The Role of IL-1 and Tumor Necrosis Factor-α in Pathogenesis of Rheumatoid Arthritis

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ABSTRACT:

BACKGROUND:

Rheumatoid Arthritis (RA) is a common chronic inflammatory and destructive arthropathy that cannot be cured.Interlukin-1 (IL-1), IL-6 and tumor necrosis factor (TNF- α) are the key cytokines that drive inflammation in the disease.

OBJECTIVE:

The study was established to shed light on the possible role of IL-1 and TNF- α in pathogenesis of RA.

PATIENTS AND METHODS:

Rheumatoid Factors (RF),TNF-α and IL-1 were detected by Enzyme-Linked Immunosorbent Assay (ELISA) .C-reactive protein(CRP) on the other hand has been detected by using latex agglutination kit in the serum of 50 Iraqi patients with RA in comparison with 50 healthy individuals.

RESULTS:

Significant high levels of IL-1and TNF- α were observed in RA patients sera with P-value (0.04 and 0.001) respectively compared to healthy control group, in addition a positive linear association was found between the concentration of IL-1and TNF- α and CRP with P-value (0.05 and 0.001) respectively.

CONCLUSION:

IL-1and TNF- α play a role in pathogenesis of RA.

KEY WORDS: rheumatoid arthritis. interlukin-1. tumor necrosis factor- α.

INTRODUCTION:

Rheumatoid arthritis (RA) is a persistent, chronic systemic inflammation of uncertain cause that affects the synovial membranes of multiple joints, principally the peripheral joints, in symmetric fashion, commonly leads to cartilage destruction, bone erosion and joint deformities; extra-articular manifestation such as vasculitis and subcutaneous nodules can also occur in the body [1,2].

The cytokine expression in rheumatoid joints began to appear in pathogenesis and treatment of disease in 1985, while tumor necrosis factor (TNF) was clearly reported in 1988, which is a potent cytokine that exerts diverse effect by stimulating a variety of cells, perhaps the best studied was its ability to promote inflammation [3]. Nonetheless, in the last few years this cytokine was used in RA treatment after well understanding of its function [4].

Studies have indicated that TNF- α has the ability to promote inflammation and inducing IL-1, IL-6, IL-8 and GM-CSF [5,6]. Besides, it promotes inflammation by stimulating fibroblasts to express adhesion molecules, resulting in increased transports of leukocytes into inflammatory sites [i.e. joints with this disease] [7].

It was reported that the predominant infiltrating cells are CD4+ T cells over CD8+ T cells in the synovial membrane, but not in synovial fluid. However, these cells play a central role in initiating, perpetuating and precipitating chronic inflammation [8]. Antigen-activated CD4+T cells stimulate macrophages, monocytes and synovial fibroblasts through specific T cell receptor (TCR).This stimulation results in production of the cytokines as interleukin-1 (IL-1), IL-6 and tumor necrosis factor (TNF-α), which formed the key cytokines that drive inflammation in RA as supposed by Choy, and Panayi [9]. Therefore the most likely disease initiators are TNF- α and IL-1 [5].

It's generally accepted that C-reactive protein (CRP) is most useful index of the acute phase

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response being sensitive and rising rapidly within 6-10 hr. following the inflammatory events. In RA patients, ESR and CRP levels are nearly always elevated and in many cases, although not all, reflecting disease activity. Although in small group of patients CRP levels lie within the normal range despite clinical evidence of active disease [10].

PATIENTS AND METHODS:

PATIENTS:

A total of 50 Iraqi RA patients, who fulfilled the American Rheumatism Association criteria for diagnosis of RA(8) were involved in the study, age range 18-69 years. Those patients were attending the consultant clinic for Rheumatology in Baghdad Teaching Hospital from February 2008 to January 2009.

Apparently healthy control group with a total number of 50, who have no history or clinical evidence of RA or any other chronic disease, and no obvious abnormalities, were selected as normal control [friends, staff, in addition to Blood Bank Donors], they are age and sex matched.

