DOI: 10.5455/medarh.2013.67.237-240
Med Arh. 2013 Aug; 67(4): 237-240
Received: March 28th 2013 | Accepted: June 25th 2013

CONFLICT OF INTEREST: NONE DECLARED

ORIGINAL PAPER

Serum Levels of Il-18 in Iranian Females with Systemic Lupus Erythematosus

Miramir Aghdashi[1], Sasan Aribi [2], Siamak Salami[3]
Rheumatology and Internal Medicine Department, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran[1]
Internal Medicine Department, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran[2]
Clinical Biochemistry Department, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran[2]

IL-18 is a member of the IL-1 family of cytokines but due to its unique inflammatory and immunoregulatory properties it is suggested that it plays important roles in autoimmune and inflammatory diseases like as systemic lupus erythematosus (SLE). **Methods:** The current study was sought to evaluate serum levels of IL-18 in Iranian females with SLE. Serum samples of 25 patients with low disease activity , SLE-DAI score <7, 25 with high disease activity, SLE-DAI score ≥7 and 25 normal subjects were assessed for IL-18, anti-ds-DNA, C_3, C_4 and other lab findings using appropriate methods. Level IL-18 in patients with SLE-DAI score ≥7 was significantly higher than patients with low disease activity and controls (p=0.026 and p =0.005, respectively). **Results and discussion:** In patients with high disease activity, a positive correlation was found between serum levels of IL-18 with DsDNA antibody (r^2= 0.38, p=0.03), protein levels in 24 hours collected urine (r^2= 0.53, p= 0.007), platelet counts (r^2= 0.506, p=0.01) and it correlates negatively with serum C_3 levels (r^2=–0.42, p=0.02). **Conclusion:** The findings point to important role of IL-18 in SLE patients with disease activity higher than SLE-DAI score 7. Key words: Systemic Lupus Erythematosus; disease activity; Interleukin-18

Corresponding author: Siamak Salami, MD. Clinical Biochemistry Department, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran, E-mail: salami.si@gmail.com

1. INTRODUCTION

Lupus erythematosus was named by the physicians in 19th century due to its specific skin lesions, but as opposed to the organ-specific autoimmune diseases such as thyroiditis, it has been found that it is a systemic disease that spares no organ and then its name has been changed to systemic lupus erythematosus (SLE). The brain, blood, and the kidney are some of the vital organs and tissues that are involved in the most of the patients, who are predominantly middle aged females. The prevalence ranges from 20 to 150 cases per 100,000 populations, and appears to be increasing as the disease is recognized more readily and survival increases. SLE occurs with an increased prevalence and greater involvement of vital organs in the Americans with African, Hispanic, or Asian ancestry. Undulating disease course with large diversity of the presentation and accumulating manifestations over the time have made its broad differential diagnosis as a challenge even for the most of smart clinicians (1). Apart from the rare cases, presence of the circulating antinuclear antibodies is the unifying laboratory that could be classified to auto antibodies with high specificity such as Anti-dsDNA (in 40–90% of patients), Nucleosomes (in 60–90% of patients), Anti-Sm (in 5–30% of patients), Anti-C1q (in 40–100% of patients), Phospholipids (in 20–30% of patients) and auto antibodies with low specificity such as Anti-SSA/Ro(in 30–40% of patients), Anti-SSB/La (in 12–20% of patients), NMDA receptor (in 30–50% of patients) (2). Different diagnostic criteria were coined by the different societies of experts such as the American College of Rheumatology (ACR) that reflects the major mucocutaneous, articular, serosal, renal, neurologic features of the disease and incorporate the associated hematologic and immunologic findings (3, 4). Contribution of the genetic, epigenetic, environmental, hormonal, and immunoregulatory factors in the expression of tissue injury and clinical manifestations have been reported but the exact etiology of the disease has remained obscure (5). Regarding immunoregulatory factors, cytokines are soluble low-molecular weight glycoproteins or small polypeptides that act in an autocrine or paracrine manner between leukocytes and other cells (1). Cytokines have many biologic functions and are important for leukocyte growth and differentiation as well as activation and migration. Defense, growth, fibrosis, angiogenesis, inflammation, and neoplasm control are some examples of the process that could be orchestrate by cytokines which are synthesized by immunologic cells such as lymphocytes and monocytes/macrophages and by nonimmunologic cells such as keratinocytes and endothelial cells (6). Interleukin-1 (IL-1), IL-2, interferon-γ (IFN-γ), and tumor necrosis factor-α (TNF-α) are some ex-

