Original Article

CLINICAL EFFICACY OF ANTI-CCP AND RHEUMATOID FACTOR IN DIAGNOSIS OF RHEUMATOID ARTHRITIS

Farheen Aslam, Asma Shaukat, Sadaf Shafique, Raheel Khan, Faizan Ahmed Zakir

Quaid-e-Azam Medical College, Bahawalpur Pakistan

ABSTRACT

Objective: To determine the clinical efficacy of Rheumatoid factor (RF) and anti-CCP for diagnosis of rheumatoid arthritis in patients with symptoms of joint pains.

Material and Methods: The serum anti-CCP and RF levels were performed in pathology department from October 2019 to September 2020. Patients who visited the Rheumatology Clinic of Medicine department of Bahawal Victoria Hospital with muscular and joint pains. The quantitative parameters (RF and anti CCP) were calculated by median and interquartile range (IQR). The sensitivities, specificities, positive predictive and negative predictive levels of both parameters were performed. Receiver operator curves (ROC) analysis was done. The area under curves were calculated for RF and anti-CCP.

Results: During this study duration, 180 patients with muscular and joint pains were investigated for RF, ESR CRP and anti-CCP tests. The small joints of the hands were mainly affected. The pain and swelling of joints with morning stiffness were chief presenting symptoms of patients. The sensitivity, specificity, PPV and NPV of serum anti-CCP were 92%, 87%, 89% and 92% while that of RF were 80%, 66%, 73% and 62% respectively. The AUC of 0.89 and 0.79 were measured on ROC analysis for anti-CCP and RF.

Conclusion: This study showed that anti-CCP is more sensitive and specific serological marker for diagnosis of RA.

Key Words: Clinical efficacy, Anti CCP, RF, Rheumatoid arthritis.

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INTRODUCTION

Rheumatoid Arthritis (RA) is an autoimmune disorder with systemic manifestation. It is estimated that overall burden of disease is about 1% [1].The study conducted on adult population of Northern Areas of Pakistan recorded about 5.5 per 1000 cases of RA [2] while another study showed that about 13% of patients who visited rheumatology clinic at Agha Khan Hospital had RA with 3.5% subjects had extra-articular manifestations [3]. This disease not only affects the life expectancy but also quality of life is disturbed as half of RA patients have to quit their jobs and routine activities.

The main focus of the physician is to start early treatment to prevent the disease progression and its complications. The review of literature has proven that early treatment strategy is beneficial for patients [4,5]. Mostly, disease is diagnosed on clinical symptoms that appear 1-2 years after the start of disease process. IgM isotype of rheumatoid factor can be used for early diagnosis of RA but RF is not disease specific biomarker and has low predictive vales [6]. This serological test gives positive result in certain infectious and rheumatological diseases but even healthy individuals show false positive values [7]. Therefore, there is a need to find out a marker that gives promising results for early detection and progression of disease process.

Over the last decade, different biomarkers i,e antikeratin and antifilaggrin were evaluated but antibodies to citrullinated peptides and proteins (anti CCP) had major role in diagnosis of RA [8]. Citrullination of proteins is a post-translational modification, which may be seen in cell apoptosis process [9]. Many studies were carried out to determine influence of Anti-CCP test in detection and monitoring response of different management plans [8,9,10].

There is option in diagnostic criteria of RA that either anti-CCP or RF can be performed along with nCRP and ESR [11]. These tests are now routinely performed in laboratories on automated analyzers with fair degree of precision and accuracy. There is progressive bone and joint dysfunction in Rheumatoid Arthritis that becomes irreversible with time. With new advances in analytical technique, it is possible to diagnose RA at early stages with high reliability. So, this study was carried out in patients who presented with symptoms of joint pain to

Correspondence: Dr Farheen Aslam Assistant Professor, Department of Pathology, Quaid-e-Azam Medical College Bahawalpur Pakistan

Email: **farheenaslam75@ yahoo.com**

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compare the diagnostic efficacy of RF and anti-CCP for rheumatoid arthritis.

MATERIAL AND METHODS

This cross-sectional study was carried out after approved from ethical review committee on patients who visited the rheumatology Clinic of Medicine department of Bahawal Victoria hospital with muscular or joint pains and serum anti-CCP and RF levels were performed in pathology department.

The study was carried out from the period of October 2019 to November 2020. The demographic and data regarding clinical signs and symptoms were obtained from patients. The blood sample for normal C reactive protein (nCRP), anti-CCP and rheumatoid factor (RF) was taken in clot activated vial while EDTA tube was used for ESR estimation.

The Rheumatoid Arthritis Classification Criteria 2010 of American College of Rheumatology (ACR) was used for scoring of rheumatic patient [11]. The eligible candidates showed involvement of one or more joints with synovitis. The four categories are included in the proforma as number of joints involved in disease process, serology (Rheumatic Factor or anti- CCP), acute phase reactants and duration of symptoms. The patients with score of 6 or more were labeled as Rheumatoid Arthritis (RA) positive and with score of less than 6 were considered as RA negative group.

