# Role of Pro- and Anti-Inflammatory Cytokines in Rheumatoid Arthritis: Correlation with Disease Activity

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**Summary:**

**Background:** Rheumatoid arthritis (RA) is a chronic and debilitating autoimmune disease characterized by chronic inflammation with subsequent cartilage and bone destruction. Cytokines are key mediators of inflammation and can be found in abundance both in the joint and blood of patients. This study was designed to evaluate the role of pro- and anti-inflammatory cytokines in pathogenesis of RA, as well as study the correlation among these cytokines.

**Patients and methods**: Forty patients with RA and thirty age-matched healthy controls were included in this study. Serum cytokines were measured by enzyme-linked immunosorbent assay.

**Results:** The serum levels of pro-inflammatory cytokines (IL-1α, IL-2, IL-6, IL-8, IL-12 and TNF-α) were significantly higher in RA patients than in healthy controls ($p<0.01$, $p<0.05$). Moreover, these levels were significantly increased in active RA patients than in inactive RA ($p<0.01$, $p<0.05$). On the other hand, the serum levels of IFN-γ and anti-inflammatory cytokines (IL-4, IL-10) showed no significant differences between RA patients and healthy controls and neither between active RA patients and inactive RA ($p>0.05$). Interestingly strong positive correlation was found among each of (IL-1α, IL-2, IL-6, IL-8, IL-12 and TNF-α), $p<0.05$.While strong negative correlation was noticed between IL-6 and (IL4 and IL-0) and also between TNF-α and (IL4 and IL-0), $p<0.05$.

**Conclusion:** The current study suggests that serum levels of pro-inflammatory cytokines (IL-1α, IL-2, IL-6, IL-8, IL-12 and TNF-α) may play an important role in RA and may be used as a marker of disease activity. Moreover imbalance between pro- and anti-inflammatory cytokines may yield effective therapeutic targets in this inflammatory disease.

**Key words:** Rheumatoid arthritis, Pro-inflammatory cytokines, Anti- inflammatory cytokines.

Fac Med Baghdad
2010; Vol. 52, No. 3
Received Apr. 2010
Accepted May 2010

## Introduction:

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic joint inflammation and subsequent joint destruction. The chronic inflammatory process is mediated through a complex cytokine network. The release of specific cytokines into the systemic circulation has been observed in a variety of inflammatory disease including RA. Their concentration levels usually reflect disease severity and prognosis (1, 2). Cytokines are differentiated into two groups on the basis of their action, pro-inflammatory cytokines (TNF-α, IFN-γ, IL-1, IL-2, IL-6, IL-8, IL-12, IL-15, IL-17 and IL-18) and anti-inflammatory cytokines (IL-4, IL-10). In RA, the balance between pro-and anti-inflammatory cytokines determines the degree and extent of inflammation, and thus can lead to different clinical effects (2). IL-1, IL-2 and TNF-α are among the many cytokines that can act alone or in synergy as mediators of tissue damage or chronic inflammation and which have been implicated in the pathogenesis of arthritis (3, 4). IL-1α and TNF-α are able to act locally to induce bone and cartilage resorption, both cytokines, independently or together with IFN-γ, can induce the proliferation of synoviocytes. The main stimuli for IL-8 production are IL-1 and TNF-α. IL-8 as a neutrophile chemoattractant is responsible for the increased number of neutrophils in RA joints, and therefore for

The clinical manifestation of joint and pain. In vivo, it has been shown that inoculation of only one intra-articular injection of IL-8 induces synovial hyperplasia similar to the human RA (5). In RA excess levels of IL-6 are produced in the joints, particularly in the thin tissue layer covering the joint. It may also cause permanent damage of bone and cartilage, as it encourages the body to break down bones and blocks the formation of bones (6,7). Another important pro-inflammatory cytokine is IL-12, produced by different antigen presenting cells. It has been shown critical role in inducing Th1 phenotype, thus initiating cell- mediated immune response. It was suggested that IL-12, modulating cellular and humoral immune response, is involved in the pathogenesis of immune rheumatic diseases (8). Consequently, the potential beneficial effects of anti-inflammatory cytokines such as IL-4 and IL-10 in RA are of

