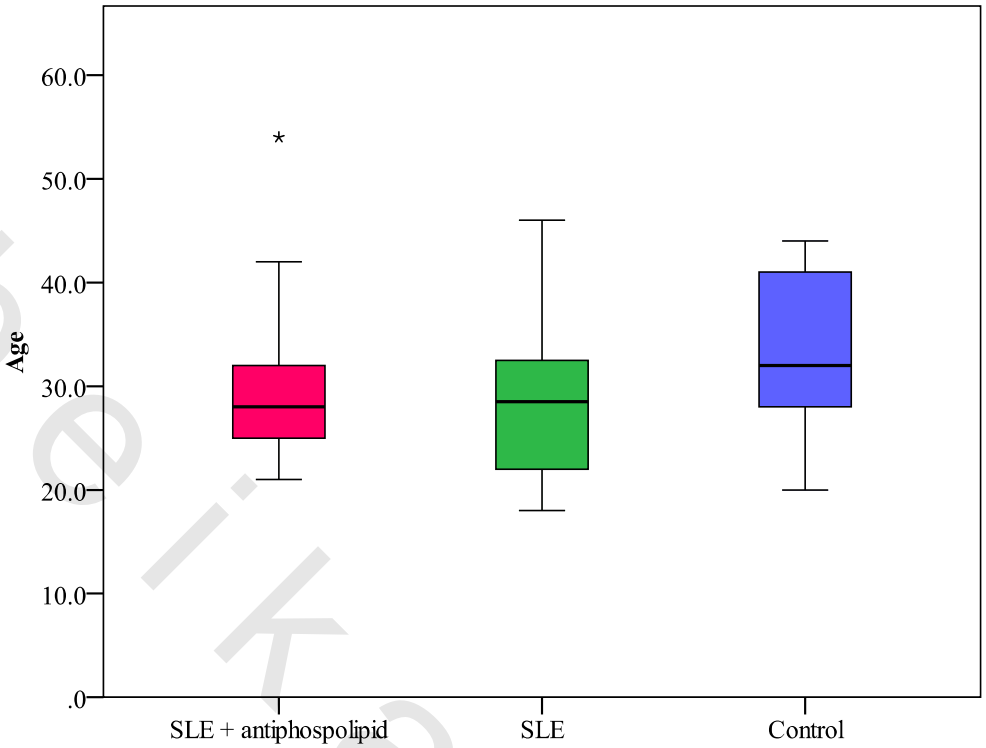

Figure (13): Comparison between the studied groups according to age

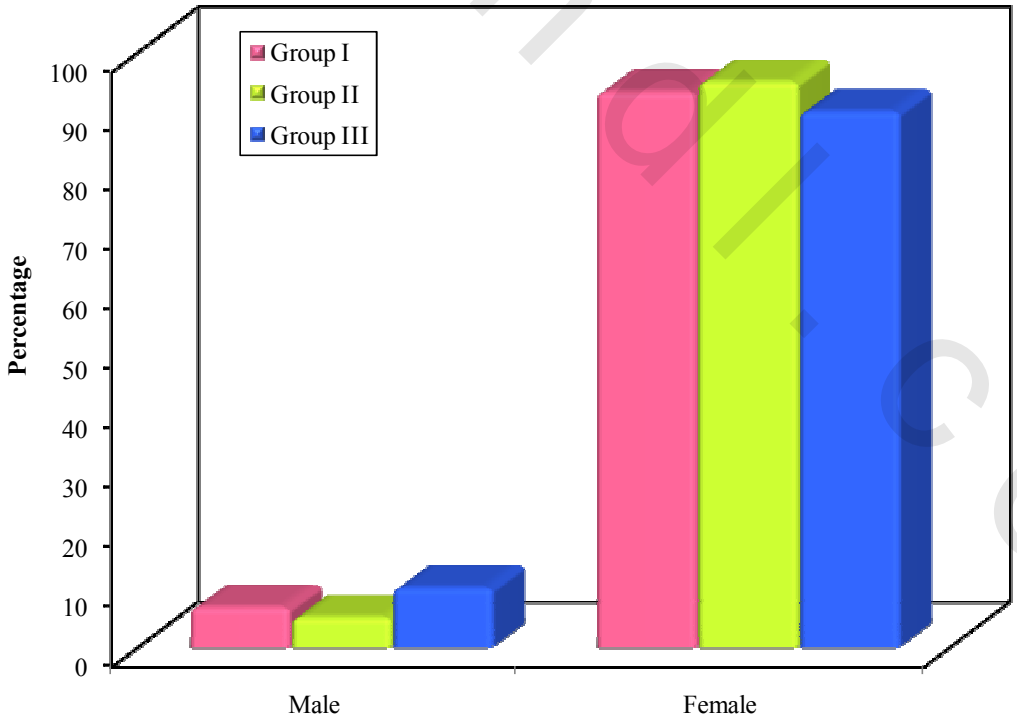

Figure (14): Comparison between the studied groups according to sex

- **Disease duration in the first two groups:** in group (I) disease duration ranges between 2-16 years, with median =5. In group (II) disease duration ranges between 2-9 years with median = 5.5.

Table (8): Comparison between the patients according to Disease duration

	Group I (n=30)	Group II (n=20)	Test of Sig.	p
Disease duration				
Min. – Max.	2.0 – 16.0	2.0 – 9.0		
Mean ± SD.	6.20 ± 4.02	5.10 ± 2.38	Z= 0.520	0.603
Median	5.0	5.50		

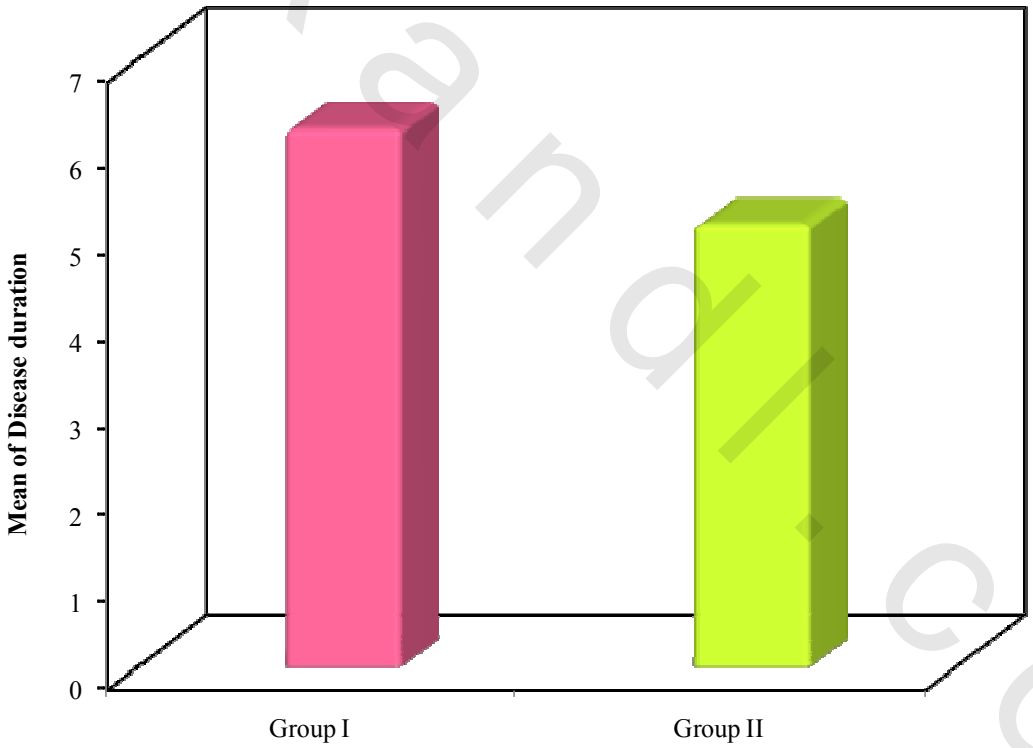

Figure (15): Comparison between the studied groups according to disease duration

- **Comparison between the studied groups according to clinical categories:**

  - Antiphospholipid syndrome (APS) in group (I) is present in all patients (100%). In group (II) APS is present in all patients (100%) and also in group (III) APS is absent in all subjects (100%). APS showed a significant difference between group (I) and group (II), between group (I) and group (III) and between group (II) and group (III). ($p < 0.001$, $x^2 = 70.000$). (Table 10) (Figure 16).

  - Abortion in group (I) is present in 10 patients (33.3%) while absent in 20 patients (66.7%). In group (II) abortion is absent in all patients (100%) and also in group (III) abortion is absent in all subjects (100%). Abortion showed a significant difference between group (I) and group (II) and between group (I) and group (III). ($^{MC}p < 0.001$, $x^2 = 15.556$). (Table 10) (Figure 17).

  - Thrombotic manifestations in group (I) is present in 22 patients (73.3%) while absent in 8 patients (26.7%). In group (II) thrombotic manifestations is absent in all patients (100%) and also in group (III) thrombotic manifestations is absent in all subjects (100%). Thrombotic manifestations showed a significant difference between group (I) and group (II) and between group (I) and group (III). ($p < 0.001$, $x^2 = 42.778$). (Table 10) (Figure 16).

  - Lupus nephritis in group (I) is present in 6 patients (20%) while absent in 24 patients (80%). In group (II) lupus nephritis is present in 6 patients (30%) while absent in 40 patients (70%). In group (III) lupus nephritis is absent in all subjects (100%). Lupus nephritis showed a significant difference between group (I) and group (III) and between group (II) and group (III). ($^{MC}p < 0.024$, $x^2 = 6.638$). (Table 10) (Figure 16).

Table (9): Comparison between the studied groups according to clinical manifestations

	Group I (n=30)		Group II (n=20)		Group III (n=20)		χ^2	p
	No.	%	No.	%	No.	%		
Antiphospolipid Syndrome								
Absent	0	0.0	20	100.0	20	100.0	70.000*	<0.001*
Present	30	100.0	0	0.0	0	0.0		
#Sig. bet. Grps	I-II***,I-III***,II-III***							
Abortion								
Absent	20	66.7	20	100.0	20	100.0	15.556*	MCp<0.001*
Present	10	33.3	0	0.0	0	0.0		
@Sig. bet. Grps	I-II**,I-III**							
Thrombotic Manifestations								
Absent	8	26.7	20	100.0	20	100.0	42.778*	<0.001*
Present	22	73.3	0	0.0	0	0.0		
#Sig. bet. Grps	I-II***,I-III***							
lupus nephritis								
Absent	24	80.0	14	70.0	20	100.0	6.638*	MCp=0.024*
Present	6	20.0	6	30.0	0	0.0		
@Sig. bet. Grps	I-III*,II-III**							

Group I: SLE + antiphospolipid
Group II: SLE
Group III: Control

χ^2: Value for Chi square test
#Sig. bet. grps was done using Chi square test
@Sig. bet. grps was done using Fisher Exact test
*: Statistically significant at $p \leq 0.05$
**: Statistically significant at $p \leq 0.01$
***: Statistically significant at $p \leq 0.001$

