ORIGINAL ARTICLE

Diagnostic Usefulness of Anti-Cyclic Citrullinated Peptide and Anti-Mutated Citrullinated Vimentin Antibodies in the Diagnosis of Seronegative Rheumatoid Arthritis Patients

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ABSTRACT

Objective: To determine the usefulness of anti-Cyclic Citrullinated Peptide and anti-Mutated Citrullinated Vimentin antibody in the diagnosis of seronegative rheumatoid arthritis.

Study Design: Descriptive Cross-sectional.

Place and Duration of Study: 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.

Materials and Methods: A total of 58 known patients of rheumatoid arthritis fulfilling the American College of Rheumatology (ACR) Criteria were included in the study. After selection of subjects, written informed consent was obtained. The venous blood sample was taken and secured in vacutainers. Serum was extracted by centrifugation and stored at -20°C till analysis. Sera of all study subjects were tested by ELISA for presence of rheumatoid factor, anti-MCV and anti-CCP antibodies. The data obtained was analyzed by using SPSS version 17. The diagnostic significance of anti-CCP and anti-MCV antibody for the diagnosis of sero-negative rheumatoid arthritis patients was determined.

Results: Serum aCCP antibody was positive in 9 out of 28 RF-ive patients. So the sensitivity of serum aCCP in RF-ive group (n=28) was 32.1%. Serum aMCV antibody was present in 11 out of 28 RF-ive patients. The sensitivity of serum aMCV in RF-ive group was 39.2%.

Conclusion: Anti-CCP and Anti-MCV had a higher sensitivity for the diagnosis of seronegative RA.

KeyWords: *Rheumatoid Arthritis (RA), Rheumatoid Factor (RF), Anti-Mutated CitrullinatedVimentin Antibody (anti-MCV), Anti-Cyclic Citrullinated Peptide Antibody (anti-CCP).*

Introduction

Rheumatoid arthritis (RA) is a common systemic autoimmune disease of multifactorial etiology characterized by chronic inflammation of synovial joints that often leads to joint destruction. Rheumatoid arthritis typically produces symmetrical swelling of peripheral joints of hand and feet, but may affect the large joints as well. Rheumatoid arthritis has a worldwide prevalence of 0.5-3%, being 2-3 times more in women than in men, most frequent during fourth and fifth decade of life. Once established, rheumatoid arthritis is a lifelong progressive disease that produces significant morbidity and premature mortality in many patients.[1]

Many studies have shown that the disease progresses rapidly within first two years of onset and can lead to irreversible erosive joint destruction.[2] The diagnosis of RA depends primarily on history and clinical findings. The gold standard for the classification of RA is the American College of Rheumatology criteria (Arnett, et al., 1988).[3] This criterion was not designed for diagnosing RA, but rather to harmonize research in population and family studies for epidemiologic purposes. But they are ubiquitously used as a diagnostic aid. Patient must satisfy four out of seven criteria to be classified as RA. ACR criteria includes: 1)Morning stiffness of more than one hour for at least six weeks 2)Arthritis and soft tissue swelling of more than 3 of 14 joints/ joint groups,present for at least six weeks. 3) Arthritis of hand joints and wrist, present for at least 6 weeks. 4) Symmetric arthritis, present for atleast 6 weeks. 5) Subcutaneous nodules 6) Rheumatoid factor at a

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level above the 95th percentile. 7) Radiological changes suggestive of joint erosion/ and or periarticular osteopenia.

By the time clinical diagnosis of RA is made, irreversible joint erosions usually have occurred. Ongoing research has shown that early therapeutic intervention results in earlier disease control and consequently less joint damage.[2]

There is no single test or finding that can diagnose rheumatoid arthritis. Rheumatoid factor is the only serological test included in the ACR criteria. However, this auto-antibody lacks specificity. It may be found in patients with other autoimmune diseases and infectious disorders. It may also be present in sera of apparently healthy elderly individuals. Upto 25% of patients with rheumatoid arthritis have negative rheumatoid factor test (seronegative).[4]Therefore, disease-specific auto antibodies are needed for early diagnosis.

