Al-Kufa University Journal for Biology / VOL.12 / NO.1 / Year: 2020
Print ISSN: 2073-8854 Online ISSN: 2311-6544

Correlation of some Immunological Markers with Systemic Lupus Erythematosus disease

Ghaneemah Malik Hamadi[1]
Prof. Dr. Manal Badi Saleh [2]

[1] Lecturer/Southern Technical University/Al-Nasiriya Technical Institute, ghaneemahm@stu.edu.iq
[2] Professor/ Thi-Qar University/College of sciences/Departments of Biology

Abstract:

SLE (Systemic Lupus Erythematosus) is a heterogeneous disease with diverse clinical manifestation disorder characterized by hyperactivity of B and T cell, creation of auto antibodies and deposition of antibody- containing immune complexes in blood vessels during body. This study aims to investigation some immunological markers related to patients with SLE. It includes the study of the level of Interleukin (IL.17), antinuclear antibody (ANA) anti dsDNA, antiphospholipid and complement C3, C4, In addition to study the complete blood picture including hemoglobin, red blood cell monocytes, white blood cells, platelets and lymphocytes, they belong to factors that may interfere with the disease. Current study included 110 patients with SLE, and 106 (96.36%) were female and 4 (3.64%) were male. Patients with an average age of 31.61 ± 8.60 years. This work also includes 70 blood specimens as control group. Our study illustrated that most infections were on Village 71.82 % compared with the City 28.18 , and patients without history disease 57.27 % while patients with history disease42.73%. The results also showed that the concentration of IL.17 was significantly higher than $p \leq 0.05$ in serum patients with lupus erythematosus (469 ± 2.25 g / ml) comparison with healthy individuals (107.39 ± 0.41 pg / ml). ELISA was used to measure some types of autoantibodies, and showed an increase in ANA and dsDNA Ab levels in SLE patients compared with healthy individuals. The results also prone that the antiphospholipid is significantly increase ($P \leq 0.05$) in patients with SLE compared to controls.Evaluating serum complement level C3, C4 in the total study, our results observed significant decrease ($p \leq 0.05$) in serum of patients with SLE (0.62 ± 0.01,0.13 ± 0.01g/l) respectively, compared with controls (1.74 ± 0.01,0.28 ± 0.01g/l) respectively.The results of the study also showed a significant decrease in hemoglobin, red blood cell monocytes, white blood cells, platelets and the number of lymphocytes for SLE patients compared with healthy individuals.The relationship between antibodies to ANA and IL-17, antibodies to dsDNA and C3, C4 and lymphocytes was identified, as well as the relationship between APA, C3 and C4 antibodies. Inconclusion ,peoples with SLE have a positive ANA, High concentration of ds DNA Ab and anti-phospholipid which plays an important role in pathogenesis and complement C3 and C4 in patients with SLE is decreased. An increased inflammatory concentration of interleukin (IL.17) can play a critical role in the pathogenesis of SLE which leads to disease progression.

***Key Words**: autoimmune disease Systemic lupus Erythematosus, autoantibodies, Cytokine, Immune complex.*

Introduction:

Systemic lupus Erythematosus (SLE) is the prototypical autoimmune disease(AD),It is a complex immune disease and clinically heterogeneous. Various immunological defects contribute to systemic lupus erythematosus, including un regulated immune response innate and adaptive.(1,2 and 3). SLE is result from the breakdown of tolerance of nuclear autoantigens, which leads to the activation of self-reactive B cells that produce antibodies against the self, nucleic acids and related proteins, Self-antibodies and auto-nucleic acids released by dead cells are bound to form immune complexes that are deposited in different parts of the body, leading to harmful inflammation and tissue damage. One of the major early events that stimulate the self-immunity in SLE is chronic innate stimulation of plasmidoid and dendritic cells (pDCs) for the secretion of type 1 of IFNs. High levels of the first type of IFNs stimulate uncompromising differentiation, monocytes in Dendritic cells which stimulate self-reactive T and B cells, and lowering the activation threshold for self-reactive B cells, Thus enhancing self-immunity in SLE (4,5 and 6). Accumulation of antibodies in tissues and organs plays a key role in the clinical signs of SLE. (7). In addition to Genetic and environmental factors, Interleukin disorders contribute to impaired immune function, as cytokines are soluble agents that can participate in differentiation, maturity, and stimulation the immune system, causing inflammation and causing damage of the organ. Cytokines Inflammatory, such as interferon type I and interferon type II, (IL-6) interleukin-6, IL-17, IL-1, and(TNF-α) Alpha Tumor Necrosis Factor in addition to immuno-cytokines, such as IL-10 and TGF-β They were Important elements in SLE (8 and 9).

The complement is part of the innate immune system (10). The genetic deficiency of the classical C1q starter predisposition strongly predisposes to SLE. Shortcomings or mutations in other complementary proteins of the classical pathway, such as` C1r, C1s`, C4, and C2, increase the risks, although to a lesser extent compared to C1q. It was initially suggested that SLE, development in patients with C1q deficiency was due to a reduced ability to remove apoptosis cells because C1q is an important opsonin for these cells (11). The prevalence of SLE up to 178 per 100,000 habitants,mostly affects women, Female to male ratio ranges from 2:1 to 15:1 (12 and 13). SLE patients have different clinical manifestations with a large number of autoantibodies. To date, more than 180 autoantibodies have been recognized in the blood of SLE patients, although different people may exhibit different antibody profiles (14). Lupus clinically diagnosis is based on the presence of at least 4 of the 11 diagnostic criteria proposed by the American College of Rheumatology (ACR) for SLE. (15). Presence of anti-nuclear antibodies (ANA) in the patient's serum is the most important standard criterion for diagnosing SLE. Major and largest properties of SLEs in SLE are nuclear fusion, anti-nucleic antibodies (ANA) and ds-DNA (antibodies against double stranded nucleic acid) which is positive in more than 90% of SLE (16). This study aims to investigation some immunogenetic markers related to patients with SLE in South of Iraq by study:- Clinical diagnosis of SLE by detection presence anti-nuclear antibodies,anti-ds DNA, antiphospholipid in patients sera . Level of cytokines (IL-17) and complement (C3, C4), in addition to studying the complete blood picture including Hemoglobine , monocytes ,Red blood cells, White blood cells, Platelets and Lymphocytes as potential factors that interfere with the disease.

Material and Method:

1.Study topic:

Our study was conducted on (110) patients with SLE (4 males and 106 females). Age range was (13- > 45) years old, in Al-Hussain Teaching Hospital in Al-Nasiriyah City during the period from April 2019 to November 2019. The inclusion criteria described by the American College of Rheumatology (ACR) for the diagnosis of SLE was used. Then immunological parameter is measured such as cytokine ,autoantibody, complement and complete blood picture in both patient and control groups. Seemingly healthy subjects (70) were chosen to participate as a normal comparison group, with the

age and gender grouping of the patients. The data for the following variables are recorded in arthritis,skin rash, butterfly-shaped rash, kidney sympathetic involvement, renal failure, and joint swelling. Diagnosis is based on these clinical exams under the supervision of doctors. All participants were asked for age, gender, living, socioeconomic status, duration of illness, symptoms of family history, history of medications or treatment with any type of antibiotic, clinical manifestations associated with SLE and severity of the disease.The severity of SLE was assessed as follows: patients were considered a "mild disease" if they presented mucous dermatitis(muco-cutaneous serositis) , and / or arthritis, and " mild severe " disease if the patients had hematological, renal and neurological manifestations. Hematological abnormalities associated with SLE included hemolysis or anemia with retinal leukemia or leukopenia (<4000 / mm3 on> two occasions) or lymphocytosis (<1500 mm3 on> two occasions) or thrombocytopenia (<100000 / Mm 3 in the absence of medication). The presence of seizures or psychosis without the use of medications or metabolic dysfunction was considered neurological symptoms associated with SLE.

