MedS Alliance
Journal of Medicine and Medical Sciences
https://doi.org/10.3126/mjmms.v1i2.46373

Original Investigation

Anti-CCP Antibody and Rheumatoid Factor among Clinically Diagnosed Rheumatoid Arthritis Patients Attending a Tertiary Hospital at Chitwan, Nepal

Fuleshwar Mandal[1], Dilip KC[2], Kishor Adhikari[3*]

[1]Department of Biochemistry, Chitwan Medical College, Tribhuvan University, Nepal
[2]Department of Laboratory Medicine, Chitwan Medical College, Tribhuvan University, Nepal
[3]School of Public Health and Department of Community Medicine, Chitwan Medical College, Tribhuvan University, Nepal

ARTICLE INFO

Article history:
Received: 11 June 2021
Revised: 3 September 2021
Accepted: 29 September 2021

***Correspondence:**
Dr. Kishor Adhikari,
Professor,
School of Public Health and Community Medicine,
Chitwan Medical College,
Tribhuvan University,
Bharatpur, Nepal

E-mail:
kishoo2006@gmail.com

ORCID:
0000-0003-2517-0834

Citation:
Mandal F, KC D, Adhikari K. Anti-CCP Antibody and Rheumatoid Factor among Clinically Diagnosed Rheumatiod Arthritis Patients Attending a Tertiary Hospital at Chitwan, Nepal. MedS. J. Med. Sci. 2021;1(2):6-10.

ABSTRACT

INTRODUCTION: Rheumatoid Arthritis (RA) is a common, chronic inflammatory multisystem autoimmune disorder of undetermined aetiology involving primarily the synovial membranes and articular structures of many joints. Rheumatoid factor (RF) is most frequently used serum biomarkers for the diagnosis of RA; however, these antibodies are detectable in many other pathological conditions and even in 5% in healthy population. Anti-Cyclic Citrullinated Peptide (Anti-CCP) antibodies is another serological biomarker having high sensitivity and specificity than RF which present in pre-clinical stage and seronegative rheumatoid arthritis patients. **MATERIALS AND METHODS:** This is a cross-sectional study conducted among the confirmed RA patients visiting orthopaedic OPD. Anti-CCP antibody was determined quantitively by CLIA on Siemen ADVIA Centaur CP immunoassay system and RA-factor were determined quantitively by Nephelometry assay on MISPA I3 and comparison was done between two serological tests, i.e., Anti-CCP and RF. **RESULTS:** Out of 112 clinically diagnosed RA patients, 23 (20.5%) were males and 89(79.5%) were females with the ratio of 1:3.9 and the mean age ± SD of the patients was 50 ± 14.56. Out of 112 clinically diagnosed RA patients, 55 patients, i.e., 49.1% were RF antibodies positive and 57 patients, i.e., 50.9% were RF antibodies negative. Among RF positive, 32 (58.2%) patients were positive for anti-CCP antibody and among RF negative, 10 (17.5%) patients were positive for anti-CCP antibody and the differences between Anti-CCP and RF groups was statistically highly significant ($p<0.001$). **CONCLUSIONS: F**emales are at high risk of developing Rheumatoid arthritis than males because male to female ratio is found to be 1:3.9. This study also showed that Anti-CCP antibody is more beneficial and better marker than RF for the diagnosis of Rheumatoid arthritis.

Keywords: Anti-cyclic citrullinated peptide antibody, rheumatoid factor, rheumatoid arthritis.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory multisystem autoimmune disorder of undetermined aetiology involving primarily the synovial membranes and articular structures of many joints [1,2]. It is most common chronic inflammatory arthritis marked by synovial inflammation and destruction of bone and cartilage, which may result in joint destruction and disability [3,4]. It is most prevalent chronic inflammatory diseases, with an incidence of 0.5%–1.0% worldwide [5]. Incidence increases with age that commonly presenting between 50 and 70 years of age, with women being affected three times more than men [6].

It is associated with several autoantibodies i.e. rheumatoid factor (RF), anti-cyclic citrullinated peptide antibody (anti-CCP), anti-perinuclear factor (APF), Anti-nuclear cytoplasmic antibody, anti-

RA33, anti-flagger antibody, anti-keratin antibody (AKA) etc.[7].

They have been reported to diagnose rheumatoid arthritis but many of them e.g., APF and AKA have high specificity but due to various technical difficulties and tedious laboratory procedure, they have not been generally adopted [8].

Together with classical clinical feature of the diseases, serum biomarkers such as Rheumatoid Factor (RF) and anti-CCP have been used as diagnostic tool for the early diagnosis of the RA [9]. Rheumatoid Factor (RF) is most frequently used serum biomarkers for the diagnosis of RA. However, the sensitivity of RF is approximately 60 to 80% in RA. The specificity of RF is low since RF is detectable in many other diseases and in approximately 5% of healthy people [10, 11].

