BRIEF RESEARCH COMMUNICATION

Frequency of Positive Anti-CCP Antibodies in Patients of Rheumatoid Arthritis with Negative Rheumatoid Factor: A Cross-sectional Retrospective Data-based Study

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Abstract

Objective: To investigate the frequency of positive anti-cyclic citrullinated peptide (CCP) antibodies in rheumatoid arthritis (RA) patients with negative rheumatoid factor (RF) from Lahore, Pakistan.

Materials and methods: This retrospective cross-sectional observational study was conducted at the Department of Medicine and Allied, Azra Naheed Medical College, Superior University Lahore. The 2010 ACR Diagnostic Criteria were used to define RA. The medical records of all patients with RF-negative RA from January 2022 to December 2023 were included in the study. Patients with incomplete data and seronegative arthritis due to other causes were excluded from the study. Retrospective data of 78 RF-negative RA patients, including basic, demographic, and clinical information such as age, sex, anti-CCP antibody status, and disease severity, were assessed and recorded. Statistical Package for the Social Sciences (SPSS) version 26 was used for data entry and analysis.

Results: The mean age was 43.8 ± 15.8 years with 10 (12.8%) males and 68 (87.2%) females. Mean duration of disease was 9.1 ± 7.8 years with 62 (79.5%) patients have disease duration more than 3 years. Mean DAS-28 score was 4.5 ± 1.7 with 48 (61.5%) patients having active disease (DAS-28 score >3.2). Anti-CCP antibodies were observed in 32 patients (41.0%). Stratification of data with regard to anti-CCP antibodies revealed a significant association with sex but not with age, duration of disease, or disease severity.

Conclusion: More than one-third of RA patients with negative RF test results had positive anti-CCP antibodies.

Keywords: Anti-CCP antibodies, DAS-28 Score, Rheumatoid arthritis, Rheumatoid factor.

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Introduction

A chronic autoimmune disease of the synovium and joints, rheumatoid arthritis (RA) may the extra-articular organ systems including lungs and kidneys.[1,2] Although seen in all ethnic racial groups, RA is more common in African and Caribbean population.[3] RA is three-fold more frequent in people who have a positive family history of RA, and a disease concordance of 20% is seen in monozygotic twins.[3] This has led to research to highlight the genetic basis of this disease. However, there are still gaps in the etiological pathways of RA. Synovial inflammation in RA is a result of various cytokines and inflammatory mediators affected by a genetic predisposition in addition to environmental factors including viral infections, cigarette smoking and trauma.[4–6] The diagnostic criteria proposed by the American College of Rheumatology (ACR) in 2010 for RA includes the following parameters: number and type of joints, anti-CCP antibodies and rheumatoid factor (RF), duration of disease and markers of inflammation (CRP and ESR).[7]

An autoantibody targeted at Fc component of IgG, RF has been extensively used in the diagnosis of RA but can be positive in other conditions as well.[8,9] Furthermore, up to 40% patients with RA may have a negative RF.[10] Anti-CCP antibodies have been shown to have sensitivity of up to 78% and specificity of up to 99% in patients with RA.[11,12] Up to 37% of patients with RF-negative disease have been shown to have positive anti-CCP antibodies.[13] Furthermore, anti-CCP antibody positivity has been linked to more aggressive and erosive disease.[14] It is not entirely known how ethnic, regional, and geographic differences affect the pathogenesis of RA and the antibodies associated with it. The present study will try to analyze and ascertain how to efficiently fix dysfunctional character in our population. Therefore, we designed the present retrospective study to find out the frequency of positive anti-CCP antibodies in RA patients who had a negative RF so that prompt diagnosis and timely management may help reduce morbidity and mortality, especially in high-risk patients.

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Materials and Methods

This retrospective observational study was undertaken at the Department of Medicine and Allied, Azra Naheed Medical College, Superior University Lahore, to investigate the frequency of positive

anti-CCP antibodies in RA patients with negative RF. The 2010 ACR Diagnostic Criteria were used to define RA.[7] In this study, medical records of the patients having RF-negative RA presenting between January 2022 to December 2023 were included. Patients with incomplete data and seronegative arthritis due to other causes (such as lupus, peripheral spondyloarthritis, and psoriatic arthritis) were excluded from the study.

