Original article

Serum interleukins 2 and 10 in juvenile systemic lupus erythematosus: relation to disease activity

Background: *Cytokines have an important immunological role in the pathogenesis of systemic lupus erythematosus (SLE) and they were considered as disease activity biomarkers. This study was conducted to evaluate serum interleukin (IL) -2 and IL-10 levels in relation to disease activity in children and adolescence with SLE.* ***Methods:*** *Sixty lupus patients were studied in comparison to 30 healthy-matched control children. Disease activity was assessed according to SLE Disease Activity Index (SLEDAI). Serum IL-2 and IL-10 were measured by ELISA and their ratio was calculated.* ***Results:*** *Thirty lupus patients had an active disease (SLEDAI ≥ 4) and 30 had low disease activity. Serum IL-2 levels were significantly lower in patients with active disease (148.90±65.88 ng/l) and patients with low disease activity (159.33±68.01 ng/l) than controls (206.40±80.08 ng/l), P<0.01, without significant relation to disease activity. In contrast, serum IL-10 levels were significantly higher in patients with active disease [4064 (1777.5-8462.75) pg/l] and patients with low disease activity (1403 (922.25-2726) pg/l] than controls [7.35 (6.55-8.50) pg/l], (P<0.01) with a significant relation to disease activity (P<0.01). The calculated IL-10/IL-2 ratio was significantly higher in patients with active disease [32.28 (11.44-49.19)] and patients with low disease activity [11.15 (5.25-21.36)] than controls [0.037 (0.031-0.045)] with a significant relation to disease activity (P<0.01). IL-10/IL-2 ratio was more specific and sensitive in evaluating disease activity than IL-10 and IL-2 with test accuracy 89%.* ***Conclusions:*** *IL-10/IL-2 ratio was elevated in lupus patients due to reduced serum IL2 and elevated serum IL-10 levels and it was associated with disease activity.*

Key words: Disease activity biomarkers, Interleukins, IL10/IL2 ratio, SLE.

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INTRODUCTION

Juvenile systemic lupus erythematosus (j-SLE) is an autoimmune inflammatory disease that affects children ≤ 18 years. It represents 15–20% of all SLE patients. It is characterized by systemic inflammation and a wide spectrum of circulating autoantibodies due to dysfunctional immune regulation.[1, 2]

Interleukins have an important role in the pathogenesis of SLE and they are considered to be disease biomarkers because their levels vary with disease activity. So, they could be used as therapeutic targets. They are produced by T helper (Th) cells.[3]

IL-2 is a monomeric glycoprotein that is produced by Th1 and CD4+ T lymphocytes. It plays a critical role in immune homeostasis and regulation. Patients lacking IL-2 expression have defective immune responses. The induction of anti-IL-2 autoantibodies and decreased its serum level may have a role in the occurrence of SLE activity.[4,5]

IL-10 is produced by regulatory Th cells (Treg), Th2, and CD8+ T lymphocytes. It plays an important role in B cell activation and autoantibody production. Also, it has direct inhibitory effects on the proliferation of CD4+ T cells and Th1 cytokines production such as IL2. IL-10 is defined as a potent stimulator of B lymphocytes and it stimulates the production of anti-DNA auto-antibodies in SLE patients. The overproduction of IL-10 may have a role in the occurrence of SLE activity.[6,7]

This study was conducted to evaluate serum levels of IL-2 and IL-10 and to calculate their ratio in relation to the disease activity in a group of children and adolescence with SLE.

METHODS

Study population:

This observational cross-sectional study was conducted on 60 children and adolescence with SLE below 18 years diagnosed according to Systemic Lupus International Collaborating Clinics Classification (SLICC) criteria.8 Patients were

recruited from the Pediatric Rheumatology clinic, Tanta University Hospitals, Egypt in the period between October 2018 and March 2020. Patients who had bronchial asthma, acute infections and sepsis were excluded.

Patients were divided into two groups according to SLE disease activity index (SLEDAI) 9 into (GI: included 30 patients with active disease (SLEDAI ≥ 4) and GII: included 30 patients with low active disease (SLEDAI < 4). Lupus patients were compared to 30 healthy children as control group. The total sample size (30 children in each group) was calculated by using G*Power 3.1.9 to achieve power 95% with effect size 0.7 and alpha error 0.05.