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).[5] All these antibodies recognize the antigenic epitope containing citrulline,[6] which is generated by post-translational modification of naturally occurring amino acid arginine by the activity of enzyme peptidyl arginine deiminase (PAD).[7] Citrullinated peptides have been synthesized as antigens for diagnostic immunoassays.[6] 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 citrullinatedvimentin (MCV-assay).[8] The Anti-MCV assay (ELISA) for the detection of antibodies against citrullinatedvimentin uses an antigen with a genetically modified sequence, which is most abundant in patients with rheumatoid arthritis.[8]

Positivity of these markers in the rheumatoid factor negative RA patients would suggest their additional benefit in the early diagnosis of this subgroup of patients, which are often diagnosed and treated late. It is expected that the results of the present study will help the clinicians in early diagnosis and timely management of this debilitating disease.

The aim of this study was to investigate the diagnostic value of antibodies against mutated citrullinatedvimentin (anti-MCV) and antibodies to cyclic citrullinated peptides (anti-CCP) in the diagnosis of seronegative rheumatoid arthritis patients.

Materials and Methods

This descriptive cross sectional study was conducted over a period of one year from January, 2010 to December, 2010. Fifty-eight subjects were recruited from Fatima Memorial Hospital, Rheumatology Outpatient Department, Lahore, by convenient sampling technique. The research work was conducted at the Department of Physiology and Cell Biology of University of Health Sciences, Lahore.

A total of 58 patients attending the Rheumatology outpatient department of Fatima Memorial Hospital, Lahore were recruited in the study. All the patients fulfilled the American College of Rheumatology criteria for RA and were diagnosed by the rheumatologist. The study was approved by the Ethical and Review Committee of University of Health Sciences, Lahore. Informed written consent was taken from each study participant. 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.

Rheumatoid factor titers were determined by ELISA (Highton, et al., 1986) using commercially available ImmuLisa anti-RF antibody IgM ELISA kit (Immco Diagnostics, USA), with an automated EIA analyzer [Coda, Bio-Rad Laboratories, Hercules, CA, USA].Results were interpreted as follows:RF-IgM value of less than 7 IU/ml was considered as negative. RF-IgM value of 7-9 IU/ml was considered as borderline. RF-IgM value of more than 9 IU/ml was considered as positive.

Serum anti-MCV antibody levels were determined by ELISA[9] 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 ELISA[10] 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.

The data was entered into SPSS version 17. Diagnostic sensitivity of anti-CCP and anti-MCV antibodies for the diagnosis of Rheumatoid arthritis in sero-negative patients were calculated by table of 2 x 2. Statistical analysis was done to determine the usefulness of the diagnostic sensitivities of anti-CCP and anti-MCV antibodies. P value of < 0.05 was considered to to be statistically significant.

Results

The study population (n=58), comprised of 58 rheumatoid arthritis patients, out of which 38 were females and 20 were males. Mean ±SEM age of the RA patients was 44±1.2 years. 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). (Table I)

Sub-grouping of RA group was done on the basis of presence or absence of RF, aCCPAb, or aMCV Ab in the sera. Subgroups were named RF+ive group (gp), RF-ivegp, aCCP+ivegp, aCCP-ivegp, aMCV+ivegp, aMCV-ivegp.

RF testing by ELISA technique, was done in a total of 58 RA patients. Out of the RA patients (n=58), 30 (52%) were RF+ive and 28 (48%) were RF-negative.

In the RA group (n=58), 34 (58%) were aCCP+ ive and 24(41.4%) were aCCP –ive. The sensitivity of serum aCCP antibodies for RA was calculated to be 58.6%. Serum aCCP antibody was positive in 9 out of 28 RF-ive patients. So the sensitivity of serum aCCP in RF-ive group (n=28) was 32.1%.

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 (Table I). The sensitivity of serum aMCV antibodies for RA was calculated to be 34.5% at the manufacturer's cutoff value of 25U/L. Serum aMCV antibody was present in 11 out of 28 RF-ive patients. The sensitivity of serum aMCV in RF-ive group was 39.2%.

