

Comparison of Serum Levels of Anti Cyclic Citrullinated Protein 2 with Rheumatoid Factor for the Diagnosis of Rheumatoid Arthritis in Gaza Strip

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Abstract

Objectives: To compare the diagnostic value and clinical utility of Anti cyclic citrullinated protein 2 antibody (ACCP2A) with IgM rheumatoid factor (IgM RF) in patients with rheumatoid arthritis (RA).

Methods: In a cross sectional study, we determined titers of these markers in 67 arthritis patients and 75 healthy controls by enzyme linked immunosorbent assay (ELISA). C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were also assayed as inflammatory parameters for all subjects. All clinical features were investigated by a rheumatologist and questionnaire interviews. And all results were analyzed by SPSS version "15".

Results: sensitivity was higher for IgM RF (85%) than ACCP2A (56%) but specificity was higher for ACCP2A (99%) than IgM RF (69%). When the two antibodies were used together, sensitivity and specificity reached 88% and 100%. A correlation with extra articular manifestations, severity of erosions and CRP was observed for both tests.

Conclusions: a combination of both may be used as "gold standard" in diagnosis of RA patients in Gaza Strip. And both assays are also a better predictor of disease severity.

Key words: Rheumatoid Arthritis, Anti cyclic citrullinated protein 2 antibody, IgM Rheumatoid Factor, Gaza Strip.

مقارنة مستوى الاجسام المضادة ضد بروتين السيتروولين 2 مع عامل الروماتزم

في تشخيص التهاب المفاصل الروماتزمي في قطاع غزة

الملخص:

هدف البحث: مقارنة القيمة التشخيصية والطبية لكل من فحص الأجسام المضادة ضد بروتين السيتروولين 2 وعامل الروماتزم في تشخيص التهاب المفاصل الروماتزمي في قطاع غزة. **أداة الدراسة وعينتها:** تم فحص هذه العلامات في 67 عينة مرضية و 75 عينة ضابطة بواسطة طريقة الإليزا. كما تم أيضاً إجراء فحوصات عامل الالتهاب البروتيني النشط "سي" وسرعة الترسيب لكرات الدم الحمراء كمعايير للالتهابات في كل عينة. تم تقييم المعلومات المتعلقة بالمرض بواسطة أخصائي الروماتزم وعن طريق استبيان. وكل النتائج تم تحليلها باستخدام "SPSS 15"

النتائج: نسبة انتشار عامل الروماتيزم في المرضى كانت (85%) وهي أعلى من نسبة انتشار الأجسام المضادة ضد بروتين السيترولين والتي بلغت (56%)، بينما نسبة عدم انتشار الأجسام المضادة ضد بروتين السيترولين في العينة الضابطة كانت (99%) وهي أعلى بكثير من نسبة انتشار عامل الروماتيزم والتي بلغت (69%). لكن علي الرغم من ذلك فقد أثبتت الدراسة أن قدرة الفحصين معاً علي إثبات المرض في المرضى هي (88%) وإثبات عدم وجودهم في العينة الضابطة هي (100%).

كما أثبت الفحص أن العلاقة مع الأعراض خارج المفاصل وشدة تآكل المفاصل و البروتين النشط "سي" قد لوحظت مع كلا الفحصين.

الإستنتاج: توصي الدراسة بعمل الفحصين معاً حيث إن هذا يعتبر أحسن دليل في تشخيص التهاب المفاصل الروماتزمي في قطاع غزة. وكذلك يعتبروا أحسن دليل علي شدة المرض.

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease characterized by joint destruction, which leads to functional decline and disability as well as increased mortality [1]. Prevalence of RA approaches 1% among adults with a female to male ratio of 3:1 [2].

RA, like all forms of inflammatory arthritis, has no single specific pathognomonic sign, symptom or laboratory feature [3]. Doctors usually base the diagnosis of RA on American criteria of rheumatology, ACR [4]. But it has limited use in early diagnosis [5].

