DOI: https://doi.org/10.3329/medtoday.v36i2.74793

Anti-CCP Antibodies in Unaffected First-Degree Relatives of Rheumatoid Arthritis Patient

Mohammad Nurul Alam*[1], Mohammed Khaled Mosharraf Hossain[2], Mohammad Mahadi Hasan Mazumder[3], Ifat Ara Begum[4], Mohammad Sabbir Hossain[5], Md Saiful Islam Patwary[6], Md. Noman Hossain[7]

Abstract

Introduction: *Rheumatoid arthritis (RA) is perhaps the most common inflammatory arthritis, affecting 0.5% to 1% of the general population worldwide. Environmental and genetic influences are important risk factors for pathogenesis of RA. First-degree relatives (FDRs) of RA patients sharing genetic and environmental risk factors for RA may represent as pre-RA state. Since anti-CCP antibody appears years before the onset of RA, it can be used to estimate risk-potential for future development of RA in unaffected FDRs of RA patient.* ***Objective:*** *To see the serum anti-CCP antibody in unaffected FDRs of RA patients.* ***Materials and Methods:*** *This cross-sectional study was conducted from July 2019 to June 2020 in Department of Biochemistry, Dhaka Medical College, Dhaka. A total number of 50 subjects were selected among which 25 were unaffected FDRs of RA patients and the other 25 were age & gender matched healthy subjects. The related RA patients were selected from Rheumatology outdoor, Department of Rheumatology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka and the healthy subjects were taken from the attendants came to the same institute who did not have RA patient in their family. The key variables (age and gender) were recorded and outcome variable (anti-CCP antibody in study subjects) was estimated. Statistical analysis was done by SPSS 26.0 and the significance was defined as $p < 0.05$.* ***Results:*** *Mean age of the FDRs of RA patient was 31.36 ± 10.51 years and that of healthy subjects was 31.56 ± 8.89 years. Most of the participants were in age group 20-29 years followed by 30-39 years. 4% of FDRs of RA patient were anti-CCP antibody positive and no positive case was recorded in healthy subjects. The median level of anti-CCP antibody in FDRs of RA patient was 0.50 U/mL and that in healthy subjects was 0.09 U/mL. The level of significance ($p = 0.002$) showed that serum anti-CCP antibody level was significantly higher (within normal range) in FDRs of RA patient than in healthy subjects, which was even more significant in FDRs of seropositive RA patients.* ***Conclusion:*** *This study revealed that serum anti-CCP antibody level was significantly higher (within normal range) in FDRs of RA patient than in healthy subjects and the proportion of positive anti-CCP antibody was more in FDRs of RA patient than in healthy subjects.*

Key words: *Anti-CCP antibodies, rheumatoid arthritis, first-degree relatives.*

Number of Tables: 06; Number of Figures: 02; Number of References: 28; Number of Correspondences: 03.

***1. Corresponding Author:**
Dr. Mohammad Nurul Alam, M. Phil (Biochemistry)
Assistant Professor
Department of Biochemistry
Chandpur Medical College, Chandpur, Bangladesh.
Email: drmnalam@gmail.com
Mobile No. 01711379317

2. Dr. Mohammed Khaled Mosharraf Hossain
M. Phil (Biochemistry)
Assistant Professor
Department of Biochemistry
Cox's Bazar Medical College, Cox's Bazar, Bangladesh.

3. Dr. Mohammad Mahadi Hasan Mazumder
M. Phil (Biochemistry)
Lecturer, Department of Biochemistry
Chandpur Medical College, Chandpur, Bangladesh.

4. Prof. Dr. Ifat Ara Begum, M. Phil (Biochemistry)
Professor (c.c.)
Department of Biochemistry
Sir Salimullah Medical College, Dhaka, Bangladesh.

5. Dr. Mohammad Sabbir Hossain
MS (Orthopedics)
Associate Professor (c.c.)
Department of Orthopedic Surgery
Chandpur Medical College
Chandpur, Bangladesh.

