Original Article

Anti-neutrophlic cytoplasmic antibody Elastase, Lactoferrin, Cathapsin G,and Lysozyme in a sample of Iraqi patients with Rheumatoid Arthritis

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Abstract

Background: Elastase, Lactoferrin, Cathapsin G and lysozyme are antigens (proteins) present in several mucosal secretions as well as in secondary granules of polymorphonuclear leukocytes. Anti-Elastase antibodies, anti-Lactoferrin antibodies,anti-Cathapsin G antibodies and anti-Lysozyme antibodies, which belong to Perinuclear Anti-Neutrophil Cytoplasmic Antibodies(pANCA) have been described in several immunomediated diseases, including Rheumatoid Arthritis .

Objectives: Investigate the prevalence of anti-Elastase antibodies, anti-Lactoferrin antibodies,anti-Cathapsin G antibodies , anti- Lysozyme antibodies and rheumatoid factor in patients with rheumatoid arthritis in comparison to healthy control.

P**atients &Methods:** The study involved 40 Rheumatoid Arthritis patients who were referred to Immunological Department in Teaching laboratory \ Medical City during period of (1st of January – 31st of June) 2011 and 25 apparently healthy individual used as a control group were investeged to rheumatic factor IgG, IgM ,IgA isotypes and Elastase antibodies, Lactoferrin antibodies, Cathapsin G antibodies , and lysozyme antibodies were measured by using enzyme immunoassay technique.

Results : Anti- Elastase, Abs anti-Cathapsin G Abs ,and anti-lysozyme Abs showed significant correlation with RF screen, and the mean concentration for these antibodies in rheumatoid arthritis patients with significant difference if compare it with the healthy control group. While anti-Lactoferrin Abs showed no significant correlation with RF screen. This study showed a association between the positive results of anti- Cathapsin G Abs with RF IgG and RF IgM only .Anti- Elastase Abs, Anti-Lactoferrin Abs and Anti-lysozyme Abs showed neither a significant correlation with RF IgG, RF IgM nor with RF IgA.

Conclusion:A significant correlation was found between Elastase antibodies, Cathapsin G antibodies , lysozyme antibodies and patients with rheumatiod arthritis Cathapsin G antibodies has a significant association with RF IgG and RF IgM

Key Word: Elastase, Lactoferrin ,Cathapsin G , Lysozyme, Rheumatoid Arthritis, Iraq.

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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.(1). It is characterized by infiltration of leukocytes (mostly neutrophils) in the joints and a proliferation of the lining synovial cells often leading to the irreversible destruction of cartilage and bone. RA also has systemic manifestations (2). Vascular inflammation is a severe complication of RA and patients with RA show an increased risk for cardiovascular events compared with the general population (3).

Reliable and earliest possible diagnosis is indispensable to keep the disease under control with suitable therapy and to avoid irreversible joint damage (1).

Rheumatoid factors (RFs or RhFs) is an autoantibodies (antibody directed against an organism's own tissues) most relevant in arthritis. RFs can be detected in normal individuals, although transiently. This dichotomy has led to questions about the origins and types of RF. Recently it has been shown that B cells that produce RFs only do so when activated by two signals, one from engagement of the B-cell receptor and the other from recognition of a pathogen-associated molecular pattern through a Toll-like receptor (TLR)(4). It are an antibodies 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 factors (IgM, IgG,and IgA)are present in the serum of 75-80%of the patients with RA at some time during the disease course .In patients with rheumatoid arthritis (RA), the reported prevalence of ANCAs has ranged from 20 to 50%, and these predominantly show p-ANCA pattern (4)

Anti-neutrophil cytoplasmic antibodies (ANCAs) are a group of autoantibodies, mainly of the IgG type, against antigens in the cytoplasm of neutrophil granulocytes and monocytes. (3) . perinuclear ANCAs (pANCAs) have been documented

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to occur in patients with necrotizing and crescent-forming glomerulonephritis,microscopicpolyangiitis(MPA)andChurg–Strauss syndrome, and the specific antigen most frequently associated with these pANCAs is myeloperoxidase(MPO). However, pANCAs have been reported in many inflammatory disorders, such as inflammatory bowel disease,primary sclerosing cholangitis, autoimmune hepatitis, and various rheumatic diseases (5). The target antigens for the pANCAs in these disorders are lactoferrin (LF), cathepsinG(CG), human leukocyte elastase (LE) and lysozyme(LZ) have been reported ,in addation to Bactericidal permeability-increasing protein (BPI) and Azurocidin(AZ)were belonging pANCA.(6)

