

# Quantitative determination of IL-2 and IL-10 levels in sera of Iraqi female patients with rheumatoid arthritis .....

Farhan A. Risan, Leen K. Mustafa, Sameer A. Jadaan, Saad M. Abdul-Wahab

## Quantitative determination of IL-2 and IL-10 levels in sera of Iraqi female patients with rheumatoid arthritis

Farhan A. Risan

Leen K. Mustafa

Sameer A. Jadaan

Saad M. Abdul-Wahab

Consultant physician /Clinical Immunology

Teaching laboratories, Baghdad Teaching Hospital, Medical

### Abstract

**Objective:** To investigate the effectiveness and the possible role of Th1 and Th2 cytokines represented by interleukin-2 (IL-2), and interleukin-10 (IL-10) in pathogenesis of rheumatoid arthritis (RA).

**Methods:** The circulating serum levels of IL-2 and IL-10 were measured by Enzyme-linked immunosorbent assay (ELISA, sandwich method) in sixty two female rheumatoid arthritis patients and twenty eight normal controls.

**Results:** Significant increase was observed on the level of both IL-2 and IL-10 when compared with control value [(1.06032±0.169 for patients, and 0.292±0.316 for controls) and (5.286±1.787, 1.104±1.258), respectively.  $p>0.0005$ ].

**Conclusion:** In rheumatoid arthritis, Th1 cytokines represented by IL-2 have increased. Th2 cytokines including IL-10 tend to be raised in order to minimize the inflammatory effects of IL-2.

**Keywords:** Rheumatoid arthritis, IL-2, IL-10.

**Rheumatoid arthritis (RA)** is a chronic, systemic, inflammatory autoimmune disease that is manifested in destructive polyarthritis in association with serological evidence of auto reactivity<sup>1</sup>.

RA is characterized by chronic pain, joint destruction, premature mortality and an elevated risk of disability with high costs for those suffering from the disease and for society<sup>2</sup>. It is distributed universally and there are no reports of areas or ethnic groups in which the disease is not found with an overall prevalence of 1-2% and female preponderance<sup>3-6</sup>.

The perpetuation of the rheumatoid process depends on the involvement of different cellular lines, including synovial cells such as fibroblast, perivascular cells, macrophages, dendritic cells and other immunocompetent cells like T and B lymphocytes<sup>7</sup>.

The inflammatory process and subsequent joint destruction are mediated by the activation of intracellular signaling pathways that stimulate the production of cytokines which are important in the regulation of the synovial inflammation<sup>8</sup>.

T cells have been implicated in RA due to their presence in the synovium and class II MHC association. Naive CD4 T cells can be differentiated into multiple effector types, including Th1 and Th2 phenotypes<sup>9</sup>. Th1 cells secrete characteristic effector molecules like (IL-2),

## Quantitative determination of IL-2 and IL-10 levels in sera of Iraqi female patients with rheumatoid arthritis .....

Farhan A. Risan, Leen K. Mustafa, Sameer A. Jadaan, Saad M. Abdul-Wahab

which are involved in the defense against intracellular pathogens and have been implicated in many autoimmune diseases<sup>10,11</sup>.

IL-10 secreted by Th2 cells, has predominantly anti-inflammatory and immunoregulatory properties through its inhibitory action on Th1 cells<sup>12</sup>. Macrophages and dendritic cells play a key role in the pathogenesis of RA<sup>13</sup>. Monocytes differentiate into dendritic cells during early stages of RA. IL-10 inhibits this transformation<sup>14</sup>. Both cytokines have been expressed in the synovium of RA patients<sup>15</sup> what suggest a potential role for these cytokines in the pathogenesis of rheumatoid arthritis.

### **Patients, Materials and Methods:**

#### **Sampling and selection of patients**

Sera were obtained from 62 female patients with rheumatoid arthritis. The age range was (30-70) years. In addition to 28 blood specimens were collected from apparently healthy individuals served as control group. The samples were collected within approximately six months, started from December 2010 till May 2011 from patients attending Baghdad teaching hospital.

All patients were proved to have RA and fulfilled four conditions or more of the revised 1987 ACR criteria published by the American college of rheumatology. The consent of the patients and/or their relatives was taken before entry into the study.

**Materials:** IL-2 ELISA kit was purchased from (Biosource, Europe, S.A. cat. No.KAP 1241) and IL-10 ELISA kit purchased from (Biosource, Europe, S.A. cat. No.KAP 132). The relevant statistical analyses were applied in order to analyze the results of this study.

