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CLINICAL ACCURACY OF ANTI-CCP AND RHEUMATOID FACTOR (RF) IN THE DIAGNOSIS OF RHEUMATOID ARTHRITIS (RA)

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ABSTRACT Rheumatoid arthritis (RA) is the commonest autoimmune disease to be encountered in practice.

The diagnosis of RA is predominantly clinical while as few serological markers may aid the diagnosis. . These markers include IgM Rheumatoid factor (RF), Anti perinuclear factor (APF), Anti keratin antibodies (AKA) and Anti cyclic citrullinated peptide (Anti CCP). We studied the clinical accuracy of Anti CCP (ELISA) and IgM-RF in the diagnosis of RA. We collected blood samples from 110 subjects. 35 subjects were diagnosed cases of RA (28 females, 7 males; with a mean age of 63.5 years) which was established using 2010 ACR/EULAR criteria. The control group consisted of 75 subjects, which included 15 subjects with connective tissue diseases (6 Sjogren syndrome, 4 Systemic Lupus Erythematosus, 4 Systemic sclerosis and 1 Mixed connevtive tissue disease), 10 subjects with psoriatic arthritis, 10 subjects with various viral infections (Hepatitis B virus, Hepatitis C virus, Epstein Barr virus, Parvovirus B 19), 10 subjects with various malignancies and 30 healthy subjects.16 among the 35 RA patients (45.71%) were documented positive for anti-CCP at very high concentration, whereas only 3 out of 75 control sera (4.0%) showed a positive reaction, which included 2 HCV seropositive patients and 2 with MGUS. None of the healthy controls or patients with connective tissue diseases or cancer was positive. IgM-RF showed a higher sensitivity (66.1% vs 45.71%) and a lower specificity (84% vs 98.1%) than anti-CCP. When assayed together, the specificity would increase from 98.7% for anti-CCP alone to 99.6%. When anti-CCP and RF determination are used collectively, the specificity for RA approaches almost 100%, which may help in distinguishing RA from other rheumatic diseases.

KEYWORDS :

INTRODUCTION

Rheumatoid arthritis (RA) is the commonest autoimmune disease to be encountered in practice.[1]

RA leads to erosive synovitis, joint destruction, joint pain, functional disability and deformities. Early diagnosis and intervention can help in preventing the joint destruction and other sequele of RA. The diagnosis of RA is predominantly clinical while as few serological markers may aid the diagnosis. These markers include IgM Rheumatoid factor (RF), Anti perinuclear factor (APF), Anti keratin antibodies (AKA) and Anti cyclic citrullinated peptide (Anti CCP).[2,3,4]The specificity of RF in the diagnosis of RA is low because it is also found in patients with other autoimmune diseases, chronic infections and even healthy immunized individuals. APF and AKA are quite specific but technical issues with substrate standardization have made these tests less popular. Anti CCP has shown very high specificity for the diagnosis of RA in few studies.[5,6]

Nowadays, 2010 ACR/EULAR criteria are widely used for the diagnosis of RA, which includes a specific scoring system of which RF and Anti CCP estimation forms an integral part. In our study we studied the clinical accuracy of Anti CCP (ELISA) and IgM-RF in the diagnosis of RA. We conclude that anti-CCP antibody assay is very efficient in the diagnosis of RA with a remarkably high specificity.

MATERIALS AND METHODS

In our study, we collected blood samples from 110 subjects. 35 subjects were diagnosed cases of RA (28 females, 7 males; with a mean age of 63.5 years) which was established using 2010 ACR/EULAR criteria. The control group consisted of 75 subjects, which included 15 subjects with connective tissue diseases (6 Sjogren syndrome, 4 Systemic Lupus Erythematosus, 4 Systemic sclerosis and 1 Mixed connevtive tissue disease), 10 subjects with psoriatic arthritis, 10 subjects with various viral infections (Hepatitis B virus, Hepatitis C virus, Epstein Barr virus, Parvovirus B 19), 10 subjects with various malignancies and 30 healthy subjects. Anti-CCP antibodies were analysed using ELISA. All sera were tested in duplicate and the average results presented. Sera were stored at -72˚C until use. The cut off value for positive reaction was set at 25 U/ml. The intraassay variation was 13.6% with a "high level serum" (1360 U/ml) and 6.6% with a "low level serum" (79 U/ml), and the interassay variation was 10.5% and 7.8%, respectively. Latex particle agglutinating IgM-RF was analysed using EIA. Statistical analysis was performed using the SPSS 6.0 for Windows statistical package (SPSS). Means, SD, and confidence intervals (CIs) were used where appropriate. Nonparametric analyses were used to compare the two different groups of patients, and multiple regression models were used to assess the importance of the different variables relative to anti-CCP status. P value <0.05 was considered significant.

RESULTS

16 among the 35 RA patients (45.71%) were documented positive for anti-CCP at very high concentration, whereas only 3 out of 75 control sera (4.0%) showed a positive reaction, which included 2 HCV seropositive patients and 2 with MGUS. None of the healthy controls or patients with connective tissue diseases or cancer was positive. None among all the four false positives had very high concentrations of anti-CCP. At a cutoff value of 50 units, sensitivity was 45.71% (CI, 36–56%) and specificity was 98.1% (CI, 95–100%). We compared the results of anti-CCP and IgM-RF for sensitivity and specificity. IgM-RF showed a higher sensitivity (66.1% vs 45.71%) and a lower specificity (84% vs 98.1%) than anti-CCP. We also analyzed how these parameters would be affected if anti-CCP and IgM-RF were combined in patients with only one of the two markers (anti-CCP or IgM-RF) positive as well as in patients with both markers (anti-CCP and IgM-RF) positive. When assayed together, the specificity would increase from 98.7% for anti-CCP alone to 99.6%.

DISCUSSION

The keratohyalin bodies present in human buccal mucosa cells contain filaggrin, a protein that is recognized by APF and AKA, which are specific antibodies present in RA subjects. These antibodies can be detected by indirect immunofluorescence techniques, but they have never become part of the diagnostic repertoire because of difficulties in the availability and storage of the antigenic substrates and difficulties associated with interpreting the results.The recent development of synthetic peptides containing citrulline, an amino acid present in the filaggrin molecule and produced after its deimination, has enabled the development of an ELISA test; from preliminary data obtained during experimental trials, this test appears to have the same high specificity as APF and AKAs and to be able to eliminate the standardization problems related to immunofluorescence procedures. In this study, we evaluated the diagnostic accuracy of this ELISA test. The diagnostic sensitivity was 45.71% and specificity was 98.1%. The low sensitivity of the test is because of the fact the fact that the synthetic peptide used in this assay represents a relatively small set of antigenic determinants that do not entirely encompasses the antigenic determinants present on the as yet unknown antigenic molecule in the joint. The high specificity of the assay make it very valuable in the diagnosis of RA.

In patients with early arthritis, the correlation with anti-CCP was highly significant, thus indicating that this assay may be used even in the early phases of disease. This aspect is important because an early diagnosis of RA may modify the therapeutic strategy substantially, suggesting the use of more aggressive pharmacological treatments that can delay the progression of joint damage and thus substantially change the natural history of the disease.

We conclude that anti-CCP antibody assay is very efficient in the diagnosis of RA with a remarkably high specificity. In view of the low sensitivity, the scope of using it as a screening test is extremely low. Because of its high specificity, especially when high antibody concentrations are present, it may become one of the most useful serologic tests for the diagnosis of RA. Also, when anti-CCP and RF determination are used collectively, the specificity for RA approaches almost 100%, which may help in distinguishing RA from other rheumatic diseases.

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