6. Dr. Md Saiful Islam Patwary
MD (Medicine)
Associate Professor (c.c.)
Department of Medicine
Chandpur Medical College
Chandpur, Bangladesh.

7. Dr. Md. Noman Hossain
FCPS (Medicine)
Assistant Professor
Department of Medicine
Chandpur Medical College
Chandpur, Bangladesh.

Received: *22 February 2024* ***Accepted revised version:*** *30 May 2024*

Introduction:

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by joint swelling, joint tenderness and destruction of synovial joints, leading to severe disability and premature mortality[1]. It is perhaps the most common inflammatory arthritis, affecting 0.5% to 1% of the general population worldwide and predominately affects females (2- or 3-fold more often than males). RA can occur at any age but it is more frequent among individuals in the fourth to sixth decades of life[2]. If untreated, 20%–30% of RA patients become so severely debilitated within the first three years following initial diagnosis that they become permanently disabled[3].

The precise causes of RA remain uncertain but environmental and genetic influences clearly are factors[4]. Smoking exposure has a dose response relationship with RA risk[5]. Genetic studies have demonstrated that a genetic predisposition resides in the human leukocyte antigen (HLA)-DR locus[6]. In monozygotic twins there is about 15% to 30% concordance rate for rheumatoid arthritis development[7].

RA is primarily considered as a disease of the joints, but multiple additional organ systems are also known to be involved including pulmonary, cardiovascular, ocular and cutaneous systems[8].

The initiation of RA begins years before the onset of clinical symptoms. The onset involves certain specific genes that can lead to the production of pathogenic antibodies that bind to modified proteins, help break tolerance, and lead to autoreactivity[4].

The standard and accepted means of defining RA is by use of classification criteria. This classification was essentially based on the criteria formulated by the American College of Rheumatology (ACR; formerly the American Rheumatism Association) in 1987[1]. These criteria exhibit 91%–94% sensitivity and 89% specificity for established RA[2], but have a significant limitation and are therefore not helpful in achieving the goal of identifying patients who would benefit from early effective intervention[1]. A systematic review of publications evaluating the performance of the ACR's 1987 clinical criteria found the sensitivity to early RA to be between 77% and 80%, with specificities between 33% and 77% based on pooled data[9]. To overcome this limitation a joint working group of the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) was formed and they developed the 2010 ACR/EULAR classification criteria for RA[1].

To meet the need for improved diagnostic and prognostic tests and algorithms, various serum biomarkers are being assessed including a wide range of autoantibodies. However, only rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP) have gained wide acceptance[9].

The first autoantibody in RA, rheumatoid factor (RF), was described by Waaler in 1940, and it was later found to be directed to the Fc region of IgG. IgM RFs are the major RF species in RA and are found in approximately 60%–80% of these patients. It is also found in patients with infections, other autoimmune diseases, and some unaffected individuals with increasing frequency in older age groups, thus limiting its specificity for RA[10]. It is found in 3-5% of the general population, and in up to 30% of the elderly[11]. Historically, RF was the main element in the serological diagnosis of RA and the only laboratory diagnostic parameter included in the 1987 American College of Rheumatology (ACR) criteria for the classification of RA[12].

Early diagnosis and treatment in RA are crucial as it can prevent disease progression and irreversible joint damage[13] but RF lacks RA specificity and has a low prevalence in new-onset disease[14]. So, anti-citrullinated protein antibodies (ACPAs) have been included in the 2010 ACR/EULAR classification criteria for RA as the second serological marker, which are focused on early diagnosis and therapy[14]. ACPAs are locally produced in RA joints, where proteins are citrullinated during the inflammatory process, which are found in 70–90% of RA patients and have high disease specificity (90–95%). Accordingly, they are rarely found in other diseases or in unaffected individuals[10].

