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Determination of Specificity and Sensitivity of Rheumatoid Factor and Anti CCP Tests in Patients with RA in Private Clinic in Tehran, Iran

MOHSEN SOROUSH[1], MOJTABA MAHMOUDI[2] and MAASSOUMEH AKHLAGHI[3]*

[1]Rheumatology section, Department of Internal Medicine,
AJA University of Medical Sciences, Tehran, Iran.
[2]AJA University of Medical Sciences, Tehran, Iran.
[3]Rheumatology Research Center, Tehran University of Medical Sciences, Tehran, Iran.
* Corresponding author E-mail: akhlaghimd@yahoo.com

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ABSTRACT

Rheumatoid Arthritis (RA) is a systemic, chronic, inflammatory and autoimmune disease that often leads to joint destruction. RA is the most common inflammatory joint disease of the world. The diagnosis of this disease has been based primarily on clinical manifestation. However the highly variable and unpredictable course of the disease suggests the need for highly sensitive and specific diagnostic tests that help to early diagnosis and treatment. We conducted a study to evaluate the sensitivity and specificity of Anti CCP and Rheumatoid Factor (RF) in our patients with RA. In this study we review documents of our patients with diagnosis of RA or other inflammatory diseases or healthy persons with simultaneous RF and Anti CCP tests. These patients visited in outpatient clinic or admitted in Imam Reza Hospital. Sensitivity and specificity of the tests were evaluated taking the clinical diagnosis as the gold standard. Our total cases were 833. 429 (51.5%) patients with RA and 404 (48.5%) non RA patients or normal population with RF and Anti-CCP test selected. There were 113 males and 316 females in RA patients and 27 males and 377 females in non RA group. Average of age in RA patents was 80 (5-88) and in non RA group was 78 (10-88). Base on statistical analysis in our patients the test sensitivity for RF and Anti CCP were 84.8% and 74.3%, respectively. Specificity for RF and Anti CCP were 72.5% and 92.8%, respectively. High specificity in the Anti CCP test support the diagnosis of RA. Anti CCP can be proved to be a powerful diagnostic tool especially in ambiguous cases or RF negative patients with RA.

Key word: Rheumatoid Factor, Anti-CCP tests, specificity and sensitivity.

INTRODUCTION

Rheumatoid Arthritis (RA) is a common systemic autoimmune disease that 1% of the *world's population is suffering from it*[1]. The disease, in addition to reducing the average life expectancy of patients, is associated with significant morbidity. So that 50% of RA patients are not able to continue their jobs after ten years of illnes[2]. In recent studies, early treatment is emphasized in order to prevent and reduce its complications. In several independent studies it has been shown that even a short delay in starting treatment can have a significant effect on the disease activity in the coming years[3,4], but given that today, RA is primarily diagnosed based on clinical symptoms, and in most cases, the characteristic symptoms of disease appear after 2-1 years of onset, early detection of disease is not often possible.

On the other hand, effective but not completely safe treatments are available today, that definitely diagnosing the disease is needed to apply them, so that their side effects not to be imposed on patients unnecessarily. So, today "how to treat RA" has been shifted to "how to recognize RA quickly and reliably"[5]. Therefore, it is obvious that a sensitive and specific serologic test is needed for diagnosing the disease in early-stages. And since RA progress differs in various patients, if the test is also associated with the severity and activity of disease, and is also able to predict the course of disease, it will be more useful[6].

IgM isotype of rheumatoid factor, conducted as serological test in laboratories today, is not useful and specific for the diagnosis of RA due to the low predictive value[7, 2]. It can be positive in infectious diseases, other rheumatic diseases, as well as in a percentage of healthy individuals. Although, AKA (anti-keratin antibodies) and APF (Anti-perinuclear factor) are not specific for RA [11-8] and also anti-Sa[9,11-13], an antibody against citrullinated vimentin (a filament protein found in mesenchymal cells), they have no practical applications because of technical difficulties[15, 14]. Above antibodies, which all are RA-specific, are measured by *immunofluorescence* method. Although antibodies are useful both in terms of diagnosis and as a hyperdiagnostic marker[17, 16], none of them is practically applicable because of the difficulty of preparing and reserving the antigenic substrate for them, as well as lack of standard pattern for the interpretation of test results, despite the high specificity for RA[18,14] and the measurement of above antibodies can be done only in specific laboratories[19].