Serum anti-CCP was performed on Architect i1000 via chemiluminescence microparticle immunoassay (CMIA) technique strictly following instructions of Abbott Laboratories. A value of greater than 5 U/ mL was considered to be positive. The within-run CVs of L1 and L2 controls were 2.9% and 3.4%. The measurement range of anti-CCP was 0.5 – 200 U/ml.

Serum RF was analyzed on AU 680 of Beckman Coulter by turbidimetric method. Both high and low controls were run to monitor accuracy and validity of patients' results. The co-efficient of variation for within run was calculated 2.03% at level of 12.1 IU/ml and 3.96% at 45.8 IU/ml. The analytical measurement range was 10-120 U/L.

The westergren method was used for estimation of ESR. The cut off levels of 20 and 15 mm/hour were set for female and male patients.

Serum CRP and RF were done on AU 680, fully automated chemistry analyzer of Beckman Coulter. The two levels of controls were run before patient samples were analyzed. The within and between run CVs of normal and high levels of CRP controls were 5.3%, 4.6%, 5.5% and 6.7% respectively while for RF 3.7%, 4.2%, 5.7% and 6.4%. The participation in external proficiency program of BIORAD is used for monitoring the accuracy of CRP results. Serum CRP of more than 5 mg/L were regarded high.

The low-positive of ant- CCP had levels between 5-10 U/ml while > 15 U/ml were marked as high-level positive values. Similarly, Rheumatoid factor values < 14 U/ml were normal, low-level and high-level positive had values in the range of 14-42 and > 42 U/ml respectively.

The statistical Package for Social Sciences (SPSS) version 21 was used for analysis of data. The quantitative parameters were calculated by median and interquartile range (IQR). The sensitivities, specificities, positive predictive and negative predictive levels of both parameters were performed. Receiver operator curves (ROC) analysis for RF and anti-CCP was done. The area under curves were calculated to evaluate the inherent capacity of the anti-CCP and RF tests to differentiate between positive and negative Rheumatoid Arthritis. The normality of data distribution was checked by Kolmogorov test (p value < 0.05). Serum anti-CCP levels of two groups were compared by applying Mann Whitney U-test. The p -value < 0.05 was considered significant.

RESULTS

During this study duration, 180 patients with muscular and joint pains were investigated for RF, ESR CRP and anti-CCP tests. The study population showed female dominance with 122 subjects and mean age was 41.6 ± 14.7 years while average age of males was 8.6±7.8 years more than females. On the basis of ACR score > 6, 105 were considered RA positive and 75 patients with ACR score <6 were labeled as RA negative [11]. The analysis of data about clinical symptomatology showed that the small joints of the hands were mainly affected. The pain and swelling of joints with morning stiffness were chief presenting symptoms of patients as represented in Table-I.

Out of 180, anti- CCP levels > 5 IU/ml were noted in 96 individuals with 18 positive patients had anti-CCP values more than 15 IU/ml. The median serum anti-CCP levels was 18.40U/ml with IQR of 20.58 U/ml. Eleven subjects with ACR score < 6 showed positive anti-CCP results while the lab results of 80 individuals were below cut off of positive limit in Table-II. The AUC of 0.89 was measured on ROC analysis for anti-CCP i.e. in Figure-I. Mann Whitney U-test revealed that RA patients with ACR score >6 had statistically significant raised anti-CCP levels in comparison to those RA negative individual on basis of < 6 ACR score (p < 0.001). The sensitivity,

specificity, PPV and NPV of the test were 92%, 87%, 89% and 92% respectively.

The samples of 74 patients were positive for RF. The median RF level was 17.65 IU/ml with IQR 17.40 in patients having ACR score >6. Out of them, 09 patients had levels >41 IU/L. The non-RA group had median of 7.8 IU/L. The positivity of RF test was 33.7% (29/86) for RA negative group. The results of 75 subjects were found to be within normal reference range. ROC analysis computed AUC of 0.79 in Figure-II. The sensitivity, specificity, PPV and NPV of serum RF were 80%, 66%, 73% and 62%.

Anti- CCP was not only sensitive but also more specific test in comparison to RF for diagnosis of Rheumatoid Arthritis. Out of total subjects, 83 patients (56 females and 27 males) were positive for both anti-CCP and RF diagnostic tests. However, positivity of both tests was not necessary to detect the disease as it had no influenced on the sensitivity or specificity.

Table-I: Presenting complaints of study subjects

Clinical symptomatology	Results
pain in joints 93.9 (92)	166 (92%)
Morning Stiffness	113(63%)
Swelling of joints	140(77%)
Small joint involvement	104(58%)
Big joint involvement	92(51%)

Table-II: Results of serum anti CCP and RF for Rheumatoid Arthritis.

