Role of Interleuken-6 & Tumor necrosis factor – α in Rheumatoid arthritis Patients in Nassiriyah governorate

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SUMMARY

The present study has been done in Al-Nassiriyha, southern Iraq, from December 2006 to August 2007,designed to determine the role of Interleuken-6 and Tumor necrosis factor – α in a group of patients with rheumatoid arthritis for specific period of time and healthy controls, and to show the diagnostic value of IL-6 and TNF-α in relation to rheumatoid factor (RF) to discriminate between those patients with and without RA . Blood samples were taken from 121 patients with rheumatoid arthritis from AL-Hussein Teaching Hospital in AL-Nassiriyah and 120 healthy controls from medical personnel and school students who were healthy . All the information were taken from the patients and controls. An Enzyme-linked immunosorbent assay was used for estimation IL-6, TNF-α and slide agglutination test for the estimation of RF. The seropositivity to IL-6 and TNF-α and RF in patients with rheumatoid arthritis was (60% , 56.1% and 57% respectively) .The study revealed that specificity of TNF- alpha was higher than IL-6 and RF (96.6 % , 92.5 % and 81.6 respectively) .

INTRODUCTION

Rheumatoid arthritis (RA) is a common chronic inflammatory disease in developed countries. The prevalence of RA varies from country to country, from 0.7% to 3%, with an average of 1% in the adult population. In the Gulf region, two studies estimated the prevalence of RA to be 1% in the Iraqi population, and 0.36% in the Omani population[1].In recent years it has become clear that early aggressive treatment in RA reduces joint damage and improves function[2].To use a potentially toxic therapy as early as possible we require an accurate diagnosis of RA and also information about prognosis in an individual patient. Today, the diagnosis of RA depends mainly on clinical criteria that may take years to fulfill [3]. Apart from clinical features, autoantibodies contribute to the diagnosis of various autoimmune diseases. In RA, rheumatoid factor (RF) has a fair sensitivity and specificity, since it is present in other rheumatic diseases, in infections, and in healthy people, especially the elderly [4]. The exact pathogenesis of RA is not fully understood, but the cytokines tumor necrosis factor (TNF)-a, IL-1b and IL-6 are involved in both inflammation and the increased bone resorption in RA patients [5].The initial trigger of the disease remains unknown;

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however, the molecular factors that perpetuate the inflammatory process are increasingly well understood[2].Disease activity in RA reflects an ongoing imbalance between pro-and anti-inflammatory cytokines. Although there are many cytokines involved, tumor necrosis factor (TNF) is a critical pro-inflammatory cytokine. TNF mediates many of the inflammatory processes in RA, including immune-cell activation and proliferation, apoptosis and regulation of leukocyte movement[6]. Elevated concentrations of IL-6 are found in serum or plasma and synovial fluid of patients with RA [7] . Serum and plasma IL-6 levels in RA patients are related to clinical measurement of disease activity and radiographic progression of joint destruction [5]. Several studies have shown that IL-6 inhibition is a therapeutic target in RA[8]. A humanized anti-IL-6Ra monoclonal IgG1 antibody (tocilizumab) has shown promising efficacy by inhibiting disease activity and joint destruction in phase I and II clinical trials of RA patients [5] . In RA patients, high concentrations of IL-6 have been detected in the synovial fluid, and serum levels of this cytokine highly correlate with markers of the disease activity, such as acute phase proteins [9].

MATERIALS& METHODS

Patients

This case-control study was carried out during the period from December 2006 till August2007.A total of 121 blood sample were collected from patients consulted the rheumatology/orthopedic and internal medicine clinics at Al-Hussein Teaching Hospital in Thi-qar governorate .This study group comprised 95 female and 26 male, those who were complained of rheumatoid arthritis according to the revised criteria formulated by the American College of Rheumatology (ACR)[10], were included in this study. Each patient fulfilled, at the time of interview, at least four of these seven criteria were included and patients were interviewed once and a blood sample was drawn from each patient. Healthy control comprised 120 persons (31 males and 89 females),collected from general population in Al-Nassiriyah (school students, medical personnel and from blood donors) with negative history of major illness and have no abnormal physical findings during clinical examination by specialist and their routine laboratory investigations were within normal .