The first immune abnormality described in patients with RA was the production of rheumatoid factor (RF) [6]. While RF is elevated in ~75% of patients with RA [7]. this antibody lacks specificity because it may be found in other autoimmune disorders or infectious diseases as well as up to 15% of the healthy elderly population [8]. Various other circulating antibodies have been reported to be of potential diagnostics value including anti perinuclear factors (APFs), anti keratin antibodies (AKAs) and anti Sa antibodies. Despite of their high specificity for RA, these antibodies never became very popular. This was mainly a consequence of the fact that testing their presence was more laborious [9]. In 1998, Schellekens developed a new serological test, anti cyclic citrullinated protein antibodies by using ELISA for its assay. He showed that this autoantibody is reactive with synthetic peptides containing the unusual amino acid citrulline, a posttranslationally modified arginine residue and is specifically present in the sera of RA patients [10].

This study will compare the diagnostic performance and clinical utility of Anti cyclic citrullinated protein 2 antibody (ACCP2A) with IgM RF in selected group residing in Gaza Strip.

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Patients and methods

Patients

In a cross sectional study, we recruited 67 arthritis patients and 75 healthy individuals as control group, later on, patients suffering from arthritis but do not comply with ACR were added to the control group. 67 arthritis patients were randomly selected from the outpatient rheumatology unit at EL SHIFA hospital. However, only 34 patients complied with criteria of ACR. And the diagnosis was confirmed by a rheumatologist. Others; two patients had osteoarthritis, one patient had systemic lupus erythematosus (SLE), and one patient had spondyloarthropathy and those patients were categorized as non RA patients. The rest 29 patients were categorized as having undifferentiated arthritis (UA).

All patients were questioned about their personal character, clinical features (onset for RA, early symptoms, classification criteria, extra-articular disease, and radiographic manifestations) and medications received. All interviews were conducted face to face.

A blood sample was drawn from each patient; serum was separated, aliquoted and stored at -82°C until use.

Methods

We used the commercially available second generation ELISA test for anti CCP (Diastat, Axis Shield Diagnostics, UK). The assay was carried out according to the manufacturer's instructions. The results of the anti CCP test were considered positive if the antibody level was greater than the cut off value (5 U/ml).

RF was measured by ELISA for IgM isotype (GA Generic Assays GmbH-Germany) and a level >6 IU/ml was considered positive. Acute phase reactants were measured by erythrocyte sedimentation rate (ESR) (mm/h) and C-reactive protein (CRP). CRP level determined by using CRP latex test (Plasmatic-UK) and ESR was determined by Westergren method.

Statistical analysis

The statistical analyses were performed using SPSS version "15". The sensitivities and specificities were calculated together with the 95% confidence intervals (CI) by receiver operating curve (ROC) which is generated by plotting sensitivity (y axis) against 1- specificity (x axis). The correlations between numerical data were analyzed by Pearson's correlation coefficient and Chi-square test of independences was used for correlation between nominal or order data. Comparison of numerical data between groups was made by Mann Whitney U test and comparison of proportions between groups was made by Z - test. Means, standard deviation (SD),

percentage and CI were used where appropriate. P-value less than 0.05 was regarded as significant.

Results

Sensitivity and specificity of ACCP2A and IgM RF for diagnosis of RA

Sensitivity expresses the percentage of RA patients that was positive for the test, whereas specificity is calculated from the percentage of test-negative non RA patients, UA patients and healthy control. Both were analysed by ROC curve at optimal cut off (>5 U/ml) for ACCP2A and at (>6 IU/ml) for IgM RF.

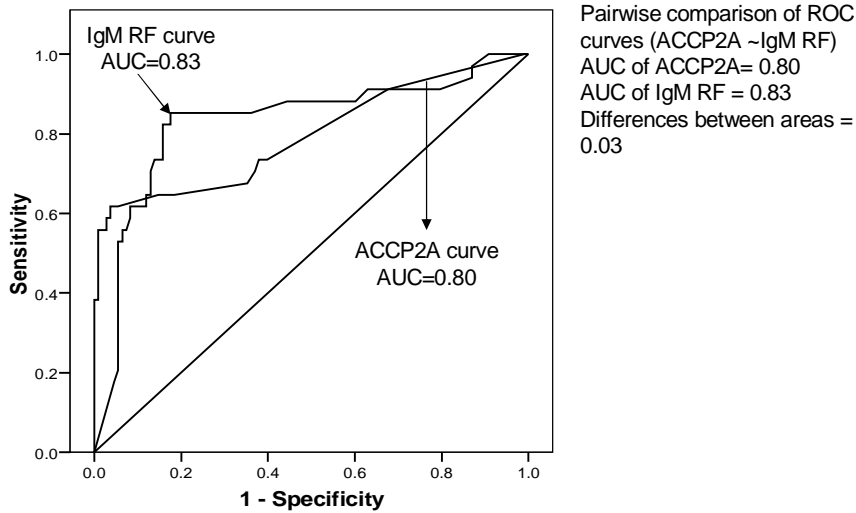
The sensitivities and specificities (with their corresponding 95% CIs) of the serologic tests are illustrated in (Table 1).

