Comparison between Anti-filaggrin, Anti-RA33 and Anti-Cyclic Citrullinated Peptide Antibodies in the Diagnosis of Rheumatoid Arthritis in Iraqi Patients

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Abstract

Background: Anti-filaggrin, Anti-RA33 and anti-CCP antibodies are highly specific markers for rheumatoid arthritis (RA), but they are not detectable in all RA patients.

Anti-filaggrin antibody are antibodies that reacts with keratinized tissue of animal esophagus(Anti-Keratin antibody) .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-filaggrin antibodies, 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.

Subjects and methods: This study was conducted in the period between April 2011 and October 2011 gathering 90 subjects. Fifty (50) 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 forty (40) subjects were apparently without any arthritis (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-filaggrin antibodies, anti-RA33 antibodies, anti-CCP antibodies, and RF (IgM) antibodies using enzyme linked Immunosorbent assay (ELISA) kit.

Results: There were forty two (42) seropositive patients (84.0%) and eight (8) seronegative patients (16.0%),with thirty four (34) (68.0 %) patients were anti-CCP antibodies positive and sixteen (16) (32.0%) patients were anti-CCP antibodies negative, while there were twenty nine (29)(58.0%) patients anti-RA33 antibodies positive and (21)(42.0%)patients were anti-RA33 antibodies negative. anti-filaggrin antibodies were positive in forty four (44) (88%) and it was negative only in six (6) (12%) patients.

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

Keywords: Rheumatoid arthritis, RA33 antibodies, anti-CCP antibodies anti-filaggrin 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)]. Auto antibodies are detected in this disease, with diagnostic and prognostic properties. One of them is AKA which reacts with the fibrous keratin in epidermis and the stratum corneum of rat esophageal epithelium with a sensitivity & specificity in rheumatoid arthritis of (85%) & (95%) respectively [(3)].

Other auto-antibodies could be implicated in the pathogenesis of the disease, 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 [(5)].

As with Anti-citrullinated peptide antibodies (ACPA) and RF, anti-RA33 antibodies may be present in the initial stages of the disease and since they do not correlate with ACPA or RF they represent additional useful markers in the diagnosis of RA especially in patients who are ACPA or RF negative [(6)].

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 [(7)].They are found in about 60-70% of RA patients with rather high specificity (almost always 97%) , 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 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]

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 April 2011 and October 2011 on 90 subjects. Fifty (50) 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 (50) 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 (6 points were mild, 7or 8 points were moderate 9or 10 points were severe). Their sex was 42 females to 8 males with ratio of female to male was 5:1, their age ranged from 18-67 years.

The control group included forty 40 subjects that have no clinical signs of arthritis or other inflammatory diseases, thirty four 34 of them were females and six 6 were males with a female to male ratio is 5:1, their ages ranged from 20-70 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-fillagrin antibodies 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**.**

Statistical Analysis

The statistical analysis was done using SPSS v-17. (P-value) of <0.05 was considered significant .P-value of <0.01 was considered highly significant, But >or=0.05 was consider non significant.

The sensitivity (among RA patients) and the specificity (among controls) were computed for each test. The sensitivity of any test in RA means the proportion of patient with RA who is test positive.

Sensitivity= (TP/TP+FN) X 100%

While the specificity mean the proportion of healthy patient without RA who are test negative.

Specificity= (TN/TN+FP) X 100%

- TP (True Positive): A positive result for patients who have the disease.
- TN (True Negative): A negative result for subject who do not have the disease.
- FP (False Positive): A positive result for subject who do not have the disease.
- FN (False Negative): A negative result for patients who have the disease.

The study underwent an ethical committee and the patients had written an informed consent

Results:

This study included 50 patients with RA,42 females & 8 males, the female to male ratio was 5:1, with mean age was 47.7 years and standard deviation (SD)was ±17.44 and 40 healthy subjects, their mean age was 46.5 years and standard deviation (SD) was ±16.3.

-Anti-fillagrin antibodies were positive in 44 (88%) patients with RA and positive in 2(5%) subject in the control group as shown in Table (1).