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great interest. IL-4 inhibit the production of IL-1 α, TNF-α and IL-6, it does not merely inhibit many pro- inflammatory cytokines; but also up-regulates the expression of anti-inflammatory mediators such as IL-1 receptor antagonist and the IL-1 type II receptor, indicating that IL-4 is an anti-inflammatory cytokine (9). IL- 10 can effectively block the production of the pro-inflammatory cytokines (TNF-α, IL-1, and IL-8) by synovial macrophages and synoviocytes. Based on its immunomodulating functions, IL-10 has been considered an attractive candidate for therapeutic applications for treatment of acute and chronic inflammation, autoimmunity, cancer and infectious disease (10). This study was designed to evaluate the role of pro- and anti-inflammatory cytokines in pathogenesis of RA, as well as study the correlation among these cytokines.

**Patients and Methods**:

Patients: Forty patients with RA, 36 females and 4 males, their age range from 25-66 years, were included in this prospective study. They are attendants of Rheumatology and Rehabilitation center at Al-Kadhumyia Teaching Hospital in Baghdad, compared with 30 age and sex matched apparently healthy individuals. Patient's group was divided in to two groups according to the disease activity (31with active RA and 9 with inactive RA). Determination of serum pro-and anti-inflammatory cytokines: The serum obtained from RA patients and healthy controls were analyzed for, IL-1α, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, TNF-α and IFN-γ using commercially available ELISA kits following the manufactures' instructions (BioSource Europe S.A. company, Belgium).

**Statistical analysis:**

Comparison of serum cytokines levels among groups were calculated by Kruskal-Wallis-test and Mann-Whitney-test. Correlation between the different parameters was calculated by the spearman test and p values of $P<0.01$and $P<0.05$ were considered significant.

**Results:**

Forty Iraqi patients with RA (36 females and 4 males) were recruited for the present study; their mean age was 48±12 years (range 25-66 years). Clinical presentation demonstrated that 31 (77%) of patients were with active RA (ARA) and 9 (23%) were with inactive RA (IARA). Thirty (75%) of patients were positive for rheumatoid factor (RF) and 10 (25%) patients had negative RF. Cytokines profiles: The serum cytokines profiles were analyzed both in RA patients and control individuals. The median serum levels of pro-inflammatory cytokines IL-1α, IL-2, IL-6, IL-8, IL-12 and TNF-α were significantly elevated in RA patients compared to controls ($p<0.01$, $p<0.05$). Interestingly, the median serum level of IFN-γ was not elevated in patients and there was no significant differences between patients and controls ($p>0.05$), table-1. On the other hand, the present results were not observe any differences in the median serum levels of anti-inflammatory cytokines (IL-4 and IL-10) between patients and controls ($p>0.05$), table-2. When the RA patients were categorized according to the disease activity, the following cytokines IL-1α, IL-2, IL-6, IL-8, IL-12 and TNF-α were noted to be significantly elevated in active RA patients as compared to inactive patients ($p<0.01$, $p<0.05$). Conversely, the median serum levels of IFN-γ, IL-4 and IL-10 was not observed any difference between active and in inactive RA patients ($p>0.05$), table-1 and table-2. Correlation among pro-and anti-inflammatory cytokines: The present study revealed significant strong positive correlation among each of (IL-1α, IL-2, IL-6, IL-8, IL-12 and TNF-α) $p<0.05$.While strong negative correlation was noticed between IL-6 and each of (IL4, IL-0) and also between TNF-α and each of (IL4, IL-0), $p<0.05$, as shown in table-3.

Table 1: The difference in median levels of serum pro-inflammatory cytokines IL-1α, IL-6, IL-8 , IL-12, TNF-α (pg/ml) and IL-2, IFN-γ (IU/ml) among the three studied groups.

