Journal of Advances in Medicine and Medical Research

33(11): 72-78, 2021; Article no.JAMMR.68036
ISSN: 2456-8899
(Past name: British Journal of Medicine and Medical Research, Past ISSN: 2231-0614, NLM ID: 101570965)

Cytokine Profile in Juvenile Systemic Lupus Erythematosus

A. Elfar[1*], Amal El-Bendary[1], M. A. Abdel-Hafez[1] and N. Abo El Hana[1]

[1]Pediatrics Department, Faculty of Medicine, Tanta University, Egypt.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMMR/2021/v33i1130927
Editor(s):
(1) Dr. Dean Markić, University Hospital Rijeka, Croatia.
(2) Dr. Syed Faisal Zaidi, King Saud bin Abdulaziz University for Health Sciences, Saudi Arabia.
(3) Dr. Mohamed Essa, Sultan Qaboos University, Oman.
Reviewers:
(1) Delong Jiao, University of Maryland, USA.
(2) Panagiotis Garantziotis, Hannover Medical University, Germany.
Complete Peer review History: http://www.sdiarticle4.com/review-history/68036

Original Research Article

Received 02 March 2021
Accepted 08 May 2021
Published 18 May 2021

ABSTRACT

Background: Cytokines have an important role in immune system dysregulation in SLE because they act on the differentiation, maturation, and activation of several effector cells, culminating in inflammation and subsequent tissue damage.The aim of the work was to evaluate cytokine profile (IL2, IL10 and IL13) in children with SLE and their possible role in the pathogenesis of lupus nephritis.
Methods: This is a cross sectional case-control study conducted on 60 children with SLE and 30 healthy children of matched age and sex served as a control group. The presence of lupus nephritis was confirmed by renal biopsy and histopathological examination. The SLE Disease Activity Index (SLEDAI) score for each patient was used to evaluate disease activity. Serum IL2, IL-10 & IL-13levels were measured using ELISA.
Results: There was a significant increase in serum IL-10 levels in SLE patients compared to healthy controls and in patients with lupus nephritis compared to patients without lupus nephritis. Also, there were significant positive correlation between IL-10 and SLEDAI Score and between IL-10 and 24-hour urinary protein collection. There was no statistically significant difference in IL-2 levels in SLE patients compared to healthy controls. However, IL2 levels were significantly lower in active patients without lupus nephritis compared to active patients with lupus nephritis. There was

**Corresponding author: E-mail: asmaa.elfar86@yahoo.com;*

no correlation between IL-2 and 24-hour urinary protein collection. The levels of IL13 were significantly higher in SLE patients compared to healthy controls and in patients with lupus nephritis compared to patients without lupus nephritis. There were significant positive correlations between Il 13 and SLEDAI Score and between IL-13and 24-hour urinary protein collection.
Conclusions: Soluble IL10 and IL-13 could be used as a measure of disease activity. Further studies are needed to evaluate SLE pathophysiology including measurement of cytokine profile.

Keywords: Cytokines; juvenile; systemic lupus erythematosus.

1. INTRODUCTION

Systemic Lupus Erythematosus (SLE) is thought to constitute a loss of tolerance in a genetically susceptible individual with progression to autoimmunity that is triggered by various environmental factors and infections [1] The etiology of juvenile SLE is multifactorial, involving genetic risk factors, epigenetic mechanisms, and environmental triggers [2]. Pathogenesis of SLE is associated with functional deficiency of multiple immunologic components, including the innate immune system, altered immune tolerance mechanisms, hyper activation of T and B cells, reduced ability of immune complexes and apoptotic cell clearance, and defects in multiple immune regulatory networks. The failure of these mechanisms could be due to the influence of variants within SLE susceptibility genes [3].

Interleukin 10 (IL-10) is an anti-inflammatory cytokine most readily associated with macrophages. The action of IL-10 results in the downregulation of major histocompatibility complex class II (MHC II) proteins and co-stimulatory molecules, such as CD80 and CD86, on the surfaces of target macrophages [4].

IL-2 is a multifunctional cytokine primarily produced by T cells and is necessary for T cell activation, proliferation, and contraction. It has been reported that production of IL-2 is decreased in patients with systemic lupus erythematosus (SLE) and this defect affects multiple aspects of host immunity [5].

Interleukin 13 (IL-13) is a protein secreted by activated T cells that inhibits proinflammatory molecule production by activated human monocytes and that modulates B-cell functions in vitro. IL-13 seems to play a significant role in B-lymphocyte proliferation, differentiation and immunoglobulin production [6].

