Detection of a Rheumatic Factor (RF,) Anti- Cyclic Citrullinated Peptides (Anti CCP) Antibodies and C- Reactive Protein (CRP) in Diagnosis and Prognosis of Rheumatoid Arthritis

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Abstract

Introduction: Rheumatoid arthritis (RA)is an autoimmune and inflammatory disease. It is characterized by arthrosynovitis with resultant joint destruction . So, an early clinical diagnosis along with simple serological tests like anti CCP antibodies, C –reactive protein (CRP) and Rheumatoid factor (RF) play an important role .An attempt was made to study and compare these parameters in OPD and IPD RA patients in our hospital setup, VMMC and Safdarjung Hospital , New Delhi.

Aims and Objectives: To study the role of RF , Anti-CCP antibodies and CRP in early detection and prognosis of Rheumatoid arthritis.

Materials and Methods: A Total of 440 samples were studied from January 2018 to December 2020 (3years). Correlation between Rheumatoid factor(RF) ,anti CCP antibodies and CRP in suspected RA patients from various departments like PMR , Orthopaedics , Medicine, Obstetrics and Gynaecology and Paediatrics was attempted. RF and CRP was performed using latex agglutination test and anti CCP antibody by ELISA.

Results and Conclusion: Of 440 samples tested, all the factors were positive in 87 patients . Anti CCP and RF alone was seen in 32 patients. RF and CRP positive in 49 patients, RF alone in 22 patients. In 250 patients all the tests were negative. So, Anti CCP along with RF and CRP showed a better positive predictive value in patients of Rheumatoid arthritis. But positivity of these parameters even in patients who lack sign and symptoms also aids in better diagnosis, treatment and prognosis.

Keywords: Rheumatic Factor, C- Reactive Protein, Rheumatoid Arthritis, Anti- Cyclic Citrullinated Peptides

Introduction

Rheumatoid arthritis (RA) is a chronic, autoimmune disease which is characterized by persistent symmetric poly-arthritis leading to joint destruction, deformity and decreased functional status. 1-2% of world population, are affected by RA with female: male ratio of 2.5:1. Etiological factors for RA are smoking, genetic factors which are believed to play a major role in leading to RA in approximately around 60% of patient population [1]. RA is considered to be one of the commonest connective tissue disorders in the world. Worldwide prevalence is estimated to be 0.8% and in India it is estimated to be around 0.75% [2].

The prominent clinical features of RA are joint pain, joint swelling, limitations in functions of joint and in some patient's joint deformities also. Grassi et al. in a study reported on the commonest triad of symptoms which included joint pain, joint swelling and motion impairment [3].

In recent years, a number of studies have demonstrated that Antibodies against Cyclic Citrullinated Peptide (Anti CCP) are more specific with a specificity up to 98% and can be considered as a predictive and prognostic marker for RA [4,5]. C-Reactive Proteins (CRP) correlate well with the degree of joint inflammation and development of bone erosion. These are also considered as inflammation indicators [1]. At present, the diagnostic criteria for RA employs RF, Anti-CCP and CRP along with erythrocyte sedimentation rate (ESR) which are considered to be biomarkers for RA with varying values [6].

This analysis was attempted to evaluate the clinical basis of the use of various markers along with combined detection and correlation of RF, Anti-CCP and CRP for the early detection and for better prognosis of Rheumatoid arthritis.

Material and Methods

This is a retrospective study conducted between January 2018 to December 2020 (3 years) in Vardhaman Mahavir Medical College and Safdarjung Hospital, New Delhi. The patients ranged from the age group of 40-75 years. This study involved analysis of 3 serological markers - Anti CCP, CRP and RF in clinically suspected indoor and outdoor patients from Departments of Physical Medical Rehabilitation, Orthopedics, Medicine and Gynecology and Obstetrics. A total of 440 patient's blood samples, who were suspected patients of RA were taken. RF and CRP tests were performed using latex agglutination test (Ozotex Diagnostic) and Anti-CCP antibody detection was done using ELISA(Immucheck). All the tests were performed using manufacturer's guidelines.

