The Correlation Between Plasma Interleukin-18 Level And Disease Activity In Jordanian Patients With Different Connective Tissue Diseases

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Abstract: Background: Interleukin 18 (IL-18) is a newly defined cytokine that has an important role in the Th1 type immune response and it shares similar functional properties with IL-12. Elevation of IL-18 levels in autoimmune diseases such as Systemic Lupus Erythematosus (SLE) has been reported in previous studies. Aims & Objectives: To study the IL-18 levels in the plasma of Jordanian patients suffering from Rheumatoid Arthritis (RA), Systemic Lupus Erythematosus (SLE) and Behcet’s disease and to correlate the disease activity and IL-18 level for the three diseases. Materials and method: The study includes one hundred and twenty three patients, forty-one patients for each disease group, and forty-one as a control group. Plasma IL-18 levels were determined using Enzyme-Linked Immuno Sorbent Assay (ELISA). Results: IL-18 levels were significantly elevated in the three study group compared to the controls (p< 0.0001). The level of IL-18 was significantly correlated with the disease activity in SLE patients (r = 0.602, p = 0.000). However, there was no significant correlation with disease activity in RA patients (r = -0.205, p=0.198, r= -0.196, p=0.22 respectively). In case of Behcet’s disease there was no significant difference between active and inactive disease. Conclusion: Elevated IL-18 levels were noticed in patients with SLE, RA and Behcet’s disease. IL-18 levels are significantly correlated with disease activity in SLE patients only. This finding may be of used for monitoring the disease activity in SLE patients.

Keywords: Plasma Interleukin-18, Rheumatoid Arthritis, Systemic Lupus Erythematosus, Behcet's disease.

Introduction

Rheumatoid Arthritis (RA) is a chronic inflammatory autoimmune disease associated with destruction of cartilage and underlying bone in the joint. It is a common autoimmune disease of joints. RA is thought to be a Th1 associated disease (Dayer, 1999). Systemic Lupus Erythematosus (SLE) is a systemic autoimmune disease characterized by the activation of Polyclonal B lymphocytes, production of auto antibodies, and formation of immune complexes causing tissue and organ damage (Amital et al., 1999). Behcet’s disease is a systemic vasculitis characterized by recurrent oral and genital ulcers, and ocular inflammation, and which may involve the joints, skin, central nervous system and gastrointestinal tract. (Sara, 2004). Interleukin (IL)-18, formerly called IFN-γ-inducing factor, is a novel Th1 cytokine produced by kuffer cells, activated macrophages, keratinocytes, intestinal epithelial cells, osteoblasts and adrenal cortex cells (Mclnnes, et al., 2000); (Dinarello, 1999). It plays an important role in the Th1 response to toxic shock and shares functional similarities with IL-12. IL-12 can induce the production of IL-18 and has a synergistic effect with IL-18 on the activation of natural killer (NK) and cytotoxic T lymphocytes (CTL) (Fehniger, et al., 1999).

The association of IL-18 with pathological condition had been evaluated in many diseases including the hemophagocytic lymphohistiocytosis (Takada, et al., 1999), Crohn's disease (Monteleone, et al., 1999) and leukemia (Taniguchi, et al., 1997). Connective tissue diseases are important causes of morbidity and mortality. They are autoimmune in nature with variable manifestations of both clinical course and management strategies. Factors that govern severity, response to treatment and outcome are largely unknown. Genetic factors, cytokines and environmental factors have been indicated in the pathogenesis and pathology of these diseases.

Aims & objectives

This study aims at measuring levels of Interleukin-18 (IL-18) in plasma samples taken from Jordanian patients suffering from different autoimmune diseases, mainly Rheumatoid Arthritis (RA), Systemic Lupus Erythematosus (SLE) and Behcet's disease, and to correlate plasma IL-18 levels with disease activity.