The aim of the work was to evaluate cytokine profile (IL2, IL10 and IL13) in children with SLE and their possible role in the pathogenesis of lupus nephritis.

2. PATIENTS AND METHODS

This is a cross sectional case-control study was conducted on 60 children with SLE from nephrology Unit, Pediatric Department, Tanta University Hospitals-Egypt from 2017-2020. Thirty healthy children of matched age and sex served as a control group. The American college of Rheumatology (ACR) SLE revised criteria was used to confirm the diagnosis of SLE. The presence of lupus nephritis was confirmed by renal biopsy and histopathological examination. The SLE Disease Activity Index (SLEDAI) score for each patient was used to evaluate disease activity. Active lupus disease was considered when SLEDAI score is more than 3.

2.1 Inclusion criteria

All patients with SLE below 18 years of age were included.

2.2 Exclusion Criteria

A. Asthma.
B. Urinary tract infection.
C. Infection.
D. Family history of immunodeficiency.
E. Family history of mixed connective tissue disorders.

Treatment was administered according to local guidelines which depends mainly on the grading of LN in the renal pathology and severity of the disease related to systemic involvements.

All patients were subjected to informed consent, history, examination and clinical examination. Venous blood samples about 10 ml were drawn from all subjects aseptically and 6 ml were used for performing routine investigations. The remaining 4 ml were stored at room temperature until coagulation occurred (usually 15-45 minutes), then centrifuged for 20 minutes at the speed of 2000-3000 run per minute to obtain the serum specimen. These specimens were kept at -20°C till analysis.

The following investigations were performed: CBC, ESR, serum creatinine, blood urea level, 24-hour urinary protein excretion, serum C3 and C4 complement levels, serum antinuclear antibodies (ANA) & anti-DNA levels (using ELISA), renal biopsy with histopathological examination. Serum interleukin 2 (IL-2), interleukin 10 (IL-10) and interleukin13 (IL-13) levels were measured by ELISA kit.

2.3 Statistical Analysis

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Qualitative data were described using number and percent. The Kolmogorov-Smirnov test was used to verify the normality of distribution Quantitative data were described using range (minimum and maximum), mean, standard deviation, median and interquartile range (IQR). Significance of the obtained results was judged at the 5% level.

The used tests were: 1) Chi-square test. 2) Fisher's Exact or Monte Carlo correction. 3) Student t-test. 4) Mann Whitney test.

3. RESULTS

There was no significant difference between patients and Controls regarding sex, age, systolic and diastolic blood pressure. However, patients were significantly shorter and high in weight Z score compared to Controls with p value 0.005 and 0.002 respectively Table 1.

There was a significantly lower hemoglobin and serum albumin level. There was a significantly higher ESR (1^{st}&2^{nd}hour), CRP, blood urea and serum creatinine level in patients compared to Controls. There was no significant difference between patients and Controls regarding platelets, total leucocytes and reticulocytes count

Table 2.

There was no significant difference in Serum IL-2 levels in patients and Controls. There was a statistically significant increase in IL-10 and IL-13 levels in patients compared to Controls Table 3.

The levels of IL-10 and IL-13 had positive correlation with 24-hour urinary protein and SLEDAI score, while S.IL-2 had no correlation with both of them Table 4.

In patients with lupus nephritis, SLEDAI score, IL-2, IL-10 and IL-13 were significantly higher compared to patients without lupus nephritis Table 5.

4. DISCUSSION

Although SLE is mainly characterized by deposition of immune complexes and autoantibody production, cytokines also have an important role in immune system dysregulation in SLE because they act on the differentiation, maturation, and activation of several effector cells, culminating in inflammation and subsequent tissue damage. Several studies revealed that cytokines are important in pathogenesis of this disorder [7].

Table 1. Demographic data of the studied groups

	Patients (n = 60)		Controls (n = 30)		Test of Sig.	P
Sex:	**No.**	**%**	**No.**	**%**		
Male	10	16.7	6	20.0	χ^2=0.152	0.697
Female	50	83.3	24	80.0		
Age: Mean ± SD	13.82 ± 2.27		13.50 ± 2.60		t=0.595	0.553
Z-score Height: Median (IQR)	-0.79 (-1.05 – -0.21)		-0.13 (-0.66 – -0.02)		U=570.0*	0.005*
Z-score Weight: Median (IQR)	-0.02 (-0.42 – 0.49)		-0.36 (-0.58 – -0.17)		U=537.0*	0.002*
Systolic(mmHg): Median (IQR)	110.0 (100.0 – 120.0)		110.0 (110.0 – 120.0)		U=820.50	0.484
Diastolic(mmHg): Mean ± SD	75.42 ± 9.49		72.67 ± 7.40		t=1.389	0.168

