Expression level of serum Interleukin-37 in Rheumatoid Arthritis patients and its correlation with Disease Activity Score

Nimrah Akram, Arshad Jamal, Sajjad Ullah, Ahmed Bilal Waqar*, Khurshid Iqbal

Abstract

Background: Interleukin-37 (IL-37) is a member of IL-1 cytokine family. IL-37 immunosuppresses the pathogenesis of rheumatoid arthritis via down-regulating pro-inflammatory cytokines. The aim of the current study was to evaluate the expression level of IL-37 in rheumatoid arthritis (RA) patients and its correlation with the disease activity score in 28 joints (DAS-28).

Methods: In the current study, forty-six RA patients, having a ratio of 19 males and 27 females, and twenty healthy controls (11 males and 9 females) were included. DAS-28 was measured on the basis of patients' clinical observations of the tender and swollen joints, physical examination and erythrocyte sedimentation rate (ESR). ESR was measured according to the Westergren method. Serum IL-37 level was measured by ELISA. Depending upon the DAS28 calculations the patients were divided in four groups as; 19 in remission, 6 had mild disease activity, 6 were in moderate state and 15 patients were found with severe disease activity.

Results: Serum IL-37 levels were found markedly raised in RA patients (mean = 862.6) than in healthy individuals (mean ± SD = 4.4 ± 1.74 pg/ml). Further, our results suggest that level of IL-37 increased significantly from mild (mean ± SD = 829.17 ± 61.40 pg/ml) to moderate (mean ± SD = 1307.5 ± 165.1 pg/ml) and severe (mean ± SD = 1607 ± 86.8 pg/ml) disease prognosis.

Conclusion: Thus we conclude, IL-37 has a positive correlation with DAS28 and thus has a potential role in RA pathogenesis.
Introduction

Rheumatoid arthritis (RA) is a systemic, chronic inflammatory autoimmune disorder characterized by immune cells infiltration, inflammatory joints and synovial hyperplasia [1]. It primarily affects the peripheral joints. The accurate pathogenesis and etiology of RA remain elusive, though cytokines are considered to play a central role in disease progression through inflammation and articular destruction [2]. An imbalance between all pro-inflammatory and anti-inflammatory cytokines leads to the progression of disease symptoms [3]. RA causes swelling of joints, tenderness of joints and also the destruction of synovial joints that leads to severe disability and premature fatality [4]. Rheumatologists don’t understand the obvious cause of RA development but the most possible risk factors include genetic background, hormones, environmental factors, smoking [5], microbes in the bowel and obesity. Middle aged mostly women are frequently affected as compared to men [6]. Estimated RA prevalence is recorded as 1% worldwide [1]. The pathogenesis of RA is a multi-step process and is divided as autoimmune, systemic inflammation and then leading to bone destruction. Certain cells of the immune system start attacking the healthy tissues causing inflammation specifically in the synovium, the tissue lining the joints. Due to the detection of auto-antibodies including rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPAs), RA is considered to be an autoimmune disease [7]. RF is not considered as RA specific as it may be detected in hepatitis C viral infection and older people whereas ACPA is RA specific and plays significant role in RA pathogenesis [8]. RF and ACPAs, cause an immune deregulation in RA patients. Due to the localization of inflammatory cells in the synovial lining of the joints, thickening and hyperplasia develop [9].

Interleukin 37 (IL-37) has been included in interleukin 1 cytokine family (IL1F7) [10,11]. IL-37 is an anti-inflammatory cytokine and has a potential role in various inflammatory diseases like ankylosing spondylitis [12], graves’ disease [13], rheumatoid arthritis [14], systemic lupus erythematosus (SLE) [15] and inflammatory bowel disease [16]. High levels of IL-37 are detected in the serum and synovial fluid of all RA patients with different ranges depending upon the severity of the disease. Elevated IL-37 level inhibits pro-inflammatory cytokines synthesis [17]. IL-37 has anti-inflammatory activity in both innate and acquired immunity. It plays a significant role in the control of RA pathogenesis by suppressing inflammation and innate immunity. Injecting IL-37 is considered to be the novel RA therapy. It functions as a down regulator of local and systemic inflammations [18]. It is expressed in various cells, tissues and organs including plasma cells, monocytes, epithelial cells, macrophages, dendritic cells, thymus, testis and uterus [19]. Its specific role is to down regulate inflammation, innate immunity and adaptive immunity. Lipo-polysaccharides (LPS) may also be induced for the maintenance of IL-37 expression levels in RA patients so that it might not be completely diminished. Treatment with LPS is found very helpful in the improvement of lungs and kidneys functioning and also in the prevention of liver damage [20].

