

Research Article



Sensitivity and Specificity of Anti-Cyclic Citrullinated Peptide Antibodies, Compared to Rheumatoid Factor in Syrian Rheumatoid Arthritis Patients

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ABSTRACT

Rheumatoid arthritis (RA) is a chronic inflammatory systemic disorder with an autoimmune pathogenesis. Rheumatoid arthritis associated auto-antibodies are helpful serological tools in stating the definite diagnosis. Therefore, we aimed in our study to evaluate the diagnostic performances of anti-cyclic citrullinated peptides antibody (anti-CCP) assay and Rheumatoid Factor (RF IgM) by enzyme linked immunosorbent assay (ELISA) in patients with RA in Syria. Anti-CCP and RF assays were performed in 70 RA patients, and healthy control patients. The cut-off values recommended by the respective manufacturers were used to determine the sensitivity and specificity. Our study shows that Sensitivity of anti-CCP was similar to RF-IgM (65.7%) with better specificity (100% vs 94.4%) The combination of rheumatoid factor (RF) and antibodies against cyclic citrullinated provide better specificity to RF-IgM. In conclusion, Anti-CCP assay could be a useful serological test for the diagnosis of Syrian patients with RA, because anti-CCP revealed higher diagnostic specificity than RF, but combination of these two markers can increase RF-IgM specificity

Keywords: Rheumatoid Arthritis, Anti-cyclic citrullinated protein, anti-CCP, Rheumatoid Factor, RF (IgM), ELISA.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, systemic, autoimmune and inflammatory disorder which affects 0.5-1% of the population worldwide¹⁻³.

The disease is diagnosed on basis of a set of clinical, serological, and radiological criteria. With significantly improved therapy options now available, early treatment could be shown to prevent irreversible joint damage reducing signs and symptoms of erosion and improving physical function. This requires a reliable diagnosis in the early stages of disease precipitation, since a substantial number of patients can undergo spontaneous remission.

Rheumatoid arthritis associated auto-antibodies are helpful serological tools in stating the definite diagnosis⁴⁻⁶. The combination of rheumatoid factor (RF) and antibodies against cyclic citrullinated peptides (CCP) is generally accepted by the majority of rheumatologists and recommended by the European League of Arthritis and Rheumatism (EULAR)⁷.

Historically, rheumatoid factor (RF) has long been the serological indicator for RA. Rheumatoid factor is an antibody directed against the Fc region of IgG that has been used as a diagnostic marker for RA. However, it is non-specific and may be present in healthy elderly persons or in patients with other autoimmune and infectious diseases⁸.

In recent years, the introduction of serum antibodies against citrulline-containing molecules showed some promise as diagnostic tools in RA. Citrulline can be formed by posttranslational enzymatic conversion of arginine residues, catalyzed by peptidylarginine deiminase enzymes⁹⁻¹⁰.

Anti-CCP antibodies are as sensitive as, but more specific than, RF isotopes for diagnosing RA and can also be found in RF-negative RA patients¹¹. However, reported diagnostic sensitivities and specificities ranged from 39% to 94% and 81% to 100%, respectively¹². We aimed in this study to determine specificity and sensitivity of both Anti-CCP and RF and its combination importance in diagnosing Rheumatoid Arthritis Syrian patient

MATERIALS AND METHODS

Patients and controls

Patient with RA: seventy Syrian patients with RA, as defined by the 1987 American Rheumatism Association (ARA) criteria, from Assad University hospital and Al-Mowasa University Hospital.

The RA patient consists of 19 male and 51, the mean age 42 years (range 16-65). Twenty nine of patients have early disease (less than year) and forty one have late disease (more than year). The controls (5 male and 13 female, mean age 39 year) were selected from healthy people how doesn't have previous join disease.

The clinical indices recorded included age, gender, duration of RA, treatment with corticosteroids, non-steroidal anti inflammatory drugs (NSAIDs), and disease modifying anti-rheumatic drugs (DRAMD).

Serology

Sera from patient with RA and healthy individual were stored° at - 80C until the assay.