The quality controls were also run simultaneously and were satisfactory for all the above tests. The collected datas were further classified and assessed with respect to the demographic profile and analyzed further for the presence of serologic markers in each group. Data was analyzed using MS excel.

Diagnostic Criteria- Revised Criteria for Diagnosis, American College of Rheumatology (ACR)

1. Morning Stiffness: This occurs in and around the joints and lasts for 1 hour before maximal improvement.

2. Arthritis of 3 or More Joint Areas: At least 3 joint areas have soft tissue swelling or fluid. These areas includes left and right proximal interphalangeal (PIP), metacarpophalangeal (MCP), elbow, wrist, ankle, knee and metatarsophalangeal (MTP) joints.

3. Arthritis of Hand Joints: At least one area in wrist, MCP or PIP joint is swollen.

4. Symmetric Arthritis: Bilateral involvement of PIPs, MCPS and MTPS.

5. Rheumatoid Nodules: Subcutaneous nodules are present over bony prominences or extensor surfaces or in juxta-articular regions.

6. Serum RF: Abnormal amounts of Serum Rheumatoid factor (RF) tested by any of the methods for which result has been positive in fewer than 5% of healthy control subjects.

7. Radiographic Changes: Typical RA is seen on postero-anterior hand and wrist radiographs, which must include erosions or unequivocal bony decalcification adjacent to involved joints.

The presence of any 4 criteria supports the diagnosis of RA. Criteria 1-4 must be present for at least 6 weeks and the physician must observe criteria's 2-5.

Results

In our study, of the 440 patients, majority of the patients were females 286 (65%) in comparison to males 154(35%). Majority of patients were confirmed RA patients from Physical Medical Re-habilitation (PMR) Department i.e., 227, 135 patients were from Orthopedics, 70 patients from Medicine and 8 patients from Obstetrics and Gynecology Department. We observed 87 patients had all the three tests namely CRP, RF and Anti-CCP all positive. 32 patients were found to be positive with RF and Anti-CCP, in 49 patients CRP and RF were found to be positive and 22 patients had only RF positive. 250 patients tested negative for all the three serological tests. Most common age groups who had all 3 serological tests positive, ranged between 42-58 years.
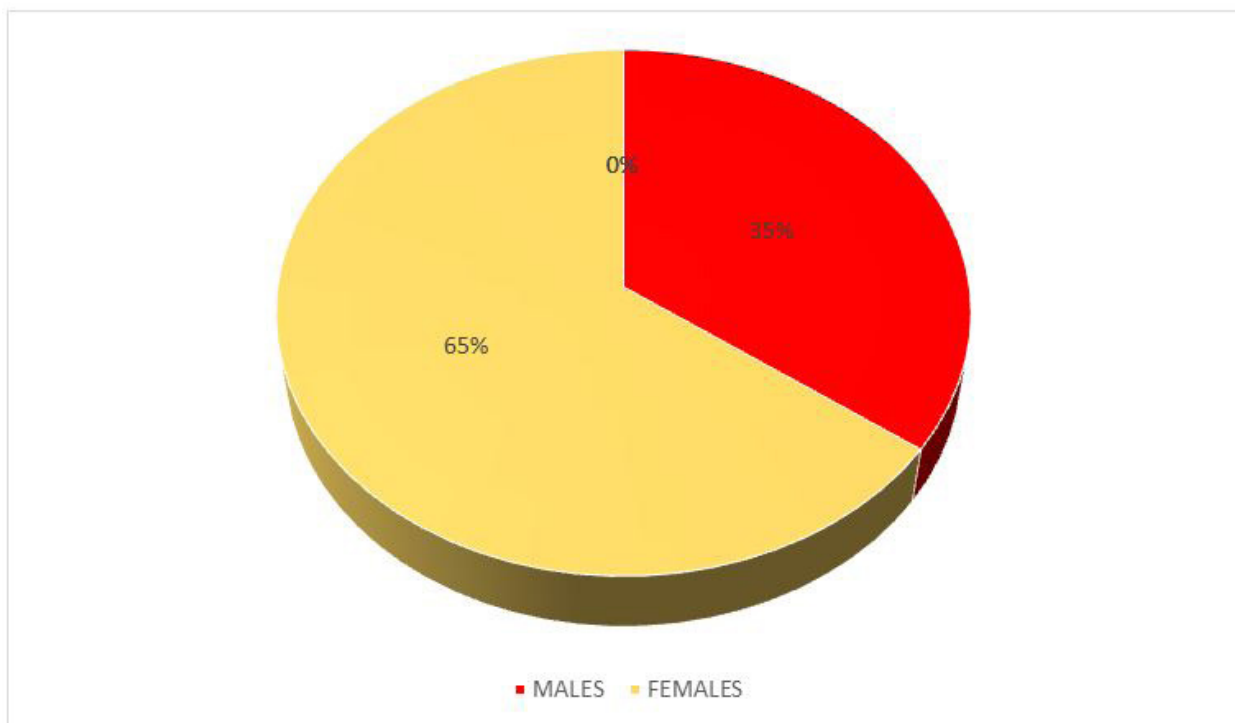

