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DIAGNOSTIC VALUE OF ANTI-PEPTIDYLARGININE DEIMINASE TYPE 4 (PADI-4) AND ANTI-CITRULLINATED PEPTIDE ANTIBODIES (ACCP) IN IRAQI PATIENTS WITH RHEUMATOID ARTHRITIS

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Abstract

This study aimed to examine the value of anti peptidylarginine deiminase (anti-PADI-4) antibody and anti-cyclic citrullinated peptide (anti-CCP) antibody among Iraqi patients with RA and to determine whether the activity and severity in RA patients are associated with anti-PADI-4 and anti-CCP antibodies positivity.

In a case control study, we determined the seropositivity of these two serological markers anti-PADI-4 and anti-CCP antibodies in100 RA patients and 100 healthy controls subject. Activity of disease was measured by disease activity score in 28 joints (DAS 28) and severity was assessed using Scott modification of the Larsen method.

Antibodies to PADI-4 were detected in (32%) among RA patients and present (1%) in controls ($p < 0.001$). Anti-CCP was present in (74%) of the RA patients and present in 2% in controls ($p < 0.001$), sensitivity was highest for anti-CCP antibody (74%) followed by anti-PADI-4 antibody (32%). Specificity was highest for anti-PADI-4 antibody (99%) followed by anti-CCP antibody (98%). A significant correlation with disease activity was observed in both markers, RA patients on remission had negative PADI-4 test (0/12) but patients with high disease activity showed higher percentage (86.4%) of PADI-4 seropositivity in compared to anti-CCP where patients on remission had (8.3%) seropositivity and RA patients with high disease activity had (91%) anti-CCP seropositivity. However, seropostivity to PADI-4 was significant correlated with RA severity ($p < 0.001$) as evaluated by scott grade (59.3%) in grade 5. Similarly, there were a significant correlation between anti-CCP seropositivity and radiological finding but less than that associated with seropositivity to PADI-4 antibody ($p < 0.05$).

In conclusion, with their excellent specificity, both anti-PADI-4 and anti-CCP antibodies can be useful in establishing the diagnosis of RA, also both markers are good predictors of disease activity and severity.

Introduction

Rheumatoid arthritis (RA) is a systemic, chronic inflammatory disease that is manifested as a destructive polyarthritis in association with serological evidence of auto-reactivity. It is characterized by chronic pain and joint destruction, premature mortality, and elevated risk of disability, with high costs for those suffering from this disease and for the society[1]. It affects up to 0.5%–1% of the world's population, with a male-to-female ratio of 3:1, and is the most common inflammatory joint disease. The onset of disease can occur at any age; however, the prevalence increases with age and the peak incidence is between the fourth and the sixth decade (Abdel-Nasser et al., 1997)[2]. In the Gulf region, three studies estimated the prevalence of RA to be 1% in the Iraqi population[3], 0.36% in the Omani population[4], and 0.22% in the Saudi Arabia population[5].

Clinically, RA is symmetrical polyarticular arthritis marked by chronic

systemic inflammation, synovial infiltrates and progressive cell-mediated destruction of the joints and their adjacent chronic inflammation of the synovium along with various clinical features of a systemic disease. The disease is characterized by persistent and progressive synovitis of peripheral joints, leading to destruction of cartilage and subchondral bone. The pathogenic basis of RA is a sustained specific immune response against yet unknown self-antigens. It is believed that in RA, the persistent autoimmune response mediates local synovial inflammation and cellular infiltration, which ultimately result in tissue damage[1]. Peptidylarginine deiminase type IV (PADI-4) is one member of PADI gene family coding for enzymes responsible for the posttranslational conversion of arginine residues into citrulline[6]. The most critical auto-antibodies in RA are directed at citrullin residues on proteins such as fibrin, filaggrin and vimentin. Noncitrullinated proteins can also be autoantibody targets in RA[7]. Recently developed citrullinated protein antibodies (ACPA) have been documented extensively over recent years as highly specific serological markers for rheumatoid arthritis, with important clinical implication for diagnosis and prognosis[8]. Although the pathophysiology of ACPA induction and the role of these antibodies in the pathogenesis of rheumatoid arthritis remains to be elucidated, it has been shown that posttranslational modification of arginine containing epitopes by deimination (citrullination) is a crucial step in the generation of antigenic targets for ACPA[9,10]. The citrullination process is mediated by the peptidylarginine deiminase (PAD) enzymes, of which subtypes 2 and 4 are found in human synovial tissue, the primary disease target of rheumatoid arthritis[11]. Autoantibodies against peptidylarginine deiminase type 4 (PAD-4) have recently been described as a specific biomarker in patients with clinically apparent RA[12]. Recently, researchers demonstrated the presence of specific anti–PAD-4 antibodies in patients with RA, as well as an association with disease severity[13-15]. However, the role of PADI-4 in the development of RA has not been fully elucidated.