The more recent autoantibodies for the diagnosis of RA are anti-cyclic citrullinated peptide antibodies (anti-CCP antibodies). Anti-CCP antibody testing is particularly useful in the diagnosis of RA and it is able to predict the severity of the disease and the irreversible damage[15]. It has been shown to be reasonably sensitive (65-80%) and highly specific for RA (up to 98%)[11]. Due to high specificity and moderate sensitivity, a positive anti-CCP antibody result means RA is likely though a negative result does not rule out the disease[16]. There is evidence that the presence of anti-CCP antibodies can be detected long (upto 15 years) before the onset of clinical manifestations which suggests their role in the pathogenesis of RA[17].

The first ACPAs in RA sera were discovered by Nienhuis et al. in 1964, and were named antiperinuclear factor (APF). In 1979, Young et al. reported that RA sera contained antikeratin antibodies (AKA)[18]. These antibodies are highly specific for RA (upto 99%), but unfortunately, the inconvenient test format and inconvenient antigenic substrates used to determine the presence of the APF/AKA antibodies have hindered their use as a serological marker[19].

In 1998, Schelleken reported that antibodies, against citrullinated peptide, are highly specific seromarker for the diagnosis as well as prognosis of rheumatoid arthritis[3]. Schellekens et al. designed a synthetic cyclic citrullinated peptide (CCP) and used it as a new antigenic substrate in anti-CCP ELISA (Enzyme Linked Immunosorbent Assay) to detect ACPAs[14].

The first generation of anti-CCP antibodies (anti-CCP1) test revealed a higher specificity for RA in comparison to the RF test. At the end of 2002, second generation anti-CCP (anti-CCP2) antibodies tests were developed, with different cyclic peptides and improved performance characteristics showing an even better specificity for RA[12].

Several studies have examined the performance characteristics of anti-CCP antibodies in RA, using both the anti-CCP1 and

anti-CCP2 assays. Sensitivity and specificity using the anti-CCP1 assay ranged from 44% to 56% and 90% to 97%, respectively. Detection of antibody with CCP2 assays resulted in improved sensitivity (64–89%), and specificity (88–99%). Rheumatoid factor sensitivity ranged from 59% to 79% and specificity from 80% to 84% in the same groups[18].

Nishimura et al. performed a meta-analysis of published studies regarding the diagnostic accuracy of anti-CCP and RF for rheumatoid arthritis. Their results showed a positive likelihood ratio of 12.46 and a negative likelihood ratio of 0.36 for anti-CCP antibody in patients with RA. The same study showed a positive likelihood ratio of 4.86 and a negative likelihood ratio of 0.38 for RF. These results indicate that anti-CCP positivity alone is more specific than IgM RF for the diagnosis of RA[20].

Studies on anti-CCP antibodies in unaffected first-degree relatives of RA patients are very few in Bangladesh. So, the aim of this study was to see the serum anti-CCP antibody in unaffected first-degree relatives of RA patients and compare with those of non-related healthy subjects.

Materials and Methods:

This cross-sectional study was conducted in the Department of Biochemistry, Dhaka Medical College, Dhaka during the period of July 2019 to June 2020. For this study, a total number of 50 subjects between 20-60 years of age were selected, among which 25 were unaffected first-degree relatives (FDRs) of rheumatoid arthritis (RA) patients and the other 25 were age & gender matched healthy subjects. The related RA patients were selected from Rheumatology outdoor, Department of Rheumatology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, and the healthy subjects were taken from the attendants came to the same institute who did not have RA patient in their family. Informed written consent was taken from the study subjects after full explanation of procedure regarding study in a prescribed form. A prefixed questionnaire was used to record detailed history, relevant physical examinations and investigations. After all aseptic precaution 5 ml of venous random blood sample was collected from each study subject. All the biochemical tests were performed in the department of Biochemistry, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, estimated by Enzyme-linked Immunosorbent Assay (ELISA) method. All data were recorded in a predesigned data collection sheet. Data was analyzed by using the statistical package for social sciences (SPSS) software version 26.0. Descriptive analysis was performed. Continuous variables were expressed as mean ± SD or median (IQR) and were compared between groups of subjects by unpaired student's t-test or Mann-Whitney U test. Categorical variables were compared using a Chi-square test or Fischer's exact test as appropriate, and presented as absolute frequencies with percentages. All 'p' values were two-tailed with significance defined as $p < 0.05$ at the level of 95% confidence interval (CI). The results were presented in the form of tables and graphs.