The present study was conducted in accordance with the ethical standards of the 1964 Declaration of Helsinki, revised in 2000. Ethical review approval and informed consent were taken before data collection. Retrospective data of 78 RA patients with negative RF, aged 21–80 years, of both sexes were assessed. Basic demographic and clinical variables, such as age, gender, anti-CCP antibody status, and disease severity (according to the DAS-28 score at the most recent visit) were assessed and recorded. Rheumatoid arthritis factors were assessed through immunonephelometry (fully automated nephelometry-NB 100) with a titer <14 IU/mL labeled as negative whereas second-generation enzyme-like immunosorbent assay (ELISA) was used to assess anti-CCP antibodies with a titer >20 IU/mL labeled as positive.[3] Disease activity was defined according to the DAS-28 score.[15] Active disease was categorized as a DAS-28 score more than 3.2 and low disease activity/remission was categorized by a DAS-28 score ≤ 3.2.

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) version 26 was used for the entry and analysis of data. Quantitative variables were presented as mean and standard deviation while frequencies and percentages were used to show qualitative variables. Confounders and effect modifiers were controlled for via stratification, with $p \leq 0.05$ significant and Chi-squared test applied. For expected counts < 5% Fisher's exact test was employed.

Results

As given in Table 1, 78 patients with RF-negative RA were enrolled, having a mean age of 43.8 ± 15.8 years, 28 (35.9%) patients aged 20–40 years, 38 (48.7%) patients aged 41–60 years and 12 (15.4%) patients aged 61–80 years. Majority of the patients ($N = 68$, 87.2%) were female having a mean duration of disease 9.1 ± 7.8 years with 62 (79.5%) patients having disease duration of more than 3 years. The mean DAS-28 score was 4.5 ± 1.7 with 48 (61.5%) patients having active disease (DAS-28 score >3.2). Positive anti-CCP antibodies were seen in 32 (41.0%) patients. Stratification of data with regard to anti-CCP antibodies is given in Table 2, revealing a significant association with sex, but not with age, duration of disease, or disease severity.

Table 1: Clinical variables of RA patients with negative RF

Clinical variables	*Frequency (n)*	*Percent (%)*
Gender		
Female	68	87.2
Male	10	12.8
Age		
20–40 years	28	35.9
41–60 years	38	48.7
61–80 years	12	15.4
Duration of disease		
≤3 years	16	20.5
>3 years	62	79.5
Disease activity		
LDA/remission	30	38.5
Active	48	61.5
Anti-CCP antibodies		
Positive	32	41.0
Negative	46	59.0

Table 2: Stratification of clinical variables according to anti-CCP antibody status

	Anti-CCP antibodies		
Clinical variables	*Present*	*Absent*	*p-value*
Gender			0.004
Female	32 (47.1%)	36 (52.9%)	
Male	00 (0.0%)	10 (100%)	
Age			0.839
20–40 years	12 (42.9%)	16 (57.1%)	
41–60 years	16 (42.1%)	22 (57.9%)	
61–80 years	04 (33.3%)	08 (66.7%)	
Duration of disease			0.009
<3 years	02 (12.5%)	14 (87.5%)	
>3 years	30 (48.4%)	32 (51.6%)	
Disease activity			0.884
LDA/remission	12 (40.0%)	18 (60.0%)	
Active	20 (41.7%)	28 (58.3%)	

Discussion

In RA, RF has been shown to have a specificity of 85% and sensitivity of 60%.[16] It should be noted that up to 40% of patients with RA may have a negative RF.[10] Furthermore, various other conditions can result in a positive RF including other autoimmune diseases (Lupus, Sjogren's Syndrome, dermatomyositis, vasculitis) and various bacterial and viral infections (tuberculosis, cytomegalovirus, Epstein-Barr virus, Hepatitis C, and HIV).[9,16] RF may also be seen in up to 30% healthy population.[17] On the other hand, anti-CCP antibodies have been shown to have sensitivity up to 78% and specificity up to 99% in patients with RA.[11,12] Various HLA associations (HLA-DR4, HLA-DR1, HLA-DR10) have been reported with anti-CCP antibodies.[18] Other factors which may predispose to positive anti-CCP antibodies include female gender, anemia, smoking, and periodontitis.[19–21] Anti-CCP antibodies have been shown to have sensitivity of up to 78% and specificity of up to 99% in patients with RA.[11,12]

