



Evaluation of Cryoglobulin Rheumatoid Factors concentration and Total IgG and IgA Levels Among Rheumatoid Arthritis Patients

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Abstract

Background: rheumatoid arthritis is a common autoimmune disease that may affect many tissues and organs, but principally attacks flexible (synovial) joints., The pathology of the disease process often leads other systemic complications, Many individuals with rheumatoid arthritis produce a group of auto –Ab, called rheumatoid factors and anti-citrullinated peptide antibodies (ACPA).

Materials and methods: from 58 rheumatoid arthritis patients , blood samples were collected and processed for manifestation on the rheumatoid factors , cryoglobulin, total IgG and IgA levels analyzed by ELISA, and erythrocytes sedimentation rate (ESR).

Results: the present study demonstrates that the difference between concentrations of R.F in whole serum and cryoprecipitable R.F of the same rheumatoid arthritis patients was non significant. additionally slight elevation of the IgG level compared with healthy persons, while the increase level of IgA in serum of Rheumatoid arthritis patients compared with those of healthy persons was statistically significant, as well as the increase of ESR level among patients group compared with the control sample was significant.

Conclusion: cryoglobulin level increase associated with severity grade of rheumatoid arthritis and the level of IgA refers to severity of the joints damage and complication of diseases .

1. Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease affecting approximately 0.5-1% of populations worldwide. Development of RA is based on both genetic susceptibility and environmental factors. Exposure to an infection may act as a trigger for RA (Silman and Pearson, 2002). Rheumatoid arthritis is characterized by synovial inflammation and hyperplasia ("swelling"), autoantibody production (particularly to FC fraction of IgG and citrullinated peptide), cartilage and bone destruction ("deformity"), and systemic features, including cardiovascular, pulmonary, psychological, and skeletal disorders.. (Koivuniemi, 2009; McInnes and Schett, 2011). Rheumatoid factor is the classic autoantibody in rheumatoid arthritis. IgM, IgG and IgA rheumatoid factors are key pathogenic markers directed against the Fc fragment of IgG. Additional (and increasingly important) types of antibodies are those directed against citrullinated peptides called anti-citrullinated peptide antibodies (ACPA). Although most, but not all, ACPA-positive patients are also positive for rheumatoid factor, ACPA seem more specific and sensitive for diagnosis and seem to be better predictors of poor prognostic features such as (progressive joint destruction. (van der Linden *et al*, 2009; Scott *et al*, 2010)

The mechanism for development RA involved the Environment–gene interactions that promote loss of tolerance to self-proteins that contain a citrulline residue, which is generated by post-translational modification. This anticitrulline response can be detected in T-cell and B-cell compartments and is probably initiated in secondary



lymphoid tissues or bone marrow that mediated by production of autoantibodies . Thereafter, localization of the inflammatory response occurs in the joint by overproduction and overexpression of TNF.9 This pathway drives both synovial inflammation and joint destruction. (Bingham, 2002 and Scott *et al*, 2010).

Cryoglobulins are immunoglobulins that precipitate in the cold and dissolve on rewarming. Three types of cryoglobulins are distinguished based on whether the cryoglobulin is monoclonal and has rheumatoid factor activity. Type I is a monoclonal antibody that does not have rheumatoid factor activity. Most commonly, type I(monoclonal immunoglobulin)which usually denote malignancy like lymphoma. Both types II and III are rheumatoid factors — antibodies that bind to the Fc fragment of IgG. Therefore, both types are called mixed cryoglobulins. In type II, the rheumatoid factor is monoclonalIgMK, whereas in type III it is polyclonal. Type II is associated with lymphoproliferative diseases, and both types can occur in patients with rheumatic diseases and chronic infections.(Erhardat *et al*, 1984; Lin and Phillips, 2002; and Shihabi,2006). cryoglobulins and immune complexes are found in synovial fluid and these are thought to play arole in the pathogenesis of the articular inflammation that occurs in that disease.(Weisman and Zvaifler, 1995).

The present study aims to evaluate the immunoglobulin concentration , RF concentration and it relationship with cryoglobulin level among certain group of Rheumatoid Arthritis.

2-Material and methods

2-1- Blood sample processing

Between January and September 2010, blood samples were collected from fifty eight patients(the age range15-55 years)were clinically diagnosed as rheumatoid arthritis patients attending in Al- Mahaweel hospital, as well as fifteen person choosing as a control subject . Serum were separated from each sample and divided for three parts for working Rheumatoid factor assay (R F),Cryoglobulin test and total IgG and IgA ELISA.