This study was approved by Tanta Faculty of Medicine ethical committee (32614/ 10/ 18). An informed, written consent to participate in the study was signed by the parents or the legal guardians of the study subjects.

Study measurements:

Clinical evaluation of lupus patients and healthy children: This was based on clinical history taking from caregivers and clinical examination.

Routine laboratory investigations of SLE:

Blood samples

Ten milliliters of venous blood were collected, 6 ml were used for performing the routine investigations of lupus and the remaining 4 ml were stored at room temperature until coagulation occurred. Separation of serum was done rapidly by centrifugation for 20 minutes at a speed of 2000-3000 run/ min and stored at -20 C until assay of IL-2 and IL-10.

CBC and levels of ESR, CRP, serum creatinine, blood urea, serum C3 and C4, antinuclear antibodies (ANA), anti-double-stranded DNA (anti-dsDNA)] were assessed. Also, 24-hour urinary proteins were also assessed.

Renal biopsies: They have been done for 41 patients (68.3%) who met any of the following criteria: Increasing serum creatinine without alternative causes, proteinuria of ≥ 1g/24 hr, proteinuria ≥ 0.5 g/24 hr with hematuria or cellular casts.10 Renal biopsies have been evaluated according to the International Society of Nephrology Renal Pathology Society (ISN/RPS) grading system.[11]

Assessment of Serum IL-2 and IL-10 levels: Test principle; serum IL-2 and IL-10 kits use a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) with one original standard reagent which was diluted according to the instruction of manufacturer (Shanghai Sunred Biological Technology CO, Shanghai, China). The samples, standard, and controls were added to wells followed by the incubation buffer. After incubation, biotin-conjugated anti-IL-2 and IL-10 antibodies were added to each well. After 2 h of incubation, streptavidin–HRP working conjugate was added. Finally, chromogen was added followed by the stop solution then absorbance was read at 450 nm.

Statistical methods

The results were analyzed by using the commercially available SPSS statistical software version 21. The parametric data were presented as mean and standard deviation (SD). In addition, non-parametric data were presented as median and interquartile range (IQR). Student's t-test was used for comparison of parametric data, while Mann–Whitney test was used for comparison between non-parametric data. ANOVA test was used to compare data of more than 2 groups. Pearson coefficient "r" was used to determine the relationship between different variables. ROC curve evaluated the sensitivity and specificity of studied tests. For all tests, a probability (P) of less than 0.05 was considered significant.

RESULTS

Clinical and basic laboratory data of lupus patients and healthy controls were shown in table 1. Lupus patients comprised 10 males and 50 females. Their ages ranged between 9 and 18 years (mean ± SD = 13.46 ± 2.75 years). Lupus patients with active disease included 6 males and 24 females. Their ages ranged between 9 and 17 years (mean ± SD = 13.13 ± 2.16 years). Lupus patients with low disease activity comprised 4 males and 26 females. Their ages ranged between 10 and 18 years (mean ± SD = 14.03 ± 2.41 years). The control group included 6 males and 24 females. Their ages ranged between 8 and 18 years (mean ± SD = 13.50 ± 2.60 years).

Lupus patients with active disease had significant decrease of hemoglobin level, platelets and leukocytic count, C3 and C4 than patients with low disease activity. ANA was positive in 96.7% of patients with active disease and in 66.7% of patients with low disease activity. In addition, anti-ds DNA was positive in 93.3% of patients with active disease and in 43.3% of patients with low disease activity. On the other hand, 24hr urinary proteins levels were significantly higher in lupus patients with active disease than patients with low disease activity. Forty-one patients (68.3%) had LN class III (41.7%), 16.7% had LN class IV, 12.5% had LN Class II and only 1.7% had LN Class V (table 1).

Serum IL-2 levels were significantly lower in patients with active disease (148.90±65.88 ng/l) and patients with low disease activity (159.33±68.01 ng/l) than healthy controls (206.40±80.08 ng/l), P<0.01 (tables 2,3). On the other hand, there was no significant difference between patients with active disease and patients with low active disease in serum levels of IL-2 (P > 0.5), table 4. There was no significant correlation between serum IL-2 levels and SLEDA (P > 0.5).