Eker et al.[22] reported anti-CCP antibodies to be present in 22.1% of RF-negative RA patients. Korkmaz et al.[23] observed anti-CCP antibodies in only 20% of RF-negative RA patients. These studies are in contrast to our study, in which positive anti-CCP antibodies were more frequent. In the present study, anti-CCP antibodies were present in more than one-third (41.0%) of RA patients with negative RF. The results of our study are in accordance with those of previous studies. Gabriel et al.[24] reported anti-CCP antibodies to be present in 50% of RF-negative RA patients. Qaiser et al.[3] demonstrated anti-CCP presence in 40.6% of patients with negative RF results,

with no significant association with sex, age, disease duration, and BMI. Similarly, Eker et al.[22] did not find any association of anti-CCP antibody positivity with the severity of disease or extra-articular manifestations. None of the male patients with negative RF had positive anti-CCP antibodies as compared with 47.1% of females ($p = 0.004$), highlighting gender differences in etiology and antibody association of RA in the present study. No statistical association was seen between anti-CCP antibodies and age, duration, or severity of disease. We recommend using anti-CCP antibodies first-line for RA diagnosis based on the findings of our study. Specificity of anti-CCP antibodies is also more as compared with that of RA factor. Anti-CCP antibodies may also be screened in asymptomatic family members of RA patients for early detection of the occurrence of RA. It has been postulated that autoantibody presence may precede clinical disease by up to two decades.[25,26]

The present study had some limitations that need to be considered. Our study was based in a single institution, had a relatively small sample size, and assessed only retrospective data. However, we recommend using the results of our study as a baseline so that further studies should be planned to study the impact, differences, and disease courses of various antibody-positive versus antibody-negative RA, so that prompt diagnosis and treatment may result in lowering disease burden, disability, and morbidity in these patients.

Conclusion

In our study, more than one-third (41.0%) of RA patients with negative RFs tested positive for anti-CCP antibodies. However, none of the male patients with negative RF had positive anti-CCP antibodies as compared with 47.1% females ($p = 0.004$), highlighting sex differences in pathogenesis and antibody association of RA. No statistical association of anti-CCP antibodies was seen with age, disease duration, and disease severity.