Laboratory investigation:-

The sera were tested for Rheumatoid Factors (RF),TNF- and IL-1 using Enzyme-Linked Immunosorbent Assay (ELISA) technique which uses human IgG Fc as the antigen coating the microwells plate and isotype-specific horse antibodies coupled to radish peroxidase(8) results were expressed as the optical density. A level > 25 IU/ml was considered positive for RF.

CRP, on the other hand has been detected by using latex agglutination kit

RESULTS:

Age and gender distribution of patients and control is listed in table-1.

The higher frequencies of disease were observed at second (14.6%) and fourth decade (56%) respectively. Higher incidence was noticed among the female (74.0%) rather than the male; in 2.8:1 ratio.

Table 1: The distribution of Rheumatoid Arthritis patients by age and gender in comparison with healthy controls:

Age Groups (years)	Study groups			
	RA patients		healthy controls	
	No.	%	No.	%
< 20	4	8	7	14.0
20-29	7	14.6	10	20.0
30-39	6	12.0	10	20.0
40-49	* 28	56.0	4	8.0
50-59	3	6.0	13	26.0
60-69	2	4.0	6	12.0
Female	*37	74.0	31	62.0
Male	13	26.0	19	38.0
Total	50	100	50	100

* Higher frequency

The demographic features of the RA Iraqi patients in addition to the healthy normal individuals are listed in the table-2.

Table 2: Clinical and demographic parameters of RA patients and healthy controls.

Disease in years:	RA Patients	Healthy Controls
Age of subjects	41.9(±12.51)	37 (±13.3)
Duration of RA	0.1-20(±7.06)	--
Age of RA onset years	34.7(12.5)	----
RF test positivity %	42 (86.3)	2(4.0)
CRP test positivity %	72.4	8.0

The average of the patient's age was between 18-69 years with a mean of 41.9±12.51.
Healthy control group on the other hand, with a mean
age of 37±13.3 years. The range of theirs age was 17-67 years.
RF positivity was found as high as 86.3% among patients, while all the healthy individuals were negative for this test except for few numbers.

The results of CRP test included 72.4% and 2.6% for RA patient and healthy controls respectively.

RF screening Test:

Table-3 shows that there is highly significant differences between the serum concentrations of RF estimated by ELISA in the two study groups (P value < 0.001). Levels of RF concentration were higher in patients sera than in normal healthy control.

Table 3: The concentration of RF(IU/ml) in the sera of the study groups

Serum RF by ELISA IU/ ml	RA patients	Healthy group
Minimum value	0-	0
Maximum value	600	200
Mean	113.7	19.6
SD	164.38	55.29
SE	18.49	6.3
No. of positive cases	42	6
P value = < 0.001		

The levels of CRP in study group are shown in table-4 which revealed highly significant statistical differences in comparison with healthy group.

Table 4: The concentration of CRP(IU/ml) in the sera of study groups

Serum CRP (mg/dl)	RA patients	Healthy group
Minimum value	5.0	5.0
Maximum value	80.0	5.0
Mean	20.7	5.0
SD	17.3	0
No. of positive cases	48	4
P value = < 0.001		

The overall frequency of clinical manifestations are shown in table-5. RA patients revealed joint stiffness, and swelling in high percentages (88.8%, and 86.3% respectively).

Table 5: Distribution of the main clinical features in RA patients

Clinical features	RA patients No.	%
Joint swelling	40	86.3
Joint stiffness	35	88.8
Ocular disturbances	28	56.5
Sandy eyes	1	2.0
Weight loss	33	66.3
Joint effusion	2	8.0
Subcutaneous nodules	18	36
Skeletal deformity	9	18.5

Estimation of IL-1 Level in the Serum:

Table-6 revealed a significant ($p<0.05$) elevation in serum IL-1 among RA patients (30.5 pg/ml) compared to that of control group (11.3 pg/ml).