In contrast, serum IL-10 levels were significantly higher in patients with active disease [4064 (1777.5-8462.75) pg/l] and patients with low disease activity (1403 (922.25-2726) pg/l] than healthy controls [7.35 (6.55-8.50) pg/l], (P<0.01), tables 2,3. Also, patients with active disease had a significant increase of serum IL-10 levels than patients with low disease activity (P<0.01), table 4.

The calculated IL-10/IL-2 ratio median was significantly higher in patients with active disease [32.28 (11.44-49.19)] and patients with low disease activity [11.15 (5.25-21.36)] than healthy controls [0.037 (0.031-0.045)] tables 2,3. Also, IL-10/IL-2 ratio was significantly elevated in patients with active disease than patients with low disease activity (P<0.01), table 4.

There were significant positive correlations between SLEDAI and both IL-10 (P<0.01) and IL-10/IL-2 ratio (P<0.01), figures 1, 2. In contrast, there were significant negative correlations between IL-10 and both C3 (P<0.01) and C4 (P<0.01).

IL-10/IL-2 ratio had higher specificity (92%) and accuracy (89%) than IL-10 (80 and 77%, respectively) and IL-2 (50% and 46%, respectively) in evaluating the disease activity (table 5 and figure 3).

Table 1. Clinical and basic laboratory data of lupus patients and healthy controls

	Lupus patients with active disease (n=30)	**Lupus patients with low disease activity (n=30)**	**Healthy controls (n=30)**	**P-value**
Age (years) Range: Mean ±SD	9 - 17 13.13 ± 2.16	10 -18 14.03 ± 2.41	8 - 18 13.50 ± 2.60	0.348
Sex Female Male	24 6	26 4	24 6	0.738
Hb (g/dl) (Mean ±SD)	9.82 ± 1.50	11.33 ± 1.12	12.84 ± 1.12	0.001
PLT ($x10^3$/ml) (Mean ±SD)	149.87 ± 57.80	286.87 ± 84.80	303.30 ± 81.35	0.001
TLC ($x10^3$/ml) (Mean ±SD)	3.06 ± 0.74	7.73 ± 2.60	6.57 ± 2.63	0.001
ESR (mm) 1st hr Median (IQR)	40 (23.75 - 61.25)	17.5 (10 - 36.25)	10 (5 – 15)	0.001
Positive CRP (n%)	14 (46.7%)	7 (23.3%)	0	0.001
Creatinine (mg/dl) (Mean ±SD)	0.98 ± 0.15	0.69 ± 0.18	0.56 ± 0.15	0.006
BUN (mg/dl) (Mean ±SD)	18.70 ± 4.76	15.07 ± 5.50	14.60 ± 2.93	0.003
Albumin (g/dl) (Mean ±SD)	3.13 ± 0.67	3.78 ± 0.34	4.36 ± 0.69	0.001
24 hr urinary protein (mg) Median (IQR)	3675 (2522.75 – 4500)	150 (100 – 287.5)	71 (45.5 – 89.25)	0.001
C3 (mg %)(Mean ±SD)	49.5 ± 9.15	95.0 ± 17.84	119.57 ± 30.49	0.001
C4 (mg %)(Mean ±SD)	7.18 ± 6.52	18.27 ± 7.92	28.50 ± 9.28	0.001
positive ANA (n%)	29 (96.7%)	20 (66.7%)	-	0.001
positive Anti-dsDNA	28 (93.3%)	13 (43.3%)	-	0.001
SLEDAI Median (IQR)	14 (8 - 22)	1 (0 - 3)	-	0.001
LN classes: (n%) ▪ LN Class II ▪ LN Class III ▪ LN Class IV ▪ LN Class V	 2 (6.7%) 12 (40%) 5 (16.7%) 1 (3.3%)	 3 (10%) 13 (43.3%) 5 (16.7%) 0	-	

ANA: Antinuclear antibody, Anti-ds DNA: Anti-double strand DNA antibody, C3, C4: Complement 3, 4, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, Hb: Hemoglobulin, LN: Lupus nephritis, Plt: Platelets, SLEDAI: Systemic lupus erythematosus disease activity index, TLC: Total leucocytic count; P-value < 0.05: significant.