Materials and methods

Sample selection

This research was conducted on 123 Blood samples obtained from patients referred to the rheumatology Out Patient Clinic of the Jordan University Hospital. The blood samples were distributed according to diagnosis into three groups. First group consists of 41 patients (32 females and 9 males) with an age range between 16 and 69 years, all patients within this group had confirmed diagnosis of SLE, according to the 1982 revised American Rheumatism Association criteria (EM Tan et al., 1982). Second group consists of 41 patients including 28 females and 13 males with an age range between 22 and 70 years, all had confirmed diagnosis of RA, according to the 1987 revised American Rheumatism Association criteria for the classification of RA (Arnett, et al., 1988). The third group also consists of 41 patients including 28 males and 13 females with an age range between 17 and 64, according to...
the internationally agreed diagnostic criteria for Behcet's disease, all patients had confirmed diagnosis of Behcet's disease. Apparently healthy individuals with 41 age and sex matched, were included as a control.

**Data collection methods:**

1-Blood specimen collection
Seven ml of peripheral blood were collected from each patient and control individual into EDTA containing tube using Vacutainer system (Becton Dickinson Vacutainer System, France). Plasma was separated within 4 hours by centrifugation at 2000g at 4°C for 10 minutes, then it was aliquoted and stored at -70°C until it was analyzed (Wong, et al., 2000). [13]

2-Evaluation of disease activity
The evaluation of the disease activity of SLE patients was performed according to the SLE Disease Activity Index (SLEDAI) (Bombardier, et al., 1992). [14] The disease activity of RA patients was evaluated in line with the clinical examination depending on the number of swollen joints and their tenderness. The disease activity of Behcet's patients was evaluated according to the persistence of oral and/or genital ulcers, eye involvement and skin disease or other disease manifestation. The disease was considered active when one or more of these clinical manifestations were present, and inactive in the absence of these clinical manifestations.

3-IL-18 Determination assay
Plasma IL-18 concentrations of patients and control subjects were measured by Enzyme Linked-Immunosorbent Assay (ELISA) using human IL-18 ELISA Kit manufactured by Medical and Biological Laboratories, Nagoya, Japan.

**Data analysis and interpretation**
Quantitative continuous measurements were expressed as mean, median, standard deviation (SD) and range. Two-tailed T-test was used for comparison between two independent sample populations at 95% confidence level (P<0.05). Spearman's correlation coefficient was used to test the correlation between IL-18 concentration and SLE Disease Activity Index and Pearson correlation to assess the correlation between IL-18 concentrations and swollen and tender joint count in Rheumatoid Arthritis patients. All analyses were performed using the Statistical Package for the Social Sciences (SPSS) statistical software for windows, Version 9.0 (SPSS, Chicago, IL, USA). A probability (P) value of <0.05 was considered as indicating a significant difference.

**Results**

SLE patients and their control group
Forty-one Jordanian patients with SLE consists of 32 females and 9 males, (mean age ± SD = 32.5±10.1 yr), were recruited for this study. The mean duration of the disease at the time of patient’s evaluation was 8.5 ± 6.6 yr, ranging from 6 months to 25 yr. Forty-one, sex-and-age-matched, apparently healthy, 28 females and 13 males, (mean age ± SD = 34 ± 7.2 yr), ranging from 23 to 57 yr were recruited as control subjects.

RA patients and their control group
Forty-one Jordanian patients with RA consists of 28 females and 13 males, (mean age ± SD = 46.4 ± 14.7 yr), were recruited for this study. The mean duration of the disease at the time of patient's evaluation was 10.2 ± 8.9 yr, ranging form 1-32 yr. Forty-one, sex-and-age-matched, apparently healthy, 28 females and 13 males, (mean age ± SD = 39.2 ± 8.1 yr), ranging from 23 to 57 yr were recruited as control subjects.

Behcet’s patients and their control group
Forty-one Jordanian patients with confirmed Behcet's disease consists of 28 males and 13 females, (mean age ± SD = 35.1 ± 10.3 yr), were recruited for this study. The mean duration of the disease at the time of the patient's evaluation was 8.5 ± 6.6 yr, ranging from 6 months to 25 yr. Forty-one, sex-and-age-matched, apparently healthy, 28 males and 13 females, (mean age ± SD = 34 ± 7.2 yr), ranging from 23 to 57 yr were recruited as control subjects.

