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RESEARCH ARTICLE

EVALUATION OF ANTI-CCP FOR EARLY DIAGNOSIS AND ASSESSMENT OF DISEASE SEVERITY IN RHEUMATOID ARTHRITIS PATIENTS

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ABSTRACT

Rheumatoid Arthritis is a severe, progressive, systemic inflammatory disease of unknown etiology characterized by chronic and erosive polyarthritis by abnormal growth of synovial tissue or pannus and causes irreversible joint disability. The diagnosis of RA, particularly early in the course of disease is empirical and imprecise. A study was carried out on 100 patients who were presented with polyarthritis within one year of presentation. The patients were divided into two groups. 50 patients who fulfilled the ARA criteria for RA were included in Group A. Again 50 patients who had polyarthritis but not fulfilled the ARA criteria were included in Group B. The patients were evaluated clinically and investigated. The anti CCP antibody was analyzed in relation with its sensitivity and specificity and also with different activity markers of RA. It was found that the specificity and sensitivity of Anti CCP was 86% and 64% respectively in early presentation of RA. The activity marker of RA were significantly correlated (DAS 28 score r=0.912; CRP, r=0.323; and VAS, r=0.382) with the increasing titre of Anti CCP ($p<0.01$). ESR were not correlated with the increasing titre of anti-CCP levels (r=0.016, $p>0.05$). Anti – CCP has high specificity and moderate sensitivity in the early presentation of RA. So it can be used as a reliable serological marker for early diagnosis of RA. The increasing titre also significantly correlated with increasing disease activity markers. So anti-CCP can be used as a reliable serological marker for early diagnosis and assessment of disease severity.

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INTRODUCTION

Rheumatoid Arthritis is a severe, progressive, systemic inflammatory disease of unknown etiology characterized by chronic and erosive polyarthritis by abnormal growth of synovial tissue or pannus and causes irreversible joint disability.

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The diagnosis of RA, particularly early in the course of disease is empirical and imprecise. RA treatment may be efficient if the treatment starts early. At the same time an early and accurate diagnosis may protect other patients who do not have RA, from aggressive therapies with potential toxicity (O'Dell, 2005). Although rheumatoid factor (RF) remains one of the American College of Rheumatology (ACR) classification criteria for RA, its value as diagnostic tool is suboptimal, due to its lack of specificity (Bizzaro, 2001 and Schellekens, 2003).

In 1998, Schllekens et al. showed that citrullin is an essential constituent of antigenic determinant recognized by the RA specific autoantibodies (Schellekens et al. 1998). This discovery led to the development of anti- cyclic citrullinated peptide antibody (anti – CCP by ELISA method) test to measure auto-antibodies recognizing citrullinated antigens as a diagnostic test of RA (Schellekens, 2003). This test for anti-CCP antibodies was made commercially available, and was previously known as the anti-CCP1 assay. A second generation assay was devised by screening a large library of citrulline-containing peptides with RA sera to identify the epitopes with the highest yield. This assay is now known as the anti-CCP2 assay, and has slightly better performance characteristic than anti- CCP1. Anti –CCP 2 is currently the widely used anti-citrullinated peptide assay. The third generation peptide used in this ELISA, CCP3 was developed by testing a large number of RA and control patients on a variety of citrullinated peptides. By ELISA method, CCP3 shows approximately 5% greater sensitivity at detecting RA patients than the second generation CCP2 while maintaining a very high specificity. Additional improvement in the CCP3 ELISA include color coded break way wells and the ability to use either serum or plasma patient sample. Although several studies have assessed the anti- CCP reactivity in RA, for many of them a significant number of the control sera was derived from the normal population and showed high specificity (88- 98%) low sensitivity (64-89%) for RA (Lee, 2003). Rheumatoid factor sensitivity ranges from 59% to 79% and specificity from 80% to 84% in the same group.