amples of pro-inflammatory cytokines include, and anti-inflammatory cytokines include IL-1 receptor antagonist, IL-4, IL-10, and transforming growth factor-β (TGF-β) (6). A complex process was reported in the immunopathogenesis of SLE that involved the interaction and synergistic effect of various cytokines, chemokines, and signaling molecules which perpetuate the disease activity in SLE. IL-18 is a member of the interleukin (IL)-1 family and it is closely related to IL-1β, and both require the intracellular cysteine protease caspase-1 for biological activity (7). Further to several autoimmune and non-immune diseases, it is also a key player in models of atherosclerosis, graft versus host disease and hepatitis (7). It has been suggested that IL-18 may play an important role in the cytokine hierarchy in cutaneous lupus erythematosus (CLE), indicating the potential benefit of a local agent that blocks IL-18 activity in the treatment of the manifestations of CLE (8). It has been also reported that IL-18 was increased in all SLE cases but IL-18 BP was increased only in patients with glomerular infiltration (9, 10). The current study was sought to evaluate serum level of IL in Iranian patients with SLE and its impact on activity and severity of disease.

2. MATERIALS AND METHODS

Subjects

Systemic lupus erythematosus (SLE) suffering females who admitted to the rheumatology clinic of university hospital were examined using the University of Toronto SLE Disease Activity Index (SLE-DAI) and 25 SLE patients with high disease activity (SLE-DAI score ≥7) and 25 patients with low disease activity (SLE-DAI score <7) were included in this study and 25 subject without known history of autoimmune or systemic disease were also selected as control group. The study was designed to comply with the Declaration of Helsinki and was approved by local board of medical ethics and written consent was obtained from subjects.

Anti-Ds-DNA antibody

Serum level of Anti-Ds-DNA was measured using Trinity Biotech dsDNA Enzyme-Linked Immunosorbent Assay test (Trinity Biotech Plc, Ireland) that detects antibodies to dsDNA antigen. Briefly, test sera, calibrator and control sera were dilute 1:21 in serum diluent, dispensed into wells and incubated at room temperature for 30 minutes. Liquid was aspirated from all wells they were washed with Wash Buffer for a total of three times. Conjugate was dispensed to each well, and was incubated at room temperature for 30 minutes and washed for three times, subsequently. Chromogen/Substrate Solution (TMB) was added to each well and incubated at room temperature for 15 minutes. Finally, reaction was stopped by addition of Stop Solution (1N H2SO4) and the developed color was read on an ELISA plate reader equipped with a 450 nm filter and a 620 nm reference filter. Values less than 40 IU/ml were assigned as negative. Coefficient of Variation (CV) for Intra-Assay and Inter-Assay Study were 6.1 and 5.8%, respectively.

Inerlukine-18 assay

Serum level of IL-18 was measured using Human IL-18 Platinum ELISA kit (Bender MedSystems GmbH, Austria). Briefly, diluted samples (1:2 with Sample Diluent) were dispensed in duplicate to the sample wells. Biotin-Conjugate was dispensed into each well and they were incubated at room temperature (18 to 25°C) for 2 hours. Plate was washed 6 times using wash buffer and diluted Streptavidin-HRP was added to all wells, including the blank wells, covered with an adhesive film and incubated at room temperature for 1 hour. TMB Substrate Solution was added to all wells after 6 times wash and it the plate was incubated at room temperature for 10 min in dark. Finally, reaction was stopped by quickly addition of Stop Solution into each well. Plate was read immediately after the Stop Solution is added by a plate reader using 450 nm as the primary wave length and 620 nm as the reference wave length. The limit of detection of human IL-18 was 9.0 pg/ml and Coefficient of Variation (CV) for Intra-Assay and Inter-Assay Study were 6.5 and 8.1%, respectively.

C3 and C4 Complement assay

Serum level of C3 and C4 were measured using MININEPH™, a laser nephelometer (Binding Site, UK). Detection range and sensitivity of the kit for C3 were 0.28–4.44 g/L and 280 mg/L, respectively. In case of C4, they were 0.08–1.23 g/L and 80mg/L, respectively.

