Original Article

The Role of Anti- Cathapsin G among Patients with Rheumatiod Arthritis

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Summary:

Background: Perinuclear Antineutrophil Cytoplasmic Autoantibodies (pANCA) have been found in patients with rheumatiod arthritis. Cathepsin G was the major target antigen.The present study was to investigate the unknown target antigen of ANCA (Cathapsin G) in patients with rheumatiod arthritis

Objective: This study is to investigate the prevalence of anti-Cathapsin G and rheumatoid factor in Iraqi patients with rheumatiod arthritis**.**

Patienties&Methods: From1st January until 30 June of 2011 fourty five rheumatiod arthritis patients referred to the immunological department in the teaching laboratory of medical city and twenty five apparently healthy individual used as a control group were investeged to rheumatic factor IgG, IgM ,IgA isotypes and anti-Cathapsin G were measured by using enzyme immunoassay technique.

Result: This study showed that the age of patients with rheumatiod arthritis ranged between eigthen years and sixty six years with mean age of (36.08) , while the mean level for RF screen, RF IgG, RF IgM, RF IgA isotypes and anti-Cathapsin G were (160.71, 91.7, 136.64, 95.03 and > 9.2) respectively. There were 17(37.8%) males and 28(62.2%) females of RA patients. Immunologically, this study revealed that the **serum** RF screen was found more than 25 U/ml in all rheumatiod arthritis patients (100%, P: 0.0001 HS). On the other hand RF isotypes IgG ,RF IgM and IgA level were elevated more than 20 U/ml and it was detected in 31 (68.9%, P: 0.0001 HS), 31 (68.9%, P : 0.0001 HS) ,and 32 (71.1%, P: 0.0001 HS) patients respectively, while the rheumatiod factors (IgG,IgM, and IgA)were normal in healthy control group . Statistically RF screen has a highly significant association with RF IgG, IgM, and IgA (P= 0.0001). Anti-Cathapsin G was found in 12(26.7%, P value: 0.019) of patients with rheumatic arthritis patients. This study showed a association between the positive result of anti-Cathapsin G with RF IgG and RF IgM (35.5%, P: 0.047) the results were the same for both, while no association found with RF IgA (25%, P: 0.692).

Conclusion: A significant correlation was found between anti-Cathapsin G and patients with rheumatiod arthritis and has a significant association with RF IgG and RF IgM while RF IgA had no relation with anti-Cathapsin G.

Keyword: Rheumatiod arthritis, rheumatiod factor and Cathapsin G.

Introduction:

Rheumatoid arthritis (RA)is one of the most common autoimmune diseases and also the most frequent chronic inflammatory arthropathy..The disease affects around 1% of the world population, 75%of which are females. It is characterized by inflammation of the synovial membrane, which spreads symmetrically from small to large joints leading to the destruction of the joints in the late phase accompanied by a systemic involvement of the soft tissue. Rheumatoid arthritis is a chronic disease, characterized by periods of disease flares and remissions Initial symptoms include painful swelling of the metacarpophalangealjoints with morning stiffness of the joints (1). Reliable and earliest possible diagnosis is indispensable to keep the disease under control with suitable therapy and to avoid irreversible joint damage (2). Rheumatoid factor (RF or RhF) is an autoantibody (antibody directed against an organism's own tissues) most relevant in arthritis. It is an antibody against the Fc portion of IgG, which itself is an antibody. RF and IgG join to form immune complexes that contribute to the disease process(1,3).Rheumatoid factor (RF)are present in the serum of 75-80 %of the patients with RA at some time during the disease course .However ,RFs are also found in the serum of the patients with infectious and autoimmune diseases hyperglobulinemias , B-cells lymph-proliferative disorders and in age population. this suggests that RF may be a finding associated with B-cell hyperactivity (4). In established RA, high titer serum IgM RF correlates with the presence of articular disease and nodules but not with systemic disease activity. The presence of either IgA or IgG RF in patients with long standing RA may be a good prognostic indicator of the systemic manifestations..The availability of IgG and IgA RF

