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DIAGNOSTIC VALUE OF ANTI-CYCLIC CITRULLINATED PEPTIDE AND ANTINUCLEAR ANTIBODY IN RHEUMATOID FACTOR POSITIVE PATIENTS WITH CLINICAL SUSPICION OF RHEUMATOID ARTHRITIS.

Clinical Microbiology

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ABSTRACT

Aim: Serological biomarkers used for the diagnosis for Rheumatoid arthritis (RA) can also be seen in other conditions. This study aims to assess the diagnostic roles of anti- cyclic citrullinated peptides (CCP) and antinuclear antibodies(ANA) in clinically suspected RA patients with positive rheumatoid factor. **Results:** 100 patients having circulating rheumatoid factor irrespective of age and gender were enlisted in the study. Anti- CCP level and ANA status were determined in each patient. 56% had high level of rheumatoid factor (RF) (>3 times the upper limit of normal). Anti-CCP and ANA were observed in 14% and 36% respectively. Positivity for all the three biomarkers was seen in 16.67%. **Conclusion:** Our results outlined the importance of performing anti- CCP and ANA along with RF in suspected Rheumatoid arthritis that will help in the exclusion of other autoimmune diseases and treatment modalities.

KEYWORDS

Anti- CCP, Anti- nuclear antibodies, rheumatoid factor, rheumatoid arthritis.

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic inflammation with the involvement of multiple joints. In India, the prevalence of RA has been reported to be around 0.28- 0.7%. [1] Rheumatoid factor (RF) are antibodies that are directed against the Fc portion of immunoglobulin G and are known to be associated with severe disease at high titers. [2] Anti citrullinated protein antibodies are produced when there is loss of tolerance of proteins having citrulline reside. This results in the production of autoantibodies like anti- cyclic citrullinated protein/ peptide antibody (Anti- CCP). American College of Rheumatology (ACR)/ European League Against Rheumatism (EULAR), 2010 included both RF and anti- CCP antibody as serological tests for diagnosis of RA. [3] RF though considered to be the immunological hallmark have modest sensitivity of about 60% and specificity of 79%. Anti- CCP on the other hand is found to have high specificity of 98% and good prognostic value for disease severity. [4] The sensitivity and specificity increase substantially when both RF and anti-CCP are positive.

In addition, other autoimmune diseases like systemic lupus erythematous (SLE), Sjogren syndrome, mixed connective tissue disease and some non- autoimmune conditions also demonstrate elevated RF. [5, 6] Antinuclear antibodies are found in approximately 50% of RA patients. Despite this, ANA testing is often missed out during the laboratory workup for many patients who are ultimately diagnosed with RA. [7] Its role in RA becomes significant in order to differentiate it from other autoimmune disorders like Lupus that is known to share various similar symptoms. The purpose of this study is to assess the diagnostic values of anti-CCP antibody and antinuclear antibodies in suspected rheumatoid arthritis patients having positive rheumatoid factor.

MATERIALS AND METHODS

This study was an observational prospective study conducted for a period of 6 months (2022- 2023). It was conducted in the Immunology laboratory, Department of Microbiology, New Delhi, India. A total of 100 clinically suspected rheumatoid arthritis with positive RF were included in the study. These samples were further subjected to anti-CCP antibody and anti- nuclear autoantibodies (ANA) tests.

Rheumatoid factor IgM Enzyme linked immunosorbent assay (DRG Instruments GmbH, Germany) was used for the quantitative measurement of IgM class rheumatoid factor. Commercially available Qualisa™ anti-CCP ELISA was used for the quantitative detection of IgG autoantibodies against cyclic citrullinated peptide in human serum. Detection of antinuclear antibodies (ANA) was done either by ANA screen ELISA (Calbiotech Inc.) or IMTEC-ANA- Line immunoassay XL (Human Diagnostic Worldwide, Germany).

RESULTS

A total of 100 clinically suspected RA patients that were found be positive for RF were included as our study population. Among the 100 patients, majority of the population were females (76%) as compared to males (24%). 82% of the samples were received from out-patient departments while only 18% were from admitted patients. As per ACR/ EULAR, high positivity of RF (>3 times the ULN) was observed in 56% while 44% had low positive titers.

All these samples were tested for the presence of anti-CCP. Out of the total 100 samples, only 14% (n=14) were found to have antibodies against cyclic citrullinated peptides. High positive anti-CCP was seen in 10 patients while only 4 among the 10 had low positive titers. High positivity of both RF and anti-CCP was observed in 9 patients. On the contrary, 2 patients presented with low positivity of both the tests. [Table 1]

Table 1: Serological tests as per ACR/EULAR 2010 criteria.

