Original Article

Comparism between Anti-RA33, Anti-CCP antibodies and Rheumatoid Factor in the Diagnosis of Rheumatoid Arthritis in Iraqi Patients

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Summary:

Background: Anti-RA33 antibodies and anti-CCP antibodies are highly specific markers for rheumatoid arthritis (RA), but are not detectable in all RA patients.
Anti-RA33 antibodies are directed to the heterogeneous nuclear ribonucleoprotein A2 (hnRNP-A2),while anti-CCP antibodies are directed to modified epitope on proteins that undergo conversion of amino acid arginine to citrullin by citrullination.

Objectives: The aim of this study was to show the correlation between anti-RA33 antibodies, anti-CCP antibodies and rheumatoid factor (RF) in terms of sensitivity and specificity for the diagnosis of rheumatoid arthritis in Iraqi patients.

Fac Med Baghdad
2012; Vol. 54, No. 1
Received Dec., 2011
Accepted Feb.2012

Subjects and methods: This study was conducted in the period between December 2010 and April 2011 on 70 subjects. Forty five (45) of them were diagnosed as established RA patients and they were on treatment (the patients group) who were attending the rheumatology out patients clinic or admitted to Baghdad teaching hospital ,and twenty five (25) subjects were healthy(the control group). From each subject 5mls of venous blood was aspirated and centrifuged to separate the serum. The sera were stored at -20C and tested for anti-RA33 antibodies, anti-CCP antibodies, and RF (IgM) antibodies using enzyme linked Immunosorbent assay (ELISA) kit.Statistical analysis in this study was done according to spss version 17.

Results: There were thirty eight (38) seropositive (RF positive) patients (84.4%) and seven (7) seronegative (RF negative) patients (15.6%),with thirty(30) (66.6%) patients were anti-CCP antibodies positive and fifteen (15) (33.4%) patients anti-CCP antibodies negative, while there were twenty five(25)(55.6%) patients anti-RA33 antibodies positive and (20)(44.4%) patients were anti-RA33 antibodies negative.

Conclusion: Anti-CCP antibodies have the highest specificity and rheumatoid factor has the highest sensitivity than anti-RA33 antibodies, but anti-RA33 antibodies are helpful in patients who are anti-CCP negative and RF negative.

Keywords: Rheumatoid arthritis,anti RA33 antibodies, anti-CCP antibodies.

Introduction:

Rheumatoid arthritis (RA) is a chronic systemic autoimmune inflammatory disease that affects many organs and tissues and principally attack synovial joints. (1).Inflammation is mediated in part by stromal micro-environment, but the exact underlying causes still unclear. (2)(3). Many auto-antibodies could be implicated in the pathogenesis of the disease; of these auto-antibodies are anti-RA33 antibodies which are directed to the heterogeneous nuclear ribonucleoprotein A2 (hnRNP-A2),the (hnRNP-A2) is over expressed in inflamed synovial tissues ,but its expression in normal joints is very low ,so anti-RA33 antibodies has been described as highly specific antinuclear antibody for RA.(4). Anti-RA33 antibodies were positive in about 35% of rheumatoid arthritis patients, also they were present in 20-25% of systemic lupus erythematosus (SLE) and in 35-40% of patients with mixed connective diseases (MCTD), and they were rare or absent in other arthritic conditions as osteoarthritis and ankylosing spondylitis.(5). As with ACPA and RF,anti-RA33 antibodies may be present in the initial stages of the disease and since they do notcorrelate with ACPA or RF they represent additional useful markers in the diagnosis of RA especially in patients who are ACPA or RF negative, Furthermore patients who are anti-RA33 positive are with more favorable prognosis.(5).Anti-citrullinated peptide antibodies (ACPA) or anti-cyclic citrullinated protein antibody (anti-CCP) are auto antibodies that are frequently detected in the blood of RA patients, they target modified epitope on proteins and the main epitope for these antibodies is filaggrin. (6).Citrullinated proteins were detected in many inflammed tissues as arthritic joints, lungs. (7).They are found