Ethical clearance:

Ethical clearance was obtained from the Ethical Review Committee, Dhaka Medical College, Dhaka with memo no. ERC-DMC/ECC/2020/37 dated 03.02.2020. All the research data were used solely for the study and remained confidential.

Result:

The present study was a cross sectional study, which was conducted in the Department of Biochemistry, Dhaka Medical College, Dhaka. The study was aimed to evaluate the serum anti-CCP antibody in unaffected first-degree relatives (FDRs) of rheumatoid arthritis (RA) patients to compare it with those of healthy subjects. Total study subjects were fifty (50) which were divided into two groups. Group A consists of 25 FDRs of RA patient and Group B consists of 25 healthy subjects. The key variables (age and gender) were recorded and outcome variable (anti-CCPA in study subjects) was estimated. Statistical analysis was done according to the data. Results are presented by tables and figures in the following pages.

Table-I showing demographic profile of the study subjects. There were no significant differences between two groups in terms of mean age and gender distribution.

Table-I: Demographic profile of the study subjects (N=50)

	FDRs of RA patient (n=25)	Healthy subjects (n=25)	p-value
Mean age (years) ± SD	31.36 ± 10.51	31.56 ± 8.89	0.942[a]
(range) years	(20-55)	(20-55)	
Gender distribution			
Male, n (%)	16 (64.0%)	14 (56.0%)	0.773[b]
Female, n (%)	9 (36.0%)	11 (44.0%)	

[a]Unpaired t test was done to measure the level of significance

[b]Chi-Square test was done to measure the level of significance.

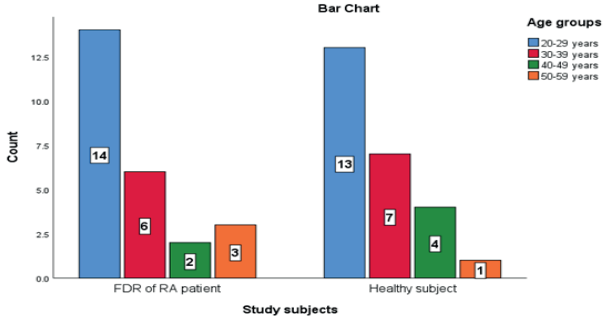

Figure-1: Bar diagram showing distribution of subjects according to their age group.

Maximum participants were in age group 20-29 years in both groups of subjects followed by age group 30-39 years.

Table-II showing that anti-CCP antibody level was significantly higher in FDRs of RA patient than in healthy subjects.

Table-II: Comparison of anti-CCP antibody level between FDRs of RA patients and healthy subjects (N=50).

	FDRs of RA patient (n=25)	Healthy subjects (n=25)	p-value
Anti-CCPA level (U/mL) Median (IQR)	0.50 (0.10-0.65)	0.09 (0.08-0.09)	0.002[a]

[a]Mann-Whitney U test was done to measure the level of significance

Table-III showing that there was no significant difference in anti-CCP antibody level between male and female.

Table-III: Comparison of anti-CCP antibody level in FDRs of RA patients according to gender (n=25).

	Male FDRs (n=16)	Female FDRs (n=9)	p-value
Anti-CCPA level (U/mL) Median (IQR)	0.33 (0.10-0.60)	0.50 (0.10-0.85)	0.598[a]

[a]Mann-Whitney U test was done to measure the level of significance

Table-IV showing that 4.0% of FDRs of RA patients were anti-CCPA positive while none of healthy subjects was anti-CCPA positive.

Table-IV: Frequency of anti-CCP antibodies in FDRs of RA patients and healthy subjects (N=50).

	FDRs of RA patients (n=25)	Healthy subjects (n=25)
Anti-CCPA positive, n (%)	1 (4.0%)	0 (0.0%)
Anti-CCPA negative, n (%)	24 (96%)	25 (100%)

Table-V showing that anti-CCP antibody level is significantly higher in FDRs of RF positive patient than in FDRs of RF negative patient.

