ORIGINAL ARTICLE

COMPARISON OF ANTI-CYCLIC CITRULLINATED PEPTIDE ANTIBOBY AND RHEUMATOID FACTOR AS A DIAGNOSTIC MARKER IN RHEUMATIOD ARTHRITIS

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ABSTRACT

Rheumatoid arthritis (RA) is a common chronic autoimmune disease affecting many systems, predominantly synovial joints. Early diagnosis and instillation of disease-modifying anti-rheumatic drugs treatment is mandatory to prevent its progression to joint destruction and permanent disability. To achieve an early diagnostic accuracy clinical application of American College of Rheumatology (ACR 1987) criteria along with (CRP) and (RF) have been practiced for a long period. Recently test for the antibodies directed to citrullinated peptides (anti-CCPA) has been widely accepted as a biomarker for diagnosis of RA.

This cross sectional study for the measurement of anti-CCPA and RF was conducted on 100 patients with chronic arthritis attending in rheumatology clinic in DMCH, Dhaka during the period July 2009 to August 2010. The specificity and sensitivity of anti-CCPA and RF were 90.24%, 100% and 73.17%, 77.78% respectively. Positive predictive value and negative predictive value of anti-CCPA and RF were 100%, 69.23% and 93.75%, 38.89%. The accuracy, positive likelihood ratio and negative likelihood ratio of anti- CCPA were 92%, 1 and 0.01. On the other hand these results of RF were 74%, 3.29 and 0.34. All the mentioned performance tests of this study support anti-CCPA to be a better diagnostic biomarker than RF in rheumatoid arthritis.

Thereby measurement of anti-CCPA by itself is useful and widely accepted as indispensible tools for diagnosis of rheumatoid arthritis. However use of anti-CCPA and RF might be more strengthened than either method alone.

Key words: Rheumatoid arthritis, anti-CCPA, RF, Diagnosis, Sensitivity, Specificity

Introduction

Rheumatoid arthritis (RA) is one of the most common progressive autoimmune systemic disease marked by inflammation of synovial joints. If remains untreated, it causes permanent destruction of cartilage, ligaments and bones leading to joint deformity and irreversible dysfunction along with systemic manifestations[1,2]. RA is more common in female and mostly diagnosed in individuals aged 40 to 60 years[3]. RA severely affects the quality of life of a patient and also has a major economical consequence on the society. Therefore every

attempt should be made to prevent the erosive process to occur. Currently the classification of RA mainly is based on ACR criteria[4]. Although these criteria were formulated many years back and rely on clinical parameters, it is also evident that the ACR criteria are not suitable for early diagnosis of RA[5]. On the other hand early diagnosis and prompt application of standard protocol of management are essential to break the progressive destruction of involved joints. For facilitating the clinical diagnosis many laboratory investigations like ESR, CRP, RF and radiological findings have been practiced for a long period. Although RF is included as a diagnostic criterion in ACR, it is not highly sensitive or specific for RA[6]. Approximately 3% of general population has low level of RF which increases with age up to 20%. It is also present in many other chronic infections and inflammatory disorders[7].

The specific autoantibody for RA directed to citrullinated antigen known as anti-CCPA was suggested by discovery of this antibody in the involved joints in RA[8]. This anti-CCPA has clinical utility for establishing early diagnosis of RA as they have excellent sensitivity and specificity for RA. For this reason anti-CCPA is considered as a novel biomarker of erosive arthritis[9]. The ideal diagnostic biomarker of early RA should fulfill at least four requirements namely i) good sensitivity ii) good specificity iii) early presence and iv) prognostic abilities. Considering these requirements the anti-CCPA is considered as a good diagnostic marker for RA. Therefore, anti-CCPA level was added to the '2010 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) diagnostic criteria' for RA[10].

A good number of patients having chronic arthritis come to the government hospital like DMCH for treatment and many of them are suffering from rheumatoid arthritis. Proper diagnosis is mandatory to prevent the irreversible changes which usually results if diagnosis becomes late and the disease process remains untreated. Recently detection of serum anti-CCPA in rheumatoid arthritis has been established in our country even in some government hospitals. On this background an observational cross sectional study was carried out to find out the sensitivity, specificity, positive predictive value and negative predictive value, accuracy, positive likelihood ratio (LR+), negative likelihood ratio (LR-) of anti-CCPA and compared with those of RF in rheumatoid arthritis.