The present study is designed to evaluate the diagnostic value of PADI-4 and anti-CCP in Iraqi RA patients and to determine whether these biomarker are associated with disease activity and severity.

Patients and methods

This case control study was carried out during the period from September 2012 till June 2013. A total of 100 unselected RA patients male to female proportion of 1:4, mean age 47.96 years (range 18 to 70), disease duration 7.6 years (range 0.1 to 40), who were diagnosed as Rheumatoid arthritis case according to fulfilling the revised 1987 ACR criteria, then blood sample was drawn from each patient. The unselected RA patients was made consecutively, taking into account the Disease Activity Score of 28 joints (DAS 28) in order to include patients with all levels of disease activity. We used the EULAR activity criteria, based on the DAS 28 (clinical remission values below 2.6, low activity between 2.6 to 3.2, moderate activity from 3.2 to 5.1 and high activity values over 5.1) as we wanted to explore the relationship among anti-CCP and PADI-4 antibodies and DAS-28. Severity of the disease was measured as described by Scott modification of the Larsen method[16,17]. Posteroanterior radiographs (x ray) of multiple joints in the hands, wrists, and feet are evaluated (the following joints are generally counted: PIP, MCP, MTP, IP, and joints of the wrist). Each joint is given a grade between 0 and 5, with 0 representing a normal joint. Grade 1 reflects slight, early, or non-specific findings of RA. Periarticular osteopenia/joint swelling must be major, and /or suggested erosions /cysts at two sites in the joint must be

smaller than 1 mm. Grade 2 reflects a definite early abnormality; one or more erosions larger than 1mm must be present, with a break in the cortical margin. Grade 3 reflects medium destructive abnormality; erosions at both sides of the joint must be of significant size with preservation of some joint surface. Grade 4 reflects severe abnormality; subluxation must be present. Grade 5 reflects mutilating abnormalities. One hundred healthy subjects with male to female proportion of 1:3, mean age 49.5 years (range 28 to 70), who were matched by age, sex, occupation, residency and level of education to cases. The control group collected from general population in Al-Basra government (school student, medical personnel, from blood donors) with no history of chronic disease and normal at physical examination were consecutively sent to our laboratory for the detection of serum RF, ACCP, PADI-4 antibodies. Rheumatoid factor and CRP were detected by slide agglutination test as described by the manufacturer,s instruction using specialized kits provided by plasmatic, UK. ACCP and PADI-4 antibodies were detected by ELISA from AESKU. Diagnostics, Germany, and ESR were estimated by the use of Westergren method.

Statistical analysis was performed using the Statistical Package for the social sciences (SPSS 15). Chi-square test was used for testing the significance of association between anti-CCP antibodies and PADI-4 antibodies. Significance was assumed at $P < 0.05$ and highly significance at $p < 0.001$, when at least one cell of chi- square table less than five use fisher's exact test for testing the significant. Sensitivity, specificity and predictive values were calculated according to the appropriate formula.

Results

Some of the socio-demographic characteristic including age, gender, level of education, residency and occupation of 100 patients with rheumatoid arthritis and 100 healthy control subject. Table 1 The age of study group ranged from 18-70 years with (mean ± SD 48.14 ± 12.96). For comparison purposes, the study population was grouped into three groups; less than 30 year, 30-50 year and above 50 year. According to the age in the RA patients (study group), the results showed that the highest percentage of patients was in age group of above 50 years (48%) followed by 30-50 years (38%) and least in those less than 30 years (14%), similarly the age group above 50 years old represent the highest percentage among control group 48%. The sex distribution showed that females were more common than males in all the study population groups among RA patient (82%). There are no significant differences in sex distribution between patients and control in the study with p value > 0.05. Most of study population came from an urban background, (78%) of RA patients and (22%) of RA patients were from rural areas with no significant differences in distribution from those in control group with p value > 0.05.Table I.

