Original Article

Comparison Of Serum Anti-mutated Citrullinated Vimentin Antibody With Anti-cyclic Citrullinated Peptide Antibody As A Diagnostic Marker In Local Pakistani Rheumatoid Arthritis Patients

Bushra Gohar Shah, Hamid Javaid Qureshi and Izaz ur rehman

Objective: To compare the diagnostic value of antibodies against mutated citrullinated vimentin (anti-MCV) and antibodies to cyclic citrullinated peptides (anti-CCP) in patients with rheumatoid arthritis.

Material and Methods: A total of 88 subjects were included in the study, comprising of 58 known patients of rheumatoid arthritis (fulfilling the American College of Rheumatology Criteria).Thirty age and sex matched normal healthy volunteers were included as controls in the study. Sera of all study subjects were tested by ELISA for presence of anti-MCV and anti-CCP antibodies.

Results: The sensitivity and specificity of serum anti CCP antibodies for RA was calculated to be 58.6% and 86.7% respectively. The sensitivity and specificity of serum anti MCV antibodies for RA was calculated to be 34.5% and 70.6% respectively at the manufacturer's cutoff value of 25U/L.

Conclusion: Anti-cyclic citrullinated peptide antibodies have higher sensitivity and specificity for the diagnosis of RA as compared to anti-mutated citrullinated vimentin antibodies.

Key words: Rheumatoid Arthritis (RA), Anti-Mutated Citrullinated Vimentin Antibody (anti-MCV), Anti-Cyclic Citrullinated Peptide Antibody (anti-CCP)

Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease of multifactorial etiology characterized by chronic joint inflammation that often leads to joint destruction.[1] Early diagnosis of rheumatoid arthritis is a challenge for physicians especially for early implementation of treatment with disease modifying drugs.[2] Diagnosis of RA relies chiefly on clinical manifestations of the disease and the presence of several diagnostic markers. The American College of Rheumatology (ACR)[3] criteria of RA are rarely met in early arthritis cases, and rheumatoid factor positivity is present in fewer than 50% of all patients with early RA in the first few months after disease onset. Therefore, additional diagnostic markers with higher sensitivity and specificity for the diagnosis of RA are required. Currently available data suggest that the diagnosis of RA can be made by testing antibodies to citrulline- containing peptides such as anti-perinuclear factor(APF), anti-keratin antibody (AKA), anti-fillagrin antibody and anti-cyclic citrullinated peptides (anti-CCP) antibody. These all belong to the family of anti-citrullinated protein/ peptide antibody (ACPA).[4] All these antibodies recognize the antigenic epitope containing citrulline,[5] which is generated by post-translational modification of naturally occurring amino acid arginine by the activity of enzyme peptidyl arginine deiminase (PAD).[6] Citrullinated peptides have been synthesized as antigens for diagnostic immunoassays.[5] Several assays for detecting anti-citrullinated peptide antibodies (ACPA´s) have been developed employing filaggrin derived peptides (CCP-assay), viral citrullinated peptides (VCP-assay), mutated citrullinated vimentin (MCV-assay).[7] The Anti-MCV assay (ELISA) for the detection of antibodies against citrullinated vimentin uses an antigen with a genetically modified sequence, which is most abundant in patients with rheumatoid arthritis.[7] Anti-CCP antibodies seemed to fulfill the requirements of an ideal marker for diagnosis of early RA, but in recent years, the interest has focused on anti-MCV antibodies because a higher diagnostic value in comparison with anti-CCP antibodies and rheumatoid factor.[7] The aim of this study was to investigate and compare the diagnostic value of antibodies against mutated citrullinated vimentin (anti-MCV) with antibodies to cyclic citrullinated peptides (anti-CCP) in patients with rheumatoid arthritis.

Materials and Methods

Study Design: Cross-sectional analytical study.
Duration and Settings: This study was conducted over a period of one year from January, 2010 to

December, 2010. Subjects were recruited from Fatima Memorial Hospital, Rheumatology Outpatient Department, Lahore. The research work was conducted at the Department of Physiology and Cell Biology of University of Health Sciences, Lahore.

Subjects: A total of 88 subjects were included in the study, comprising of 58 known patients of rheumatoid arthritis (fulfilling the ACR Criteria) diagnosed by the rheumatologist. Thirty age and sex matched normal healthy volunteers were included in the study.

