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Value of Anti-PeptidylArginine Deiminase-4Antibodies in Rheumatoid Arthritis Patients Compared to Anti-cyclic CitrullinatedPeptide

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ABSTRACT

Background:*Rheumatoid arthritis (RA) as a chronic multisystem disease isdistinguished by chronic polyarthritis and can lead to deformities and disabilities* ***[1].****Antibodies to anti-cyclic citrullinated peptidewere found to be specific for RA, including the early form, demonstrating a possible prognostic value* ***[2].****In addition to the widely used RF and ACCP autoantibody assay, several tests have emerged to help in RA diagnosis, such as serum anti PADI4****[3],*** *which is the enzyme that causescitrullination of proteins leading to production of autoantibodies****[4]****.* ***Objectives:****To assess the role of serum Anti-PADI4 Ab. in patients with RA and its relation with clinical parameters, disease activity and comparing it with ACCP level in RA patients.****Subjects and Methods:****A case-control study comprising 100 subjects, 50 RA patients and 50 apparently healthy subjects asa control was carried out at Department of Physical Medicine, Rheumatology and Rehabilitation, Zagazig University Hospitals. All included patients underwent detailed history taking, clinical examination, and disease activity assessment using DAS-28, CRP and ESR. Serum Anti-PADI4 and ACCP were determined in RA patients and healthy controls utilizing quantitative ELISA technique.* ***Results:****We found a significant positive correlation between Anti-PADI4 and ACCP level (P = 0.02). We found no correlations between serum Anti-PADI4, ACCP and DAS28, number of tenders, swollen joints, and ESR.* ***Conclusion:****Significant increase existed in Anti-PADI4 serum level of RA patients over healthy controls subjects. Sensitivity and specificity of Anti-PADI4 increased in the presence of ACCP, indicating that Anti-PADI4 may helpto diagnoseRA patients lacking ACCP.*

Keywords: *Rheumatoid arthritis, PeptidylArgenineDeiminase- 4, Cyclic citrullinated peptide, DAS28, diagnostic.*

I. INTRODUCTION

Asa chronic multisystem autoimmune disorder, rheumatoid arthritis (RA) diseaseis identified by its chronic joint inflammation,causingbone and cartilagedestruction, in addition to autoantibodies existence such as rheumatoid factor (RF) as well ashighly RA-specific antibodies called anti-cyclic citrullinated peptide (ACCP) **[5].**

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RF, as well as ACCP antibodies,were found in RA patients before they have clinical symptoms, and they may indicate early immune dysregulation in patients years before symptoms appear[6].Furthermore, ACCP isdemonstratedasa preciseprognostic RA marker and anticipateswhether disease progression is erosive or non-erosive. Consequently, ACCPis beneficialtowards optimal therapeutic management of patients with RA [7].

UnlikeconventionalRFdetection, new specific peptidyl arginine deiminase-4 (anti-PADI4) autoantibodies to citrullinated antigens contribute to RA diagnosis and are recommended as early disease indicators[8].

Citrullinationexemplifiesa post-translation modification in whicharginine is modified to citrulline (non-standard residue). Such achange is performedby peptidyl arginine deiminase (PADI)enzymewhen combined withcalcium[9].

PAD4 is positionedin monocytecytoplasm, T and B cells, neutrophils, eosinophils and NK cells, and upon cell activation,it can move to nucleus. PAD4 holds a physiological functionin regulating genesthrough histones'citrullination. In RA, PADI4 generatesanti-citrullinated peptide antibodies (ACPA) specific substrates and is vulnerableto autoantibodies. Such autoantibodies against PADI4 areconsideredas a specific biomarker in patientsexhibitingclinically apparent RA[10].Such aprocess is possibleto be highlysignificant in RA patients provided the established association of anti-PADI4 antibodies with disease existenceand activity[11].

II. AIM OF THE WORK

This work's objective was to assess the role of serum Anti-PADI4 Ab. in patients with RA and its relation with clinical parameters, disease activity and comparing it with ACCP level in RA patients.

III. SUBJECTS AND METHODS

In the current study, 50 consecutive patients,including 43 females(86%) and 7 males(14%) attending outpatients clinic,were subjected to random recruitment. thirty-six of such patients tested positive against IgM-RF(seronegative RA). All included patients met The American college of Rheymatology (ACR) criteria towards RA[11].Patients suffering from other rheumatic diseases, patients with concomitant autoimmune disease,associated with malignancy or infections e.g HCV were excluded.

