Original Article

The Contribution of Serum Anti–cyclic Citrullinated Peptide Antibody and Matrix Metalloproteinase-3 in Predicting the Activity of Rheumatoid Arthritis Disease

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Abstract:

Background: Rheumatoid arthritis is an autoimmune disease characterized by chronic synovial inflammation. The insufficient immune clearance of the apoptotic cell results into the formation of anti-cyclic citrullinated peptide antibodies which may play a critical role in the initiations of inflammatory responses. These antibodies together with Matrix Metalloproteinase-3 play an important role in joint destruction in rheumatoid arthritis disease.

Objectives: to study the value of anti-cyclic citrullinated peptide antibodies, and Matrix Metalloproteinase-3 in differentiation between active and inactive rheumatoid arthritis.

Patients and Methods: A cross- sectional study was conducted on 60 Iraqi patients with rheumatoid arthritis (16 males and 44 females) aging from 29 to 74 years who presented to the Rheumatology Consultation Clinic of Baghdad Teaching Hospital / Medical City during the period of July 2013 to the end of October 2013. The patients were divided, according to rheumatoid arthritis activity depending on Disease Activity Score 28 and erythrocyte sedimentation rate, into two groups: 30 patients with active rheumatoid arthritis and the other 30 patients with inactive rheumatoid arthritis. Quantitative sandwich enzyme immunoassay technique was used to measure serum level of anti-cyclic citrullinated peptide antibodies, and Matrix Metalloproteinase-3.

Results: The means concentration of anti-cyclic citrullinated peptide antibodies, and Matrix Metalloproteinase-3 were significantly higher in rheumatoid arthritis cases with active disease compared to those with inactive disease. The disease activity index DAS28 showed a statistically significant strong positive (direct) linear correlation with serum MMP3 (r=0.762) and serum ACCP (r=0.806). In addition, serum MMP3 showed a statistically significant moderately strong positive (direct) linear correlation with serum ACCP (r=0.64). Both serum MMP3 and ACCP had a higher validity than blood ESR (which is used itself in calculating DAS28) is predicting an active disease status.

Conclusion: Anti-cyclic citrullinated peptide antibodies and Matrix Metalloproteinase-3 could be useful biological markers for assessment of rheumatoid arthritis disease activity.

Key words: Rheumatoid Arthritis, Anti–cyclic citrullinated Peptide Antibody, and Serum Matrix Metalloproteinase-

Introduction:

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic synovial inflammation, leading to destruction of joint cartilage and bone resulting into significant disability. Although the exact etiology was not determined till now, it is thought that RA results from a breach in immune tolerance, where T cell responses to several joint-associated autoantigens, even including 'altered self ' citrullinated peptides can be detected in a proportion of RA patients [1-3]. Moreover, the function of peripheral blood regulatory T cells has been found to be impaired in RA patients with active disease [4].

Regarding the genetic background for this disease, the strongest genetic association with RA is the human leucocyte antigen (HLA), particularly the HLA-DRB1 'shared epitope' [5]. On the other hand, various environmental factors have also been implicated in the etiology of RA. These include the exposures to pollutants and silica [6] exposure to Parvovirus B19 [7]. Moreover, smoking has been associated with increase in the risk and severity of RA [8]. This possibly because smoking may induce citrullination of peptides that leads in turns to the formation of anti-cyclic citrullinated peptide antibodies (ACCP) [9]. However, ACCP was discovered in 1998, where it has been found that patients with RA produce antibodies against peptides and proteins containing citrulline, a modified form of the amino acid arginine [10]. Anyhow, during inflammation, arginine amino acid residues can be enzymatically converted into citrulline residues in proteins such as vimentin, by a process called citrullination. If their shapes are significantly altered, the proteins may be seen as antigens by the immune system, thereby generating an immune response resulting in the production of ACCP [11].

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Regarding citrullination, it is a normal physiological process that occurs inside many dying cells of the body. The dying cell is ingested by macrophages in the specific clearance of apoptotic cells. When the clearance system is inefficient, peptidylarginine deiminase (PAD) enzymes and citrullinated proteins can leak from the necrotizing cell and 'meet' the immune system. The released PAD enzymes will citrullinate many extracellular proteins containing arginine, thus creating a large pool of citrullinated antigens [12].The generation of ACCP is major histocompatibility complex dependent, where the conversion of arginine into citrulline generates 'altered self ' peptides that can be bound and presented by DRB1*1001 [1]. The ACCPs have proved to be powerful biomarkers that allow the diagnosis of RA to be made at a very early stage [13].

