Determining Levels of Rheumatoid Factor (RF) and C - reactive protein (CRP) in a Blood Sample of Iraqi Patients with Rheumatoid Arthritis (RA)

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Abstract

Rheumatoid arthritis (RA) is a common autoimmune disease characterized by chronic inflammation and joint destruction. The aim of this study was to investigate levels of Rheumatoid factor (RF) and C- Reactive protein in Iraqi RA patients. Blood samples were obtained from 50 RA patients (males and females) referred to Rheumatology consultation clinic in Baghdad teaching hospital, Medical city, with a mean age of 40.88±18.75 years. On the other hand, blood samples were obtained from 50 healthy controls with a mean age of 38.90±14.73 years. Results showed that Rheumatoid factor (RF) was detected in all serum samples taken from RA patients (100% positive results) with 100% negative results in healthy controls while C- Reactive protein (CRP) was detected in 70% of serum samples taken from the same patients.

Keywords: Rheumatoid arthritis; Rheumatoid factor; C-Reactive Protein

تحديد مستويات عامل الروماتويد (RF) و بروتين سي الفعال (CRP) في عينة دم من المرضى العراقيين المصابين بالتهاب المفاصل الروماتويدي (RA)

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الملخص

التهاب المفاصل الروماتويدي (RA) هو احد امراض المناعة الذاتية الشائعة والذي يسبب التهاب مزمن يصيب المفاصل. كان الهدف من هذه الدراسة هو التحقيق من مستويات العامل الروماتويدي (RF) والبروتين سي الفعال (CRP) في المرضى العراقيين المصابين بألتهاب المفاصل الروماتويدي. تم الحصول على عينات دم من 50 مريض (ذكور و إناث) من عيادات استشارية المفاصل في مستشفى بغداد التعليمي، مدينة الطب، بمتوسط عمر تتراوح مابين 40.88 ± 18.75 سنة. من ناحية أخرى، تم الحصول على عينات الدم من 50 عينة ضبط صحيحة بمتوسط عمر 38.90 ± 14.73 سنة. تم الكشف عن العامل الروماتويدي (RF) في جميع عينات المصل المأخوذة من مرضى التهاب المفاصل الروماتويدي (100٪ نتائج إيجابية) مع 100٪ نتائج سلبية في عينات الضبط الصحيحة بينما تم الكشف عن البروتين سي الفعال (CRP) في 70٪ من عينات المصل المأخوذة من نفس المرضى.

1. Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease characterized by synovial inflammation and structural damage of joints [1]. The diagnosis of rheumatoid arthritis is based on clinical manifestations of joints and serological markers [2]. Patients with RA are characterized by presenting some circulating auto-antibodies in their blood. In clinical practice, the most common diagnostic test is a Rheumatoid factor (RF), C- Reactive Protein (CRP) and anti-cyclic citrullinated peptide (anti-CCP) is generally accepted by the majority of rheumatologists and recommended by the European League of Arthritis and Rheumatism (EULAR) [3]. These autoantibodies are valuable biomarkers for the diagnosis of RA, articular manifestations and disease activity [4].

Although the cause of RA remains unknown, the excessive production of cytokines that play a fundamental role in the processes that cause inflammation, articular destruction and extra-articular manifestations associated with RA [5], occupies a critical pathogenic role in the development and progression of the disease [6, 7].

The pathological roles of cytokines have also been clarified in various disease conditions, such as inflammatory, autoimmune, and malignant diseases [8]. This cytokine plays a potential role in immune modulation of several studies suggest that reactive oxygen species (ROS) and oxidative stress may be involved in the pathogenesis of various diseases, including RA. ROS have been implicated as mediators of tissue damage in patients with RA. Under normal conditions, a variety of antioxidant mechanisms serve to control this ROS production. In recent years, increasing attention has been given to the role of reactive oxygen metabolites in the pathogenesis of inflammatory disease such as RA. It has been suggested by several studies that enzymatic and/or non-enzymatic antioxidant systems are impaired in RA and that RA patients are thus exposed to oxidant stress [9, 10].

2. Materials and Methods

2.1. Materials

Rheumatoid factor latex kit contains latex reagent coated with human gamma globulin, positive and negative control serum. C- Reactive Protein latex kit contains a suspension of latex particles coated with anti-human C-reactive protein, positive and negative control serum. All reagents contain 0.9 g/L Sodium azide as a preservative.