Table 1: Baseline characteristics of the patient's rheumatoid arthritis (RA) and control

	RA group	Control group
Age	N=70	N=18
Gender	(19 male, 51 female)	(5 male, 13 female)
Age	18-65 year	55-20
	12.83± SD:42.8±X	SD± X:8.9±39.6
Disease duration	1 month-25 year	

Detection of anti-CCP antibodies

The levels of Anti-CCP in sera were determined by Elisa kit (Euro Diagnostica, using Anti-CCP detection kit provided in strict accordance with the instructions of the kit. The concentration of Anti-CCP in the sera is presented as relative value compared to OD of the stander serum. Anti-CCP antibody cut off value of the 25 U/mL according to the manufacturer's instructions.

Detection of IgM-RF

The levels of RF IgM in sera were determined by Elisa kit (IBL, Germany) using RF IgM detection kit provided by in strict accordance with the instructions of the kit. The concentration of RF IgM in the sera is presented as relative value compared to OD of the stander serum (RF IgM) antibody cut off value of the 15 U/mL according to the manufacturer's instructions.

Statistical analysis

Data were analyzed using Excel (2007), SPSS version 19, Test was used to evaluate the relation between (RA, healthy group), correlation between two variable was performed using Pearson correlation, P-value less than 0.05 was regarded as significant.

RESULTS

Serum titer of Anti-CCP in rheumatoid arthritis and control group

The average level Anti-CCP were significantly higher ($P<0.0001$), in patient with RA. ($X\pm SD: 462.93\pm 663.38$ U\ML) than control ($X\pm SD: 17.58\pm 6.02$ U\ML), Figure (1)

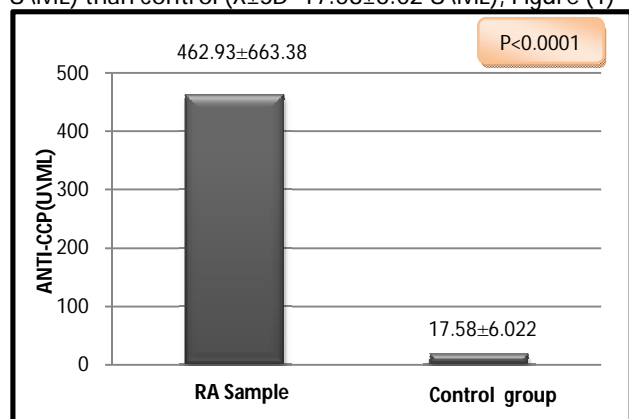


Figure 1: Levels of Anti-CCP in RA and control groups

Serum titer of RF IgM in rheumatoid arthritis and control group

The average level RF IgM were significantly higher ($P<0.001$) in patient with RA ($X\pm SD: 96.03\pm 97.62$ U\ML) than control ($X\pm SD: 3.91\pm 5.83$ U\ML), Figure (2).

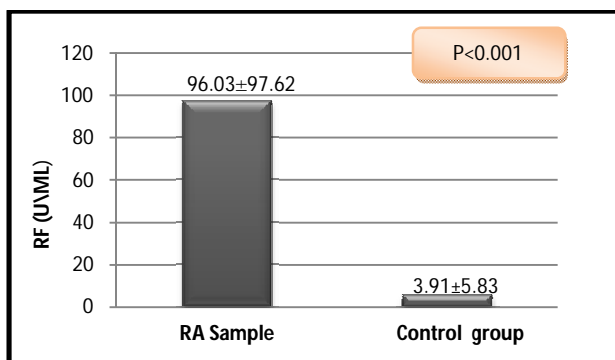


Figure 2: Levels of (RF IgM) in RA and control groups

At a cut-off value 25 U/ml, anti-CCP was positive in 67.5% (47 of 70) of the RA cases and none of (18) controls. Therefore, the assay featured 67.5% sensitivity and 100% specificity.

At a cut-off value 15 U/ml, IgM-RF was positive in 65.7% (46 of 70) of the RA cases and in 5% (1 of 18) of the controls. Therefore, the assay featured 65.3% sensitivity and 94.4% specificity.

We compared the results of anti-CCP and IgM-RF in terms of sensitivity and specificity; IgM-RF showed the same sensitivity (65.7% vs 67.5%) and a lower specificity (94.4% v s. 100%) than anti-CCP.

Among patients with RA, 40/70 (60%) were positive for both antibodies (anti-CCP and IgM RF). Among 24 RF negative patients, 6 (25%) were anti-CCP positive. Among 23 anti-CCP negative patients, 5 (21.7%) were positive for IgM RF. By combination between Anti-CCP and RF, sensitivity of RA 58.57%, specificity 100%, also we found small but significant correlation between the levels of anti-CCP and RF ($r=0.301$, $p<0.001$) (Figure3).

