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Anti-Sa Antibody, Cyclic Citrullinated Peptide Antibody and Rheumatoid Factor As Diagnostic Markers Of Rheumatoid Arthritis

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Abstract
Citrullinated peptide Antibodies are novel diagnostic markers for Rheumatoid Arthritis (RA).Anti-Sa Antibody is one of the recently discovered Citrullinated peptide Antibody. Immunoassays which use synthetic citrullinated peptide are highly effective tests for the detection of auto antibodies against citrullinated peptides In this study, the clinical utility of Anti-Sa Antibody as a diagnostic marker was evaluated along with the established markers Anti cyclic citrullinated peptide (ACCP) & Rheumatoid factor (RF) on clinically suspected cases of Rheumatoid Arthritis.

Key words
Rheumatoid Arthritis (RA), Anti-Sa Antibody, Anti cyclic citrullinated peptide (ACCP), Rheumatoid factor(RF), Chemiluminescence micro particle Immunoassay (CMIA) ,Immunonephelometry.

Introduction

Rheumatoid Arthritis is one of the commonest Systemic autoimmune diseases affecting the joints. The disease severity varies from mild inflammation in the joints to serious complications. Besides the conventional marker Rheumatoid Factor (RF), many serological markers belonging to the class of citrullinated peptide Antibodies are associated with the disease.

Anti-Sa Antibody is a new marker for Rheumatoid Arthritis. As per various studies, Anti-Sa is found to be strictly linked to disease or disease onset and belongs to the class of Anti Citrullinated peptide Antibodies (ACPA)[1.] Antigens bound by ACPAs include a variety of naturally citrullinated proteins, including vimentin (anti-Sa)), filaggrin, or fibrinogen, as well as synthetic cyclic citrullinated peptides (CCPs)[2,3]. The second generation assays (CCP2) are now widely used for the diagnosis of Rheumatoid arthritis. Anti CCP antibodies are found to remain positive during the course of treatment and have less prognostic significance. Anti-Sa ,even though less prevalent in Rheumatoid Arthritis is said to be more predictive of midterm severe outcomes and is hence useful in prognosis[4]. The Sa antigen is found in the synovium and comprises of citrullinated Vimentin. It is also considered as a hapten carrier complex with Vimentin as carrier and citrulline as the hapten[5]. Vimentin comprises of 43 arginine residues which could be citrullinated in Rheumatoid arthritis. It is hence possible that Sa antigens could provide more citrullinated epitopes for diagnosis[6]. Anti-Sa could predict more severe presentations of Rheumatoid Arthritis when compared to ACCP and RF[7].In another cohort study, it was also found that only a very small percentage of ACCP positive/Anti-Sa Negative healthy individuals later developed Rheumatoid Arthritis[8].

There are studies indicating increased severity of Arthritic complications in Anti-Sa positive patients demanding more aggressive treatment options especially in male patients.[9,]10. Currently available Immunological techniques for Anti cyclic citrullinated peptides are considered more sensitive than Anti Sa .Newer Assays for Anti Sa with higher sensitivity would facilitate the identification of Antibodies undetected by currently available test systems [11,12].

In this study the clinical utility of Anti-Sa antibody is compared to the established markers ACCP and RF in patients with early symptoms of RA.

Materials and Methods

The study was carried out at Educare Institute of Dental Sciences .A total of 60 patients with suspected RA as per the American Council of Rheumatology (ACR) criteria were selected for the study. Age and Sex matched with normal individuals were included as controls. Five mL of venous blood was collected from all the subjects after getting their informed consent, sera separated and were tested for Anti Sa by Euroimmun Anti Sa IgG ELISA, ACCP (second generation) by Automated Abbott Architect Chemiluminescence microparticle Immunoassay (CMIA)

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and RF by Immunonephelometry on Siemens BNProspec analyzer. The results were analyzed using Minitab & SPSS statistical software.

Results and Discussion

60 patient samples were analyzed for Anti-Sa, ACCP and RF during a six month period.60 age and sex matched controls were also included. Female constituted 75% of the patients and controls. The Distribution of ACCP, RF and CRP in the patients and the control subjects are given in Table 1&2.

Out of 120 subjects (both patients and controls) 32(27%) were Anti Sa-positive, 50(42%) ACCP-positive and 56(47%) were positive for RF, while in RA patients, Anti Sa, ACCP and RF were positive in 30(50%), 44(73%) and 48(80%) cases respectively (Table 5). There was significant correlation between the Anti-Sa, ACCP, and RF ($p<0.01$) as mentioned in table 4.

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Table 1. Descriptive Statistics of the study population (n=120).

