Original Article

Cytokine Profile in Patients with Rheumatoid Arthritis

Falah S. Manhal* **PhD**

Summary:

Background: Cytokines produced by inflammatory cells play a pivotal role in synovial inflammation and joint destruction in rheumatoid arthritis.

Patients and Methods: The cytokine serum levels were measured by EASIA (Enzyme amplified sensitivity immunoassay) in sera from 50 RA patients, and 40 healthy donors. Cytokine levels were compared in different RA subpopulations (positive or negative rheumatoid factor (RF), long term or recent onset disease, high or low disease activity). In addition, the possible association with other demographic and clinical parameters (gender, age, etc) was also analyzed.

Results: It was demonstrated that IL-2, IL-6 and IFN-δ levels were elevated in serum samples of RA patients as compared with apparently healthy controls. Maximum elevation of TNF-α was recorded in a few number of patient's sera. There were non significant differences between control and RA patient groups in serum TNF-α level.

Conclusions: Assessing the serum IL-2, IL-6, IFN-δ and TNF-α levels may be helpful in the confirmation of the RA activity. Due to the chronic course of this disease, other inflammatory markers must be identified in order to provide early therapeutic strategies to these patients.

Key words: Rheumatoid Arthritis, Interleukine-2, Interleukine-6, Tumor Necrosis Factor- α, Interferon- δ.

Fac Med Baghdad 2009; Vol. 51, No. 4
Received Mar.2009
Accepted June 2009

Introduction:

Rheumatoid arthritis is a chronic inflammatory disease characterized by synovial inflammation and structural damage of joints. Although the cause of rheumatoid arthritis (RA) remains unknown, the excessive production of proinflammatory cytokines such as tumour necrosis factor-α (TNF-α), interferon gamma, interleukin-6 (IL-6), interleukin-1 (IL-1) and others by intra-articular macrophages occupies a critical pathogenic role in the development and progression of the disease [1, 2]. The key role of TNF-alpha led to the development of highly effective new therapies. TNF-alpha inhibitors, such as monoclonal anti-TNF-alpha antibody, infliximab (Remicade), have demonstrated efficacy in clinical trials [3]. It is now clear that TNF-alpha blockade, in addition to reducing joint inflammation and leukocyte infiltration, also results in decreased formation of new blood vessels in the synovium. Such mechanism of action is now paving the way for the development of the next generation of drugs for treatment of rheumatoid arthritis [4, 5]. Recent data were presented mainly from laboratories illustrating the importance of IL-15 and IL-18 in the induction and perpetuation of chronic inflammation during experimental and clinical rheumatoid synovitis. These findings suggest that antagonists to these cytokines may have a potential therapeutic role against organ-specific autoimmune diseases [6]. The ability of IL-1 to drive inflammation and joint erosion and to inhibit tissue repair processes has been clearly established in vitro systems and animal models [5]. Although there are elevated levels of IL-17 in synovial fluid of patients with rheumatoid Arthritis, the pathogenic role of IL-17 in the development of rheumatoid arthritis remains to be elucidated. There are observations suggest that IL-17 plays a crucial role in T cell activation, downstream of IL-1, causing the development of autoimmune arthritis [7]. The pathological roles of IL-6 have also been clarified in various disease conditions, such as inflammatory, autoimmune, and malignant diseases. On the basis of the findings, a new therapeutic approach to block the IL-6 signal using humanized anti-IL-6R antibody for rheumatoid arthritis, Castleman's disease, and multiple myeloma has been attempted [8]. Sufficient evidence exists that establishes a key role for the kallikrein-kinin cascade in inflamed joints. In addition, there appears to be an inter-relationship between cytokines and kinins in the inflammatory process. Kinins induce the release of cytokines, and cytokines have been shown to augment the effects of kinins. This may lead to an enhancement and perpetuation of the inflammatory process [8]. Interleukin 7 (IL7), a T cell growth factor and a regulator of Th1 and Th2 cytokine production, is produced by synoviocytes from patients with RA [9]. IL-12 is a proinflammatory cytokine produced by different antigen presenting cells. It has been shown to exert a critical role in inducing Th1 phenotype, thus initiating cell-mediated immune responses. It was suggested that IL-12, modulating cell and humoral immune responses, is involved in the pathogenesis of immune rheumatic diseases [10].Little has been reported about the cytokine profiling in Iraq, although data concerning the investigation of RA are substantial. This study was designed to investigate the serum levels of selected cytokines in a group of Iraqi patients with rheumatoid arthritis (RA) compared with apparently healthy controls.