Table 1, Diagnostic value of ACCP2A and IgM RF for RA

Criterion	ACCP2A	IgM RF	Combination of ACCP2A and IgM RF
Sensitivity % 95% CI	56%	85 %	88%
Specificity % 95% CI	99 %	69%	100%

For comparison of the diagnostic value of each assay, area under curve (AUC) was calculated. As shown in (fig 1) AUC was 0.83 (95% CI, 0.74-0.92) for IgM RF and 0.80 (95% CI, 0.70-0.90) for ACCP2A with p-value (sig) = 0.000 for both. Therefore both tests exhibited similar diagnostic value in Palestinian patients with RA.

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(Fig 1) Comparison between ACCP2A and IgM RF ROC

Comparison between the mean levels of ACCP2A and IgM RF in RA and controls plus others groups

Since P- value (sig.) = 0.000 which is smaller than the level of significance ($\alpha = .05$), then there exists a significant differences between the two groups (sig. = 0.000) for each ACCP2A and IgM RF. Therefore based on this result and the mean rank of ACCP2A and IgM RF which are higher in patients than in control, we concluded that means of ACCP2A and IgM RF for patients are significantly higher than for controls. The results are summarized in (Table 2).

(Table 2) Comparison of ACCP2A and IgM RF between RA and controls

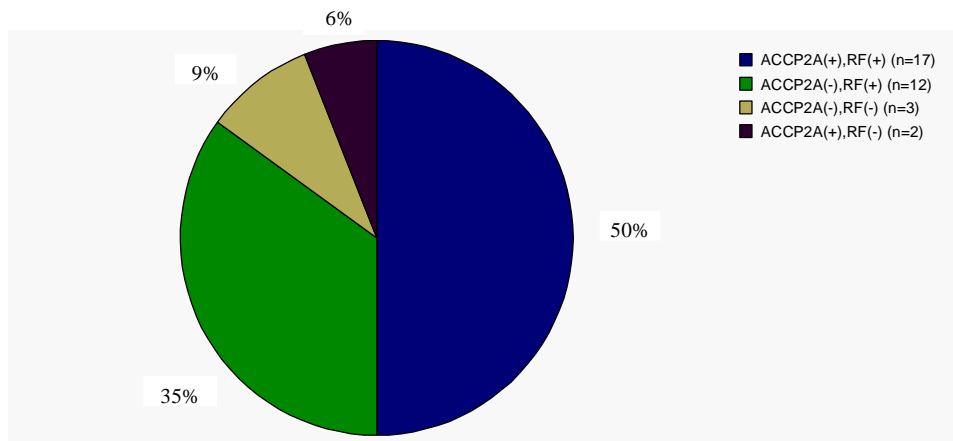
Groups	NO	ACCP2A (U/ml) Mean ±SD	IgM RF (U/ml) Mean ±SD
RA patients	34	34.5±42.7	98±76.9
Non RA patients	4	1.1±0.58	19.9±21
UA patients	29	2.0±3.8	48.0±73
Healthy controls	75	1.1±0.57	9.2±23.7
Mean rank			
RA patients		103.4	107.1
Controls		61.4	60.3
P value		0.000	0.000
Significant level P< 0.05			

between them was significant correlation (sig = 0.000). This means that the two tests have the ability to identify the same patients as positive or negative.

Occurrence of ACCP2A and IgM RF in RA patients

The percentage of RA patients who are positive for ACCP2A or IgM RF and both are demonstrated in (Fig 2)

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(Fig 2) Frequency of ACCP2A and IgM RF in RA patients

Occurrence of ACCP2A and IgM RF in controls group

Non RA patients were not positive for ACCP2A while 50% of them showed positive result with IgM RF. In UA patients, only 3.4% were positive for ACCP2A and 48% for IgM RF. Healthy individuals were not positive for ACCP2A whereas 23% of them were positive for IgM RF.

