TO ASSESS THE SENSITIVITY AND SPECIFICITY OF SERUM ANTI-CYCLIC CITRULLINATED PEPTIDE ANTIBODY IN PATIENTS WITH RHEUMATOID ARTHRITIS IN A COMMUNITY HOSPITAL IN LAHORE, PAKISTAN

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ABSTRACT

OBJECTIVES: To compare the sensitivity and specificity of serum anti-Cyclic Citrullinated Peptide antibody (anti-CCP) with serum rheumatoid factor in diagnosing rheumatoid arthritis (RA) in a community hospital in Lahore, Pakistan.

DESIGN: Cross-sectional analytical study.

PLACE & DURATION OF STUDY: Subjects were recruited from Fatima Memorial Hospital, Rheumatology Outpatient Department from January, 2010 to December, 2010. The research work was conducted at Department of Physiology and Cell Biology of University of Health Sciences, Lahore.

SUBJECTS & METHODS: Eighty diagnosed patients of rheumatoid arthritis and thirty normal healthy controls 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. Titers of anti-CCP and rheumatoid factor were determined by ELISA. The data obtained was analyzed by using SPSS version 16.0.

RESULTS: The sensitivity and specificity of serum anti-CCP was found to be 58.6% and 86.7% respectively as compared to 47.5% and 83.3% for serum rheumatoid factor (RF) in diagnosing rheumatoid arthritis. Sensitivity of anti-CCP antibody in RF negative sub-group was 32.1%.

CONCLUSION: Serum anti-CCP antibody is more sensitive and specific marker for diagnosis of rheumatoid arthritis as compared to the rheumatoid factor.

KEY WORDS: Rheumatoid Arthritis, Anti- Cyclic Citrullinated Peptide Antibody (ACCP), Rheumatoid Factor

INTRODUCTION

Rheumatoid arthritis (RA) is a common systemic autoimmune disease of unknown 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. Early diagnosis of rheumatoid arthritis rests mainly on clinical symptoms which are usually mild and nonspecific, and patients usually do not fulfill the American College of Rheumatology (ACR) criteria for the diagnosis.²

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.³

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, detection of disease-specific auto antibodies are needed for early diagnosis.

Other RA associated antibodies which have been described are anti-perinuclear factor (APF), anti-keratin antibody (AKA), anti-filaggrin antibody and anti-cyclic citrullinated peptide antibody. These all belong to the family of anti-citrullinated protein/ peptide antibody (ACPA).⁵ 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 peptidylarginine deiminase (PAD). Several citrullinated proteins proposed as antigens include fibrin, Epstein-Barr virus nuclear antigen, alpha-enolase and vimentin.⁶ Process of citrullination is augmented in inflammatory conditions. Synovial fluid of rheumatoid arthritis patients has shown ACPA and their production by local plasma cells. Citrullinated peptides have been synthesized as antigens for diagnostic immunoassays.⁷ 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).⁶

Research has shown that ACPA have a higher specificity than rheumatoid factor in diagnosing rheumatoid arthritis.^{7,8} Studies have shown that

ACPA's can be detected years before the appearance of first symptoms of RA.⁹ These also help to differentiate rheumatoid arthritis from other types of arthritis, in early phase of the disease.^{10,11} These have also shown to be predictive for a severe course of the disease. Several studies have shown that ACPA positive early rheumatoid arthritis patients develop a more erosive disease than those without these antibodies.¹²

The aim of this study was to access the diagnostic value of serum anti-CCP in local Pakistani RA patients as compared to serum rheumatoid factor in diagnosis of the RA.

PATIENTS AND METHODS

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

A total of 110 subjects, both male & female, between the age of 30-60 years were included in the study, comprising of 80 known patients of rheumatoid arthritis (fulfilling the ACR Criteria) diagnosed by the rheumatologist. Additionally, patients with neoplastic and chronic viral diseases such as hepatitis C were excluded from the study because of the possibility of rheumatoid factor positivity in these diseases. Thirty (30) age and sex matched normal healthy volunteers were recruited over the same period and included in the study. Informed written consent was obtained from all study subjects. The venous blood samples were taken and secured in vacutainers. Serum was extracted by centrifugation and stored at -20°C till analysis. The data obtained was analyzed by using SPSS version 16.0

Presence of rheumatoid factor in the serum was determined by latex agglutination method.¹³ Serum Rheumatoid factor titers were determined

by ELISA¹⁴ using commercially available Immulisa anti-RF antibody IgM antibody ELISA kits (Immco Diagnostics, USA), with an automated EIA analyzer [Coda, Bio-Rad Laboratories, Hercules, CA, USA] according to manufacturer's instructions. RF- IgM value of more than 9 IU/ml was considered as positive. 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.

Serum anti-CCP antibody levels were determined by ELISA¹⁵ 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=110), comprised of 80 rheumatoid arthritis patients and 30 normal healthy (age and sex matched) controls. Mean age $\pm$ SEM of the RA group was 44 $\pm$ 1.2 years and the mean age $\pm$ SEM of the control group was 44.1 $\pm$ 1.58 years. In the control group (n=30), 23 were females and 7 were males. In the RA group (n=80), 69 were females and 11 were males. In RA group (n=80), median (Interquartile range {IQR}) disease duration was 5(4-8) years. Median (IQR) RF titer (IU/ml) was 27.76 (2.51-32.9). Median (IQR) anti-CCP titer (IU/ml) was 10.8(0.00-340.5). All the patients (n=80) were using methotrexate, while 35 were using steroids (Table-1).