2. Methods:-

ANA Screen is an ELISA(Enzyme Immunometric assay) -based test system f0r the qualitative measurement 0f IgG class autoantibodies against SS- A 60, SS-A 52, SS-B, RNP-70, Sm, RNP/Sm, Scl-70, centromere B, J0-1 in human serum or plasma samples. Used Demeditec (Germany) Kits. The dsDNA Screen is an ELISA test system for the quantitative measurement 0f IgG, IgM and IgA class autoantibodies against double-stranded DNA in human serum or plasma.. Used Human ds-DNA Screen ELISA Kit from Demeditec (Germany). Phospholipid Screen IgG/IgM is an ELISA test system to screen for the presence of IgG and IgM class autoantibodies against cardiolipin, phosphatidyl serine, ph0sphatidyl inositol, phosphatidic acid and beta-2-glycoprotein I in human serum or plasma.

Estimation 0f the Serum Level of Complement C3 andC4 in vitro quantitative determination of complementsC3 and C4 content in human serum . used C0mplement C3 and C4 Detection Kit (Nephelometry). Reverence range for C3 and C4=0.9-8 g/L and 0.1-0.4g/L respectively.

Statistical analysis

Statistical analysis was performed using SPSS version 23.0. The data is presented as mean standard error (SE). The patient-control comparison was analyzed using the T test. Pearson correlation coefficients were used.

Results

Distribution of SLE disease according to Gender and Age:

This study included (110) patients with SLE and (70) controls. In the patient group (110), 106 (96.36%) were female and 4 (3.64%) were male. 31.61 ± 8.60 years

mean age of patients. Our study showed that most of the infections were in the village of 71.82% compared to the city of 28.18, and the patients who had no historical disease 57.27% while the patients of history 42.73%.

Characteristics and clinical manifestations:

(Table 1) Clinical manifestation and ACR classification criteria

ACR Classification	SLE	
	No.	%
Malar rash	71	64.54
Discoid	8	7.27
Arthritis	96	87.2
Serositis	34	30 .90
Haematological abnormalities	72	65.45
Immunological abnormalities	81	73.63
ANA	89	80.91
Photosensitivity	78	70.91

ACR: American College of Rheumatology. Tan, *et al*.(17) .

Psychological condition in patients with SLE disease:

patients with Psychological condition were 74.64% while patients without Psychological condition were 26.36,and patients with Depression were 34.57 while patients with Anxiety were 65.43.(Table 2).

Table (2) Distribution of SLE disease according to Psychological condition for patients:

According to Psychological condition		SLE Patients	
		No.	%
Without Psychological condition		29	26.36 %
With Psychological condition	Total	81	73.64 %
	Depression	28	34.57 %
	Anxiety	53	65.43 %
Total		110	100%

Autoantibody Detection:

1. Antinuclear antibody level serum of patients with SLE:

Results of current study have illustrated a significant difference in the serum level of antinuclear Antibody (ANA) in patients with SLE disease and healthy group. It is clear that anti ANA Ab has been raised in the serum of SLE patients (3.14± 0.08pg/ml) as in the control group (0.50±0.01pg/ml) $P \leq 0.05$. Table(3)

Table(3) The concentration of serum ANA in patient group in comparison with control group.

Groups	ANA Mean ±SE
Patient	3.14±0.08
Controls	0.50±0.01
P value	The value of p is < .00001. The result is significant at p < .05

2. Anti dsDNA Antibody Level in Serum of Patients with SLE :

The results of current study have illustrated a significant difference in the serum level of anti dsDNA Ab in patients with SLE disease and control group. It was clear that dsDNA Ab was raised in serum of SLE patients (44.89 ± 0.32pg / ml) compared with control (7.48 ± 0.22pg / ml) P ≤ 0.05. (Table4)

Table (4) Anti dsDNA Antibody Level in Serum of SLE Patients and control Groups.

Groups	ds DNA (Mean ±SE)
Patient	44.89±0.32
Controls	7.48±0.22
P value	The value of p is < .0001. The result is significant at p < .05

3. Antiphospholipid antibodies Class IgG and IgM Level in Serum of Patients with SLE

The current study showed that APA IgG / IgM was elevated in serum of SLE patients (9.98 ± 0.09, 10.58 ± 0.19 IU / ml), respectively, as in comparison with controls (1.41 ± 0.02, 1.44 ± 0.02 IU / ml), respectively at ≤ 0.05. (Table 5).

Table (5) Anti Phospholipid Antibodies class IgG and IgM Level in Serum of patients with SLE:

Groups	Patient	Control	P value
ACP IgG Mean ±SE	9.98±0.09	1.41±0.02	The value of p is < .0001. The result is significant at p < .05
ACP IgM Mean ±SE	10.85±0.19	1.44±0.02	The value of p is < .0001. The result is significant at p < .05

Concentration of Interlukin-17 in Serum of Patients with SLE :

Results of the current study showed a significant difference P≤ 0.05 in serum IL-17 level for SLE patients and controls. It was clear that IL-17 was increased in serum for SLE patients (469.96 ± 2.25 pg / ml) compared to the control group (107.39 ± 0.41 pg / ml).(Figure 1).

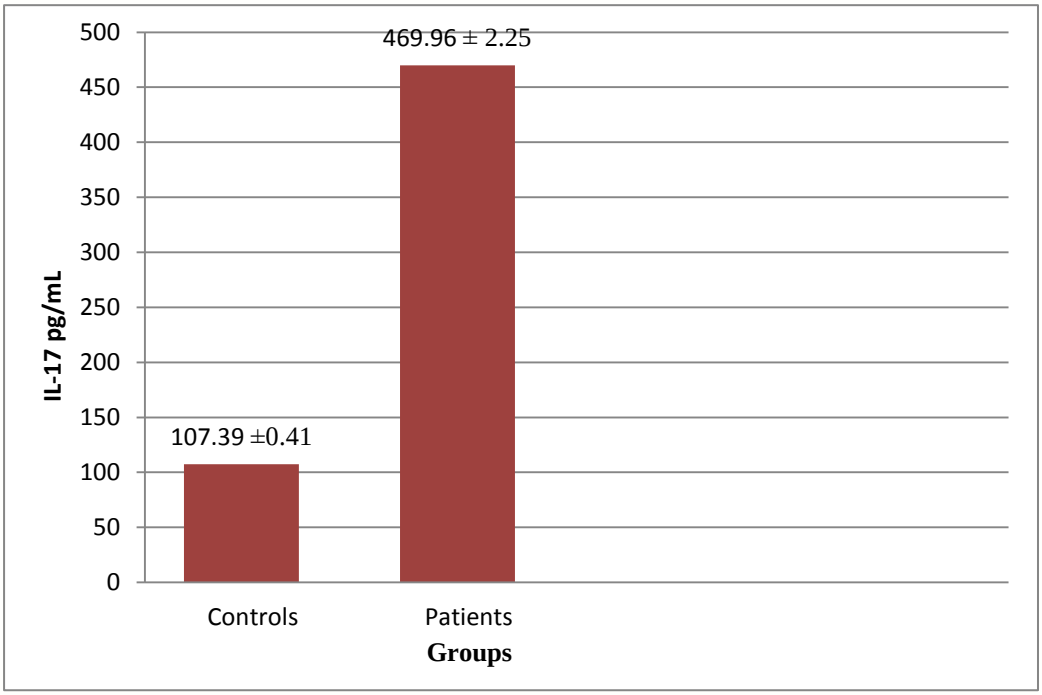


Figure (1) Mean Serum concentration of IL-17 in patients with SLE and control group.