The seropositivity to Anti- CCP antibody as estimated by ELISA was (74%) among RA patients compared to 2% only among healthy control subject. Sera tested for detection of anti- PADI-4 antibody by the use of ELISA showed that (32%) were positive among RA patients compared to (1%) positive in healthy control subject. By the use of latex agglutination test (82%) of RA were positive for RF compared to (6%) were seropositive among healthy control subject. There were highly significant differences between RA patients and control groups for all the three parameter tested ($p < 0.001$). Table II The relation between the seropositivity of serological biomarker (RF, ACCP, PADI-4 antibodies) and disease activity (DAS-28) among RA patients, where DAS -28 showed highly significant correlation to seropsitivity of ACCP and PADI-4 antibodies ($P < 0.001$) which is

more evident with PADI-4 antibodies where patient on remission had negative PADI-4 test (0/12) but highly active RA patients showed high percentage of seropositivity (86.4%) in comparison to ACCP seropositive (8.3%) among patients on remission and in (91%) among highly active disease group. Table III.

The presence of antibodies to RA, ACCP, PADI-4 are not significantly correlated to duration of disease ($p > 0.05$). However, PADI-4 seropositivity was the least related to duration of illness as percentages among those of < 2 years and those of greater than 10 years almost the same; 30%, and 30.5% respectively. Table IV. The relation between the serological marker, RF, ACCP, PADI-4 antibodies and the radiological finding as estimated by scott modification of the Larsen method grade among RA patients where the seropositivity to PADI-4 antibody highly correlated with the severity of RA ($p<0.001$). There were no or low percentages of RA patient in first four grades 0%, 16.7%, 5%, and 12.5% respectively and high percentage of RA patient was found in grade 5 (59.3%). However, the other two marker also significantly correlated to radiological finding but to less extent than that with PADI-4 antibody ($p< 0.05$).Table V.

The validity and ability of serological biomarker to predict the positive and negative cases through measurement of variation in sensitivity, specificity and both positive and negative predictive value (PPV, NPV). Both PADI-4, ACCP antibodies tested showed high specificity among RA patients 99%, 98% respectively and powerful prediction ability to detect RA cases in tested population 97%, 97.3% respectively, but both test with low sensitivity compared to RF test, 32%, 74% respectively, versus 82% to RF. Table VI.

Discussion

The prevalence of Rheumatoid Arthritis in Iraq reported to be 1%.3 there is growing evidence that diagnosis and prompt therapeutic intervention in the course of RA represent an important tool to obtain more efficient disease control, less long-term joint damage and subsequent functional disability and better disease outcome. This study showed the predominant age of RA patients was in the fifty years old and above, this agreed with several studies, from whom Abdel-Nasser et al., 1997 found the peak incidence is between the fourth and the sixth decade.2 Although RA can affect any age group, its onset has reproducibly been shown to peak during the sixth decade of life[18,19]. This may result from immune senescence with the decline in host immunity with advancing age promoting immune reactivity to self-antigens[20].

Serological studies of PADI-4 antibody in RA in Arab population are rare. Only a study in Egypt Zagazig University on PADI-4 polymorphisms and related haplotype in rheumatoid arthritis patients was reported[21]. So far, this study is first about the serological anti-PADI-4 antibody in RA patients, at 2008 my colleague study the diagnostic value of antibodies to citrulline containing peptide in patients with RA in Nassiriyah, Iraq[22]. The current study is the attempt to present the validity of PADI-4 and ACCP antibodies among RA patients in Basra, South Iraq with outcome in the activity and severity of RA disease.