Lactoferrin (LF) LF which belongs to the pANCA is an iron-binding protein, which occurs in high concentrations in secretions at mucosa surfaces, in tears and in milk. LF also resides in the specific granules of polymorphnuclear neutrophil leukocytes (PMN) and becomes exocytosed upon PMN activation during active inflammatory disease. LF antibodies have experimentally been shown to increase both the magnitude and duration of hydroxyl radical formation at site of inflammation . Hypothetically, LF-ANCA could therefore have pathogenic importance by counteracting the anti-inflammatory effect of LF, thereby aggravating and prolonging the inflammatory process.(7)

Cathepsin expression in normal tissues is restricted to granulocytes, especially neutrophils. However, mononuclear phagocytes have been demonstrated to bind and internalize proteases from neutrophils. Cathepsin G is located in neutrophilic polymorphonuclear leukocytes which contain specialized azurophil granules together with two other serine proteases; elastase and hepsin. These three proteases may participate in the killing and digestion of engulfed pathogens and in connective tissue remodelling at sites of inflammation(8) .Cathapsin G belong to a group of intracellular proteases mainly found in lysosomes is a serine protease and a further p-ANCA 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 (9).

Neutrophil elastase is a serine proteinase released by activated human neutrophils, can degrade a wide variety of biomacromolecules including elastin and collagen fibers. Elastase has a crucial mediator of inflammatory tissue damage and is considered a marker of inflammatory diseases such as idiopathic pulmonary fibrosis, rheumatoid arthritis, adult respiratory distress syndrome, and cystic fibrosis(10). Macrophages secrete many substances either constitutively or after stimulation . Amongst these substances are different proteolytic enzymes, lysozyme and reactive oxygen intermediates, important for cartilage and bone destruction in RA .Monocytes and neutrophils contain intracellular granular proteins, which are important for the host defence. Lysozyme is an enzyme that hydrolyses glycosidic bonds and is thus able to hydrolyse the cell wall peptidoglycans of some micro-organisms and thereby kill the organism (11).

Material and Methods:

A cross-sectional study was conducted on two main groups ,45 patients with Rheumatoid arthritis who were diagnosed by physician and 30 apparently healthy control referred to immunological department in teaching laboratory \ medical city during period of (1st of January – 31st of June) 2011 . Base line data about subjects were obtained from their history &clinical examination, a previously arranged questionnaire was used for this purpose. 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). From each individual 5ml of venous blood was collected and the serum stored at -20c^{o} till used for the quantitative estimation of serum human RF screen Abs, IgG , IgM , IgA isotypes Elastase Abs, Lactoferrin Abs, CathapsinG Abs , and lysozyme Abs were detected by using enzyme immunosorbent assay (ELISA) technique company (IMMUCHEM-France) kits.

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 study involved 45 Rheumatoid arthritis patients 28(62.2%) of them were females and 17(37.8%) males their age ranged between (18 - 66) years with mean age of 36.08 ±.14.71 years. A control matched group include 25 apparently healthy individual 8 (32%) males and 17 (68%) females with mean age of 30.28±14.22 years.

The rheumatiod factor screen levels was found ≥ 25 U/ml in all patients of RA 45(100%), , 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) . In other hand we detected that the serum of RF IgM, RF IgG and IgA were elevated more than 20 U/ml in 31 (68.9%) ,31 (68.9%),and 32 (71.1%) patients in arthritis patients respectively but it was found less than 20 U/ml in all control group, with a significant P value (0.0001).Table(1)

Table(1) Distribution of Rheumatoid factor screen ,RF IgM, RF IgG and RF IgA in rheumatoid arthritis patients and healthy control.

Study groups		RF screen		RF IgM		RF IgG		RF IgA	
		+ve ≥25 u/ml	-ve <25 u/ml	+ve ≥20 u/ml	-ve <20 u/ml	+ve ≥20 u/ml	-ve <20 u/ml	+ve ≥20 u/ml	-ve <20 u/ml
Rheumatic Arthritis No.45	NO.	45	0	31	14	31	14	32	13
	%	100	0	68.9	31.1	68.9	31.1	71.1	28.9
Healthy control No.25	NO.	0	25	0	25	0	25	0	25
	%	0	100	0	100	0	100	0	100
χ^2 test	P value	0.0001*		0.0001*		0.0001*		0.0001*	
	Sig.	H.S*		H.S*		H.S*		H.S*	

*P-value: 0.0001 HS (significant < 0.05)

Anti-Elastase Abs, anti Cathapsin G, and anti- Lysozyme Abs were positive their concentration more than 10 U /ml found 9 (20%) ,12 (26.7%) and 9(20%) in rheumatoid arthritis patients respectively in association to RF screen (45) with a significant p value(0.017,0.019,0.017) respectively while anti-Lactoferrin Abs found 4 (8.9 %) with non significant association to RF screen (p value 0.899).Table (2).