In this work, 50 apparent healthy coulteers, including 37(74%) females and 13(26%) males, were enrolled. The normal controls exhibited no history of connective tissue diease,chronic infection/inflammation, cancer, or organ failure. The patients, as well as controls, demonstrated age and sex matching. All participents provided written informed consent and the study got approval from local ethics committee. Both patients and controls were subjected to provide 5 ml blood into plain vacutainer tubes. After certification, serum was attained and kept at -20 for further test.

Laboratory investigations: Comprised erythrocyte sedimentation rate (ESR) using Westergren method recorded mm/hr[12], C-reactive protein (CRP) by BN prospecnephelometersemines with normal range (1-6 mg/dl) [13]. The nephelometric method was utilized to determine RF existence. ELISA techniques were employed for

detecting anti-CCP and anti-PADI4 antibodies whose cut-off values were 16 U/mL and 8 U/mL, respectively, as recommended by manufacturers.

Statistical Analysis: Data analysis was conducted by means of SPSS version 20 software (SPSS Inc., Chicago, IL, US). Due to the lack of normal data distribution, a nonparametric test was utilized, and medians having interquartile ranges are displayed. We deployed Mann-Whitney test for testing if medians of two unpaired sets of measurement are different from each other. The significance level at $P < 0.05$ was taken at 95% confidence interval (CI).

IV. RESULTS

Regarding demographic characteristics, RA patients were (43) females (86%) and (7) males (14%), with ages ranging from 22 to 60 years and a mean of (39.24±9.83), while controls were (37) females (74%) and (13) males (13%), with ages ranging from 24 to 55 years and a mean of (37.62±8.4), with no statistically significant difference between both groups. Of 50 RA patients, 47 patients tested positive towards anti-PADI4 antibodies (94%), 30 patients tested positive towards anti-CCP antibodies (60%) and 36 patients tested positive towards RF (72%). In contrast, of 50 apparent healthy controls, five persons tested positive for anti-PADI4 antibodies (10%), and 7(14%) was positive for ACCP antibodies.We found that ACCP serum levels in RA patients were with a median of 80 with interquartile range of (17-200ng/ml), compared with that of control group 7.5 (5-14ng/ml). The ACCP serum level was higher in case group than in control group with statistically significant difference (P <0.001) **(Table 1).**

Anti-PADI4 antibodies showed good specificity (90 vs. 88%) and sensitivity (97 vs. 80%) when compared to ACCP antibodies, respectively, demonstrating a positive predictive value of 90.9% and accuracy of 95% **(Table 2).**

As shown in **Table 3**, of 50 RA patients, 47 patients tested positive against anti-PADI4 antibodies, and three patients had negative anti-PADI4 antibodies. Of 47 RA patients with positive anti-PADI4 antibodies, 30 (63.8%) were ACCP antibodies positive. Of 20 RA patients with negative ACCP, negative RF, 9 patients were positive for anti-PADI4. As a consequence, anti-PADI4 was regarded as the only positive marker for such a group, demonstrating that its assay may assist in diagnosing RA in patients exhibiting negative tests against ACCP as well as RF. As **Table 4 and 5** displays, we correlate levels of anti-PADI4 and ACCP antibodies with ESR, DAS28 and CRP, without observing significant associations between such variables.

Table (1): Demographic information and laboratory investigations of Rheumatoid Arthritis (RA) patients and control groups.

			Control N=50	RA patients N=50	P. value
Demographic data	Age		37.62±8.4	39.24±9.83	0.3
	Sex	Female	37(74.0%)	43(86.0%)	0.1

		Male	13(26.0%)	7(14.0%)	
	Smoking	No	41(82.0%)	45(90.0%)	0.2
		Yes	9(18.0%)	5(10.0%)	
	Durations / year		-	3.0(2.0- 8.0)	N.A
Laboratory investigation	ACCP Titer		7.5 (5.0- 14.0)	80.0 (17.0- 200.0)	0.001**
	Anti PAD4 Ng/ml		2.3 (1.9- 3.7)	31.6 (12.1- 39.6)	0.001**
	Serum Anti- PADI4	**Negative**	45.0 (90.0%)	3.0 (6.0%)	0.001**
		Positive	5.0 (10.0%)	47.0 (94.0%)	
	Serum ACCP	**Negative**	43 (86.0%)	20.0 (40.0%)	0.001**
		Positive	7 (14.0%)	30.0 (60.0%)	

Age is represented as Mean ± SD; the data were analyzed by student t test. While Sex, smoking, Disease Scale and activity are represented as F (%) frequency and percent; the data were analyzed by X^2 test. But Durations, Anti CCP andPAD4 are represented as Median with Interquartile range (25% -75%), the data were analyzed by Mann-whitney U test.