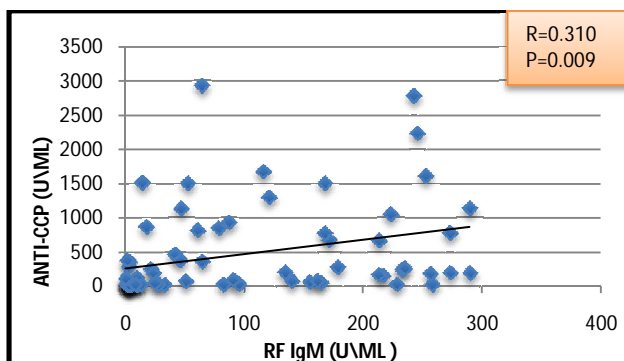


Figure 3: correlation between the levels of anti-CCP and RF

Mean value of anti-CCP between male and female (485.9 vs 455.1 U\ml) had no significant difference in RA ($p=0.86$), also mean value of RF-IgM between male and

female (94.07 vs 96.76U/ml) had no significant difference in RA ($p=0.92$).

DISCUSSION

In recent years, the introduction of anti-cyclic citrullinated peptide antibodies (anti-CCP) showed some promise as a diagnostic tool in RA. These antibodies are detected by ELISA technique where a synthetic cyclic citrullinated peptide is used as a substrate¹³⁻¹⁴. The first generation of the test showed reasonable sensitivity (48–68%) and excellent specificity (98%)¹⁵. The second generation of the test was introduced in 2002. Cyclic citrullinated peptide was used as an epitope in order to achieve a better sensitivity. The sensitivity of the anti-CCP2 test varied from 64.4% to 96%. The specificity values reported ranged from 88.9% to 100%⁽¹⁶⁾.

In our study The diagnostic performance of anti-CCP sera obtained from patients with RA and healthy control showed the anti-CCP sensitivity (67.5%) and specificity (100%), respectively this result was similar with Sthaneshwar P, et al.,¹⁷ reported the diagnostic sensitivity and specificity of anti-CCP (65%, 96%), Manole1 C, et. al.,¹⁸ showed that the diagnostic sensitivity and specificity of anti-CCP (69%, 99%), Serdaroflu M et. al.,¹⁹ find that diagnostic sensitivity and specificity of anti-CCP (65%, 98%). The mean value of in our study anti-CCP between male and female had no significant difference in RA ($p=0.761$), similar to Choi et. al.,²⁰ study reported no significant difference in the mean value between male and female ($p=0.761$).

RF –IgM sensitivity did not significantly differ from that of anti-CCP (65.7% vs 67.5%) with lower specificity (94.4% vs 100%). This result was similar to Damjanovska L⁽²¹⁾ reported the diagnostic sensitivity and specificity of anti-CCP and RF-IgM (69%,67%),(99%,92%).These result different from Frempong et al.,²² which reported sensitivity of anti-CCP and RF-IgM (54.3%, 54%), and specificity (96.67%, 69.5%). This differentiation can be due to different cut-off values or type of RF-IgM assay. In our study mean value of RF-IgM between male and female had no significant difference in RA ($p=0.924$), as reported in Golender I et. al.,²³ study.

In our study, (25%) RF negative RA patients were anti-CCP positive these result was similar to Sthaneshwar P et. al.,¹⁷ (28%) but less than Mobini M et. al.,²⁴ (33%). This suggests that in anti-CCP could be an additional diagnostic marker for RA, especially in RF negative patient.

As shown in other studies. Anti-CCP and IgM RF are correlated and it is considered that 58–72% the IgM RF-positive patients are also anti-CCP-positive¹⁶. In our study, combined IgM RF and anti-CCP obtained a sensitivity (60%) and a better specificity 100%. This result was similar to Jansen et. al.,²⁵ reported combined IgM RF and anti-CCP in RA and obtained a sensitivity of 5.4% and a specificity of 96.7%.

In general we noticed that anti-ccp sensitivity did not significantly differ from RF-IgM but it had better specificity, also it can be useful marker especially in RF negative patient .

CONCLUSION

Anti-CCP assay could be a useful new serological test for the diagnosis of Syrian patients with RA, because anti-CCP revealed higher diagnostic specificity than RF, but combination of these two markers can increase RF-IgM specificity.

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