In the synovium the citrulline act as an antigenic stimulant to induce anti citrullinated protein antibodies (ACPA) locally produced by plasma cell (Van Venrooij, 2000). Patients with RA show considerable variability in disease activity, which can be difficult to predict at the onset of disease. Anti-CCP antibody has proven useful in identifying those patients who are likely to have clinically significant disease activity. In a study, anti –CCP assays were done on sera from 242 RA patients who were followed for 3 years. The patients were treated at the physician's discretion, and the physicians were blinded to the patient's anti- CCP status. Anti- CCP antibodies were positively correlated with higher erythrocyte sedimentation rate (ESR), C- reactive protein (CRP), swollen joint count and worse physician global assessment ratings. Presence of rheumatoid factor was positively correlated with increased ESR and CRP but there was no association with other disease activity markers. So Anti-CCP could be regarded as a reliable serological marker in early RA. It can be used as a diagnostic test for early diagnosis and assessment of disease severity in RA.

MATERIALS AND METHODS

A Cross sectional descriptive study was done at Medicine Department, Chittagong Medical College Hospital (CMCH), Chittagong, Bangladesh from May 2010 to April 2011. Patients who were admitted in Medicine wards, attended in the Outpatient Department of Medicine and the Rheumatology Clinic in CMCH.

Classification criteria of RA (2010 ACR-EULAR criteria)

Criteria		Score
Joint involvement	1 large joint (shoulder, elbow, hip, knee, ankle)	0
	2–10 large joints	1
	1–3 small joints (MCP, PIP, Thumb IP, MTP, wrists)	2
	4–10 small joints	3
	>10 joints (at least 1 small joint)	5
Serology	Negative RF and negative ACPA	0
	Low-positive RF or low-positive anti-CCP antibodies (<3 times ULN)	2
	High-positive RF or high-positive anti-CCP antibodies (>3 times ULN)	3
Acute-phase reactants	Normal CRP and normal ESR	0
	Abnormal CRP or abnormal ESR	1
Duration of symptoms	<6 weeks	0
	>6 weeks	1

Early in the disease process, RA is often difficult to distinguish from other types of inflammatory arthritis and systemic inflammatory conditions as initial presentations may be similar. Several studies have examined the utility of anti- CCP antibody testing indistinguishing RA from other inflammatory diseases, by studying cohorts of patients who presented with non-specific early inflammatory arthritis. In one such study, 524 patients with early undifferentiated arthritis of <2 years duration had anti- CCP antibody testing at inception and were followed longitudinally for 2 years. After 2 years 60% had self-limited inflammatory arthritis, 16% had persistent non-erosive arthritis and 24% had persistent erosive arthritis. Anti-CCP positivity conferred an odds ratio of 4.58 for persistent vs. self- limited arthritis, as well as an odds ratio of 4.58 for erosive vs, non erosive disease. Rheumatoid factor conferred an odds ratio of 2.99 for persistent vs self- limited arthritis, and an odds ratio of 2.99 for erosive vs non- erosive disease. The pathogenesis of anti-CCP antibodies in the Rheumatoid arthritis patients has been shown to be attributable to the body's humoral response to citrulline.

Approval was taken from the ethical committee of Chittagong Medical College, Chittagong. Patients with polyarthritis having duration of symptoms more than 6 weeks and less than one year irrespective of sex, treatment and presence of deformity or extraarticular manifestations and who fulfilled the inclusion criteria. Inclusion criteria are patients with polyarthritis, duration of polyarthritis more than 6 weeks and than one year irrespective of sex, treatment, and presence of deformity and extraarticular manifestations .Exclusion criteria are patient Age <18 years, patients unwilling to sign informed written consent, inactive disease, and another identifiable clinical syndrome of arthritis – eg septic arthritis, and TB arthritis. In our study, 100 patients were recruited. Patients were grouped in two categories. Group A: Patients with polyarthritis and who had RA (ARA criteria fulfilled) and group B: Patients with polyarthritis but not fulfilled the ARA criteria. The study was focused on the following parameters:

- RA clinical and biological activity: DAS -28(Disease activity score) using 4 variables (Number of tender

joints, number of swollen joints, ESR and assessment of disease activity), C reactive protein.
- RA factor with titre
- Anti- CCP and its titre.