#### **Methods:**

Blood samples (5 ml) were drawn by venipuncture and have been centrifuged (after being clotted) at 4000 rpm for 5 minutes. Serum aliquots were frozen and stored at -20C until analysis. The specimens were thawed immediately before analysis and used at volumes indicated by the manufacturer. The analyses were performed using 96-well microtiter plate enzyme immunoassay kits (ELISA, sandwich method).

#### **Estimation of IL-2**

Interleukin-2 ELISA is a solid phase enzyme with amplified sensitivity immunoassay performed on microtiter plate by using monoclonal antibodies (mAbs) directed against distinct epitopes of IL-2. One hundred  $\mu$ l of incubation buffer was pipetted into wells. Equal volumes of 100  $\mu$ l of calibrator, control and sample were added into appropriate wells. Fifty  $\mu$ l of anti IL-2 HRP conjugate was added. After 2 hours incubation at room temp. on a horizontal shaker set at 700 rpm, liquid was aspirated from each well, then plate was washed 3 times by dispensing 0.4 ml of wash solution into each well. Later, 100  $\mu$ l of chromogenic solution was placed followed by incubation for 15 minutes at room temp. direct sunlight was avoided. Two hundred  $\mu$ l of stop solution was added. The absorbance was read at 450 nm, a standard curve was drawn.

Four-parameter logistic curve fitting was used to build up the calibration curve. By interpolation on the calibration curve, IL-2 concentration in samples was determined.

#### **Measurement of IL-10**

One hundred  $\mu$ l of incubation buffer was pipetted into wells. Equal volumes of 100  $\mu$ l of calibrator, control and sample were added into appropriate wells. After 2 hours incubation at room temp. on a horizontal shaker set at 700 rpm, liquid was aspirated from each well, then plate was washed 3 times by dispensing 0.4 ml of wash solution into each well. One hundred  $\mu$ l of specimen diluents and then 50  $\mu$ l of anti IL-10 HRP conjugate was added. The plate was

## Quantitative determination of IL-2 and IL-10 levels in sera of Iraqi female patients with rheumatoid arthritis .....

Farhan A. Risan, Leen K. Mustafa, Sameer A. Jadaan, Saad M. Abdul-Wahab

incubated again for 2 hours at room temp. followed by three times washing. Later, 200 µl of freshly prepared revelation solution was pipetted into each well within 15 minutes following washing step. The microtiter plate was incubated for 30 minutes at room temp. Direct sunlight was avoided. Fifty µl of stop solution was added and the absorbance was read at 450 nm after making standard curve.

Four-parameter logistic curve fitting was used to build up the calibration curve. By interpolation on the calibration curve, IL-10 concentration in samples was determined.

### Results:

Results obtained from the present study indicated that the number and percentage of rheumatoid arthritis patients with the age group (40-49) years were the most prevalent among other age groups (table 1) followed by the age group (50-59) years [(40.322%) and (25.8%), respectively].

**Table1: Distribution of rheumatoid arthritis Patients according to Age range groups**

| Age groups ( Years ) | Patients number (No. ) | Percentage (%) |
|----------------------|------------------------|----------------|
| (30-39)              | 13                     | 20.967         |
| (40-49 )             | 25                     | 40.322         |
| ( 50-59 )            | 16                     | 25.806         |
| 60-70 )(             | 8                      | 12.903         |
| Total                | 62                     | 100            |

### • IL-2 levels:

By comparison with IL-2 levels of control group, the patients' mean concentration of IL-2 revealed a prominent increase (table 2) with a highly significant difference [1.06032±0.169 for patients , and 0.292±0.316 for controls; p>0.0005].

**Table 2 : the mean concentration of serum IL-2 (pg/ml) in patients and controls**

| Studied groups | No. | Mean of IL-2 | SD       | significance    |
|----------------|-----|--------------|----------|-----------------|
| patients       | 62  | 1.06032      | 0.169129 | P>0.0005<br>HS* |
| controls       | 28  | 0.29268      | 0.316680 |                 |
| total          | 90  |              |          |                 |

\*HS= High significant

### • IL-10 assay:

The mean concentration of IL-10 in patients has demonstrated a highly significant increase (table 3) when compared with its level within the healthy individuals [5.286 ± 1.787, 1.104 ± 1.258, respectively; p>0.0005].