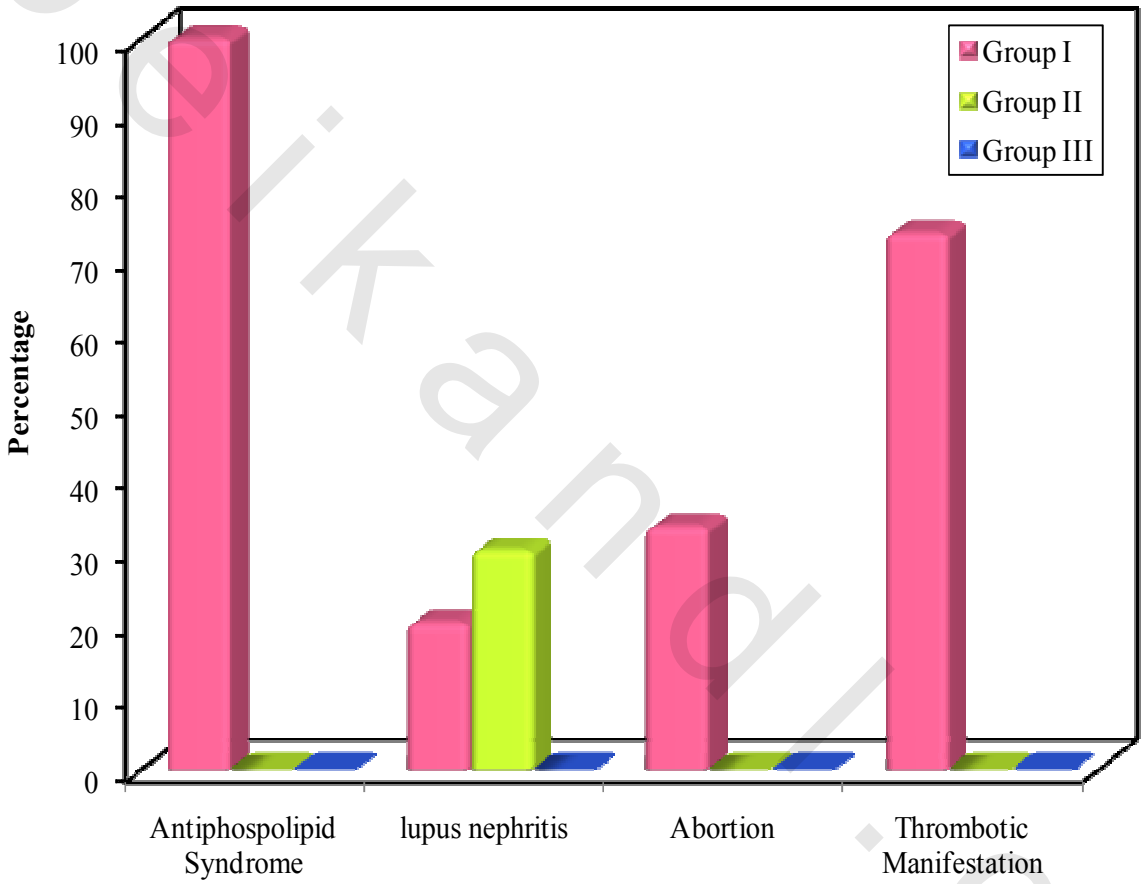

Figure (16): Comparison between the studied groups according to clinical manifestation

➢ **Comparison between the studied groups according to clinical manifestation:**

- The following table and figure shows number of patients in the different groups that had complications.

Table (10): Comparison between the studied groups according to complications

	Group I (n=30)		Group II (n=20)		Group III (n=20)		χ^2	p
	No.	%	No.	%	No.	%		
Complication								
Absent	4	13.3	9	45.0	20	100.0	36.223*	<0.001*
Deep vein thrombosis (DVT)	13	43.3	0	0.0	0	0.0	21.287*	MCp<0.001*
Stroke	6	20.0	0	0.0	0	0.0	8.750*	MCp=0.004*
Pulmonary embolism	3	10.0	0	0.0	0	0.0	4.179	MCp=0.072
Other lung complication	2	6.7	0	0.0	0	0.0	2.745	MCp=0.509
Pregnancy morbidity	10	23.3	0	0.0	0	0.0	10.370*	MCp=0.002*
Nephritis	6	16.7	5	25.0	0	0.0	5.347*	MCp=0.019*
Cardiac complication	3	10.0	1	5.0	0	0.0	2.254	MCp=0.200
Thrombocytopenia	3	10.0	0	0.0	0	0.0	4.179	MCp=0.072
Psychosis	1	3.3	2	10.0	0	0.0	2.554	MCp=0.223
Seizures	3	10.0	0	0.0	0	0.0	4.179	MCp=0.072
Vasculitis	1	3.3	3	15.0	0	0.0	4.729	MCp=0.083
Hypertension (HTN)	1	3.3	1	5.0	0	0.0	0.944	MCp=0.483

Group I: SLE + antiphospolipid
Group II: SLE
Group III: Control

χ^2: Value for Chi square test
MC: Monte Carlo test
*: Statistically significant at $p \leq 0.05$

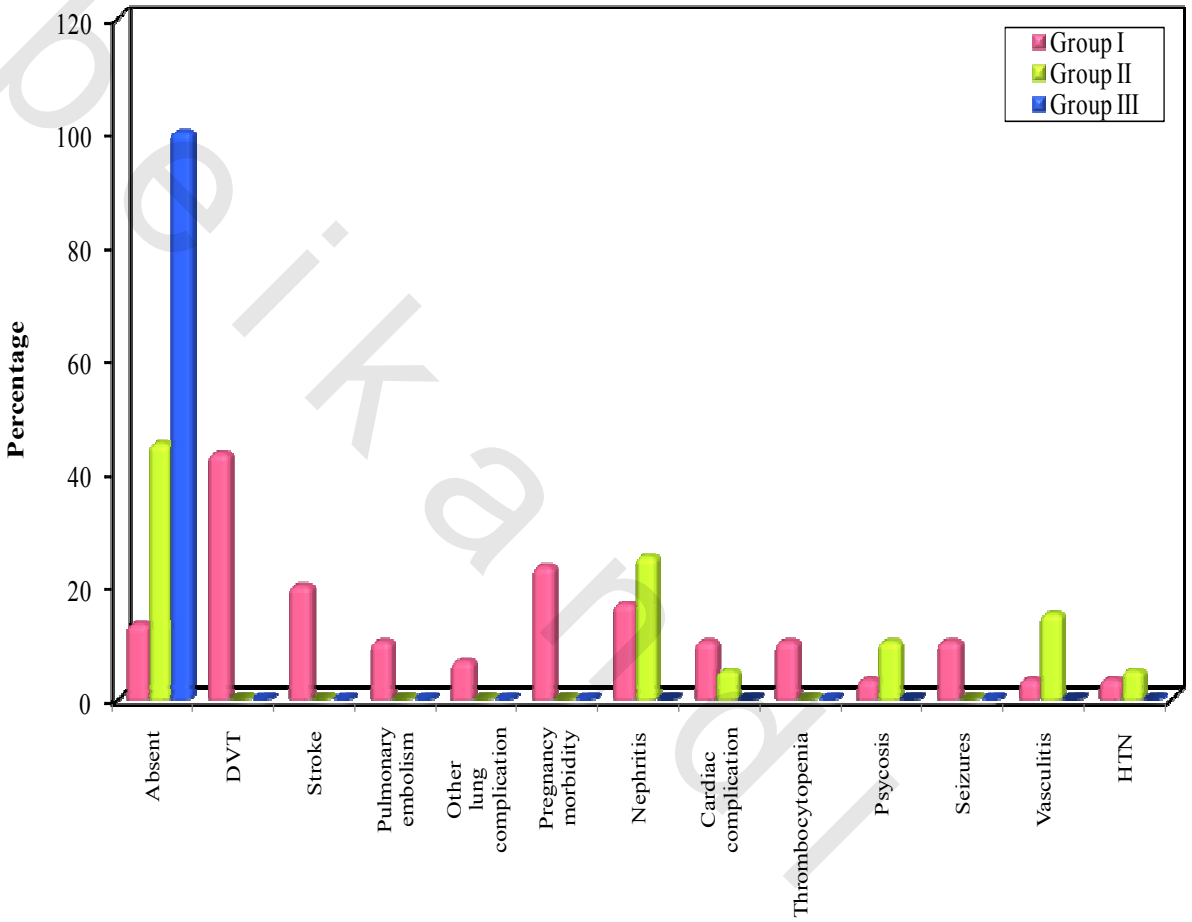

Figure (17): Comparison between the studied groups according to complications

➢ **Illustration of the medications received by patients (group I and II):**

- **Corticosteroids:** in group (I) 29 patient (96.7%) were taking it, and in group (II) 20 patient (100.0%).
- **Cyclophosphamide**: in group (I) 4 patient (13.3%) were taking it, and in group (II) 3 patient (15.0 %).
- **Hydroxychloroquine**: in group (I) 27 patient (90.0%) were taking it, and in group (II) 13 patient (65.0 %).
- **Azathioprine**: in group (I) 6 patient (20.0%) were taking it, and in group (II) 6 patient (30.0 %).
- **Oral anticoagulant**: in group (I) 19 patient (63.3%) were taking it, and in group (II) 0 patient (0.0 %).
- **Antiplatlet**: in group (I) 11 patient (36.7%) were taking it, and in group (II) 0 patient (0.0 %).
- **Heparin**: in group (I) 1 patient (3.3%) were taking it, and in group (II) 0 patient (0.0 %).

Table (11): Comparison between the studied groups according to medications received

	Group I (n=30)		Group II (n=20)		Test of Sig.	p
	No.	%	No.	%		
Corticosteroids	29	96.7	20	100.0	χ^2= 0.680	^{FE}p=1.000
Cyclophosphamide	4	13.3	3	15.0	χ^2= 0.028	^{FE}p=1.000
Hydroxychloroquine	27	90.0	13	65.0	χ^2= 4.688	^{FE}p=0.067
Azathioprine	6	20.0	6	30.	χ^2= 0.658	^{FE}p=0.506
Oral anticoagulant	19	63.3	0	0.0	-	-
Antiplatlet	11	36.7	0	0.0	-	-
Heparin	1	3.3	0	0.0	-	-

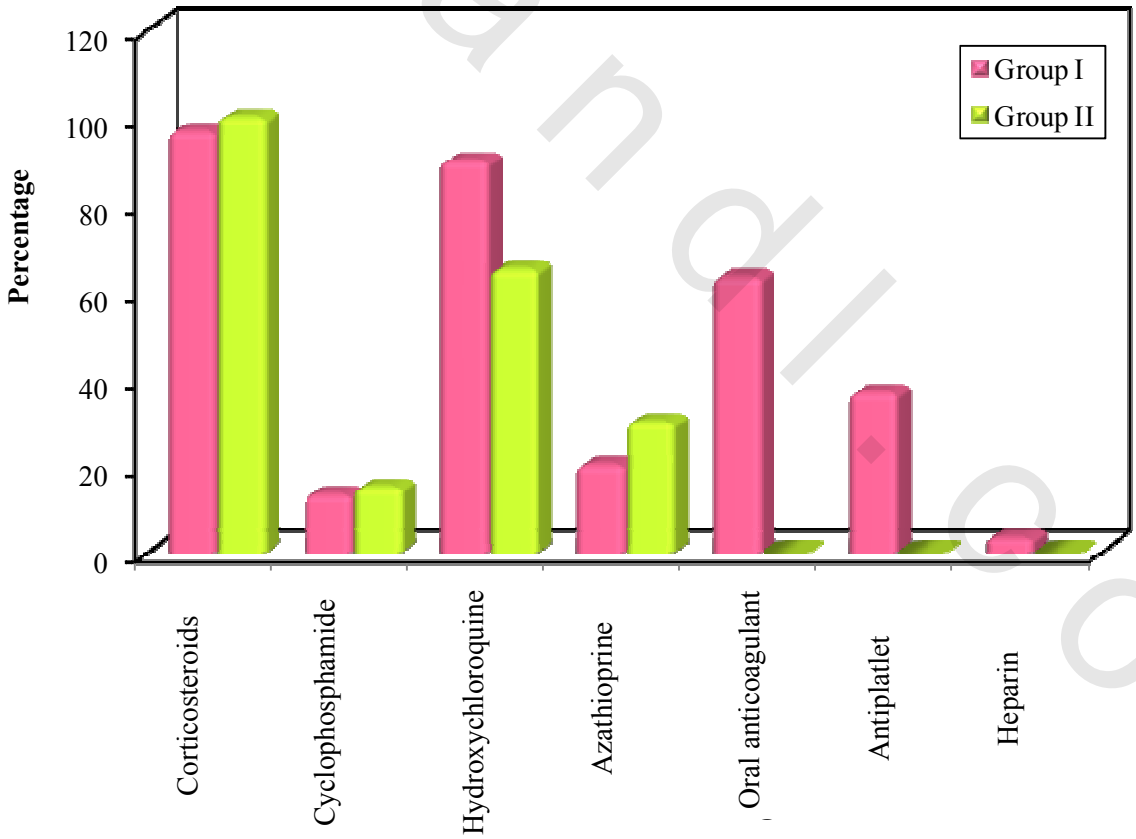

Figure (18): Comparison between the studied groups according to medications received

2- Comparison of laboratory findings among the studied groups:

A. comparison of immunological investigations among the studied groups:

- Anti-dsDNA in group (I) is positive in 26 patients (86.7%) while negative in 4 patients (13.3%). In group (II) 17 patients have positive anti-dsDNA (85.0%) and 3 patients have negative anti-dsDNA (15%). In group (III) all subjects were anti-dsDNA negative (100%). anti-dsDNA showed a significant difference in frequency in group (I) compared to group (III) and group (II) compared to group (III) ($p < 0.001$, $x^2 = 44.607$). (Table13) (Figure 19).
- ANA in group (I) ranged between 160-640 with median = 16.00 In group (II) ANA ranged between 80-640 with median =16.00, whereas ANA of the control group (III) ANA in all patients was < 80. ($Z = 2.310$, $p = 0.129$) (Figure 20) (Table 13).
-