A correlation of ACCP2A and IgM RF with inflammatory parameters (CRP and ESR) and disease activity parameters (radiological manifestation and extra-articular manifestation)

As shown in (Table 3), a significant correlation was found between ACCP2A and radiological manifestations, extra-articular manifestations and CRP and no correlation existed between ACCP2A and ESR. In addition there is a significant correlation between IgM RF and radiological manifestation, extra-articular manifestation, CRP and ESR.

(Table 3) Correlation between Abs assay and inflammatory and disease activity parameters

Criterion	ACCP2A Pearson correlation Sig (2- tailed)	IgM RF Pearson correlation Sig (2- tailed)
Radiological manifestation Pearson chi square Sig. (2-tailed)	22.045 0.000**	17.260 0.001**
Extra-articular manifestation Pearson chi square Sig. (2-tailed)	15.068 0.001**	16.169 0.001**
CRP Pearson chi correlation Sig. (2- tailed)	7.468 0.024*	36.679 0.000**
ESR Pearson correlation Sig. (2- tailed)	0.005 0.952	0.190 0.023*
Significant level P< 0.05		

Relation of ACCP2A and IgM RF with personal characters of RA patients (age, gender, family history and smoking)

There was no significant relationship between both ACCP2A and IgM RF with age. On the other hand, there was a significant relationship between IgM RF and gender. However, this relation wasn't significant for ACCP2A. In addition, there was no significant relationship between ACCP2A and IgM RF with family history or smoking.

Discussion

RF assay is known to have suboptimal sensitivity and specificity for the diagnosis of RA. Therefore, easier, more convenient, higher specific serological methods have been required for the diagnosis of RA [11]. Now the diagnostic properties of ACCP2A in the specific diagnosis of RA may outplay other available antibody tests, especially IgM-RF [12].

In the present study, the sensitivity of IgM RF was moderately high 85% and it is in agreement with most studies which resulted in sensitivity of about 80% [13,14]. ACCP2A showed good sensitivity (56%), however there is considerable variation in diagnostic sensitivity of ACCP2A among studies, ranging from 41-80% [15,16]. The reasons of the wide range of sensitivity of reported results of ACCP2A may be due to the variation in

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determination of cut off value between articles (some articles determined it from manufactures and some from ROC curve) so some standardization between manufactures would appear desirable. Characteristics and ethnicity of patients may be other reason. However some studies suggested that the sensitivity of ACCP2A may improve by using microarrays and multiplex cytokine assay or by using new ACCP3A assay [17,18].

The specificity of the ACCP2A test (99%) was significantly higher than that obtained for the IgM RF test (69%). The specificity of IgM RF is in concordance with other studies which is about 70% [19, 20]. There is also consensus of the different studies about the high specificity of ACCP2A ranging between 95% and 100% [21,22]. The specificity is the most valuable aspect of this assay, so much so that it may be proposed as the most important examination in the diagnosis of RA.

Our study also showed that the combination of ACCP2A with IgM-RF could increase the sensitivity (to 88%) and specificity (to 100%). The complementarity of RF to ACCP2A is controversial. Some reports suggested that RF and ACCP2A should be combined to reach optimum diagnostic properties, whereas others found only little additional diagnostic value when combining RF and ACCP2A [23]. Further studies should be executed to investigate this issue.

AUC of ACCP2A and IgM RF was 0.80 and 0.83, respectively and this indicated that the diagnostic accuracy of ACCP2A and IgM RF was similar, this result is similar to other studies [24]. and differ from other [25]. However, the difference in mean levels of ACCP2A and IgM RF between RA patients and control groups was significant and this is another point which emphasizes that ACCP2A and IgM RF are having similar diagnostic accuracy.

The diagnosis of sero-negative RA is a more challenging diagnosis, and more likely to include misclassified reactive, psoriatic, viral and crystalline arthritides [26]. Our study shows that; although the two Abs were significantly correlated, ACCP2A was present alone in 6% of IgM-RF negative sera of RA patients. This result confirms the hypothesis that "ACCP2A and RF are distinct antibody systems yielding different information" [27]. And also indicate that ACCP2A estimations may be useful in the disease diagnosis of RA patients who are RF-negative or where the diagnosis may be uncertain and this added diagnostic and prognostic value for ACCP2A.