**Table 3: the mean concentration of serum IL-10 (pg/ml) in both studied groups**

| Studied groups | No. | Mean of IL-10 | SD    | Significance   |
|----------------|-----|---------------|-------|----------------|
| patients       | 62  | 5.286         | 1.787 | P>0.0005<br>HS |
| controls       | 28  | 1.104         | 1.258 |                |

# Quantitative determination of IL-2 and IL-10 levels in sera of Iraqi female patients with rheumatoid arthritis .....

Farhan A. Risan, Leen K. Mustafa, Sameer A. Jadaan, Saad M. Abdul-Wahab

|       |    |
|-------|----|
| total | 90 |
|-------|----|

## Discussion

Rheumatoid arthritis is a global disease. It is distributed universally and its prevalence does not appear to significantly vary among different studied groups around the world<sup>3,4</sup>. In the current study, the highest rate of RA patients was at the fourth decade of age, precisely among the patients within the age group (40-49) years followed by the age group (50-59).

The previously conducted international studies stated that the highest proportion of RA cases have shown to be occurred between the ages of 40 and 60 years<sup>16</sup>.

There is no good explanation for such results respecting the age of RA patients but according to the information retrieved from the available literatures, there is a significant gap between the preliminary observation of the signs and symptoms and the time of diagnosis of RA which takes long time to be decided perhaps due to the misleading between rheumatoid arthritis, rheumatic fever and other forms of arthritis<sup>17,18</sup>. Additionally, the most widely used method for diagnosis is the 1987 ACR criteria which are based on several criterions take long time to be happened<sup>19</sup>.

Further, the rheumatoid factor (RF) which is not considered as quite reliable as other alternative tests like anti-cyclic citrullinated peptide antibody (anti-ccp) which is deemed in 2010 ACR/EULAR criteria<sup>20</sup> what makes the possibility of reaching an early diagnosis something difficult.

Current results have showed a significant increase in the mean concentration of sera IL-2 of RA patients compared with controls. T-helper cells (CD4+) have been thought to differentiate into one of two primary effector cell types: T-helper 1 (Th1) and T-helper 2 (Th2) cells<sup>10</sup>. IL-2 secreted by (Th1) has a pivotal role in the disease process of RA. IL-2 is important for CD8+ memory cell generation and maintenance<sup>21</sup>. Lack of CD4+ or IL-2 during primary stimulation of CD8+ T- cells can promote the expression of molecules like PD1, which negatively regulates proliferation<sup>22</sup>.

Moreover, IL-2 promotes differentiation of B-cells into plasma cells which are important in RF production<sup>23</sup>.

Another significant increase was detected in RA patients at the level of IL-10 compared with control value.

The importance of IL-10 has been underscored by the finding that IL-10 knockout mice develop severe autoimmune disease<sup>22</sup>.

There are numerous studies of serum levels of IL-10 in patients with RA. The findings are contradictory, with investigators finding elevated, normal, reduced or undetectable levels of IL-10<sup>15, 24-26</sup>.

It is known that IL-4 promotes the differentiation of naïve CD4+ T-cells into Th2 cells, which in turn produce IL-10. IL-10 inhibits macrophage antigen presentation and decreases expression of MHC I molecules. Macrophages and dendritic cells play a key role in the immunopathogenesis of RA. Monocytes differentiation into dendritic cells during early stages of RA is inhibited by IL-10<sup>27-29</sup>.

Logically, IL-10 level is inversely proportional with the level of IL-2 but according to the current outcomes which have proclaimed an elevation in the level of IL-10, the presumed level of IL-2 should be diminished. However, our results have declared an increase in the level of IL-2 by comparison with the level of healthy individuals. The present results seem to be contradictory with that fact but to a certain extent, results of both cytokines did not register contrast with that rule especially if we took into consideration the clear impact of elevated IL-10 on diminishing IL-2 level

(5.286+<sub>-</sub>1.787, 1.06032+<sub>-</sub>0.169) but that decrease still to show high records when compared with the control value what explained the significant difference in IL-2 value of both studied

# Quantitative determination of IL-2 and IL-10 levels in sera of Iraqi female patients with rheumatoid arthritis .....

Farhan A. Risan, Leen K. Mustafa, Sameer A. Jadaan, Saad M. Abdul-Wahab

groups.

In conclusion, In rheumatoid arthritis Th1 cytokines represented by IL-2 have increased. Th2 cytokines including IL-10 tend to be raised in order to minimize the inflammatory effects of IL-2.