Table (12): Comparison between the studied groups according to anti-dsDNA and ANA

	Group I (n=30)		Group II (n=20)		Group III (n=20)		Test of Sig.	p
	No.	%	No.	%	No.	%		
Anti-dsDNA								
Negative	4	13.3	3	15.0	20	100.0	$\chi^2=44.607^*$	$<0.001^*$
Positive	26	86.7	17	85.0	0	0.0		
Sig. bet. grps	I-III***,II-III***							
ANA (unit)								
Min. – Max.	160.0 – 640.0		80.0 – 640.0		-			
Mean ± SD.	29.867 ± 200.34		21.20 ± 122.50		-		Z=2.310	0.129
Median	16.00		16.00		-			

Group I: SLE + antiphospolipid
Group II: SLE
Group III: Control

χ^2: for Chi square test
Z: Z for Mann Whitney test
Sig. bet. grps was done using Fisher Exact test
*: Statistically significant at $p \leq 0.05$
**: Statistically significant at $p \leq 0.01$

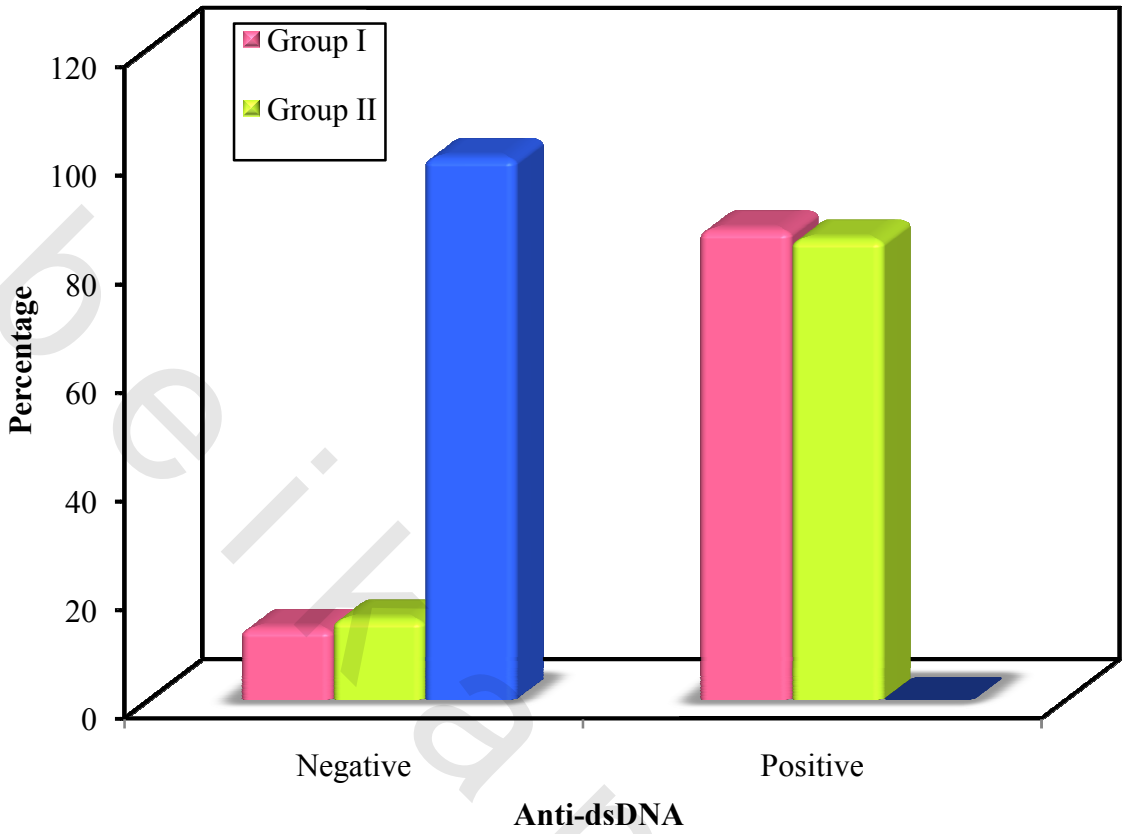

Figure (19): Comparison between the studied groups according to anti-dsDNA

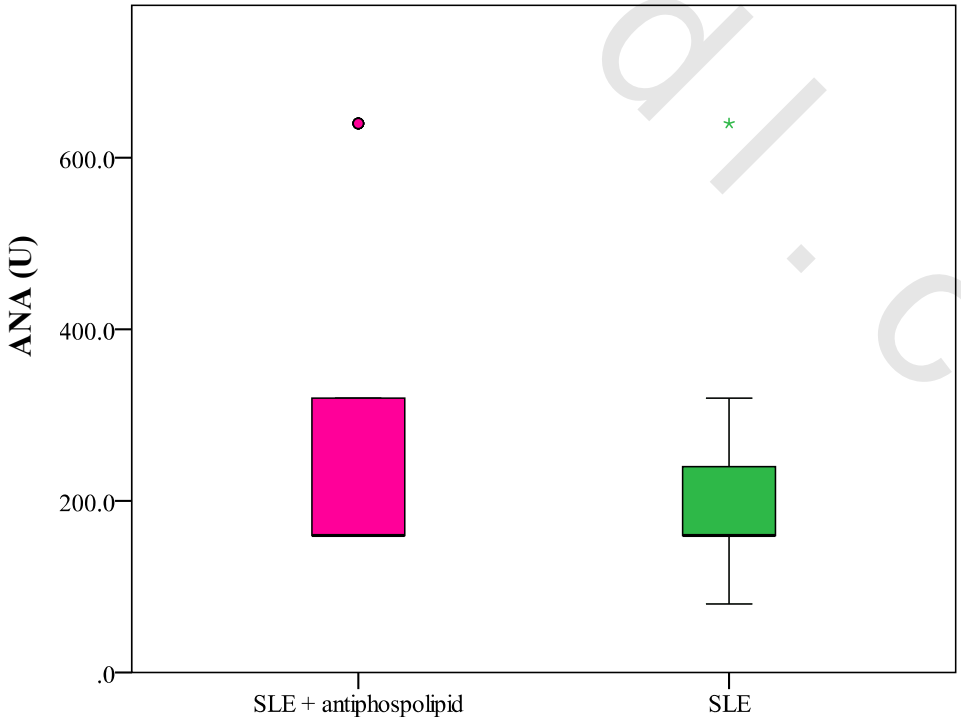

Figure (20): Comparison between the studied groups according to ANA

B. comparison of coagulation investigations among the studied groups:

- Prothrombin Time (PT) in group (I) is abnormal in 8 patients (26.7%) (3 patients < 11 second and 5 patients > 13.5 second) while normal in 22 patients (73.3%). In group (II) 5 patients have abnormal PT (25%) (2 patients < 11 second and 3 patients > 13.5 second) and 15 patients have normal PT (75%). In group (III) all subjects had normal PT (100%). PT showed a significant difference in frequency in group (I) compared to group (III) and in group (II) compared group (III) (MCp =0.028 ,x^2=6.408).(Table 14) (Figure 21)

- Partial Thromboplastin Time (aPTT) in group (I) is abnormal in only 1 patient (3.3%) (> 13.5 second) while normal in 29 patients (96.7%). In group (II) only 1 patient had an abnormal aPTT (5%) (> 13.5 second) and 19 patients have normal aPTT (95%). In group (III) all subjects had normal aPTT (100%). aPTT showed a significant difference in frequency in group (I) compared to group (III) only (MCp =1.000, x^2=0.944). (Table 13) (Figure22).

Table (13): Comparison between the studied groups according to Prothrombin Time and aPTT

	Group I (n=30)		Group II (n=20)		Group III (n=20)		Test of Sig.	P
	No.	%	No.	%	No.	%		
Prothrombin Time (Normal:11-13.5 second)								
Normal	22	73.3	15	75.0	20	100.0	χ^2=6.408*	MCp = 0.028*
Abnormal	8	26.7	5	25.0	0	0.0		
#Sig. bet. grps	I-III*, II-III*							
Min. – Max.	1.90 – 29.0		10.20 – 21.90		11.40 – 13.40		KWχ^2=0.832	0.660
Mean ± SD.	13.35 ± 4.29		13.03 ± 2.53		12.44 ± 0.69			
Median	12.60		12.55		12.50			
Partial Thromboplastin Time (Normal:25-35 second)								
Normal	29	96.7	19	95.0	20	100.0	χ^2=0.944	MCp = 1.000
Abnormal	1	3.3	1	5.0	0	0.0		
Min. – Max.	26.0 – 56.40		21.40 – 47.80		22.80 – 36.0		F=4.697*	0.012*
Mean ± SD.	33.57 ± 5.78		30.21 ± 5.62		29.06 ± 4.71			
Median	33.0		30.0		28.45			
@Sig. bet. grps	I-III*							

Group I: SLE + antiphospolipid
Group II: SLE
Group III: Control

F: F test (ANOVA)
KWχ^2: Chi square test for Kruskal Wallis test
χ^2: value for Chi square test
Sig. bet. grps was done using Fisher Exact test or Chi square test
@Sig. bet. grps was done using Post Hoc test (Scheffe)
*: Statistically significant at $p \leq 0.05$