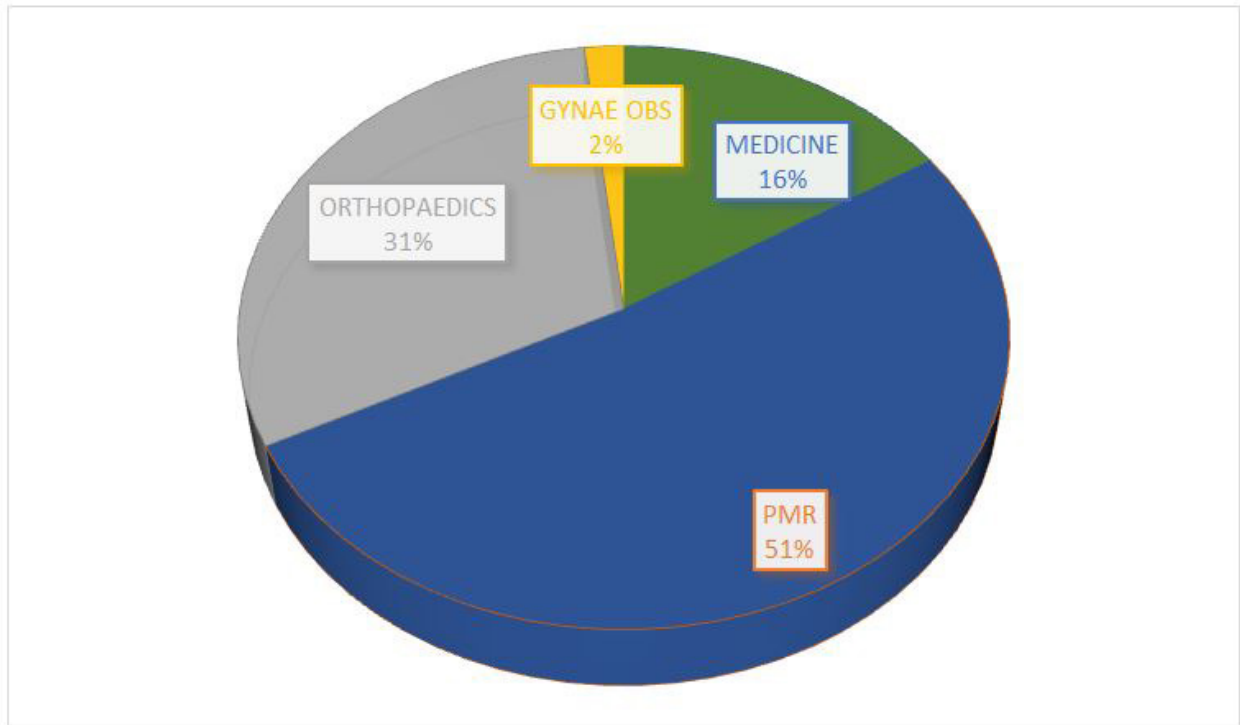**Figure 1:** Male: female ratio in our study