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in about 60-70% of RA patients with rather high specificity (almost always 100%) , and in only about 2% of healthy population are ACPA positive and relatively few patients with other systemic inflammatory diseases are also ACPA positive. (8).In July 2010, ACPA testing has become part of the 2010 ACR/EULAR classification criteria for the early diagnosis of RA which overruled the old ACR criteria of 1987. (9). Rheumatoid factor RF is the most studied antibody in RA and its discovery back at 1930, leads to the logical view that RA is an autoimmune disease. (10) Rheumatoid factor (RF) is an antibody that is detectable in the blood; it is commonly used as a blood test for diagnosis of rheumatoid arthritis. It is present in 70% to 90% of adults (but to a much lower proportion in children) who have RA. They are auto antibodies of predominantly immunoglobulin M (IgM type) which are reactive with the FC (constant) portion of IgG resulting in RF-IgG immune complexes, that may be deposited in tissues and activate the classical complement pathway and lead to tissue damage. (11).The presence of RF may be of prognostic significance because patients with high titer tend to have more severe disease and a positive RF result is a strong predictor of radiological progression in early RA. (12) (1) RF is present in 5% of healthy individuals and in other pathological conditions as systemic lupus erythematous, Sjogren's syndrome, chronic liver diseases and tuberculosis. (1)

Subjects and Methods:

This study was conducted in the period between December 2010 and May 2011 on 70 subjects. Forty five (45) of them were diagnosed as established RA patients and they were on treatment (the patients group) that were attending the rheumatology out patient's clinic or admitted to Baghdad teaching hospital.All (45) patients met 2010 American College Criteria (ACR)/European League Against Rheumatism (EULAR) classification criteria for diagnosis of RA, (include scoring from zero to ten points) where each patient should get at least 6points to be considered as an RA patient. There were 39 females to 6 males with ratio of female to male was 6.5:1, their age ranged from 15-64 years. The controls include twenty five (25) healthy subjects that have no clinical signs of arthritis or other disease, nineteen (19) of them were females and six(6) were males with a ratio of females to males was 3:1 and their ages ranged from 20-51 years. From each subject 5ml of venous blood was aspirated ,sera were separated by centrifugation and were stored at -20 centigrade and tested for anti-RA33 antibodies ,anti-CCP antibodies ,and for RF by enzyme- linked Immunosorbent assay(ELISA) kit from Human co. Germany according to manufacturer's protocol.

Results:

This study included 45 patients with RA, 39 females & 6 males, the female to male ratio was 6.5:1, with mean age was 44.7 and standard deviation (SD)was ±11.43 and 25 healthy subjects, their mean age was 45.3 and standard deviation (SD) was ±6.7.Anti-RA33 antibodies were positive in 25 patients (55.6%) with RA and positive in 1(4%) subject in the control group as shown in Table (1).

Table (1): Results of Anti-RA33 antibodies, anti-CCP antibodies and rheumatoid factor by ELISA technique in patients and control groups.

Parameter	Result	Patient	Control	P-Value
RA33	Positive	25(55.6%)	1(4.0%)	<0.005 Highly sign.
	Negative	20(44.4%)	24(96.0%)	
CCP	Positive	30(66.7%)	0(0%)	<0.005 Highly sign
	Negative	15(33.3%)	25(100.0%)	
RF	Positive	38(84.4%)	4(16.0%)	0.05 not sign.
	Negative	7(15.6%)	21(84.0%)	

Rheumatoid factor has the highest sensitivity and anti-CCP has the highest specificity as shown in Table (2).

Table (2): Comparison of sensitivity and specificity between Rheumatoid factor, anti-CCP and anti-RA33 in patients with Rheumatoid arthritis.

Parameter	Sensitivity	Specificity
Rheumatoid factor	84.44%	84%
Anti-CCP	66.66%	100%
RA33 antibodies	55.55%	96%

There was significant correlation between anti-RA33 antibodies and disease severity (scoring) in patients with rheumatoid arthritis as shown in Table (3).

Table (3): Relation between RA33 and age, duration and scoring (severity of disease according to 2010 ACR/EULAR classification criteria).

Type	RA33	No.	Mean	Std. Deviation	P-Value
Age	Positive Negative	25 20	45.2000 44.2500	12.16210 10.75505	0.785 Not sign.
Duration (years)	Positive Negative	25 20	5.1664 6.3500	5.37253 4.90193	0.450 Not Sign.
Scoring severity	Positive Negative	25 20	9.1400 8.3250	0.94119 1.41677	0.026 Significant relation

There was significant correlation between anti-CCP antibodies and disease severity (scoring) in patients with rheumatoid arthritis as shown in Table (4).

Table (4): Relation between anti-CCP antibodies and age, duration and scoring (severity of disease according to 2010 ACR/EULAR classification criteria).