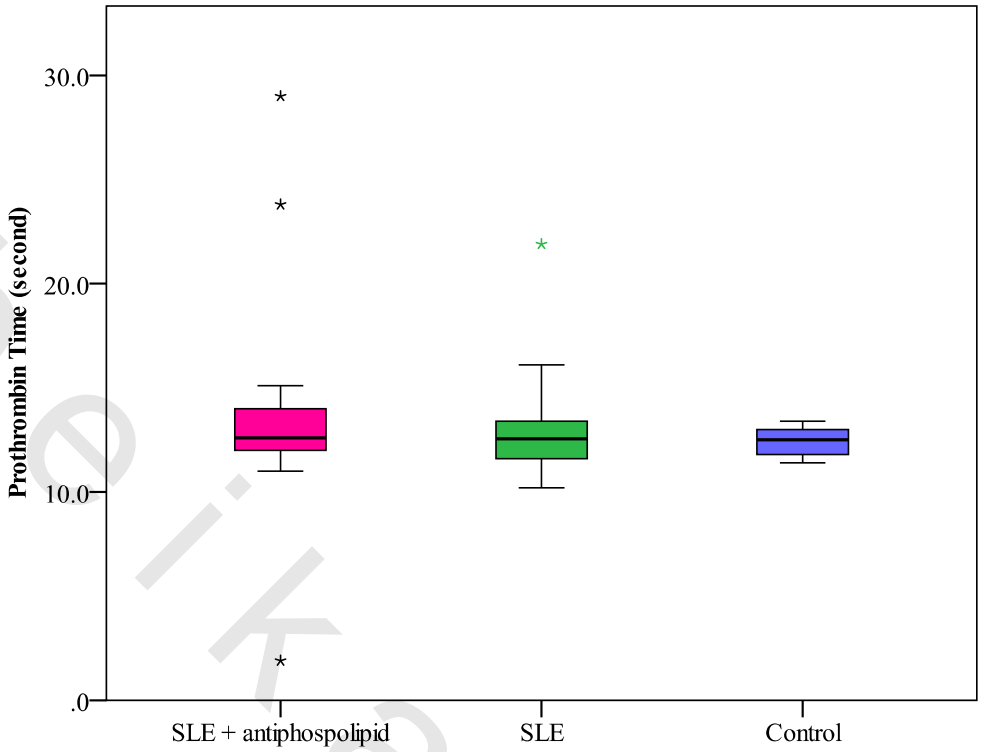

Figure (21): Comparison between the studied groups according to Prothrombin Time

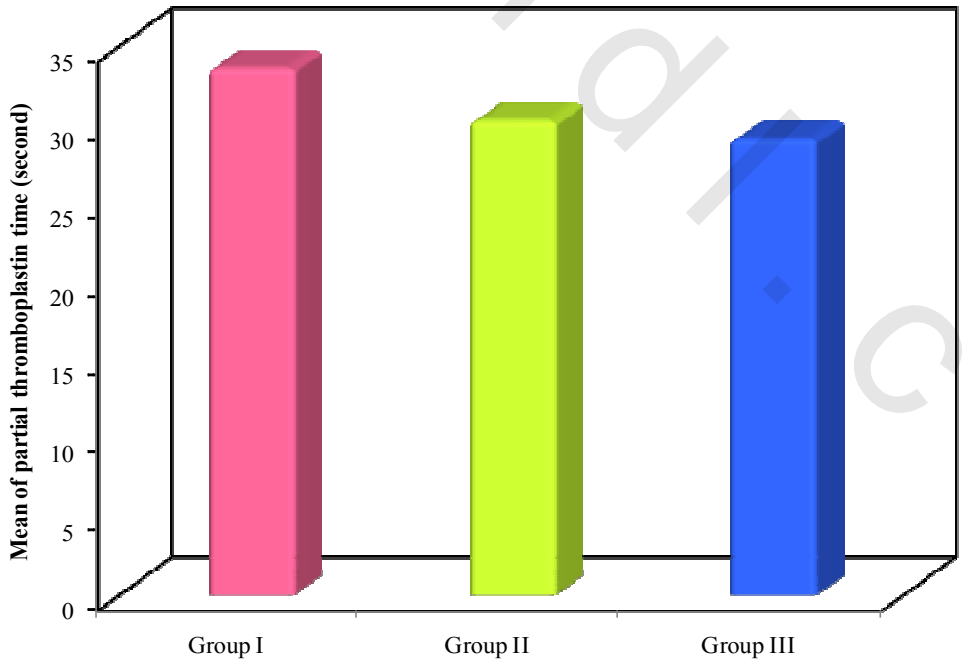

Figure (22): Comparison between the studied groups according to aPTT

C. Comparison of haematological investigations among the studied groups:

- Haemoglobin (Hb) in group (I) is abnormal in 24 patients (80%) (23 patients < 13 gm/dl and only one patient > 16 gm/dl) while normal in 6 patients (20%). In group (II) 12 patients have abnormal Hb (60%) (11 patient < 13 gm/dl and 1 patient > 16 gm/dl) and 8 patients have normal Hb (40%). In group (III) all subjects had normal Hb (100%). Hb showed a significant difference in frequency in group (I) compared to group (III) and group (II) compared to group (III) ($p < 0.001$, $x^2=31.569$). (Table 14) (Figure 23).

- White blood cells (WBCs) in group (I) is abnormal in 2 patients (6.7%) (1 patient < 4×10^9/L, 1 patient > 4×10^9/L) while normal in 28 patients (93.3%). In group (II) 7 patients have abnormal WBCs (35%) (3 patients < 4×10^9/L, 3 patients > 4×10^9/L) and 13 patients have normal WBCs (65%). In group (III) all subjects had normal WBCs (100%). WBCs showed a significant difference in frequency in group (I) compared to group (II) and group (II) compared to group (III) (MCp =0.002, $x^2=12.729$). (Table 14) (Figure 24).

- Platelet count in group (I) is abnormal in 7 patients (23.3%) (5 patients < 150×10^9/L and 2 patients < 150×10^9/L) while normal in 23 patients (76.7%). In group (II) 2 patients have abnormal Platelet count (10%) and 18 patients have normal Platelet count (90%). In group (III) all subjects had normal Platelet count (100%). Platelet count showed a significant difference in frequency in group (I) compared to group (III) only (MCp =0.042, $x^2=6.035$). (Table 14) (Figure 25)

Table (14): Comparison between the studied groups according to CBC

	Group I (n=30)		Group II (n=20)		Group III (n=20)		Test of Sig.	P
	No.	%	No.	%	No.	%		
Heamoglobin (Normal:13-16 gm/dl)								
Normal	6	20.0	8	40.0	20	100.0	χ^2 = 31.569*	p <0.001*
Abnormal	24	80.0	12	60.0	0	0.0		
#Sig. bet. Grps	I-III***, II-III***							
Min. – Max.	6.10 – 13.80		8.0 – 15.0		11.80 – 14.0			
Mean ± SD.	10.47 ± 1.47		11.09 ± 1.71		12.66 ± 0.69		F=15.415*	<0.001*
Median	10.35		11.10		12.60			
@Sig. bet. Grps	I-III***,II-III**							
WBCs (Normal:4-11×10^9/L)								
Normal	28	93.3	13	65.0	20	100.0	χ^2 = 12.729*	MCp = 0.002*
Abnormal	2	6.7	7	35.0	0	0.0		
#Sig. bet. Grps	I-II*, II-III**							
Min. – Max.	2.70 – 11.20		2.98 – 16.0		4.90 – 9.60			
Mean ± SD.	6.87 ± 2.12		8.16 ± 3.67		7.86 ± 1.39		F=1.831	0.168
Median	6.80		7.86		8.15			
Platelets (Normal:150-450 ×10^9/L)								
Normal	23	76.7	18	90.0	20	100.0	χ^2 = 6.035*	MCp = 0.042*
Abnormal	7	23.3	2	10.0	0	0.0		
#Sig. bet. Grps	I-III*							
Min. – Max.	10.0 – 589.0		122.0 – 478.0		170.0 – 379.0			
Mean ± SD.	274.97 ± 134.10		253.75 ± 88.88		278.40 ± 70.79		KWχ^2=1.470	0.480
Median	247.0		241.0		267.50			

Group I: SLE + antiphospolipid
Group II: SLE
Group III: Control

F: F test (ANOVA)
KWχ^2: Chi square test for Kruskal Wallis test
χ^2: Value for Chi square test
Sig. bet. grps was done using Fisher Exact test or Chi square test
@Sig. bet. grps was done using Post Hoc test (Scheffe)
*: Statistically significant at $p \leq 0.05$
**: Statistically significant at $p \leq 0.01$
***: Statistically significant at $p \leq 0.001$

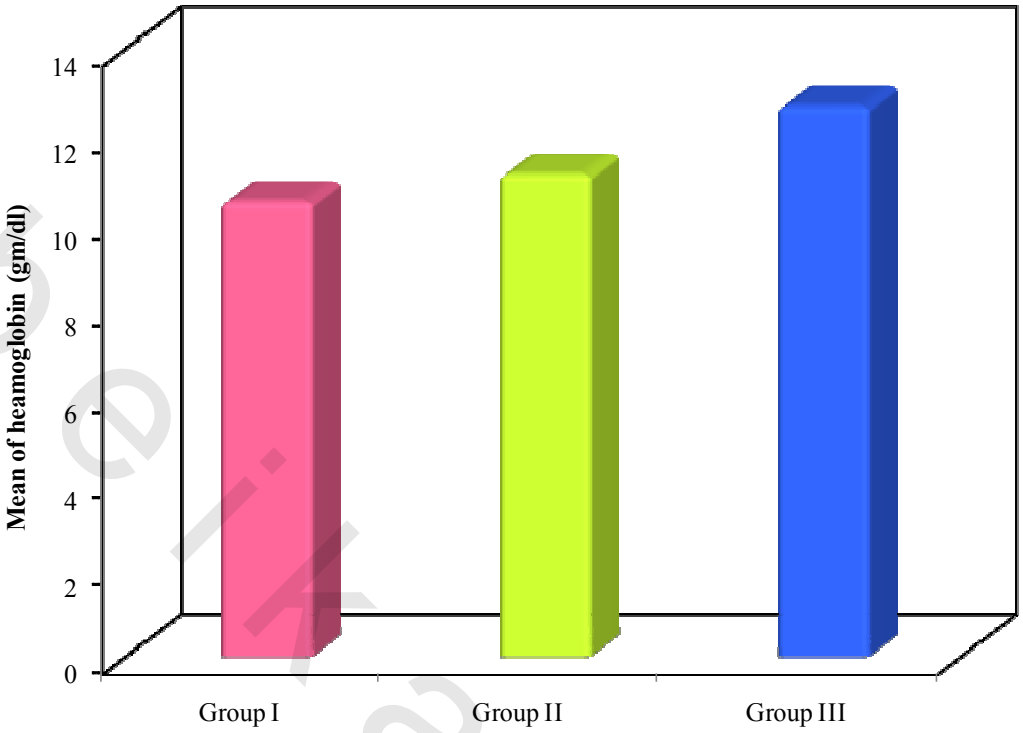

Figure (23): Comparison between the studied groups according to haemoglobin

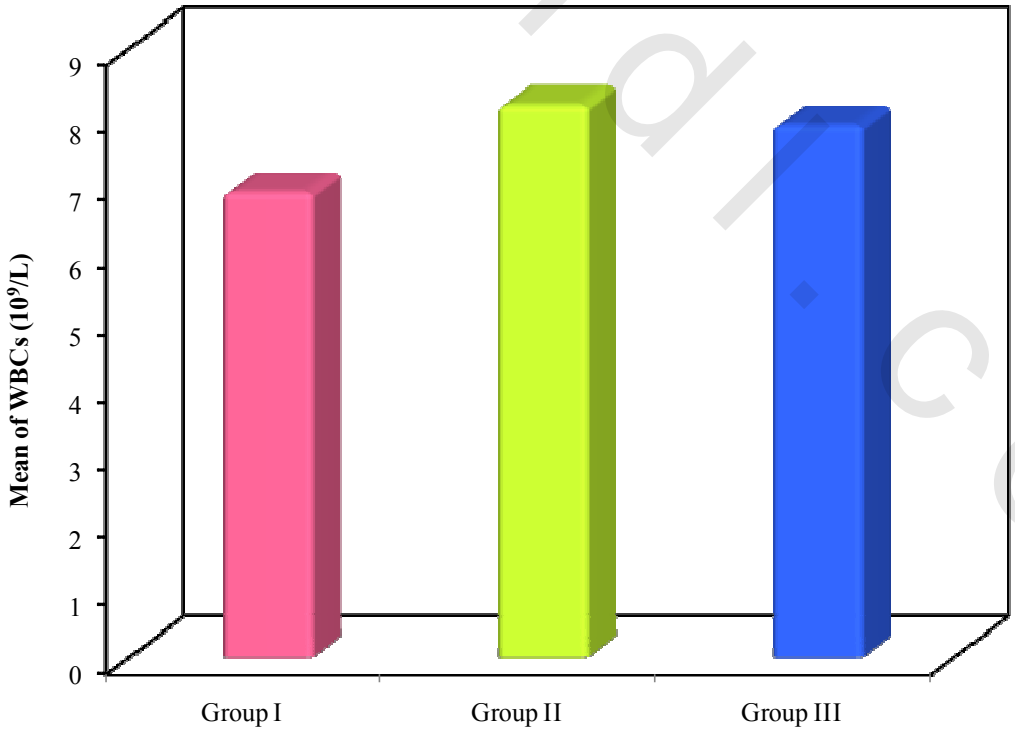

Figure (24): Comparison between the studied groups according to WBCs

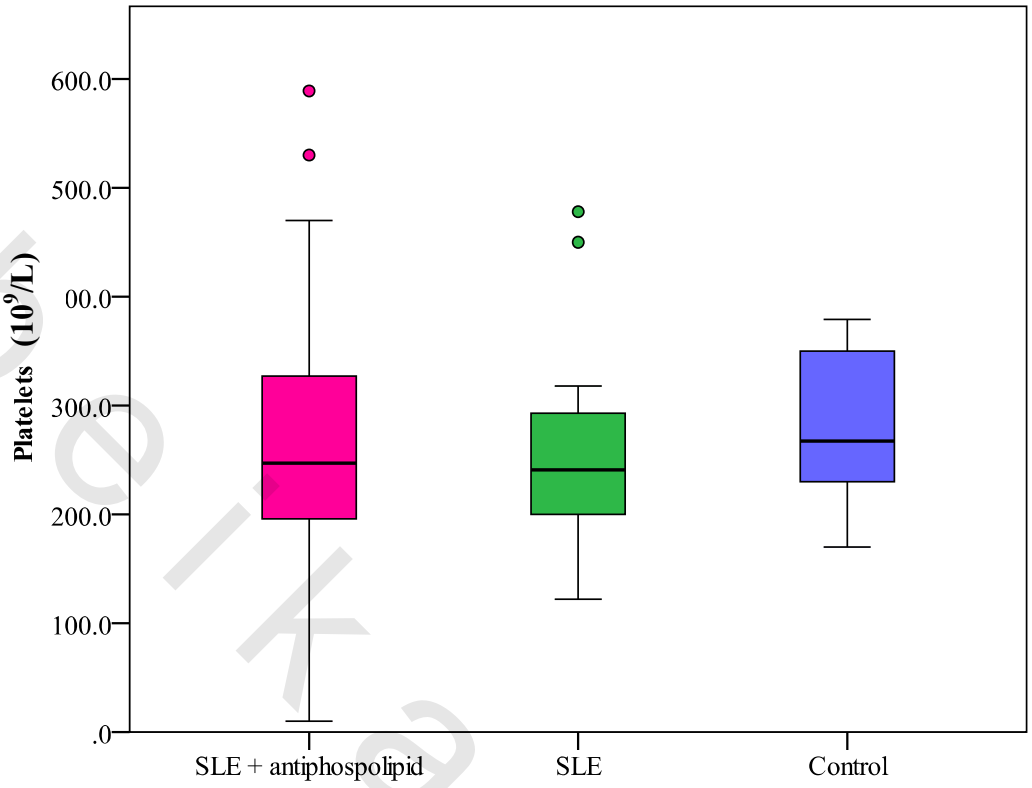

Figure (25): Comparison between the studied groups according to platelets

D. comparison of blood chemistry investigations among the studied groups:

- Serum urea in group (I) is abnormal in 6 patients (20%) (3 patients < 15 mg/dl and 3 patients < 45 mg/dl) while normal in 24 patients (80%). In group (II) 7 patients have abnormal s.urea (35%) (5 patients < 15 mg/dl and 2 patients < 45 mg/dl) and 13 patients have normal abnormal s.urea (65%). In group (III) all subjects had normal s.urea (100%). S. urea showed a significant difference in frequency in group (II) compared to group (III) only (MCp =0.011, x^2=8.171). (Table 15).