Parameter	Rheumatoid Arthritis positive patient	Rheumatoid Arthritis negative patient
Anti-CCP positive	89	11
Anti-CCP negative	7	73
RF positive	76	29
RF negative	18	57

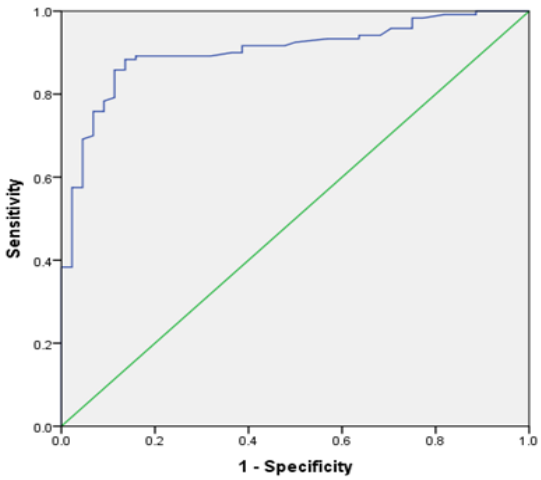

Figure-I: ROC curve of serum anti-CCP.

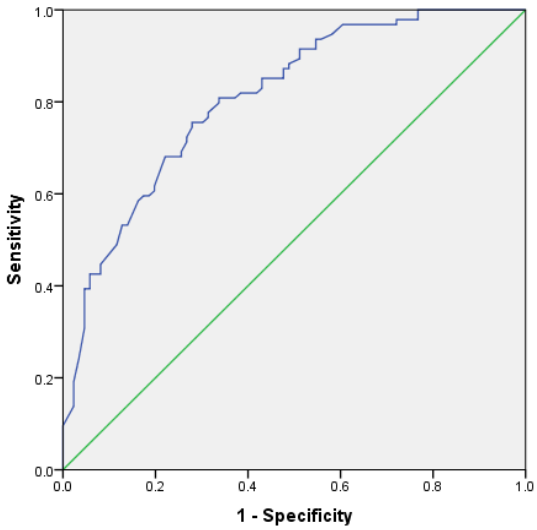

Figure-II: ROC curve of serum rheumatoid (RF).

DISCUSSION

RA is a systemic inflammatory disorder and its main target organ is joint. A survey conducted on poor and affluent areas of Karachi, Pakistan about joint symptoms reported that 0.9 and 1.98 per thousand respectively had this complaint [12].

In our study, the disease mostly involved female population but disease pattern is more aggressive in male subjects as high anti CCP and RF levels were observed in these patients. However, both groups exhibited similar joint involvement pattern, systemic manifestation and serological testing. Similar results were replicated in other studies and so it's beneficial to start early aggressive treatment effect for better outcome [13,14].

Earlier RF was performed as screening test to diagnose the disease. Some studies claimed that it had some prognostic role and could predict the disease severity but this marker has low specificity for RA and its levels are raised in other autoimmune disorders [7,15].

The review of scientific literature established the sensitivity of RF in diagnosis of RA in range from 26 to 90 percent [7]. In our study, the sensitivity and specificity of 79.2 and 66.29% were noted for RF test. These levels were in accordance with Aflaki where analysis was done by latex fixation method but Hodkinson's *et al* produced quite different results [16,17].

The patient selection criteria, racial and geographical variation and difference in analytical technique i.e. turbidimetry, latex fixation or ELISA might be responsible for wide sensitivity range.

The results of our study showed higher sensitivity (92%) of the anti-CCP antibody that is consistent with Çiftci *et al*. as duration of disease in our study subjects was at least one year and anti-

CCP was analyzed by sensitive technique [18]. The specificity and sensitivity of Anti-CCP were calculated 74.3% and 92.8% in another study [19].

Similarly, our study demonstrated higher specificity of anti-cyclic citrullinated peptide in comparison to RF (87% vs 66%) and AUC for anti-CCP was 0.89 proving its major role in the diagnostic efficiency of RA. Similar patterns were noted in Iranian and Chines studies [6,20]. However false positive results were also recorded as some patients with other autoimmune diseases also exhibited high anti CCP levels [21,22]. The possible explanation of different study results would be variation in suggested cut off level, different study population and performing the test on analyzers with different working principles and reagents.

The subjects with high anti-CCP antibodies showed severe disease pattern as compared to the patients whose results falls within normal reference ranges. This finding is consistent with Khan *et al*. that early aggressive treatments always exhibit significant advantages for the overall outcomes of RA patients [13].

CONCLUSION

Therefore, anti-CCP measurement is an important diagnostic tool in early diagnosis but also helpful in evaluating the outcome of RA.

AUTHOR CONTRIBUTIONS

Farheen Aslam: Concept, Data collection, drafting and data analysis.
Asma Shaukat: Reviewed and finally approved the manuscript for submission to the journal.
Sadaf Shafique: Data interpretation and work design
Raheel Khan: Study design, Data collection.
Faizan Ahmed Zakir: Questionnaire design, revision and finalization of results.

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