In 1998 it became apparent that all the RA-specific antibodies target *citrulline*-containing proteins[21, 20]. Recently, *citrulline*-containing peptides have been provided synthetically and led to the emergence of anti-CCP test by ELISA [20]. In this test, antibodies, which are RA-specific and against citrullinated peptides in patients' serum, are measured using citrulline- containing synthetic protein[9,20,22].

S. Dubucquoi and colleagues compared the sensitivity and specificity of AKA, RF, linear *anti-citrullinated filaggrin antibody* and Anti-CCP peptide in a recent study conducted on the serum of 140 RA patients and 131 controls (consisting 33 healthy subjects and 98 patients with autoimmune diseases other than RA). In their study, the sensitivity of Anti-CCP was 65%, linear *anti-citrullinated filaggrin antibody* 45%, anti-keratin antibody 48% and RF 60%. Specificity of Anti-CCP was 96.4%, linear anti-filaggrin antibody 97.1%, anti-keratin antibody 93% and RF 69%. They suggest that Anti-CCP for high sensitivity (65%) and specificity (96%) could be a useful alternative for anti-keratin antibodies and linear anti- citrullinated peptide for RA diagnosis[17].

J. Vencovsky and colleagues, in a prospective study conducted on 104 patients with rheumatoid arthritis with duration <2yr, showed that Anti-CCP positive predicts the emergence of early erosions with progressive disease in patients[23].

According to previous studies, this test has advantages such as the following: It is performed by ELISA and able to be standardized[9]. It results are expressed quantitatively. It detects numbers more than 1000 in positive cases and below 50 in negative cases[14], as well as, RA-specific antibodies, namely, AKA and APF and has a high specificity. It doesn't require to prepare antigen from buccal mucosa or esophageal mucosa and can therefore be applied in practice [9]. Because its antigen is produced a synthetically, it is positive in the early stages of the disease and even before symptoms appearance. It is positive in 40% of RF-negative RA patients. Also, since it doesn't have high concordance with RF, doing it with RF increases the sensitivity of both tests and associates with severe and erosive cases[24, 22].

Therefore, due to the positive and promising results in studies conducted previously, Anti-CCP test is addressed as an optimal serological test which is able to detect RA in early-stages and with high reliability, and even able to predict the course of disease, and thus helps to determine treatment strategies in different patients, and it is predicted to be included in RA diagnosis criteria and performed as a routine test in laboratories in the near future. Therefore, the sensitivity and specificity of this test was evaluated

in RA patients in Rheumatology Research Center, Shariati Hospital in this study, so that it's practical application to be provided.

METHODS

This study is a cross-sectional study on patients Presented to a private clinic. If patients referred to rheumatology clinic consented to participate in the research, they entered the study and were placed in either rheumatoid arthritis or non-rheumatoid arthritis group on the basis of having or not having rheumatoid arthritis according to ACR criteria and relevant information was extracted from their files and analyzed. The sample size was 833 patients referred to rheumatology clinic from May 2006 to February 2009. Personal information and data relating to RA diagnostic tests in patients, including RF and Anti-CCP test results were collected in questionnaires. After recording the data, it was imported into Excel 2007 and desired variables were analyzed using Student's T-paired test and finally the results of this survey were reported.

How to test RF

IgG-, IgA- and IgM-RF isotypes were performed by ELISA test according to the protocol introduced by Jonsson *et al.,*[25]. The results were reported in U/ml. ELISA results were considered positive with value greater than 15 U/ml and this amount of value was considered for all RF isotypes. The results of these tests were used for statistical analysis.

How to test Anti-CCP

This test was conducted by CCP ELISA industrial kits. Results were reported as an arbitrary unit. Samples with antibody titers greater than 25 units were considered positive. The results of these tests were analyzed.

RESULTS

Among patients in the study, 429 subjects were RA (51.5%), 113 (26.3%) males and 316 females (73.3%), and 404 subjects were non-RA (48.5%), 27 males (6.7%) and 377 females (93.3%). In general, the study is constituted of 140 males (16.9%) and 693 females (83.1%). The age range for RA group was 5 to 88 years with a mean age of 44.9 years and the age range for non-RA group was 10 to 88 years with a mean age of 45.6 years. According to the results of Anti-CCP and RF tests in both RA and non-RA groups using the following calculation formula, the sensitivity and specificity were calculated for each test.

$$\text{Sensitivity} = \frac{\text{real positive}}{\text{real positive} + \text{false negative}}$$

Table 1: Results of combining two tests as the positivity of both RF and Anti-CCP tests

	Negative	Positive	SUM
RA	130 (30.3%)	299 (69.7%)	429 (100%)
Non-RA	293 (72.7%)	111 (27.3%)	404 (100%)
SUM	423	410	833

Table 2: Results of combining two tests as the positivity of one of them (RF or Anti-CCP)

	Negative	Positive	SUM
RA	345 (80.3%)	84 (19.7%)	429 (100%)
Non-RA	130 (32.2%)	274 (67.8%)	404 (100%)
SUM	475	358	833

Anti-CCP test results in RA and non-RA groups were as the following.