Elisa & Serological Tests

Blood was drawn from the veins of patients and control persons using a sterile disposable syringe. 5 ml of the venous blood was taken, and then emptied into disposable tubes which were left standing oblique for one hour at room temperature allowed to clot and then centrifuged at 3000 rpm for 5 minutes and serum drawn by clean pipette into other sterile plastic tubes, and the sera kept frozen at –20C° until the serological examination was performed for detection of tumor necrosis factor-α (TNF-α),Interleukin–6(IL-6) by Enzyme Amplified Sensitivity Immunoassay(EASIA) methods, no serum sample was refreeze and thawed more than once .

Determination of Serum IL-6 EASIA Kit:

This test kit has been produced for research use only not for use in diagnostic procedures. It is produced by: BIOSOURCE EUROPE S.A. Rue de I'Industrie , 8 B-1400 Nivelles Belgium/ the catalogue No. KAC1261. Normal values of IL-6 less than 7.0 pg/ ml

Determination of Serum TNF-α EASIA Kit

This test kit has been produced for research use only not for use in diagnostic procedures . It produced by : BIOSOURCE EUROPE S.A. Rue de I'Industrie , 8 B-1400 Nivelles Belgium/ the catalogue No. KAC1751. Normal values of TNF-α less than 21 pg/ ml

RESULTES:

Some of the sociodemographic characteristics of 121 patients of rheumatoid arthritis and 120 healthy controls involved in this study are shown in Table .1.The age of the studied group ranged from 15 to 65 years (mean45.61and ±14.18 standard deviation) according to the age in rheumatoid arthritis patients. The results showed that the prevalence of the disease is more common in age group of 50 years followed by 30– 49 years and least in the less than 29 years, while the age group between 30-49 years old is the most frequent one among healthy control groups .The sex distribution showed that females were more common than males in all the studied population groups. There are no significant differences in sex distribution between the two groups. Most of the study population in the two groups comes from urban background, and the RA patients included in this study were from Al-Nassiriyah governorate and the surrounding area, yet 87(71.9 %) cases are from urban areas and 34(28.1 %) cases are from rural areas. On the other hand, most of the studied persons were unemployed. By the use of slide agglutination test for the detection of RF 57% (69/121) were positive. The seropositivity to RF among healthy control group was 13.3 %. Sera tested for TNF-α and IL-6 by the use of EASIA showed that 56.1% and 60% among RA group were positive for TNF-α and IL-6 respectively. On the other hand, sera from healthy control groups showed lower percentages of seropositivity to these cytokines , 3.3%for TNF-α and 5.0% for IL-6 (Table.2).The ability of each test to predict a positive or a negative case is presented in table 3. Although there were a variation in the sensitivity and specificity of various tests,TNF- α detection has a powerful prediction ability to detect RA positive cases with specificity (96.6%) and sensitivity was (47.1%) of the tested population. However, IL-6 and RF seropositivity showed closed ability in prediction of positive RA (54.5% and 57% respectively) and higher specificity for IL-6 (92.5 %) in comparison with RF (81.6%) in RA cases.

DISCUSSION

Rheumatoid arthritis is a relatively common disease in our country. The diagnosis of RA depends primarily on clinical manifestations of the disease, whereas serological tests provide an aid in terms of confirmation. Hence it is the early period of development of the disease for which a specific and sensitive serologic test is needed. This study showed that the predominant age affected by RA was the 50 years old and above , this agreed with several studies [11,12]; also Fabiola Atzeni(2006) found the peak age of onset at the 56 years in their series of 57patients [13]while Ewa Beglin,2006 found age-peak between 4th and 6th decades[14]. On the other hand Michael M. Frank (1995) found that the frequency of RA varies considerably with age, it appears in the early teenage years, increases moderately in the third decade , and rises sharply during the fourth[15].However, there are differences in the female: male ratio in different studies ranged from 3:1[15,16], 2:1 to 4:1[17] up to 5:1[13] .Similar to the general agreement