The sensitivity of Anti-CCP is 75-80% and specificity is 97% in the diagnosis of rheumatoid arthritis [12]. Anti-CCP antibody is an important diagnostic tool, particularly in the early stages of the disease diagnosis and predictive of the disease progression since it is detected at an early stage of seronegative rheumatoid arthritis patients [13]. The study was designed to compare between anti-CCP antibodies and RF among clinically diagnosed RA patient.

MATERIALS AND METHODS

Study design and setting

The cross-sectional study was carried out in Department of Biochemistry in collaboration with Department of Orthopaedic, Chitwan Medical College and Teaching Hospital, Bharatpur, Nepal.

Patients and procedure

A total of 112 clinically diagnosed RA patients were included following convenient sampling technique in this research after taking informed consent. Clinical details of all patients along with the serology reports (RF, Ante-CCP antibody) were entered in a proforma constructed in Microsoft excel.

Data collection procedure

A structured questionnaire was constructed to record clinical details including duration of symptom, pattern of joint involvement, early morning stiffness, extra articular and systemic involvement were recorded as per the 2010 EULAR/ACR criteria[14].

Socio-demographic data, i.e., age and sex were also recorded. The samples were collected from 1st January 2017 to 1st August 2017. All clinically diagnosed rheumatoid arthritis patients either sex above 20 years were included in the study whereas pregnant, patient with known-chronic illness other than the ailment under study were excluded from the study.

To analyze Rheumatoid factor and Anti-CCP antibody, 5 ml blood sample were drawn from antecubital vein in vacuum gel tube. Blood sample was centrifuged at 3000 RPM for 5 minutes and serum were separated. Rheumatoid factors (RF) were determined quantitively by Nephelometry assay on MISPA I3 [15]. Anti-CCP antibody were determined quantitively by CLIA on Siemen ADVIA Centaur CP immunoassay system [16, 17]

Study variables and its measurement

Explanatory variables: Age and Sex

Output variables:

Rheumatoid factor, Anti-CCP antibody

Statistical analysis and data management

IBM SPSS (version 20) was used for data analysis. Data entered in Microsoft excel were transferred into SPSS. Mean value of Rheumatoid factor (RF), and Anti-CCP antibody were analyzed. Chi square test was used to determine significance of differences in categorical variables. Fisher exact test was used to measure associations of ACPA with various variables.

Ethical considerations

Ethical clearance was obtained from institutional review committee of Chitwan Medical College prior the data collection. (Ref no. 2073/74 - 84).

RESULTS

Out of 112 clinically diagnosed RA patients, 23 (20.5%) males and 89(79.5%) females were taken for this study. The ratio of male to female was 1:39 and the mean age ± SD of the patients was 50 ± 14.56. Out of 112 clinically diagnosed RA patients, 55 patients, i.e., 49.1% were RF antibodies positive and 57 patients, i.e., 50.9% were RF antibodies negative. Majority of respondents, i.e. 64.3% were tested RF positive from 60 years and above age group followed by 20-39 years age group and 40-59 years age groups. Slightly high percentage of female respondents were tested Positive for RF. Though, the differences in age and sex was not statistically significant in terms of RF status (Table 1).

Majority of respondents, i.e., 53.6% were tested Anti-CCP positive from 60 years and above age group followed by 40-59 years age group and 20-39 years age groups, i.e., 38.0% and 23.5% respectively. Slightly high percentage of female respondents (40.4%) were tested Positive for Anti-CCP. Though, the differences in age and sex was not statistically significant in terms of Anti-CCP status (Table 2).

Highest proportion of patients, i.e., 50.38% who reported moderate level of morning stiffness were found Anti-CCP positive followed by mild level of morning stiffness, i.e., 33.33%. Least percentage of patients (14.28%) reported severe level of morning stiffness who tested positive for Anti-CCP positive (Table 3).

Table 4 highlights the comparison between the test finding of RF and Anti-CCP. Among RF positive, 32 (58.2%) patients were positive for anti-CCP antibody and among RF negative, 10 (17.5%) patients were positive for anti-CCP antibody and the differences between Anti-CCP and RF groups was statistically highly significant (p<0.001) (Table 4).