TableI: Serum RF, aCCP and aMCV status in the RA group (n=58)

Serum RF+ive	Serum RF-ive	Serum RF titer (IU/ml)
30 (52%)	28 (48%)	27.76 (2.51-32.9)
Serum aCCP +ive	Serum aCCP –ive	Serum aCCP titer (IU/ml)
34 (58.5%)	24 (41.4%)	10.8 (0.00-340.5)
Sensitivity of aCCP in RF-ivegp 32.1%		
Serum aMCV +ive	Serum aMCV– ive	Serum aMCV titer(IU/ml)
20 (34.5%)	38 (65.5%)	19.7 (14.2-30.06)
Sensitivity of aMCV in RF-ivegp 39.2%		

Discussion

A close relationship exists between autoimmunity and antibodies; despite this, some patients are persistently negative for disease specific autoantibodies. These conditions have been defined as seronegative autoimmune diseases. Although the prevalence of seronegative autoimmune diseases is low, they may represent a practical problem because they are often difficult and challenging cases for the clinicians/rheumatologist.[11] About 80% of the patients affected by RA are positive for RF, the rest 20-25% being seronegative. A more disease specific marker for RA may help in diagnosing early disease and seronegative RA patients resulting in reduced joint damage. It is therefore important to differentiate between RA and other forms of arthritis early 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. Keeping in view the need of a more sensitive marker, especially in the seronegative cases, the present study aimed to evaluate the sensitivity of anti-CCP and anti-MCV antibodies in local Pakistani seronegative RA subjects.

In seronegative cases of arthritis, the differential diagnosis is not easily established in the early disease course. Especially seronegative patients need the determination of an additional marker for RA besides rheumatoid factor to confirm diagnosis. The high specificity of anti-CCP antibodies has been reported in RF-neative RA patients. In the present study, 9 out of 28 seronegative patients were positive for anti-CCP antibodies. So, the sensitivity of anti-CCP antibodies in the seronegative sub-group was 32.1%.

In a study, conducted by Alexiou, et al., sensitivity in seronegative group was reported to be 34.9%, which is almost comparable to our results.[12] Similarly, Mobini, et al.,[13] found sensitivity in seronegative group to be 33.3%, whereas Vanichapuntu, et al.,[14] found sensitivity value of 20% in the seronegative sub-group whereas Serdaroglu, et al.,[15] reported sensitivity of 14.3% in seronegative group. Thus, anti-CCP antibody serves as a better diagnostic marker in the diagnosis of RA, especially in the seronegative group.

The sensitivity of anti-MCV in the sero-negative RA group was 39.2%, as 11 out of the 28 RF-ive patients were anti-MCV positive. This finding is supported by recent results of other authors, that the higher sensitivity of anti-MCV especially in the sero-negative patients makes it a more valuable marker in the diagnosis of RA.[16] Wagner, et al.,[17] reported sensitivity of anti-MCV in the sero-negative group of 43% and sensitivity of anti-CCP to be 30.8%. In seronegative RA patients, the sensitivity of anti-MCV was superior over anti-CCP. Soos, et al.,[18] reported sensitivity of anti-MCV in the sero-negative group to be 29.4%. Narvaez, et al.[19] documented sensitivity of 23% in their series of sero-negative RA patients.

Limitations of the Study

Small sample size was the limitation of this study. Further studies with greater number of RA patients are recommended.

Conclusion

Both anti-CCP and anti-MCV antibodies can be used for the early diagnosis of sero-negative patients of Rheumatoid Arthritis. Moreover anti-MCV antibody has a significantly higher sensitivity as compared to anti-ccp antibodies for the diagnosis of seronegative RA

Recommendations

Clinicians must be aware of the implications of delayed diagnosis in RA. Keeping in view the cost-effectiveness, this study emphasizes the utility of RF initially for the diagnosis of RA, reserving ACPA's for seronegative RA patients where strong clinical suspicion exists.

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