We have found that autoantibodies against the PAD-4 enzyme are present in (32%) among RA patients (OR = 2.38, 95% CI 1.96-2.88). Other studies reported approximately (23%) of Caucasian RA patients have serum IgG antibodies against hPAD4[23] and in the Japanese RA patients the serum anti-PAD4 was reported in 21 out of 42[24], the different frequency of anti-PAD4 in that study may be due to differences in disease phenotype or genetic background. The seropositivity of anti-CCP and anti-PADI-4 showed highly significant correlation to disease activity among RA patients as estimated

by DAS-28 ($p < 0.001$). These observation is more evident with anti-PADI-4 test where all patients on remission had negative anti PADI-4 test but those with highly active RA patients showed higher percentage of seropositivity to anti-PADI-4 (86.4%), compared to (8.5%) of anti-CCP seropositive patients on remission and 91% among patients with highly active disease. Accordingly, the present study reveals that the disease activity tends to increase in cases where anti-PADI-4 and anti-CCP test results were positive. However, no any other study was found demon-strating the presence of specific anti-PADI-4 antibodies in patients with RA relating this biomarker to disease activity, so this study may refer to this high association between anti-PADI-4 and anti-CCP test and disease activity of RA patients. On the other hand, this study agreed with a study showed a median of disease duration of 12 years where the activity was greater in patients who had anti-CCP antibodies than in anti-CCP negative patients[25]. Two other studies found an association between the presence of anti-CCP and higher scores for the swollen joint, erythrocyte sedimentation rate (ESR) and C - Reactive protein[26,27], that almost agreed with our results.

The current study showed that the seropostivity to anti- PADI-4 and anti-CCP test are not significantly correlated to disease duration were (p value > 0.05), the seropositivity of anti -PADI-4 test is less related than anti-CCP test to variability of symptom duration among RA patients, where the percentage at duration of < 2 year and > 10 year almost similar (30%,30.5% respectively) This is in agreement with a study an RA patients in whom serum anti-hPAD4 IgG levels were stable over 10 years. However patients who were negative for anti-PAD4 IgG at baseline had become positive after 10 years[28].

The seropositivity to anti-PADI-4 showed highly significant correlation with the severity of RA disease ($p < 0.001$), there were no or low percentages of Scott modification of the Larsen method in first four grade 0%, 16.7%,5%, 12.5% respectively, which high percentage of Scott Score associated with in the grade 5 (59.3%).The anti CCP test also significantly correlated to radiological finding but less than that with PADI-4 antibody ($p < 0.05$). Our results showed that serum anti-PAD4 is rather infrequent, but it may contribute to a more severe disease. Recently studies a large RA cohort showed that the presence of anti-hPAD4 auto-antibodies at baseline is associated with radiological damage after 10 years[29]. It is not known why serum anti- PADI-4 antibodies are associated with a more aggressive disease course. The present study is in agreement to a study found that anti-CCP predict structural damage which was evaluated in 273 patients with RA of less than 1 year's duration[30] and Positive anti-CCP tests were associated with greater structural damage after 6 years. However, the association of radiological finding to anti-PADI-4 observed in this study was not reported before.

Both serological biomarkers anti PADI-4 anti-CCP in Iraqi RA patients showed high specificity than RF (99%, 98% and 94% respectively) but less sensitivity (32%, 74% and 82% respectively). These results are in agreement with another study where 18.1% of RA was positive for anti- PADI-4 and 1.2% of control subjects were positive for anti PADI-4 test, resulting in sensitivity and specificity of18.1% and 98.8% respectively[31]. However, our results are comparable to the results of anti-CCP reported by previous Iraqi study where the sensitivity was 61.1% and specificity of 97.6%. The positive predictive value and negative predictive value were reported to be 90.2% and 90.3% respectively which is lower than that observed in our study (97%, and 97.3% respectively). When we consider the sensitivity of anti- CCP those varies with peptides used as antigens. First

generation ELISA kit in which walls are coated with single CCP have sensitivity of 41-68%, where second generation ones coated with many CCPs raised the sensitivity to 75-94%. In addition the characteristic of patients studied and cut-off values differed widely from a study to other even when tests were provided by the same manufacture[32]. From there observation, we can conclude that PADI-4 has a very high specificity and powerful prediction ability to a sign early the RA cases.

Table I: General characteristic of patients with Rheumatoid Arthritis as compared to the healthy controls subject.