Disease Activity Score (DAS) is a scoring strategy implies for the evaluation of disease severity of RA [21]. DAS28 includes 28 joints to be examined. DAS28 is calculated on the basis of number of all swollen and tender joints [21], visual assessment of patients’ disease severity and erythrocyte sedimentation rate (ESR). ESR is required for indicating the degree of inflammation. RA treatment therapies can slow down the joint deterioration and inflammation leading to disability. On the basis of DAS28, RA patients can be categorized in four levels; in remission DAS28<2.6, in low disease activity DAS28≤3.2, in moderate disease activity DAS≤5.1 and in high or severe disease activity DAS>5.1 [21]. DAS28 evaluation every four weeks can help your doctors and you to decide whether your treatment needs any changes for further improvements [22]. Disease activity scoring was made to evaluate the disease severity of the patients clinically to minimize other related complications and also to modify the treatment therapy according to the severity level of the disease. Originally DAS was developed to assess the disease onset in early affected RA patients. Newly developed indices for DAS evaluation are to investigate the severity of patients in early stage as well as in the severe diseased. DAS28 is now considered and more preferable and simple method for the evaluation of RA subjects.

The current study demonstrates, the serum level of IL-37 in RA patients and its correlation with DAS28 score in inactive and severely diseased patients.
Methods

Patients and clinical evaluations:
Forty-six RA patients from Sharif Medical City hospital Lahore-Pakistan were included in this study. Patients fulfilling the Rheumatology criteria of American College of Rheumatology were selected. In addition, twenty healthy individuals of more than 30 years of age were taken as controls. This study was first approved by the institutional Ethical Review Board (ERB) of Imperial College of Business Sciences (ICBS), Lahore-Pakistan. Informed consent was signed from all the study participants and counseling, related to our further investigations, was done before subjecting the patients.

All patients were presented with varying symptoms and complaints. The total count of tender and swollen joints of each patient was calculated on DAS28 score; ESR was tested on immediate basis and disease severity was assessed for DAS28 calculations. DAS28 grouping was done according to the disease severity of the patients. On the basis of DAS28 evaluations, all patients (n=46) were divided in four groups: remission (DAS28≤2.6), mild (2.6<DAS28≤3.2), moderate (3.2<DAS28≤5.1) and severe (DAS28>5.1). While examining the patient clinically, blood samples were collected for ESR and interleukin level measurement.

Serum IL-37 level measurement:
Clotted blood samples were centrifuged at 1300 g (RCF) for 10 minutes and serum was separated in specifically labeled serum cups. All the samples were stored at freezing temperature until analysis. Serum IL-37 level of all the samples was detected via enzyme linked immunosorbent assay (ELISA) (Cusabio CSB E16185h). IL-37 specific antibody was pre-coated on microplate. 100 μl standard and samples were loaded in allocated wells and incubated for 2 hours at 37°C. Wells were washed twice to remove unbound proteins. 100 μl of biotin antibody was added and plate was wrapped in aluminum foil and incubated at 37°C for 1 hour. After incubation and washing, 100 μl horse radish peroxidase (HRP) avidin solution was added and incubated for one hour. Wells were again washed; 90 μl TMB substrate was added and kept in dark for 30 minutes. Color reaction developed was directly proportional to the presence of IL-37 levels in it. After incubation, 50 μl of stop solution was added. OD values of the microplate were recorded at 450nm.

Statistical analysis:
Prism Graph Pad was used to analyze the data. Unpaired t-test was employed to determine statistically significant difference among various diseased groups. The difference was significant when p value was less than 0.05. The correlation between IL-37 level and DAS28 was analyzed by Pearson’s test. Significant correlation was evaluated as P<0.05.