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assays has significantly improved the diagnostic specificity of the test compared to latex agglutination and turbidimetry (5). Moreover, the occurrence of RF IgA together with RF IgM was reported to precede the development of RA by several years(6). RF IgG,when measured as F(ab')2 after pepsin digestion, has been shown to have a high specificity for RA(5,7). Antineutrophil cytoplasmic antibodies (ANCAs) are directed against lysosomal enzymes of human neutrophils and monocytes.perinuclear ANCAs (pANCAs) have been documented to occur in patients with necrotizing and crescent-forming glomerulonephritis, microscopic polyangiitis (MPA) and Churg–Strauss syndrome, and the specific antigen most frequently associated with these pANCAs is myeloperoxidase(MPO) (8). However, pANCAs have been reported in many inflammatory disorders, such as inflammatory bowel disease,primary sclerosing cholangitis, autoimmune hepatitis, and various rheumatic diseases (9,10). The target antigens for the pANCAs in these disorders are usually unclear, although several antigens such as lactoferrin (LF), cathepsinG(CG), human leukocyte elastase (LE) and lysozyme have been reported (11). In patients with rheumatoid arthritis (RA), the reported prevalence of ANCAs has ranged from 20 to 50%, and these predominantly show a pANCA pattern (12). Cathepsin G belong to a group of intracellular proteases mainly found in lysosomes , especially of the spleen, liver and the kidney .Cathapsin G is a serine protease and a further PANCA antigen. It participates to a great part in the destruction of osteid tissue as of its hydrolytic properties. The auto-antibodies against Cathapsin G occur in collagenosis and inflammatory rheumatic arthritic diseases. (13). Cathepsin G, made by the neutrophils is a regulates enzyme, is also an attractive target because it belongs to a class of enzymes known as proteases. Proteases are anew class of cytokines that may play a significant role in chronic inflammation and joint destruction. (14)

Material and Methods:

A cross section study was conducted in a period between (1st January – 30 th June) 2011. The patients were individual attending the ward of Baghdad Teaching Hospital (Rheumatology Clinic) diagnosed then referred to immunological department in the teaching laboratory/ medical city for RF IgG, IgM, IgA isotypes and Cathapsin G assessed in 70 individual (45 patients with Rheumatiod arthritis and 25 apparently healthy control).The RA patients includes 45 patients (17 males, 28 females) with an age range 18 - 66 years old .For purpose of comprise , a control matched group include 25 apparently healthy individual with an age ranged from 14-56 years old (8 males, 17 females). Blood of study groups were collected and the serum stored at -80 until used for the quantitative estimation of serum human RF IgG ,IgA , IgM and antibodies to Cathapsin G by using enzyme immunoassay kits (ELISA technique) method (indirect type).The intensity of coloration produced was proportional to the RF IgG ,IgA , IgM isotypes and anti-Cathapsin G concentration in the sample or standard. The absorbance of each well was read at 450 against substrate blank and the result were calculated by interpolation from standard curve which was constructed in the same assay as the sample, then location of the average absorbance for each sample on the vertical axis was done and the corresponding RF IgG ,IgA , IgM isotypes and anti-Cathapsin G antibodies concentration was read on the horizontal axis. The student T test and chi-sequar test were used to compare soluble factor level among patients and control group and to test for associations between variables. A p-value of 0.05 or less was designated as significant.

Results:

The age of patients with RA in this study range from (18 - 66) years with mean age was 36.08(P-value: 0.115) while the mean of RF screen, RF IgG, RF IgM, RF IgA levels and anti-Cathepsin G were 160.71(P-value: 0.0001 highly significant), 91.70(P-value: 0.02), 136.64(P-value: 0.002), 95.03(P-value: 0.001) and 9.20(P-value: 0.003) respectively with significant difference if compare it with the healthy control group, Table(1). There were 17(37.8%) males and 28(62.2%) females of RA patients meaning there is a considerable difference in sex of the patients study sample.

Table(1) show the mean& P-value of age, RFscren ,RF IgG, RF IgM, RF IgA levels and anti-Cathapsin G in RA patients in compare to healthy control .

	Type	N	Mean	P-value
Age	Patient	45	36.08	
	Control	25	30.28	0.115
Rf screen level	Patient	45	160.71	
	Control	25	8.11	0.000
RF IgG level	Patient	45	91.70	
	Control	25	7.83	0.020
RF IgM level	Patient	45	136.64	
	Control	25	8.06	0.002
RF IgA level	Patient	45	95.03	
	Control	25	8.66	0.001
CathepsinG	Patient	45	>9.20	
	Control	25	3.60	0.003

The anti Cathapsin G antibody was found more than 10 U/ml in rheumatiod arthritis patients and healthy control as 12(26.7%) and 1(4%), respectively with a statistically significant P value (0.019), so this detection consider a significant result for rheumatiod arthritis .Table (2). In all patients of RA 45(100%), the rheumatiod factor screen levels was found ≥ 25 U/ml, while in healthy control group the rheumatiod factor screen was found < 25 U/ml all of them with a highly significant P value(0.0001) as showed in tables(2). when we comparisum the results of RF screen with the results of RF IgG in study groups we detected that serum RF IgG was elevated more than 20 U/ml in 31