- Serum creatinine in group (I) is abnormal in 10 patients (33.3%) (7 patient < 0.06 mg/dl and 3 patients > 1.3 mg/dl) while normal in 20 patients (66.7%). In group (II) 15 patients have abnormal s. creatinine (75%) (10 patient < 0.06 mg/dl and 5 patients > 1.3 mg/dl) and 5 patients have normal abnormal s. creatinine (25%). In group (III) 2 subjects have abnormal s. creatinine (10%) while 18 subjects have normal s. creatinine (90%). S. creatinine showed a significant difference in frequency in group (I) compared to group (III) and in group (II) compared to group (III) (MCp < 0.001, x^2=18.440). (Table 15).

- Albumin/creatinine ratio in urine in group (I) is abnormal (> 30) in 7 patients (23.3%) while normal in 23 patients (76.7%). In group (II) 6 patients have abnormal (> 30) albumin/creatinine ratio in urine (30%) and 14 patients have normal abnormal albumin/creatinine ratio in urine (70%). In group (III) all subjects had normal albumin/creatinine ratio in urine (100%). Albumin/creatinine ratio in urine showed a significant difference in frequency in group (I) compared to group (III) and in group II compared to group (III) (MCp =0.023, x^2=6.739). (Table 15) (Figure 26).

Table (15):Comparison between the studied groups according to renal function

	Group I (n=30)		Group II (n=20)		Group III (n=20)		Test of sig.	P
	No.	%	No.	%	No.	%		
S. Urea (Normal:15-45 mg/dl)								
Normal	24	80.0	13	65.0	20	100.0	χ^2=8.171	$^{MC}p = 0.011^*$
Abnormal	6	20.0	7	35.0	0	0.0		
Sig. bet. grps	II-III**							
Min. – Max.	12.0 – 340.0		10.0 – 254.0		18.0 – 34.0		$^{KW}\chi^2 = 4.207$	0.122
Mean ± SD.	39.10 ± 57.99		47.05 ± 52.50		25.10 ± 5.96			
Median	26.0		31.0		24.0			
S. Creatinine (Normal:0.6-1.3 mg/dl)								
Normal	20	66.7	5	25.0	18	90.0	χ^2=18.440*	$^{MC}p < 0.001^*$
Abnormal	10	33.3	15	75.0	2	10.0		
Sig. bet. Grps	I-II**, II-III***							
Min. – Max.	0.40 – 5.20		0.40 – 9.50		0.50 – 1.0		$^{KW}\chi^2 = 1.752$	0.416
Mean ± SD.	1.03 ± 0.92		1.55 ± 2.01		0.80 ± 0.16			
Median	0.80		1.05		0.80			
Albumin/Creat. Ratio (Normal: < 30)								
Normal	23	76.7	14	70.0	20	100.0	χ^2= 6.739*	$^{MC}p = 0.023^*$
Abnormal	7	23.3	6	30.0	0	0.0		
Sig. bet. Grps	I-III*, II-III*							
Min. – Max.	0.10 – 1123.0		0.10 – 5043.50		3.80 – 22.0		$^{KW}\chi^2 = 2.320$	0.313
Mean ± SD.	112.32 ± 278.11		371.68 ±1133.16		12.43 ± 6.28			
Median	12.45		16.35		12.60			

Group I: SLE + antiphospolipid
Group II: SLE
Group III: Control

$^{KW}\chi^2$: Chi square test for Kruskal Wallis test
χ^2: value for Chi square
MC: Monte Carlo test
#Sig. bet. grps was done using Fisher Exact test or Chi square test
*: Statistically significant at $p \leq 0.05$
**: Statistically significant at $p \leq 0.01$
***: Statistically significant at $p \leq 0.001$

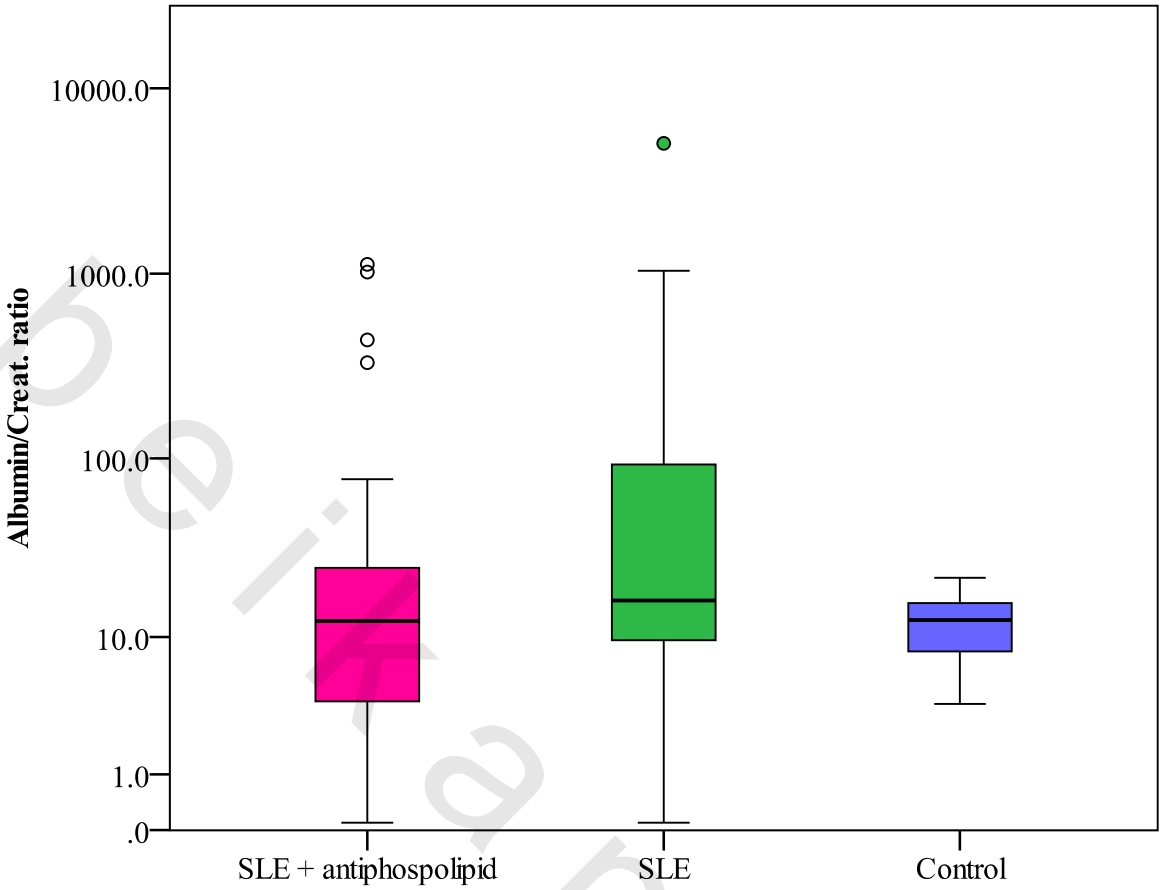

Figure (26): Comparison between the studied groups according to albumin/creat. ratio

Regarding urine analysis:

- Proteinuria in group (I) is present in 8 patients (26.7%) while absent in 22 patients (73.3%). In group (II) proteinuria is present in 8 patients (40%) while absent in 12 patients (60%). In group (III) proteinuria is absent in all subjects (100%). Proteinuria showed a significant difference between group (I) and group (III) and between group (II) and group (III). ($^{MC}p = 0.004$, $x^2 = 9.506$). (Table 16).
- Urinary casts in group (I) is present in 9 patients (30%) while absent in 21 patients (70%). In group (II) urinary casts is present in 4 patients (20%) while absent in 16 patients (80%). In group (III) urinary casts is absent in all subjects (100%). urinary casts showed a significant difference between group (I) and group (III) and between group (II) and group (III). ($^{MC}p = 0.020$, $x^2 = 7.179$). (Table 16).

Table (16): Comparison between the studied groups according to urine analysis

Urine Analysis	Group I (n=30)		Group II (n=20)		Group III (n=20)		χ^2	^{MC}p
	No.	%	No.	%	No.	%		
Proteinuria								
Absent	22	73.3	12	60.0	20	100.0	9.506*	0.004*
Present	8	26.7	8	40.0	0	0.0		
Sig. bet. Grps	I-III**,II-III***							
Casts								
Absent	21	70.0	16	80.0	20	100.0	7.179*	0.020*
Present	9	30.0	4	20.0	0	0.0		
Sig. bet. Grps	I-III**,II-III*							

Group I: SLE + antiphospolipid
Group II: SLE
Group III: Control

χ^2: Value for Chi square test
Sig. bet. grps was done using Fisher Exact test
*: Statistically significant at $p \leq 0.05$
**: Statistically significant at $p \leq 0.01$

3- Comparison of the frequency of antiphospholipids positive subjects among the studied group:

A. Comparison between the studied groups according to antibeta2 glycoprotein antibodies IgM and IgG:

The frequency of antibeta2 glycoprotein antibodies among the studied groups is shown in Table (12).

- Regarding the frequency of antibeta2 glycoprotein IgM in group (I): 28 patients were antibeta2 glycoprotein IgM positive (93.3%) while only 2 patients were antibeta2 glycoprotein IgM negative (6.7%). In group (II) 18 patients were antibeta2 glycoprotein IgM positive (90%) while only 2 patients were antibeta2 glycoprotein IgM negative (10%). In group (III) 12 subjects were antibeta2 glycoprotein IgM positive (60%) while 8 subjects (40%) were antibeta2 glycoprotein IgM negative. antibeta2 glycoprotein IgM showed a significant difference in frequency in group (I) compared to group (III) and in group II compared to group (III) (MCp 0.007, $x^2 = 10.393$). (Table 17) (Figure27).
- The frequency of antibeta2 glycoprotein IgG in group (I): 21 patients were antibeta2 glycoprotein IgG positive (70%) while 9 patients were antibeta2 glycoprotein IgG negative (30%). In group (II) 20 patients were antibeta2 glycoprotein IgG positive (100%) while no patients were antibeta2 glycoprotein IgG negative (0%). In group (III) 6 subjects were antibeta2 glycoprotein IgG positive (30%) while 14 subjects (70%) were antibeta2 glycoprotein IgG negative. antibeta2 glycoprotein IgG showed a significant difference in frequency in group (I) compared to group (III) and in group II compared to group (III) (MCp < 0.01, χ^2=22.405). (Table 17) (Figure 28).