Table 2. Comparison between lupus patients with active disease and healthy controls in serum IL2 and IL-10 levels and IL-10/IL-2 ratio

	Lupus patients with active disease (n=30)	**Healthy controls (n=30)**	**P-value**
Serum IL-2 (ng/l) Mean ±SD	148.90 ± 65.88	206.40 ± 80.08	0.003
Serum IL-10 (ng/l) Median (IQR)	4064 (1777.5 - 8462.75)	7.35 (6.55 - 8.50)	0.001
IL-10/IL-2 ratio Median (IQR)	32.28 (11.44 - 49.19)	0.037 (0.031 - 0.045)	0.001

P-value < 0.05: significant

Table 3. Comparison between lupus patients with low disease activity and healthy controls in serum IL2 and IL-10 levels and IL-10/IL-2 ratio

	Lupus patients with low disease activity (n=30)	**Healthy controls (n=30)**	**P-value**
Serum IL-2 (ng/l) Mean ±SD	159.33 ± 68.01	206.40 ± 80.08	0.013
Serum IL-10 (ng/l) Median (IQR	1403 (922.25 - 2726)	7.35 (6.55 - 8.50)	0.003
IL-10/IL-2 ratio Median (IQR)	11.15 (5.25 - 21.36)	0.037 (0.031 - 0.045)	0.005

P-value < 0.05: significant

Table 4. Comparison between lupus patients with active disease and patients with low disease activity in serum IL2 and IL-10 levels and IL-10/IL-2 ratio

	Lupus patients with active disease (n=30)	**Lupus patients with low disease activity (n=30)**	**P-value**
Serum IL-2 (ng/l) Mean ±SD	148.90 ± 65.88	159.33 ± 68.01	0.574
Serum IL-10 (ng/l) Median (IQR	4064 (1777.5 - 8462.75)	1403 (922.25 - 2726)	0.001
IL-10/IL-2 ratio Median (IQR)	32.28 (11.44 - 49.19)	11.15 (5.25 - 21.36)	0.001