Table 1 | Age and sex wise distribution of Rheumatoid Factor (RF)

Parameters	Rheumatoid Factor (RF) Positive (%)	Rheumatoid Factor (RF) Negative (%)	p-value
Age Group			0.179
20-39 years	15(44.1)	19(55.9)	
20-39 years	22(44.0)	28(56.0)	
20-39 years	18(64.3)	10(35.7)	
Mean (SD)	50(±14.56)		
Sex			0.052
Male Female	11(47.8) 44(49.9)	12(52.2) 45(50.6)	
Total	55(49.1)	57(50.9)	

Table 2 | Age and sex wise distribution of anti-cyclic citrullinated peptide (Anti-CCP) findings

Parameters	Anti- cyclic citrullinated peptide Positive n(%)	Anti- cyclic citrullinated peptide Negative n(%)	p-value
Age Group			0.539
20-39 years	8(23.5)	26(76.5)	
20-39 years	19(38.0)	31(62.0)	
20-39 years	15(53.6)	13(46.4)	
Gender			0.152
Male	6(26.1)	17(73.9)	
Female	36(40.4)	53(59.6)	
Total	**42(37.5)**	**70(62.5)**	

Table 3 | Anti-CCP antibody status among the patients with levels of morning stiffness

Level of Morning stiffness	Anti-CCP Positive n(%)	Anti-CCP Negative n(%)	N (%)
Mild	14 (33.33)	30 (42.85)	44(39.30)
Moderate	22 (52.38)	26 (37.14)	48(42.85)
Severe	6 (14.28)	17 (40.47)	20(17.85)
Total	42 (37.5)	70 (62.5)	112 (100)

Table 4 | Comparison between rheumatoid factor and Anti-CCP antibody groups

RF status	Anti-CCP Positive N (%)	Anti-CCP Negative N (%)	p-value
Positive	32 (58.2)	23 (41.8)	0.001*
Negative	10 (17.5)	47 (82.5)	
Total	**42(37.5)**	**70(62.5)**	

*Statistically significant

DISCUSSION

This study was done in 112 clinically diagnosed RA patients where 55 (49.9%) were tested RF positive and 57 (50.1%) were tested RF negative. Out of 55 RF positive patients, 32 (58.2%) were positive for

Anti-CCP antibody and 23(41.8%) were negative for Anti-CCP antibody. This finding is quite similar to the research finding conducted by Afzal A et al [18] and Kastbom et. Al [19] where 50% Anti-CCP antibodies was found positive among RF positive patients. Among 57 RF negative patients, 10 (17.5%) were positive for Anti-CCP antibody which is almost similar to the study conducted by Korkmaz C et al. [20], who showed that 20% Anti-CCP antibodies was positive in RF negative patients.

This study also showed that the comparison between RF and Anti-CCP antibody groups were found to be statistically significant ($p<0.001$). So, the results of this study have almost similar finding to the study conducted by Münevver S, Haşim Ç et al [21]. Likewise, Out of 112 clinically diagnosed RA patients, 42 patients were Anti-CCP antibodies positive and 70 patients were Anti-CCP antibodies negative and the comparison between Anti-CCP positive and Anti-CCP negative was statistically significant ($p<0.001$) which is almost similar to the finding of the study conducted by Nadeem Afzal et al [18]. Highest proportion of patients, i.e., 50.38% who reported moderate level of morning stiffness were found Anti-CCP positive followed by mild level of morning stiffness, i.e., 33.33% in the present study which is similar to study conducted by Yazici et al. [22] who confirmed that morning stiffness in early stage of RA was associated with more functional disability [48]. The major limitation in the present study is that other important serological biomarkers such as ANCA, CRP, ESR, etc. were not taken into consideration.

CONCLUSIONS

From this study, it is concluded that females are at high risk of developing Rheumatoid arthritis than males because male to female ratio is found to be 1:3.9. This study also showed that Anti-CCP antibody is more beneficial and better marker than RF for the diagnosis of Rheumatoid arthritis. The RF positive and Anti-CCP negative results showed the patients might not be suffering from the RA as it may rises in the no. of pathological conditions like connective tissue diseases, chronic inflammatory disease, malaria etc. However, RF is still the reliable marker for diagnosis of RA. So, if these two tests are performed together, the sensitivity and specificity of the tests increases which provides strong evidence for the diagnosis of Rheumatoid arthritis.

ADDITIONAL INFORMATION AND DECLARATIONS

Acknowledgements: We would like to thank the departmental heads of Orthopedic department and biochemistry department. We would like to extend our thanks to Dr. Sanjay Yadav, Head, Department of laboratory medicine, CMCTH.

Funding: Self-funded

Competing Interests: The authors declare no competing interests.

Author Contributions: Concept and design: FM, KA; statistical analysis: KA, FM; writing of the manuscript: FM, DKC and KA; revision and editing the manuscript: FM, DKC and KA. All authors contributed to all-analysis, interpretation of results, literature review, and revision of the manuscript, and all have read and agreed with the contents of the final manuscript.

Data Availability: Data will be available upon request to corresponding authors after valid reason.