* p. value <0.05 is significant, ** p. value <0.01 is highly significant.

Table 2:-. Diagnostic performances of the studied biomarkers.

Markers	Cutt-off	Sn.	Sp.	PPV	NPV	Accuracy	AUC	95% C.I	p value
ACCP	16.0	80.0%	88.0 %	87.0 %	81.5%	84.0%	91.0%	85.6% - 96.3%	0.001**
Serum Anti-PADI4	8.0	97.0%	90.0 %	90.9 %	97.0%	95.0%	94.2%	89.2% - 99.2%	0.001**

Sn.: Sensitivity, Sp.: Specificity, PPV: Positive predictive value, NPV: Negative predictive value and AUC Area under curve. * P-value < 0.05 is significant, ** P-value < 0.01 is highly significant.

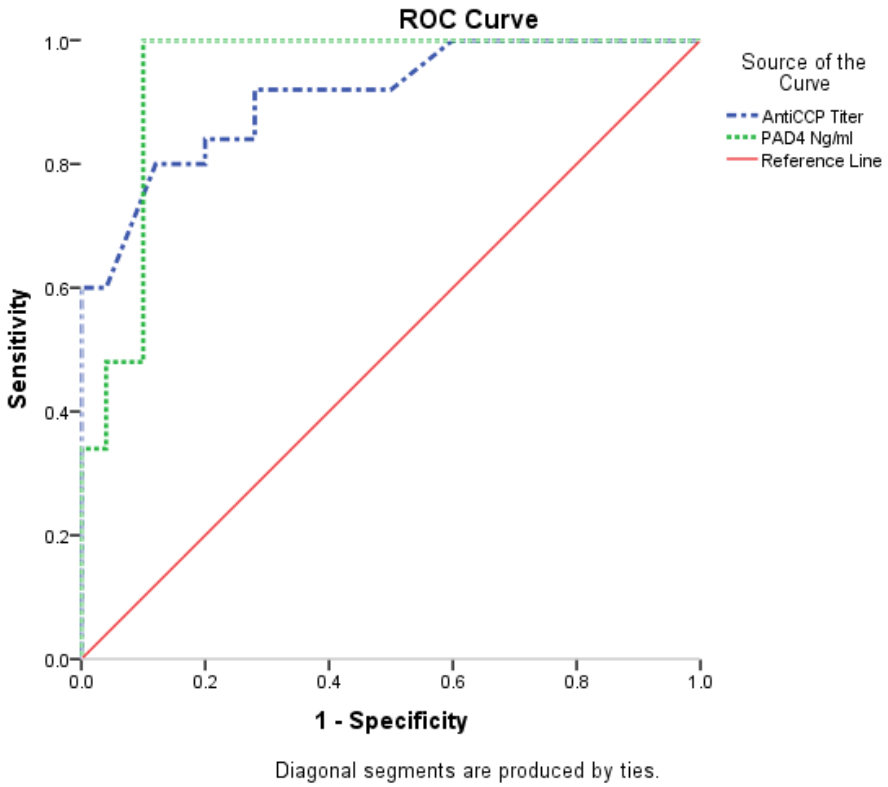

Table (3): association of anti-PADI4 antibodies, ACCP antibodies and RF.

		ACCP positive N=30	ACCP negative N=20	P value
Anti –PADI4	**Positive**	30(100.0%)	17(85.0%)	0.03*
	Negative	0(0.0%)	3(15.0%)	
RF positive	**Anti –PADI4 +ve**	28(100.0%)	8(100.0%)	N.A
	Anti –PADI4 –ve	0(0.0%)	0(0.0%)	
RF negative	**Anti –PADI4 +ve**	2(100.0%)	9(75.0%)	0.4
	Anti –PADI4 –ve	0(0.0%)	3(25.0%)	

ACCP: anti-cyclic citrullinated peptide, anti-PADI4: anti-peptidyl arginine deaminase antibody and RF: rheumatoid factor.

