# Effect of Methotrexate on Serum Levels of IL-1α and IL-8 in Rheumatoid Arthritis

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## ABSTRACT:

**BACKGROUND:**

Rheumatoid arthritis is a chronic systemic inflammatory disease affects many tissues and organs, but principally attacks flexible (synovial) joints**.** Methotrexate is the most commonly used disease-modifying antirheumatic drug for the treatment of rheumatoid arthritis.

**OBJECTIVE:**

The aim of this study was to evaluate the effect of methotrexate on serum levels of IL-1α and IL-8 in rheumatoid arthritis.

**SUBJECTS AND METHODS:**

Blood samples were collected from 50 patients with rheumatoid arthritis (25 patients without treatment and 25 patients are received methotraxate) and from 30 healthy age and sex matched individuals served as controls. Serum IL-1α and IL-8 were measured by means of enzyme-linked immune-sorbent assay.

**RESULTS:**

The present results showed that serum levels of IL-1α and IL-8 were significantly higher in RA patients than in healthy controls (P<0.01), furthermore, level of IL-1α was significantly decrease in patients treated with methotraxate as compared to those patients who have received no treatment (P<0. 01). On the other hand serum level of IL-8 did not showed any significant differences between patients treated with methotraxate and those patients without treatment (P>0. 05).

**CONCLUSION:**

These finding demonstrate that methotrexate turns out to be a good inhibitor for IL-α production. In addition, IL-1α and IL-8 may have a significant role in the pathogenesis of rheumatoid arthritis, and could be use as .


**KEY WORDS:** rheumatoid arthritis, methotrexate, cytokines.

## INTRODUCTION:

Rheumatoid arthritis (RA) is an autoimmune disease causing preclinical systemic abnormalities that finally lead to synovial inflammation and destruction of joint architecture. T-cell activation and migration occur as an early consequence of RA, and these cells adopt a proinflammatory phenotype. The activation and infiltration of T cells and macrophages in the synovium result in production of cytokines[(1)].

Pathogenetic therapy of RA is a complex task, according to modern views on the development of autoimmune inflammation in RA, cytokine imbalance plays a great part in RA pathogenesis [(2,3)].Studies by Elliott *et al*. have shown thatregulation of cytokine levels in patients with RA may also be a novel approach for treatment of these diseases [(4,5)]. They described successful treatment of refractory arthritis in patients with RA by infusion of antibodies to tumor necrosis factor-alpha (TNF-α), suggesting a key role for this cytokine in the pathogenesis of chronic arthritis.Methotrexate is an anchor drug for the treatment of RA because of its efficacy, acceptable safety, and cost. MTX is used in mono-therapy or in combination with either biological agents or other small molecule anti-rheumatic drugs [(6)].

Regarding its anti-rheumatic mechanisms, it has been reported that MTX promotes adenosine release, inhibits pro-inflammatory cytokine

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production, suppresses lymphocyte proliferation, and reduces serum immunoglobulin via the inhibition of folic acid metabolism [7,8]. However, loss or reduction of its efficacy is a major problem in the treatment of RA. The efficacy of MTX varies among treated patients, and approximately 30% of patients discontinue administration within one year [9,10]. Therefore the aim of this study was to evaluate the effect of MTX on the levels of IL-1α and IL-8 in RA.

**Table 1: Demographical picture of the studied groups.**

| No. | Demographical Parameters | RA Cases | Healthy Control | P-Value |
|---|---|---|---|---|
| 1 | Age (years)[Mean ±SE] | 42.3± 2.01 | 43.60±2.54 | P=0.17[NS] |
| 2 | Females [No (%)] | 40 (80%) | 24 (80%) | P=0.15[NS] |
| 3 | Males [No (%)] | 10 (20%) | 6 (20%) | P=0.88[NS] |
| | Total number | 50 | 30 | |

SE: standard error, NO: number, %: percentage, NS: non significant

## SUBJECTS AND METHODS:

Fifty patients with RA (25 patients without treatment and 25 patients were received MTX at a stable dose for at least 4 weeks) were enrolled in this study their age range from 25 – 62 years. They were from attendants seeking treatment in the Rheumatology and Rehabilitation center at Baghdad Teaching Hospital in Medical city in Baghdad.The diagnosis of each case was established by clinical examination done by specialist rheumatologist in the Hospital. Apparently healthy volunteers their ages and gender were matched with patients group and consisted of 30 individuals who were considered as control. All of them had no history or clinic evidence of RA. Their age ranged from 20- 60 years. Levels of IL1α and IL-8 have been estimated by using commercially available Enzyme-Linked Immunosorbent Assay (ELISA) and performed as recommended in leaflet with kits (Immunotech, France). Statistical analyses were computer assisted using SPSS version 13 (Statistical Package for Social Sciences). The outcome quantitative variable (IL1α and IL-8) were non-normally distributed. Such variable is described by median.