After getting the informed written consent socio-demographic data were collected in a pretested data sheet, primary clinical evaluation was done and physical examinations were carried out. Patients who fulfilled the ARA criteria for RA were included in Group A and patients who had polyarthritis (at least 5 more tender or swollen joints) but had insufficient criteria for RA were included in Group B. Disease activity was assessed by DAS 28 score. ARA criteria and DAS 28 and VAS were evaluated in presence of an independent observer at least Assistant Professor level. To analyze the Anti CCP and other serological and hematogical investigations 10 ml of venous blood was collected with all aseptic precaution from the medial cubital vein of the patients after his/her consent. The blood was kept in a 20 ml vial mixed with anticoagulant and was sent for analysis. Kits used for analysis of anti-CCP was QUANTA Lite CCP3 IgG ELISA (Appendix-IV).

DAS 28 score: The DAS 28 score is a validated index of RA disease activity. The DAS 28 is continuous, has a Gaussian distribution with a theoretical range from 0 – 10. DAS 28 values <3.2 are regarded as representing low disease activity and DAS 28 values >5.1 as representing high disease activity. The DAS 28 is calculated using the results of the 28 tender joint count (TJC 28), swollen joint count (SJC 28) and ESR:

DAS 28 = [0.56√(TJC28) + 0.28√(SJC28) + 0.70In(ESR)] 1.08 + 0.16

In our study total test positive and test negative cases of Anti CCP among RA patients(Group A) and non RA patients (Group B) were calculated and Sensitivity and Specificity of Anti CCP were calculated by the following formula.

	Affected	Unaffected
Positive test	True +ve (a)	False +ve (b)
Negative test	False -ve (c)	True -ve (d)

Sensitivity (%) = [a/(a + c)] × 100

Specificity (%) = [d/(b + d)] × 100

Data analysis

Data was processed and analyzed by using computer based software SPSS- 18(Chicago, Illinois). Statistical method was applied were Chi- square test, Pearson's correlation coefficient and specificity and sensitivity. Significance in age group, sex, extra-articular manifestations and anti CCP positivity in both groups were calculated by Chi-square test, sensitivity and specificity of anti CCP were calculated after obtaining disease and test positive cases of RA and disease and test negative cases of RA. Correlation of anti CCP with DAS 28 score, CRP, ESR and were done by Pearson's correlation test. P values were considered as statistically significant when it was less than 0.05 and for correlation coefficient of r, it was considered weak correlation when r was 0.2-0. 4, moderate when it was 0.4- 0.7 and strong when it was 0.7-1.

RESULTS

Sex of the patients: Both male and female patients were studied. Out of 100 patients, 61 were female and 39 were male patients. The female-male ratio was1.56:1. In group A, 40(80%) were female and 10(20%) were male. In group B, 21(42%) were female and 29(58%) were male.

Table. 1. Distribution of the patients according to sex (n=100)

		Group		P value*
		Group-A	Group-B	
Sex	Male	10(20%)	29(58%)	<0.001
	Female	40(80%)	21(42%)	
Total		50	50	

* Chi square test

Clinical profile of the patients: Regarding analysis of clinical profile fever, tender and swollen joints were common findings in both groups. Cutaneous manifestations, deformity and rheumatoid nodule were some less common findings. No patients with normal CRP was found in Group A whereas 7(14%) patients was found normal CRP in group B. Anti CCP and RA factor was found positive in 32(64%) and 28(56%) respectively in Group A and 7(14%) and 21(42%) respectively in Group B.