Table (4): Associations of RA patients regarding the Positivity of ACCP and Serum Anti-PADI4.

			ACCP			Anti-PADI4		
			Negative N=20	**Positive N=30**	**P. value**	**Negative N=3**	**Positive N=47**	**P. value**
Demographic data	Age		35.9±10.4	41.5±8.9	0.05*	47.0±6.9	38.7±9.8	0.2
	Sex	Female	20(100.0%)	23(76.7%)	0.02*	10(77.0%)	30(81.1%)	0.2
		Male	0(0.0%)	7(23.3%)		3(23.0%)	7(18.9%)	
	Durations/years		2.5(1.0- 6.8)	3.0(2.0- 9.0)	0.1	2.0(2.0- 2.0)	3.0(2.0- 8.0)	0.6
Laboratory investigations	ESR		19.0 (12.0- 24.8)	21.0 (9.5- 25.0)	0.9	10.0 (3.0- 16.0)	22.0 (13.0- 25.0)	0.1
	CRP	Negative	12(60.0%)	12(40.0%)	0.2	6(46.2%)	18(48.6%)	0.8
		Positive	8(40.0%)	18(60.0%)		7(53.8%)	19(51.4%)	
	CRP Titer		4.0(3.4- 12.7)	5.7(3.4- 6.0)	0.5	3.5(2.5- 5.0)	5.3(3.4- 10.0)	0.1
	RF	Negative	12(60.0%)	2(6.7%)	0.001**	10(76.9%)	6(16.2%)	0.001**
		Positive	8(40.0%)	28(93.3%)		3(23.1%)	31(83.8%)	
	RF Titer		10.4 (10.0- 297.0)	184.0 (54.5- 227.8)	0.01*	10.7 (10.0- 18.0)	184.0 (20.0- 256.0)	0.01*
Clinical findings	Morning stiffness/ min		15.0 (10.0- 30.0)	15.0 (5.0- 30.0)	0.9	1.0 (0.5- 2.0)	15.0 (10.0- 30.0)	0.1
	Number of Tender joints		4.0(3.3- 10.0)	4.0(2.8- 9.3)	0.3	4.0(1.5- 8.9)	5.0(3.0- 10.0)	0.7
	Number of		2.0 (1.0 -	2.0(0.0- 4.0)	0.6	1.0(1.0- 3.0)	2.0(1.0- 4.0)	0.08

	Swollen joints	2.0)					
DAS28		3.9±1.2	4.1±1.4	0.6	3.4±0.9	4.1±1.3	0.1
ACCP Titer		16.2 (11.3- 17.0)	190.5 (90.0- 451.0)	0.001**	8.9 (5.0- 19.5)	90.0 (17.0- 200.0)	0.001**
PAD4 Titer Ng/ml		11.9 (11.5- 13.5)	38.5 (34.9- 40.7)	0.001**	7.9 (7.7- 8.0)	35.9 (12.2- 39.7)	0.001**

Age and DAS2 are represented as Mean ± SD; the data were analyzed by student t test. While Sex, CRP and RF are represented as F (%) frequency and percent; the data were analyzed by X^2 test. But Durations, ESR, CRP Titer, RF Titer, Morning stiffness, Number of Tender joints, Number of Swollen joints, ACCP and PADI4 are represented as Median with Interquartile range (25% -75%), the data were analyzed by Mann-whitney U

Table (5):- Correlation between serum antiPAD4, ACCP and different disease characters of RA patients.

	ACCP		Anti-PADI4	
	Correlation Coefficient	P. value	Correlation Coefficient	P. value
Age	0.170	0.237	0.319*	0.024
Duration / year	0.129	0.372	-0.030	0.837
ESR	0.027	0.851	0.144	0.320
CRP Titer	0.098	0.498	0.105	0.469
RF Titer	0.351*	0.012	0.367*	0.011
Duration of morning stiffness	0.074	0.608	0.111	0.441
Tender J C	-0.016	0.913	0.009	0.952
Swollen joints	0.069	0.635	0.063	0.663
DAS28	0.161	0.263	0.158	0.272
Anti-PADI4 level	0.792**	0.001		
ACCP serum level	-	-	0.853**	0.001

*. Correlation is significant at the 0.05 level. **. Correlation is significant at the 0.01 level.

