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Study of newer diagnostic techniques for Rheumatoid Arthritis (By Line Immuno Assay profiling).

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Abstract

Background : Arthritis is an umbrella term that refers to approx 200 rheumatic diseases and conditions that affect various joints of the body, which including lupus and rheumatoid arthritis. Rheumatoid arthritis (RA) is a chronic autoimmune and common systemic inflammatory disease that results in joint deformity and functional disability when not properly managed. The early diagnosis and treatment of RA are imperative for optimal disease management, a greater probability of remission, and prevention of permanent clinical and radiographic damage.

Objectives: The present study was conducted to study the value of rheumatoid factor (RF), anti-cyclic citrullinated peptide autoantibodies (anti-CCP), and anti-RA33 autoantibodies for diagnosis of RA and prediction of outcome in patients with very early arthritis.

Methods: This was a prospective study carried out in a tertiary care centre situated at western part of India which included 30 patients aged between 18 – 65 years who presented with multiple joints pain. Inclusion criteria included Population aged between 18 to 65 years with clinically suspected patients of Rheumatoid Arthritis and Duration of signs and symptoms more than 6 weeks. Exclusion criteria included Patients <18 years and >65 years, patients with other chronic diseases such as Carcinoma, Diabetes mellitus, HIV, etc. A p-value of <0.05 was considered statistically significant.

Results: Out of 30 patients, 57% of patients were females and rest 43% males. Out of which 66.66% patients were positive for antibodies and 33.33% were negative. Rheumatoid arthritis positivity was seen in 60% cases while other arthropathies were observed in 40% of cases. Specific antibody wise distribution shows 75% positivity with RF – IgM plus ACPA (Anticitrulline protein antibody) and 25% positivity with RA – 33 antibody.

Conclusion: LIA based autoantibody testing in early inflammatory joint disease, should be used as a sensitive and effective strategy for distinguishing patients with RA at high risk for poor outcome. Anti – CCP is an extremely helpful diagnostic marker being not only highly specific for RA but also strongly associated with erosive disease.

Keywords: ACPA (Anticitrulline protein antibody), RA – 33, Rheumatoid arthritis, RF – IgM Ab.

INTRODUCTION:

Arthritis is an umbrella term that refers to around 200 rheumatic diseases and conditions that affect joints, including lupus and rheumatoid arthritis. It is clinically defined as swelling and tenderness of one or more joints [1]. Injury, abnormal metabolism, genetic makeup, infections,

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and immune system dysfunction are some of the factors that play a role in the development of arthritis.

Rheumatoid arthritis (RA) is a chronic autoimmune and common systemic inflammatory disease that results in joint deformity and functional disability when not properly managed. The early diagnosis and treatment of RA are imperative for optimal disease management, a greater probability of remission, and prevention of permanent clinical and radiographic damage[2].

Early symptoms of RA may appear as a vague pain, gradual in appearance without classic symptoms of joint swelling or tenderness. These unusual symptoms are usually non-specific and may persist for a prolonged period. Early articular manifestations of RA may be indistinguishable from other rheumatic diseases. Prolonged duration of morning stiffness with arthralgia or arthritis in a limited number of joints may be a clue for considering RA diagnosis[3].

Identification of RA at initial presentation and treatment at an earlier stage can affect disease course, prevent the development of joint erosions or retard progression of erosive disease. Recognizing early RA from non-RA at the onset of the disease is not straightforward. There is a limitation in the use of the American College of Rheumatology revised criteria (ACR criteria) for early diagnosis. Since due to inadequate clinical or laboratory evidence at the onset of arthritis, this criteria is not sensitive enough to identify early RA [4].

The main autoantibodies markers for the diagnosis of RA include rheumatoid factors (RF), anti-keratin antibodies (AKA), and anti-perinuclear factor (APF) [5]. Although these serum biomarkers have diagnostic value for RA, they still have some deficiencies, e.g. the rheumatoid factor (RF) has been identified in other connective tissue diseases and in elderly individuals, which signifies a lack of specificity [6].

This study focuses on detecting antibodies against ACPA (Anti-citrullinated protein antibodies), RA33, RF-IgM, Antinuclear antibodies (Anti dsDNA, SS-A, SS-B) and correlate with synovial tissue biopsies [7].

Multiple types of biomarkers are being investigated for the purpose of RA disease activity monitoring: serum acute phase reactants, genetic factors, and tissue-specific markers from cartilage, bone, and synovium [8]. IL-6, a prominent acute phase reactant in RA, remains under investigation but unfortunately has not been found to correlate with the radiographic progression of the disease. Ultimately, the combined use of multiple biomarkers may prove to be a more effective measure of disease activity [9].

METHODS:

Patient Selection

This was a prospective study carried out in a tertiary care centre situated at western part of India. The study duration was 2 years, and the sample size was 30. All patients patients aged between 18 – 65 years who presented with multiple joints pain attending tertiary care centre were included. Inclusion criteria included Population aged between 18 to 65 years with clinically suspected patients of Rheumatoid Arthritis and Duration of signs and symptoms more than 6

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weeks. Exclusion criteria included Patients <18 years and >65 years, patients with other chronic diseases such as Carcinoma, Diabetes mellitus, HIV, etc.

Sample Processing

The study was performed using the patient's serum and subjecting it to line immunoassay for the qualitative detection of 10 different autoantibodies in human serum and plasma to diagnose Rheumatoid Arthritis [Figure 1].

Reagents and specimens should be at room temperature before use. Use rocking shaker during all incubation steps.