Table 2. Distribution of patients according to clinical profile (n=100)

Clinical Profiles	Group – A N (%)	Group- B N (%)
Fever	35(70%)	31 (62%)
Swollen joint	49 (98%)	28 (56%)
Tender joint	50 (100%)	39 (78%)
Cutaneous manifestations	6(12%)	7(14%)
Deformity present	4(8%)	5(10%)
Rheumatoid nodule	9(18%)	1(2%)
Anti CCP +ve	32(64%)	7(14%)
RA factor +Ve	28(56%)	21(42%)
CRP normal*	0	7(14%)
ESR (Median and Range)	79(53-112)	78(47-109)
Positive family history	7(14%)	3(6%)
On DMARD	21(42%)	16(32%)

*<6 u/ml was considered normal CRP

Diagnosis of the patients: In Group A, 50 patients were RA and in the Group B, SLE were 13 (26%), Ankylosing spondylosis were 14 (28%), undifferentiated arthritis were 14 (28%), dermatomyositis were 4 (8%), gout were 1(2%), mixed connective tissue disease (MCTD) were 1 (2%), psoriatic arthritis were 2 (4%) and enteropathic arthritis were1 (2%).

Table 3. Distribution of the patients according to diagnosis (n=100)

Diagnosis	Group		Total
	Group-A	Group-B	
RA	50	0	50
SLE	0	13(26%)	13
Ankylosing spondylosis	0	14(28%)	14
Undifferentiated arthritis	0	14(28%)	14
Dermatomyositis	0	4(8%)	4
Gout	0	1(2%)	1
MCTD*	0	1(2%)	1
Psoriatic arthritis	0	2(4%)	2
Enteropathic arthritis	0	1(2%)	1
Total	50	50	100

* Mixed connective tissue disease

Anti - CCP antibody status of the patients: In group A 32(64%) of patients were Anti CCP positive and 18(36%) patients were test negative. In group B 7(14%) patients were test positive and 43(86%) were test negative. Table 4: Distribution of Anti CCP Antibody in different groups (n=100)

Table 4. Distribution of Anti CCP Antibody in different groups (n=100)

Anti-CCP Ab		Group		P value
		Group-A	Group-B	
	Positive	32(64%)	7(14%)	
	Negative	18(36%)	43(86%)	<0.001*
Total		50	50	

* Results are statistically significant (Chi square test)

Specificity and sensitivity of Anti-CCP and RA test: Sensitivity of the Anti-CCP in RA was 64% and specificity was 86% whereas it was 56% and 58% for RA test respectively. ARA criteria were considered gold standard in evaluating the validity of RA and anti-CCP within one year of presentation.

Table 5. Validity test of anti CCP and RA test (n=100)

Validity	RA test	Anti - CCP
True Positive	28	32
True Negative	29	43
False Positive	21	7
False Negative	22	18
Sensitivity	56 %	64%
Specificity	58 %	86 %
PPV*	57.14%	82.05%
NPV**	56.86%	70.49%
Accuracy	54%	75%

*PPV= Positive predictive value, **NPV= Negative predictive value
Anti CCP test was considered negative when titre was <20 IU/L
RA test was considered negative when titre was <10 IU/L

Correlation of Anti-CCP titre with DAS 28 score: In the correlation study there were found strong correlations between increasing titre of Anti CCP and increasing activity marker DAS 28 score(r=0.912)(p =<0.001).

Table 6. Correlation of titre of Anti-CCP and DAS 28 score

		Titre of Anti –CCP	DAS 28 Score
Titre of Anti –CCP	Pearson Correlation	1	.912(**)
	Sig. (2-tailed)		.000
	N	100	100
DAS 28 Score	Pearson Correlation	.912(**)	1
	Sig. (2-tailed)	.000	
	N	100	100

