P- ISSN 1991-8941 E-ISSN 2706-6703 *Journal of University of Anbar for Pure Science (JUAPS)* Open Access
2012,(6), (3) :01-06

Diagnostic value of Anti-CCP antibodies compared with Rheumatoid factor in Rheumatoid arthritis patients.

Hanaa N. Abdullah* **Amina N.Al-Thuwani **** **Mohamed I. Nadir**** **Khudhair Al-Badri*****

* Foundation of technical education - College of Health and medical technology
** University of Baghdad- Institute Genetics Engineering and biotechnology for Postgraduate .
*** University of Baghdad - College of Medicine.

ARTICLE INFO

Received: 20 / 6 /2012
Accepted: 7 / 10 /2012
Available online: 29/8/2013
DOI: 10.37652/juaps.2012.78249

Keywords:
Diagnostic value ,
Anti-CCP antibodies ,
Rheumatoid.

ABSTRACT

Citrullinated proteins have been discovered in the joints of patients with rheumatoid arthritis but not in other forms of joint disease. The citrullinated proteins in the joints correspond to the presence of the citrulline antibodies in the blood and suggest a possible role for these antibodies in the development of rheumatoid arthritis. The present study assessed the recent diagnostic value of anti-cyclic citrullinated antibodies (CCP) compared with rheumatoid factor (RF) in patients with rheumatoid arthritis. One hundred blood samples were collected from RA patients and thirty from apparently healthy group. Sera from each subject were tested for anti-CCP and RF by enzyme linked immunosorbent assay (ELISA). The majority of patients were females (84%), with a female: male ratio 5.2:1. The results indicated that anti-CCP postivity for RA patients was (69%) when compared with the healthy group (0.0%), which was highly significant in RA in comparison with control groups ($P<0.001$). The current study revealed that the sensitivity and specificity of Anti-CCP appeared the highest (69%) in comparison with low (47%) sensitivity for RF. Moreover, the specificity of Anti-CCP was very high (100%), while the specificity of RF was high (90%).

Introduction

Rheumatoid factor are antibodies directed to the FC fragment of human IgG molecules resulting in RF-IgG immune complexes which could be deposited in tissues and activate the classical complement pathway, and lead to tissue damage (1). It is not clear whether RF is directly related to the symptoms of RA, although RF is found significantly more often in cases of aggressive joint inflammation. Since the presence of RF is one of the American College of Rheumatology criteria for RA, the test is performed on a routine basis in most clinical laboratories. Some reports mention raised IgA-RF levels as a parameter for disease activity (2). A combined routine determination of IgM-RF, IgG-RF and IgA-RF is recommended for an improved sensitivity, for diagnostic specificity and for predictive value(3). Finally Citrulline antibody directed against a circular peptide (a ring of amino acids) called Citrulline is a nonstandard amino acid, as it is not incorporated into proteins during protein synthesis.

* Corresponding author at: Foundation of technical education - College of Health and medical technology-
.E-mail address:

It can, however, be generated via post-translational modification of arginine residues by peptidylarginine deiminase (PAD) enzymes (4,5). Conversion of arginine into citrulline involves the replacement of an amine group by an oxygen atom in the side chain of this amino acid, and is associated with the loss of a positive charge (at neutral pH). Although this conversion results in a relatively small chemical alteration of the protein involved, the reactivity of autoantibodies reactive with citrulline-containing epitopes seems to be critically dependent on the presence of a citrulline residue(6). The citrulline antibody is known more formally as cyclic citrullinated peptide antibody as anti-CCP antibody IgG (7). Citrullinated proteins have been discovered in the joints of patients with rheumatoid arthritis but not in other forms of joint disease. The citrullinated proteins in the joints correspond to the presence of the citrulline antibodies in the blood and suggest a possible role for these antibodies in the development of rheumatoid arthritis (8). The anti-CCP antibodies are mainly of the IgG class, although IgM and IgA anti-CCP can also be detected, albeit at a much lower

prevalence (9). Anti-CCP represents a superior serological marker for RA. Anti-CCP is (i) highly specific for the disease, (ii) able to distinguish RA from other arthritis that mimic RA, (iii) present in the majority of patients (good sensitivity), (iv) detectable very early in the disease, and (v) helpful in predicting disease outcome. Its prognostic potential may aid the rheumatologist in reaching decisions on the most optimal treatment strategies. Moreover, anti-CCP can be detected with producible and easily performed ELISA, the CCP2 test, which is important from the perspective of laboratory management (10).