* *Dept. of Clinical Laboratories*
College of Health and Medical Technology/ Baghdad.

Patients and Methods:

This case-control study was conducted during the period from March 2007 to June 2007. Blood samples were collected from 50 patients with rheumatoid arthritis (RA). All patients have been submitted to a complete clinical and radiological examination by specialist doctors. The selection of study patients was achieved according to the original criteria of the American Rheumatism Association revised by Klippel *et al*. [11]. Pain intensity and functional disability were evaluated by visual analogue scale (VAS) scored by a health professional. A questionnaire form was formulated that involved name, age, gender, clinical history, disease stage, disease duration, family history, residence, socio-economic status, general health condition, smoking, drinking, and any possible previous therapies. Patient specimens were gathered from Baghdad Teaching Hospital. A group of 40 apparently healthy blood donors was included as a control from National Bank for Blood Transfusion. Blood specimens from patients and healthy controls were properly conveyed to the location of processing and testing at the Department of Immunology, Central Laboratories for Public Health. The serum specimens were obtained and distributed in Eppendrof vials and saved in deep freezing at -20 C° until testing. Additional laboratory tests for general health assessment were conducted that's including: ESR, CRP, and RF tests.

IL-2 EASIA Test: EASIA (Enzyme amplified sensitivity immunoassay was applied using The BioSource IL-2 test (IL-2 EASIA Kits, BIOSOURCE, Europe, S.A, Belgium) on microplates according to manufacturer instructions. Reference interval of IL-2 is 0-0.1 U/ml (detection limits: 0.05 U/ml) [12].

IL-6 EASIA Test: EASIA (Enzyme amplified sensitivity immunoassay was applied using The BioSource IL-6 test (IL-6 EASIA Kits, BIOSOURCE, Europe, S.A, Belgium) on microplates according to manufacturer instructions. Standards or samples containing IL-6 react with capture monoclonal antibodies (MAbs-1) coated on the microtiter well. Reference interval of IL-6 is 3.0-8.5 pg/ml [13].

TNF-α EASIA Test: The principle of BioSource TNF-α test TNF-α EASIA Kits, BIOSOURCE, Europe, S.A, Belgium) on microplates is similar to IL-6 according to manufacturer instruction. Reference interval of TNF-α is 0-20 pg/ml.

IFN-δ EASIA Test: The principle of BioSource IFN-δ test IFN-δ EASIA Kits, BIOSOURCE, Europe, S.A, and Belgium) on microplates is similar to IL-6 according to manufacturer instruction. Reference interval of IFN-δ is 0.1-1.2 IU/ml.

Statistical analysis: Data are presented as means ± SD for continuous variables or as number with percentage for categorical variables. Differences between continuous variables were assessed by Student's *t* test and differences between categorical variables were assessed by Chi-square test. For all analysis, statistical significance was considered at highly significant level *P* values of < 0.01, significant level *P* values of < 0.05, and insignificant level *P* values of > 0.05. All the statistical analysis was done by using SPSS computer program version 10 and Excel application.

Results:

Demographic and clinical characteristics of the study patients are shown in Table1. Fifty patients were included in this study, 22 (44%) males and 28 (56%) females. Distribution of patients among age groups showed that 13 (26%) patients were in 16-30 yrs age group, 27 (54%) patients were in 31-50 yrs age group, and 10 (20%) patients were in 51-70 yrs age group. It was shown that patients with long term duration of illness were more than those with recent onset. All serum specimens obtained from study patients showed positive RF results. Clinical presentation demonstrated equal number and percentage of study patients with high and low disease activity.

Table1: Demographic and clinical characteristics of the study patients

Characteristics	No.	%
Gender:		
- Male	22	(44%)
-Female	28	(56%)
Age in years:		
- 16-30	13	(26%)
- 31-50	27	(54%)
- 51-70	10	(20%)
Duration of illness:		
- Long term	34	(68%)
- Recent onset	16	(32%)
Rheumatoid factor (RF):		
- Positive	50	(100%)
- Negative	0	0
Disease activity:		
- High	25	(50%)
- Low	25	(50%)

The results shown in table 2 indicate that mean of serum IL-2 levels in sera from study patients was above normal reference interval, 0.845 IU/ml versus 0-0.1 IU/ml. Comparison of differences in serum IL-2 levels among patient and control groups indicated highly significant differences between control and RA patient groups. In the same table, it was shown that mean of serum IFN-δ (IU/ml) levels in sera from patients and healthy controls did not rise above normal reference interval**,** 0.605 (IU/ml) versus 0.1-1.2 IU/ml, although there were remarkable differences between patient and control levels**.** Comparison of differences in serum IFN-δ (IU/ml) levels among patient and control groups indicated only significant differences between control and RA patient groups.