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

Parameter	Result	Patient	Control	P-Value
Anti-Fillagrin Abs	Positive	44(88.0%)	2(5.0%)	<0.005
	Negative	8(12.0%)	38(95.0%)	Highly sign.
Anti- RA33Ab	Positive	29(58.0%)	3(7.5%)	<0.005
	Negative	21(42.0%)	37(92.5%)	Highly sign.
Anti- CCP Abs	Positive	34(68.0%)	1(2.5%)	<0.005
	Negative	16(32.0%)	39(97.5%)	Highly sign
RF	Positive	42(84.0%)	8(20.0%)	>0.05
	Negative	8(16.0%)	32(80.0%)	Not sign.

Anti-fillagrin antibodies have the highest sensitivity and anti-CCP has the highest specificity as in Table (2).

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

Parameter	Sensitivity	Specificity
Anti-fillagrin Antibodies	**88.0%**	95%
Anti-RA33 antibodies	58.0%	92.5%
Anti-CCP Antibodies	68.0%	**97.5%**
Rheumatoid factor IgM	84%	80%

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

Table (3): Relation between anti-fillagrin antibodies & anti-RA33 antibodies with the scoring (severity of disease according to 2010 ACR/EULAR classification criteria).

Type	No. of Patients	Mean±SD of Scoring (severity)	P-Value
Anti-Fillagrin Antibodies	Positive 44(88%) Negative 8(12%)	9.3224±1.10324 7.3245± 0.93321	**<0.001** **Highly Significant**
Anti-RA33 antibodies	Positive 29(58%) Negative 21(42%)	9.2500 ± o.92133 8.2233 ± 152311	**<0.05** **Significant difference**

Discussion:

This study included 50 patients with RA who were attended the rheumatology outpatient clinic or admitted to Baghdad teaching hospital in the period from April 2011 to October 2011 and 40 control healthy persons.

The mean age of patients was 47.7±17.44 yrs, this is in accordance with other study which mentioned that RA affects usually people at 40 years of age & starts usually after middle age as other Auto-Immune Diseases [14]. RA starts after 40 yrs due to many reasons that depress immunity as stress, thyme depression, and 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 5:1 and this agree with other study that showed a female predominance conducted by AL-Rawi et al at 1977 [16]. The female predominance may 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) shows that RF (IgM) was positive in 42 patients (84.0%) with sensitivity and specificity of (84.0% & 80%) respectively, which is in agreement with other study [1].

Anti-CCP antibodies were positive in 34 patients (68.0%) 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 than anti-RA33 because they remained positive even 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 39 (97.5%) subject of the control group 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 [18,19].

Anti-RA33 antibodies were positive in 29 patients (58.0%) while other study by Ronnelid et al showed its sensitivity ranges between 30-50 % [18] , this difference may be due to there is a 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 92.5% in accordance with other studies especially if SLE and mixed connective tissue disease were excluded as in this study [21].

The relation between RA33 and age had shown no significance in accordance with other study [22] .In contrast anti-RA33 antibodies had significant relation with severity (scoring) of the disease as shown in table (3), 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].

Anti-Fillagrin antibodies were positive in 44 patients (88.0%) like other study which showed its sensitivity ranges between 80-100 % [19]. Specificity of anti-fillagrin antibodies was 95.0% which is also in accordance with other studies [19,21].

The relation between anti-fillagrin antibodies and age had shown no significance in accordance with other study [19]. In contrast anti- fillagrin antibodies had a significant relation with severity (scoring) of the disease as shown in table (3), that's mean most of patients with severe RA(10 points) had positive anti-fillagrin antibodies

Table (2) showed that anti-CCP had the highest specificity than anti-fillagrin antibodies, anti-RA33 and RF in accordance with other studies [8,22].

The highest sensitivity was in anti-fillagrin antibodies than anti-CCP and anti-RA33 and remains positive even if the patient had remission or on treatment these results are in accordance with other studies [3,19].

Patients with positive anti-fillagrin antibodies, anti-RA33 antibodies and anti-CCP antibodies had a more severe disease, this might be due to that auto antibodies played an important role in the disease and joint inflammation, especially anti- fillagrin antibodies & anti-RA33 antibodies which is positive in the initial phase so plays an important role in the initiation of the disease [22, 23].

Conclusion:

Anti-CCP antibodies have the highest specificity and anti- fillagrin antibodies has the highest sensitivity than anti-RA33 antibodies, but anti-RA33 antibodies are helpful in the diagnosis of patients who are anti- fillagrin antibodies, anti-CCP and RF negative

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