Concentration of Complement C3 and C4 in Serum of patients with SLE:

Our analysis has confirmed that C3 and C4has been decreased in the serum of SLE patients (0.62 ± 0.01,0.13 ± 0.01g/l) respectively as in comparison with control group (1.47 ± 0.01,0.28 ± 0.01g/l) respectively P ≤ 0.05.(Table 6).

Table (6): Concentration complement C3 and C4 in serum SLE patients.

Groups g/l	Patient	Controls	P value
C3 Mean ±SE	0.62±0.01	1.74±0.01	The value of *p* is 0.005. The result is significant at $p < .05$
C4 Mean ±SE	0.13±0.01	0.28±0.01	The value of *p* is < .00001. The result is significant at $p < .05$

Complete Blood Picture for SLE patients:

Our study data also shows low level significantly in hemoglobin, red blood cell monocytes, white blood cells, platelets and the number of lymphocytes among SLE patients. Table (7).

Table(7) Concentration and Numbers of some hematological parameters in SLE patients:

Groups	Patients	Controls	P value
RBC Mean ±SE	3.05±0.01	4.92±0.2	The value of *p* is <.00001. The result is significant at *p* <.05
WBC Mean ±SE	3.89±0.02	6.86±0.49	The value of *p* is 0.02. The result is significant at *p* <.05
Lymphocyte Mean ±SE	0.87±0.01	2.25±0.04	The value of *p* is <.001. The result is significant at p <.05
Monocytes Mean ±SE	0.55±0.01	0.21±0.01	The value of *p* is < .001. The result is significant at *p* < .05
Haemoglobin Mean ±SE	7.97±0.03	12.81±0.03	The value of *p* is 0.01. The result is significant at *p* < .05
Platelet Mean ±SE	152.49±0.57	211.30±1.28	The value of *p* is 0.02. The result is significant at *p* < .05

Correlation between the Immunological Parameters:

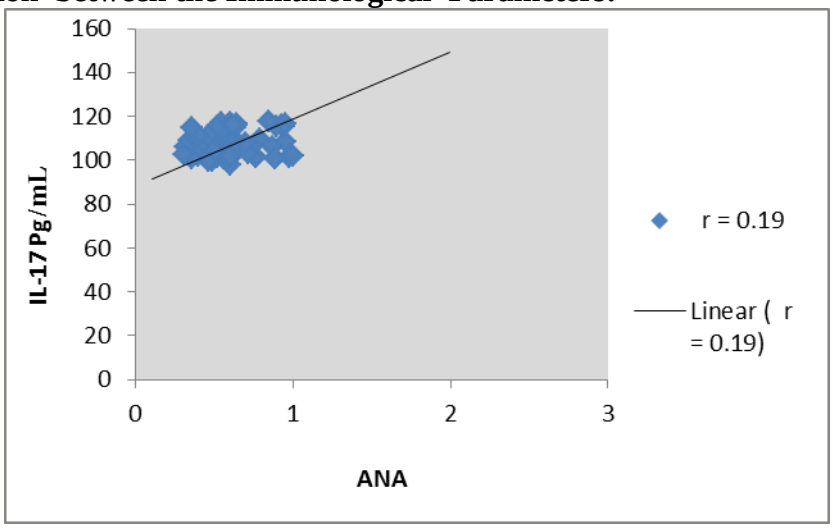


Figure (2) Correlation between anti ANA Ab and IL-17 in patients with SLE:

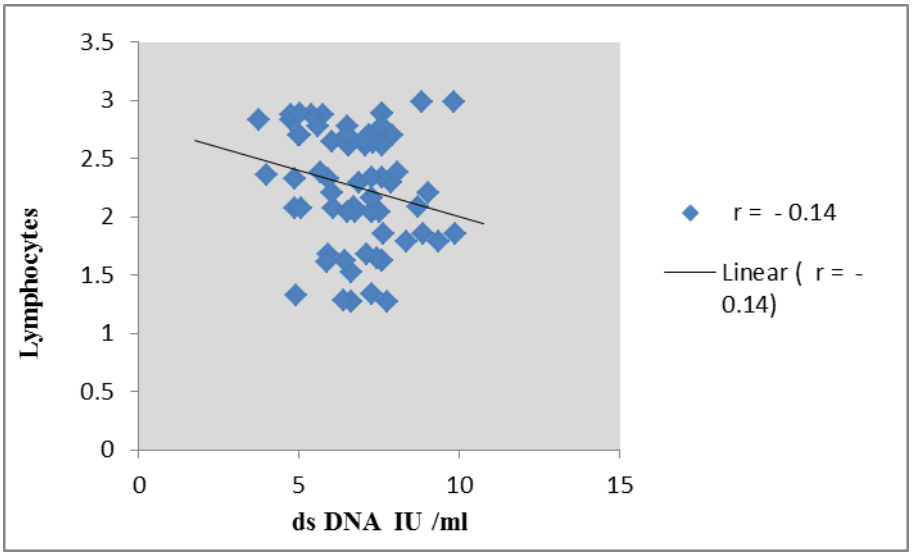
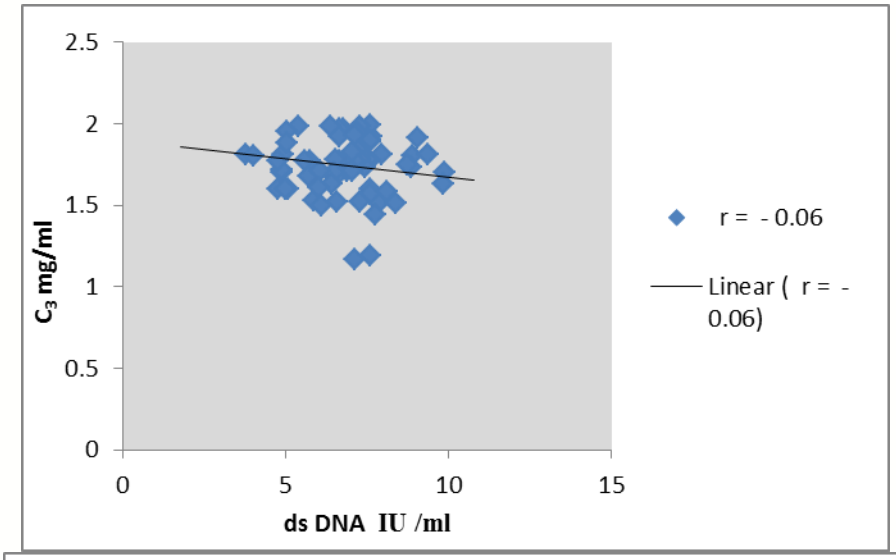


Figure (3) Correlation between anti dsDNA Ab and lymphocytes in patients with SLE:



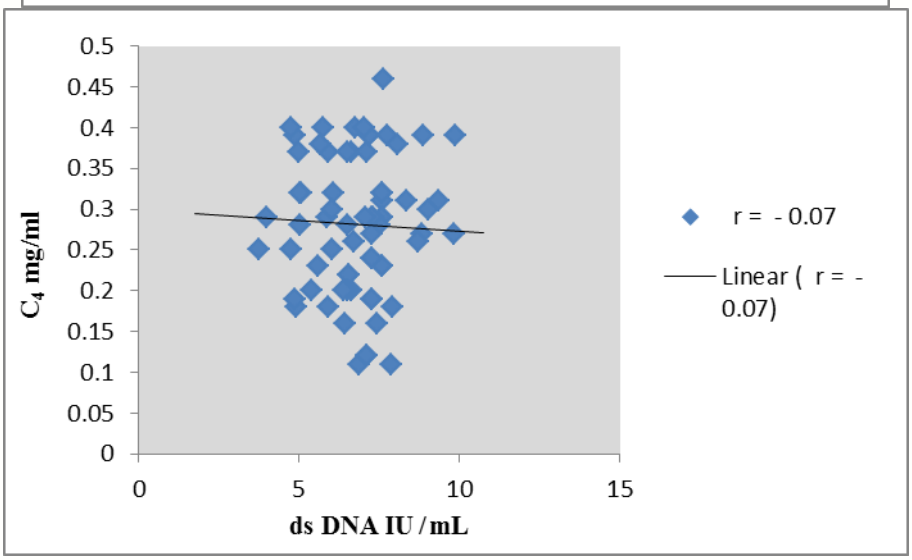


Figure (4) Correlation between anti dsDNA Ab and C3 and C4 in patients with SLE:

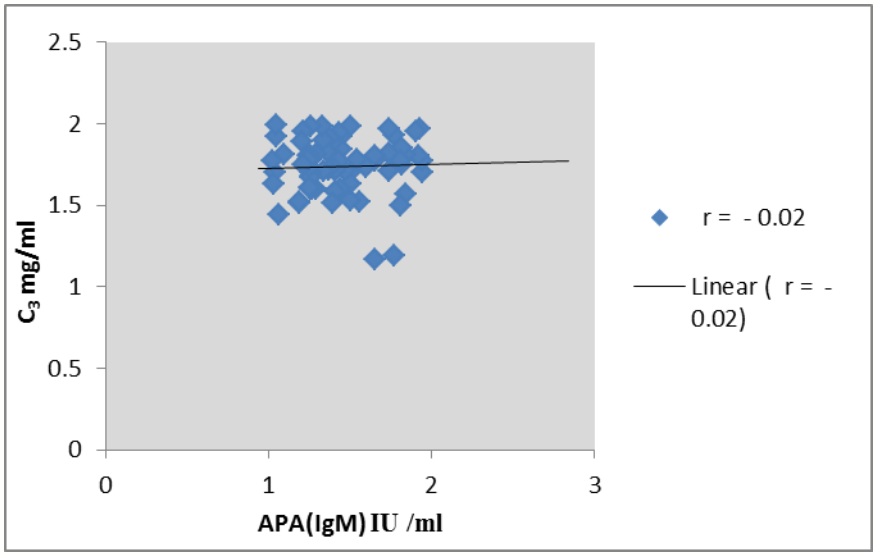


Figure (5) Correlation between anti APA and C3 and C4 in patients with SLE:

Discussion:

Psychological condition in patients with SLE disease: These results are in agreement with a study of Postal, *et al*.(18), who found Depression symptoms were classified in 70 patients withSLE and 10 in healthy people. symptoms of Anxiety 0bserved in 93 in SLE patients and 16 in controls. There is important evidence, inflammation as an important mediator, is involved in the pathophysiology of mood and anxiety disorders. Peripheral cytokines, which are produced during the inflammatory response, may be useful biomarkers when investigating a possible relationship between inflammation, mood, and anxiety disorders (19).

Antinuclear antibodies (ANA) in this study were positive with percentage of (80.91%).while (19.09 %) were negative. This was in agreement with Choi, *et al*.(20),who have found from 1137 patients,1049(92.3%) were p0sitive to ANA ,71(6.2%) were negative. A total of 1,137 patients were included; 1,049 (92.3%) were ANA positive, 71 (6.2%) were antinuclear antibody negative.ANA is SLE's immunomodulatory trait and its widely use for its detection based on its high sensitivity, despite its low specificity.Joseph et al,(21) found ANA p0sitivity was seen in 97.9% of SLE patient`s at 1:100 dilution. and 97.8% at 1:80 dilution noted by systematic review by Leuchten *e*t al.(22). Tarazi,*et al*.(23) Found 301 patients were with biopsy, and they had CLE (cutaneous Lupus erythematosus) and ANA were known, 111 had negative ANA (36.9%), 190 had positive results, their results showed that ANA is not always present in patients with Systemic disease, This should be borne in mind when determining the SLE rating, the standard that should be used for clinical trials, however, Passive ANA testing in the context of high clinical suspicion, for SLE is the clinical challenge. Since the creation of HEp-2 cells as the mainstay of the ANA test worldwide, The negative prevalence of ANA decreased significantly in SLE (24). However, a small subset of Lupus patients already negative in ANA may present difficulties;it may also be due a false-negative ANA test due to laboratory technical error and interobserver variability. (25).ANA positivity in healthy controls may probably be due to difference in ethnicity, environmental factors, and infection load.(26). In their systematic literature review of` ANA in SLE of 25 years conclude that in healthy individuals, ANA positivity increases with age to 23%;thus, proving that Positive ANA will result in significantly higher specificity of SLE in the number of healthy y0ung adults than typical sick population in the Rheumatology

Departments.The nuclear dense fine-speckled ANA pattern noted exclusively in healthy individuals was also noted by Au,(27).

Systemic lupus erythematosus disease is characterized by a huge immune system deviations that include T cells, B cells, and monocytic lineage cells, resulting in autoantibody production, polyclonal B cell activation and increased numbers of antibody producing cells(28). Our study illustrated that positive anti dsDNA antibody were (78.18 %) while (21.82%) were negative. Antibodies to dsDNA in serum of patients are elevated compared with the control. The results of this study are compatible to results that obtained by Javier et al.,(29) who have found 72% of patients tested positive for anti-double stranded DNA antibodies and 76.5% had hypocomplementemia at some time during the course of the disease. Barnado, *et al*. (30) have found Race/ethnicity (%)Caucasian 45 +,55 -. African American 61+,39-. Hispanic 76+,25-. Asian 88+,12- for ds DNA. They found dsDNA was the the most strongly associated with nephritis, chronic kidney disease and multiple ACR SLE criteria suggested that dsDNA, compared to other autoantibodies, may be the most relevant in assessing a patient's prognosis for major SLE manifestations. Mosca, *et al*. (31) found Data were collected on 389 with early SLE Compared to patients with mimicking conditions, patients with early SLE were much more likely to have antibodies to dsDNA (71.7% vs. 6.9% of non-SLE).Compared withto 227 patients who were referred to the risk of SLE who were eventually diagnosed after clinical and serological examinations were performed in the same centers. They identified parameters that could assist in identifying early SLE and patients, and could direct the doctor in a differential diagnosis with simulated conditions. In addition, they identified items relevant to the development of a new classification criteria for SLE, with particular attention to improving sensitivity and specificity of the early disease classification.Many studies such as: Ciccacci *etal*., (32)It indicates that Antibodies to phospholipid (APA) are autoimmune, a condition of hypercoagulable, caused by antibodies to phospholipids in cellular membranes, which raise clots in blood (`thrombosis) in vens and arteries , as well as pregnancy-related complications such as miscarriage , stillbirth or premature birth or severe preeclampsia (33). These antibodies are present in a small percentage of the population but occur more commonly in patients with connective tissue disorders such as systemic lupus erythematosus (34). This observation is compatible with that of Chighizola, and Meroni,. (35) who have found that According to 2015, a systematic review of APL analysis in patients below, 50 years, a positive APL test can be found in 17.4% of people with any cardiovascular event, 17.2% of stroke patients, and 11.7% of, individuals with From transient ischemia, attack.Also, it is in line with Pons-Estel, *et al*. (36) Who found SLE patient`s (40%) have antibodies to phospholipids, while 40% of them will eventually develop thrombosis state. The APL can stimulate the pro-thrombotic state, and reduce the threshold thrombosis, by inducing the phenotype of protein and inflammatory cells in the endothelial cells. Urowitz *et al* (37) determined that SLE can be considered a risk factor for thrombosis. Indeed, the classic risk factor for Framingham reduces CV and risk for SLE patients, in particular, for coronary artery disease. Thus, presence of simultaneous SLE and APL, increases risk of thrombosis, as well as organ damage, and reduces survival.Takeno *et al*.,(38) have mentioned that self-reactive T cell cloning from SLE patient`s also produce a large amounts of IL-6, thus enhancing B- cell activation and production autoantibodies.Mok *et al*.,(39) showed the presence of APA a leading indicator of fetal death in SLE.In studies of Pierangeli and Harris.,(40) There is an association