Materials and Methods

An observational cross-sectional study for detection of serum anti-CCPA as a biomarker for the diagnosis of RA was carried out in the department of biochemistry of DMCH during the period of July 2009 - December 2010. A total number of hundred patients (age ranges 21-70 years) clinically having chronic arthritis attending in the rheumatology clinic of the department of internal and physical medicine of DMCH were recruited according to the selection criteria. The gouty arthritis, traumatic arthritis and psoriatic arthritis patients were excluded. The baseline study of each patient was evaluated by detailed history, physical examinations, radiology of affected joints and relevant laboratory investigations like CBC, ESR, CRP. These all suspected patients were categorized into two groups on the basis of ACR criteria-patients who fulfill the ACR criteria and patients who did not fulfill the ACR criteria.

Serum anti-CCPA and RF were estimated in both groups. Informed written consent was taken from all patients. Ethical clearance was taken

from research ethical committee of DMCH. Maintaining all aseptic precautions 5 mL of venous blood was collected from each patient. Serum was separated from clot within three hours from the time of collection. The sample was centrifuged at 4000 rmp for 10 minutes and stored at -200C up to 7 days before test. Serum was used for the estimation of anti-CCPA and RF by ELISA and Latex nephlometry respectively.

Data were collected in a pre-designed sheet which included particulars of the patients, history, results of clinical examinations and relevant investigations. Statistical analysis was performed using SPSS (Statistical Package for Social Science) software for windows version 12.0 (SPSS inc., Chicago, Illinois, USA). Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV), accuracy, positive likelihood ratio (LR+), negative likelihood ratio (LR-) of anti-CCPA and comparative performance of RF in rheumatoid arthritis patients were determined by kappa agreement test.

Results

Out of total 100 patients of chronic arthritis 82 patients were RA +ve and 18 patients were RA –ve on the basis of ACR criteria. The age range of the RA +ve patients was 21-70 years and RA -ve patients was 21-56 years. Among RA +ve patients 28(34.1%) were within the age group 41-50 years, 22(26.8%) patients were within 31-40 years of age. The RA –ve (18) patients were distributed more or less equally in different age groups.

Table I: Distribution of study population according to age (N=100)

Age (years)	RA+ve (n=82)		RA-ve (n=18)	
	Frequency	Percentage	Frequency	Percentage
21–30	14	17.1	6	33.3
31–40	22	26.8	4	22.2
41–50	28	34.1	4	22.2
51–60	10	12.2	4	22.2
61–70	8	9.8	0	0
Range	21.00-70.00		21.00-56.00	

Table II shows that among the study subjects 78 were female and only 22 were male. Among the female patients 68 were RA +ve and 14 male patients were RA +ve. Table III shows the number of joints involved. Table IV shows comparison between serum anti-CCPA +ve and serum anti-CCPA -ve cases among serum RF positive and negative cases.

Table II: Distribution of subjects according to sex and test results

Sex	RA +ve (n=82)		RA -ve (n=18)	
	Frequency	Percentage	Frequency	Percentage
Male (n=22)	14	55.6	8	44.4
Female (n=78)	68	82.9	10	17.1

Table III: Number of joints involved

Number of Joints	RA +ve (n=82)		RA -ve (n=18)	
	Frequency	Percentage	Frequency	Percentage
0–4	0	0	14	77.8
5–8	62	75.6	2	11.1
≥9	20	24.4	2	11.1

Table IV: Comparison between serum anti-CCPA +ve and serum anti-CCPA -ve cases among serum RF positive and negative cases

Serum RF	Anti-CCPA positive (n=74) Frequency (%)	Anti-CCPA negative (n=26) Frequency (%)	Kappa (k) value
Positive	60 (81.1)	4 (15.4)	0.584
Negative	14 (18.9)	22 (84.6)	

Table V shows performance of serum anti-CCPA test in diagnosing rheumatoid arthritis and Table VI shows Performance of serum RF test in diagnosing rheumatoid arthritis. The performance of serum anti-CCPA test in the diagnosis of rheumatoid arthritis is expressed as sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy, positive likelihood ratio (LR+) and negative likelihood ratio (LR-). It indicates high diagnostic efficacy of anti-CCPA than that of RF in rheumatoid arthritis (Table VII).