Statistical Analysis

Collected data was entered into MS-Excel and was analyzed using Statistical package for Social Sciences (SPSS) version 25.0. Categorical data was expressed as frequency or proportions and quantitative data as mean and standard deviation. Chi-square test or Fisher's exact test were used as test of significance for categorical variables and unpaired t-test was used for mean and standard deviation. A p-value of <0.05 was considered statistically significant.

RESULTS:

The mean age (mean ± standard deviation) of the patients was 41.50 ± 10.71 years with range 18 -65 years and median age was 24.50 years. Majority of patients were female (57%) and rest were males (43%). Out of which 66.66% patients were positive for antibodies and 33.33% were negative. Rheumatoid arthritis positivity was seen in 60% cases while other arthropathies were observed in 40% of cases. Specific antibody wise distribution shows 75% positivity with RF – IgM plus ACPA (Anticitrulline protein antibody) and 25% positivity with RA – 33 antibody. There were total 9 patients positive with RF – IgM plus ACPA (Anticitrulline protein antibody) out of which 3 patients show early erosive disease progression with rheumatoid arthritis, while rest 6 patients did not show any erosive disease progression. There were total 3 patients show positivity with RA – 33 out of which none of the patients shows any erosive disease.

Table 1 shows specific antibody wise distribution of patients with clinical suspicion of rheumatoid arthritis. Table 2 shows gender wise distribution of cases. Table 3 shows prognostic value of autoantibodies for development of erosive disease in patients with rheumatoid arthritis. Table 4 shows Comparison of statistical analysis with various authors.

Figure 1 shows line immune assay strip. Figure 2 shows chart based diagram for diagnosis of rheumatoid arthritis and related risk of developing erosive disease.

DISCUSSION:

The proposed LIA diagnostic algorithm in our study for autoantibody testing in patients with very early inflammatory joint disease is not only helpful in establishing a diagnosis of RA but also allows definition of patients at increased risk of developing erosive disease. Overall, in this study it should be noted that despite the excellent performance of high titre RF, ACPA proved slightly better, both for disease specificity and prognostic value. Proposed algorithm may help to establish an effective diagnosis and prognosis in the majority of patients with very early inflammatory joint disease.

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Anti – RA 33 on other hand, despite its limited specificity , may be useful in patients negative for high titre RF and anti CCP, allowing identification of patients with a good prognosis who will respond well to treatment with DMARDs. Thus, autoantibody signatures convey diagnostic and prognostic insights that may allow appropriate therapeutic strategies to be designed even at the first visit, a time point most challanging in the course of RA.

No study is totally complete in itself and therefore, the present study also has its own limitations. Firstly, the sample size of the present study was too small and therefore, to establish a stronger association between early diagnosis of rheumatoid arthritis and its prognosis, a study with larger sample size needs to be carried out. Secondly, the results can not be generalized as the sample size is small.

CONCLUSION:

LIA based autoantibody testing in early inflammatory joint disease, should be used as a sensitive and effective strategy for distinguishing patients with RA at high risk for poor outcome. Anti – CCP is an extremely helpful diagnostic marker being not only highly specific for RA but also strongly associated with erosive disease.

ETHICAL CONSIDERATIONS:

Prior to start of the study, consent for conducting the study was obtained from the institutional ethics committee (IEC) and it was approved by the IEC with IEC approval number DHR-EC/2022/SC/07/25. Consent was also obtained from the patients prior to their participation in the study.

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Table 1 – Specific antibody wise distribution.

ANTIBODIES	TOTAL
RF – IgM + ACPA	09 (75%)
ONLY RA - 33	03 (25%)
TOTAL	12

Table 2 – Gender wise distribution.

RESULT	MALE	FEMALE	TOTAL
POSITIVE	**04 (33.33%)**	**08 (66.66 %)**	**12**
NEGATIVE	**09 (50 %)**	**09 (50 %)**	**18**
TOTAL	**13**	**17**	**30**

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Table 3 : Prognostic value of autoantibodies for development of erosive disease in patients with RA.

ABs	EROSIVE	NON-EROSIVE	TOTAL
ACPA +RF - IgM	03	06	09
RA - 33	00	03	03
TOTAL	03	09	12

Table 4 - Comparison of statistical analysis with various authors

	Present Study	Van Gaalen FA et al.	Sumeet Agrawal et al.	O Meyer et al.	Abhishek kumar et al.
Sensitivity (%)	90.91	93.91	90.23	94.32	91.6
Specificity (%)	94.44	98.44	96.32	92.19	98.3
PPV (%)	91.32	90.91	99.23	83.19	89.6
NPV (%)	97.29	98.44	94.83	97.2	94.3
Accuracy (%)	93.1	97.33	96.20	95.83	92.9

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Figure 1 – Line immuno assay strip with 10 different antibodies.

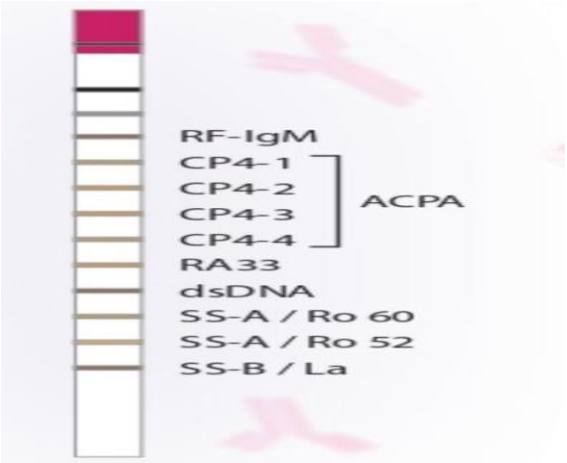

Figure 2 - chart based diagram for diagnosis of rheumatoid arthritis and related risk of developing erosive disease.