Materials and Methods

Sample collection

One hundred blood samples were collected from RA patients and thirty samples from SLE patients and thirty samples from apparently healthy controlindividuals. The RA and SLE patients were chosen according to the definition by the American Rheumatism Association 1987 criteria. In our study groups age, sex, disease duration and clinical characteristics were reordered. The diagnosis was made by the consultant medical staff in Baghdad teaching hospital from March 2008 to March 2009.

Serology

Serum samples from patients and control groups were kept at (-20˚C). Rheumatoid factor (RF) and RF isotypes (IgG , IgM, IgA) were detected using ELISA kit (Euroimmun, Germany). Anti-CCP antibodies were detected by ELISA (Euroimmun, Germany) . These assays were performed according to the manufactures instructions (cut off value , 5 IU/ml).

Statistical Methods

The mean ± SD were given, difference between means of patients and healthy control group were assessed by least significant differences (LSD). These statically analyses were done by using Pentium four computer through the SSPS program. Receiver operating characteristic (ROC) was done. Curves when drawn and the area under the curve (AUC) along with corresponding confidence intervals were calculated. Diagnostic characteristics were determined by means of sensitivity and specificity.

Results

The quantitative determination for RF isotypes has been applied for studied groups, as mentioned in table (1). A rheumatoid factor positivity in the current study showed (47%) for RA cases with a strong significant difference (P<0.001). Moreover, RF-IgG (67 %) RF-IgM (85%) and RF- Anti-CCP antibody which has been detected in the sera of the RA patients, SLE and healthy control groups. High frequency of anti-CCP positivity for RA patient's sera (69%) rather than the control groups 6.7% and 0.0% for SLE and healthy cases respectively. Anti-CCP Antibodies showed highly significance in RA patients in comparison with the control groups (P<0.001),as summarized in table (2). IgA (76%) revealed a highly significant difference (P<0.001). The current study revealed that the sensitivity and specificity of Anti-CCP as recorded in table (3) which presents in the validity test that appeared the highest (69%) in comparison with low (47%) sensitivity for RF. Moreover, the specificity of Anti-CCP was very high (100%), while the specificity of RF was high (90%). For further comparisons of the diagnostic value of each assay, we undertook an ROC (Receiver operating characteristic) analysis and calculated the area under the (AUC) (Figure 1 and 2). The ROC analysis displays the pairs of sensitivity and specificity for different tests (anti-CCP and RF). It could clearly be shown that anti-CCP ELISA provided the best combination of sensitivity and specificity for detecting RA.

By analyzing the benefit of single or combined use of all six antibody assays to find an impressive additional diagnostic value of CCP compared with the single use of RF isotypes alone. Frequencies of anti-CCP antibodies, RF and RF-isotypes in patients are shown in table (4). The majority of RA patients, (47.8%) were anti-CCP and RF positive but (45.2%) were anti-CCP negative and RF positive. In addition, among these patients with RA, (81.2%) were positive for both tests and (93.5%) were anti-CCP negative and RF-IgM positive. Moreover, (71%) of patients were positive for Anti-CCP and RF-IgA, while (87.1%) of patients were Anti-CCP negative and RF-IgA positive. In this study, only (13%) were positive for Anti-CCP and ANA but (16.1%) of patients were Anti-CCP negative and ANA positive.

Discussion:

The percentage of RF (47%) in our RA group, was relatively low.These findings disagreed was with Abbas who mentioned that RF-IgG (47.3%) RF-IgM