Table-1 Characteristics of 80 patients with RA

Characteristics	Mean $\pm$ SEM/ Median(IQR)
Drug treatment	
Methotrexate (MTX)	80
Steroid	35
Disease duration (years)	5(4-8)
Serum RF titer (IU/ml)	27.76 (2.51-32.9)
Serum anti-CCP titer (IU/ml)	10.8 (0.00-340.5)

By Latex agglutination test, out of the RA patients (n=80), 38(47.5%) were RF positive; 42(52.5%) were RF negative and of the controls (n=30), 5(16.6%) were RF positive and 25(83.3%) were RF negative. Further, the RF titers were determined in the RA group (n=80). Out of the 80 RA patients, 49(63.6%) RA patients turned out to be RF positive and 28(36.3%) were RF negative and 3 cases were borderline positive with a titer between 7-9 IU/ml.

Out of 88 subjects, 58 were RA patients and 30 were controls. Out of the RA patients (n=58), 30 were RF+ive and 28 were RF-negative. In the RA group (n=58), 34 (58%) were anti-CCP+ ive and 24(41.4%) were anti-CCP -ive. In the control group (n=30), 26(86.7%) were anti-CCP-ive and only 4(13.3%) were anti-CCP +ive (Table-2). Out of the 28 RF-ive patients, 9 were anti-CCP +ive.

Table-2 Serum RF and anti-CCP status in the RA and control groups

Parameters	RA group	Controls
RF positive(by	38 (47.5%)	5 (16.6%)
RF negative(by	42 (52.5%)	25 (83.3%)
	RA group	Controls
Serum anti-CCP	34 (58.5%)	4 (13.3%)
Serum anti-CCP -	24 (41.4%)	26 (86.7%)

Sensitivity and specificity of serum RF by agglutination method for RA was calculated to be 47.5% and 83.3% respectively. The sensitivity of RF by ELISA method for RA was calculated excluding borderline cases and was 63.6%.The sensitivity and specificity of serum anti-CCP antibodies for RA was calculated to be 58.6% and 86.7% respectively. The positive predictive value (PPV), negative predictive value (NPV) with a 95% CI, likelihood ratio (LHR) and diagnostic accuracy of serum rheumatoid and anti-CCP antibodies for diagnosis of RA are presented in Table-3. The sensitivity of anti-CCP antibodies in the seronegative sub-group was 32.1%.

Table-3 Diagnostic characteristics of serum RF and anti-CCP for the diagnosis of rheumatoid arthritis

	Rheumatoid factor	Anti-CCP
Sensitivity	47.5%	58.6%
Specificity	83.3%	86.7%
PPV (95% CI)	88.3% (0.77-0.96)	89.5%(0.76-0.96)
NPV(95% CI)	37.3% (0.28-0.49)	52% (0.39-0.65)
Diagnostic accuracy	57.2%	68.2%
Positive LHR	2.85	4.39
Negative LHR	0.88	0.48

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 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 and specific marker, present study aimed to find out the sensitivity and specificity of anti-CCP antibodies in local Pakistani RA subjects.

In the present study, it was found that 34 out of the 58 RA patients were positive for anti-CCP antibodies, whereas 4 of the controls had a positive serum for anti-CCP antibodies. Diagnostic sensitivity of anti-CCP reactivity was 59%, which is comparable with other studies conducted indifferent Asian countries. Samanci, et al. (2005) reported sensitivity of 49% in a cohort of 76 Turkish RA patients¹⁶. Whereas Mobini, et al. (2009) found a lower sensitivity of 50.9% in a cohort of 55 Irani RA patients¹⁷. Whereas Vanichapuntu, et al. (2010) detected a comparable sensitivity of 58.4% in 125 Thai RA patients¹⁸. Study conducted on a group of 51

Malaya RA patients by Sockalingam. (2009) reported a higher sensitivity value of 80.4%¹⁹. The different sensitivities of anti-CCP antibodies in various RA cohorts can be explained on the basis of the fact that anti-CCP antibodies are directed against different epitopes in citrulline-containing molecule. Discrepancy in sensitivity might reflect different cutoff levels, racial and genetic and environmental backgrounds, as well as the differences of the used antigens and detection techniques.

In the present study, 4 of the 30 controls were positive for anti-CCP antibodies with a specificity of 87%. It was slightly lower than that reported in other studies, where the specificity of anti-CCP in RA is more than 90% in almost all reports. But the titers of all the positive controls in the present study were in the lower range with the titers being 27, 30, 38 & 44 IU/ml. In a study on 136 Irani RA patients with disease duration of less than two years, the sensitivity and specificity of anti-CCP were 62.5% and 89.1%, respectively (Sharif, et al., 2007)²⁰. In another study from Iran by Heidari, et al. (2009), specificity of 87.5% was reported, when 208 non-RA patients were taken as the control group²¹.

In this study, nine out of 28 seronegative patients were positive for anti-CCP antibodies. So, the sensitivity in seronegative sub-group was 32.1%. In the Greek study, conducted by Alexiou, et al. (2007), sensitivity in seronegative group was 34.9%, which is comparable to our results²². Thus, anti-CCP antibody testing may be useful in patients with suspected RA who have had a negative RF test.

CONCLUSION

This study concludes that serum anti-CCP antibody is a more sensitive and specific marker as compared to RF for the diagnosis of RA in local Pakistani RA patients. Furthermore, anti-CCP antibody testing may be useful in patients with suspected RA who have had a negative RF test.

ACKNOWLEDGEMENTS

This research was funded by the Higher Education Commission of Pakistan.

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