

Original Research Article

Anti-citrullinated peptide antibodies (ACPA): Possible role in determining disease activity and severity in rheumatoid arthritis of less than one year duration

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Abstract: This study was done to evaluate the possible role of Anti-Citrullinated Peptide Antibody (ACPA) in determining disease activity and severity in patients of rheumatoid arthritis (RA) of less than one year duration. This hospital based observation study was done among 150 newly diagnosed RA cases of less than one year duration, diagnosed as per 2010 ACR/EULAR Classification Criteria for RA. Patients who were on DMARD or refused to give consent were excluded from the study. Disease activity was measured according to disease activity score (DAS-28). Blood sample was taken from each study participant and sent for complete blood count, erythrocyte sedimentation rate, rheumatoid factor, C-reactive protein and ACPA. ACPA positivity was found in 100/150(75%) cases of RA. RF titer was higher in ACPA positive (33.59 + 15.88 IU/ml) compared to ACPA negative cases (15.24 + 10.68 IU/ml) ($p < .001$). Among ACPA positive patients 82 (82.00%) had high DAS-28 score (>5.1) and 18 (18.00%) had moderate DAS-28 (5.1-3.2). ACPA positivity was associated with high disease activity ($p < 0.01$). RF positivity was higher in ACPA positive patients compared to ACPA negative patients (85% v/s 42%) ($p < 0.001$). All individual DAS-28 parameters were significantly higher in ACPA positive patients ($p < .001$). ACPA titer had statistically significant positive correlation with DAS-28 ($r + 0.372$, $p < 0.05$), duration of disease ($r + 0.176$, $p < 0.05$) and RF titer ($r + 0.451$, $p < 0.01$). We concluded that in RA, ACPA positivity was associated with higher disease activity (DAS-28). ACPA titer had positive correlation with disease activity (DAS-28). Thus ACPA positivity may be an indicator of active RA. ACPA positivity had no significant effect on severity (erosions) in RA of less than one year duration.

Keywords: Anti-citrullinated peptide antibodies, Rheumatoid Arthritis, Disease activity.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic inflammatory, auto immune disease characterized by persistent symmetric polyarthritis that commonly affects joints of hands and feet. The hallmark autoantibody of RA, rheumatoid factor (RF), is directed against immunoglobulin G (IgG). In fact, the most specific auto antibodies are directed to citrullinated peptide such as fibrinogen, vimentin, or α -enolase in RA. These auto antibodies recognizing citrullinated epitopes are named as anti-citrullinated peptide antibodies (ACPA) [1]. Citrullinated epitopes were first identified in filaggrin, a protein that is highly abundant in squamous epithelial cells, where it promotes aggregation of intermediate filaments [2]. ACPA appear early in the course of the disease and

similarly to RF, they may also precede the clinical onset of RA [3]. ACPA are now generally considered to be the most valuable serologic markers of RA.³ These antibodies are useful for diagnosis and are also predictors of more aggressive disease marked by bone and cartilage destruction.³ ACPAs have at least 95% specificity for RA and sensitivity of approximately 75% in established RA. In all studies, ACPA and IgM-RF were found to be highly associated with each other. In view of the above, this study was an effort to evaluate the possible role of ACPA in determining disease activity and severity in rheumatoid arthritis (of less than one year duration) patients.

MATERIAL AND METHODS:

This was a hospital based observation study among 150 newly diagnosed rheumatoid arthritis cases (with duration of RA less than one year, diagnosed as per 2010 ACR/EULAR Classification Criteria for RA) [4] who attended the Rheumatology clinic, S.M.S. hospital, Jaipur during May 2012 to April 2013, after taking permission of the institutional ethics committee and informed consent from the study participants. Patients who were on DMARD or refused to give consent were excluded from the study.

A complete history including present illness, past history, family history and drug history was noted and complete rheumatological assessment was done. Parameters studied were number of joints involved, number of swollen joints, tender joints, deformities, range of movements, and evidence of joint effusion. The 28 joints assessed were 10 proximal interphalangeal joints, 10 metacarpophalangeal joints, two wrists, two elbows, two shoulders and two knee joints. Disease activity was measured according to disease activity score (DAS-28).