Table (17): Comparison between the studied groups according to antibeta2 glycoprotein

Antibeta2 Glycoprotein (U/ml)	Group I (n=30)		Group II (n=20)		Group III (n=20)		Test of sig.	P
	No.	%	No.	%	No.	%		
IgM (Normal: < 5 U/ml)								
Negative	2	6.7	2	10.0	8	40.0	χ^2= 10.393*	MCp= 0.007*
Positive	28	93.3	18	90.0	12	60.0		
#Sig. bet. Grps	I-III**, II-III*							
Min. – Max.	3.60 – 135.0		4.20 – 182.0		3.60 – 7.70		KWχ^2 = 18.540*	<0.001*
Mean ± SD.	20.88 ± 30.53		18.33 ± 38.91		5.52 ± 1.39			
Median	9.10		7.80		6.10			
@Sig. bet. Grps	I-III***,II-III**							
IgG (Normal: < 5 U/ml)								
Negative	9	30.0	0	0.0	14	70.0	χ^2=22.405*	<0.001*
Positive	21	70.0	20	100.0	6	30.0		
#Sig. bet. Grps	I-II**, I-III**, II-III***							
Min. – Max.	3.0 – 51.10		5.20 – 54.0		3.40 – 6.70		KWχ^2 = 22.026*	<0.001*
Mean ± SD.	11.35 ± 11.79		11.99 ± 11.93		4.66 ± 1.13			
Median	6.20		7.85		4.60			
@Sig. bet. Grps	I-III**,II-III***							

Group I: SLE + antiphospolipid
Group II: SLE
Group III: Control

KWχ^2: Chi square test for Kruskal Wallis test
χ^2: Chi square test
MC: Monte Carlo test
#Sig. bet. grps was done using Monte Carlo test
@Sig. bet. grps was done using Mann Whitney test
*: Statistically significant at $p \leq 0.05$
**: Statistically significant at $p \leq 0.01$
***: Statistically significant at $p \leq 0.001$

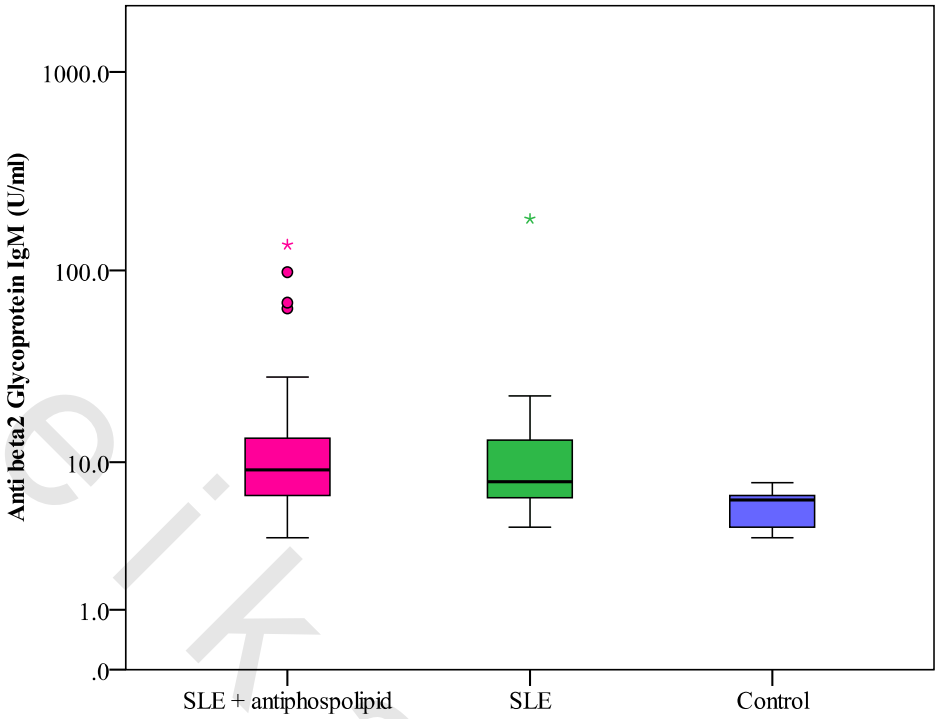

Figure (27): Comparison between the studied groups according to antibeta2 glycoprotein IgM

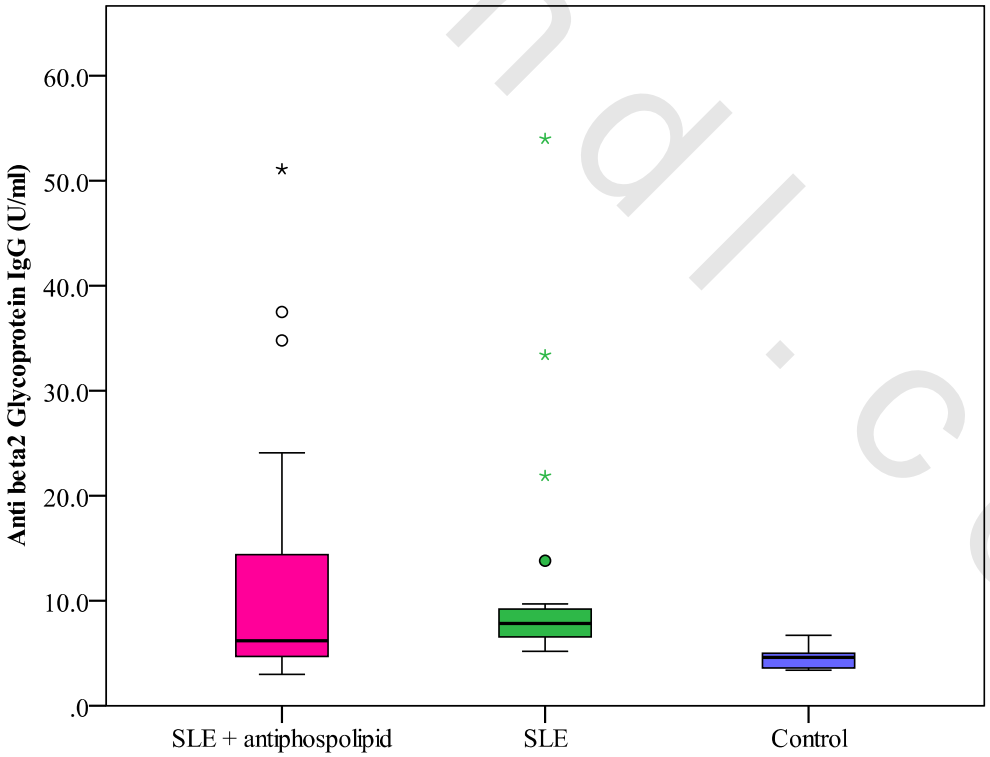

Figure (28): Comparison between the studied groups according to antibeta2 glycoprotein IgG

B. Comparison between the studied groups according to lupus anticoagulant, anticardiolipin antibodies IgM and IgG:

- Frequency of Lupus anticoagulant (LA) in group (I): no patients were LA strongly positive (0%), 11 patients were LA moderately positive (36%), 10 patients were LA weakly positive (33.3%), while 9 patients were LA negative (30%). In group (II) only one patient was LA strongly positive (5%), 6 patients were LA moderately positive (30%), 7 patients were LA weakly positive (35), while 6 patients were LA negative (30%). In group (III) the twenty subjects were LA negative (100%). Lupus anticoagulant showed a significant difference in frequency in group (I) compared to group (III) and in group II compared to group (III) (MCp <0.001, x^2=30.333). (Table 18) (Figure 29).

– Regarding the frequency of anticardiolipin IgM in group (I): 6 patients were anticardiolipin IgM positive (20%) while 24 patients were anticardiolipin IgM negative (80%). In group (II) 3 patients were anticardiolipin IgM positive (15%) while 17 patients were anticardiolipin IgM negative (85%). In group (III) no subjects were anticardiolipin IgM positive (0%) while the 20 subjects (100%) were anticardiolipin IgM negative. Anticardiolipin IgM showed a significant difference in frequency in group (I) compared to group (III) and in group II compared to group (III) (MCp= 0.096, x^2=4.399). (Table 18) (Figure 30).

– The frequency of anticardiolipin IgG in group (I): 9 patients were anticardiolipin IgG positive (30%) while 21 patients were anticardiolipin IgG negative (70%). In group (II) 3 patients were anticardiolipin IgG positive (15%) while 17 patients were anticardiolipin IgG negative (85%). In group (III) only 2 subjects were anticardiolipin IgG positive (10%) while 18 subjects (90%) were anticardiolipin IgG negative. Anticardiolipin IgG showed a significant difference in frequency in group (I) compared to group (III) only (MCp =0.240, χ^2= 3.438). (Table 18) (Figure 31).

Table (18): Comparison between the studied groups according to lupus anticoagulant, anticardiolipin antibodies IgM and IgG

	Group I (n=30)		Group II (n=20)		Group III (n=20)		Test of Sig.	P
	No.	%	No.	%	No.	%		
Lupus anticoagulant								
Negative	9	30.0	6	30.0	20	100.0		
Weakly positive	10	33.3	7	35.0	0	0.0	$\chi^2=30.333^*$	$^{MC}p<0.001^*$
Moderately positive	11	36.7	6	30.0	0	0.0		
Strongly positive	0	0.0	1	5.0	0	0.0		
#Sig. bet. Grps	I-III**, II-III**							
Anticardiolipin antibodies (U/ml) IgM (Normal: < 7 U/ml)								
Negative	24	80.0	17	85.0	20	100.0	$\chi^2=4.399^*$	$^{MC}p = 0.096$
Positive	6	20.0	3	15.0	0	0.0		
Min. – Max.	3.10 – 44.0		3.20 – 44.0		2.80 – 6.10		$^{KW}\chi^2=10.167^*$	0.006^*
Mean ± SD.	8.84 ± 10.75		8.32 ± 11.59		3.88 ± 1.05			
Median	4.10		4.35		3.45			
@Sig. bet. Grps	I-III**, II-III**							
IgG (Normal: < 10 U/ml)								
Negative	21	70.0	17	85.0	18	90.0	$\chi^2=3.438$	$^{MC}p = 0.240$
Positive	9	30.0	3	15.0	2	10.0		
Min. – Max.	3.60 – 50.40		3.60 – 54.0		3.0 – 42.0		$^{KW}\chi^2=6.578^*$	0.037^*
Mean ± SD.	11.44 ± 11.59		9.82 ± 13.02		8.26 ± 11.59			
Median	5.70		5.30		4.80			
@Sig. bet.Grps	I-III*							

Group I: SLE + antiphospolipid
Group II: SLE
Group III: Control

χ^2: Value for Chi square test
MC: Monte Carlo test
$^{KW}\chi^2$: Chi square test for Kruskal Wallis test
@Sig. bet. grps was done using Mann Whitney test
#Sig. bet. grps was done using Monte Carlo test or Fisher Exact test or Chi square test
*: Statistically significant at $p \leq 0.05$
**: Statistically significant at $p \leq 0.01$
***: Statistically significant at $p \leq 0.001$