Table-V: Comparison of anti-CCP antibody level in FDRs of RA patients according to RF in related patient (n=25).

	FDRs of RF positive RA patient (n=22)	FDRs of RF negative RA patient (n=3)	pvalue
Anti-CCPA level (U/mL) Median (IQR)	0.50 (0.14-0.73)	0.07 (0.07-0.07)	0.010[a]

[a]Mann-Whitney U test was done to measure the level of significance

Table-VI showing that anti-CCP antibody level is significantly higher in FDRs of anti-CCPA positive patient than in FDRs of anti-CCPA negative patient.

Table-VI: Comparison of anti-CCP antibody level in FDRs of RA patients according to anti-CCPA in related patient (n=25).

	FDRs of anti-CCPA positive RA patient (n=19)	FDRs of anti-CCPA negative RA patient (n=6)	pvalue
Anti-CCPA level (U/mL) Median (IQR)	0.50 (0.17-0.80)	0.075 (0.07-0.12)	0.010[a]

[a]Mann-Whitney U test was done to measure the level of significance

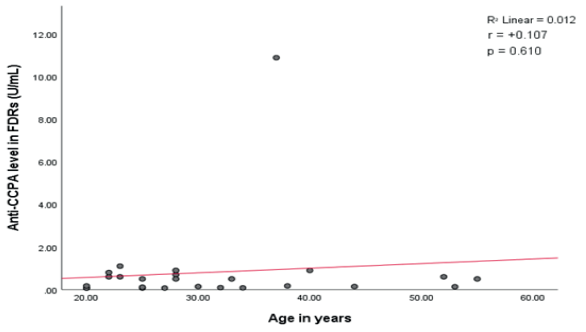

Figure-2: A positive correlation without significance between the age of the FDRs of RA patients and the anti-CCP antibody level in them.

Pearson's correlation was done

Figure-2: showing a positive correlation without significance between the age of the FDRs of RA patients and the anti-CCP antibody level in them.

Discussion:

Several studies have revealed that clinically symptomatic rheumatoid arthritis (RA) is preceded by a long period of autoimmunity characterized by the presence of antibodies, elevated cytokine levels, and other markers of inflammation[21]. The factors and mechanism causing initiation of disease are not well understood. Studies of the pre-RA period are useful for understanding the pathogenesis of RA. One way to study pre-RA may be to investigate unaffected first-degree relatives of RA patients, who should share some of the genetic risk and may also share environmental risk factors for RA. Studies on anti-CCP antibodies in unaffected first-degree relatives of RA patients are very few in Bangladesh. No data have been found for the Bangladeshi population regarding pre-clinical autoimmunity, including anti-CCP seropositivity in FDRs of established RA patients prior to the development of RA. So, this cross-sectional study was conducted to see the serum anti-CCP antibody level in unaffected first-degree relatives (FDRs) of RA patients to compare with that of healthy subjects. For this purpose, 25 FDRs of RA patients and another 25 healthy subjects were enrolled as per inclusion and exclusion criteria. In this study mean age of the FDRs of RA patients was 31.36 ± 10.51 years and that of the healthy subjects was 31.56 ± 8.89 years. Minimum age of the study subject was 20 years and the maximum was 55 years in both groups. These results were supported by Smolik et al. (2013)[22] where the mean age of FDRs was 35 ± 13 years and that of healthy control was 33 ± 11 years. In current study males were predominant in both groups of subjects. In our sociocultural condition male attendants are usually predominant in outpatient department and our sampling method was purposive and convenient as well, so males were included more in the study. Taking a large sample size enrolled by a random sampling method could overcome this limitation.

This study revealed that FDRs of RA patients have a significantly higher level of ant-CCP antibodies (within normal range). This finding was in agreement with a study done by Ärlestig et al. (2011)[23]. These finding established that, a positive family history of RA is a risk factor for high titer of anti-CCP antibody in FDRs.