Statistical Analysis

All tests were done at least three times and the results were expressed as mean ± SD or Median ± SE. Differ-

FORM OF DISEASE	Age (Years)	IL-18 concentration (pg/ml)	dsDNA (IU/ml)	C4 (g/L)	C3 (g/L)	ESR 1st h (mm)	WBC (cell /mm3)	24h urinary protein (mg/Uv)	Duration (Months)	
High Activity SLE	Mean±Std. Deviation	33.48 ±10.46	281.15 ±318.38*§	3.02 ±1.87	0.17 ±0.1	0.82 ±0.55§	59.68 ±34.22	4926 ±1933	2106 ±1940§	24.52 ±29.37
	Median± Std. Error	32.0 ±2.09	189.69 ±63.67	3.10 ±0.37	0.13 ±0.02	0.96 ±0.11	52.0 ±6.84	4800 ±386	1500 ±388	12.0 ±5.87
Low activity SLE	Mean±Std. Deviation	30.56 ±12.25	184.24 ±184.86*	1.93 ±1.10	0.35 ±0.42	1.2 ±0.33	15.84 ±10.46	5643 ±2333	225 ±421	57.48 ±56.42
	Median ± Std. Error	27.0 ±2.45	145.13 ±36.97	2.10 ±0.22	0.2 ±.084	1.10 ±0.06	14.0 ±2.09	4800 ±466	20±86	36.0 ±11.28
Controls	Mean±Std. Deviation	30.28 ±5.53	85.12 ±57.94*	——-	——-	——-	——-	——-	——-	——-
	Median± Std. Error	29.0 ±1.10	67.66 ±11.58	——-	——-	——-	——-	——-	——-	——-

Table 1: demographic and lab findings in different groups. * Significant difference was found between serum level of IL-18 in patients with high disease activity and other groups (p=0.026 and p =0.005, respectively). § Significant correlation was found between serum IL-18 levels with dsDNA, C3 complement and 24 hours urinary protein levels (r2= 0.38, p=0.03; r2=–0.42, p=0.02 and r2= 0.53, p= 0.007, respectively).

ences or correlations among the variables were statistically analyzed using appropriate tests by SPSS 17 and p<0.05 was accepted as significant.

3. RESULTS

Age: As it shown in Table 1, the mean age of active, no active and control subjects were 33.48±10.46, 30.56±12.25 and 30.28±5.53, respectively. No significant difference was found among the groups (ANOVA, P > 0.5).

Inerlukine-18 assay: Mean concentration of serum IL-18 in SLE patients with high and low activity and control subjects were 281.15±63.67, 184.2493 ± 36.97 and 85.12±11.58, respectively. A significant difference was found between patients with high activity SLE and patients with low activity disease and control subjects (ANOVA, Tukey test, p=0.026 and p =0.005, respectively) but significant difference was not found between patients with low disease activity and control subjects (ANOVA, Tukey test, p >0.25) (Table 1 and Figure 2).

In patients with high disease activity, a positive correlation was found between serum levels of IL-18 with DsDNA antibody (r^2= 0.38, p=0.03), protein levels in 24 hours collected urine (r^2= 0.53, p= 0.007), platelet counts (r^2= 0.506, p=0.01) and it correlates negatively with serum C_3 levels (r^2=–0.42, p=0.02), but no significant correlation was found between IL -18 concentration and patients' age, duration of disease, serum levels of C4, erythrocyte sedimentation rate and white blood cell count (p>0.5). Such correlations were not found in patients with low activity SLE too.

Anti-Ds-DNA antibody: A significant difference was found in serum level of Anti-Ds-DNA antibody between SLE patients with high and low disease activity (t-test, p=0.016) (Table 1). Detectable level of Anti-Ds-DNA antibody was not found in the controls. In SLE patients with high activity, a positive correlation was found between serum levels of Anti-Ds-DNA antibody and protein levels of 24 hours collected urines, platelet and white blood cell counts (0.012, 0.004 and 0.007 respectively).

C3 and C4 Complement assay: Serum levels of C3 and C4 in patients with high disease activity were significantly lower than patients with low disease activity (t-test, p=0.006) (Table 1). No significant correlation was found between serum levels of C3 or C4 with other factors (p>0.5).