Another biomarker that plays an important role in joint destruction in RA is the matrix metalloproteinase-3 (MMP-3) [14]. The MMP-3 is a member of matrix-metalloproteinases family and it is produced by articular synovial cells, fibroblasts and chondroblasts. The MMP-3 is the predominant metalloproteinase synthesized in the human articular cartilage [15]. It also has a wide range of substrates including collagens (III–V, and IX), gelatin, , decorin, laminin, elastin, casein, osteonectin, entactin, plasminogen [16], and it activates other degrading enzymes such as procollagenase and progelatinase B [17].

In RA patients, MMP-3 is over expressed, therefore their synovial fluid contains large amounts of MMP- 3 and, accordingly, serum MMP-3 levels of these patients are also highly elevated and correlate with the amount of MMP-3 in the synovial fluid. Many studies have demonstrated that MMP-3 levels rose as joints were increasingly affected and destroyed in RA patients, and that MMP-3 elevation in serum represents disease activity regardless of age or disease duration. This led to the conclusion that serum MMP-3 is a useful marker of inflammatory activity in the joints of RA patients [18]. Thus, using MMP- 3 possibility can predict Anti-cyclic citrullinated peptide antibodies (ACCP) negative patients, and specifying prognosis in ACCP positive patients [19].

Methods:

This study was conducted at the Rheumatology Consultation Clinic of Baghdad Teaching Hospital / Medical City during the period of July 2013 to the end of October 2013, where 60 Iraqi patients with RA (16 males and 44 females) aging from 29 to 74 years have been submitted to a cross- sectional study. Ethical permission to conduct the research was obtained from rheumatology unit /department of medicine and a signed consent was taken from all patients. Selection of the patients was accomplished with assistance of rheumatology specialists in the department. The patients were divided, according to RA activity depending on Disease Activity Score 28 (DAS28) and erythrocyte sedimentation rate (ESR), into two groups: 30 patients with active RA and the other 30 patients with inactive RA.

Quantitative sandwich enzyme immunoassay technique was used to measure serum level of ACCP and MMP-3. A standard curves were created for each of the two biomarkers by reducing the data using software capable of generating a four parameter logistic (4-PL) curve-fit by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph.

Statistical Analysis:

Data were translated into a computerized data base structure. An expert statistical advice was sought for. Statistical analysis was done using statistical package for social sciences version 18 (SPSS V.18, Chicago, IL, USA). Chi square test and Student's T test were used to test the association between discrete variables. Regression analysis was used to examine the association of ACCP and MMP-3 with other variables. P value less than 0.05 were considered significant. A cut off value for ACCP and MMP-3 to identify the disease activity was determined by using 95% confidence interval.

Results:

The results were based on the analysis of 60 cases with RA. The age ranged between 29 and 74 years with a mean +/- standard deviation of 50+/-10 years. The disease activity index DAS28 ranged between a minimum of 1.7 and a maximum of 6.6 with a mean +/- standard deviation of 3.8+/-1.3. Female patients constituted 73.3% of the cases with a female to male ratio of 2.8:1. The study sample was equally divided according to DAS28 into cases with active disease (n=30) and those with inactive disease (n=30).

There was no obvious or statistically significant difference in mean age between cases with active and inactive RA. The mean ESR was significantly higher (37.3 mm/hour) in RA cases with active disease compared to those with inactive disease (21.2 mm/hour). The mean ACCP was significantly higher (190.7 IU/ml) in RA cases with active disease compared to those with inactive disease (88.3 IU/ml). In addition, the mean MMP3 was also significantly higher (515.6 Pg/ml) in RA cases with active disease compared to those with inactive disease (233Pg/ml), as demonstrated in table 1.

Table1: The difference in mean of selected measurements between RA cases with active and inactive disease.

