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

Mohammed Marrof Al-Ani. MSc, FICMS

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 erythematosus, 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 6 points to be considered as an RA patient (6 points were mild, 7 or 8 points were moderate 9 or 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-filaggrin 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 considered 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 are test positive.

Sensitivity = $(TP / (TP + FN)) \times 100\%$

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

Specificity = $(TN / (TN + FP)) \times 100\%$

- 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-filaggrin 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-filaggrin 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-Filaggrin Ab	Positive	44(88.0%)	2(5.0%)	<0.005
	Negative	8(12.0%)	38(95.0%)	Highly sign.
Anti- RA33 Ab	Positive	29(58.0%)	3(7.5%)	<0.005
	Negative	21(42.0%)	37(92.5%)	Highly sign.
Anti- CCP Ab	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-filaggrin antibodies have the highest sensitivity and anti-CCP has the highest specificity as shown in Table (2).

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

Parameter	Sensitivity	Specificity
Anti-filaggrin 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-filaggrin 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-filaggrin antibodies & anti-RA33 antibodies with the scoring (severity of disease according to 2010 ACR/EULAR classification criteria).

Type	Number Of Patients	Mean & Std. Deviation Of Scoring (severity)	P-Value
Anti-Filaggrin Antibodies	Positive 44(88%) Negative 8(12%)	9.3224±1.10324 7.3245± 0.93321	<0.001 Highly Significant difference
Anti RA33 antibodies	Positive 29(58%) Negative 21(42%)	9.2500 ± 0.92133 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, thymic 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-Filaggrin antibodies were positive in 44 patients (88.0%) like other study which showed its sensitivity ranges between 80-100% [19]. Specificity of anti-filaggrin antibodies was 95.0% which is also in accordance with other studies. [19, 21]

The relation between anti-filaggrin antibodies and age had shown no significance in accordance with other study [19].

In contrast anti-filaggrin 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-filaggrin antibodies

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

The highest sensitivity was in anti-filaggrin 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 study. [3,19].

Patients with positive anti-filaggrin 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-filaggrin 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-filaggrin antibodies has the highest sensitivity than anti-RA33 antibodies, but anti-RA33 antibodies are helpful in the diagnosis of patients who are anti-filaggrin antibodies, anti-CCP and RF negative

References:

- 1- Lipsky P. Rheumatoid arthritis. In: Fauci AS. Harrison's Rheumatology. 16th ed. New York, NY, 2006: 85-104.
- 2- Mc Innes IB, Schett G: Cytokines in the pathogenesis of rheumatoid arthritis. *Nat Rev Immunol* 2007; 7: 429-42.
- 3- Abedian F, Baradaran H, S.A. Rezaee M. Designing of Anti keratin Antibody kit by Immuno fluorescent assay (IFA) and it's evaluation in Rheumatoid Arthritis (RA) patients *Journal of Mazandaran University of Medical Sciences* 2005, 15: 18-25
- 4-- Steiner G, Hochberg MC, Silman AJ. *Rheumatology* London. Elsevier sciences. 2003: 833-42.
- 5- Christian V, Guy S , Frederic L , Bernard F, Christian A, Antone F, and Jean-Pierre S . High diagnostic value in rheumatoid arthritis of antibodies to the stratum corneum of rat oesophagus epithelium, so-called 'antikeratin antibodies. *Annals of the Rheumatic Diseases* 1989; 48: 712-722
- 6- Kinloch A, Lundberg K . .Synovial fluid is a site of citrullination of auto antigen in inflammatory arthritis .*Arthritis Rheum* 2008; 120:1-5.
- 7- Borgeurtz T, Cantaert T, Frederic L, Bernard F. Citrullination in extra-articular manifestations of rheumatoid arthritis. *Rheumatology (Oxford)*, 2007; 46:70-5.
- 8- Klareskog L, Ronneild J, Lundberg K. Immunity to citrullinated protein in rheumatoid arthritis. *Annu Rev Immunol* 2008; 26:651-75.
- 9- Aletaha D, Neogi T, Silman AJ. The 2010 rheumatoid arthritis classification criteria: An ACR/EULAR. *Ann. Rheum. Dis.* 2010; 69(9): 1580-8. Retrieved Feb, 2011.
- 10- Waaler E. On the occurrence of a factor in human serum activating the specific agglutination of sheep red blood cell corpuscles. *Acta Pathol Microbial Scand* 1940; 17:172-73.
- 11- Christian D, Werner Klotz, Heike L, Christina D Michael Schirmer and Manfred H. Diagnostic value of antibodies against a modified citrullinated vimentin in rheumatoid arthritis *Arthritis Research & Therapy* 2008; 8(4):1-6.
- 12- Jansen LM, Van Der Host-Bruinsma IF. Predictor of radiologic joint damage in patients with early rheumatoid arthritis. *Ann Rheum Dis.* 2006. 924-927.
- 13- Silman AJ. And Pearson JE. Epidemiology and genetics of rheumatoid Res. 4(Suppl.3): 2002; S265-72.
- 14- Alamanos Y, Voulgari PV, Heike L Lundberg K. Incidence and prevalence of rheumatoid arthritis based on 1987 American college of rheumatology: a systemic review .*Semin Arthritis Rheum.* 2006.36(3):182-88.
- 15- Flouris A. D.; Metsios G. S.; Carrillo A. E.; Jamurtas A. Z.; Gourgoulis K.; Kiriopoulou T.; Tzatzarakis M. N.; Tsatsakis A. M.; and Koutedakis Y. "Acute and short-term effects of secondhand smoke on lung function and cytokine production". *Am. J. Respir. Crit. Care Med.* (2009); 79, 1029-33.
- 16- AL-Rawi ZS, AL-Shackarchi HA, Marjana NH .. Rheumatoid Arthritis in Iraq. *Rheumatol Rehabil* 1977; 16(2):128-323.
- 17- Ansar Ahmed S, Dauphinee, MJ, Talal , N. Effects of short term administration of sex hormones on normal and autoimmune mice. *J Immunol* 1985; 134: 204.
- 18- Ronnelid J, Wick MC.. Longitudinal analysis of citrullinated protein/ peptide antibodies (anti-ccp) in 5yrs follow up in early rheumatoid arthritis: anti-ccp status predicts worse disease activity & greater radiological progression. *Ann Rheum Dis.* 2005; 64:1744-9.
- 19-Khitam Y .Diagnosis value of Anti-filaggrin antibody in comparison with Rheumatoid factor in Iraqi patients with rheumatoid arthritis .a thesis submitted to council of pathology in partial fulfillment of the requirements for the degree of fellowship of the Iraqi Board for medical specializations in pathology /immunology 2011.
- 20- Majithia V.; Geraci S. A. "Rheumatoid arthritis: diagnosis and management". *Am. J. Med.* (2007); 120 (11): 936-9.
- 21- Hassfeld W, Studincka-Benke A. Autoimmune response to the splice some antigens. An immunologic link between RA, MCTD and SLE. *Arthritis Rheum* 1995; 45: 777-785.
- 22- Van Gaalen F, Toes RE, Huizinga TW.The emerging role of anti-citrullin autoimmunity in rheumatoid arthritis. *J. Immunol* 2005; 175 (9):5575-80.
- 23- Paimela L.; Gripenberg M.; Kurki P; and Leirisalo-Repo M. "Antikeratin antibodies: diagnosis and prognostic markers for early rheumatoid arthritis". *Ann. Rheum. Dis.* (1992); 51:743-746.

*Path-Immunology/ Department of Microbiology/ College of Medicine-Baghdad