Type	Anti-CCP	No.	Mean	Std. Deviation	P-Value
Age	Positive Negative	30 15	44.2667 45.8000	10.58930 13.31594	0.677 Not sign.
Duration (years)	Positive Negative	30 15	5.3720 6.3333	5.15377 5.24631	0.561 Not sign.
Scoring (severity)	Positive Negative	30 15	9.2000 7.9333	1.12648 0.99762	0.001 Highly Sign.

Discussion:

RA is a chronic inflammatory disorder manifesting typically as a symmetrical polyarthritis,it is characterized by chronic inflammation of synovial joints that leads to progressive joint destruction & disability with reduction in quality of life.(13). This study included 45 patients with RA who were attended the rheumatology outpatient clinic or admitted to Baghdad teaching hospital in the period from December 2010 to April 2011 and 25 control healthy persons. The mean age of patients was 44.7±11.43 yrs, this is in accordance with other study which mentioned that RA affects usually people 40 years of age & starts usually after middle age as other which AIDs usually start after middle age.(14) RA starts after 40 yrs due to many reasons that depress immunity as stress , thymic depression, exposure to different antigens as smoking (tobacco) that leads to activation of auto-reactive lymphocytes.(15).This study shows that female are more predominant for RA than males with ratio of 6.5:1 and this agree with other study that showed a ratio of 3:1 conducted by AL-Rawi et al at 1977.(16),this female predominance in Iraq could be related to many reasons as the high number of females compared to males due to wars. The female predominance also could be due to hormonal factors such as estrogen which enhances the function of T-helper cells and inhibits the function of T-suppressor cells. (17). Table (1) showed that RF (IgM) was positive in 38 pts. (84.44%) with sensitivity and specificity of (84.44% & 84%) respectively in agreement with other study.(1) Anti-CCP antibodies were positive in 30 patients (66.66%) as shown in table (1), although our patients were on treatment but this did not affect the results of CCP and they are more sensitive markers than anti-RA33 because they remained positive even when the patient had treatment for long time while anti-RA33 usually positive in the initial phase of the disease.(8)(18) Anti-CCP antibodies were negative in all control groups while other study stated that they were positive in 2% of healthy persons, this difference may be due to small number of the healthy subjects. Anti-RA33 antibodies were positive in 25 patients (55.6%) with a sensitivity of 55.55% while other study showed its sensitivity ranges between 40-60 %. (19) This is due to strong racial variations and depends on the duration of the disease as it is mostly positive in the initial stages of the disease. (20) Specificity of anti-RA33 antibodies was 96% and this is in accordance with other studies especially if SLE and mixed connective tissue disease were excluded as in our patients. (21) The relation between RA33 and age had shown no significance and this isin accordance with other study as shown in Table (3). (22) Duration also showed no significance to anti-RA33 antibodies in this study as most of the patients had RA for more than 1 year so anti-RA33 antibodies showed no significance as it is positive in the initial stage of the disease Table (3). (5) In contrast anti-RA33 antibodies had significant relation with severity (scoring) of the disease that>s mean most of patients with severe RA(10 points) had positive anti-RA33 antibodies so patients with positive RA33 had more severe disease than seronegative patients.(5) Table (4) showed that anti-CCP antibodies had no significance with age and duration of the disease while had significant correlation with severity of the disease so patients with positive anti-CCP antibodies have a more severe rheumatoid arthritis than patients with negative anti-CCP antibodies and this is in accordance with other studies. (23) (24) Tables (2) showed no significant correlation between anti-RA33 antibodies, anti-CCP antibodies and rheumatoid factor in relation to positivity and negativity and since they do not correlate with ACPA and rheumatoid factor so anti-RA33 antibodies represent useful additional markers antibodies especially in patients who are negative for ACPA and rheumatoid factor this is in accordance with other study. (5). Table (2) showed that anti-CCP had the highest specificity than anti-RA33 and RF and this is in accordance with other studies. (25)(8) While highest sensitivity was in RF than anti-CCP and anti-RA33 because RF remains positive even if the patient had remission or on treatment so it remains positive and this is in accordance with other study, this is due to cross reaction with non- specific antigens. (1) While anti-RA33 antibodies are positive only in the initial phase of the disease. (20) So patients with positive anti-RA33 antibodies and anti-CCP antibodies had a more severe disease, this might be due to that these auto antibodies played an important role in the disease and joint inflammation, especially anti-RA33 antibodies which is positive in the initial phase so plays an important role in the initiation of the disease.

Conclusion:

Anti-CCP antibodies have the highest specificity and rheumatoid factor has the highest sensitivity than anti-RA33 antibodies, but anti-RA33 antibodies are helpful in patients who are anti-CCP negative and RF negative.

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