between antibody to APL and fetal loss in detected SLE patients, APA found in 35% -40% of pregnant with SLE , and more diffuse in recurrent miscarriage patients.
The results of this study are consistent with the results obtained by Elewa et al., (41), and have documented, that the IL-17 serum level has been shown to be significantly higher in SLE, and can be used as biomarkers, renal activity. Yamei Tang,*et al*.,(42) have determined the IL-17 serum was higher significantly than those in the HCgroup. They found serum IL-6, serum IL-17, and high sensitivity C-reactive proteins (h s CRP) levels were correlated with the disease activity. Tanaka,*et al*.,(43) have determined IL-17 were significantly higher in patients with active SLE. compared with healthy donors or patients with inactive SLE.Pan, et al. (2) Found interleukin 17 is the major interleukine which encourages Th 17 to participate in SLE. It has been confirmed that the level of Interleukin 17 in the kidney, in patients with lupus nephritis increases, as genetic expression of IL-17 in urinary sediments increases.
Moreover, the Th17 expression is also found in skin, lung and tissues of Kidney in SLE patients. Further,increased Th17 was associated with disease activity in SLE. IL-17 and B-cell stimulation factor (BLS) together, regulate B cells, their differentiation and their survival, thereby increasing humeral immunity to produce autoantibodies. Increased serum IL-17 levels in SLE patients have been reported in other studies (44,45, 46 and 47), but the exact role for these cytokines in causing lupus has not yet been determined. In addition, Abdel Galil's research reported that receiver operating characteristic (ROC) curve analysis of IL-6 and IL-17 proved that both these 2 cytokines can act as sensitive biomarkers of disease activity and could be used for early detection of disease exacerbation (48).Our results are consistent with the results of Tang, *et al*., (49) who found that C3 and C4 level`s in patients with SLE were (0.80-0.28,0.21-0.08 g/L)significantly lower than healthy group(1.49-0.08, 0.36-0.02 g/L), and compatibal with Idborg, *et al*,.(50) who have Found C3 and C4 was (0.88 , 0.15) in patients compared to control C3 1.04 , C4 0.21 . Aysha, *et al*,.(51)have found C3 and C4 decreas in patients compared to control.Our study is consistent with the results of Li *et al*. , (52) who found low levels 0f C3 and C4 in Chinese SLE patients. These results also indicate hypocomplementemia is of diagnostic and valuable significance for SLE by improving the sensitivity of the diagnosing SLE.Consumption of complement factors induced by complement activation it may lead to low levels of these factors. Although serum C3 and C4 levels were not different among the 3 subgroups, hypocomplementemia has shown important diagnostic value for SLE by improving the sensitivity of the diagnosis of SLE, so these indicators could be the trait marker, not the state marker, of SLE. Complement testing levels were a standard, and a laboratory evaluation component to help evaluate activity of disease through observing SLE patient`s. Low and often integrated level s indicate active lupus, especially lupus nephritis. However, It is difficult to ascertain if complement levels are low, due to consumption during inflammation, or because of an inherent deficiency of one or more alleles, more disturbingly, two scenarios can exist in one individual (53). The complement system is a major influence mechanism, the innate immune system and plays an important role in immune defense, and the biological functions of supplement, are opsonization and phagocytosis, stimulating, and inflammatory reactions, byanaphylatoxins and complement mediated cytolysis of microbes, especially encapsulated bacteria (54).

Our study data also showed a significant depletion in level of hemoglobin, red blood cell monocytes, white blood cells, platelets and the number of lymphocytes

among SLE patient s (Table 7). The present study is consistent with Sabry *et al*. (55) report, who found that the level of platelets and hemoglobin significant depletion in active SLE patients compared to inactive patients.

Hemoglobin deficiency can be occurred due to autoantibodies to RBCs as a part of autoimmunity, or it may occur as a result of erythropoietin production impairment that involved by kidney in SLE ,GI blooding due to Anti- inflammatory treatment, high destruction of red blood cells from hyper splenism or drug-induced immune phenomena (56).Patients with autoimmune hemolytic anemia are associated with renal disease, thrombocytopenia and thrombosis, often in the context of secondary phospholipid syndrome, as well as other mechanisms, that can lead to anemia such as autoantibodies, abnormalities, the cytokine network that affects the bone marrow, erythrocytes Activity of T-lymphocytes (57) Another study conducted by AL-Alfy *et al*.,(58) has been approached with the current study. Their study has confirmed that there is a statistical different in the mean level of white blood cell , hemoglobin and platelets, among SLE without nephritis and with nephritis. Current results have also approached Santos, *et al*,. (59) who found that according to the ACR classification, Characteristics of the patients included in the study 19.1% had mucocutaneous manifestations, 35.3% had hematological disorders (15.4% had leukopenia ,32.4% had lymphopenia, 2.9% had Thrombocytopenia), 6.6% had arthritis and 0.7% had serositis (pleurisy) . Three mechanisms involved in thrombocytopenia occurrence were : impaired production of platelets in the bone marrow, sequestration of platelets in the spleen or accelerated destruction of platelets in the peripheral circulation, the major cause of thrombocytopenia in SLE patients are increased peripheral destruction that is mediated by antiplatelet antibodies(60). Chang-Hee Suh,*et al*.,(61) have found Serositis, hematologic involvement, and use of higher than the low dose of GCs are risk factors for serious infections in patients with SLE.

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by autoantibodies production and immune complex deposition with systemic clinical manifestations. Interleukin (IL)-17-producing cells play a crucial role in disease pathogenesis and represent an attractive therapeutic target (62). In this study Figure (2) showed a correlation between anti ANA Ab and IL-17 in patients with SLE . Regarding the interaction between autoantibodies and cytokines, the absence of autoantibodies was associated with a low frequency of cytokines (63). The central pivot role of Th17cells as primary driver autoimmune responses to SLE by secreting proinflammatory cytokines including IL-17, IL-22 and IL-23 causing local inflammation and tissue destruction was supported by some studies. Accordingly, recently reported elevated levels of IL-17 and IL-17 produced T cells circulating in SLE. Furthermore, T-cells produced with IL-17 have also been shown to infiltrate the lungs, skin and kidneys in lupus patients contributing to organ damage (64).

This study results Figure (3), correlation between anti dsDNA Ab and lymphocytes in patients with SLE . Show Vila *et al*. (65) that lymphocytopenia is associated with antibody to DNA not with Antinuclear antibody, Anticardiolipin IgG or Anticardiolipin IgM. Association of abnormal deficiency in blood lymphocytes and dsDNA antibodies is particularly noticeable due to lymphoid toxicity, and the activity of endogenous antibodies due to a possible mutual interaction between nuclear material and lymphatic membrane (66).