2.2. Methods

Blood samples (5 ml) were collected in plain tubes from 50 patients with Rheumatoid arthritis, and from 50 healthy control volunteers. Then blood samples were centrifuged at 3000 rpm for 15 minutes. After centrifugation, serum samples were taken and immediately stored at -20˚C for detecting RF and CRP levels.

2.3. Detection of Rheumatoid factor and C- Reactive Protein by latex agglutination

All reagents and specimens were brought to room temperature. One drop (50 µl) of the positive control and 50 µl of the patient serum were placed into separate circles on the glass slide. The latex reagent was shaken gently and one drop (45 µl) was added to each circle next to the sample to be tested and control. Mixing well by using disposable stirrer, the mixture was spread over the whole test area and the slide was tilted gently and agitates for about two minutes with rotator or by hand and observe for the presence or absence of agglutination.

Serial dilutions (1:2, 1:4, 1:8, 1:16,1:32, 1:64, 1:128) of the positive test sample in the qualitative method was prepared by using normal saline 0.9%. For each specimen to be tested, 100 µL of 0.9% saline was added to test tubes numbered 1 to 5. Then, 100 µL of the specimen was added to test tube 1. After that, the mixture was mixed. 100 µL of mixed sample was transferred from tube 1 to 2. This serial dilution procedure repeated in tube 3 to 4, and then 5. 100 µL from test tube 5 was disposed of after mixing. Tubes 1 to 5 represent a dilution series as follows:

Tube Number	1	2	3	4	5
Dilution	1:2	1:4	1:8	1:16	1:32

The qualitative test procedure was performed by using each dilution as a test specimen. The serum RF and CRP titer can be defined as the highest dilution showing a positive result. The approximate RF or CRP level (IU/ml) present in the sample can be obtained by the following formula: RF or CRP Titer (IU/ml) = Highest dilution with positive reaction x Reagent sensitivity (10 IU/ml), e.g. if the agglutination is present up to a titer 1:8, the approximate serum RF or CRP level is 8 x 10 = 80 IU/ml.

3. Results and Discussion

3.1 Rheumatoid Factor (RF)

Rheumatoid Factor (RF) has been widely used as a screening test for patients with arthritis. A Recent study revealed that RF titer reflected RA disease activity [11]. However, RF is present in patients with other autoimmune and infectious diseases, and even in a noticeable proportion of normal healthy subjects, particularly in aged individuals [1].

In this study, Results showed that Rheumatoid factor (RF) was detected in all serum samples taken from RA patients (100% positive results) with 100% negative results in healthy controls (Table 1). Rheumatoid factor values in patients seem inconsistent, where Artur and Brikena demonstrated that (77.1 %) of RA patients with positive and (22.9 %) patients were negative [12], but several Iraqi studies demonstrated that all patients were a positive result for RF [13] [14]. High serum levels of RF are a hallmark of rheumatoid arthritis and can be used to monitor disease activity [15]. In another study, the presence of RF has been proved to be predictive of radiological disease progression, which is a clinical hallmark of aggressive disease [16].

3.2. C- Reactive Protein (CRP)

C-reactive protein, an acute phase protein, is synthesized by hepatocytes in response to pro-inflammatory cytokines, in particular, IL-6. It has been shown to be of a great value as an inflammatory marker in RA and has been suggested to mediate part of the complement activation in RA [17].

In this study, RA patients 35/50 (70%) showed positive for CRP, while 15/50 (30 %) were negative for CRP (Table 1). It is indicated that CRP can be used as a serum marker for RA [18]. It is concluded, therefore, that C-reactive protein and rheumatoid factor in patients with rheumatoid arthritis might be sensitive inflammation markers for reflecting the presence and activity of the disease.

Table 1: Rheumatoid factor and C - reactive protein percentage in RA patients and healthy controls

Parameter		**Healthy controls N (%)**	**RA patients N (%)**
RF	Positive (+ve)	0 (0.0)	50 (100.0)
	Negative (-ve)	50 (100.0)	0 (0.0)
CRP	Positive (+ve)	0 (0.0)	35 (70.0)
	Negative (-ve)	50 (100.0)	15 (30.0)

4. Conclusion

Rheumatoid arthritis is a common autoimmune disease characterized by chronic inflammation and joint destruction. In clinical practice, the most common diagnostic test is a rheumatoid factor and C-reactive protein and are generally accepted by the majority of rheumatologists. Rheumatoid Factor and C- Reactive Protein are Immunological markers confers susceptibility to RA Iraqi patients.

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