4. DISCUSSION

Autoimmune diseases such as systemic lupus erythematosus, macrophage activation syndrome, rheumatoid arthritis, Crohn's disease, psoriasis and ischemia, including acute renal failure in humans, are thought to be mediated, in part, by IL-18 (7). The current study was sought to evaluate serum levels of IL-18 in Iranian females SLE patients with high disease activity, i.e. SLEDAI-2K score ≥7with, low disease activity and control subjects. Measurement of disease activity in systemic lupus erythematosus (SLE) plays a vital role in evaluating of outcomes, differences, responses to a new drug proposed, and also for assessing disease longitudinally for observational and clinical trials /11). Since the early 1980s, several validated and updated instruments have been available that SLEDAI-2K is one of them with high reliability and validity (12, 13). SLEDAI-2K was used in the current study as an instrument for evaluation of disease activity. We found that its levels in SLE patients with high disease activity, i.e. SLEDAI-2K score ≥7, is significantly higher than patients with low disease activity and control subjects. Similar findings of several studies are in favor of our findings (14, 15, 16). Park et al were reported that serum level of IL-18 significantly correlate with disease activity (16), but no significant correlation was found in the current study that might be due to difference in study design while they followed the patients up after treatments for about forty days and significant correlation was only reported for the patients with active form of disease (16). In the other study, a significant difference was also reported between SLE patients and normal subjects but the difference between patients with high and low activity score was not statistically different (9). Instead of score 7 in the current study, they used score 5 to grouped the patients (9) and it could be assumed as a reason for the observed dissimilarly. As it reported by Park et al (16), we also found a significant correlation between serum level of IL-18 and anti ds-DNA in the patients with high disease activity. A significant inverse correlation between serum level of IL-18 and C3 complement were also found in the current study which was not reported by the others (9, 16). A significant correlation between serum level of IL-18 and

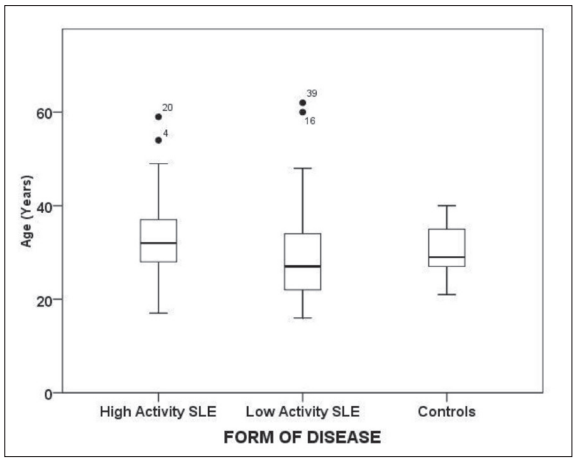

Figure 1. age distribution in different groups. Median, 25 and 75 % quartiles and outlier data is showed in the figure and no significant difference was found among groups (p > 0.2)

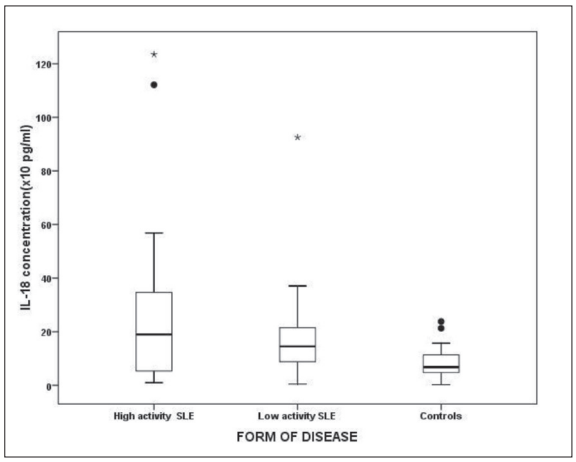

Figure 2. Serum levels of IL-18 in different groups. Median, 25 and 75 % quartiles and outlier data are showed in the figure. Significant difference was found between SLE patients with high disease activity and low disease activity and controls (p=0.026 and p =0.005, respectively). No significant difference was found between SLE patients with low disease activity and controls.

protein content of 24 h urine samples was found in the current study which points to the reported role of IL-18 in lupus related nephritis (10).

5. CONCLUSION

The current findings are in good agreement with the recently reported findings that shed lights to the role(s) of IL-18 in the pathogenesis of SLE, mainly in the form of disease with high activity and it revealed that grouping of the patients using SALDI score of 7 has better discriminative power than other scores which reveals correlation of serum level of IL-18 with well-known role players of SLE pathogenesis like as anti-ds-DNA antibodies, C3 and C4 components of serum complements and renal involvements. Therefore, therapeutic manipulation of IL-18 or its receptor as it suggested by Novick et al (9) seems reasonable which may leads to new approach in the clinical management of SLE patients.