A: Rheumatoid factor assay

The measurement of the RF in a serum patient performed by using latex fixation test for the qualitative screening according to the recommendations of the manufactured company(Genix technology Vancouver, Canada).

The principle of this test is based on the immunologic reaction between the RF in serum with the corresponding IgG coated onto latex particles resulting in visible agglutination.

B: Cryoglobulin assay

Cryoglobulins are predominantly immunoglobulin complexes precipitate at 4°C and dissolved at 37°C. This test was performed according to Ferri(2008) methods, which includes the following steps:

1-2 ml of serum must be obtained from clotted blood at body and putted in graduated Khan tube or cryocrit tube .

2-Fill the cryocrit tube up to 100 mark with serum .

3-leave it in cool place (refrigerator at 2-4°C for 2-4 days .

4-After 4 days measure the precipitating layer (Crycrit layer) . The negative result refer to completely clear of serum .

5-Spin crycrit tube at 2000 rpm / 4°C for 10 min.

6-Determine the percent by reading calibrator , from 1-100 according to this steps :



- Read the total amount of serum (2ml) .
- Read the amount of cryocrit .
- Divide the cryocrit reading to the total serum reading and multiply by 100 to obtain the cryocrit result .

Cryoglobulin R.F assay (Modified method)

After cryoglobulin percentage calculation discard the supernatant and retain the cryoglobulin layer then reconstitute the volume up to origin volume (2ml) by normal saline .Repeat the Rh. Factor by titration methods to monitor the titer of positive result same as the whole serum .

C-Total IgG and IgA ELISA

The quantitative determination of total IgG and IgA in serum of patient with positive for rheumatoid factor assay by enzyme linked immunoassay (ELISA) are performed according to the manual procedure of immunotech company.

The principle for these test depend on the IgG in samples and standards binds to antibodies which are coated to the microtiter plate. After a washing step a peroxidase labeled detection antibody is added. A second washing step is followed by the addition of the substrate which is converted to a colored product by the peroxidase. The reaction is terminated by the addition of an acidic stop solution. The optical densities are read at 450 nm in a microtiter plate reader. The IgG concentration can be calculated from the standard curve.

D-Erythrocytes sedimentation rate (ESR) test

Blood samples were collected with anticoagulant from patients .This test was performed according to method submitted by Brown 1976.

3-Results and discussion

1-Age of patients

The age range of Rheumatoid arthritis (R.A) patients was ranging from (15-55)years .It was divided into four age groups (table-1) .The highest detection rate of disease was noted at the age group of (26-35)years ,which constituted (41.3).This result might be refer to Relation between ,social behavior , stress factors and immune reaction among both male and female patients .(MacGregoret *al*, 2000; Silman and Pearson,2002; McInnes and Schett, 2011)

Table-1-the distribution of patients according to the age groups

No.	Age group	Total no.	%
1.	15-25 years	6	10.5
2.	26-35	24	41.3
3.	36-45	17	29.3
4.	46- 55	11	18.9

2- R.F and cryoglobulin

The comparison between the concentrations of R.F in whole serum and cryoprecipitable R.F of the same rheumatoid arthritis patients were given in Table-2 , which show the statically difference(using *t* test) was non significant (N.S.) ($P>0.05$).This finding referred to slight elevation of cold antibody among this group of patients , additionally the level of cryoglobulin associated with grades of

rheumatoid arthritis. In rheumatoid arthritis (RA), cryoglobulins and immune complexes (20) are found in synovial fluid and these are thought to play a role in the pathogenesis in that disease (Weisman and Zvaifeler, 1975 and Shihabi, 2006). Muller, (2012) used flow cytometry analysis for detection cryoglobulin and referred to that low CG levels may escape detection by the current diagnostic methods

Table-2- The Descriptive statistic of RF concentration (I.U /ml) in whole serum compared with the RF concentration in cryoglobulin layer of rheumatoid arthritis patients

Mean	Whole serum	Cryoglobulin layer
	107.4	126.2
No.	21	18
S.D	21.2	22.7
$t=0.79$ $P>0.05$ N.S.		