4% of FDRs of RA patients were found to be positive in present study which was comparable to a study done by Kim et al., (2013)[24] on 202 FDRs from Korea and found 5% seropositivity for anti-CCP antibody. Earlier and higher expression of RA-related antibodies, especially anti-CCP antibody, may be related to increased risk for RA in the unaffected FDRs of RA patients.

Barra et al. (2013)[17] worked on a Caucasian population from Southwestern Ontario, Canada and found very high prevalence of anti-CCP antibody in FDRs of RA patient (48%). Another study done by Ärlestig et al. (2011)[23] on a Caucasian population from Sweden revealed 21.7% anti-CCP positivity in FDRs. Kolfenbach et al., (2009)[25] reported anti-CCP positivity in FDRs at a very low rate of 1.7% among the Caucasian population from the US. Another study was done by El-Gabalawy et al. (2009)[26] over a North American native population from central Canada which showed 17% of FDRs to be anti-CCP positive. The rate of positive anti-CCP in this study (4%) was comparable to that in a US study (1.7%)[25], but much lower than that in other studies.

The differences in the prevalence of ACP antibody in FDRs from the various studies may be due to the characteristics of the study subjects, the assays used, or the study design. It has been evident that RA is much more prevalent in some groups (eg, in Native American groups) and much less prevalent in others (eg, in southern Europe)[27]. For some unknown reason, in certain parts of the African continent RA has been never found[28]. This ethnic differences clearly had an effect on different results in different studies.

Conclusion:

In conclusion, this study describes unaffected first-degree relatives (FDRs) of rheumatoid arthritis (RA) patients. It had been found that anti-CCP antibody (anti-CCPA) level was significantly higher (within normal range) in FDRs of RA patient in comparison to healthy subjects, which was even more significant in FDRs of seropositive RA patient. 4% of the FDRs were anti-CCPA positive and none of the healthy subjects was positive for anti-CCPA. Anti-CCPA levels were weakly correlated with age of the FDRs. There was no significant difference in anti-CCPA level between male and female FDRs.

Conflict of Interest: None.

Acknowledgement:

We are grateful to all the teachers and staffs of the Department of Biochemistry, Dhaka Medical College and all doctors, nurses and staffs of the Department of Rheumatology, Banga bandhu Sheikh Mujib Medical University (BSMMU), Dhaka. We are also grateful to all the patients who willingly participated in our research work with their kind consent and cooperations.

References:

1. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO, et al. 2010 rheumatoid arthritis classification criteria: An American College of Rheumatology/European League against rheumatism collaborative initiative. Arthritis &amp Rheumatism. 2010 Aug 10;62(9):2569-81. https://doi.org/10.1002/art.27584 PMid:20872595

2. Henrique da Mota LM, Afonso Cruz B, Viegas Brenol C, Alves Pereira I, Rezende-Fronza LS, Barros Bertolo M, et al. Guidelines for the diagnosis of rheumatoid arthritis. Revista Brasileira de Reumatologia (English Edition). 2013 Mar;53(2):141-57. https://doi.org/10.1016/S2255-5021(13)70019-1

3. Bala K, Kumar N, Rna A, Sharma M, Aggarwal R, Griwan A. Anti-CCP antibody vs rheumatoid factor: A comparison of diagnostic characteristics for rheumatoid arthritis. International Journal of Current Microbiology and Applied Sciences. 2018 Oct 10;7(10):1095-9. https://doi.org/10.20546/ijcmas.2018.710.120

4. Firestein GS, Gabriel SE, McInnes IB, O'Dell JR. Kelley and Firestein's textbook of rheumatology. 10th ed. Philadelphia, PA: Elsevier; 2017;141-57.