REFERENCES

1. Van Boekal M, Vossenaar ER, Van den Hoogen FHJ, and WJ Venrooij. Autoantibody systems in rheumatoid arthritis: sensitivity and diagnostic value. Arthritis Res. 2001; 4(1):87-93.
2. Xiaochun Yang , Yue Cai, Bin Xue and Bo Zhang. Diagnostic value of anti-cyclic citrullinated peptide antibody combined with rheumatoid factor in rheumatoid arthritis in Asia: a meta-analysis. J of Intl Med Research. 2021;49(9) 1–16.
3. Ashley Nicole Lee,Carolyne Beck, Margot Hall ARGOT. Rheumatoid Factor and Anti-CCP Autoantibodies in Rheumatoid Arthritis: A Review. Clinical Laboratory Science, Winter. 2008;21(1):15-8)
4. McInnes, I.B.; Schett, G. The pathogenesis of rheumatoid arthritis. N. Engl. J. Med. 2011; 365: 2205–19.
5. Smolen JS, Aletaha D, McInnes IB. Rheumatoid arthritis. Lancet. 2016;388: 2023–38.
6. Biemond P, Swaak AJ, Koster JF. Protective factors against oxygen free radicals and hydrogen peroxide in rheumatoid arthritis synovial fluid. Arthritis Rheum. 1984; 27:760–5.
7. Bas S, Perneger TV, Seitz M, Tiercy MJ, Lombard RP, Guerne AP. Diagnostic tests for rheumatoid arthritis. Comparison of anticyclic citrullinated peptide antibodies, anti-keratin antibodies and IgM rheumatoid factor. Rheumatol. 2002;41:809-14.
8. Khosla P, Shankar S, Duggal L. Anti-CCP antibodies in rheumatoid arthritis. J Indian

Rheumatol Assoc. 2004;12:143-6.

9. Mayer,O.,C. Labarre, M.Dougados,P. Goupille and A. Cantagrel et al. Anticitrullinated protein/peptide antibody assay in early rheumatoid arthritis for predicting five year radiographic damage. Ann Rheum Dis. 2003;62:120-26.

10. Eggel and T, Munthe E. The role of the laboratory in rheumatology, rheumatoid factors. Rheum Dis Clin. 1983;9:135-60.

11. FC Arnett, SM Edworthy, DA Bloch, DJ McShane, JF Fries, NS Cooper, LA Healey, SR Kaplan, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum. 1988; 31(3):315-24.

12. Van Venrooij WJ, Hazes JM, Visser H. Anticitrullinated protein/ peptide antibody and its role in the diagnosis and prognosis of early rheumatoid arthritis. Neth J Med. 2002;60(10):383.

13. Quinn AM, Gough SA, Green JM, et al. Anti-CCP antibodies measured at disease onset help identify seronegative rheumatoid arthritis and predict radiological and functional outcome. Rheumatol. 2005;45:478-80.

14. Daniel Aletaha , Tuhina Neogi, Alan J Silman, Julia Funovits, David T Felson, Clifton O Bingham. Rheumatoid arthritis classification criteria: an American College of Rheumatology / European League Against Rheumatism collaborative initiative. Ann Rheum Dis. 2010;69(9):1580-8.

15. Frederick, Wolfe et al.Arthritis and Rheumatism. 1991: 34; 951-60.

16. Banseok Kim, Yongjung Park, Jin-Su Park, Kyoung Ja Jang, Hyo Jun Ahn, Min Hyuk Choi , Chan Hee Lee. Laboratory and Clinical Performance of the ADVIA Centaur Anticyclic Citrullinated Peptide Assay for Rheumatoid Arthritis Diagnosis. Arch Pathol Lab Med. 2018;142(12):1554-59.

17. Siemens ADVIA Centeur anti-CCP IgG assay. Available from: http://www.healthcare.siemens.com/ autoimmune.

18. Afzal N, KarimS, MahamudT, SamiW,Arif M,Abbas S,Evaluation of Anti-CCP Antibody for Diagnosis of Rheumatoid Arthritis. Clin. Lab. 2011;57:895-98.

19. Kastbom A, Strandberg G, Lindroos A, Skogh T. Anti-CCP antibody test predicts the disease course during three years in early rheumatoid arthritis. Ann Rheum Dis. 2004; 63:1085-9.

20. Karkmaz C, US T,Kasifoglu T,Akguin Y. Anticyclic citrulinated peptide antibodies in Patients withlong standing arthritis and their relationship with extra articular manifestation. Clinical Biochemistry. 2006;39:961-5.

21. Serdaroglu M, Cakrbay H, Deger O, Cengiz S, Kul S.The association of anti-CCP antibodies with disease activity in rheumatoid arthritis. Rheumatology international. 2008,28:965.

22. Yazici Y, Pincus T, Kautiainen H, Sokka T. Morning stiffness in patients with early rheumatoid arthritis is associated more strongly with functional disability than with joint swelling and erythrocyte sedimentation rate. J Rheumatol. 2004;31:1723-6.