Acknowledgement

This study has been partially granted by the Deputy of Research and Technology, Urmia University of Medical Sciences.

REFERENCES

1. Tsokos G C. Systemic lupus erythematosus. N Engl J Med. 2011; 365: 2110-2121.
2. Marks SD, Tullus K. Autoantibodies in systemic lupus erythematosus. Pediatr Nephrol. 2012; 27: 1855-1868.
3. Freire EA, Souto LM, Ciconelli RM. Assessment measures in systemic lupus erythematosus. Rev Bras Reumatol. 2011; 51: 70-80.
4. Lattanzi B, Consolaro A, Solari N, Ruperto N, Martini A, Ravelli A. Measures of disease activity and damage in pediatric systemic lupus erythematosus: British Isles Lupus Assessment Group (BILAG), European Consensus Lupus Activity Measurement (ECLAM), Systemic Lupus Activity Measure (SLAM), Systemic Lupus Erythematosus Disease Activity Index (SLEDAI), Physician's Global Assessment of Disease Activity (MD Global), and Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index (SLICC/ACR DI; SDI). Arthritis Care Res (Hoboken). 2011; 63(Suppl 11): S112-S117.
5. Crispin JC, Liossis SN, Kis-Toth K, Lieberman LA, Kyttaris VC, Juang YT, Tsokos GC. Pathogenesis of human systemic lupus erythematosus: recent advances. Trends Mol Med. 2010; 16: 47-57.
6. Commins SP, Borish L, Steinke JW. Immunologic messenger molecules: cytokines, interferons, and chemokines. J Allergy Clin Immunol. 2010; 125: S53-S72.
7. Dinarello CA. Interleukin-18 and the pathogenesis of inflammatory diseases. Semin Nephrol. 2007; 27: 98-114.
8. Wang D, Drenker M, Eiz-Vesper B, Werfel T, Wittmann M. Evidence for a pathogenetic role of interleukin-18 in cutaneous lupus erythematosus. Arthritis Rheum . 2008; 58: 3205-3215.
9. Novick D, Elbirt D, Miller G, Dinarello CA, Rubinstein M, Sthoeger ZM. High circulating levels of free interleukin-18 in patients with active SLE in the presence of elevated levels of interleukin-18 binding protein. J Autoimmun. 2010; 34: 121-126.
10. Shimizu C, Fujita T, Fuke Y, Ito K, Satomura A, Matsumoto K, Soma M. High circulating levels of interleukin-18 binding protein indicate the severity of glomerular involvement in systemic lupus erythematosus. Mod Rheumatol. 2012; 22: 73-79.
11. Romero-Diaz J, Isenberg D, Ramsey-Goldman R. Measures of adult systemic lupus erythematosus: updated version of British Isles Lupus Assessment Group (BILAG 2004), European Consensus Lupus Activity Measurements (ECLAM), Systemic Lupus Activity Measure, Revised (SLAM-R), Systemic Lupus Activity Questionnaire for Population Studies (SLAQ), Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K), and Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index (SDI). Arthritis Care Res (Hoboken). 2011; 63(Suppl 11): S37-S46.
12. Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang CH. Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. Arthritis Rheum. 1992; 35: 630-640.
13. Isenberg DA, Gordon C. From BILAG to BLIPS - disease activity assessment in lupus past, present and future. Lupus. 2000; 9: 651-654.
14. Wong CK, Ho CY, Li EK, Lam CW. Elevation of proinflammatory cytokine (IL-18, IL-17, IL-12) and Th2 cytokine (IL-4) concentrations in patients with systemic lupus erythematosus. Lupus. 2000; 9: 589-593.
15. Amerio P, Frezzolini A, Abeni D, Teofoli P, Girardelli CR, De Pita O, Puddu, P. Increased IL-18 in patients with systemic lupus erythematosus: relations with Th-1, Th-2, pro-inflammatory cytokines and disease activity. IL-18 is a marker of disease activity but does not correlate with pro-inflammatory cytokines. Clin Exp Rheumatol. 2002; 20: 535-538.
16. Park MC, Park YB, Lee SK. Elevated interleukin-18 levels correlated with disease activity in systemic lupus erythematosus. Clin Rheumatol. 2004; 23: 225-229.