** r= 0.912 and p= .000 (results are statistically significant)

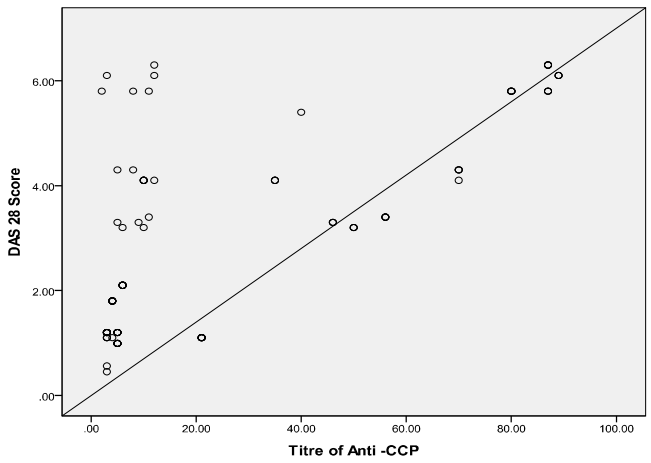

Figure 1. Correlation between Anti-CCP and DAS 28 score

Correlation of Anti-CCP with visual analogue scale: In the correlation study there were found weak correlations between increasing titre of Anti-CCP and increasing activity marker visual analogue scale (VAS) (r=0.382) (p =<0.001)

Table 7. Correlation of Anti-CCP and VAS

		Titre of Anti –CCP	Visual analog scale(VAS)
Titre of Anti –CCP	Pearson Correlation	1	.382(**)
	Sig. (2-tailed)		.000
	N	100	100
Visual analog scale	Pearson Correlation	.382(**)	1
	Sig. (2-tailed)	.000	
	N	100	100

** r= 0.382 and p= .000 (results are statistically significant)

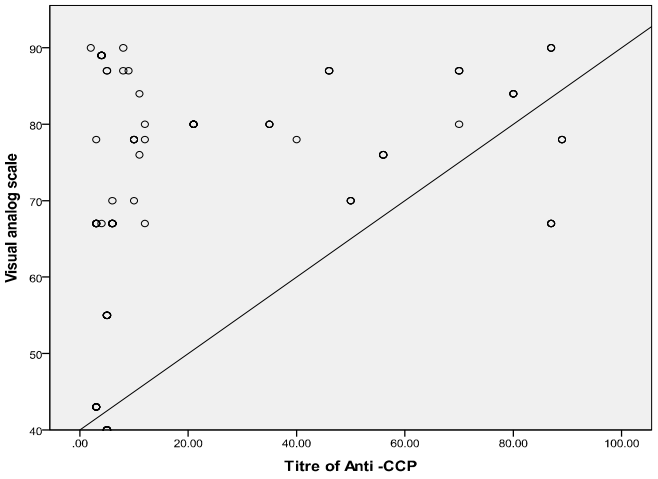

Figure 2. Correlation of Anti- CCP with VAS

Correlation of Anti-CCP with C- reactive protein (CRP): In the correlation study there were found weak correlation between increasing titre of Anti CCP and activity marker CRP (r=0.323)(p =<0.001)

Table 8. Correlation of anti CCP and CRP

		Titre of Anti –CCP	Titre of CRP
Titre of Anti -CCP	Pearson Correlation	1	.323(**)
	Sig. (2-tailed)		.001
	N	100	100
Titre of CRP	Pearson Correlation	.323(**)	1
	Sig. (2-tailed)	.001	
	N	100	100