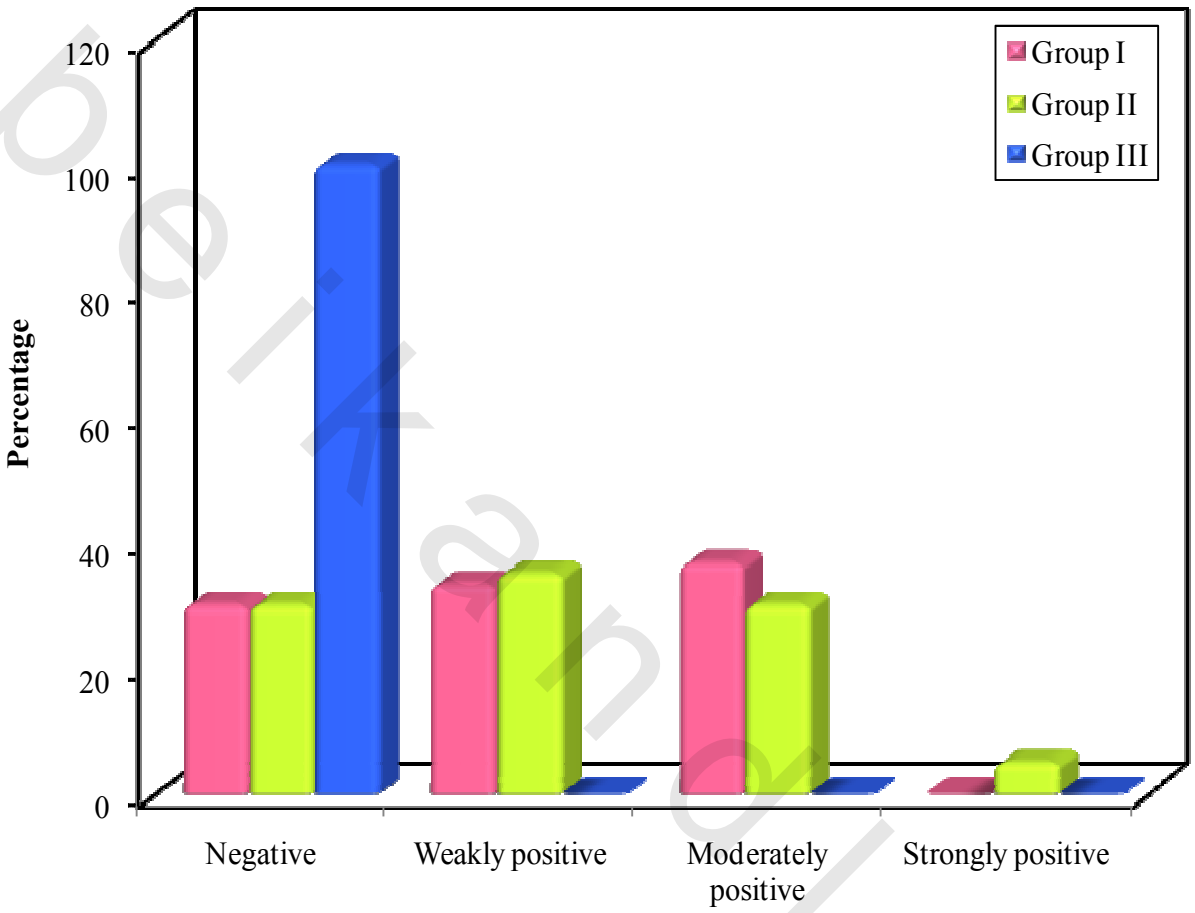

Figure (29): Comparison between the studied groups according to lupus anticoagulant

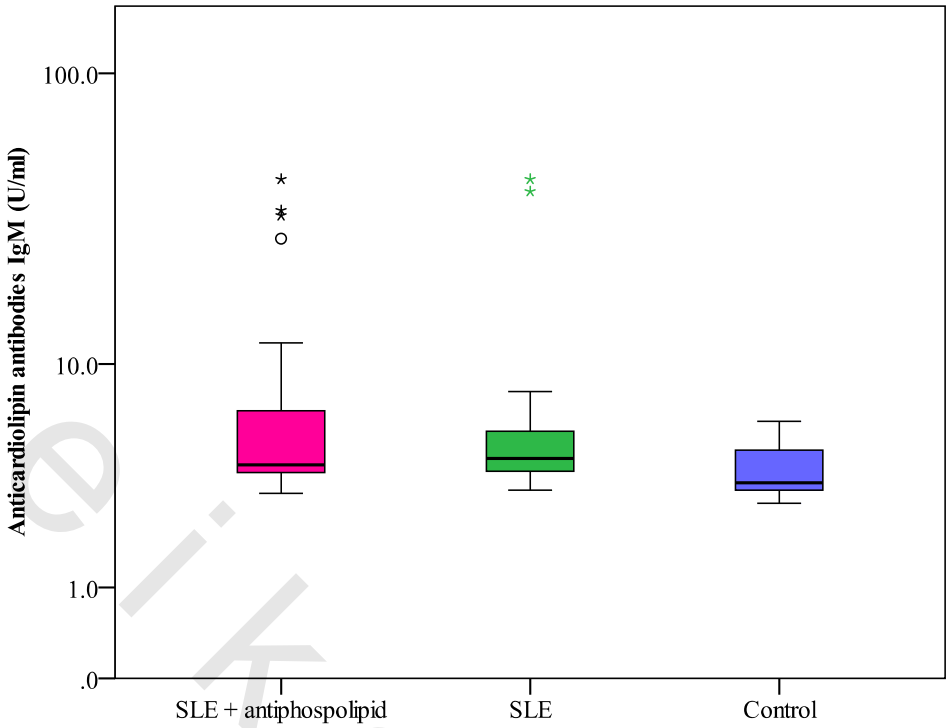

Figure (30): Comparison between the studied groups according to anticardiolipin antibodies IgM

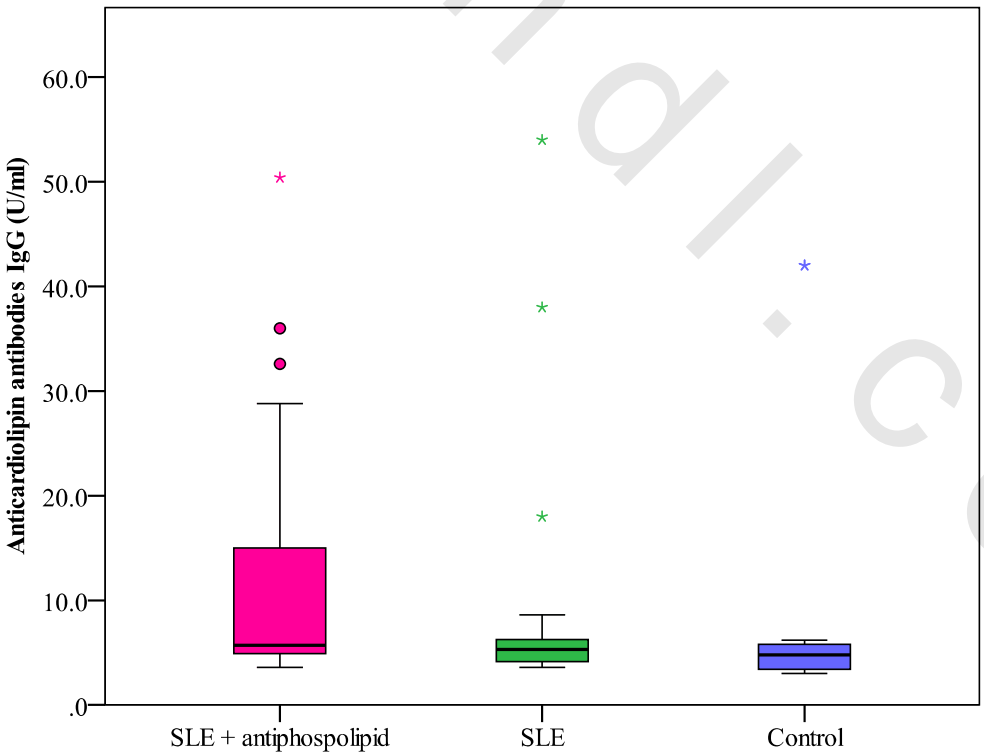

Figure (31): Comparison between the studied groups according to anticardiolipin antibodies IgG

4. Sensitivity, specificity and accuracy for antibeta2 Glycoprotein (IgM and IgG) and Anticardiolipin antibodies (IgM and IgG) with control group:

A. Sensitivity, specificity and accuracy for antibeta2 Glycoprotein (IgM and IgG) and Anticardiolipin antibodies (IgM and IgG) with control group and SLE + antiphospholipid:

- Antibeta2 glycoprotein IgM is positive in 12 subjects and negative in 8 subjects of the control group (group III) , while positive in 28 patients and negative in 2 patients of SLE with secondary APS (group I) giving a sensitivity of (93%) and specificity of (40%) with accuracy =(72.0). (Table 19).

- Antibeta2 glycoprotein IgG is positive in 6 subjects and negative in 14 subjects of the control group (group III) , while positive in 21 patients and negative in 9 patients of SLE with secondary APS (group I) giving a sensitivity of (70%) and specificity of (70%) with accuracy =(70.0). (Table 19).

- Anticardiolipin IgM is positive in only 2 subjects and negative in 18 subjects of the control group (group III) , while positive in 6 patients and negative in 24 patients of SLE with secondary APS (group I) giving a sensitivity of (20%) and specificity of (90%) with accuracy= (50.0). (Table 19).

- Anticardiolipin IgG is positive in only 2 subjects and negative in 18 subjects of the control group (group III) , while positive in 9 patients and negative in 21 patients of SLE with secondary APS (group I) giving a sensitivity of (30%) and specificity of (90%) with accuracy = (54.0). (Table 19).

Table (19): Sensitivity, specificity and accuracy for antibeta2 Glycoprotein (IgM and IgG) and Anticardiolipin antibodies (IgM and IgG) with control group and SLE + antiphospholipid

			Control	SLE + antiphospholipid	Sensitivity	Specificity	PPV	NPV	Accuracy
Antibeta2 Glycoprotein (U/ml)	IgM	Negative	8	2	93.0	40.0	70.0	80.0	72.0
		Positive	12	28					
	IgG	Negative	14	9	70.0	70.0	77.78	60.87	70.0
		Positive	6	21					
Anticardiolipin antibodies (U/ml)	IgM	Negative	18	24	20.0	90.0	75.0	45.45	50.0
		Positive	2	6					
	IgG	Negative	18	21	30.0	90.0	81.82	46.15	54.0
		Positive	2	9					

B. Sensitivity, specificity and accuracy for antibeta2 Glycoprotein (IgM and IgG) and Anticardiolipin antibodies (IgM and IgG) with control and SLE:

- Antibeta2 glycoprotein IgM is positive in 12 subjects and negative in 8 subjects of the control group (group III), while positive in 28 patients and negative in 2 patients of SLE (group II) giving a sensitivity of (90%) and specificity of (40%) with accuracy =(65.0). (Table 20).
- Antibeta2 glycoprotein IgG is positive in 6 subjects and negative in 14 subjects of the control group (group III), while positive in all patients in SLE (group II) giving a sensitivity of (100%) and specificity of (70%) with accuracy =(85.0). (Table 20).
- Anticardiolipin IgM is negative in all subjects of the control group (group III), while positive in 3 patients and negative in 17 patients of SLE (group II) giving a sensitivity of (15%) and specificity of (100%) with accuracy = (57.50). (Table 20).
- Anticardiolipin IgG is positive in only 2 subjects and negative in 18 subjects of the control group (group III), while positive in 3 patients and negative in 17 patients of SLE (group II) giving a sensitivity of (15%) and specificity of (90%) with accuracy =(52.50). (Table 20).

Table (20): Sensitivity, specificity and accuracy for antibeta2 Glycoprotein (IgM and IgG) and Anticardiolipin antibodies (IgM and IgG) with control and SLE

			Control	SLE	Sensitivity	Specificity	PPV	NPV	Accuracy
Antibeta2 Glycoprotein (U/ml)	IgM	Negative	8	2	90.0	40.0	60.0	80.0	65.0
		Positive	12	18					
	IgG	Negative	14	0	100.0	70.0	76.92	100.0	85.0
		Positive	6	20					
Anticardiolipin antibodies (U/ml)	IgM	Negative	20	17	15.0	100.0	100.0	54.05	57.50
		Positive	0	3					
	IgG	Negative	18	17	15.0	90.0	60.0	51.43	52.50
		Positive	2	3					

5. Correlation between antibeta2 Glycoprotein IgM and IgG with different parameters:

A. Correlation between antibeta2 Glycoprotein IgM and IgG with different parameters in SLE + antiphospolipid syndrome group:

- The correlations between antibeta2 glycoprotein IgM and IgG, lupus anticoagulant, age, albumin creatinine ratio in urine, haemoglobin, WBCs, platelets, prothrombin time, partial thromboplastin time in SLE patients with secondary APS (group I) are shown in (Table 21). Significant positive correlation was detected between antibeta2 glycoprotein IgM and platelets (r_s=-0.408, p=0.025) (Figure 32).