5. Ngian GS. Rheumatoid arthritis. Australian Family Physician. 2010 Sept 1;39(9):626-628.

6. Kurkó J, Besenyei T, Laki J, Glant TT, Mikecz K, Szekanecz Z. Genetics of rheumatoid arthritis - a comprehensive review. Clinical Reviews in Allergy & Immunology. 2013 Jan 5;45(2):170-9. https://doi.org/10.1007/s12016-012-8346-7 PMid:23288628 PMCid:PMC3655138

7. Gibofsky A. Overview of epidemiology, pathophysiology, and diagnosis of rheumatoid arthritis. Am J Manag Care. 2012 Dec;18(13 Suppl):S295-302.

8. Deane KD, Holers VM. The Natural History of Rheumatoid Arthritis. Clin Ther. 2019 Jul;41(7):1256-1269. https://doi.org/10.1016/j.clinthera.2019.04.028 PMid:31196652

9. Taylor P, Gartemann J, Hsieh J, Creeden J. A systematic review of serum biomarkers anti-cyclic citrullinated peptide and rheumatoid factor as tests for rheumatoid arthritis. Autoimmune Diseases. 2011;2011:1-18. https://doi.org/10.4061/2011/815038 PMid:21915375 PMCid:PMC3170888

10. Song YW, Kang EH. Autoantibodies in rheumatoid arthritis: rheumatoid factors and anticitrullinated protein antibodies. QJM. 2010 Mar;103(3):139-46. https://doi.org/10.1093/qjmed/hcp165 PMid:19926660 PMCid:PMC2825384

11. Miriovsky BJ, Michaud K, Thiele GM, O'Dell JR, Cannon GW, Kerr G, et al. Anti-CCP antibody and rheumatoid factor concentrations predict greater disease activity in men with rheumatoid arthritis. Annals of the Rheumatic Diseases. 2010 May 3;69(7):1292-7. https://doi.org/10.1136/ard.2009.122739 PMid:20439294 PMCid:PMC3122886