Results

Clinical assessment of research subjects:
For the evaluation of serum IL-37 expression levels, forty-six RA patients (19 males and 27 females) average age of 51 years (ranged from 33 to 79 years) and twenty healthy controls (11 males and 09 females) average age of 43 years (ranged from 36 to 53 years) were included. DAS28 results were analyzed on the basis of tender and swollen joints count, ESR and the physical severity of the disease regarding patients’ pain (table 1). On the basis of DAS28 results, 46 RA patients were divided: 19 remission, 6 mild, 6 moderate and 15 in severe disease activity group.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>RA patients (n=46)</th>
<th>Healthy controls (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>51 (33-79)</td>
<td>43 (36-53)</td>
</tr>
<tr>
<td>Gender (male: female)</td>
<td>19:27</td>
<td>11:09</td>
</tr>
<tr>
<td>Swollen joints</td>
<td>4 (0-18)</td>
<td>—</td>
</tr>
<tr>
<td>Tender joints</td>
<td>3 (0-18)</td>
<td>—</td>
</tr>
<tr>
<td>ESR results (mm/hour)</td>
<td>35 (10-99)</td>
<td>10 (4-17)</td>
</tr>
<tr>
<td>DAS28 grouping</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Remission</td>
<td>19 patients</td>
<td>—</td>
</tr>
<tr>
<td>(DAS28 ≤2.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>06 patients</td>
<td>—</td>
</tr>
<tr>
<td>(2.6&lt;DAS28≤3.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>06 patients</td>
<td>—</td>
</tr>
<tr>
<td>(3.2&lt;DAS28≤5.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>15 patients</td>
<td>—</td>
</tr>
<tr>
<td>(DAS28&gt;5.1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Clinical features of all research subjects

Gender ratio:
Among healthy individuals, 11 were males and 9 were females. Accordingly the gender ratio of RA patients included, 19 patients were male and 27 were of female gender. RA remission patients were 10 males and 9
Expression level of serum Interleukin-37 in Rheumatoid Arthritis patients and its correlation with Disease Activity Score

You’re reading females. Out of six patients from mild group, 2 were males and 4 were females. Moderate group patients included 2 males and 4 females. In severe diseased patients, 5 were males and 10 were females. (Figure 1)

Figure 1: Graphical representation of gender distribution of healthy individuals and all RA groups

Serum IL-37 measurement:
The level of IL-37 in healthy controls’ serum was remarkably low, mean ± SD = 4.4 ± 1.7 pg/ml. On contrary, it was markedly raised in RA patients with mean = 862.6 pg/ml. Interestingly, IL-37 level increased significantly with disease progression from mild to moderate and severe conditions. Concisely, in remission patients the IL-37 level (mean ± SD = 145.16 ± 48.39 pg/ml) was lowest among the four categories. Additionally, the level of IL-37 in the mild, moderate and severe patients was observed to be [mean ± SD] 829.17 ± 61.40 pg/ml, 1307.5 ± 165.1 pg/ml and 1607 ± 86.8 pg/ml respectively (Table 2) (Figure 2).

Figure 2: Graphical representation of the serum level of IL-37 detected among different disease group patients i.e., Remission (n=19), Mild (n=6), Moderate (n=6) and Severe (n=15). The data is statistically analyzed by unpaired t-test. Data is statistically significant when p < 0.05.

Table 2: IL-37 ELISA results of Healthy Controls and RA patients

<table>
<thead>
<tr>
<th>IL-37 ELISA results (pg/ml)</th>
<th>Control Group</th>
<th>Remission Group</th>
<th>Mild Group</th>
<th>Moderate Group</th>
<th>Severe Diseased Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>4.46</td>
<td>145.16</td>
<td>829.17</td>
<td>1307.5</td>
<td>1607</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>1.74</td>
<td>48.39</td>
<td>61.40</td>
<td>165.1</td>
<td>86.8</td>
</tr>
</tbody>
</table>

Table 2: IL-37 ELISA results of Healthy Controls and RA patients

Serum IL-37 level and DAS28 correlation:
Serum IL-37 level was found markedly low in all healthy controls having no chronic or inflammatory diseases, while it was found strongly elevated in all RA patients. Interleukin levels and disease severity was correlated. The P value of correlation between serum IL-37 patients and DAS28 of RA subjects was significant (P<0.05) (Table 3). Our results demonstrated that the correlation between remission (-0.018), mild (-0.077) and moderate (-0.307) patient groups and IL37 was negative. However, the IL37 level was positively correlated with severe patients (0.168).

