The Relationship among Interlukin-10 ,Interlukin-6 with anti-cyclic citrullinated peptide antibody, C-reactive protein ,Rheumatoid factor in patients with Rheumatoid Arthritis

Ahmed Abbas Hasan*
*MSc .Immunology ,Department of Medical analysis /College of Health and Medical Technology , Kufa/ University of Al furat al –Awsat techniques/ Foundation of Technical Education
E-mail:ahmedabbashasan@gmial.com
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الخلاصة : يعتبر التهاب المفاصل الريثاني من اشد الأمراض التي تصيب المفاصل وكذلك هو أكثر أمراض المناعة الذاتية شيوعا"، والذي يصيب حوالي 1% من الأشخاص البالغين ،نفذت هذه الدراسة على 40 حالة مرضية (30 حالة سريريه للمرضى الإناث و 10 للمرضى الذكور) مع أعمار تتراوح بين 20-70 سنة، جمعت العينات من مدينة الحسين الطبية في محافظة كربلاء ،احتوت مجموعة السيطرة على 20 شخص خالين تماما من أعراض وعلامات الإصابة بالتهاب المفاصل الريثاني وقد تطابقوا من حيث الجنس والعمر مع المرضى ،أيضا" اخذ بنظر الاعتبار خلوهم تماما من تاريخ الإصابة بالمرض.تم فحص الانترلوكين 10 ، الانترلوكين 6 ،مضاد السايكلك سترولينيتد ببتايد و عامل الروماتزم بطريقة الامتزاز المناعي المرتبط بالأنزيم ، بينما تم قياس البروتين المتفاعل C باستخدام طريقة مقياس الكدورة الكمي ، حللت البيانات إحصائيا" الرزمة الإحصائية (SPSS version 22 -ANOVA- Pearson correlation)، وكذلك تم مقارنة القيم بواسطة العينة المستقلة (T-test)، إذا كان مستوى المعنوية اصغر أو يساوي0,05مع المقارنة بمجموعة السيطرة فتعتبر عالية المعنوية . بينت النتائج ارتفاع مستوى المعنوية للانترلوكين10،الانترلوكين 6،مضاد السايكلك سترولينيتد ببتايد ،البروتين المتفاعل Cوعامل الروماتزم في المرحلة المعتدلة (41.4±1.3)، المرحلة الحادة(54.8±0.8)،المرحلة الحادة (953.3±114)، المرحلة الحادة (181.3±11) بالتتابع بين هذه المجاميع ،من ناحية أخرى ليست هناك فروقات معنوية لهذه العوامل بالنسبة لمجاميع العمر والجنس، بينما كانت هنالك فروقات معنوية عالية بين العوامل المذكورة .
الكلمات المفتاحية :التهاب المفاصل الريثاني الانترلوكين 10، النترلوكين 6.

Abstract:
Rheumatoid arthritis (RA), the most severe disease affect the joint and also the most common systemic autoimmune disease, affecting approximately 1% of the adult population .We investigate the serum levels of IL-10 and IL-6 in patients with Rheumatoid arthritis. About forty patients with Rheumatoid arthritis (30 female and 10 male) with ages ranged between (20-70) years were taken from (Al-Hussein Medical City/Kerbala).Control group consisted of 20 healthy people were free from signs and symptoms of arthritis were matched in age and gender with patients, and had no history for any arthritis problem .IL-10 (IL-10-EASIA Kit, DIAsource) (IL-6 ELISA kit ,immunotech),(Anti CC-P ELISA kit ,Medizym/Germany) ,(RF ELISA Kit Euroimmun/Germany) were measured using the enzyme-linked immunosorbent assay (ELISA) method and CRP Vital kit using quantitative turbidimetry method . t-test , ANOVA and Pearson correlation were used to analyze results by using SPSS version 22. P-value $\leq$ 0.05 was considered significant. IL10,IL6,Anti-CCP,CRP and RF were increased significantly ($p< 0.05$) in patients compared with control group, So increasing of IL10,IL6,Anti-CCP,CRP and RF values at mild stage (41.4±1.3),sever stage(54.8±0.8),sever stage(952±114), server stage(49.3±1),sever stage (181.3±11) respectively increased significantly value ($p< 0.05$) among the stages, The results revealed no significant value ($p>0.05$) among the age groups and according the gender groups, While there were highly significant correlation ($p< 0.05$) found among studded parameters .
Keyword: Rheumatoid Arthritis,Interlukin10,Interlukin 6.

Introduction

Rheumatoid arthritis (RA), the most severe diseases affect the joint and also the most common systemic autoimmune disease, affecting approximately 1% of the adult population [**1**] .The major features of RA are the activation and proliferation of synovial tissue and the degradation of articular cartilage. Synovial fibroblasts and inflammatory cells, such as macrophage, play

key roles in this process. Innate immunity also plays an important role in the pathogenesis of RA [**2**].develop an antibody response to citrullinated proteins (anti citrullinated protein antibodies, ACPAs) and to IgG (rheumatoid factors, RFs) combined with a raised systemic inflammatory response. Many years later the joints are affected. It is not known which autoimmune and inflammatory factors are important for this shift that shortly precedes clinical onset of RA. It is known however that the polyclonal ACPA antibodies shifts in epitope specificity [**3**].