Parameter	ESR (mm/hr)		ACCP (IU/ml)		MMP3 (Pg/ml)	
	Inactive	active	Inactive	Active	Inactive	Active
Range	(8.1 -37.8)	(8.1- 65.2)	(49-143.6)	(81.9-385)	(16-494.3)	(397.2-695.6)
Mean	21.2	37.3	88.3	190.7	233	515.6
SD	10.1	17	26.1	90.8	148.1	91.9
SE	1.9	3.1	4.8	16.6	27	16.8
N	30	30	30	30	30	30
P-value	<0.001		<0.001		<0.001	

A multiple linear regression model was used to study the net and independent effect of disease activity on each of serum ACCP and MMP3 after accounting for the possible effect of age and gender. Having an active disease is expected to increase serum ACCP by a mean of 102.3 compared to RA cases with inactive disease after adjusting for the possible confounding effect of age and gender. The other form of disease activity which is the original DAS28 was also tested for its effect on ACCP. For each one unit increase in DAS28 the serum ACCP is expected to increase by a mean of 51.3 after adjusting for the possible confounding effect of age and gender. Age and gender on the other hand had no important or statistically significant association with serum ACCP after adjusting for disease activity. The model was statistically significant and able to explain 37.8% of variation in the dependent variable (ACCP), table 2.

Table 2: Multiple linear regression model with serum ACCP as the dependent (outcome) variable and disease activity, age and gender as independent (explanatory) variables.

ACCP	Unstandardized Partial Regression Coefficient	P
(Constant)	85.6	0.08[NS]
Active disease compared to inactive	102.3	<0.001
Age in years	0.1	0.94[NS]
Male compared to female	-2.3	0.91[NS]
R2=0.378		
P (Model)<0.001		
DAS28	51.3	<0.001

Regarding MMP3, having an active disease is expected to increase serum MMP3 by a mean of 282.9 compared to RA cases with inactive disease after adjusting for the possible confounding effect of age and gender. The other form of disease activity which is the original DAS28 was also tested for its effect on MMP3. For each one unit increase in DAS28 the serum MMP3 is expected to increase by a mean of 108.6 after adjusting for the possible confounding effect of age and gender. Age and gender on the other hand had no important or statistically significant association with serum MMP3 after adjusting for disease activity. The model was statistically significant and able to explain 57.6% of variation in the dependent variable (MMP3), table 3.

Table 3: Multiple linear regression model with serum MMP3 as the dependent (outcome) variable and disease activity, age and gender as independent (explanatory) variables.

MMP3	Unstandardized Partial Regression Coefficient	P
(Constant)	239.6	0.009
Active disease compared to inactive	282.9	<0.001
Age in years	-0.2	0.93[NS]
Male compared to female	5.3	0.89[NS]
R2=0.576		
P (Model)<0.001		
DAS28	108.6	<0.001

As shown in figure 1, DAS28 showed a statistically significant strong positive linear correlation with serum ACCP (r=0.806) and serum MMP3 (r=0.762).

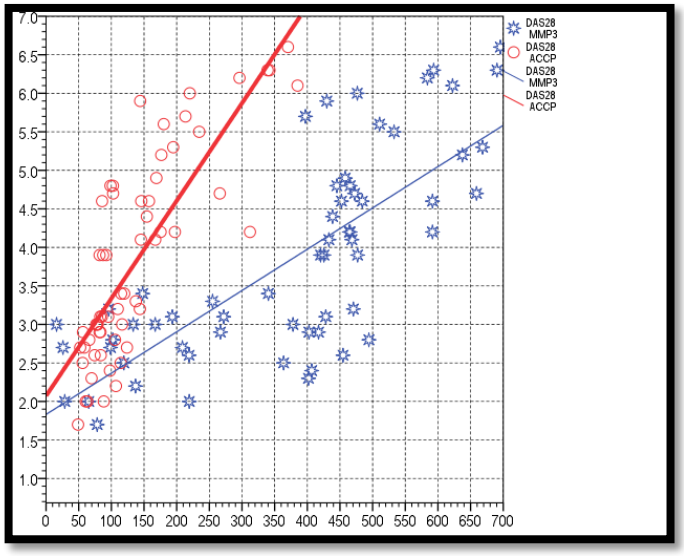

Figure 1: Scatter diagram with fitted regression line showing the linear correlation between DAS28 and each of ACCP and MMP3

In addition, serum ACCP showed a statistically significant moderately strong positive linear correlation with serum MMP3 (r=0.64), figure 2.