** r= 0.323 and p= .001 (results are statistically significant)

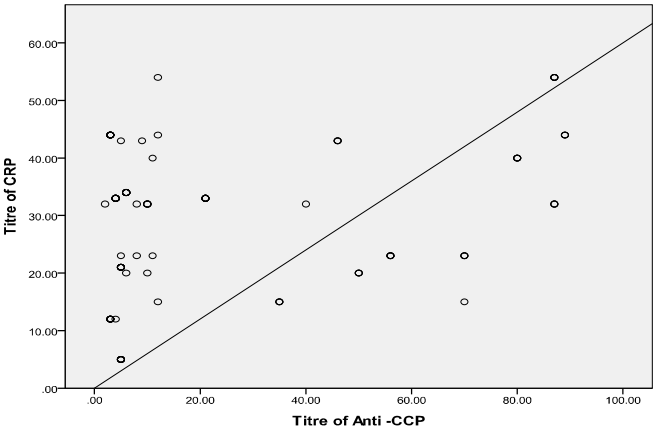

Figure 3. Correlation of anti CCP with CRP

DISCUSSION

Throughout the last decades several autoantibody systems have been described that are associated with Rheumatoid Arthritis (RA). Rheumatoid Factor (RF) is the oldest and most widely known of these autoantibody. Another autoantibody with the greatest clinical potential for RA is found which is anti-CCP antibody (Vossenaar, 2003). In our study patients were included within one year of disease presentation. It was revealed that sensitivity and specificity of Anti CCP (64% and 86%) were higher than the sensitivity and specificity of RA test (56% and 58%) in early diagnosis of RA. So it can be said that Anti CCP is a more specific and sensitive serological marker than RA factor in early diagnosis RA. In the study there was a statistically significant correlation found between the increasing titre of anti CCP and increasing activity marker (DAS 28 score). The other activity markers- visual analog scale and C-reactive protein also showed a statistically significant correlation between the anti- CCP antibody. Anti-CCP antibodies have proven useful in identifying those patients who are likely to have clinically significant disease activity. In a study which was done on 150 patients with long-standing RA, a strong correlation was found between greater disease activity and anti-CCP positivity (Schellekens, 2001). In our study most of the patients were female (61%) and female to male ratio was 1.56: 1. Diseases related with polyarthritis are commonly autoimmune in origin and these diseases are common in female. Findings also consistent with the previous literature (Vossenaar, 2003). Along with the articular manifestations, some extra articular features also observed. In this study, fatigue ability was the most common extra-articular manifestation involving both groups. In a study (Rantappa, 2003), it was found that among all extrarticular manifestations fatigue ability was the most common; causing functional impairment. In our study positive anti CCP was found 64% in RA group and 14% in non RA group within one year of disease presentation. In a study more than 70% of the were anti CCP positive at their first visit to the rheumatology clinic (Rantappa, 2003). So it can be said that anti CCP has significant role in detecting the RA in early stage of the disease.

Conclusion

In this small study anti - CCP antibody had higher sensitivity and specificity compared to RA test. It helps in early diagnosis of RA as it becomes positive earlier than other markers. Quantitively the titre of the anti - CCP is significantly correlated with the activity and severity markers. Higher titre of anti-CCP was observed in patients with higher level of inflammatory marker like CRP and clinical activity markers like DAS 28 score and visual analog scale. So it can be said that anti-CCP may serve as a powerful serologic marker for early diagnosis and severity predictor of RA.

Disclosure: All the authors declare no competing interest.

REFERENCES

Bizzaro N, Mazzanti G, Tonutti E. 2001. Diagnostic accuracy of the anti- citrulline antibody assay for rheumatoid arthritis. Clin Chem, 47:1089-93

Lee DM, Schur PH. 2003. Clinical utility of Anti-CCP, assay in patient with rheumatic disease. *Ann Rheum Dis.* 62: 870-4.

O'Dell JR. 2005. Rheumatoid arthritis: the clinical picture. In:Koopman WJ, Moreland LW, eds. Arthritis and Allied Condition. A text book of Rheumatology Lippincott Williams & Wilkins, Baltimore, MA. p.1165- 94

Rantappa D. 2003. Antibodies against citrullinated peptides and Ig A rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis Rheum.* 48(10):1741-2749

Schellekens GA, de jong BA, van den Hoogen FH. 1998. Citrullin is an essential constituent of antigenic determinant recognized by RA, specific autoantibodies. *J clin Invest.* 101: 273-8

Schellekens GA, Visser H, de jong BA. 2003. The diagnostic properties of RA antibodies to cyclic citrullinated peptide. Arthritis rheum.43: 155-63.

Schellekens GA. 2001. The diagnostic properties of RA antibodies to cyclic citrullinated peptide. Arthritis Rheu.43:155-63.

Van Venrooij WJ, Pruijin GJ. 2000. Citrullination: a small change for a protein with great consequences for rheumatoid arthritis. *Arthritis Res.* 2(4):249-251.

Vossenaar ER, Zendman AJW. 2003. Growing family of citrullinating enzymes Genes, features and involvement in diseases. Bioassays. 25: 1006-1118

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