Rheumatoid Factor n (%)	Anti- CCP (High Positive) n(%)	Anti- CCP (Low positive) n(%)	Anti- CCP Negative n(%)
High positive: 56 (56%)	9 (90%)	2 (50%)	45 (52.33%)
Low positive: 44 (44%)	1 (10%)	2 (50%)	41 (47.67%)
Total	10	4	86

ANA test was performed among which 36 out of total 100 were found to be positive. The positivity was found to be higher among the female population (n=30) as compared to male which comprises of only 6 patients. Among the 36 that showed ANA positivity, 80.56% (n= 29) and 19.44% (n= 7) presented with high RF and low RF titer respectively. [Table 2] Positivity for all (RF+, anti- CCP +, ANA+) were observed only in 6 samples while the remaining 30 presented with a negative anti- CCP antibody. [Table 3]

Table 2: Antinuclear antibodies (ANA)

Rheumatoid factor	ANA positive n(%)	ANA negative n(%)	Total
High positive	29 (80.56%)	27 (42.19%)	56
Low positive	7 (19.44%)	37 (57.81%)	44
Total	36	64	100

Table 3: Status of three biomarkers- RF, anti-CCP and ANA.

RF and Anti- CCP	ANA + n(%)	ANA – n(%)	Total
RF+, Anti- CCP +	6 (16.67%)	8 (12.5%)	14
RF+, Anti- CCP -	30 (83.34%)	56 (87.5%)	86
Total	36	64	100

DISCUSSION:

Serological markers like RF and anti-CCP play an important role in the

diagnosis and prognosis of rheumatoid arthritis. RA is an autoimmune disease hence, the sex ratio in our study was more in favor of female supported by the findings of Soroush M et al. [8] RF though an important biomarker for RA, is reported to have poor specificity. Before the modifications of ACR/EULAR 1987, RF was considered as the only serological marker for the diagnosis owing to its high sensitivity. Anti- CCP was added along with RF owing to it being a good indicator of predicting severity as evident from various studies. [9, 10] High positive RF or anti-CCP are given 3 points as per the ACR/EULAR diagnostic criteria for RA. In our study, 56% and 10% presented with high positive RF and anti- CCP respectively. High positivity of both RF and anti-CCP were observed in 9 patients. At the same time, low positive RF and anti-CCP were noticed in 44 and 4 patients respectively earning 2 points as per ACR/EULAR criteria. Dogan M et al. in their study found the co-existence of both RF and anti-CCP antibodies to be 50.4% among the RA patients. [11] However, in contrast, our study showed only 14% to be positive for both. Our findings corroborated well with a study conducted by Kirti M et al. [12] This could be due to the fact that these patients do not have rheumatoid arthritis and the positivity of RF could be due to other autoimmune disorders or unspecified causes.

Studies have been conducted that denote the usefulness of anti-CCP in predicting the severity of disease. As bony erosion and structural damage once ensued is irreversible, early recognition and determining the presence of serological markers like anti-CCP becomes essential. Anti- CCP antibodies have been demonstrated to be a good indicator of predicting bony erosion as evident from various study. Our study showed that majority of those presented with high anti-CCP antibody titer among the total positive anti-CCP samples. Individuals with disease remission are more likely to show lower anti-CCP antibody level as compared to those with existing disease activity, and high titer are associated with more severe disease as compared to low antibody titer. [9, 10, 13]

There are other autoimmune disorders that can also present with circulating RF. For this reason, presence of antinuclear antibodies was also tested among the RF positive patients. In our study, 36% of the seropositive RF were found to have ANA in their system. This finding corroborated with a study conducted in India where 38.9% were found to have ANA among the rheumatoid disease group. [14] Positivity of all three (RF, anti- CCP, ANA) regardless of their titre were noted in 16.67% (n=6).

Diagnosing autoimmune disease can be difficult due to similar clinical presentations like joint pain, swelling and morning stiffness especially in cases of rheumatoid arthritis and Lupus. It becomes imperative to rule out other autoimmune conditions to guide in the management of the patients. In RA patients with positive ANA, hydroxychloroquine (HCQ) is considered to be a better choice of treatment over methotrexate (MTX) which is the first- line therapy for RA.

Additionally, HCQ is considered to have good treatment outcome in SLE diagnosed patients. [5, 15] The possibility of drug- induced Lupus is also a matter of concern. Yukawa N et al. had found significant rise of ANA titer from 25% to 40% in RA patients who were treated with infliximab. [16] Knowledge of ANA status can also help us reconsider the use of bDMARDs as it is evident from various studies that tumor necrosis inhibitors (TNFi) especially Infliximab showed poor treatment efficacy and induction of autoantibodies (high seroconversion). [16, 17, 18]

CONCLUSION:

Anti-CCP and RF are recognized biomarkers associated with RA that are incorporated in the current ACR/EULAR criteria guidance for RA diagnosis. As evident from our findings, anti- CCP is not always present in all positive RF patients. For this reason, anti- CCP should be performed in conjunction with RF to increase its diagnostic values. ANA positivity was also noticed among our study population proposing RA may be present with other autoimmune disorders. Therefore, ANA should also be taken into consideration that could help in better understanding of the health outcomes and managements of patients with suspected RA.

Limitations:

The study faced some limitations as it was performed on a small group of populations, thereby lacking diversity. It was also conducted for a short duration of time hence, follow up research could not be done.

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Conflicts of interest: The author declares that he/she has no competing interests.

Abbreviations:

1. RA- Rheumatoid arthritis
2. CCP- Cyclic citrullinated peptide
3. ACR/ EULAR- American College of Rheumatology/ European League Against Rheumatism
4. RF- Rheumatoid factor
5. ANA- Antinuclear antibodies
6. bDMARDS- Biological Disease modifying antirheumatic drug

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