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References

1. Benahmed D, Abbadi M, Zaoui D, et al. Tumor necrosis factor alpha induced protein 3, interleukin 10, tumor necrosis factor alpha, and interleukin 17 F genes polymorphisms in Algerian patients with rheumatoid arthritis. Mol Biol Rep 2024;51(1):545. DOI: 10.1007/s11033-024-09525-2.
2. Butt NI, Rasheed MB, Ghoauri MSA, et al. Prevalence of carpal tunnel syndrome in patients with rheumatoid arthritis and its association with disease severity. Pak J Phsyiol 2023;19(4):32–35.
3. Qaiser F, Masood A, Ujala S. Frequency of positive anti-CCP antibodies in rheumatoid arthritis patients with negative rheumatoid factor. J Islamabad Med Dental Coll 2023;12(4):242–247. DOI: 10.35787/jimdc.v12i4.665.
4. Lina G, Cuixin Q, Xia Z, et al. CCR9+CD4+ T cells are associated with disease activity in patients with rheumatoid arthritis. Medicine (Baltimore) 2024;103(16):e37803. DOI: 10.1097/MD.0000000000037803.
5. Khan Tareen MQ, Butt NI, Khan S, et al. Neutrophil lymphocyte ratio as an inflammatory marker in rheumatoid arthritis. PAFMJ 2023;73(5):1509–1512. DOI: 10.51253/pafmj.v73i5.7588.
6. Yang Z, Bai G, Ding H, et al. Development and validation of the rheumatoid arthritis scale among the system of quality of life instruments for chronic diseases QLICD-RA (V2.0). Sci Rep 2024;14(1):8954. DOI: 10.1038/s41598-024-58910-1.
7. Kay J, Upchurch KS. ACR/EULAR 2010 rheumatoid arthritis classification criteria. Rheumatology (Oxford) 2012;51 Suppl 6:vi5–9. DOI: 10.1093/rheumatology/kes279.
8. Elghali M, Bannour I, Touil I, et al. Increased Rheumatoid Factor production in patients with severe COVID-19. Diagn Microbiol Infect Dis 2024;109(3):116284. DOI: 10.1016/j.diagmicrobio.2024.116284.
9. Ashfaq F, Butt NI, Mahmood K, et al. Does vitamin D deficiency affect severity of rheumatoid arthritis? RMJ 2023;48(4):830–834.
10. de Brito Rocha S, Baldo DC, Andrade LEC. Clinical and pathophysiologic relevance of autoantibodies in rheumatoid arthritis. Adv Rheumatol 2019;59(1):2. DOI: 10.1186/s42358-018-0042-8.
11. Aggarwal R, Liao K, Nair R, et al. Anti-citrullinated peptide antibody assays and their role in the diagnosis of rheumatoid arthritis. Arthritis Rheum 2009;61(11):1472–1483. DOI: 10.1002/art.24827.
12. Szekanecz Z, Szabó Z, Zeher M, et al. Superior performance of the CCP3.1 test compared to CCP2 and MCV in the rheumatoid factor-negative RA population. Immunol Res 2013;56(2–3):439–443. DOI: 10.1007/s12026-013-8425-8.
13. Zhu JN, Nie LY, Lu XY, et al. Meta-analysis: compared with anti-CCP and rheumatoid factor, could anti-MCV be the next biomarker in the rheumatoid arthritis classification criteria? Clin Chem Lab Med 2019;57(11):1668–1679. DOI: 10.1515/cclm-2019-0167.
14. Hammam N, El-Husseiny PN, Al-Adle SS, et al. Clinical implications of seropositive and seronegative autoantibody status in rheumatoid arthritis patients: A comparative multicentre observational study. Rheumatol Immunol Res 2024;5(1):57–65. DOI: 10.1515/rir-2024-0007.
15. van Riel PL, Renskers L. The disease activity score (DAS) and the disease activity score using 28 joint counts (DAS28) in the management of rheumatoid arthritis. Clin Exp Rheumatol 2016;34(5 Suppl 101):S40–S44.
16. Jiang Y, Li Z, Li Z, Shi X, Chang L, Yan C. Performance evaluation of newly-developed tumor markers assay kit based on flow fluorescence assay. Clin Lab 2024;70(4). DOI: 10.7754/Clin.Lab.2023.230813.
17. Korpilähde T, Heliövaara M, Kaipiainen-Seppänen O, et al. Regional differences in Finland in the prevalence of rheumatoid factor in the presence and absence of arthritis. Ann Rheum Dis 2003;62(4):353–355. DOI: 10.1136/ard.62.4.353.
18. Zhao J, Xu L, Wei K, et al. Identification of clinical characteristics biomarkers for rheumatoid arthritis through targeted DNA methylation sequencing. Int Immunopharmacol 2024;131:111860. DOI: 10.1016/j.intimp.2024.111860.
19. van Wesemael TJ, Ajeganova S, Humphreys J, et al. Smoking is associated with the concurrent presence of multiple autoantibodies in rheumatoid arthritis rather than with anti-citrullinated protein antibodies per se: A multicenter cohort study. Arthritis Res Ther 2016;18(1):285. DOI: 10.1186/s13075-016-1177-9.
20. Di Matteo A, Mankia K, Garcia-Montoya L, et al. Utility of testing for third-generation anticyclic citrullinated peptide (anti-CCP3) antibodies in individuals who present with new musculoskeletal symptoms but have a negative second-generation anticyclic citrullinated peptide (anti-CCP2) antibody test. RMD Open 2024;10(2):e003927. DOI: 10.1136/rmdopen-2023-003927.
21. Butt NI, Ashfaq F, Habib O, et al. Clinico-demographic differences and severity of rheumatoid arthritis. Professional Med J 2023;30(03):342–347. DOI: 10.29309/TPMJ/2023.30.03.7319.
22. Eker YÖ, Pamuk ÖN, Pamuk GE, et al. The frequency of anti-CCP antibodies in patients with rheumatoid arthritis and psoriatic arthritis and their relationship with clinical features and parameters of angiogenesis: a comparative study. Eur J Rheumatol 2014;1(2):67–71. DOI: 10.5152/eurjrheumatol.2014.022.
23. Korkmaz C, Us T, Kaşifoğlu T, et al. Anti-cyclic citrullinated peptide (CCP) antibodies in patients with long-standing rheumatoid arthritis and their relationship with extra-articular manifestations. Clin Biochem 2006;39(10):961–965. DOI: 10.1016/j.clinbiochem.2006.06.004.

24. Gabriel SE, Crowson CS, O'Fallon WM. The epidemiology of rheumatoid arthritis in Rochester, Minnesota, 1955–1985. Arthritis Rheum 1999;42(3):415–420. DOI: 10.1002/1529-0131(199904)42:3<415::AID-ANR4>3.0.CO;2-Z.
25. Hajiesmaeili Y, Tamhankar P, Stranges S, et al. Factors associated with incident cardiovascular disease in patients with rheumatoid arthritis: A scoping review. Autoimmun Rev 2024;23(5):103539. DOI: 10.1016/j.autrev.2024.103539.
26. Keleşoğlu Dinçer AB, Şahan HF. Increased epicardial adipose tissue thickness as a sign of subclinical atherosclerosis in patients with rheumatoid arthritis and its relationship with disease activity indices. Intern Emerg Med 2024. DOI: 10.1007/s11739-024-03542-6.