| | ARA cases | IARA cases | Healthy control | P (kruskalwallis) |
|---|---|---|---|---|
| Serum IL-1α | | | | |
| Minimum | 3 | 0 | 0 | |
| Maximum | 73 | 30 | 10 | |
| Median | 21 | 1.5 | 4.00 | p<0.01 |
| NO. | 31 | 9 | 30 | |
| P (Mann-Whitney) | | | | |
| ARA X IARA p<0.05 | | | | |
| ARAX Healthy control p<0.01 | | | | |
| Serum IL-2 | | | | |
| Minimum | 0.00 | 0.00 | 0.00 | |
| Maximum | 77.2 | 30.00 | 50.00 | |
| Median | 33.00 | 0.00 | 7.5 | p<0.01 |
| NO. | 31 | 9 | 30 | |
| P (Mann-Whitney) | | | | |
| ARA X IARA p<0.05 | | | | |
| ARAX Healthy control p<0.01 | | | | |
| Serum IL-6 | | | | |
| Minimum | 1.8 | 1.8 | 1.5 | |
| Maximum | 161.0 | 60.0 | 30.0 | |
| Median | 19.0 | 1.8 | 4.0 | p<0.01 |
| NO. | 31 | 9 | 30 | |
| P (Mann-Whitney) | | | | |
| ARA X IARA p<0.05 | | | | |
| ARAX Healthy control p<0.01 | | | | |
| Serum IL-8 | | | | |
| Minimum | 7 | 7 | 2 | |
| Maximum | 296 | 21 | 44 | |
| Median | 24.0 | 7.0 | 2.0 | p<0.01 |
| NO. | 31 | 9 | 30 | |
| P (Mann-Whitney) | | | | |
| ARA X IARA p<0.01 | | | | |
| ARAX Healthy control p<0.01 | | | | |
| Serum IL-12 | | | | |
| Minimum | 10 | 10 | 6 | |
| Maximum | 292 | 65 | 37 | |
| Median | 70.0 | 11 | 8.5 | p<0.01 |
| NO. | 31 | 9 | 30 | |
| P (Mann-Whitney) | | | | |
| ARA X IARA p<0.05 | | | | |
| ARAX Healthy control p<0.01 | | | | |
| Serum TNF-α | | | | |
| Minimum | 3.2 | 3 | 1.5 | |
| Maximum | 256 | 63.2 | 32 | |
| Median | 40.1 | 4 | 4 | p<0.01 |
| NO. | 31 | 9 | 30 | |
| P (Mann-Whitney) | | | | |
| ARA X IARA p<0.01 | | | | |
| ARAX Healthy control p<0.01 | | | | |
| Serum IFN-γ | | | | |
| Minimum | 0.00 | 0.00 | 0.00 | |
| Maximum | 10 | 6 | 6 | |
| Median | 0.00 | 0.00 | 1.00 | p>0.05 |
| NO. | 31 | 9 | 30 | |
| P (Mann-Whitney) | | | | |
| ARA X IARA p>0.05 | | | | |
| ARAX Healthy control p>0.05 | | | | |

**Table 2: The difference in median levels of serum anti-inflammatory cytokines IL-4 and IL-10 )pg/ml) among the three studied groups.**

| | ARA CASES | IARA CASES | HEALTHY CONTROL | P (KRUSKAL-WALLIS |
|---|---|---|---|---|
| Serum IL-4 | | | | |
| Minimum | 0 | 0 | 0 | |
| Maximum | 1 | 4 | 9 | |
| Median | 2.00 | 0.00 | 3.50 | p>0.05 |
| NO. | 31 | 9 | 30 | |
| P (Mann-Whitney) | | | | |
| ARA X IARA p>0.05 | | | | |
| ARAX Healthy control p>0.05 | | | | |
| Serum IL-10 | | | | |
| Minimum | 0.00 | 0.00 | 0.00 | |
| Maximum | 47.2 | 10 | 41.0 | |
| Median | 2.0 | 5.00 | 1.00 | p>0.05 |
| NO. | 31 | 9 | 30 | |
| P (Mann-Whitney) | | | | |
| ARA X IARA p>0.05 | | | | |
| ARAX Healthy control p>0.05 | | | | |

**Table 3: Correlation among pro-and anti-inflammatory cytokines**

| spearman's correlation | IL-1α | IL-2 | IL-4 | IL-6 | IL-8 | IL-10 | IL-12 | TNF-α | IFN-γ |
|---|---|---|---|---|---|---|---|---|---|
| IL-1α | 0 | | | | | | | | |
| IL-2 | 0.62 | 0 | | | | | | | |
| IL-4 | 0.47 | 0.57 | 0 | | | | | | |
| IL-6 | 0.75* | 0.58 | -0.78* | 0 | | | | | |
| IL-8 | 0.85* | 0.49 | 0.55 | 0.74* | 0 | | | | |
| IL-10 | 0.52 | 0.59 | 0.65 | -0.71* | 0.66 | 0 | | | |
| IL-12 | 0.74* | 0.35 | 0.41 | 0.55 | 0.87* | 0.67 | 0 | | |
| TNF-α | 0.80* | 0.84* | -0.87* | 0.85* | 0.74* | -0.85* | 0.51 | 0 | |
| IFN-γ | 0.61 | 0.41 | 0.53 | 0.66 | 0.89* | 0.44 | 0.58 | 0.84* | 0 |