	Minimum	Maximum	Mean	SD
ACCP	0.00	200.0	56.42	81.4
ANTI- SA	2.0	200.0	33.33	56.2
RF	9.0	1022.0	112.5	202.7
Age	30.0	73.0	48.84	10.7

Table 2:Sex wise group statistics of the study population

	Sex	N	Mean	SD	SE Mean
ACCP	Male	30	54.91	79.19	14.46
	Female	90	56.92	82.57	8.70
ANTI SA	Male	30	37.45	65.44	11.95
	Female	90	31.96	53.12	5.60
RF	Male	30	120.1	237.6	43.39
	Female	90	109.9	191.1	20.15
Age	Male	30	49.57	8.20	1.50
	Female	90	48.60	11.44	1.21

Table 3: Group Statistics and t test of Test vs Control groups

	Category	Mean	SD	t-test p-Value
ACCP	Control (n=60)	1.75	3.94	0.000
	Test (n=60)	111.09	85.28	
ANTI SA	Control (n=60)	8.27	5.11	0.000
	Test (n=60)	58.39	71.19	
RF	Control (n=60)	12.10	8.71	0.000
	Test (n=60)	212.92	249.64	
Age	Control (n=60)	50.92	11.19	0.033
	Test (n=60)	46.77	9.83	

Table 4: Correlation statistics and significance of ACCP, Anti SA and RF

		ACCP	ANTI SA	RF
ACCP	Pearson Correlation	1	0.749**	0.664**
	Sig. (2-tailed)		0.000	0.000
ANTI-SA	Pearson Correlation	0.749**	1	0.306**
	Sig. (2-tailed)	0.000		0.001
RF	Pearson Correlation	0.664**	0.306**	1
	Sig. (2-tailed)	0.000	0.001	

**. Correlation is significant at the 0.01 level (2-tailed).

The clinical sensitivity and specificity for Anti-Sa, ACCP and RF were obtained as 66.67% & 96.77%; 78.95% & 90.91% and 83.3% & 88.24% respectively. The clinical sensitivity of Anti-Sa was found to be lower when compared to ACCP and RF. Studies by Goldbach etal[9] & Hayem et al [10] indicated a clinical sensitivity for Anti Sa ranging from 20%-45% and a specificity close to 95% depending on

varying degree of disease severity. ACCP by the second generation CMIA was found to have higher combined disease predictive value. As radiological evidence of join erosion is found to be more extensive in CCP2 positive RA patients, testing for ACCP could be vital in RA diagnosis.

Table 5: Patient characteristics and test outcome.

	R A patients (n=60; 50%)	Healthy controls (n=60; 50%)	Total (n=120)
Female	45 (75%)	43 (71%)	88 (73%)
Age (mean (SD)) (years)	46.77 (9.83)	50.9 (11.19)	48.8 (12.8)
RF positive (n(%))	48 (80%)	8 (13%)	56 (47%)
ACCP positive (n(%))	44 (73%)	6 (10%)	50 (42%)
Anti SA positive (n(%))	30 (50%)	2 (3%)	32 (27%)

The higher specificity of Anti-Sa highlights its importance as a promising biomarker as there are studies which shows that Anti-Sa positive RA patients might require more aggressive therapy.

Figure 2. Histogram of RF Values of Test and Control population.