3- IgG ELISA

In this study we demonstrated that the statistic difference between the IgG concentration in patients compared with health persons was non significant, (Table-3). This result may be due to the formation of the immune complexes by rheumatoid factors that directed against the Fc fragment of IgG and trapped then precipitate it in target organs. The production of IgG RF is particularly important due to the ability of IgG RF to self-associate and form large aggregates with high complement-fixing potential. (scott, 2010)

Table-3- The descriptive statistic of the serum IgG level among the rheumatoid arthritis patients compared with control study .

Mean	Test	Control
	1693	1693
No.	15	15
S.D	390	386
$t=0.09$ $P>0.05$ N.S.		

4- IgA ELISA

The present study also includes a manifestation on the IgA level in the serum of Rheumatoid arthritis patients and compared with those of health persons . The result was revealed of IgA level among the patients compared with the control group. The increase was statistically significant, (Table 4). This result was consistent with those demonstrated by Veys and Claessens, (1968) and [Jónsson](#) (1992), who found that elevation in IgA level among young Rheumatoid arthritis patients at severe disease course, possibly due to immune complex formation (Badcock *et al*, 2003)

Table-4- The descriptive statistic of the serum IgA level among the rheumatoid arthritis patients compared with control study

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Mean	Test	Control
	427	326
No.	15	15
S.D	132.9	108
$t= 0.03$ $P<0.05$.S.		

5- Erythrocyte sedimentation rate (ESR) level

The ESR level is a diagnostic and prognostic marker to arthritis and most types of infection as well as chronic diseases. Table-5- explains that a significant increase ($P<0.05$) of ESR level among patient group compared with the control sample. Sfriso and his colleagues found that the rheumatoid patients with high IgA level accompanied with increase in erythrocyte sedimentation rate (ESR),

Table-5- The ESR level of rheumatoid arthritis patients compared with control group

Mean	Test	Control
	60.7	6.5
No.	58	15
S.D	24.9	1.88
$t= 0.01$ $P<0.05$.S.		

4- Conclusions

1-Cryoglobulin associated with a sever course of rheumatoid arthritis in patients at winter compared with other seasons .

2- Increase level of total IgA may be refers to the abundance of rheumatoid factor class IgA and associated with a severity of the tissue damage.

3-ESR level is useful as prognostic indicator for chronicity of disease and follow up the treatment.

5- References

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Weisman M., and Zvaifler N., (1995) : Cryoglobulinemia in rheumatoid arthritis . Significance serum of patients with rheumatoid vasculitis. *J. Clin. Invest.* 56(3):725-739

**في مرضى التهاب IgA و IgG تقييم تركيز العوامل الرئوية المترسبة بالبرودة والمستوى الكلي للضدين
المفاصل الرثوي**

عبيد فوزي الربيعي كلية العلوم للبنات جامعة بابل	عروبة كطوف البيرماني كلية العلوم للبنات جامعة بابل	رحيم طعمة المعموري مستشفى المحاويل مختبر الصحة العامة
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الخلاصة

مقدمة: التهاب المفاصل الرثوي هو احد امراض المناعة الذاتية الشائعة والتي تؤثر على عدد من الانسجة والاعضاء و بشكل اساسي تهاجم المفاصل (السائل المفصلي). ان عملية الامراضية هذه غالبا ما تمتد الى مضاعفات جهازية اخرى.

المواد وطرائق العمل: تم جمع عينات الدم من ثمانية وخمسون لمرضى التهاب المفاصل الرثوي وتم معاملتها لغرض التحري عن وجود العامل الرثوي والكلوبيولين المناعي البارد وكذلك الاليز التقدير مستوى تركيز كل من الضد IgG و IgA ومعدل ترسيب خلايا كريات الدم الحمراء

النتائج: لم تشر نتائج الدراسة الحالية الى وجود اختلاف معنوي بين تركيز العامل الرثوي في عينة المصل الكامل وتركيز العامل الرثوي المرسب بالبرودة لمرضى التهاب المفاصل الرثوي . بالاضافة الى ارتفاع بسيط في مستوى IgG بالمقارنة مع الاسوياء ، في حين كان هناك زيادة معنوية في مستوى الضد IgA في المرضى مقارنة بالاسوياء. في ما يخص معدل ترسيب كريات الدم الحمراء فقد كان هناك ارتفاع معنوي في المرضى مقارنة بالاسوياء .

الاستنتاج: اثبتت الدراسة الحالية ان زيادة مستوى تركيز الضد البارد يكون مرتبط بدرجة شدة المرض كما ان مستوى الضد IgA يشير الى شدة تضرر المفاصل ومدى مضاعفات المرض