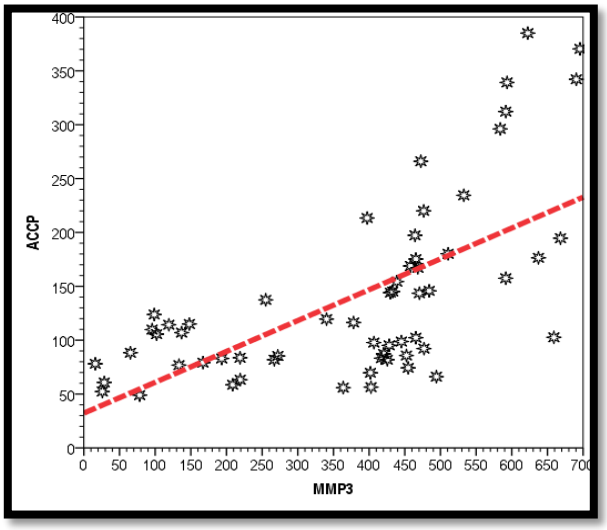

Figure 2: Scatter diagram with fitted regression line showing the linear correlation between ACCP and MMP3.

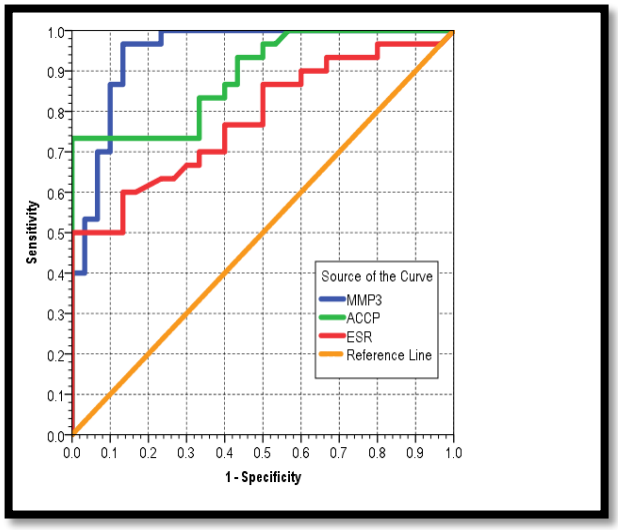

Figure 3: ROC curve showing the trade-off between sensitivity (rate of true positive) and 1-specificity (rate of false positive test) for selected measurements when used as tests to predict subjects with active disease among cases with RA

Serum ACCP, MMP3, and blood ESR were tested for their validity in predicting a subject with active disease among cases with RA as shown in figure 3 and table 4,5.

Table 4: ROC area for selected measurements when used as tests to predict subjects with active disease among cases with RA.

	AUROC	P
ACCP	0.889	<0.001
MMP3	0.947	<0.001
ESR	0.781	<0.001

Table 5: Validity parameters for selected measurements when used as tests to predict subjects with active disease among cases with RA.

Positive if ≥ cut-off	Sensitivity	Specificity	PPV at pretest Probability=		NPV at pretest Probability=10%
			50%	90%	
ACCP					
80.5 (Highest sensitivity)	100.0	43.3	63.8	94.1	100.0
143.9 (Optimum cut-off and highest specificity)	73.3	100.0	100.0	100.0	97.1
MMP3					
387.7(Highest sensitivity)	100.0	76.7	81.1	97.5	100.0
412.0 (Optimum cut-off)	96.7	86.7	87.9	98.5	99.6
502.5 (highest specificity)	40.0	100.0	100.0	100.0	93.8

Discussion:

In this study the mean age for RA patients was 50 years, which was in accordance with other studies as that of AL- Ubaidi et al. (2012), and Abdul-Wahid et al. (2013) [20,21] who mentioned that most of the patients with RA were above the age of 40 years. This could be due to the accumulation of many reasons that lead to decrease immunity, such as stress, thymic depression and increase in chance and duration of exposure to different antigens, drugs and chemicals as well as smoking, which lead to activation of auto-reactive lymphocytes that interact with self-antigens [22]. Regarding gender, This study showed a female to male ratio of 2.8:1 and this was in agreement with the finding of other studies which reported a higher prevalence of RA in female in comparison to male [20,21]. The female predominance may be due to the hormonal effect such as estrogen which enhances the function of T-helper cells and inhibits the function of T-suppressor cells. In addition, estrogen receptors have been found to

be present on the memory T-cells and on synovial cells [23].