Blood sample was taken for each study participant and sent for complete blood count, erythrocyte sedimentation rate (ESR, measured by Westergren method), fasting blood sugar, serum urea and serum creatinine, liver function test, rheumatoid factor (RF, measured by nephelometry test, value ≥ 20 IU/ml was taken as positive), C-reactive protein (CRP, measured by Latex agglutination test) and ACPA. The radiographs of both hands in PA view were taken and reported by experienced radiologist for presence of erosions. The radiologist was blinded to the study and to the clinical status of the patients. ACPA was measured by Enzyme Linked Immuno Sorbent Assay (ELISA) kit. Cut off value of ≥ 20 U/ml was taken as positive result.

Statistical analysis

Microsoft Excel® and SPSS® 17.0 for Windows® were used for data storage and analysis. Continuous variables were expressed as mean \pm standard deviation. Student's t test and Chi-Square test were used to determine statistical difference between variables. Pearson's coefficient was used to investigate the correlation between the two variables. Statistical significance was set at P value ≤ 0.05 .

RESULTS:

In our study of 150 RA patients mean age was 43.77 ± 12.95 years (age range 17-73 years) with male: female ratio 1:2.95. There was no statistically significant difference in age in ACPA positive and ACPA negative patients ($p > 0.05$). ACPA positive cases had significantly longer duration RA ($7.11 + 2.28$ months) compared to ACPA negative patients ($5.72 + 2.11$ months) ($p < .001$). RF titer was higher in ACPA positive ($33.59 + 15.88$ IU/ml) compared to ACPA negative cases ($15.24 + 10.68$ IU/ml) ($p < .001$). In ACPA positive patients, 42 (42.00%) patients had disease duration between 1-6 months and 58 (58.00%) patients had disease duration between 7-12 months. (Table no 1)

Among ACPA positive patients 82 (82.00%) had high DAS-28 (>5.1) and 18 (18.00%) had moderate DAS-28 (5.1-3.2). Low disease activity (DAS-28 <3.2) was not found in our patients. ACPA positivity was associated with high disease activity (high DAS-28) ($p < 0.01$). RF positivity was higher in ACPA positive patients compared to ACPA negative patients (85% v/s 42%) ($p < 0.001$). Statistically there was no significance difference in ACPA positive and ACPA negative patients for the presence of erosions (11% v/s 2%) ($p > .05$). (Table no. 1)

Table no. 1: Characteristic features of RA patients

		Total	ACPA		p-value
			Positive (100)	Negative (50)	
Age (years)		43.77 ± 12.95	43.88 ± 12.60	43.54 ± 13.75	> 0.05
Duration of disease (months)		$6.65 + 2.31$	$7.11 + 2.28$	$5.72 + 2.11$	< 0.001 HS
Duration of disease (months)	1-6	80	42 (42%)	38 (76%)	
	7-12	70	58 (58%)	12 (24%)	
ACPA (U/ml)		168.02 ± 116.82	$161.82 + 119.87$	$7.48 + 2.37$	< 0.001 HS
RF (IU/ml)		$27.47 + 16.74$	$33.59 + 15.88$	$15.24 + 10.68$	< 0.001 HS
Positive CRP (n %)		123 (82.00%)	83 (83%)	40 (80%)	> 0.05 NS
Positive RF (n %)		106 (70.67%)	85 (85%)	21 (42%)	< 0.001 HS
Erosions (n %)		12 (8.00%)	11 (11%)	1 (2%)	> 0.05 NS
DAS-28 (n, %)	Moderate (3.2-5.1)	39 (26.00)	18 (18)	21 (42.00)	< 0.01 HS
	High(>5.1)	111 (74.00)	82 (82)	29 (58)	

The RF positive and the RF negative patients did not differ for the presence of erosions ($p > .05$) (table no.2). All individual DAS- 28 parameters were significantly higher in ACPA positive patients ($p < .001$). (Table no.3). ACPA titer had statistically