Discussion
RA is a systemic, chronic inflammatory and autoimmune disease having cell infiltration, uncontrolled synovial cells proliferation and cartilage destruction [23]. Numerous studies have reported the elevated levels of pro inflammatory cytokines such as TNF, IL-1 and IL-6 in RA patients. Inhibition of these inflammatory cytokines is considered to be the treatment goal for such patients [24]. Most of the pro inflammatory cytokines have a potential role in the pathogenesis of the disease, as their serum and synovial fluid levels are significantly raised [4,25]. In various inflammatory autoimmune diseases IL-37, an anti-inflammatory cytokine, levels increases with the increase of inflammatory cytokines [17]. Thus IL-37 has been emerged as a novel anti-inflammatory cytokine that possesses extra- and intracellular properties. It suppresses inflammation and the innate immune responses [26]. Its potential protective role is noticed in several animal models such LPS induced shock [27], dextran sulphate sodium induced colitis [28], insulin resistance and obesity induced inflammation, hepatitis B virus infection [29], Aspergillus infection [30], Graves’ disease and concanavalin A-induced hepatitis. On the other hand, down-regulation of IL-37 expression is observed in Bechet’s disease [31], invertebral disc regeneration [32] and Vogt-Koyanagi-Harada disease [33].
In the current study we are reporting the correlation of IL-37 level with the prognosis of RA in Pakistani population, which was not reported before. Our data demonstrated the increased serum IL-37 level all RA patients (mean = 862.6 pg/ml) and was reduced considerably in healthy individuals (mean ± SD was 4.4 ± 1.7 pg/ml). Published studies suggest the significant relation between the IL-37 level and the disease activity and pro-inflammatory cytokines production. Yang L et al, reported a positive correlation of elevated IL-37 with pro-inflammatory cytokines and disease activity in all RA patients [8, 13]. It has been reported that immune histochemical staining of synovial lining of RA patients have elevated IL-37 level as compared to healthy controls [20]. The current study demonstrated that serum IL-37 level was markedly low in remission RA (mean ± SD = 145.16 ± 48.39 pg/ml) patients than severely diseased active RA patients (1607 ± 86.8 pg/ml) suggesting that remission RA patients had low pro-inflammatory cytokines and decreased symptoms and joint complications. This suggests that elevated production of pro-inflammatory cytokines might have triggered the elevated levels of IL-37 [3]. Both pro- and anti-inflammatory cytokines levels are known to be raised in RA patients. Sequentially, the presence of pro-inflammatory cytokines may up regulate the production of IL-37 [17] and IL-37 may down regulate the excessive level of pro-inflammatory cytokines [19]. Although the expression level of IL-37 is high in severe active RA patients, however as compared to the level of pro-inflammatory cytokines it is still low, and one of the most probable reasons of disease progression in such patients [34]. Thus the uncontrolled inflammation in RA patients is due to inadequate working of antagonistic cytokines, specifically IL-37 [19]. A previous study has shown that in all the inactive remission state patients there was significantly decreased level of serum IL-37 and was majorly regulated by pro-inflammatory cytokines [3]. Based on our results and previously reported results, it is hypothesized that IL-37 produced due to activated immune responses has a vital role in RA pathogenesis, and promote inflammation via suppression of pro-inflammatory cytokines.

In current study, we demonstrated that serum IL-37 level had positive correlation with DAS28 in RA patients (p < 0.05). Consistent with the results, a positive correlation of IL-37 and DAS28 was previously recorded in China among 50 RA patients [3] and in Changhai among 34 RA patients [19]. Thus, IL-37 is the most probable biomarker of RA diagnostics, disease activity evaluation and remedial response observations [35]. The results of the present study demonstrated that women were more frequently affected by RA as compared to men (27 females and 19 males). It was reported in Oslo that the prevalence of RA in females was two times greater than in men [36]. Jacqueline has also reported that females are affected two to three times greater as compared to males. Moreover it has been noticed that in majority of the autoimmune diseases females are predominantly affected [37].
In conclusion, the level of IL-37 in serum was markedly high in RA patients, in Pakistani population, specifically in the severe diseased patients. Positive correlation was demonstrated between IL-37 levels and disease severity (with P value less than 0.05). The limitation of the current study is that, we could not investigate the serum level of other anti and pro-inflammatory cytokines and their correlation with the level of IL-37. However, the current data highlights the fact that increased serum IL-37 has a potential anti-inflammatory role in RA pathogenesis.

Conflict of Interests
Authors declare that there is no conflict of interest for publishing this study.

Acknowledgement
The Study was supported by Faculty of Health and Allied Sciences, Imperial College of Business Studies.

References
22. Fransen J, Stucki G, van Riel PLCM. Rheumatoid arthritis measures: Disease Activity Score (DAS), Disease Activity Score-28 (DAS28), Rapid Assessment of Disease Activity in Rheumatology (RADAR), and Rheumatoid Arthritis Disease Activity Index (RADAI). Arthritis & Rheumatology, (2003); 49(S5): S214–24.
24. Hurkmans E, van der Giesen FJ, Vlieet Vlieland TP, Schoone J, Van den Ende ECHM. Dynamic exercise programs (aerobic capacity and/or muscle strength training) in patients with
Expression level of serum Interleukin-37 in Rheumatoid Arthritis patients and its correlation with Disease Activity Score


This work is licensed under a Creative Commons Attribution-Non Commercial 4.0 International License. To read the copy of this license please visit: https://creativecommons.org/licenses/by-nc/4.0/