Table 6: The difference in mean serum IL-1 levels (pg/ml) between the study groups

	Study groups		
Values pg/ml	Healthy control	RA patients	P value
Minimum	8.7	18.00	0.04
Maximum	14	70.0	
Mean	11.3	30.5	
SD	2.03	14.2	

Estimation of TNF- Level in the Serum:
Statistical analysis revealed that there was a significant elevation ($p<0.001$) of TNF- α serum levels in RA patients when compared to control group, table-7.

Table 7: The difference in mean serum of TNF- α levels (pg/ml) between the study groups

	Study Groups		
Values Pg/ml	Healthy control	RA patients	P value
Minimum	1.1	53	0.001
Maximum	16	350	
Mean	5	100.9	
SD	2	53.7	

An attempt to find a possible association in this study between mean serum level of IL-1 and TNF-α with Concentration of CRP, table -8 shows an increasing trend of IL -1 and TNF-α serum levels with Concentration of CRP with p-values 0.05 and 0.001 respectively.

Table 8: The difference in mean serum IL-1 levels and TNF-α (pg/ml) with CRP Concentration between the study groups

Cytokines	CRP Conc.	P value
IL-1	0.289	0.05
TNF-α	0.320	0.001

DISCUSSION:

Rheumatoid arthritis (RA) is a world wide disease with a wide variation in the incidence, severity and clinical manifestation between different racial and ethnic groups (1). In Iraq reported studies showed that the disease was relatively common [11,12].

In this study, age incidence was in accordance to other previous reported studies in Iraq [11,12], which showed the predominant age is in the second and third decade, while discrepancies have been observed with abroad reports, the result where age incidence were shown to be in the fourth and fifth decade [13,14] This difference in age incidence is probably due to genetic, environmental or other precipitating factor[8].

The above results denoted a high prevalence of RA among women rather than men, which may be due to the hormonal differences between them and in turn, their effects on the immune responses. Those make women normally tend to mount more robust IRs and these responses tend to be more TH1 - like responses rather than TH2 response, which are pro-inflammatory, hence may enhance the development of autoimmunity [15].

The female to male ratio was 2.8:1 which is nearly comparable to most other studies in Iraq in which age range resides between 2.7:1 [11] to 2.9:1 [12].

T cell abnormalities, B-cell hyperactivity and abnormal cytokine production have been well implicated in the pathogenesis of this disease [8]. Among the cytokines which is accepted to play a crucial role in pathogenesis of RA are TNF-**α** and IL-1. Therefore, the present study analyzed serum levels of these cytokines as indicator of immune response in different clinical courses of RA.

Table (6 and 7), revealed a significant high levels of TNF-α and IL-1 in sera of RA patients as compared to control subjects.

These results were in agreement with the previous results [16,17] they found that high levels of TNF-α and IL-1 were frequently observed in RA patients. Actually, this finding is attributed to the production of this cytokines by activated monocytes and

macrophages [5]. The most likely explanation of these results is that these two cytokines, as part of cytokines network, may be involved in pathogenesis of RA, through their stimulation of the mesenchymal cells, such as synovial fibroblasts, osteoclasts and chondrocytes, that release tissue-destroying matrix metalloproteinases which are responsible for bone degradation.

In relation to CRP concentration, our data revealed positive linear association between TNF-α and IL-1 and concentration of CRP with p-value 0.05 and 0.001 respectively. Similar interesting finding were clearly reported by Snaxne and Chikanza [18]. They found that the highest level of CRP was demonstrated in sera of RA patients with advanced stage of the disease and highest level of IL-1 and TNF-α, while the lowest were demonstrated in patients with stable disease [18].

CONCLUSION:

It is now clear that these cytokines play a fundamental role in the processes that cause inflammation, articular destruction, and the comorbidities associated with RA.

Furthermore, agents that target inflammatory cytokines may provide relief to the millions of sufferers of RA.

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