(68.9%) patients in arthritis patients but it was found less than 20 U/ml in all control group,with stastically significant Pvalue (0.002).Table(3) On other hand ,the results of RF screen comparing to the results RF IgM ,we found the same results of RF IgG with same P-value (0.002). Table (4).while when it comparing the results of RF IgA concentration they were found RF IgA > 20 U/ml in 32 (71.1%)patients and all the healthy groups were < 20 U/ml with statistically significant P value (0.0001).Table(5)

Table (2): The comparison of RF screen concentration & anti-Cathapsin G in patients with Rheumatiod arthritis patients and healthy control.

Study groups		RF screen				Anti- Cathapsin G			
		Positive (≥25 U/ml)		Negative (<25 U/ml)		Positive (≥10 U/ml)		Negative (<10 U/ml)	
		No.	%	No.	%	No.	%	No.	%
Rheumatiod arthritis No.45		45	100	0	0.	12	26.7	33	73.3
Healthy control No.25		0	0.	25	100	1	4	24	96
χ^2 test	P-value	0.0001				0.019			
	Sig.	HS				HS			

Table (3): The comparison of RF screen concentration & RF IgG in patients with Rheumatiod arthritis patients and healthy control.

Study groups	RF screen				RF IgG			
	Positive (≥25 U/ml)		Negative (<25 U/ml)		Positive (≥20 U/ml)		Negative (<20 U/ml)	
	No.	%	No.	%	No.	%	No.	%
Rheumatiod arthritis No.45	45	100 Within patient group	0	0.	31	68.9	14	31.1
Healthy control No.25	0	0.	25	100	0	0.	25	100

P-value: 0.0001 (significant < 0.05)

Table (4): The comparison of RF screen concentration & RF IgM in patients with Rheumatiod arthritis patients and healthy control.

Study groups	RF screen				RF IgM			
	Positive (≥25 U/ml)		Negative (<25 U/ml)		Positive (≥20 U/ml)		Negative (<20 U/ml)	
	No.	%	No.	%	No.	%	No.	%
Rheumatiod arthritis No.45	45	100 Within patient group	0	0.	31	68.9	14	31.1
Healthy control No.25	0	0.	25	100	0	0.	25	100

P-value: 0.0001 (significant < 0.05)

Table (5): The comparison of RF screen concentration & RF IgA in patients with Rheumatiod arthritis patients and healthy control.

Study groups	RF screen				RF IgA			
	Positive (≥25 U/ml)		Negative (<25 U/ml)		Positive (≥20 U/ml)		Negative (<20 U/ml)	
	No.	%	No.	%	No.	%	No.	%
Rheumatiod arthritis No.45	45	100	0	0.	32	71.1	13	28.9
Healthy control No.25	0	0.	25	100	0	0.	25	100

P-value: 0.0001 HS (significant < 0.05)

In this study, the positive results of rheumatic factor IgG, IgM, and IgA levels comparing to positive results of anti-Cathapsin G in rheumatiod arthritis patients, were for RF IgG(31 (68%)) and RF IgM(31 (68%))about one-third(11 (35.5)) of both of them had anti-Cathapsin G level more than 10 U/ml with a significant p value (0.047). While in the patients who had positive RF IgA (32 (71.1%)) only eight patients their serum anti-Cathapsin G concentration more than 10 U/ml, statistically not significant P value (0.692). table (6)

Table (6): the positive results of RF IgG, IgM and IgA comparing to positive results of anti-Cathapsin G Ab in RA patients

Positive RF > 20 U/ml	Anti-Cathipsin G		TOTAL	P value
	Positive >10 U/ml	Negative < 10 U/ml		
RF IgG NO.	11	20	31	0.047*
%	35.5	64.5	100	
RF IgM NO.	11	20	31	0.047*
%	35.5	64.5	100	
RF IgA NO.	8	24	32	0.692
%	25	75	100	

***P value significant (< 0.05)**

Discussion:

The thrust of many researches had been in the direction of detecting the immune abnormalities that are responsible for the damage in rheumatoid arthritis (RA). There is a complex cellular interaction among different cells in rheumatiod arthritis patients such as T lymphocytes, B lymphocytes, macrophages, plasma cells, fibroblasts, dendritic cells, and neutrophils, this cellular interaction leads to the manufacture and release of many chemical messengers or enzymes. Recently an enzyme known as Cathepsin G which regulates the ability of immune cells known as neutrophils to secrete chemicals that attract other immune cells and start the local inflammatory process. Over time, the excessive accumulation of immune cells can lead to tissue and cartilage damage in joints, causing pain and limiting mobility (14). In this study we found that rheumatiod arthritis occurs relatively more in females(62.2%) than in males(37.8%) , so it is similar to other studies which referred that the rheumatiod arthritis founds around 1% of the world population, 75%of which are female (1)**.** On other hand in this study showed that the mean age for rheumatoid arthritis patients compared to healthy control group was(36.02%) which is statistically not significant P value (0.115). while the mean level of RF screen was 160.71 with highly significant P-value(0.0001),moreover the mean level of RF IgG, RF IgM, RF IgA isotypes and Cathepsin G were 91.70(P-value: 0.02), 136.64(P-value: 0.002), 95.03(P-value: 0.001) and 9.20(P-value: 0.003) respectively . This finding is in consistence with *Swedler W, et al* (5).The prevalence of RF in RA patients based on the results of ELISA technique was varied 70-80% . We found that 100% (45 /45) of our RA patients were positive, which indicate that, the RF is autoantibody (antibody directed against an organism's own tissues) most relevant in rheumatoid arthritis. It is an antibody against the Fc portion of IgG, which is itself an antibody. RF and IgG join to form immune complexes that contribute to the disease process, with highly significant P value (0.0001)which is in agreement with so many study (2,3) . In addition to IgM RF, this study showed that there is a significant relationship between the presence of IgG and IgA RF isotypes and RF antibodies in the likelihood of making a diagnosis of RA, with a highly significant P value (0.001) for all RF isotypes and this was in accordance with *Halldorsdottir HD et al* (6) . Indeed when all RF isotypes were positive (IgM+ IgG+ IgA+), the patients were very likely to have RA; this is in agreement with the 96% PPV for patients who were RF IgM+ IgG+ IgA+ in a hospital population a definite diagnosis (5). The reason for variable presence of RF isotypes in addition to IgM has not been clarified, but may consequences have a response to therapy. There is probably an overlap between natural and pathological IgM RF in the individual as well as in the population. Since the prevalence of IgA and Ig RF in the RA population is lower than that of IgM RF, the isotype switch does not always occur, for unknown reasons. (15) Positive RF IgA is significantly more severe and has worse out-come than negative RF IgA. Thus, appropriate identification of RF(positive) patients in the screening process is essential, followed by isotype determination, , may lead to early diagnosis of RA even before the onset of clinical symptoms (5) .For those very likely to have or develop RA, early diagnosis may change the outcome and may significantly reduce the cost of treatment (16) . In the present study, the rate of anti-Cathapsin G in RA patients was 26.7% with a significant P value (0.019) and it is in accordance with *Anu Mustila et al.* (17) Who showed that positivity of anti-Cathapsin G was associated with clinical and laboratory findings indicating increased inflammatory activity. Moreover progressive X-ray erosive changes were associated with an increase in anti-Cathepsin G (18) .In other hand one of the healthy group (4%) had anti-Cathepsin G, in which the presence or absence of RF was not related to ANCA positivity or the presence of any of its subsets. (19). In this trial a significant correlation was found between RF IgG , RF IgM isotypes and anti-Cathapsin G (P value (0.047) . Perinuclear ANCA (Cathapsin G) has been shown to induce an increased release of reactive oxygen species and granule contents by granulocytes (17, 19).. Another pathogenetic mechanism is may be cross-reactivity between epitopes on the granulocyte and endothelial cell surface. Such cross reactivity has been suggested by demonstration of shared antigen between granulocytes and endothelial cells (11, 20). This hypothesis is supported by the finding that the granulocyte stimulated with p-ANCA in combination with tumor necrosis factor α (TNF-α) and lipopolysaccharide are capable of inducing damage to cultured endothelial cells (21). On the other side of this study there was no correlation between RF IgA isotype and Anti-Cathipsin G (P value 0.0692) which is in agreement with Swedler W et al and Hagen EC etal .

Conclusion:

Anti-Cathapsin G was found among 26.7% of patients with rheumatiod arthritis with a significant P value ,also it has a significant association with RF IgG and RF IgM ,while RF IgA has no significant association with anti-Cathapsin G .

References:

1-Gabriel SE. The epidemiology of rheumatoid arthritis .Rheum Dis Clin North Am 27(2001)269.