Nakabayashi *et al*.,(67) have reported that SLE patients with active nephritis have high concentration of anti-T-cell antibodies, especially in proliferative

glomerulonephritis.In compatibility with these findings, our results figure (4) correlation between anti-ds DNA Ab and C3 and C4 in patients with SLE . Bernstein *et al*. (68) found significantly increased serum dsDNA Ab levels in serum patients with active SLE from patients with inactive SLE.The increase in dsDNA antibody levels is associated with disease, and flares are usually combined, with reduced levels of complement and C3 and C4 proteins (69,70).

Patients with a positive anti-ds DNA had statistically depletion level for C3 and C4 in comparison to passive anti-ds DNA (71). Fabrizio, *et al*. (72) found correlation among persistence or previous positive for anti- ds DNA and immunomodulatory traits (reduced serum levels of C4). Recent evidence indicates the effect of complement receptors on the development of anti-dsDNA, by participating in the removal of immune complexes and / or modification of B cell activation in response to the antigen. Both antibody for dsDNA and lowcomplement are related with SLE activity they had been suggested that receptors of complement may regulate antibody production for ds DNA (73).

This study results Figure (5) expressed a Correlation between anti APA and C3 and C4 in patients with SLE. Garabet, *et al*. (71) have determined statistical correlation among APL presence in SLE patient`s and lowcomplement C3 and C4 levels indicating an increase activation of complements, possibly caused by acivation of APL, another possible explanation is that APL pathogen leads to activation of complement ,and hence consumption of complement . Pierangeli, *et al*., (74) suggested that in an vivo murine model were thrombosis of microcirculation where APL injection was used. Complement consumption may therefore not be specific for APL antibodies. The relative importance, of different autoantibodies, in complement, consumption, should be investigated in future studies.

Conclusions

Based on this study, the following conclusions can be reached: Individuals with SLE have a positive ANA, increased for anti-ds DNA antibody and antiphospholipids concentration which play an important role in pathogenesis of disease and serum complement C3 and C4. It decreases in lupus patients.The high concentration of inflammatory cytokines (IL.17) can play crucial role for causing SLE that leads to the development of the disease.

References:

1- Stojan G.,Petri M. Epidemiology of systemic lupus erythematosus: An update. Curr Opin Rheumatol. 2018;30(2):144-50.

2- Pan, L., Lu, M.-P., Wang, J.-H., Xu, M., & Yang, S.-R.. Immunological pathogenesis and treatment of systemic lupus erythematosus. World Journal of Pediatrics. 2019; doi:10.1007/s12519-019-00229-3

3- Manuel Rojas, Yhojan Rodríguez, Kelly Joan Leon, Yovana Pacheco, Yeny Acosta- Ampudia, Diana M. Monsalve, Carolina Ramírez-Santana, Juan-Manuel Anaya. Cytokines and Inflammatory Mediators in Systemic Lupus Erythematosus . EMJ Rheumatol. 2018 ;5[1]:83-92.

4- Lande, R., Ganguly, D., Facchinetti, V., Frasca, L., Conrad, C., Gregorio, J., … Gilliet, M. Neutrophils Activate Plasmacytoid Dendritic Cells by Releasing Self-DNA-Peptide Complexes in Systemic Lupus Erythematosus. Science Translational Medicine, 2011; 3(73), 73ra19. doi:10.1126/scitranslmed.3001180

5- Tipton CM, Fucile CF, Darce J, et al. Diversity, cellular origin and autoreactivity of antibody-secreting cell population expansions in acute systemic lupus erythematosus. Nat Immunol.2015;16:755-765.

6- Halliley JL, Tipton CM, Liesveld J, et al. Long-lived plasma cells are contained within the CD19(-) CD38(hi)CD138(+) subset in human bone marrow. Immunity. 2015;43:132-145.

7- Crispin JC, Liossis SN, Kis-Toth K, Lieberman LA, Kyttaris VC, Juang YT, et al. Pathogenesis of human systemic lupus erythematosus: recent advances. Trends Mol Med. 2010;16(2):47-57. doi: 10.1016/j.molmed.2009.12.005.

8- Ohl, K., & Tenbrock, K. Inflammatory Cytokines in Systemic Lupus Erythematosus. Journal of Biomedicine and Biotechnology, 2011; 1–14. doi:10.1155/2011/432595.

9- Santos, F. M. M., Telles, R. W., Lanna, C. C. D., Teixeira, A. L., Miranda, A. S., Rocha, N. P., & Ribeiro, A. L.. Adipokines, tumor necrosis factor and its receptors in female patients with systemic lupus erythematosus. Lupus. 2016 ; 26(1), 16. doi:10.1177/0961203316646463.

10- Ricklin D, Hajishengallis G, Yang K, et al. Complement: a key system for immune surveillance and homeostasis. Nat Immunol 2010;11:785–97.

11- Leffler, J., Bengtsson, A. A., & Blom, A. M. The complement system in systemic lupus erythematosus: an update. Annals of the Rheumatic Diseases, 2014; 73(9), 1601– 1606. doi:10.1136/annrheumdis-2014-205287.

12- Jordan N. Management of pregnancy in systemic lupus erythematosus: What a GP should know. Lupus. 2018;27:40-43.

13- Rees F, Doherty M, Grainge MJ, et al. The worldwide incidence and prevalence of systemic lupus erythematosus: a systematic review of epidemiological studies. Rheumatology; 2017; 56: 1945-1961.

14- Chen Z, Zhang H, Xia B, Wang P, Jiang T, Song M, et al. Association of PTPN22 gene (rs2488457) polymorphism with ulcerative colitis and high levels of PTPN22 mRNA in ulcerative colitis. Int J Colorectal Dis. 2013;28(10):1351-8. doi: 10.1007/s00384-013- 1671-3.

15- Gregersen PK, Olsson LM. Recent advances in the genetics of autoimmune disease. Annu Rev Immunol. 2009;27:363-91.

16- Zhu H, Luo H, Yan M, Zuo X, Li QZ. Autoantigen Microarray for High-throughput Autoantibody Profiling in Systemic Lupus Erythematosus. Genomics Proteomics Bioinformatics. 2015;13(4):210-8. doi: 10.1016/j.gpb.2015.09.001.

17- Tan, E. M., Cohen, A. S., Fries, J. F., Masi, A. T., Mcshane, D. J., Rothfield, N. F., Winchester, R. J. The revised criteria for the classification of systemic lupus erythematosus. Arthritis & Rheumatism, 1982;25(11), 1271–1277. doi:10.1002/art.1780251101

18- 18-Postal, M., Lapa, A. T., Sinicato, N. A., de Oliveira Peliçari, K., Peres, F. A., Costallat, L. T. L., Appenzeller, S.. Depressive symptoms are associated with tumor necrosis factor alpha in systemic lupus erythematosus. Journal of Neuroinflammation, 2016; 13(1). doi:10.1186/s12974-015-0471-9

19- Rosenblat JD, Cha DS, Mansur RB, McIntyre RS. Inflamed moods: a review of the interactions between inflammation and mood disorders. Prog Neuropsychopharmacol Biol Psychiatry. 2014;53:23–34.

20- Choi, M. Y., Clarke, A. E., St.Pierre, Y., Hanly, J. G., Urowitz, M. B., Romero-Diaz, J., Fritzler, M. J. Antinuclear Antibody-Negative Systemic Lupus Erythematosus in an International Inception Cohort. Arthritis Care & Research. 2018; doi:10.1002/acr.23712

21- Joseph Sushil Rao1, Vineeta Shobha2, Tinku Thomas3, Usha Kini. Standardizing initial dilution titers of antinuclear antibodies for the screening of systemic lupus erythematosus Phys.Rev. 2019; 47,777-780.