Written informed consent was taken from each study subject. A purposefully designed proforma was used to record data of the subjects including age, gender, disease duration, clinical characteristics and medication used. The venous blood samples were taken and secured in vacutainers. Serum was extracted by centrifugation and stored at -20°C till titer of anti-CCP and anti-MCV antibodies. The data obtained was analyzed by using SPSS version 16.0

Serum anti-MCV antibody levels were determined by ELISA8 using ELISA kit (Cusabio Biotech Co., Ltd, China), with an automated EIA analyzer [Coda, Bio-Rad Laboratories, Hercules, CA, USA].

Serum anti-CCP antibody levels were determined by ELISA8,9 using commercially available ELISA kit (Immco Diagnostics, USA), with an automated EIA analyzer [Coda, Bio-Rad Laboratories, Hercules, CA, USA]. 25U/ml was taken as cut-off value for anti-CCP antibodies.

Results

The study population (n=88), comprised of 58 rheumatoid arthritis patients and 30 normal healthy (age and sex matched) controls. Mean ±SEM age of the RA group was 44±1.2 years and that of the control group was 44.1±1.58 years. In the control group (n=30), 23 were females and 7 were males. In the RA group (n=58), 38 were females and 20 were males.

In RA group, median (IQR) disease duration was 5(4-8) years. Median (IQR) anti-CCP antibodies titer (IU/ml) was 10.8(0.00-340.5). Median (IQR) anti-MCV antibodies titer (IU/ml) was 19.7(14.2-30.06). All the patientswere using methotrexate, while 35 were using steroids. **(Table-1)**

In the RA group (n=58), 34 (58%) were aCCP+ ive and 24(41.4%) were aCCP ive. In the control group (n=30), 26(86.7%) were aCCP-ive and only 4(13.3%) were aCCP +ive. In the RA group (n=58), 20(34.5%) patients were aMCV+ and 38(65.5%) were aMCV ive, at cutoff value of 25U/L. In the control group (n=30), 9 (30%)were aMCV+ive and 21(70%) were aMCV-ive **(Table 2).**

The sensitivity and specificity of serum aCCP antibodies for RA was calculated to be 58.6% and 86.7% respectively. The PPV of serum aCCP antibodies for RA was found to be 0.895 (89.5%) with 95% CI of 0.76-0.96. The NPV of serum aCCP antibodies for RA was 0.52 (52%) with 95% CI of 0.39-0.65. Positive likelihood ratio was 4.39(43.9%) and negative likelihood ratio was 0.48 (48%) **(Table-3).**

The sensitivity and specificity of serum anti-MCV antibodies for RA was calculated to be 34.5% and

Table-1: Characteristics of patients with RA.

Characteristics	Mean±SEM/Median(IQR)
Drug treatment	
Methotrexate (MTX)	58
Steriod	35
Disease duration (years)	5 (4-8)
Serum aCCP ti ter (IU/ml)	10.8 (0.00-340.5)
Serum aMCV ti ter (IU/ml)	19.7 (14.2-30.06)

Table-2: Serum aCCP and aMCV status in the RA and control groups.

Parameters	RA group (n=30)	Controls (n=30)
Serum aCCP +i've	34 (58.5%)	4 (13.3%)
Serum aCCP -i've	24 (41.4%)	26 (86.7%)
Serum aCCP +i've	20 (34.5%)	9 (30%)
Serum aCCP -I've	38 (65.5%)	21 (70%)

Table-3 Diagnostic characteristics of anti-CCP and anti-MCV.

	aCCP	aMCV
Sensitivity	58.6%	34.5%
Specificity	86.7%	70.6%
PPV (95% CI)	89.6% (0.76-0.96)	68.9% (0.51-0.83)
NPV (95% CI)	52% (0.39-0.65)	35.6% (0.25-0.48)
Diagnostic accuracy	68.2%	46.6%
Positive LHR	4.39%	1.15%
Negative LHR	0.48%	0.94%

70.6% respectively at the manufacturer's cutoff value of 25U/L. The PPV of serum anti-MCV antibodies for RA was found to be 68.9% with 95% CI of 0.51-0.83. The NPV of serum anti-MCV antibodies for RA was 35.6% with 95% CI of 0.25-0.48. Positive likelihood ratio (LHR) was 1.15 and negative likelihood ratio was 0.94. The diagnostic accuracy was calculated as 46.6% (table-3). At a cutoff value of 20U/L, the sensitivity improved to 51.7% but the specificity decreased to 56.6%.