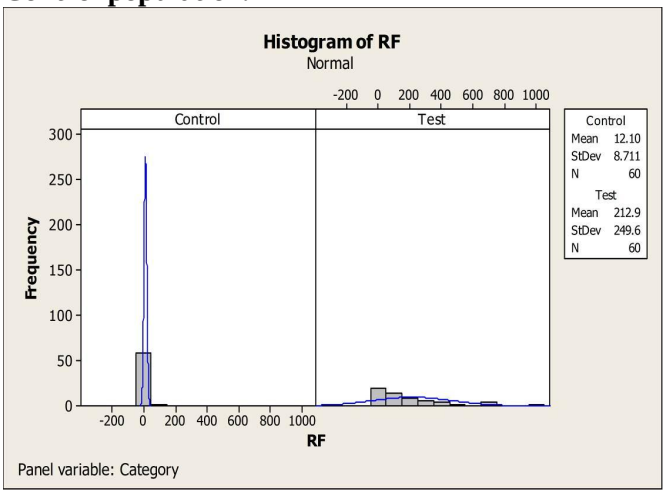


Figure 1 : ROC Curve of ACCP, Anti SA and RF

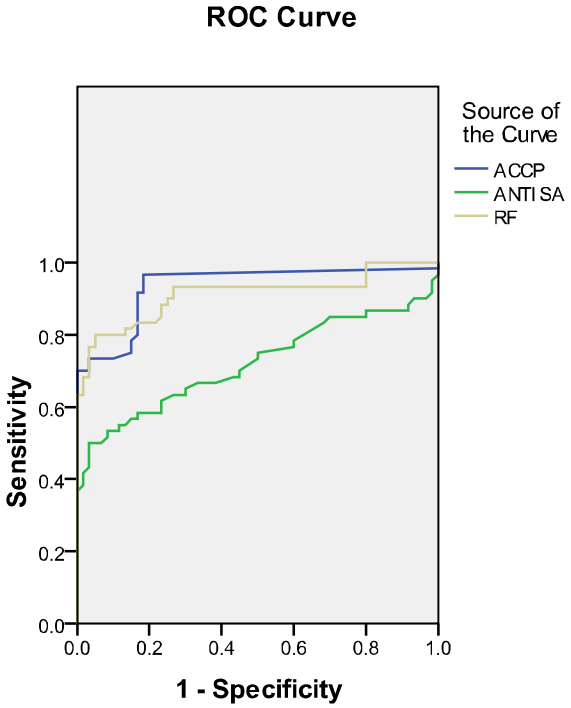


Figure 3. Histogram of Anti-SA Values of Test and Control population.

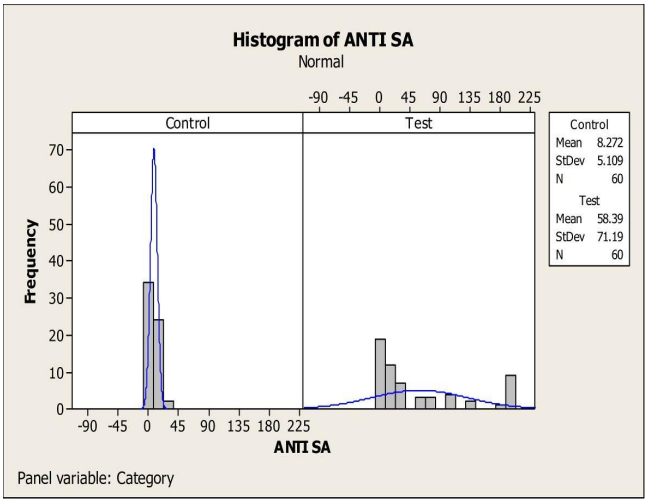
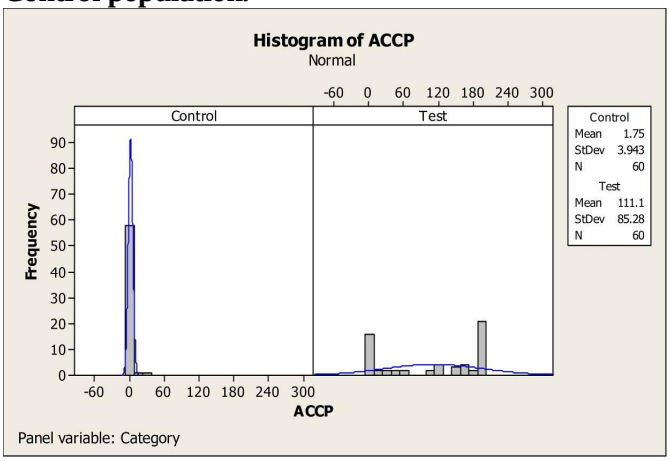


Figure 4. Histogram of ACCP values of Test and Control population.



Anti CCP by the CCP2 assay showed higher sensitivity among the three markers [13].

In a comparative study with Rheumatoid factor had reported a higher sensitivity and specificity for CCP2 assay especially in conditions of erosive arthritis. Greiner etal[14] had earlier reported a sensitivity of 98 % for Anti CCP compared to other citrullinated peptide antibodies and Rheumtoid factor.

Rheumatoid Arthritis patients exhibit variable response to therapy. Early detection is possible by the use of different serological markers in conjunction. Our study also showed a very high disease predictive value when Anti CCP & Anti-Sa were tested in conjunction .This finding is also in agreement with the study by Rodriguez etal[15] which emphasized the significance of combination testing for RA diagnosis.

Anti-Sa had the highest specificity for RA in our study. The clinical utility of Anti-Sa as a marker for RA is yet to be established. The study by Boire et al[4] highlighted the usefulness of Anti-Sa in erosive Arthritis in a significant group of RA patients. The high sensitivity of ACCP and RF and high specificity of Anti-Sa might be helpful in early diagnosis and treatment when the tests are used in combination.

Conclusion

Anti-Sa is a new serological marker for Rheumatoid Arthritis belonging to the class of citrullinated peptide Antibodies. The high specificity and prognostic value of Anti-Sa, combined with the higher sensitivity of existing markers like Anti CCP and RF would be beneficial in the early diagnosis and management of Rheumatoid Arthritis.

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