IL-18 level in the plasma of SLE patients and their controls
The mean concentration of IL–18 in SLE patients was 620 ± 269 pg/ml ranging from 179 to 1229 pg/ml. the levels of plasma IL–18 of the control group were ranging from 66 to 452 pg/ml with a mean value of 282 ± 73. The comparison of both patients versus controls IL – 18 levels was statistically significant, (p< 0. 0001). When the levels of IL–18 was studied according to the disease activity, there were significant differences between the levels of IL-18 as shown in table 1.

Correlation between IL-18 level and SLE Disease Activity Index (SLEDAI) Score
When we studied the correlation between IL-18 level and SLE disease activity, a significant positive correlation was demonstrated (r= 0.602, p= 0.000) as shown in Figure 1.
IL-18 levels in the plasma of RA patients and their control group

The mean concentration of IL-18 in RA patients was $519 \pm 268$ pg/ml ranging from 67 to 1156 pg/ml. The levels in the plasma of control group were ranging from 68 to 420 pg/ml with a mean value of $287 \pm 77$. The comparison of both patients versus controls IL-18 levels was statistical significant, ($p<0.0001$). When the levels of IL-18 were studied according to the disease activity, there were significant differences between the levels of IL-18 as shown in table 1. When we studied the correlation between IL-18 level and the swollen and tender joint count, we were unable to demonstrate any statistical significance with a correlation factor of ($r = -0.205$, $p = 0.198$) and ($r = -0.196$, $p = 0.220$) as shown in Figure 2–a and b respectively.

![Figure 2a](image)

**Figure (2a):** correlation between IL-18 level and swollen joint count in RA patients.

IL-18 levels in the plasma of Behcet’s patients and their controls

The mean concentration of IL-18 in Behcet’s Disease patients was $623 \pm 353$ pg/ml ranging from 144 to 1409 pg/ml. The levels of IL-18 in the plasma of the control group ranging from 66 to 406 pg/ml with a mean value of $280 \pm 79$. The comparison of both patients versus controls IL-18 levels was statistically significant, ($p<0.0001$). When the levels of IL-18 were studied according to the disease activity, there were significant differences between the levels of IL-18 as shown in table 3.

Discussion

In the current study our findings showed that the plasma IL-18 levels were significantly elevated in patients with SLE as a group and as active and inactive subgroup in comparison to the control group (621 pg/ml VS 280 pg/ml respectively with $p<0.0001$). Moreover, there was a significant difference in IL-18 level between patients of active and inactive disease. These findings were in agreement with those reported by other authors (Wong, et al., 2000)$^{[13]}$. They estimated the plasma, but there was no significant difference between the levels in SLE patients without renal involvement and the control group. Amerio, et al.$^{[16]}$ showed that Th1 and Th2 cytokine can be elevated in SLE patients and that only IL-18 level correlated with disease activity. The findings for RA showed that the plasma IL-18 levels were significantly elevated in patient with RA as a group, and in patients with active and inactive disease as subgroup, in comparison to the control group the mean concentration 402, 388, 619, and 305 Pg/ml respectively. However, there was no significant statistical difference between the patients with active and inactive. Similar to this study’s findings, Bresnihan$^{[17]}$ reported that the serum levels of IL-18 was significantly higher in RA in comparison to psoriatic arthritis ($p<0.001$), and also the found no statistical significant correlations between serum levels of IL-18 and the number of swollen joints, which confirmed our results. Gracie et al.$^{[18]}$ also showed an elevation of IL-18 in 67% of synovial fluids obtained from patients with RA. Munakata et al.$^{[19]}$ also found IL-18 significantly elevated levels in both serum and synovial fluid obtained from patients with RA in comparison to those obtained from patients with osteoarthritis (OA) patients and normal volunteers. Yamamura$^{[20]}$ and his co-workers also reported similar results. The results obtained for Behect’s disease were similar to those demonstrated in SLE and RA groups, were IL-18 levels significantly elevated (610-pg/ml) in comparison to the control group (280-pg/ml). However, there was no significant difference between patients with active and inactive disease. To the best of our knowledge, we were unable to find any published data about the IL-18 in Behcet’s disease.
Recommendation:
We recommended to evaluate other types of autoimmune diseases rather than Rheumatoid Arthritis (RA) and Behcet’s disease, and correlate plasma interleukin 18 level with disease activity. Further study can be conducted using large sample.