The difference in median of quantitative non-normally distributed variable groups was calculated by Kruskal-Wallis-test and Mann-Whitney-test. P values of $P<0.001$and $P<0.05$ were consideredsignificant.

## RESULTS:

In the present study the age of RA patients ranged between 25-62 years with a mean age of 42.3±2.01 years. Furthermore, there was female's predominance, as shown in table (1).

The current findings showed that there is significant elevation in the median serum level of IL-1α in RA patients without treatment (44pg\ml) in comparison with RA patients treated with MTX (23pg\ml) and healthy control group (11pg\ml), $p<0.001$, table (2). On the other hand, result presented in table (3) failed to showed any significant differences in serum IL-8 level of RA patients who have received no treatment (880pg/ml) in comparison to RA patients treated with MTX (820pg\ml) and to healthy controls (250 pg\ml), ($P>0.05$). In addition, both groups of patients have significant elevation in the median serum level of IL-8 as compared to healthy control ($p<0.001$).

**Table 2: The differences in median serum levels of IL-1α (pg/ml) in three studied groups.**

| Serum IL-1α | RA Cases (No treatment) | RA Cases(With treatment) | Healthy control | P(Kruskal-Wallis-test) |
|---|---|---|---|---|
| Minimum | 18 | 10 | 10 | |
| Maximum | 205 | 146 | 35 | |
| Median | 44 | 23 | 11 | <0.001 |
| NO. | 25 | 25 | 30 | |
| P (Mann-Whitney) | | | | |
| RA with no treatment X Healthy control <0.001 | | | | |
| RA with treatment X Healthy control <0.001 | | | | |

RA: rhumatoid arthritis, IL-α: interleukin 1-alpha

**Table 3: The differences in median serum levels of IL-8 (pg/ml) in three studied groups.**

| Serum IL-8 | RA Cases (No treatment) | RA Cases(With treatment) | Healthy control | P(Kruskal-Wallis-test) |
|---|---|---|---|---|
| Minimum | 180 | 180 | 150 | |
| Maximum | 1900 | 1796 | 490 | |
| Median | 880 | 820 | 250 | >0.05 |
| NO. | 25 | 25 | 30 | |
| P (Mann-Whitney) | | | | |
| RA with no treatment X Healthy control <0.001 | | | | |
| RA with treatment X Healthy control <0.001 | | | | |

IL-8:interleukin-8

## DISCUSSION:

Cytokines that are abundantly produced in the inflamed rheumatoid synovial fluid, such as IL-1, IL-6, IL-8,TNF-α and granulocyte macrophage colony stimulating factor (GM-CSF), play crucial roles in the pathophysiology of RA [(11)]. Several inflammatory cytokines have been evaluated for their potential to predict MTX treatment response, especially in early stages of the disease [(12)].In this study we determine the serum levels of IL-1α and IL-8 in RA patients and found that serum concentrations of these cytokiones were substantially higher as compared with that of healthy control. Similar results were reported by other researchers [(13,14)], who pointed out to the important role of IL-1α and IL-8 in inflammatory processes of RA, and may provide clinically useful markers for the diagnosis of disease activity.Another interesting finding in this work is the reduction in serum levels of IL-1α in RA patients treated with MTX when compared to those patients without treatment and these results are in accordance with the observations of the previous researchers [(8,15,16)], who mentioned that MTX seems to be an efficient inhibitor of cytokine production, this is may be due to inhibition of the de novo synthesis of purines and pyrimidines. Similarly Kraan and colleagues reported that down-regulation of inflammatory cytokines such as TNF-α and IL-1α in rheumatoid synovium has been observed during treatment with MTX [(17)]. In contrast to the present study other results observed that MTX treatment did not affect serum IL-1 levels, and repoted that the MTX response can be enhanced when the agent is used in combination with anti-IL-1therapy, such as anakinra [(18,19)]. Furthermore, Maillefert and co-workers noticed that that serum IL-1 level did not correlate with response to MTX [(20)].Regarding the effect of MTX on serum levels of IL-8, this study did not showed any effect of MTX on the levels of IL-8, this result is in agreement with other studies [(20,21,22)].

## CONCLUSION :

These finding demonstrate that IL-1α and IL-8 may have a significant effect in the pathogenesis of RA, and may be use as an indicators of disease activity.

In addition, MTX turns out to be a good inhibitor for some cytokines production.

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