Concisely speaking, both cytokines have manifested paramount importance in pathogenesis of the target disease what shed more lights towards emphasis on treatment by cytokines which has been actually established in developed countries.

## References

- 1-Almanos, Y.; Voulgari, P.V. and Drosos, A.A. 2006. " Incidence and prevalence of RA based on the 1987 ACR criteria: a systematic review". *Semin. Arthritis Rheum.* 36 (3):182-188.
- 2- Klippel, J.H. ; Crofford, L. J. and Stone J. H. 2001. " Primer on Rheumatic Diseases ". 11 th edition. Arthritis foundation, Atlanta, Georgia.
- 3-Hirsch, R. ; Lin, J. and Scott, W.W.J. 1998. " Rheumatoid Arthritis in the Pima Indians: the intersection of epidemiologic , demographic, and genealogic data". *Arthritis Rheum.*41:1464-1469.
- 4-Paget, S.A. ; Gibofsky, A. and Beary, J. F. 2000. " Manual of Rheumatology and outpatient orthopedic disorders : diagnosis & therapy: Division of rheumatic diseases, department of orthopedic surgery, Cornell university Medical college, 4 th edition . New York city.
- 5-Shoenfeld, Y. ; Cervera, R. and Gershwin , M. E. 2008. "Diagnostic criteria in autoimmune diseases". 2<sup>nd</sup> edition. Humana Press, Springer Science, Totowa; USA
- 6-Wingrave, S.J. and Kay, S.R. 1998." Reduction in incidence of RA associated with oral contraceptives". *Lancet I*: 569-571.
- 7-Cambridge, G. and Edwards, J.C. 2006." B-cell targeting in rheumatoid arthritis and other autoimmune diseases". *Nat Rev Immunol* 6:394-403.
- 8-Golbach-Mansky ,R. ; Lee, J. and McCoy, A. 2000. "Rheumatoid Arthritis associated autoantibodies in patients with synovitis of recent onset". *Arthritis Res* 22: 236-243.
- 9-Hatada ,E.N.; Krappmann, D. and Scheidereit, C. 2000. " NF-κB and the innate immune response". *Curr Opin Immunol* 12: 52-58.
- 10-Arend, W.P. 2001. " Physiology of cytokine pathways in rheumatoid arthritis". *Arthritis Rheum* 45:101-106.
- 11- Schrijver, I.A. ; Melief, M. J. and Tak, P.P. 2000. "Antigen-presenting cells containing bacterial peptidoglycan in synovial tissues of rheumatoid arthritis patients coexpress costimulatory molecules and cytokines". *Arthritis Rheum* 43: 2160-2168.
- 12-Keystone, E.; Wherry, J. and Grint, P.1998." IL-10 as a therapeutic strategy in the treatment of rheumatoid arthritis". *Rheum Dis Clin North Am*; 24:269-39.
- 13- Jorgensen, C. ; Apparilly, F. and Sany, J.1999." Immunological evaluation of cytokine and anticytokine immunotherapy in vivo: what have we learnt?". *Ann Rheum Dis*; 58:136-41.
- 14- Allavena, P. ; Piemonti, L. and Longoni, D. 1998. " IL-10 prevents the differentiation of monocytes in dendritic cells but promotes their maturation to macrophages. *Eur J Immunol*; 28: 359-69.
- 15-Cush, J.J.; Splawski, J.B. and Thomas, R. 1995." Elevated IL-10 levels in patients with rheumatoid arthritis". *Arthritis Rheum*;38:69-104.
- 16- Klippel, J.H.; Stone, J. H. and Leslie, J.C. 2008. "Primer on the Rheumatic Diseases". 13 th edition . Arthritis Foundation. Springer.
- 17-Symmons, D.P.M. 2007. " Classification criteria for rheumatoid arthritis –time to abandon rheumatoid factor ?". *Rheumatol*;46:725-6.
- 18-Young, A. 2005. " Early rheumatoid arthritis ".*Rheum Dis Clin North Am* ; 4: 659-79.
- 19- Schellekens, G.A.; Visser, H. and de Jong ,B.A.W. 2000. " The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide". *Arthritis Rheum* 43: 155-63.
- 20- Aletaha, D.; Neogi, T. and Silman, A.J. 2010. "2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative". *Ann. Rheum. Dis.* 69 (9): 1580-1588.
- 21- Laky, K. and Fowlkes ,B.J. 2005." Receptor signals and nuclear events in CD4 and CD8 T cell lineage commitment". *Curr Opin Immunol*;17:116-121.
- 22-Weaver, C.T.; Harrington, L.E. and Mangan, P.R. 2006. " Th17: an effector CD4 T cell lineage with regulatory T cell ties". *Immunity*;24:677-688.
- 23-Kalia, V.; Sarkar, S. and Gourley ,T.S. 2006." Differentiation of memory B and T cells". *Curr Opin Immunol*;18:255-264.
- 24-Lapadula, G.; Iannone, F. and Dell' Accio, F.1995. " Interleukin -10 in rheumatoid arthritis". *Clin Exp Rheumatol*; 13:629-32.
- 25-Cicuttini, F.M.; Byron, K.A. and Maher, D. 1995. " Serum IL-4, IL-10, IL-6 levels in inflammatory arthritis". *Rheumatol Int* 14:201-6.
- 26-Lacki, J.K.; Klama, K. and Mackiewicz, S.H.1995. "Circulating interleukin-10 and interleukin-6 serum levels in rheumatoid arthritis patients treated with methotrexate or gold salts: preliminary report. *Inflamm Res*; 44: 24-6.
- 27-Howard, M.; O'Garra, A. and Ishida, H. 1992." Biological properties of IL-10 ". *J Clin Immunol*;12:239-47.
- 28-De Wall Malefyt, R.; Haanen, J. and Spits, H. 1991. " Interleukin 10 (IL10) and viral IL-10 strongly reduce antigen-specific human T cell proliferation by diminishing the antigen presenting capacity of monocytes via downregulation of class II major histocompatibility complex express ". *J Exp Med*; 174: 915-24.
- 29-Mottonen, M.; Isomaki, P. and Saario, R . 1998." Interleukin-10 inhibits the capacity of synovial macrophages to function