*χ^2:Chi square test, t: Student t-test, U: Mann Whitney test, p: p value for comparing between the studied groups *: Statistically significant at p ≤ 0.05*

Table 2. Routine laboratory investigations of the studied groups

	Patients (n = 60)		Controls (n = 30)		Test of sig.	P value
	No.	%	No.	%		
Hb (g/dl) Mean ± SD.	10.39 ± 1.74		12.84 ± 1.12		t=7.031*	<0.001*
PLT (10^3/ Cmm) Mean ± SD.	274.97 ± 105.41		303.30 ± 81.35		t=1.291	0.200
TLC (10^3/Cmm) Median (IQR)	6.50 (5.05 – 9.10)		6.70 (5.0 – 8.60)		U=896.50	0.976
Reticulocytes count (%)						
Normal	0.50 – 8.0		0.50 – 1.50		U=853.50	0.689
High	1.0 (0.90 – 1.30)		1.10 (0.70 – 1.30)			
ESR 1st hour (mm) Mean ± SD.	31.65 ± 21.55		10.67 ± 5.21		U=260.50*	<0.001*
ESR 2nd hour (mm) Mean ± SD.	55.51 ± 29.68		19.67 ± 8.30		U=167.50*	<0.001*
CRP (mg/L)						
Negative	43	71.7	30	100.0	χ^2=10.479*	0.001*
Positive	17	28.3	0	0.0		
BloodUrea (mg/dl) Mean ± SD.	31.97 ± 10.18		27.60 ± 2.93		t=3.077*	0.003*
S.Creatinine (mg/dl) Median (IQR)	0.70(0.6 – 0.8)		0.50(0.4 – 0.7)		U=507.0*	0.001*
S.Albumin (g/dl) Mean ± SD.	3.46 ± 0.59		4.45 ± 0.58		t=7.488*	<0.001*
24-hour urinary PTN (mg/day) Median (IQR)	304.0 (152.5 –1170.0)		71.0 (47.0 – 89.0)		U=75.00*	<0.001*

*Hb(Hemoglobin), PLT(Platelets), TLC(Total leukocytic counts), Retics (Reticulocytes counts), ESR (Erythrocytic sedimentation rate),CRP (C - Reactive Protein), t: Student t-test, U: Mann Whitney test, p: p value for comparing between the studied groups, *: Statistically significant at p ≤ 0.05*

Table 3. Cytokines profile of the studied groups

	Patients (n = 60)	Controls(n = 30)	U	P
	Median (IQR)			
S.IL 2 (ng/l)	114.5 (37.0 – 372.0)	163.5 (133.0 – 271.0)	782.00	0.312
S.IL 10 (pg/ml)	1860.0(785.0 – 3329.0)	7.35(6.6 – 8.5)	1.000*	<0.001*
S.IL 13 (pg/ml)	285.50 (84.50 –587.5)	4.0 (2.0 –6.0)	20.00*	<0.001*

U: Mann Whitney test, p: p value for comparing between the studied groups, *: Statistically significant at p ≤ 0.05

Table 4. Correlation between 24-hour urinary protein and SELDAI with cytokines profile in patients group

	24-hour PTN		SELDAI	
	r_s	p	r_s	P
S.IL 2 (ng/l)	0.210	0.108	0.239	0.066
S.IL 10 (pg/ml)	0.276	0.033*	0.425	0.001*
S.IL 13 (pg/ml)	0.396	0.002*	0.432	0.001*

*r_s: Spearman coefficient, *: Statistically significant at p ≤ 0.05*

Table 5. SlEDAI score and cytokine profile in patients with and without lupus nephritis

	No (n = 15)	Yes (n = 45)	U	P
	Median			
SELDAI	0.0	16.0	22.500*	<0.001*
S.IL 2 (ng/l)	47.0	223.0	261.50*	0.010*
S.IL 10 (pg/ml)	1309.0	2907.5	213.00*	0.001*
S.IL 13 (pg/ml)	114.0	560.5	144.00*	<0.001*

*U: Mann Whitney test, p: p value for association between different categories, *: Statistically significant at p ≤ 0.05*

In our study IL10 levels were significantly higher in SLE patients compared to healthy controls and in patients with lupus nephritis compared to patients without lupus nephritis. Also, there were significant positive correlation between Il 10 and SLEDAI Score

Several studies were compatible with the results of the present study [7-14].