Table 2: Mean of serum IL-2 and IFN-δ (IU/ml) in sera from patients and healthy controls

Studied groups	No.	Mean	SD[2]	Min.	Max.	Student's *t* test	
						P-value	Significance
IL-2							
Control	40	0.767	0.127	0.40	0.99	0.043	Highly Sig. (P< 0.01)
RA[1]	50	0.845	0.183	0.37	1.17		
IFN-δ							
Control	40	0.231	0.054	0.26	0.76	0.013	Sig. (P< 0.05)
RA[1]	50	0.605	0.427	0.31	2.48		

RA[1]: Rheumatoid arthritis. SD[2]: Standard deviation.

Table 3 indicates that mean of serum IL-6 levels in sera from study patients was above normal reference interval, 30.50 pg/ml versus 3.0-8.5 pg/ml. Comparison of differences in serum IL-6 levels among patient and control groups indicated highly significant differences between control and RA patient groups. In the same table, it was shown that mean of serum TNF-α (pg/ml) levels in sera from patients and healthy controls did not rise above normal reference interval, although there were remarkable differences between patient and control levels**.** Comparison of differences in serum TNF-α (pg/ml) levels among patient and control groups indicated no significant differences between control and RA patient groups.

Table 3: Mean of serum IL-6 and TNF-α (pg/ml) in sera from patients and healthy controls

Studied groups	No.	Mean	SD[2]	Min.	Max.	Student's *t* test	
						P-value	Significance
IL-6							
Control	40	6.471	2.05	3.04	9.98	0.00	Highly Sig. (P< 0.01)
RA[1]	50	30.50	19.19	9.21	85.20		
TNF-α							
Control	40	11.35	4.80	3.35	19.97	0.216	Non Sig. (P>0.05)
RA[1]	50	14.54	9.43	6.18	48.62		

RA[1]: Rheumatoid arthritis. SD[2]: Standard deviation.

Discussion:

The role of cytokines in the pathogenesis of rheumatoid arthritis (RA) and their significance in clinical monitoring of the disease advancement has been attracting much attention in recent years [14]. The recorded elevations in our study for IL-2 and IL-6 were absolute and 94%, respectively. It was shown that concentration of IL-6 in blood serum of RA patients is many folds higher than those in healthy individuals and might be correlated with the disease activity and its duration. These results were consistent with the observations made by Madhok *et al* [15], and van Leeuwen *et al*, [16]. Our study showed that there were no significant differences between healthy control and RA patient groups in serum TNF-α (pg/ml) levels. This observation could be inconsistent with various studies and might be attributable to the limitations of our study, that's including: some of study patients were referred while they in remission stage rather than active phase of the disease, TNF-α levels were not measured serially by quantitative image analysis, or the clinical diagnosis of the studied cases might be doubtful. On the other hand, it was suggested from another study that TNF-α and lL-2 levels are up-regulated in RA patients but did not significantly differ from the control group [17]. In contrast, substantial researches indicated that (TNF-α) plays a central role in rheumatoid arthritis (RA) pathogenesis [18]. However, its overproduction may also lead to pathologic changes. The latter situation occurs often in chronic inflammatory diseases such as rheumatoid arthritis. The concept suggesting tumor necrosis factor-alpha as a potential target emerged from experiments showing its key role in inducing many cytokines and mediators of inflammation. Several clinical trials targeting this cytokine in rheumatoid arthritis patients with a novel group of anti- TNF-α factor agents demonstrated reduced synovial inflammation and inhibition of bone and cartilage degradation [19]. Little has been reported about the cytokine profiling in Iraq, although data concerning the investigation of RA are substantial. Al-Badry *et al* showed that there was a significant elevation in IL-2 in a group of 80 Iraqi patients with RA as compared with systemic lupus erythematosus (SLE) and controls. It was concluded from Al-Badry study that elevation of IL-2 was observed in active chronic RA cases rather than in recently diagnosed RA cases [20]. In our study, certain cytokines were selected due to clinical relevance of laboratory routine work in checking these cytokines in our hospitals. In conclusion: IL-2, IL-6 and IFN-δ levels were elevated in serum samples of RA patients. We suggest that assessing the serum IL-2, IL-6, IFN-δ and TNF-α levels may be helpful in the confirmation of the RA activity. Due to the chronic course of this disease, other inflammatory markers must be identified in order to provide early therapeutic strategies to these patients.

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