*: strong significant if p>0.70

**Discussion:**

Several studies in recent years were conducted to confirm the role of cytokines in the pathogenesis of RA. Disequilibrium between stimulatory and inhibitory factors has a fundamental role in pathogenesis of this disease (11, 12, and 13). In line with present findings, high levels of TNF-α, IL-1α, IL-2, IL-6, IL-8 and IL-12 have also been reported by other studies (14, 15). Rostamian et al., found that serum levels of TNF-α and IL-1α in active patients with bone erosion were higher than that in inactive patients and healthy control. So they concluded that serum levels of inflammatory factors such as TNF-α and IL-1α could be associated with RA pathogenesis. However; anti-inflammatory drugs against these cytokines would be a useful treatment mode (12). High concentration of IL-2 in active RA patients than inactive patients and healthy control were observed in current study, and was consistent with Manhal (16), but at variance with Altomonte and colleagues, who reported that IL-2 significantly reduce in RA patients compared to control and explained that the reduce levels of IL-2 may be an expression of deficiency of T-cells to produce IL-2 in the active phases of RA or may be due to a possible absorption of IL-2 by lymphocyte receptors (17). It is well known that IL-6 required for the development of autoimmune arthritis; IL-6 deficient mice are resistant to the induction of autoimmune disease (18). Tchorzewski and associates mentioned that slightly diminished IL-6 production by non stimulated lymphocytes from RA patients and enhanced IL-6 production after PHA stimulation has proved the possibility that IL-6 can play a key role in the development of arthritis in these patients. Moreover reduced severity of RA was observed

in IL-6 deficient mice (19). IL-8 was detected in the sera of most our RA patients, Correspondingly Peichl et al, pointed out to the important role of IL-8 and its auto-antibodies in inflammatory processes of RA, and may provide a clinically useful marker for the diagnosis of disease activity (20). On the other hand the present result was in contrast to other studies (21, 22). Similar to published findings, the current study was found increase in serum levels of IL-12 in RA patients when compared to controls (23, 24). Ebrahimi and colleagues study 43 Iranian patients with RA, and observed that serum IL-12 was significantly high in those patients when compared to healthy controls, thus the authors concluded that serum IL-12 may be more important and predictive factor in RA course and in the active form of the disease (23), as we confirmed in the present study. Regarding the serum levels of IFN-γ, previous studies showed that serum IFN-γ was increased in RA patients as compared to controls (24, 16). On the other hand Sakito et al, observed that serum IFN-γ correlated well with the number of peripheral lymphocytes but not reflect the activity of RA (25). However; our findings failed to show any significant increase or correlation with disease activity in RA patients. Conversely, serum levels of anti-inflammatory cytokines (IL-4 and IL-10) were not increase in our patients as reported by many papers (2, 14, and 15). Normal levels of anti-inflammatory cytokines may support the result in the present study regarding the negative correlation between pro-inflammatory (TNF-α, IL-6) and anti-inflammatory cytokines because one of the normal biological activities of IL-4 and IL-10 is down regulates of pro-inflammatory cytokines production by monocyte (9, 26). Similarly Lacki and colleagues observed inverse correlation between IL-10 and IL-6, and conducted that IL-10 decrease IL-6 production (27). Finally, this variation among different cytokines in the same sample reflects the intricate cytokine network and its regulatory functions. The balance between pro-inflammatory and anti-inflammatory cytokines in RA determines the degree and extent of inflammation which can lead to major clinical effects (14). In conclusion the current study suggests that serum levels of pro-inflammatory cytokines (IL-1α, IL-2, IL-6, IL-8, IL-12 and TNF-α) may play an important role in RA and may be used as a marker of disease activity. Moreover imbalance between pro- and anti-inflammatory cytokines may yield effective therapeutic targets in this inflammatory disease.

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