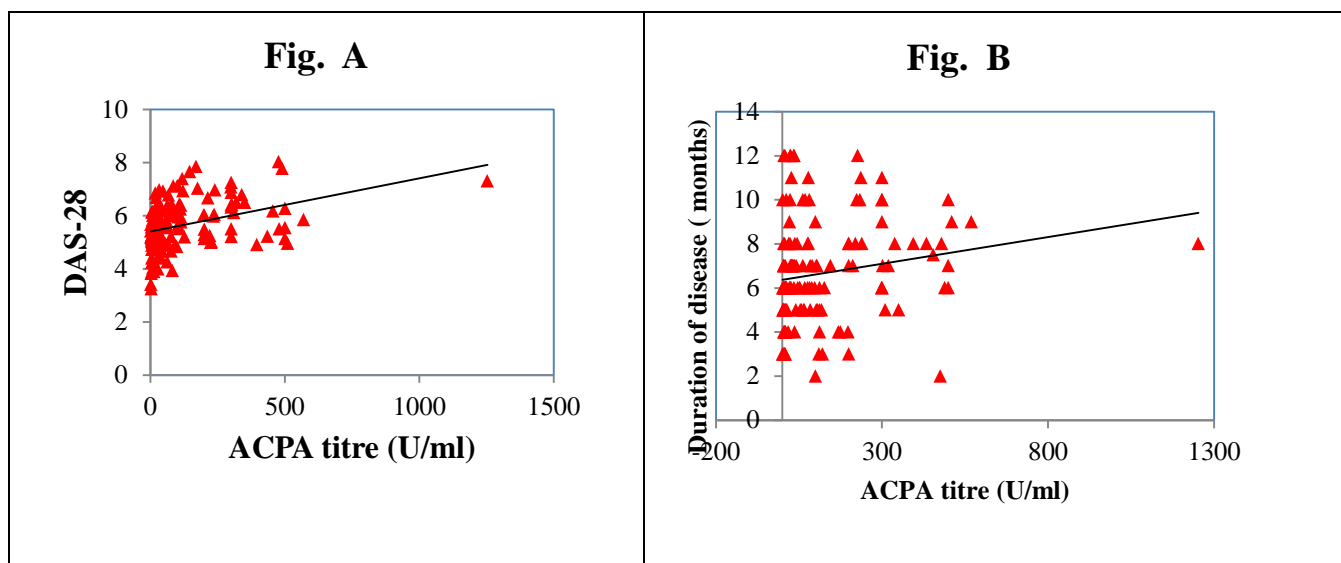
significant positive correlation with DAS-28 ($r + 0.372$, $p < 0.05$), duration of disease ($r + 0.176$, $p < 0.05$) and RF titer ($r + 0.451$, $p < 0.01$). RF titer also had positive correlation with DAS 28. ($r + 0.312$, $p < 0.05$) (Fig 1).