12. Vos I, Van Mol C, Trouw LA, Mahler M, Bakker JA, Van

Offel J, et al. Anti-citrullinated protein antibodies in the diagnosis of rheumatoid arthritis (RA): diagnostic performance of automated anti-CCP-2 and anti-CCP-3 antibodies assays. Clin Rheumatol. 2017 Jul;36(7):1487-1492.
https://doi.org/10.1007/s10067-017-3684-8
PMid:28578492 PMCid:PMC5486477
13. Martinez-Prat L, Nissen MJ, Lamacchia C, Bentow C, Cesana L, Roux-Lombard P, et al. Comparison of Serological Biomarkers in Rheumatoid Arthritis and Their Combination to Improve Diagnostic Performance. Front Immunol. 2018 Jun 6;9:1113.
https://doi.org/10.3389/fimmu.2018.01113
PMid:29928272 PMCid:PMC5997814
14. Puszczewicz M, Iwaszkiewicz C. Role of anti-citrullinated protein antibodies in diagnosis and prognosis of rheumatoid arthritis. Arch Med Sci. 2011 Apr;7(2):189-94.
https://doi.org/10.5114/aoms.2011.22067
PMid:22291756 PMCid:PMC3258718
15. Binesh F, Soleimani Salehabadi H, Behniafard N, Ranginkaman K, Behniafard N. A Comparative Assessment of the Diagnostic Value of Anti-cyclic Citrullinated Peptide Antibodies and Rheumatoid Factor in Rheumatoid Arthritis. Journal of Clinical & Experimental Pathology. 2014;4(2):1-5.
https://doi.org/10.4172/2161-0681.1000158
16. Braschi E, Shojania K, Allan GM. Anti-CCP: a truly helpful rheumatoid arthritis test? Can Fam Physician. 2016 Mar;62(3):234.
PMID: 26975916; PMCID: PMC4984588.
17. Barra L, Scinocca M, Saunders S, Bhayana R, Rohekar S, Racapé M, et al. Anti-citrullinated protein antibodies in unaffected first-degree relatives of rheumatoid arthritis patients. Arthritis Rheum. 2013 Jun;65(6):1439-47.
https://doi.org/10.1002/art.37911
PMid:23450693
18. Niewold TB, Harrison MJ, Paget SA. Anti-CCP antibody testing as a diagnostic and prognostic tool in rheumatoid arthritis. QJM. 2007 Apr;100(4):193-201.
https://doi.org/10.1093/qjmed/hcm015
PMid:17434910
19. Schellekens GA, De Jong BA, Van den Hoogen FH, Van de Putte LB, Van Venrooij WJ. Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies. J Clin Invest. 1998 Jan 1;101(1):273-81.
https://doi.org/10.1172/JCI1316
PMid:9421490 PMCid:PMC508564
20. Nishimura K, Sugiyama D, Kogata Y, Tsuji G, Nakazawa T, Kawano S, et al. Meta-analysis: diagnostic accuracy of anti-cyclic citrullinated peptide antibody and rheumatoid factor for rheumatoid arthritis. Ann Intern Med. 2007 Jun 5;146(11):797-808.
https://doi.org/10.7326/0003-4819-146-11-200706050-00008
PMid:17548411
21. Kokkonen H, Mullazehi M, Berglin E, Hallmans G, Wadell G, Rönnelid J, et al. Antibodies of IgG, IgA and IgM isotypes against cyclic citrullinated peptide precede the development of rheumatoid arthritis. Arthritis Res Ther. 2011 Feb 3;13(1):R13.
https://doi.org/10.1186/ar3237
PMid:21291540 PMCid:PMC3241357
22. Smolik I, Robinson DB, Bernstein CN, El-Gabalawy HS. First-degree relatives of patients with rheumatoid arthritis exhibit high prevalence of joint symptoms. J Rheumatol. 2013 Jun;40(6):818-24.
https://doi.org/10.3899/jrheum.121016
PMid:23504380
23. Ärlestig L, Mullazehi M, Kokkonen H, Rocklöv J, Rönnelid J, Dahlqvist SR. Antibodies against cyclic citrullinated peptides of IGG, IGA and IGM isotype and rheumatoid factor of IGM and IGA isotype are increased in unaffected members of multicase rheumatoid arthritis families from northern Sweden. Annals of the Rheumatic Diseases. 2011 Nov 29;71(6):825-9.
https://doi.org/10.1136/annrheumdis-2011-200668
PMid:22128080 PMCid:PMC3371219
24. Kim SK, Bae J, Lee H, Kim JH, Park SH, Choe JY. Greater prevalence of seropositivity for anti-cyclic citrullinated peptide antibody in unaffected first-degree relatives in multicase rheumatoid arthritis-affected families. Korean J Intern Med. 2013 Jan;28(1):45-53.
https://doi.org/10.3904/kjim.2013.28.1.45
PMid:23345996 PMCid:PMC3543960
25. Kolfenbach JR, Deane KD, Derber LA, O'Donnell C, Weisman MH, Buckner JH, et al. A prospective approach to investigating the natural history of preclinical rheumatoid arthritis (RA) using first-degree relatives of probands with RA. Arthritis Rheum. 2009 Dec 15;61(12):1735-42.
https://doi.org/10.1002/art.24833
PMid:19950324 PMCid:PMC2795101

26. El-Gabalawy HS, Robinson DB, Hart D, Elias B, Markland J, Peschken CA, et al. Immunogenetic risks of anti-cyclical citrullinated peptide antibodies in a North American Native population with rheumatoid arthritis and their first-degree relatives. J Rheumatol. 2009 Jun;36(6):1130-5. Epub 2009 May 1. PMID: 19411392.
https://doi.org/10.3899/jrheum.080855
PMid:19411392
27. Abdel-Kader Martín N, Cardiel MH. Geographical factors in rheumatoid arthritis. International Journal of Clinical Rheumatology. 2011 Jun;6(3):259-61.
https://doi.org/10.2217/ijr.11.21
28. Bajraktari IH, Teuta BÇ, Vjollca SM, Bajraktari H, Saiti V, Krasniqi B, et al. Demographic features of patients with rheumatoid arthritis in kosovo. Med Arch. 2014 Dec;68(6):407-10.
https://doi.org/10.5455/medarh.2014.68.407-410
PMid:25649180 PMCid:PMC4314179