The mechanisms that give rise to RA are only partly understood, and several different immune cells are involved, including lymphocytes, macrophages and neutrophils.

Furthermore, a number of inflammatory mediators are implicated in the establishment and progression of arthritis, including proinflammatory cytokines such as tumor necrosis factor alpha (TNFα), interleukin (IL)-6 [**4**], IL-6 stimulates the inflammatory and auto-immune processes in for rheumatoid arthritis.[**5**]

Interleukin-10 (IL-10), also known as human cytokine synthesis inhibitory factor (CSIF), is an anti-inflammatorycytokine. In humans, IL-10 is encoded by the *IL10* gene.[**6**]

CRP an acute phase protein is synthesized by hepatocytes in response to proinflammatory cytokines in particular IL-6. It has been shown to be of great value as an inflammatory marker in RA and has been suggested to mediate part of the complement activation in RA[**7**].

PATIENTS AND METHODS:

Selection of patients

During the period 1/July /2014 to 1/September /2014, forty patients with Rheumatoid arthritis (20 female and 20 male) with ages ranged between (20-70) years were taken from (Al-Hussein Medical City/Kerbala).

Control group consisted of 20 healthy people who were free from signs and symptoms of Rheumatoid arthritis who matched in age and gender with patients, and had no history for any arthritis problems .

Sample collection and assay procedure

Blood sample (5ml) were collected and left at room temperature and then centrifuge for 15 min. at (3000 rpm). Serum was then separated and store until time of analysis. Estimation of IL10 ELISA kit(Cusabio/China),IL-6 ELISA kit (immunotech),Anti CC-P ELISA kit (Medizym/Germany) ,RF ELISA Kit (Euroimmune/Germany) , CRP Vital quantitative turbidimetry kit in serum using commercially available and performed as recommended in leaflet with kit.

Statistical analysis:

Results are expressed as mean ± standard error (SE), student t-test, ANOVA and Pearson correlation were used to analyze results by using SPSS version 22, p-value ≤ 0.05 was considered significant.

Results:

A total of 40 patients with rheumatoid arthritis divided into three groups according to the age (20-40) yrs 7(17.9%), (41-50)yrs 8(20.5%) and age(51-70)yrs24(61.5%) ,about 30(75%) patients were females. The distribution of the patients according to pathological evaluation Mild, Moderate, were16(41%),12(30.8%),11(28.2%) respectively table(1) .

Table 1. demographic and clinical characteristics in Patients with Rheumatoid arthritis.

Characteristic	N	Percentage (%)
No of patients	40	100
Gender Male Female	 10 30	 25 75
Age(years) (20-40)year (41-50)year (51-70)year	 7 9 24	 17.5 22.5 60
Severity of disease Mild Moderate Sever	16 12 12	40 30 30

Serum Rheumatoid factor, Anti CC-P,CRP ,IL10 and IL-6 were estimated in 40 patients (30 females,10 males) compared with 20 healthy subjects ,these parameters were increased significantly ($p \leq 0.05$) in patients in compare with control group as shown in table (2)

Table2: Mean values of serum concentration of IL-10,IL-6,AntiCC-P,CRPand Rheumatoid factor in both studied groups .

parameters	Patients N(40)	Control N(20)	p-Value
IL-10 Mean ±SE	37.5±1	8.7±0.1	0.001
IL-6 Mean ±SE	47.8±1	22.5±0.4	0.001
Anti CC-P Mean ±SE	416.5 ±69	1.9±0.3	0.001
CRP Mean ±SE	41.7±1.4	1.8±0.2	0.001
Rheumatoid factor Mean ±SE	111.4±9	2.8±0.3	0.001

While table (3) revealed that the increasing value of IL-10,IL-6,AntiCC-P,CRP and RF were at stage 1 for IL-6 and Stage 3 for other parameters as shown in table 3.

Table3: Mean values of parameters according to the severity of Rheumatoid Arthritis.

parameters	Mild N(16)	Moderate N(12)	Sever N(12)	p-Value
IL-10 Mean ±SE	41.4±1.3	37.8±1.3	31.8±1.6	0.001
IL-6				

Mean ±SE	42.8±0.6	47.3±1.6	54.8±0.8	0.001
Anti CC-P Mean ±SE	68.6±7	344±54	952±114	0.001
CRP Mean ±SE	35.5±2	42.2±2	49.3±1	0.001
Rheumatoid factor Mean ±SE	60.9±5	108.8±12	181.3±11	0.001

*Highly significant

Table 4 Show there is no significant effects of age difference on studded parameters.