In our study 3.4% of patients could not fulfill ACR criteria and were classified as UA who showed positivity toward ACCP2A. This means that

these antibodies appear in the serum many years before the onset of clinical disease [28]. So monitoring ACCP2A activity in individuals that may have an increased risk for the development of RA, e.g. based upon genetic factors, may eventually allow earlier treatment of ACCP2A positive individuals in which the antibodies titers are increasing. As a consequence, the lag time between first visit to the rheumatology clinic and start of therapeutic intervention may be importantly reduced and joint erosion may already be inhibited at the very early stages [29].

All non RA patients (SLE, osteoarthritis and spondyloarthropathy patients) were negative for ACCP2A test but 50% of those patients were positive for IgM RF. This means that ACCP2A may be useful in distinguishing RA from other similar diseases. This finding is in consensus with other studies [30, 31]. These data and the absence of ACCP2A in healthy individuals and in other rheumatic diseases confirm their high propensity to be associated with RA.

ACCP2A and IgM RF were found to have significant correlation with radiological manifestation and this is in agreement with Meyer [32]. and the relation of both ACCP2A and IgM RF with extra articular manifestation was also significant and this finding is in accordance with Turesson [33]. So these findings revealed that the presence of ACCP2A and IgM RF act as indicators of disease severity in RA, and the presence of both suggest a role for these Abs in the pathogenesis of radiological and extra-articular manifestation.

The correlations of ACCP2A and IgM RF with CRP and ESR were studied. CRP and ESR, along with the number of swollen and painful joints, one of the major criteria for the “clinical” disease activity score and the ACR scores [34]. There were significant correlations between ACCP2A and IgM RF with CRP. This is in accordance with some reports [35, 36].

The significant correlation of ESR was found only with IgM RF and this in consensus with Aldifran [36]. and the correlation of ESR with ACCP2A wasn't significant, this is in consensus with Shovman [37].

It is interesting to note that IgM RF has more correlation with CRP than ACCP2A. In addition it has significant correlation with ESR rather than ACCP2A. It has been concluded that IgM RF and ACCP2A are two different systems of Abs reacting differently to the treatment prescribed [38]. However the patients included in this study were under the different regimes of treatment (disease modifying anti rheumatic drugs (DMARD), steroids, anti-inflammatory drugs). So further studies should be made to demonstrate the reactions of ACCP2A with each drug.

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Most studies have shown that there was no correlation between ACCP2A with age or gender [39, 40]. This has also been confirmed in our study. There was also no correlation between IgM RF and age. Similar results were obtained [39, 40]. However, significant correlation was found between IgM RF and gender and this is in consensus with Masi [41]. The strong correlation of IgM RF with gender is an indication that gender could act as a risk factor for RA and the weak correlation of ACCP2A with gender may be due to the moderate sensitivity of ACCP2A in our study. In addition, variations in hormonal levels between male and female may influence the results.

Various studies have shown that smoking is a predictor of the development of RA [42, 43]. In our study there was no correlation of ACCP2A and IgM RF with smoking. This may be due to the fact that most of the patients in this study were female and smoking habit was more among male patients than females. In addition epidemiologic studies have revealed that the risk of cigarette smoking for seropositive RA is higher in men (odd's ratio: 4.77) than in women (odd's ratio: 1.14) [44].

Our study showed that there is no correlation of ACCP2A and IgM RF with family history. This is in concordance with van Gaalen who found that other groups at risk of developing RA, such as family members of RA patients or even the general public are less likely to benefit from ACCP2A testing due to the low prevalence of RA 40% [45]. In addition, Svendsen showed that Genes are of minor importance in the development of RA [46]. This point needs further research on all family members to reach concrete conclusion. Finally, RA is still of unknown etiology. Environmental and genetic risk factors have been identified, but no single risk factor has emerged as necessary or sufficient to cause the disease.

Conclusions

ACCP2A assay is a very valuable tool for the diagnosis of RA in Gaza Strip, the use of ACCP2A and RF tests combined is considered to be the 'gold standard' in the detection of RA, ACCP2A is important in diagnosis of early RA, these marker could be of use for prognosis towards disease severity.

Acknowledgments

We would like to thank the deanship of graduate studies at the Islamic university in Gaza for supporting this research.

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