Rianthavornl et al reported that serum IL-10 levels did not significantly differ in JSLE patients with nephritis when compared to patients without nephritis. The difference in ethnic groups and genetic backgrounds may partially explain the changes in results with lupus nephritis patients [11].

While IL-10 is generally considered an anti-inflammatory cytokine, serum levels are often raised in lupus patients where it can act as a potent B-cell growth factor, driving autoantibody production, class switching, and plasma cell differentiation Thus, IL-10 may be important for suppressing tissue damage from activated pro-inflammatory macrophages and neutrophil responses to immune complex deposits, but can also contribute indirectly to the development of pathogenic immune complexes by raising high-affinity anti-nucleic acid and nucleoprotein autoantibody levels, a process remote from the inflammatory lesion [15].

Th2 response is predominant in SLE and have the capacity to secrete copious amounts of IL-13. Nowadays, IL-13 has been widely used as a therapeutic target for Th2 disease because it can drive inflammation and fibrosis independently in many diseases [16,17].

Several studies reported that the production of cytokine IL- 13 level was significantly higher in lupus nephritis patients compared to patients with SLE without renal involvement and healthy group. Also, found that serum levels of IL13was significantly higher in SLE than control group [18-20].

Cavalcanti et al and Viallard et al reported the same results of the present study with the exception that Viallard.et al study found that statistically significant correlations were present between IL-2 and disease activity [7,21].

The correlation of IL2 and disease activity reported by Viallard might be due to small sample size (only 24 SLE patients) and also clinical disease activity was assessed by applying the systemic lupus activity measure (SLAM) not SLEDAI score.

Several studies observed a decrease in IL 2 level in SLE patients compared to controls and the low cytokine levels did not correlate with clinical or serologic activity of disease [22-26].

5. CONCLUSIONS

There was an increased levels of serum IL-10 and IL-13 in SLE patients compared to controls, while IL-2 levels were not statistically significant. There was an increased levels ofserumIL-10, IL-2 and IL-13 in SLE patients with lupus nephritis compared to patients without lupus nephritis. There were significant positive correlation between serum IL-10 and IL-13 levels and SLEDAI score. There were significant positive correlation between serum IL-10 and IL-13 levels and 24 hour urinary protein collection. However, IL-2 levels had no correlation neither to SLEDAI score nor to 24-hour urinary protein collection.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Schwartz N, Goilav B, Putterman C. The pathogenesis, diagnosis and treatment of lupus nephritis. Curr Opin Rheumatol. 2014;26:502-9.
2. Rekvig OP, Van der Vlag J. The pathogenesis and diagnosis of systemic lupus erythematosus: still not resolved. Semin Immunopathol. 2014;36:301-11.
3. Chai HC, Phipps ME, Chua KH. Genetic risk factors of systemic lupus erythematosus in the Malaysian