that the incidence of the disease is more common in females than in males[15], our study also showed that female comprises 78.5% from the studied RA patients in comparison to males (21.5 %). The reason for that is attributed to possibility that sex hormones or a factor associated with the inheritance of two X chromosomes could enhance the expression of RA in females [15]. After thorough search in the locally available conventional and grey literature and the Internet we obtained only few studies investigating serum levels of TNF-α and IL-6 in RA patients . A study carried out by Lene S.et al (2004) who observed the circulating levels of IL-6 were significant elevated in RA patients compared with healthy subjects (p<0.001) and showed in the early RA patients serum IL-6 reached at (42% , p=0.025) compared to patients with erosive RA serum IL-6 was significantly higher (62%, p=0.0026)[5] . Also there are no strong correlation between age and the IL-6 concentration and no difference in IL-6 concentrations between men and women [5]. Other study carried out by Frédéric A. et al (2005) reveal that IL -6 was detected in about one-third of the serum samples from patients with RA[17].Others have found that only 10—21% of RA patients had normal serum or plasma IL-6 [18]. In contrast it has been reported that approximately 66% of RA patients had normal serum IL-6[19]. Some of these differences are probably due to different assay used and different RA populations with different disease activity. The degree of expression of these cytokines displays a vast heterogeneity between different individuals with RA, as demonstrated in studies of synovia[20]. The current study shows that the seropositivity and titers of cytokines IL-6 and TNF-α were significantly higher in RA patients compared to control groups. On the other hand , our study reveal that the specificity of TNF-α is higher in comparison with that IL-6 and RF .At the same time the sensitivity of the three markers in the present study appeared to be low. However, cytokines other than IL-6 and TNF-α might be involved in immunological mechanism which constitutes another limitation of this study, as we were unable to conduct assays for additional cytokines. Based on the recent findings of immune abnormalities in the pathogenesis of RA, the present findings are of considerable significance that highlights the needs of further research in this area.

CONCLUSION

In conclusion, our results show an increased production of the proinflammatory cytokines, IL-6, and TNF-α in rheumatoid arthritis patients. Also the higher specificity of TNF-α and IL-6 in comparison with RF which appear in this study might need further overall agreement studies of these markers which might alter the attitude to the traditional criteria of the diagnose of RA in early stages of the disease which can result in early use of disease modifying agents and immune modulators (Anti- Tumor necrotic factor-α) that might result in the reduction of disabilities , morbidity and mortality in RA patients .

Table .1 :The Characteristics of the study population:

character	Rheumatoid arthritis No.tested (%) N= 121		Healthy control No.tested (%) N= 120	
	N	%	N	%
1. Age :				
< 29	12	9.9	20	16.6
30 - 49	52	42.9	54	45.0
> 50	57	47.1	46	38.3
Mean ± SD X^2 = 0.005	45.61±14.18 df = 1		43.98±14.21 P> 0.05	
2.Sex :				
Male	26	21.5	31	25.8
Female :	95	78.5	89	74.2
X^2 = 0.005	df = 1		P> 0.05	
3.Residency				
Urban areas	87	71.9	82	68.3
Rural areas	34	28.1	38	31.6
X^2 = 0.000	df = 1		P> 0.05	
4.Occupation:				
Employed	29	24.0	33	27.5
Unemployed Or free trading	92	76.0	87	72.5
X^2 = 0.005	df = 1		P> 0.05	

Table 2 Serological parameters in patients with rheumatoid arthritis andhealthy controls .

Study groups / Test	Rheumatoid arthritis N+ve / Tested(%) N = 121 N	%	Healthy control N+ve/Tested(%) N = 120 N	%
RF				
Positive	69	57.0	16	13.3
Negative	52	43.0	104	86.7
	χ^2 = 64.151 df= 1		P = 0.001	
IL-6 Positive	72 60%		6	5.0
Negative	49	40	114	95
	χ^2 = 110.880 df= 1		P = 0.001	
TNF-α				
Positive	68	56.1%	4	3.3
Negative	53	43.8 %	116	96.7
	χ^2 = 63.593 df= 1		P = 0.001	

Table 3Comparison of the sensitivity and specificity between serological parameters .

Test	sensitivity	specificity
TNF- α	56.1 %	96.6%
IL- 6	60%	92.5 %
RF	57.0 %	81.6 %

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