P-value < 0.05: significant

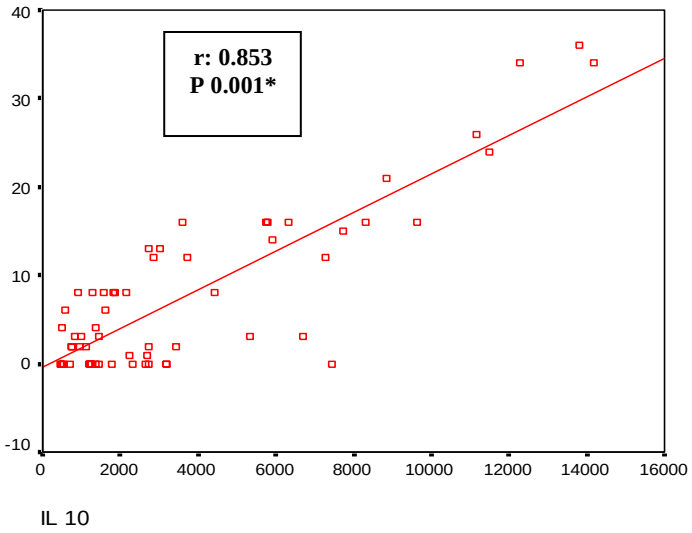

Figure 1. Correlation between serum IL-10 level and SLEDAI

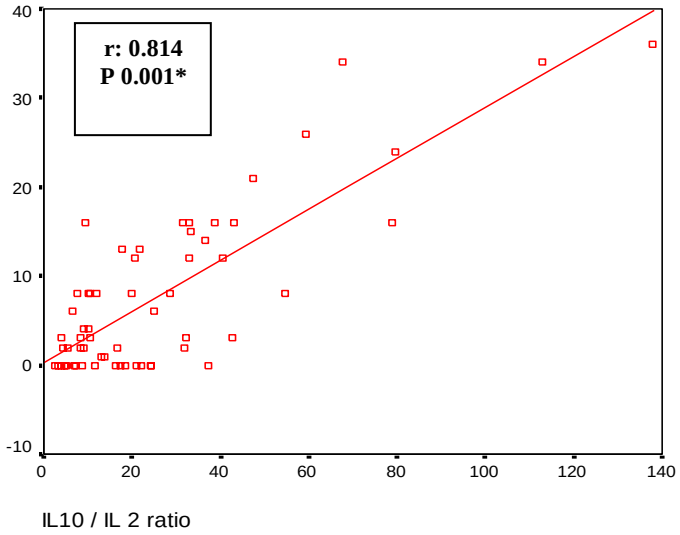

Figure 2. Correlation between IL-10/IL-2 ratio and SLEDAI

Table 5. Sensitivity, specificity, positive, negative predictive values and accuracy of serum level of IL-2, IL-10 and IL-10/IL-2 ratio as markers of SLE activity

	Cut off value	AUC	Sensitivity	Specificity	PPV	NPV
IL-2	143	0.46	57	50	53	54
IL-10	2795	0.77	63.3	80	76	71
IL-10/IL-2 ratio	24.6	0.89	80	92	81	86.7

AUC: Area under curve (Test accuracy)**, PPV:** Positive predictive value, **NPV:** Negative predictive value

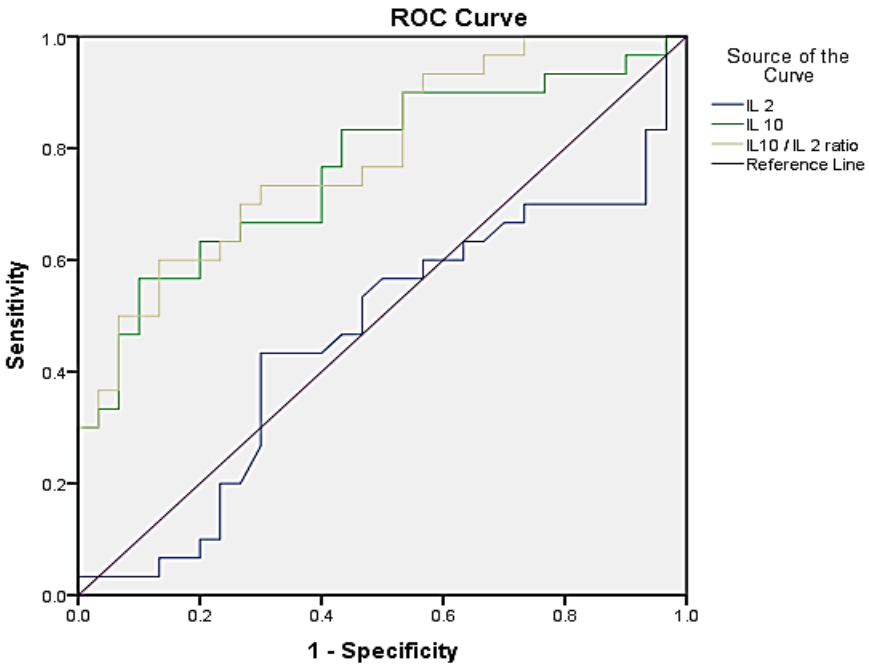

Figure 3. ROC curve of serum IL-2, IL-10 and IL-10/IL-2 ratio

DISCUSSION

Many interleukins regulate SLE activity as IL-2 and IL-10. Th1 cells produce IL2, IFN gamma, IL12 and IL18 while Th2 cells produce IL4, 5, 6, 10 and 13. The balance of Th1/Th2 is essential for the normal human immunity and so changes in the levels of Th1/Th2 cytokines might be involved in the pathogenesis of autoimmune diseases.[12]

In the current study, serum IL-2 levels were significantly lower in lupus patients with active disease and patients with low disease activity than healthy controls without a significant relation to disease activity. A previous study[13] also reported reduced serum IL-2 levels in lupus patients with no relation to the disease activity. These results were compatible with previous finding that SLE T cells produce diminished amounts of IL-2 after antigenic stimulation in vitro. In contrast, another study[14] reported normal levels of serum IL-2 in lupus patients. The contradiction between the results of these studies might be attributable to the difference in the assessment technique and the study sample size.