Table V: Performance of serum anti-CCPA test in diagnosing rheumatoid arthritis

Serum anti-CCPA findings (n=100)			Grouping based on ACR criteria			
			Positive		Negative	
	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
Positive	74	74.0	74	100.0	0	0
Negative	26	26.0	8	30.8	18	69.2
Total	100	100	82	82.0	18	18.0

Table VI: Performance of serum RF test in diagnosing rheumatoid arthritis

Serum RF finding (n=100)			Grouping based on ACR criteria			
			Positive		Negative	
Frequency		Percentage	Frequency	Percentage	Frequency	Percentage
Positive	64	64.0	60	93.8	4	6.3
Negative	36	36.0	22	61.1	14	38.90
Total	100	100	82	(82.0)	18	(18.0)

Table VII: Diagnostic efficacy of serum anti-CCPA and rheumatoid factor (RF)

Diagnostic points	anti-CCPA (%)	RF (%)
Sensitivity	90.24	73.17
Specificity	100	77.78
Positive predictive value (PPV)	100	93.75
Negative predictive value (NPV)	69.23	38.89
Accuracy	92	74
Positive likelihood ratio (LR^+)	1	3.29
Negative likelihood ratio (LR^-)	0.10	0.34

Discussion

The diagnosis of rheumatoid arthritis is primarily based on clinical symptoms. In this study, clinical diagnosis is done on the basis of ACR criteria supported by serological markers. RF is the most common diagnostic marker which has been used for the last 4 decades. This RF is non-specific and present in other inflammatory conditions. In the recent past anti-CCPA have been practiced as a most sensitive and specific biomarker for early diagnosis of RA. Here the diagnostic performance of these two commonly used serological tests (anti-CCPA and RF) has been studied and statistically compared.

Anti-CCPA is more useful than RF in the diagnosis of rheumatoid arthritis. In early stage of rheumatoid arthritis anti-CCP antibodies are detected in significant number of cases and long-term studies have shown its association with its severity[11]. In this study, we have assessed the relative role of anti-CCPA and RF in the field of diagnosis of rheumatoid arthritis.

The study shows that serum anti-CCPA has a sensitivity of 90.24% where as the sensitivity of serum RF is 73.17% in RA patients. Van Boekel et al reported the sensitivity of anti-CCPA to be 91%, whereas RF was 75% in RA patients. Similar types of findings were observed in the studies of many researchers[12-14]. But Stropuviene et al[15] carried out a study on RA patients and found the sensitivity of anti-CCPA and RF to be 79% and 93%. In our study we found that the specificity of anti-CCPA and RF are 100% and 77.78% respectively. Schellekens et al and Quill et al, reported the specificity of anti-CCPA to be 94% and 98% and that of RF was 64% and 78% respectively[13,16]. Many other studies showed the similar findings regarding the specificity of these two antibodies for RA[17-20]. The sensitivity and specificity of our study is a bit higher than other studies because most of our patients attended in Rheumatology clinic as established and late cases.

In the present study the PPV of serum anti-CCPA is 100% and that of serum RF is 93.75%, NPV of anti-CCPA and RF are 69.23% and 38.89% respectively. Accuracy of anti-CCPA is 92% whereas that of RF is 74%. The LR+ of anti-CCPA and RF are 1 and 3.29. The LR- of anti-CCPA and RF are 0.10 and 0.34 respectively. These findings are supported by the study carried by Nishimura et al[21].

The Kappa (Agreement) test was done for comparison between serum anti-CCPA and RF in rheumatoid arthritis which revealed fair agreement (K=0.584). All the mentioned performance tests of this study support anti-CCPA to be a better diagnostic marker than RF.

However, in the present study the performance of anti-CCPA and RF as an early diagnostic marker could not be evaluated due to unavailability of RA patients in the early stage in our hospital. But other researchers in their studies had shown anti-CCPA as an early diagnostic marker than RF[19]. As the RA is an autoimmune, progressive and morbid disease, early appropriate diagnosis and proper treatment can reduce the complications and morbidity. So anti-CCPA may be estimated in all suspected cases of rheumatoid arthritis for appropriate diagnosis.

Conclusion

Early diagnosis and prompt aggressive management of RA reduce the progressive destruction of involved joints and thereby prevent the irreversible joint damage. Our study shows the superiority of anti-CCPA over RF for the diagnosis of RA. So anti-CCPA may be done in all suspected cases of RA for establishment of correct diagnosis.

Limitation

This study failed to evaluate anti-CCPA as an early diagnostic marker of RA because of majority of our study patients attended to our hospital in late stage of disease. This study could not show prognostic value of anti-CCPA in the management of RA as because after starting of treatment this test is not done during follow up.

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