22- Leuchten, N., Hoyer, A., Brinks, R., Schoels, M., Schneider, M., Smolen, J. Performance of Antinuclear Antibodies for Classifying Systemic Lupus Erythematosus: A Systematic Literature Review and Meta-Regression of Diagnostic Data. Arthritis Care & Research, 2018; 70(3), 428–438. doi:10.1002/acr.23292.

23- Tarazi, M., Gaffney, R. G., Kushner, C. J., Chakka, S., & Werth, V. P. Cutaneous lupus erythematosus patients with a negative antinuclear antibody meeting the American College of Rheumatology and/or Systemic Lupus International Collaborating Clinics criteria for systemic lupus erythematosus. Arthritis Care & Research. 2019; doi:10.1002/acr.23916

24- Cross LS, Aslam A, Misbah SA. Antinuclear antibody-negative lupus as a distinct diagnostic entity – Does it no longer exist? QJM. 2004;97:303-8.

25- Schmajuk G, Hoyer BF, Aringer M, Johnson SR, Daikh DI, Dörner T. Multi-center Delphi Exercise Reveals Important Key Items for Classifying Systemic Lupus Erythematosus. Arthritis Care & Research .2017; doi:10.1002/acr.23503.

26- Ghosh P, Dwivedi S, Naik S, Agarwal V, Verma A, Aggarwal A, et al. Antinuclear antibodies by indirect immunofluorescence: Optimum screening dilution for diagnosis of systemic lupus erythematosus. Indian J Med Res. 2007;126:34-8.

27- Au EY. ANA testing: What should we know about the methods, indication and interpretation? Hong Kong Bull Rheumatic Dis. 2017;2:53-7.

28- Hahn, B. H.Antibodies to DNA.‖ The New England Journal of Medicine., 1998; 338(19):1359–1368.

29- Javier Narváez,Helena Borrell,Fernando Sánchez-Alonso,Iñigo Rúa-gueroa,Francisco Javier López-Longo,María Galindo-Izquierdo,Jaime Calvo Alén,Antonio FernándeznNebro,Alejandro Olivé, José Luis Andreu,Víctor Martínez- Taboada,Joan Miquel Nolla, José María Pego-Reigosa . Primary respiratory disease in patients with systemic lupus erythematosus: data from the Spanish rheumatology society lupus registry . Arthritis Research & Therapy.2018;20:280.

30- Barnado, A., Carroll, R. J., Casey, C., Wheless, L., Denny, J. C., & Crofford, L. J. Phenome-wide association study identifies dsDNA as a driver of major organ involvement in systemic lupus erythematosus. Lupus, 2018; 096120331881557. doi:10.1177/0961203318815577

31- Mosca, M., Costenbader, K. H., Johnson, S. R., Lorenzoni, V., Sebastiani, G. D., Hoyer, B. F. How Do Patients with Newly Diagnosed Systemic Lupus Erythematosus Present? A Multicenter Cohort of Early Systemic Lupus Erythematosus to Inform the Development of New Classification Criteria. Arthritis & Rheumatology. 2018; doi:10.1002/art.40674

32- Ciccacci, C.; Perricone, C.and Ceccarelli, F. A multilocus genetic study in a cohort of Italian SLE patients confirms the association with STAT4 gene and describes a new association with HCP5 gene. PLoS ONE. 2014;9(11): Article ID e111991.

33- Asherson ,R.A. and Cervera, R.Antiphospholipid syndrome. J Invest Dermatol., 1993; 100: 21-27.

34- Petri, M. Epidemiology of the antiphospholipid antibody syndrome. J Autoimmun ., 2000;15:145–51.

35- Chighizola, C. B., & Meroni, P. L. Thrombosis and Anti-phospholipid Syndrome: a 5-Year Update on Treatment. Current Rheumatology Reports, 2018; 20(7). doi:10.1007/s11926- 018-0741-5

36- Pons-Estel, G. J., Andreoli, L., Scanzi, F., Cervera, R., & Tincani, A. The antiphospholipid syndrome in patients with systemic lupus erythematosus. Journal of Autoimmunity, 2017; 76, 10–20. doi:10.1016/j.jaut.2016.10.004

37- Urowitz M.B, D. Ibanez, J. Su, D.D. Gladman, Modified framingham risk factor score for systemic lupus erythematosus, J. Rheumatol. 2016; 43. 875e879.

38- Takeno, M.; Nagafuchi, H.; Kaneko, S.; Wakisaka, S.; Oneda, K.; Takeba, Y.; Yamashita, N.; Suzuki, N.; Kaneoka, H. and Sakane, T. Autoreactive T cell clones from patients with systemic lupus erythematosus support polyclonal autoantibody production. J Immunol ., 1997;158(7):3529-3538.

39- Mok, M.Y.; Leung, P.Y. and Lao, T.H. Clinical predictors of fetal and maternal outcome in Chinese patients with systemic lupus erythematosus. Ann Rheum Dis. 2004;63:1705–6.

40- Pierangeli, S.S. and Harris, E.N. A quarter of a century in anticardiolipin antibody testing and attempted standardization has led us to here, which is? Semin Thromb Hemost. 2008 ;34(4):313–28.

41- Elewa ,E. A. ;Zakaria, O.;Mohamed ,E.I. and Boghdadi .G. The role of interleukins 4, 17 and interferon gamma as biomarkers in patients with Systemic Lupus Erythematosus and their correlation with disease activity. The Egyptian Rheumatologist . 2014;36: 21–27.

42- Yamei Tang, Huai Tao, Yuji Gong, Fang Chen, Cunyan Li, and Xiudeng Yang. Changes of Serum IL-6, IL-17, and Complements in Systemic Lupus Erythematosus Patients Journal of Interferon & Cytokine Research. 2019; 10.1089. jir. 0169

43- Tanaka A , T Ito, K Kibata. Serum high-mobility group box 1 is correlated with interferon-α and may predict disease activity in patients with systemic lupus erythematosus. Sage Journals. 2019; ISSN: 0961-2033

44- Umare, V., Pradhan, V., Nadkar, M., Rajadhyaksha, A., Patwardhan, M., Ghosh, K. K., & Nadkarni, A. H. Effect of Proinflammatory Cytokines (IL-6, TNF-α, and IL-1β) on Clinical Manifestations in Indian SLE Patients. Mediators of Inflammation, 2014; 1–8. doi:10.1155/2014/385297

45- Hammad A, Osman E, Mosaad Y, Wahba M. Serum interleukin-17 in Egyptian children with systemic lupus erythematosus: is it related to pulmonary affection? Lupus.2017; 26(4):388–395.

46- Pan Q, Gong L, Xiao H, Feng Y, Li L, Deng Z, Ye L, Zheng J,Dickerson CA, Ye L, An N, Yang C, Liu HF. Basophil activation-dependent autoantibody and interleukin-17 production exacerbate systemic lupus erythematosus. Front Immunol2017; 8:348.

47- Wu Y, Cai B, Zhang J, Shen B, Huang Z, Tan C, Baan CC, Wang L. IL-1beta and IL-6 are highly expressed in RF+IgE+ systemic lupus erythematous subtype. J Immunol Res.2017;:5096741.

48- Abdel Galil SM, Ezzeldin N, El-Boshy ME. The role of serum IL-17 and IL-6 as biomarkers of disease activity and predictors of remission in patients with lupus nephritis. Cytokine. 2015; 76(2):280–287.