Defective IL-2 production is a factor involved in the immune dysregulation responsible for SLE pathogenesis.[15] The transcriptional regulators responsible for suppression of IL-2 production are imbalanced in SLE T cells and this explains the reduced IL-2 levels that were found in lupus patients.[16, 17]

Decreased IL-2 production in lupus patients may result in a reduction of the number of regulatory T cells in these patients. IL-2 is also necessary for CD8+ T cell effector function and the development of CD8+T memory cells. A defect in CD8+T cell cytotoxic function leads to an increased chance of intra-cellular infection which could be fatal for SLE patients.[18]

IL-10 is a multifunctional cytokine that plays a central role in the pathogenesis of SLE, including regulation of growth and differentiation of B cells with auto-antibody production,[6,7] class switching and plasma cell differentiation[19]. In the present study, serum IL-10 levels in lupus patients were higher than healthy controls with a significant relation to the disease activity. Furthermore, serum levels of IL-10 showed positive correlation with SLEDAI. These results were similar to the findings reported by previous studies.[13,14,18,19]

IL-10 is considered to be an anti-inflammatory cytokine that plays a role in the inhibition of pro-inflammatory cytokines. Thus, serum IL-10 overproduction in lupus patients with an active disease occurs to inhibit the production of these pro-inflammatory cytokines.[18,19] Furthermore, IL-10 contributes indirectly to the development of pathogenic immune complexes by raising high-affinity anti-nucleic acid and nucleoprotein autoantibody levels, a process remote from the auto-inflammatory lesions in lupus patients.[20]

In agreement to a previous study,[13] the present work revealed a significant negative correlation between serum levels of IL-10 and both C3 and C4. These results may indicate that serum IL-10 could be used as a biomarker of disease activity in patients with SLE. The current study revealed an elevation of the calculated IL-10/IL-2 ratio in lupus patients, due to IL-10 overproduction and low IL-2 production, with a significant relation to the disease activity. Moreover, a significant positive correlation between IL-10/IL-2 ratio and SLEDAI was found in this study. Similar findings were reported by a previous study.[20] In addition, this study reported a higher specificity (92%) and accuracy (89%) of the calculated IL-10/IL-2 ratio than both IL-10 (80% and 77%, respectively) and IL-2 (50% and 46%, respectively). Therefore, IL-10/IL-2 ratio could be used as a disease activity biomarker.

CONCLUSIONS

Serum IL-2 levels were reduced in lupus patients with no relation to disease activity. In contrast, serum IL-10 and IL-10/IL-2 ratio were elevated in lupus patients with a significant relation to disease activity. IL-10/IL-2 ratio is specific and sensitive in evaluating the disease activity with high accuracy, so it is considered a disease activity biomarker.

REFERENCES

1. **Hassan A, Farghal N, Hegab D, Mohamed W, Abd-Elnabi H.** Serum-soluble CXCL16 in j-SLE: a promising predictor of disease severity and lupus nephritis. Clin Rheumatol 2018;37(11):3025 -32.
2. **Sadun RE, Ardoin SP, Schanberg LE.** Systemic lupus erythematosus. In: Kliegman RM, Stanton BF, Geme JW, Schor NF, Berhrman RE, editors. Nelson Textbook of Pediatrics, 21st edition. Philadelphia: WB Saunders Co; 2019. pp:1176-80.
3. **Guimaraes P, Scavuzzi B, Stadtlober N, Santos L, Franchi L, Lozovoy M, et al.** Cytokines in SLE: for beyond Th1/Th2 dualism Lupus: Cytokines Profile. Immunol Cell Biol 2017;95(9):824-31.
4. **Rezaei A, Ziaee V, Sharabian FT, Harsini S, Mahmoudi M, Soltani S, et al.** Lack of association between interleukin-10, transforming growth factor-beta gene polymorphisms and j-SLE. Clin Rheumatol 2015;34(6):1059-64.