For direct comparison of the diagnostic values of anti-MCV and the anti-CCP assays, we performed Receiver operating characteristic (ROC) analysis and its accuracy was measured by area under the curve (AUC). The calculated area under the curve (AUC) for anti-MCV was 0.513. The area under the curve (AUC) for anti-CCP was 0.77 and was considered to be good. **(Figure1 and 2)**

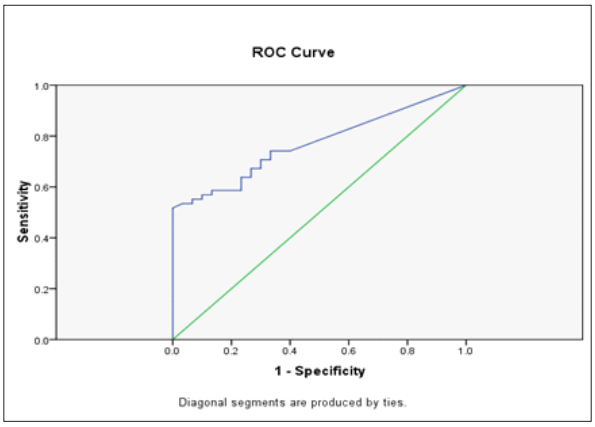

Fig-1: Receiver Operating Characteristic Curve for anti-CCP in the diagnosis of RA.

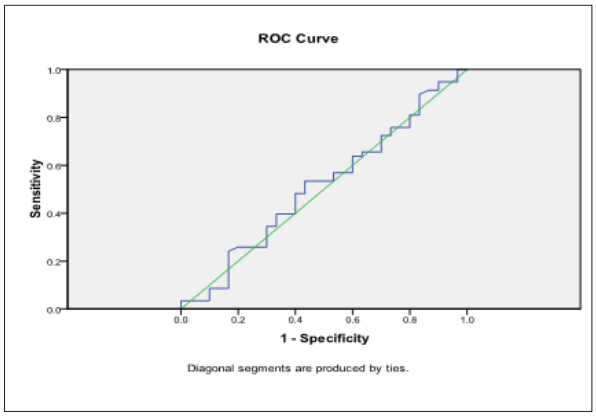

Fig-2: Receiver Operating Characteristic Curve for anti-MCV.

Discussion

The modern trend of RA treatment has been changed to start it as early as possible. Early control of inflammation in RA results in reduced joint damage. It is therefore important to differentiate between RA and other forms of arthritis earlier after the onset of symptoms. Therefore, a specific and sensitive serological marker, which is present very early in the disease, is needed so that the rheumatologist are able to target the use of potentially toxic and expensive drugs to those patients, where the benefits clearly outweighs the risk. Anti-CCP antibodies were the first citrullinated antibodies that were established as diagnostic tools in clinical use. Anti-MCV is also described as an antibody with high specificity for RA. The clinical performance of this marker has not been evaluated thoroughly. We therefore designed this study to evaluate the clinical value of anti-MCV determination as compared to anti-CCP in the diagnosis of RA in local Pakistani subjects.

In the present study RA group (n=58), 20 were positive for anti-MCV with diagnostic sensitivity for RA to be 35%, at a manufacturer cutoff of 25U/L. When value of 20U/L was considered as the cutoff, the sensitivity improved to 52%. Out of 58 RA patients 34 were positive for anti-CCP antibodies. So diagnostic sensitivity of anti-CCP reactivity was 59%. The results of this study show that anti-CCP has a higher sensitivity for the diagnosis of RA as compared to anti-MCV (59% *vs*35%). The results of our study were not in concordance with the studies of most of other authors who reported higher sensitivity of anti-MCV antibody as compared to anti-CCP antibody. Result of study by Bang et al[7](sensitivity 82% vs 72% of anti-CCP), Coenen et al[8] (several assay tested: 74.5% vs 70-77% of anti-CCP), Soos et al[10] (75.6% vs 66.4% of anti-CCP). Similar results have been found in study by Dejaco[11] et al. who reported a slightly higher sensitivity of 70.1% of anti-CCP vs 69.5% for anti-MCV.