Table (21): Correlation between antibeta2 Glycoprotein IgM and IgG with different parameters in SLE + APS group

	Antibeta2 Glycoprotein			
	IgM		IgG	
	r_s	P	r_s	P
Lupus anticoagulant	0.111	0.561	0.062	0.743
Age	-0.068	0.722	-0.222	0.239
Albumin/Creat. Ratio	0.190	0.315	0.126	0.507
Heamoglobin	-0.288	0.122	-0.117	0.538
WBCs	-0.150	0.428	-0.003	0.986
Platelets	0.408*	0.025	-0.033	0.862
Prothrombin Time	-0.171	0.365	-0.031	0.871
Partial Thromboplastin Time	-0.087	0.646	-0.191	0.311
Anti-dsDNA	0.074	0.699	0.255	0.173

r_s: Spearman coefficient
*: Statistically significant at $p \leq 0.05$

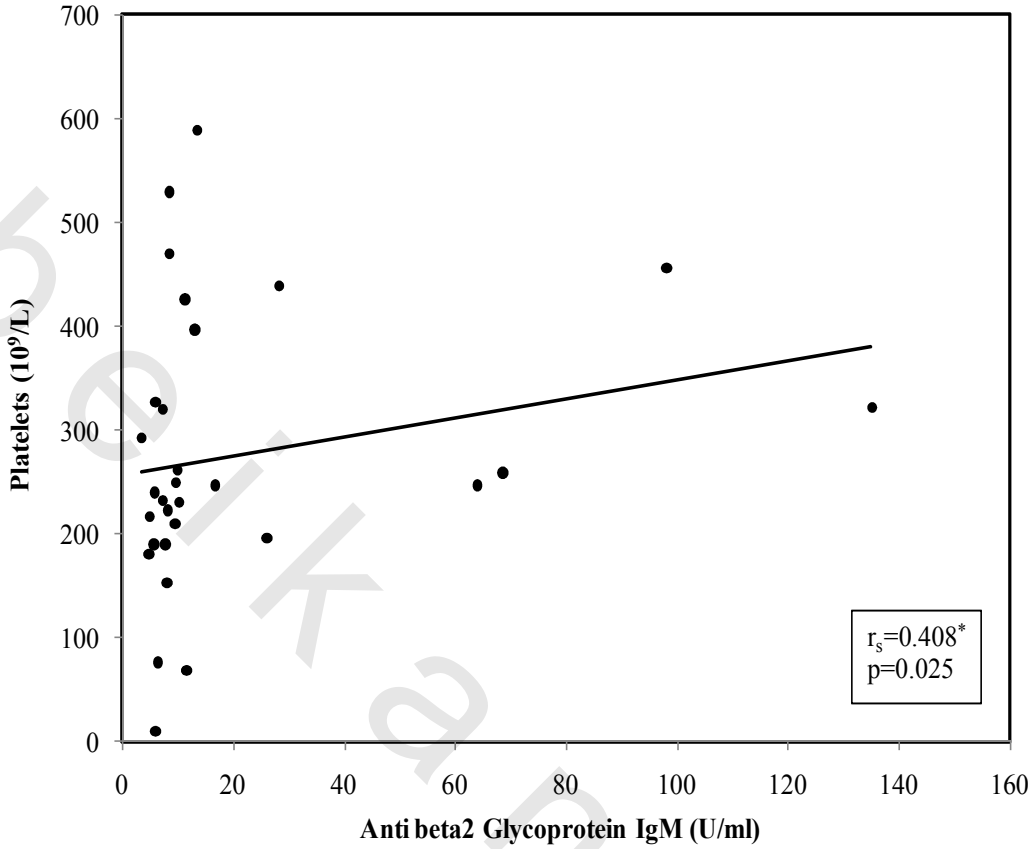

Figure (32): Correlation between antibeta2 Glycoprotein IgM and IgG with platelets in SLE + antiphospolipid group

B. Correlation between antibeta2 Glycoprotein IgM and IgG with different parameters in SLE group:

- The correlations between antibeta2 glycoprotein IgM and IgG, lupus anticoagulant, age, albumin creatinine ratio in urine, haemoglobin, WBCs, platelets, prothrombin time, partial thromboplastin time in SLE patients (group II) are shown in (Table 22). Significant positive correlation was detected between antibeta2 glycoprotein IgM and partial thromboplastin time (r_s=-0.521, p=0.018). (Figure 33).

Table (22): Correlation between antibeta2 Glycoprotein IgM and IgG with different parameters in SLE group

	Beta2 Glycoprotein			
	IgM		IgG	
	r_s	p	r_s	P
Lupus anticoagulant	0.310	0.184	-0.169	0.476
Age	0.195	0.410	0.162	0.494
Albumin/Creat. ratio	0.145	0.541	-0.082	0.731
Heamoglobin	-0.143	0.549	-0.177	0.455
WBCs	0.033	0.890	0.271	0.248
Platelets	-0.219	0.354	0.283	0.226
Pro thrombin Time	0.238	0.312	-0.124	0.602
Partial Thromboplastin Time	0.521*	0.018	-0.020	0.933
Anti-dsDNA	0.085	0.721	0.085	0.721

r_s: Spearman coefficient
*: Statistically significant at $p \leq 0.05$

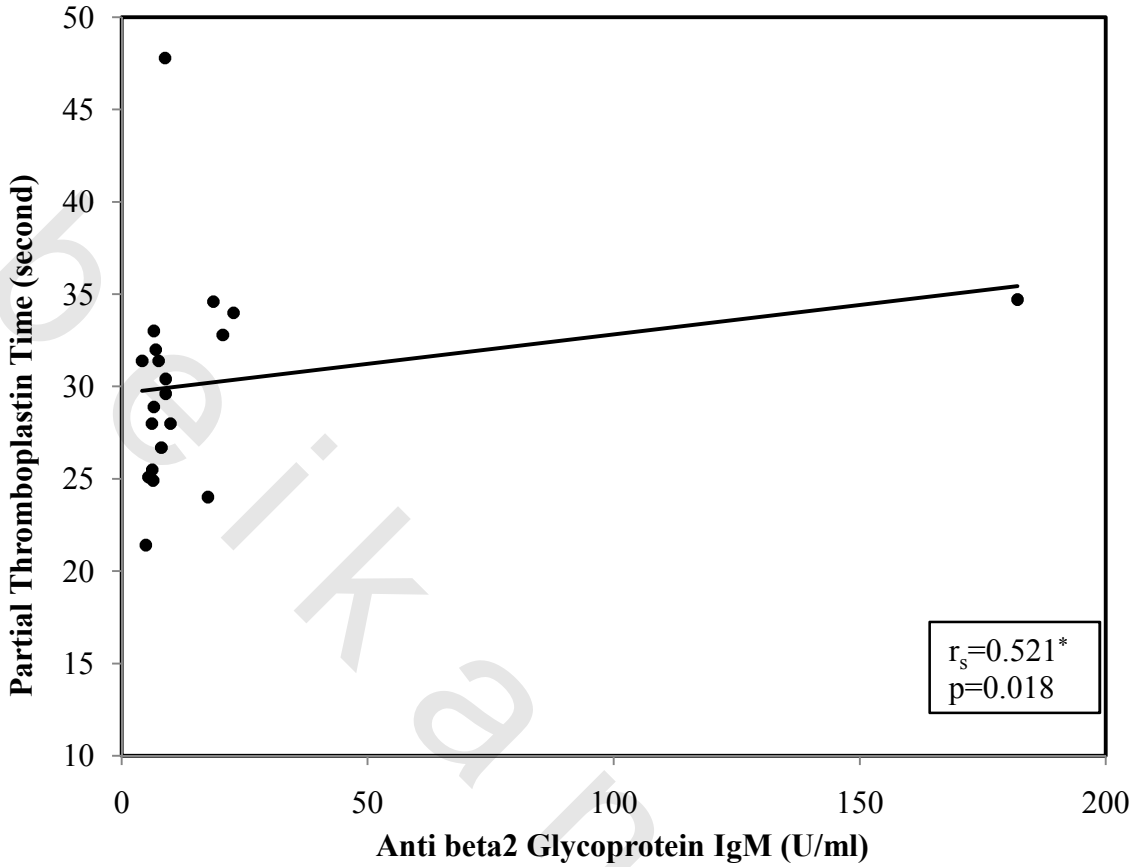

Figure (33): Correlation between antibeta2 Glycoprotein IgM and IgG with partial thromboplastin time in SLE group

6. Correlation between antibeta2 Glycoprotein IgM and IgG and clinical categories:

A. Correlation between antibeta2 Glycoprotein IgM and IgG and clinical presentation in SLE + antiphospholipid group:

- The following table shows a descriptive analysis for antibeta2 glycoprotein IgM and IgG antibodies in different clinical manifestation in SLE patients with secondary APS (group I).

Table (23): Correlation between antibeta2 Glycoprotein IgM and IgG with clinical presentations in SLE + APS group

	N	Antibeta2 Glycoprotein			
		IgM		IgG	
		Min. – Max.	Mean ± SD	Min. – Max.	Mean ± SD
Lupus nephritis					
Absent	**24**	3.60 – 135.0	20.79 ± 32.29	3.0 – 34.80	9.41 ± 8.28
Present	**6**	5.80 – 68.60	21.25 ± 24.69	4.0 – 51.10	19.12 ± 20.02
Z (p)		0.156 (0.876)		1.505 (0.132)	
Abortion					
Absent	**20**	3.60 – 135.0	22.14 ± 35.57	3.40 – 51.10	11.59 ± 12.75
Present	**10**	6.10 – 64.0	18.37 ± 17.94	3.0 – 37.50	10.88 ± 10.23
Z (p)		0.880 (0.379)		0.683 (0.495)	
Thrombotic Manifestation					
Absent	**8**	6.10 – 64.0	18.65 ± 19.44	3.0 – 16.0	8.24 ± 4.58
Present	**22**	3.60 – 135.0	21.69 ± 34.04	3.40 – 51.10	12.48 ± 13.42
Z (p)		0.868 (0.385)		0.211 (0.833)	

Z: Z for Mann Whitney test

B. Correlation between antibeta2 Glycoprotein IgM and IgG and clinical presentation in SLE group:

- The following table shows a descriptive analysis for antibeta2 glycoprotein IgM and IgG antibodies in clinical manifestations in SLE patients (group II).

Table (24): Correlation between antibeta2 Glycoprotein IgM and IgG with clinical presentations in SLE group

	N	Antibeta2 Glycoprotein			
		IgM		IgG	
		Min. – Max.	Mean ± SD	Min. – Max.	Mean ± SD
Lupus nephritis					
Absent	**14**	4.20 – 182.0	20.31 ± 46.67	5.70 – 54.0	13.03 ± 13.75
Present	**6**	6.10 – 22.70	13.70 ± 7.44	5.20 – 21.90	9.55 ± 6.21
Z (p)		1.114 (0.265)		0.413 (0.679)	

Z: Z for Mann Whitney test

7. Correlation between antibeta2 Glycoprotein IgM and IgG and clinical manifestations:

A. Correlation between antibeta2 Glycoprotein IgM and IgG and clinical manifestations in SLE + antiphospholipid syndrome group:

- The following table shows a descriptive analysis for antibeta2 glycoprotein IgM and IgG antibodies in different complications in SLE patients with secondary APS (group I).

Table (25): Correlation between antibeta2 Glycoprotein IgM and IgG and clinical manifestations in SLE + APS group

	N	Anitbeta2 Glycoprotein			
		IgM		IgG	
		Min. – Max.	Mean ± SD	Min. – Max.	Mean ± SD
Complication					
DVT	13	3.60 – 135.0	24.33 ± 41.67	3.0 – 34.80	9.64 ± 9.81
Stroke	6	4.90 – 68.60	16.62 ± 52.50	4.0 – 51.10	13.10 ± 18.66
Pregnancy morbidity	7	6.10 – 28.40	12.96 ± 7.79	5.0 – 37.50	11.69 ± 11.83
Nephritis	5	5.80 – 68.60	25.26 ± 25.67	5.0 – 51.10	21.52 ± 21.36
Cardiac complication	3	3.60 – 28.40	15.17 ± 12.48	4.0 – 37.50	21.63 ± 16.82
Thrombocytopenia	3	4.90 – 11.70	7.57 ± 3.63	5.40 – 24.10	12.07 ± 10.44
Seizures	3	4.90 – 8.60	7.37 ± 2.14	3.40 – 5.40	4.60 ± 1.06

B. Correlation between antibeta2 Glycoprotein IgM and IgG and clinical manifestations in SLE group:

- The following table shows a descriptive analysis for antibeta2 glycoprotein IgM and IgG antibodies in different complications in SLE patients (group II).

Table (26): Correlation between antibeta2 Glycoprotein IgM and IgG and clinical manifestations in SLE group

	N	Antibeta2 Glycoprotein			
		IgM		IgG	
		Min. – Max.	Mean ± SD	Min. – Max.	Mean ± SD
Complication					
Nephritis	5	6.20 – 22.70	12.06 ± 7.58	6.10 – 21.90	10.70 ± 6.35
Psychosis	2	4.90 – 8.80	6.85 ± 2.76	6.40 – 13.80	10.10 ± 5.23
Vasculitis	3	5.40 – 18.60	10.03 ± 7.43	5.70 – 54.0	21.80 ± 27.89