5. **Rojas M, Rodriguez Y, Leon K, Pacheco Y, Acosta-Ampudia Y, Monsalve D, et al.** Cytokines and Inflammatory Mediators in SLE. EMJ Rheumatol 2018;5:83-9.
6. **Zhang R, Wang J, Yang R, Sun J, Chen R, Luo H, et al.** Effects of Pro12Ala polymorphism in peroxisome proliferator-activated receptor-γ2 gene on metabolic syndrome risk: a meta-analysis. Gene 2014;535(1):79-87.
7. **Song G, Choi S, Ji J, Lee Y.** Associations between Interleukin-10 polymorphism and susceptibility to SLE: A meta-analysis. Human Immunol 2013;74(3):364-70.
8. **Petri M, Orbai A, Alarcón GS, Gordon C, Merrill JT, Fortin PR, et al.** Derivation and validation of Systemic Lupus International Collaborating Clinics Classification Criteria for systemic lupus erythematosus. Arthritis Rheum 2012;64(8):2677-86.
9. **Pope J.** Measuring flares in systemic lupus erythematosus. Rheumatology (Oxford) 2014:53(12):2134-5.
10. **Hahn BH, McMahon MA, Wilkinson A, Wallace WD, Daikh DI, Fitzgerald JD, et al.** American College of Rheumatology guidelines for screening, treatment, and management of lupus nephritis. Arthritis Care Res (Hoboken). 2012; 64 (6):797-808.
11. **Bajema I, Wilhelmus S, Alpers C, Bruijn J, Colvin R, Cook T, et al.** Revision of the International Society of Nephrology/Renal Pathology Society classification for lupus nephritis: clarification of definitions, and modified National Institutes of Health activity and chronicity indices. Kidney Int 2018;93(4):789-96.
12. **Harsinia S, Ziaeeb V, Tahghighic F, Mahmoudi M, Rezaei A, Soltani S, et al.** Association of interleukin-2 and interferon-Ɣ single nucleotide polymorphisms with j-SLE. Allergol Immunopathol (Madr) 2016;44(5):422-6.
13. **Chun H, Chung J, Kim H, Yun J, Jeon J, Ye Y, et al.** Cytokine IL-6 and IL-10 as Biomarkers in SLE. J Clin Immunol 2007;27(5):461-5.
14. **Cavalcanti A, Santos R, Mesquita Z, Duarte AL, Lucena-Silva N.** Cytokine Profile in Childhood Onset Systemic Lupus Erythematosus: across Sectional and Longitudinal Study. Braz J Med Biol Res 2017;50(4):5738.
15. **Li P, Li Y, Zhou A, Chen S, Li J, Wen X, et al.** Association study of a proliferation-inducing ligand, spermatogenesis associated 8, platelet-derived growth factor receptor-alpha, and POLB polymorphisms with SLE in Chinese Han population. Chin Med J (Engl) 2016;129:2085-90.
16. **Lashine Y, Salah S, Aboelenein H, Abdelaziz A.** Correcting the expression of miRNA-155 represses PP2Ac and enhances the release of IL-2 in PBMCs of juvenile SLE patients. Lupus 2015;24:240-7.
17. **Yang T, Li Y, Lyu Z, Huang K, Corrigan C, Ying S, et al.** Characteristics of proinflammatory cytokines and chemokines in airways of asthmatics: Relationships with disease severity and infiltration of inflammatory cells. Chin Med J (Engl) 2017;130:2033-40.
18. **Abou El-Fetouh S, Mohammed R, Abozaid H.** Serum interleukin-18 and interleukin-10 levels in SLE: correlation with SLEDAI score and disease activity parameters. Egypt Rheumatol Rehab 2014;41:160-6.
19. **Orozco-Barocio G, Mercado M, Munˆoz-Valle J.** Association of interleukin-10 promoter haplotypes with disease susceptibility and IL-10 levels in Mexican patients with SLE. Clin Exp Med 2015;15:439-46.
20. **Uzrail A, Assaf A, Abdalla S.** Correlations of Expression Levels of a Panel of Genes (IRF5, STAT4, TNFSF4, MECP2, and TLR7) and Cytokine Levels (IL-2, IL-6, IL-10, IL-12, IFN-γ, and TNF-α) with SLE Outcomes in Jordanian Patients. Biomed Res Int 2019:170384.