49- Tang, Y., Tao, H., Gong, Y., Chen, F., Li, C., & Yang, X. Changes of Serum IL-6, IL-17, and Complements in Systemic Lupus Erythematosus Patients. Journal of Interferon & Cytokine Research. 2019;doi:10.1089/jir.2018.0169

50- Idborg, H., Eketjäll, S., Pettersson, S., Gustafsson, J. T., Zickert, A., Kvarnström, M., Svenungsson, E. TNF-α and plasma albumin as biomarkers of disease activity in systemic lupus erythematosus. Lupus Science & Medicine, 2018;5(1), e000260. doi:10.1136

51- Aysha I.Z. Badawi a, Alaa M. Abd El-Hamid a, Noha Kh. Mohamed a, Enas M.M. Darwish a, Mona Wassef, Hala Elfirganica . Serum tumor necrosis factor (TNF)-

like weak inducer of apoptosis (TWEAK) and leptin as biomarkers of accelerated atherosclerosis in patients with systemic lupus erythematosus and antiphospholipid syndrome. The Egyptian Rheumatologist . 2017; 39 (2),P:75-81.

52- Li H, Lin S, Yang S, Chen L, Zheng X. Diagnostic value of serum complement C3 and C4 levels in Chinese patients with systemic lupus erythematosus. Clin Rheumatol. 2015; 34(3):471–477.

53- Vaneet Sandhu , Michele Quan, SLE and Serum Complement: Causative, Concomitant or Coincidental?, The Open Rheumatology Journal, 2017; DOI: 10.2174/1874312901711010113, 11, 113-122 .

54- Potlukova, E. and Kralikova, P. Complement component C1q and Anti-C1q Antibodies in Theory and in clinical P ractice. Scandinavian Journal of Immunology. 2008; 67(5): 423 – 430.

55- Sabry,A.; Kalil,M. ; El-Rahim,M.; El-Shahat, F.; Elbasyouni,R. Proinflammatory Cytokines (TNF alpha and IL-6) in Egyptian SLE patients with Lupus Nephritis is it correlated with disease activity.Eur J Gen Med., 2005; 2(4):153-158.

56- Lam, S.K and Quah, T.C. Anemia in systemic lupus erythematosus. J Singapore Paediatr Soc., 1990; 32(3-4):132-6.

57- Giannouli, S.; Voulgarelis, M.; Ziakas, P.D. and Tzioufas, A.G. Anaemia in systemic lupus erythematosus, from pathophysiology to clinical assessment. Ann Rheum Dis. 2006 ;65(2):144-8.

58- AL- Alfy,M .N.; AL- Hakim, M. S.; Abd Elmouttaleb, A .T.; Bayomy, E. M.; Abonar, A. A. and aAbood, M. A. Study of Interleukin-12 Cytokine and Anti-C1q Antibodies in Lupus Nephritis Patients. International Journal of Internal Medicine . 2014 ;3(1): 13-26.

59- Santos, F. M. M., Telles, R. W., Lanna, C. C. D., Teixeira, A. L., Miranda, A. S., Rocha, N. P., & Ribeiro, A. L. Adipokines, tumor necrosis factor and its receptors in female patients with system iclupus erythematosus. Lupus, 2016; 26(1), 10– 16. doi:10.1177/0961203316646463.

60- Kapouzas, G.A.Hematological and Lymphoid Abnomalities in SLE. In: Wallace, D.J and Hahn, B.H. DUBOIS' Lupus erythmatosus and Related Syndomes. 8th edn. Philadelphia: Elsevier., 2013; 426–37.

61- Chang-Hee Suh, Ju-Yang Jung,Dukyong Yoon, Hyoun-Ah Kim and Sang-Heon Lee . Serositis, hematologic involvement, and steroid dose are risk factors for serious infections in patients with systemic lupus erythematosus. 2019; 6 (1)

62- Koga, T., Ichinose, K., Kawakami, A., & Tsokos, G. C. The role of IL-17 in systemic lupus erythematosus and its potential as a therapeutic target. Expert Review of Clinical Immunology. 2019; doi:10.1080/1744666x.2019.1593141.

63- Pacheco, Y., Barahona-Correa, J., Monsalve, D. M., Acosta-Ampudia, Y., Rojas, M., Rodriguez, Y.,. Anaya, J-M. Cytokine and autoantibody clusters interaction in systemic lupus erythematosus. Journal of Translational Medicine, 2017; 15. https://doi.org/10.1186/s12967-017-1345-y

64- López, P., de Paz, B., Rodríguez-Carrio, J., Hevia, A., Sánchez, B., Margolles, A., & Suárez, A. Th17 responses and natural IgM antibodies are related to gut microbiota composition in systemic lupus erythematosus patients. Scientific Reports, 2016; 6(1). doi:10.1038/srep24072.

65- Vila, L.M.; Alarcon, G.S.; Mcgwin, G. J.; Bastian, H.M.; Fessier, B.J. and Reveille, J.D.Systemic lupus erythematosus in a multiethnic US cohort, XXXVII,association of lymphopenia with clinical manifestations, serologic abnormalities, disease activity, and damage accrual .Arthritis Rheum. 2006; 55:799–806.

66- Shoenfeld, Y.; Zamir, R. a`12nd Joshua, H. Human monoclonal anti-DNA antibodies react as lymphocytotoxic antibodies. Eur J Immunol ., 1985; 15:1024-8.

67- Nakabayashi, K.; Arimura, Y.; Yoshida, M. and Nagasawa, T. Anti-T cell antibodies in primary glomerulonephritis. Clin Nephrol., 1985;23: 74–80.

68- Bernstein, K.A.; Kahl, L.E.; Balow, J.E. and Lefkowith, J.B. Serologic markers of lupus nephritis in patients: use of a tissue based ELISA and evidence for immunopathogenetic heterogeneity. Clin Exp Immunol ., 1994;98:60–5.

Linnik, M.D.; Hu, J.Z.; Heilbrunn, K.R.; Strand ,V.; Hurley, F.L. and Joh, T. Relationship between anti double-stranded DNA antibodies and exacerbation of renal disease in patients with systemic lupus erythematosus. Arthr Rheum., 2005;52(4):1129–37.

69- Ng, K.P.; Manson, J.J.; Rahman, A. and Isenberg, D.A. Association of antinucleosome antibodies with disease flare in serologically active clinically quiescent patients with systemic lupus erythematosus. Arthr Care Res. 2006 ;55(6):900–4.

Garabet, L., Gilboe, I.-M., Mowinckel, M.-C., Jacobsen, A. F., Mollnes, T. E., Sandset, P. M., & Jacobsen, E.-M. Antiphospholipid Antibodies are Associated with Low Levels of Complement C3 and C4 in Patients with Systemic Lupus Erythematosus. Scandinavian Journal of Immunology, 2016; 84(2), 95–99. doi:10.1111/sji.12445.

70- Fabrizio, C., Fulvia, C., Carlo, P., Laura, M., Elisa, M., Francesca, M., Guido,V. Systemic Lupus Erythematosus with and without Anti-dsDNA Antibodies: Analysis from a Large Monocentric Cohort. Mediators of Inflammation, 2015; 1–6. doi:10.1155/2015/328078.

71- Giles BM, Boackle SA. Linking complement and anti-dsDNA antibodies in the pathogenesis of systemic lupus erythematosus. Immunol Res 2013;55:10–21.

72- Pierangeli SS, Girardi G, Vega-Ostertag M, Liu X, Espinola RG, Salmon J. Requirement of activation of complement C3 and C5 for antiphospholipid antibody- mediatedthrombophilia. Arthritis Rheum 2005;52:2120–4.