(48.6%) and RF-IgA (54.1%) (11). However, Bas *etal* reported that the RF-IgM and RF-IgA isotypes were noticed predominated among early RA patient's sera (12). These variations may be related to differences in population and timing of sample collection. These results may be explained by the fact that RA patients encompass the fall range of RA patients severity /activity because of the health system in Iraq. Raised level of RF-IgG, RF-IgA, and RF-IgM have been reported in patients with RA. Several groups have reported that a high level of RF-IgA is prognostic for more severe disease outcome. High level of RF-IgA during three years of onset of symptoms have been associated with more severe disease after six years of onset (13). Other references denoted RF-IgM typically is seen not only in RA but also in other various conditions. RF-IgA is more easily detected than RF-IgG which may be a better indicator of T-cell dependent affinity matured antibodies directed to FC–γ epitopes relevant to RA than RF-IgM. The combined detection of RF-IgM and RF-IgA in a serum is a strong indicator of RA (14). Recently, a highly specific autoantibody system has been described for RA, in which patients develop antibodies to citrullinated, and this has resulted in the development of the anti-cyclic citrullinated peptide (anti-CCP) antibody test (15). Anti-CCP was a good serological marker for RA and should be highly specific for the disease and be able to distinguish RA from other arthritis that mimic RA. The frequency of anti-CCP positivity among the RA patients Sera (69%), this percentage is higher than that of other researchers who proposed 50-60 % detection of anti-CCP in RA cases (16,17), while, the present study comparable to Iraqi study is (68.09%) (18). Additionally, there is a highly significance in RA and control groups ($P<0.001$). This result agrees with these studies, which had positivity reached to (68%) (15) and indicated the anti-CCP which was a good marker for RA disease. The interpretation for these variations is that the level of anti-CCP decreases during therapy and those with positive results had more destructive disease than those without anti-CCP (7). It was noticed that the result of different studies were heterogeneous. This may be caused by the manufacturing companies with different serum dilutions and different CCPs used in the assays. These all may alter the result and necessitate the need for international standardization. Also, high specificity of Anti-CCP antibodies had been investigated by other researchers. Most of them reported specificity of (90-99%), and sensitivity (64-74%) (19;20). Anti-CCP antibody can be detected very early in RA although with somewhat lower sensitivity (40-60%) (21). From this outcome it can be concluded that Anti-CCP antibody is a significant diagnostic marker with high specificity (100%). The explanation of difference is due to the variations in the characteristics of patients (early or late RA) that was included, this difference can probably be related to the fact that few patients are newly diagnosed but others are late RA. The combination of anti-CCP and IgM-RF yields higher sensitivity for diagnosis of RA than either test alone. This result confirms previous studies with combination of RF plus Anti-CCP (6;21). Anti-CCP is a prognostic indicator for RA progression, although generally not more useful than RF-IgM (22). These parameters for rheumatoid arthritis are considered as superior to other RF-isotypes. Positivity in highly specific CCP ELISA supports the diagnosis of disease, CCP proved to be a powerful diagnostic tool, especially in ambiguous cases or negative patients with RA (14). Furthermore, the presence of anti-CCP and IgM-RF was associated with a higher probability of radiological signs of joint damage and that RF is with higher functional disability (23). Rantaapaa-Dalqvist *et al.* showed that CCP and IgA-RF predict, the development of RA, with CCP having the highest predictive value of all antibodies(8), whereas the study by Bas *et al*., also observed an association of RF-IgA and CCP with clinical signs of disease activity (12).

Conclusions:

Anti-CCP was a good serological marker for RA and should be highly specific for the disease and be able to distinguish RA from other arthritis that mimic RA.The combination of anti-CCP and IgM-RF yields higher sensitivity for diagnosis of RA than either test alone. In addition combination of CCP and IgA-RF predict the development of RA.

References

1.Smolen, J. (1996). Autoantibodies in rheumatoid arthritis. In: Manual of Biological Markers of Disease. Edited by van Venrooij ,W.& Maini ,R. Section C 1. Dordrecht: Kluwer Academic Publishers. PP:1-18.

2.Pai, S.; Pai, L. & Birkenfeldt, R. (1998). Correlation of serum IgA rheumatoid factor levels with disease severity in rheumatoid arthritis. Scand J. Rheumatol.;27 : 252–256.

3.Houssien, D.;Jonsson, T.; Daview, E. & Scott, D. (1998). Rheumatoid factor isotypes, disease activity and the outcome of rheumatoid arthritis. Comparative effects of different antigens. Scand J. Rheumatol. ;27 : 46–53.

4.Steiner ,G. and Smolen , J. (2002) . Autoantibodies in rheumatoid arthritis and their clinical significance. *Arthritis Res*.; 4 Suppl 2S:15.

5.Vossenaar, E.; Zendman, A.; Van Venrooij, W. & Pruijn G. (2003). PAD, a growing family of citrullinating enzymes: Genes, features and involvement in disease. Bioessays; 25:1106–1118.

6.Schellekens, G. ; De Jong, B.;Van den Hoogen, F.; Van de Putte ,L. & Van Venrooij ,W. (1998). Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis specific autoantibodies. J. Clin .Invest.; 101:273-281.

7.Rantapaa-Dahlavist, S. (2005). Diagnosis and prognostic significance of antibodies in early rheumatoid arthritis. Scand. J. Rheumatol.; 34: 83-96.

8.Rantapaa-Dahlqvist, S.;De Jong, B.; Berglin, E.; Hallmans, G.;Wadell, G.; Stenlund, H.; Sundin, V. & van Venrooij, W. (2003). Antibodies against Cyclic citrullinated peptide and IgA rheumatoid factor predict the development of Rheumatoid arthritis. Arthritis Rheum.;48: 2741-2749.