Table4:Mean values of studied immunological markers in Rheumatoid Arthritis according to age groups .

parameters	(20-40)year N(7)	(41-50)year N(9)	(51-70)year N(24)	p-Value
IL-10 Mean ±SE	34.3±2	38.2±1.8	38.1±1.4	0.3
IL-6 Mean ±SE	52.3±2	48.3±2	46.2±1.2	0.07
Anti CC-P Mean ±SE	602±112	536.8±194	317±82	0.2
CRP Mean ±SE	47±1.5	43.3±3.1	39±2	0.1
Rheumatoid factor Mean ±SE	124.7±13	115.3±21	106±13	0.7

*Non significant

While table(5) shown no significant ($p \geq 0.05$) effects for the gender on the studded parameters.

Table5: Means values of studies parameters in Rheumatoid Arthritis according to the gender.

parameters	Male N (10)	Female N(30)	p-Value
IL-10 Mean ±SE	38.1±1.5	36.8±1.4	0.5
IL-16 Mean ±SE	48.5±1.3	47±1.5	0.4
Anti CC-P Mean ±SE	378.3±78	451±113	0.6
CRP Mean ±SE	40.3±2.3	42.9±2	0.3
Rheumatoid factor Mean ±SE	102.5±12	119.5±15	0.3

*Non significant

The correlation between the parameters in patients were shown in table(6) the results revealed a strong correlation ($p \leq 0.05$) between all parameters .

Table6: Correlation among studded parameters.

Parameters	r	p-Value
IL-10 vs. IL-16	-0.69	0.001
IL-10 vs. Anti CC-P	-0.68	0.001
IL-10 vs. CRP	-0.64	0.001
IL-10 vs. Rheumatoid factor	-0.67	0.001
IL-16 vs. Anti CC-P	0.7	0.001
IL-16 vs. CRP	0.693	0.001
IL-16 vs. Rheumatoid factor	0.61	0.001
Anti CC-P vs. CRP	0.612	0.001
Anti CC-P vs. Rheumatoid factor	0.856	0.001
CRP vs. Rheumatoid factor	0.581	0.001

Discussion

In the current study the mean serum level of CRP was significantly higher in the RA group than the control group (table 2), and mean serum level of CRP in severe RA group was significantly higher than in moderate RA which was significantly higher than in mild RA (Table 3). These results were supported with **[8],** who had found CRP is a protein produced by the liver in response to tissue injury, infection and inflammation .

Furthermore **[9],** showed that serum CRP level was higher in RA and reflected a higher inflammatory activity in RA and CRP level increase by increasing Rheumatoid Factor RF is a very old serological marker for diagnosis of RA. RF is taken as a nonspecific marker of RA because it is also seen in other collagen vascular disease [**10]**.

In the present study RF was significantly higher in RA than in the control group. This data was agreed with [**11],** who had found association between RA and the presence of rheumatoid factor in the serum .

The results were supported with **[12, 13],** who found that there was a difference in RF level between RA group and non- RA groups .

Anti-CCP antibodies and RF are shown as an essential serological marker for diagnosis and as a likely prognostic marker for the progress of erosive disease **(14 , 15).**A new study showed that in patients with synovitis of many months duration, a combination of anti-CCP antibodies and RF has a high specificity (97%) for development of persistent RA **[16].**also ,anti-CCP have been included into newly proposed diagnostic criteria for rheumatoid arthritis and proved to be strongly related with erosive arthritis**.[17].**

It appears that anti-CCP antibodies have predictive relevance similar to RF **[18, 19and 20]**found that anti-CCP positively was better than RF at predicting progression of Larsen score over two years .As well ,in a prospective cohort study many patients with early RA followed up for three years , the anti-CCP antibody results correlated with RF ,But were better than RF as prophet of a more aggressive disease course **[21].** Kroot et al **[22],** in a study of patients with early rheumatoid arthritis ,found that anti-CCP positive patients at follow up had developed notably more radiological harm than patients without this antibody.

IL-6 is a multifunctional cytokine that regulates immune response and induces acute phase response.

Despite the significant physiological activities of IL-6, deregulated overproduction of IL-6 is pathologically involved in different

immune-mediated inflammatory diseases including RA **[23]**.

In the present study serum level of IL-6 was significantly higher in RA than in the control group and the level of IL-6 in the severe RA group was significantly higher than in the moderate RA group which was significantly higher than in the mild RA group. These results were supported with [**24],** as they found that IL-6 is a cytokine that can assist autoimmune phenomena, increase acute inflammation and promote the progress into a chronic inflammatory state in RA patients also [**25],** had found that IL-6 is an important cytokine, present at high levels in patients with rheumatoid arthritis. The biological actions of IL-6 contribute to both systemic and local RA symptoms .

Increase level of IL-10 in RA has been previously revealed in many studies .We could also clearly shown this in our study .The expression of IL-10 was high in serum of patients with RA **[26].**

Conclusion

Significantly correlation of IL10,IL6,Anti-CCP, CRP and RF with rheumatoid arthritis ,So no significantly (P≥0.05) according to the age and gander groups, While there is highly significant correlation (p≤ 0.05) found in the studding parameters.

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