2-Bukhari MA, Wiles NJ, Lunt M, et al, Influence of disease-modifying therapy on radiographic outcome in inflammatory polyarthritis at five years :results from large observational inception study .Arthritis Rheum 48(2003)46-53.

3- Barland P, Lipstein E. Selection and use of laborarory tests in rheumatic diseases. Am J Med 1996,100:165-325.

4- Borretzen M, Mellbye OJ, Thompson KM, et al, Rheumatoid Factors. IN :Peter JB, Shoenfeld Y ,

eds. Autiantibodies . 1 ed .Amsterdam :Elsevier ,1996:706-715.

5- Swedler W, Wallman J, Froelich CJ, et al . Routine measurement of IgM, IgG and IgA rheumatoid factors :high sensitivity, specifity and productive value for rheumatoid arthritis J.Rheumatol .1997;24:1037-1044.

6- Halldorsdottir HD, Jonsson T, Thorsteinsson J, et al;.A prospective study on the incidence of rheumatoid arthritis among people with persistent increase of rheumatoid factor. Ann Rheum Dis 2000;59:149-51.

7- Pope RM, McDuffy SJ. IgG rheumatoid factor. Relationship to seropositive rheumatoid arthritis and absence in seronegative disorders. Arthritis Rheum 1979;22:988-98.

8- Issei Kida , Shigeto Kobayashi Ken Takeuchi , et al Antineutrophil cytoplasmic antibodies against myeloperoxidase, proteinase 3, elastase, cathepsin G and lactoferrin in Japanese patients with rheumatoid arthritis: Japan College of Rheumatology 2010

9- Snook JA, Chapman RW, Fleming K, et al; Anti-neutrophil nuclear antibody in ulcerative colitis. Crohn's disease and primary sclerosing cholangitis. Clin Exp Immunol. 1989;76:30–3.

10-Braun MG, Csernok E, Schmitt WH,et al; Incidence and specificity of p-ANCA in rheumatic diseases. In: Gross WL, editor. ANCA-associated vasculitis: immunological and clinical aspects. London: Plenum; 1993. p. 371–73.

11. Hagen EC, Ballieux BEPB, Van Es LA,et al; Antineutrophil cytoplasmic antibodies: a review of the antigens involved, assay, and the clinical and possible pathogenic consequence. Blood. 1993;81:1996–2002.

12- Manolva I, Dantcheva M. Antineutrophil cytoplasmic antibodies in Bulgarian patients; with rheumatoid arthritis: characterization and clinical association. Rheum Int. 2005;26:107–14.

13- Schmitt, W. H. and Gross, W. L. Antineurophile Cytoplasmatic Antigen (ANCA). Internist 1995. No.36.pp. 282-290.

14- Raptis SZ, Shapiro SD, Simmons PM, et al ;Serine protease Cathepsin G regulates adhesion-dependent neutrophil effector functions by modulation integrin clustering. Immunity, June 7, 2005, 679-691.

15- Bobbio-Pallavicini F, Caporali R, Alpini C, et al. High IgA rheumatoid factor levels are associated with poor clinical response to tumour necrosis factor alpha inhibitors in rheumatoid arthritis. Ann Rheum Dis 2007;66:302-7.

16- Majka DS, Holers VM. Can we accurately predict the development of rheumatoid arthritis in the preclinical phase? Arthritis Rheum 2003;48:2701-5.

17-Anu Mustila, Markwu Koppela, Jukka Mustonen,et al : Perinuclear anti-neutrophil cytoplasmic antibody in rheumatoid arthritis. Arth Rheum;1997: 40(4): 710-717

18-Coremans IEM, Hagen EC, Daha MR, et al. : Antilactoferrin antibodies
in patients with rheumatoid arthritis are associated with vasculitis. Arthritis
Rheum.;1992: 35 : 1466 – 1475

19-Falk RJ, Terrel RS, Charles LA, et al: anti-neutrophil cytoplasmic autoantibodies induce neutrophils to degranulate and produce oxygen radicals in vitro. Proc Natl Acad Sci USA A; 1990:87: 4115-4119.

20- De Bandt MD, Meyer O, and Haim T (1996): Antineutrophil cytoplasmic antibodies in rhematiod arthritis patients . Br. J. Rheumatol; 35 :38 -43 . Anti-Elastase, Anti-Cathapsin G & Anti-Lactoferrin in Rheumatic Disorders.

21-Ewert B, Falk R, Jennette JC : Anti-neutrophil cytoplasmic antibodies
stimulate neutrophils to injure endothelial monolayers in vitro. Third
Workshop on ANCA. Washington DC, November;1990: 29-30.