population: A minireview. Clin Dev Immunol. 2012;963730.
4. Cyktor JC, Turner J. Interleukin-10 and immunity against prokaryotic and eukaryotic intracellular pathogens. Infect Immun. 2011;79:2964-73.
5. Lieberman LA, Tsokos GC. The IL-2 defect in systemic lupus erythematosus disease has an expansive effect on host immunity. J Biomed Biotechnol. 2010;740619.
6. Spadaro A, Rinaldi T, Riccieri V, Taccari E, Valesini G. Interleukin-13 in autoimmune rheumatic diseases: Relationship with the autoantibody profile. Clin Exp Rheumatol. 2002;20:213-6.
7. Cavalcanti A, Santos R, Mesquita Z, Duarte A, Lucena-Silva N. Cytokine profile in childhood-onset systemic lupus erythematosus: A cross-sectional and longitudinal study. Brazilian Journal of Medical and Biological Research. 2017;50.
8. Godsell J, Rudloff I, Kandane-Rathnayake R, Hoi A, Nold MF, Morand EF, et al. Clinical associations of IL-10 and IL-37 in systemic lupus erythematosus. Scientific reports. 2016;6:34604.
9. Talaat RM, Alrefaey SA, Bassyouni IH, Ashour ME, Raouf AA. Genetic polymorphisms of interleukin 6 and interleukin 10 in Egyptian patients with systemic lupus eythematosus. Lupus. 2016;25:255-64.
10. McCarthy EM, Smith S, Lee RZ, Cunnane G, Doran MF, Donnelly S, et al. The association of cytokines with disease activity and damage scores in systemic lupus erythematosus patients. Rheumatology (Oxford). 2014;53:1586-94.
11. Rianthavorn P, Chokedeemeeboon C, Deekajorndech T, Suphapeetiporn K. Interleukin-10 promoter polymorphisms and expression in Thai children with juvenile systemic lupus erythematosus. Lupus. 2013;22:721-6.
12. Chun HY, Chung JW, Kim HA, Yun JM, Jeon JY, Ye YM, et al. Cytokine IL-6 and IL-10 as biomarkers in systemic lupus erythematosus. J Clin Immunol. 2007;27:461-6.
13. Liu TF, Jones BM. Impaired production of IL-12 in system lupus erythematosus. II: IL-12 production in vitro is correlated negatively with serum IL-10, positively with serum IFN-γ and negatively with disease activity in SLE. Cytokine. 1998;10:148-53.
14. Houssiau F, Lefebvre C, Vanden Berghe M, Lambert M, Devogelaer J-P, Renauld J-C. Serum interleukin 10 titers in systemic lupus erythematosus reflect disease activity. lupus. 1995;4:393-5.
15. Dahal LN, Basu N, Youssef H, Khanolkar RC, Barker RN, Erwig LP, et al. Immunoregulatory soluble CTLA-4 modifies effector T-cell responses in systemic lupus erythematosus. Arthritis research & therapy. 2016;18:1-11.
16. Doran E, Cai F, Holweg CTJ, Wong K, Brumm J, Arron JR. Interleukin-13 in asthma and other eosinophilic disorders. Front Med (Lausanne). 2017;4:139.
17. Passalacqua G, Mincarini M, Colombo D, Troisi G, Ferrari M, Bagnasco D, et al. IL-13 and idiopathic pulmonary fibrosis: Possible links and new therapeutic strategies. Pulm Pharmacol Ther. 2017;45:95-100.
18. Brugos B, Vincze Z, Sipka S, Szegedi G, Zeher M. Serum and urinary cytokine levels of SLE patients. Pharmazie. 2012;67:411-3.
19. Sultan MM, Al Salam FMA, Hassan DA, Abdulla HS, El-Rahim A, Marwa M, et al. Assessment of proinflammatory Th1 cytokines (IL18-IFN□) and Th2 cytokine (IL13) concentrations in patients with autoimmune rheumatic diseases (systemic lupus erythematosus, rheumatoid artharitis and systemic sclerosis). The Egyptian Journal of Hospital Medicine. 2010;38:1-12.
20. Zhao M, Tang J, Gao F, Wu X, Liang Y, Yin H, et al. Hypomethylation of IL10 and IL13 promoters in CD4+ T cells of patients with systemic lupus erythematosus. Journal of Biomedicine and Biotechnology; 2010.
21. Viallard JF, Pellegrin JL, Ranchin V, Schaeverbeke T, Dehais J, Longy-Boursier M, et al. Th1 (IL-2, interferon-gamma (IFN-gamma)) and Th2 (IL-10, IL-4) cytokine production by peripheral blood mononuclear cells (PBMC) from patients with systemic lupus erythematosus (SLE). Clin Exp Immunol. 1999;115:189-95.
22. Avrămescu C, Biciuşcă V, Dăianu T, Turculeanu A, Bălăşoiu M, Popescu SN, et al. Cytokine panel and histopathological aspects in the systemic lupus erythematosus. Rom J Morphol Embryol. 2010;51:633-40.
23. Solomou EE, Juang Y-T, Gourley MF, Kammer GM, Tsokos GC. Molecular basis of deficient IL-2 production in T cells from patients with systemic lupus

erythematosus. The Journal of Immunology. 2001;166:4216-22.
24. Draeger AM, Swaak A, van den Brink HG, Aarden L. T cell function in systemic lupus erythematosus: Normal production of and responsiveness to interleukin 2. Clinical and experimental immunology. 1986;64:80.
25. Linker-Israeli M, Bakke A, Kitridou R, Gendler S, Gillis S, Horwitz D. Defective production of interleukin 1 and interleukin 2 in patients with systemic lupus erythematosus (SLE). The Journal of Immunology. 1983;130:2651-5.
26. Alcocer-Varela J, Alarcón-Segovia D. Decreased production of and response to interleukin-2 by cultured lymphocytes from patients with systemic lupus erythematosus. J Clin Invest. 1982;69:1388-92.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/68036