Conclusion
Elevated IL-18 levels were noticed in patients with SLE, RA and Behcet’s disease. IL-18 levels are significantly correlated with disease activity in SLE patients only. This finding may be of used for monitoring the disease activity in SLE patients.

Table (1): Plasma Levels of IL-18 in patients with SLE and the control group (values in pg/ml)

<table>
<thead>
<tr>
<th>1L-18 Pg/ml</th>
<th>All SLE N=41 (a)</th>
<th>Active SLE N=22 (b)</th>
<th>Inactive SLE N=20 (c)</th>
<th>Control Group N=41 (d)</th>
<th>Statistical comparisons (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean+ SD</td>
<td>620+ 269</td>
<td>787+ 243</td>
<td>446+ 169</td>
<td>282+ 73</td>
<td>(a)/(d)*p.&lt; 0.0001</td>
</tr>
<tr>
<td>Median</td>
<td>620</td>
<td>704</td>
<td>449</td>
<td>280</td>
<td>(b)/(d)*p.&lt; 0.0001</td>
</tr>
<tr>
<td>Range</td>
<td>(179-1229)</td>
<td>(418-1229)</td>
<td>(179-712)</td>
<td>(66-452)</td>
<td>(c)/(d)*p.&lt; 0.0001</td>
</tr>
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<td></td>
<td>(b)/(c)*p.&lt; 0.0001</td>
</tr>
</tbody>
</table>

*p(significant)

Table (2): Plasma Levels of IL-18 in patients with RA and the control group (values in pg/ml)

<table>
<thead>
<tr>
<th>1L-18 Pg/ml</th>
<th>All RA Patients N=41 (a)</th>
<th>Active RA N=22 (b)</th>
<th>Inactive RA N=19 (c)</th>
<th>Control Group N=41 (d)</th>
<th>Statistical comparisons (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean+ SD</td>
<td>519+ 268</td>
<td>480+ 256</td>
<td>564+ 281</td>
<td>287+ 77</td>
<td>(a)/(d)*p.&lt; 0.0001</td>
</tr>
<tr>
<td>Median</td>
<td>402</td>
<td>388</td>
<td>619</td>
<td>305</td>
<td>(b)/(d)*p.&lt; 0.0001</td>
</tr>
<tr>
<td>Range</td>
<td>(67-1156)</td>
<td>(225-1156)</td>
<td>(67-1087)</td>
<td>(68-420)</td>
<td>(c)/(d)*p.&lt; 0.0001</td>
</tr>
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<td></td>
<td>(b)/(c)*p.&lt; 0.0001</td>
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<td></td>
<td>(b)/(c) p.= 0.324</td>
</tr>
</tbody>
</table>

*p(significant)

Table (3): Plasma Levels of IL-18 in patients with Behcet’s disease and the control group (values in pg/ml)

<table>
<thead>
<tr>
<th>1L-18 Pg/ml</th>
<th>All Behcet’s disease N=41 (a)</th>
<th>Active Behcet’s disease N=27 (b)</th>
<th>Inactive Behcet’s disease N=14 (c)</th>
<th>Control Group N=41 (d)</th>
<th>Statistical comparisons (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean+ SD</td>
<td>623+ 353</td>
<td>675+ 345</td>
<td>522+ 359</td>
<td>280+ 79</td>
<td>(a)/(d)*p.&lt; 0.0001</td>
</tr>
<tr>
<td>Median</td>
<td>610</td>
<td>615</td>
<td>353</td>
<td>280</td>
<td>(b)/(d)*p.&lt; 0.0001</td>
</tr>
<tr>
<td>Range</td>
<td>(144-1409)</td>
<td>(219-1409)</td>
<td>(144-2226)</td>
<td>(66-406)</td>
<td>(c)/(d)*p.&lt; 0.0001</td>
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<td></td>
<td></td>
<td></td>
<td>(b)/(c) p.= 0.194</td>
</tr>
</tbody>
</table>

*p(significant)

References


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[7]. Fehniger TA. Shah MH, Torner MJ. Differential cytokine and chemokine gene by human NK cells following activation with IL-18 or IL-15 in combination with IL-12: implications for the innate immune response. *Journal of Immunology*. 1999; 162: 4511-4520