V. DISCUSSION

PADI4 has a significant functionin RA pathogenesis and progression. PADI4 expressing white blood cells are recruited to inflammation sites, become activated, and release PADI4 to the surrounding tissue. The enzymes can then citrullinate extracellular proteins, creating neoantigens for which an immune response is mounted in those who are genetically pre-disposed to RA [14].

we aimed to assess the role of serum Anti-PADI4 Abs. compared to ACCP level in RA patients.

The ACCP serum level was with 80.0% sensitivity and88.0% specificity, 87.0%PPV, 81.5%NPV and84.0%accuracy.

While ***Reyes-Castillo et al.*** [15], showed that ACCP antibodies were detected in RA patients in a percentage of 74%, with 100%specificity in their studies.

In other studies and meta-analyses, the reported ACCP sensitivity for diagnosingRA and reported specificity werein the range of 60-83% and 90-98%, respectively. Many differences were observedin patients' characteristicsassessedand cutoff value utilizedto define a positive test. Such differences could elucidate the wide range of sensitivity and specificity of the reported results [16].

In our study, we found higher Anti-PADI4 Abs. level in the RA group with a median of 31.6 with interquartile of (12.1-39.6) ng/ml compared with control group,2.3(1.9-3.7) ng/ml, with a statistically significant difference between both groups (P<0.001).This finding suggests that Anti-PADI4 Abs. holdsgreatpotentialin RApathogenesis. All RA patients were above cut-off value with significant area under curve with cutoff >8.0.

Our study was also in agreement with the study done by ***Ishigami et al.*** [17],32 RA patients and 20 healthy controls and found that PADI4 levels were 0.89 ± 1.12 ng/ml in RA patients and 0.33 ± 0.12 ng/ml in healthy controls. Significant differences were detected between RA as well ashealthy controls ($p < 0.01$).

In our study, Anti-PADI4 revealedsensitivity of97.0%, specificity of90.0%, PPV of 90.9%, NPV of 97.0% with accuracy of 95.0%.

Contradictory to our work,***El-Hallous et al.*** [18] reported that Anti-PADI4 demonstrated 60%sensitivity and 95%specificity.

Umeda et al. [4]reported thatsensitivity of Anti-PADI4 in serum of RA patients was 19.6%, and the disease specificity was 88.5% when compared with healthy controls and SLE patients.

In the present work, when we correlated Anti-PADI4 with the disease activity parameters, we observed no significant correlations between Anti-PADI4 and morning stiffness, tender jointsnumber, swollen jointsnumber, PGA, DAS28, CRP and ESR in RA patients (p >0.05).

These results were consistent with the study done by ***Umeda et al.***[4],who found that Anti-PADI4 levels were not correlated with CRP, ESR, DAS28and with clinical findings. While **Qian *et al.*** [19]reportedthat Anti-

PADI4 level in RA patients was positively correlated with DSA28, ESR (r = 0.24, P = 0.03; r = 0.23, P = 0.03), respectively. However,no positive correlations were found with number of tender joints or swollen joints.

Our results were in disagreement with the study done by **El-Hallous et al**.[18], who reported a significant positive association between serum level of anti-PAD4 antibodies and DAS 28 score as a disease activity measure.

In our study, we found a significant positive correlation when we correlated Anti-PADI4 Abs. with RF and ACCP level.

This agrees with **El-Hallous et al.**[18], who reported that anti-PAD4 antibodies showcased a strong positive association with conventional ACCP diagnostic marker.

Also, **Reyes-Castillo et al.**[15] reported that anti-PAD4 antibodies showed a marginal correlation with ACCP (rs =0.15, P=0.05).

However, such a result was in disagreement with **Qian et al**.[19] and **Umeda et al.** [4], who studied the correlation between serum Anti-PADI4 and several ACPAs in patients with RA, and reported that Anti-PADI4 levels were not correlated to RF and ACCP Abs.

In our study, we observed that 3 of 50 (6%) patients with RA are anti-PADI4 negative, while 20 of 50 (40%) RA patients are anti-CCP negative, and 17 of them are anti-PADI4-positive. This agrees with **El-Hallous et al.** [18], who detected that 5 of 40 (12.5%) RA patients are ACCP negative, while 4 of them are anti-PAD4-positive.

Such findings manifested that antiPAD4 may be valuable in diagnosing RA patients lacking ACCP.

VI. CONCLUSION

Anti-PADI4 represents a useful serologic diagnostic marker in RA patients especially in the presence of ACCP.

AntiPAD4 may be helpful for the diagnosis of RA patients lacking ACCP.

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