9.Silman, A. & Pearson , J. (2002). Epidemiology and genetics of rheumatoid. Arthritis Res.; 4 Suppl : S265–S272.

10.Pruijn, G.; Vossenaar, E.; Drijfhout,J.; Van Venrooij, W. & Zendman, A. (2005). Anti-CCP Antibody detection Facilitates early diagnosis and prognosis of rheumatoid arthritis. Current Rheum. Reviews ; 1:1-7.

11.Abbas, NR. (2003). Some crucial immunological elements in patients with rheumatoid arthritis. M.Sc. thesis. College of Medicine. University of Baghdad.

12.Bas, S.; Genevay, S.; Mayer,O. & Gabay, C. (2003). Anti-cyclic citrullinated peptide antibodies, IgM and IgA rheumatoid factors in the diagnosis of rheumatoid arthritis. Rheumatol.; 42: 677-80.

13.Jonsson,T. & Valdimarrson, H. (1993). Is measurement of rheumatoid factor isotypes clinically useful. Ann.Rheum. Dis.; 52(2):161-4.

14.Vallbracht, I. ;Rieber, J.; Oppermann, M.; Forger, F.;Siebert, U. & Helmke, K. (2009). Diagnostic and clinical value of anti-cyclic citrullinated peptide antibodies compared with rheumatoid factor isotypes in rheumatoid arthritis. Ann. Rheum. Dis. ; 63: 1079-1084.

15.Quinn, M.;Gough, A.; Green, M.; devlin, J.; Hensor,E.; et al.(2006). Anti-CCP antibodies measured at disease onset help identify seronegative rheumatoid arthritis& predictor radiological and function outcome. Rheumatol.;45 (4):478-480.

16.Khosla, P.; Shankar, S. & Duggal, L. (2004). Anti-CCP antibodies in rheumatoid arthritis. J. Indian rheumatol.Assoc.;12: 143-46.

17. Nell, V.; Machold, K.; Hueber, W. et al. (2003). The diagnostic significance of autoantibodies in patients with very early rheumatoid arthritis. Arthritis Res. Ther.; 5(suppl1):16.

18.Tofiq, D. (2007). Diagnostic value of anti-CCP 2 antibodies in comparison with IgM & IgA rheumatoid factor in rheumatoid arthritis. Board for pathology /Microbiology & Immunology. The scientific council of pathology.

19.De-Rycke, L.; Peen, I.; Hoffman, I.; Kruithof, E.; Union, A.; etal.(2004). Rheumatoid factor & Anti-CCP antibodies in Rheumatoid arthritis: diagnostic value, associations with radiological progression rate & extra-articular manifestations. Ann. Rheum. Dis.; 63: 1587-93.

20. Zandman, A.;Van-venrooij, W. & Pruijn, G. (2006). Use & significance of anti-CCP auto antibodies in rheumatoid arthritis.Rheumatol.;45:20-25.

21. Bizzaro, N.; Mazzanti, G.; Tonutti, E.;Villalta, D. & Tozzoli, R. (2001). Diagnostic accuracy of anti-CCP assay for rheumatoid arthritis. Clin.Chem.; 47(6):1089-93.

22. Kroot, E.; de jong, B.; van Leeuwen, M.;Swinkels, H.;van den Hoogen,F.;et al. (2000). The prognostic value of anti-cyclic citrullinated peptide antibody in patients with recent-onset rheumatoid arthritis. Arthritis Rheum.; 43(8): 1831-5.

23.Bas, S.; Genevay, S.; Mayer,O. & Gabay, C. (2003). Anti-cyclic citrullinated peptide antibodies,

IgM and IgA rheumatoid factors in the diagnosis of rheumatoid arthritis. Rheumatol.; 42: 677-80.

Table 1:- The frequency of RF & RF-isotype among studied groups (RA patients, SLE patients and apparently healthy control).

Immunological parameters	Studied groups (%) of positivity			Comparisons		
	RA patients	SLE patients	Healthy control	Chi-Square	df	p-value
RF	47	3.3	0.0	18.89	1	0.00 HS
RF-IgM	85	3.3	0.0	68.73	1	0.00 HS
RF-IgG	67	10	0.0	56.32	1	0.00 HS
RF-IgA	76	10	0.0	42.16	1	0.00 HS

Table 2:-The frequency of Anti-CCP Antibodies among studied groups.