There was no statistically significant difference in mean age between cases with active and inactive RA. This possibly indicate that disease activity is not affected by age. The mean ESR was significantly higher in RA cases with active disease compared to those with inactive disease. This because active disease is associated with inflammation and the ESR is a non-specific marker of inflammation [24]. The mean ACCP was significantly higher in RA cases with active disease compared to those with inactive disease. This was in accordance with the finding of another study which also reported an increase in ACCP concentrations in association with disease activity [25]. However, a multiple linear regression model in this study revealed that having an active disease is expected to increase serum ACCP by a mean of 102.3 compared to RA cases with inactive disease after adjusting for the possible confounding effect of age and gender. Furthermore, in this work there was a significant association between ACCP and DAS28 with strong positive correlation. This was similar to the finding of another study which reported a positive correlation between serum anti-CCP and DAS28 [26]. In this study, for each one unit increase in DAS28 the serum ACCP is expected to increase by a mean of 51.3 after adjusting for the possible confounding effect of age and gender. This indicates that serum ACCP level could be considered as a biological marker for the inflammatory state and disease activity of RA patients. Regarding MMP-3, its mean serum was significantly higher among patients with active RA than those with inactive disease, and this result was similar to the finding of another study which reported that patients with active RA had raised serum MMP-3 values while those with inactive RA had normal MMP-3 serum levels [27]. In this study, a multiple linear regression model revealed that having an active disease is expected to increase serum MMP3 by a mean of 282.9 compared to RA cases with inactive disease after adjusting for the possible confounding effect of age and gender. This indicates that serum level of MMP-3 correlates with disease activity and may be particularly helpful in situations where traditional markers are less accurate e.g. patients with a normal CRP and who have non-erosive disease at presentation [28]. Moreover, it has been shown that serum level of MMP-3 correlates strongly with clinical parameters of rheumatoid arthritis activity, and with the number of joint erosions, therefore it may serve as an additional biomarker for assessment of RA activity [15]. In this work, there was a significant association between MMP-3 and DAS28 with strong positive correlation. This was similar to the finding of Posthumus et al. (2000) who mentioned that patients with early RA, time-integrated values of serial serum MMP-3 measurements showed close correlations with DAS28 [29], where in this study, multiple linear regression model revealed that For each one unit increase in DAS28 the serum MMP3 is expected to increase by a mean of 108.6 after adjusting for the possible confounding effect of age and gender. In addition, serum ACCP showed a statistically significant moderately strong positive linear correlation with serum MMP3 which was in accordance with the finding of another study [30]. This indicates that the serum level of both of them may help in the judgment about the joint inflammatory state and RA disease activity. Serum ACCP, MMP3 and blood ESR were tested for their validity in predicting a subject with active disease among cases with RA. Serum MMP3 was associated with an excellent test, being the most valid among the 3 tested parameters (ROC area=0.947). ESR is included here to provide a reference against which the validity of the two tested parameters is assessed. Serum ACCP was also associated with a very good test, ranking next in its validity (ROC area = 0.889). Both serum MMP3 and ACCP had a higher validity than blood ESR (which is used itself in calculating DAS28) in predicting an active disease status. The optimum cut-off value and the most specific for serum ACCP was ≥ 143.9, which is 73.3% sensitive and 100% specific for diagnosing an active RA disease status. Testing positive at this cut-off value will establish the diagnosis of active disease status with 100% confidence in any clinical context. The most sensitive cut-off value is ≥ 80.5, which is 100% sensitive. Testing negative at this cut-off value (having serum ACCP< 80.5) would exclude active disease status with 100% confidence in any clinical context. The optimum cut-off value for serum MMP3 was ≥ 412, which is 96.7% sensitive and 83.3% specific for diagnosing an active RA disease status. Testing positive at this cut-off value will establish the diagnosis of active disease status with 87.9% confidence in a clinical context where the pretest probability of having an active disease status is equivocal (50% pretest probability, which is the situation when the patient is self-referred for testing). The predictive accuracy of a positive test at this optimum cut-off value is increased to 98.5% in a clinical context where the active disease status is highly probable based on clinical findings (90% pretest probability). The most sensitive cut-off value is ≥ 387.7, which is 100% sensitive. Testing negative at this cut-off value (having serum MMP3 < 387.7) would exclude active disease status with 100% confidence in any clinical context. The most specific cut-off value is ≥ 502.5, which is 100% specific. Testing positive at this cut-off value (having serum MMP3≥ 502.5) would indicate active disease status with 100% confidence in any clinical context.

Conclusion

In rheumatoid arthritis, MMP-3 and ACCP are useful diagnostic tests for differentiation between active and inactive RA patients especially when combined with DAS28 and ESR. Both serum MMP3 and ACCP had a higher validity in predicting an active disease status.

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