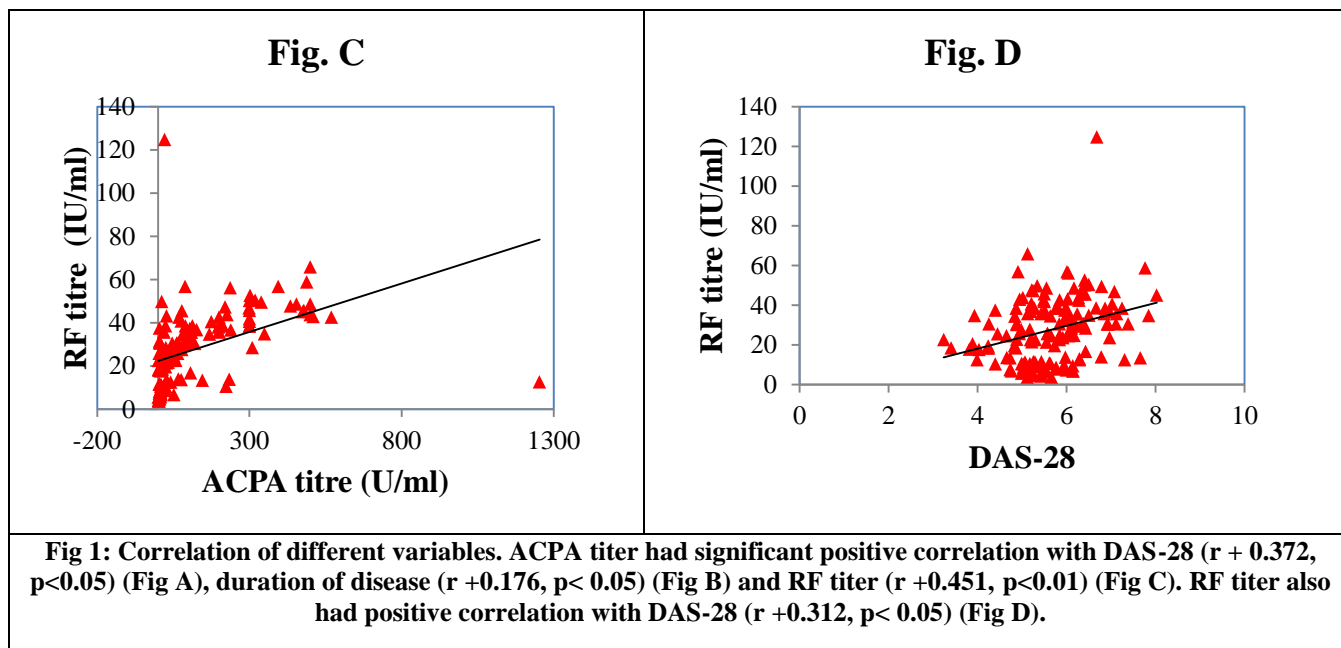
Table No.-2: Distribution of study subjects according to RF & Erosions

Erosion	RF		Total N (%)
	Positive N (%)	Negative N (%)	
Present	10 (9.43%)	2 (4.54%)	12 (8.00%)
Absent	96 (90.57%)	42 (95.45%)	138 (92.00%)
Total	106 (70.67%)	44 (29.33%)	150 (100.00%)

Table No.-3: Mean + SD of DAS-28 parameters according to ACPA

Parameter	ACPA		P	
	Positive (n=100)	Negative (n=50)		
A). TJC	12.74 + 4.78	9.58 + 3.43	<0.001	HS
B). SJC	10.34 + 3.99	7.38 + 3.21	<0.001	HS
C). ESR (mm/1 st hr)	46.47 + 24.73	32.18 + 21.17	<0.001	HS
D). VAS	33.40 + 12.49	26.80 + 9.19	<0.001	HS
E). DAS-28	5.91 + 0.86	5.09 + 0.73	<0.001	HS





DISCUSSION:

In this study the possible correlation of ACPA and disease activity in rheumatoid arthritis patients was evaluated. In our study, ACPA positivity was seen in 100/150 (66.67%) cases of RA. Similarly, Del Val del amo [5] found 64% ACPA positivity in RA. Other studies reported 50-73% positivity of ACPA in RA cases [6-12]. The ACPA positive (43.88 ± 12.60 years) and ACPA negative patients (43.54 ± 13.75 years) did not differ significantly for age at disease onset ($p > 0.05$). Age of the patients did not affect ACPA status in current study as reported in previous studies [10, 11, 13].

The statistically highly significant difference for the duration of disease was found among ACPA positive and ACPA negative cases ($p < .001$). Thus ACPA positive cases had longer disease duration. Ronnelid *et al.*; [7] also observed statistically significant difference in relation to disease duration at inclusion of study in ACPA positive (6 month) and ACPA negative patients (5 months) ($p < 0.05$). In contrast to our study, some authors found no difference in disease duration in ACPA positive and ACPA negative cases (>0.05) [8, 11, 14].