Studied groups		Anti-CCP		Total	P-value
		Positive	Negative		
RA patients	N	69	31	100	0.00
	%	69	31	100	
SLE patients	N	2	28	30	
	%	6.7	93.3	100	
Healthy group	N	0.0	30	30	
	%	0.0	100	100	

Note :- Anti-CCP: Anti-cyclic citrullinated peptide.

Table 3:- Validity test (%) of immunological parameters (Anti-CCP and RF).

Tests	Sensitivity %	Specificity %	PPV %	NPV %	Accuracy %
Anti-CCP	69	100	69	49.18	76.15
RF	47	90	94	33.75	56.92

PPV: Positive Predictive value NPV: Negative Predictive value

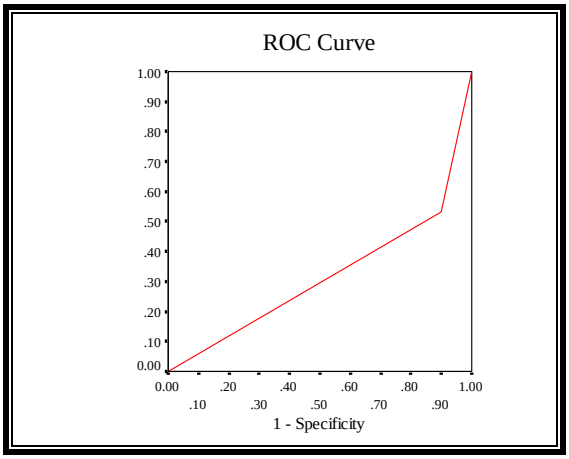

Figure 1: - Receiver Operating Characteristic (ROC)curve of anti- CCP antibodies

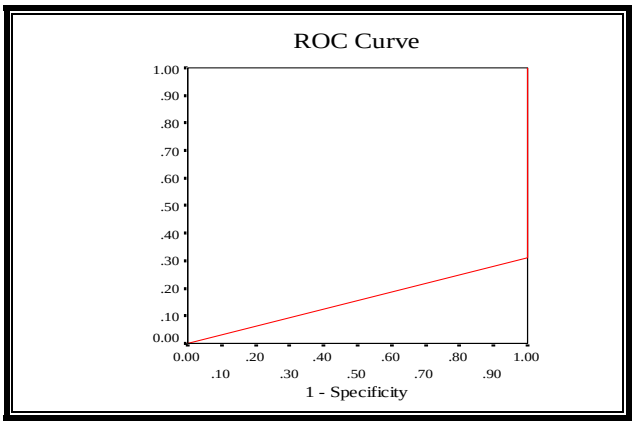

Figure 2: -Receiver Operating Characteristic (ROC) curve of RF.

Table 4:-Combinations between Anti-CCP and RF-isotypes among Rheumatoid Arthritis patients.

Serological parameters (positive result)	Anti-CCP	
	Positive	Negative
RF	47.8%	45.2%
RF-IgG	72.5%	54.8%
RF-IgA	71.0%	87.1%
RF-IgM	81.2%	93.5%
ANA	13.0%	16.1%

ANA: Antinuclear antibody

P- ISSN 1991-8941 E-ISSN 2706-6703 *Journal of University of Anbar for Pure Science (JUAPS)* Open Access
2012,(6), (3) :01-06

القيمة التشخيصية لضد الببتيد الستروليين الحلقي مقارنة بالعامل الرثوي لدى مرضى التهاب المفاصل الرثوي

هناء ناجي عبدالله أمنه نعمه الثويني محمد ابراهيم نادر خضير البدري

الخلاصة:

تهدف الدراسة الحالية الى استخدام طريقة حديثة لضد بروتين الستروليين الحلقي مقارنة بالعامل الرثوي. تم جمع مئة عينة دم من مرضى التهاب المفاصل الرثوي وثلاثون عينة من الأشخاص الأصحاء ظاهريا واختبرت العينات بطريقة ELISA. وكانت اغلبية المرضى من النساء بنسبة 84% وبمعدل 5.2:1 من الذكور. واشارت النتائج ان نسبة ايجابية لضد بروتين الستروليين الحلقي 69% والتي اظهرت معنوية عالية مقارنة بمجموعة الاصحاء, وكانت نسبة الايجابية للعامل الرثوي 47% للمرضى مع فارق معنوي. اظهرت الدراسة الحالية ان حساسية فحص اضداد الستروليين الحلقي عالية (69%) مقارنة بالعامل الرثوي (47%) إضافة الى ذلك كانت خصوصية اضداد الستروليين الحلقي عالية جدا (100%) مقارنة بخصوصية العامل الرثوي (90%).