Figure 2: Percentage of patients with RA from different departments in the present study

S.NO	SEROLOGICAL TESTS	POSITIVE	NEGATIVE	%Age positive (n= 440 PATIENTS)
1.	CRP+RF+Anti-CCP	87	353	19.7%
2.	CRP+RF	49	391	11.1%
3.	RF+ Anti-CCP	32	408	7.2%
4.	RF	22	418	5%
5.	ALL NEGATIVE	0	250	56.8%

Table 1: Positives, Negatives and percentage positive in different serological tests

S.NO	SEROLOGICAL TESTS	POSITIVE (TOTAL)	PMR	ORTHO	MEDICINE	OBS AND GYNAE
1.	CRP+RF+Anti-CCP	87	64	19	3	1
2.	CRP+RF	49	23	12	9	5
3.	RF+Anti-CCP	32	21	8	3	0
4.	RF	22	16	4	2	0

Table 2: Positivity of serological tests from different departments

Discussion

Earlier, the diagnosis of RA was based on clinical manifestations along with the clinical correlation of the disease because of the unavailability of proper diagnostic tests. RF test has been done in RA patients with increased sensitivity whereas Anti-CCP antibodies assay plays a vital role in early prediction for joint damage [7]. In a study Lee DM reported that RA was seen most commonly between the age group of 40-70 years which was similar to our study, where we found the most common age group of RA patients was between 42-68 years [8]. In another study by Shamanna P, the age group of clinically suspected RA patients was 41-50 years which was nearer to that of our study [7].

The positivity rate of RF in early RA is approximately 40%-60% and in some patients there may be the presence of RF even before the clinical onset of the disease. The specificity of RF is also poor [9]. The American College of Rheumatology/European League Against Rheumatism in 2019, jointly proposed a new diagnostic criterion for RA, establishing the importance of Anti-CCP antibodies in the diagnosis of RA [10]. Bas et has reported that Anti-CCP antibodies has been proven to be a good indicator for determining bone erosion. Anti-CCP antibodies are a specific indicator for the early prediction, identification and diagnosis of RA. It is considered to be a good indicator of bone destruction [11]. Rongchun et al. in their study of 135 patients had reported 74(55%) patients were positive with Anti-CCP antibodies along with RF [12].

In 1987, the American College of Rheumatology (ACR) defined Rheumatoid factor (RF) as the only serologic marker for the diagnosis of RA due to its high sensitivity. But the specificity of RF is relatively low. There is a 50% positivity rate of RF in patients with other connective-tissue disorders like Systemic lupus erythematosus (SLE), Primary Sjogren syndrome and dermatomyositis, in patients with certain infections and in healthy elderly persons too it can be positive which limits its diagnostic value [13]. So, Anti-CCP antibodies should always be considered as an important test which should be done along with RF in a Known case of RA or in a suspected cases of RA.

CRP is considered to be an important immune marker and not just only an infection/inflammatory marker [14,15]. It has been seen that CRP levels are often persistently elevated in patients with RA, with levels of >20 mg/L. This has been frequently reported at baseline levels in randomized clinical trials (RCTs) of drugs in the treatment of RA [16]. Retrospective and observational studies show that many patients have normal CRP levels despite exhibiting RA disease activity [17,18]. So, this study suggests that other parameters like RF, Anti-CCP antibodies should also be considered to establish the diagnosis of RA and CRP alone should not be considered as a biomarker for RA.

Conclusion

In our study, majority of the patients were positive for RA, when all the 3 tests were done together. On correlation with other studies, we strongly recommend that before diagnosing RA or for the prognosis to be assessed in RA patients, all the 3 parameters namely CRP, Anti-CCP antibodies and RF should be screened together at the same time, as no one test is reliable when done alone. On the other hand, clinical symptoms and its correlation with the biomarkers is also vital.

CRP is also an infection marker. Many a times RF is normal in known RA patients. So, testing and correlation of these two parameters together along with Anti-CCP antibodies is considered important. They are all biomarkers for bone erosion and can help to get a better picture of the disease in established RA cases as well as in suspected RA patients.

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