Disease activity and severity parameters of RA cases according to ACPA

We found higher DAS-28 in ACPA positive patients. Among 100 ACPA positive patients, 82 (82.00%) had high DAS-28 and 18 (18.00%) had moderate DAS-28. This indicates the association of ACPA positivity with high disease activity ($p < 0.05$). Forslund *et al.*; [8] also found similar results. (Table no.1)

CRP positivity was noted in 83% ACPA positive patients and 82% ACPA negative patients in this study and this difference was statistically not significant ($p > 0.05$). (Table no.1). In present study RF titer was significantly higher in ACPA positive ($33.59 + 15.88$ IU/ml) compared to ACPA negative cases ($15.24 + 10.68$ IU/ml) ($p < .001$). RF positivity was significantly higher in ACPA positive patients (85/100) compared to ACPA negative cases (21/50) ($p < 0.001$). Several studies also had similar observation [7-8,10-11]. Ibrar Ahmed *et al.*; [14] also reported statistically significant difference in RF positivity between ACPA positive (84.2%) and ACPA negative (35.8%) patients ($p < 0.05$). (Table no.1)

In current study, statistically there was no significant difference in ACPA positive and ACPA negative patients for presence of erosions ($p > .05$). (Table no.1) Munevver Serdaroglu *et al.*; [11] also observed the similar results in a study conducted on 40 patients of RA. Kroot *et al.*; [10] also did not find significant difference in erosions in ACPA positive and ACPA negative patients. But some authors observed that RA patients, positive for ACPA develop significantly more radiological damage than ACPA negative patients [8, 13, 16].

Though in this study, the finding of insignificant difference in RF positive and RF negative patients for the presence of erosions ($p > .05$) matched with the findings of Matthey *et al.*; [17] this is in contrast with the study of Mewar *et al.*¹⁸ who observed strong association of RF with radiographic severity. (Table no.2) In our study; the study cohort included RA with < 1 year duration (mean duration $7.11 + 2.28$ months), which may be too early to develop erosions. This may

be the reason that we could not find any association of erosions with RF or ACPA.

ACPA may have a prognostic value in RA. ACPAs are locally produced by synovial B cells and may therefore together with RF contribute to the inflammatory and destructive processes in the rheumatoid joint.¹⁹ ACPAs were detected in mice with collagen-induced arthritis and after administration of monoclonal antibodies against citrullinated fibrinogen in joints, whereas induction of tolerance to a citrullinated peptide led to a significant reduction in disease susceptibility. These results seem to provide direct evidence that ACPA is involved in the pathogenesis of erosive arthritis.

Individual DAS-28 parameters and ACPA

ACPA positivity in RA cases was associated with significantly higher tender joint count, swollen joint count, ESR and VAS ($p < 0.001$ for each). Mansoor Karimifar *et al.*; [13] also observed similar results. Similarly Forslind *et al.*; [8] also revealed association of ACPA and ESR. Few studies reported no association between ACPA and TJC, SJC, ESR or DAS-28 [7,11] Forslind *et al.*; and Munevver Serdaroglu *et al.*; [11] also mentioned no significant difference for VAS in ACPA positive and ACPA negative patients. (Table no.3) ACPA positivity was associated with higher disease activity (DAS-28) in our study. A recent study also revealed the association of ACPA with DAS-28 in RA patients [13]. Previous studies also reported higher DAS-28 in patients with ACPA [13, 20]. Ronnelid *et al.*; [7] did not find difference for DAS-28 among ACPA positive (4.99) and ACPA negative (5.06) patients.

Correlation between various parameters of study subjects

This study showed a statistically significant positive correlation of ACPA titer with DAS-28 ($r + 0.372$, $p < 0.05$) which is in accordance with Mansoor Karimifar *et al.*; [13] and in contrast to Munevver Serdaroglu *et al.*; Statistically significant positive correlation between ACPA titer and duration of disease also existed ($r + 0.176$, $p < 0.05$). As reported by Munevver Serdaroglu *et al.*; [11]. We also found a significant positive correlation between ACPA titer and RF titer ($r + 0.451$, $p < 0.01$). (Fig no.1) Statistically significant positive correlation between RF titer and DAS-28 ($r + 0.312$, $p < 0.05$) was also observed, this observation was similar to IbnYacoub *et al.*; [21] (Fig no.1)

CONCLUSION:

In RA, ACPA positivity was associated with higher disease activity (DAS-28). ACPA titer had positive correlation with disease activity (DAS-28). Thus ACPA positivity may be an indicator of active

RA. ACPA positivity had no significant effect on severity (erosions) in RA of less than one year duration.

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