110 individuals out of RA group (25.7%) were Anti-CCP negative and the others (74.3%) were positive. While 375 individuals out of non-RA group (92.8%) were Anti-CCP negative and the others (7.2%) were positive. Finally according to the above formula, the specificity and sensitivity of Anti-CCP were calculated 74.3% and 92.8%, respectively. The RF test results were also analyzed in both RA and non-RA groups and it was shown that 65 individuals out of RA group (15.15%) were RF negative and 364 individuals (84.8%) were RF positive. In another group 293 individuals (72.5%) were RF negative and 111 individuals (27.4%) showed a positive response to the test. As previous test results, results were placed in the formula to assess the specificity and sensitivity. The results showed that the specificity and sensitivity of this test were 84.4% and 72.5%, respectively. Results of combining two tests were analytically analyzed as the positivity of both tests to assess the impact of both tests to detect the disease. The results can be seen in Table 1.

According to the above table and the use of formula to calculate sensitivity and specificity, the sensitivity and specificity of combining two tests as the positivity of both tests were calculated 69.7% and 72.7%, respectively. Also, the results obtained from the combination of two tests were calculated as the positivity of one of them for comparison with previous results. Details of the survey can be seen in Table 2.

According to the table 2 and expressed formula, the sensitivity and specificity of combining two tests as the positivity of one of them were 19.7% and 32.2%, respectively.

DISCUSSION:

This study is an evaluation of diagnostic tests and its purpose is to evaluate the sensitivity and specificity of Anti-CCP in diagnosing rheumatoid arthritis. The mean age of the participants in this study was 45.3 years and as expected, the majority of patients was female (73%) that this result is approved in studies conducted in Brazil [26], Syria [10] and America[8]. RF test used in RA diagnosis since 75 years ago was a sensitive test in previous studies, meaning that it was more used for screening and had no needed specificity for RA diagnosis and in this study, its sensitivity and specificity were 80.48% and 72.5%, respectively that is consistent with available resources and previous articles.

In a study conducted in Brazil, 75% of patients had positive Anti-CCP [26]which is consistent with the number of positive cases in this study (72.7%) that indicates the power of this study. While 67.9% of them had positive RF, 84.8% of patients were RF positive in our study. This difference may be explained by saying that patients in our study are in more advanced stages of the disease and their disease is active. It's why RF is a sensitive test, meaning that its negativity is valuable for us. In Brazilian study, like current study, there was no relationship between gender and response to Anti-CCP, but positive response was greater in the younger group that the difference with this study can be attributed to the older mean age of the study and the wider age range in Brazilian study.

$$\text{Specificity} = \frac{\text{real positive}}{\text{real positive} + \text{false negative}}$$

In a study conducted in Syria, the sensitivity and specificity of Anti-CCP test have been reported 71.9% and 100%, respectively which is a little different with current study that can be attributed to the low sample size in Syrian study[10]. Also, the surprising result in Syrian study that is inconsistent with our and other studies is the sensitivity and specificity of RF test which have been reported 70.3% and 96%, respectively. The rate of positive Anti-CCP in RF-negative individuals was 31.6% that is consistent with the results obtained in this study (40%). Lack of association between gender and Anti-CCP response rate proved in this study has been proved in Syrian study too.

As noted in the results, when RF and Anti-CCP tests are combined, sensitivity and specificity will change in a ratio, so that together the disease can be diagnosed more accurately. In a study conducted in Norway with rheumatoid arthritis more

than one year of duration by combining these two tests the sensitivity and specificity have been achieved 37% and 92%, respectively[8], that is a little different with current study which can be attributed to the duration and activity of the disease (more than one year) and non-specified timeframe in Norwegian study. So that the more the time and the more the disease severity, the more the possibility of factor *positivities.*

CONCLUSION

In general, given to the results obtained in this study as well as the results of other studies, it can be concluded that simultaneously doing both RF and Anti-CCP tests leads to the timely diagnosis of rheumatoid arthritis, with the dramatic increase in sensitivity and specificity of test.

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