**Quantitative determination of IL-2 and IL-10 levels in sera of Iraqi female patients with rheumatoid arthritis .....**

Farhan A. Risan, Leen K. Mustafa, Sameer A. Jadaan, Saad M. Abdul-Wahab

as antigen-presenting cell". *Br J Rheumatol*; 37:1207-14.

## التقدير الكمي لمستويات الانترلوكينات 2 و 10 في مصول النساء

### العراقيات المصابات بالتهاب المفاصل الرثوي

د. فرحان عيود رسن

د. لين خلوق مصطفى

سمير عبد العباس جدعان

سعد محمد عبد الوهاب

كلية التقنيات الصحية والطبية، بغداد، طبيب استشاري لمناعة سريري، المختبرات التعليمية، مستشفى بغداد التعليمي مدينة الطب بغداد

#### الخلاصة

اجريت الدراسة الحالية لتقييم و بحث مدى التأثير المحتمل والدور الفاعل للوسائط الخلوية المفترزة من قبل الخلايا التائية المساعدة الاولى والثانية متمثلة بالوسائط الخلوية (الانترلوكين 2 و 10) على النشوء المرضي لالتهاب المفاصل الرثياني .

تم قياس مستويات الانترلوكينات 2 و 10 بطريقة الامتزاز المناعي المرتبط بالانزيم (ELISA) في مصول الدم لاثنتان وستون مريضا جميعهم من الاناث بالاضافة الى ثمانية وعشرون عينة لاشخاص اصحاء مثلو مجموعة السيطرة .

بالمقارنة مع قيم ومستويات الانترلوكينات المسجلة من قبل مجموعة السيطرة فإنه تم ملاحظة وجود زيادة هامة ومؤثرة في تراكيز الانترلوكينات .

بالنسبة للانترلوكين 2 فإن القيم المسجلة هي  $1,06032 \pm 0,169$  للمرضى  $0,316 \pm 0,292$  لمجموعة السيطرة ( $P < 0,0005$ ) اما بالنسبة للانترلوكين 10 فهي كالاتي  $1,787 \pm 5,286$  للمرضى  $1,104 \pm 1,258$  لمجموعة السيطرة ( $P < 0,0005$ ).

خلصت الدراسة الحالية الى الاتي:

في مرض التهاب المفاصل الرثوي ترتفع مستويات الانترلوكينات المفترزة من قبل الخلايا التائية ذات النمط الاول متمثلة بالانترلوكين 2 فيما تزداد مستويات الانترلوكينات المنتجة من الخلايا التائية -النمط الثاني ولاسيما الانترلوكين 10 كمحاولة لتقليل التأثير الالتهابي للانترلوكين 2.