P value	Total		Control		Patient		Characteristic
	%	No.	%	No.	%	No.	
							Age (year)
0.138	10.0	20	6.0	6	14.0	14	<30
	42.0	84	46.0	46	38.0	38	30-50
	48.0	96	48.0	48	48.0	48	>50
			49.5±9.52		48.14±12.98		Mean ± SD
							Sex
0.381	20.5	41	23.0	23	18.0	18	Male
	79.5	159	77.0	77	82.0	82	Female
							Residency
0.411	75.5	151	73.0	73	78.0	78	Urban
	24.5	49	27.0	27	22.0	22	Rural
							Education
0.491	22.0	44	20.0	20	24.0	24	Illiterate
	32.0	64	35.0	35	29.0	29	Primary
	13.0	26	16.0	16	10.0	10	Intermediate
	18.5	37	17.0	17	20.0	20	Secondary
	14.5	29	12.0	12	17.0	17	University and more
							Occupation
0.567	42.0	84	40.0	40	44.0	44	Employed
	58.0	116	60.0	60	56.0	56	Unemployed

Table II: Serological parameters in patients with Rheumatoid Arthritis and healthy control.

95% confidence interval	Odds ratio	P value	Total		Control		Patient		Test
			%	No.	%	No.	%	No.	Result
									RF
3.78-8.89	5.79	< 0.001	44.0	88	6.0	6	82.0	82	Positive
			56.0	112	94.0	94	18.0	18	Negative
									ACCP
3.29-6.54	4.64	< 0.001	38.0	76	2.0	2	74.0	74	Positive
			62.0	124	98.0	98	26.0	26	Negative
									PADI-4
1.96-2.88	2.38	< 0.001	16.5	33	1.0	1	32.0	32	Positive
			83.5	167	99.0	99	68.0	68	Negative

Table III: Seropositivity of serological biomarker in relation to disease activity parameter of RA patients.

Disease activity Level (DAS-28)								Test
High		Moderate		Low		Remission		Result
<5.1		3.2-5.1		2.6-3.2		>26		
%	No.	%	No.	%	No.	%	No.	
								RF
72.7	16	90.2	37	88	22	58.3	7	Positive
27.3	6	9.8	4	12	3	41.4	5	Negative
100	22	100	41	100	25	100	12	Total
								ACPA
91.0	20	83.0	34	76.0	19	8.3	1	Positive
9.0	2	17.0	7	24.0	6	91.7	11	Negative
100	22	100	41	100	25	100	12	Total
								PADI-4
86.4	19	29.3	12	4	1	0.0	0.0	Positive
13.6	3	70.7	29	96	24	100	12	Negative
100	22	100	41	100	25	100	12	Total

Table IV: Serological biomarker of RA patients in relation to duration of RA disease:

P value	Disease duration (year)						Test result
	>10		2-10		<2		
	%	No.	%	No.	%	No.	
							RF
0.283	87.0	20	84.2	48	70.0	14	Positive
	13.0	3	15.8	9	30.0	6	Negative
	100	23	100	57	100	20	Total
							ACPA
0.571	78.2	18	75.5	43	65	13	Positive
	21.8	5	24.5	14	35	7	Negative
	100	23	100	57	100	20	Total
							PADI-4
0.947	30.5	7	33.3	19	30.0	6	Positive

Table V: Seropositivity of serological marker in relation to radiological finding among RA patients:

P value	Disease duration (year)												Test Result
	Grade 5		Grade 4		Grade 3		Grade 2		Grade 1		Grade 0		
	%	No	%	No	%	No	%	No	%	No	%	No	
													RF
<0.05*	81.5	22	93.3	28	93.8	15	65.0	13	66.7	4	0.0	0	+ve
	18.5	5	6.3	2	6.3	1	35.0	7	33.3	2	100	1	-ve
	100	27	100	30	100	16	100	20	100	6	100	1	Total
													ACPA
<0.05*	81.5	22	86.7	26	81.3	13	50.0	10	50.0	3	0.0	0	+ve
	18.5	5	13.3	4	18.8	3	50.0	10	50.0	3	100	1	-ve
	100	27	100	30	100	16	100	20	100	6	100	1	Total
													PADI-4
<0.001*	59.3	16	40	12	12.5	2	5.0	1	16.7	1	0.0	0	+ve
	40.7	11	60.0	18	87.5	14	95.0	19	83.3	5	100	1	-ve
	100	27	100	30	100	16	100	20	100	6	100	1	Total

Fishers exact test

Table VI: Comparison of the sensitivity, specificity between serological biomarker use in diagnosis of RA

**NPV	*PPV	Specificity	Sensitivity	Test
%	%	%	%	
83.0	93.0	94.0	82.0	RF
79.0	97.3	98.0	74.0	ACCP
59.2	97.0	99.0	32.0	PADI-4

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