Lower sensitivity of anti-MCV for RA in the present study could be attributed to the difference in the anti-MCV assay kits used. Anti-MCV assay kits utilized in all the reported studies was manufactured by the ORGENTEC Diagnostica GmbH, Mainz, Germany, whereas the anti-MCV assay kits utilized in the present study was manufactured by IMMCO Diagnostics, USA. Moreover, the cutoff value recommended by the manufacturer of our kit was higher (25U/L) as compared to 20U/L which was recommended cut-off value by ORGENTEC Diagnostica in all other studies. Further studies with a larger sample size would help in better evaluation of this antibody in our population. Nine out of the 30 normal healthy controls were

positive for anti-CCP. So the specificity of anti-MCV was 70.6% while that of anti-CCP was 87%. Specificity of anti-CCP for RA was higher as compared with anti-MCV (87% vs 70.6%) in our cohort. Our results are comparable with the results of other authors: (Dejaco et al[11] anti-CCP 98.7%, anti-MCV 90.8%; Coenen et al[8] anti-CCP 93-96.4%, anti-MCV 91.5%; Soos et al[10] anti-CCP 98.3%, anti-MCV 91.5%; Wagner et al[12] anti-CCP 97.6%, anti-MCV 81.3%). Only Bang et al[7] found higher specificity of anti-MCV as compared to anti-CCP. Specificity of anti-MCV is lower in our cohort due to more frequent positivity in healthy controls. The analysis of the ROC curve confirms the finding of higher specificity of anti-CCP. These results are in agreement with that reported by Dejaco et al.[11]

Limitation of our study was the small sample size; larger sample would have been better representative of the diagnostic spectrum of these antibodies. One more limitation of the present study was the cross-sectional study design, with lack of follow up data of the controls with positive auto-antibodies. As controls with positive antibodies might end up having the disease, as it has been shown that ACPA's might be present years before the onset of the disease.[13] Prospective study design would help to evaluate the prognostic and diagnostic value of this test.

Conclusion

Anti-cyclic citrullinated peptide antibodies have higher sensitivity and specificity for the diagnosis of RA as compared to anti-mutated citrullinated vimentin antibody. So, it is stated that anti-CCP antibody is a more sensitive and specific marker for the diagnosis of RA as compared to anti-MCV antibody.

Deparment of Physiology,
Avicenna Medical College, Lahore.
www.esculapio.pk

References

1. Lee DM, Weinblatt ME. Rheumatoid Arthritis. Lancet 2001; 358: 903-11.
2. Combe B. Progression in early rheumatoid arthritis. Best Pract Res ClinRheumatol 2009; 23(1): 59-69.
3. Saraux A, Berthelot JM, Chales G, Le Henaff C, Thorel JB, Hoang S, et al. Ability of the American College of Rheumatology 1987 criteria to predict rheumatoid arthritis in patients with early arthritis and classification of these patients two years later. Arthritis Rheum 2001; 44: 2485-91.
4. Zendman AJ, van Venrooij MJ, Pruijn GJ. Use and significance of anti-CCP autoantibodies in rheumatoid arthritis. Rheumatology. 2006; 45(1): 20-5.
5. 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; 101: 273-81.
6. Vossenaar ER, Zendman AJ, van Venrooij WJ, Pruijn GJ. PAD, a growing family of citrullinating enzymes: genes, features and involvement in disease. Bioessays 2003; 25: 1106-18.
7. Bang H, Egerer K, Gauliard A, Berg W, Fredenhagen G, Feist E, et al. Mutation and citrullination modifies vimentin to a novel autoantigen for rheumatoid arthritis. Arthritis & Rheumatism. 2007; 56(8): 2503-11.
8. Coenen D, Verschueren P, Westhovens R, Bossuyt K. Technical and diagnostic performance of 6 assays for the measurement of citrullinated protein/peptide antibodies in the diagnosis of rheumatoid arthritis. Clin Chem. 2007;53(3): 498-504.
9. Bizzaro N, Mazzanti G, Tonutti E, Villalta D, Tozzoli, R.Diagnostic accuracy of the anti-citrulline antibody assay for rheumatoid arthritis. Clin Chem. 2001;47(6): 1089-93.
10. Soos L, Szekanecz Z, Szabo Z, Fekete A, Zeher M, Horvath IF, et al. Clinical evaluation of anti-mutated citrullinatedvimentin by ELISA in rheumatoid arthritis. J Rheumatol 2007; 34(8): 1658-63.
11. Dejaco, C., Klotz, W., Larcher, H., Duftner, C., Schirmer, M. andHerold, M. (. Diagnostic value of antibodies against a modified citrullinatedvimentin in rheumatoid arthritis. Arthritis Res &Ther. 2008),8:R119.
12. Wagner E, Skoumal M, Bayer PM, Klaushofer K. Antibody against mutated citrullinatedvimentin: a new sensitive marker in the diagnosis of rheumatoid arthritis. RheumatolInt 2009; 29: 1315-21.
13. Rantapaa-Dahlqvist S, de Jong BA, Berglin E, Hallmans G, Wadell G, Stenlund H, et al. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. Arthritis Rheum 2003; 48(10): 27419.