Table(2)The comparison of anti-Elastase Ab,anti-Lactoferin Ab,anti-Cathapsin G Ab and anti-Lysozyme Ab with rheumatoid factor screen in rheumatoid arthritis patients and healthy control.

Study groups		Anti-Elastasen Ab		Anti-Lactoferin Ab		Anti-Cathapsin G Ab		Anti-Lysozyme Ab	
		+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve
Patient group with RF screen NO. 45	+ve No.45	9 20 %	36 80 %	4 8.9%	41 91.9%	12 26.7%	33 73.3%	9 20%	36 80%
	-ve NO.0	٠ ٠٪	0 0%	0 0 %	0 0 %	0 0%	0 0%	0 0 %	0 0 %
Control group With RF screen NO. 25	+ve NO. 0	0 0%	0 0%	0 0%	0 0%	1 4%	24 96%	0 0%	0 0%
	-ve NO.25	0 0%	25 100%	2 8 %	23 92%	0 0%	25 100%	0 0%	25 100%
χ^2 test	P value	0.017*		0.899		0.019*		0.017*	
	Sig.	Sig.		N.Sig.		Sig.		Sig.	

*P value significant(0.047)< 0.05

In other hand anti-Elastase Abs, anti-Lactoferrin Abs , and anti- Lysozyme Abs were positive their concentration more than 10 U /ml found 8 (25.8%) ,4 (12.9%) and 6 (19.4%) in rheumatoid arthritis patients respectively in association to positive RF IgM (31) with non significant p value(0.147,0.159,0.872) respectively while anti Cathapsin G found 11 (35.5%) with significant association to positive RF IgM (p value 0.047).Table (3)

Table(3)The comparison of anti-Elastase Ab,anti-Lactoferin Ab,anti-Cathapsin G Ab and anti-Lysozyme Ab with rheumatoid factor IgM in rheumatoid arthritis patients and healthy control.

Study groups		Anti-ElastaseAb		Anti-Lactoferin Ab		Anti-Cathapsin G Ab		Anti-Lysozyme Ab	
		+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve
Patient group with RF IgM NO. 45	+ve No.31	8 25.8%	23 74.8%	4 12.9%	27 87.1%	11 35.5%	20 64.5%	6 19.4%	25 80.6%
	-ve NO.14	1 7.1%	13 92.9%	0 0%	14 100%	1 7.1 %	13 92.9%	3 21.4%	11 18.6%
Control group With RF IgM NO. 25	+ve NO. 0	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%
	-ve NO.25	0 0%	25 100%	2 8%	23 92%	1 4%	24 96%	0 0%	25 100%
χ^2 test	P value	0.147		0.159		0.047*		0.872	
	Sig.	N.Sig.		N.Sig.		Sig.*		N.Sig	

*P value significant(0.047)< 0.05

In table (4) anti Cathapsin G level detected more than 10 U/ML in 11 (35.5%) of rheumatoid arthritis patients which had a significant association to positive RF IgG (p value 0.047) while anti-Elastase Abs, anti-Lactoferrin Abs , and anti- Lysozyme Abs levels were found 8 (25.8%) ,4 (12.9%) and 8 (25.8%) in rheumatoid arthritis patients respectively in association to the positive RF IgG (31) which had non significant p value(0.147,0.159,0.147) respectively.

Table(4)The comparison of anti-Elastase Ab,anti-Lactoferin Ab,anti-Cathapsin G Ab and anti-Lysozyme Ab with rheumatoid factor IgG in rheumatoid arthritis patients and healthy control.

Study groups		Anti-Elastase Ab		Anti-Lactoferin Ab		Anti-Cathapsin G Ab		Anti-Lysozyme Ab	
		+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve
Patient group with RF IgG NO. 45	+ve No.31	8 25.8%	23 74.8%	4 12.9%	27 87.1%	11 35.5%	20 64.5%	8 25.8%	23 74.2%
	-ve NO.14	1 7.1%	13 92.9%	0 0%	14 100%	0 0%	14 100%	1 7.1%	13 92.9%
Control group with RFIgG NO. 25	+ve NO. 0	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%
	-ve NO.25	0 0%	25 100%	2 8%	23 92%	1 4 %	24 96 %	0 0%	25 100%
χ^2 test	P value	0.147		0.159		0.047*		0.147	
	Sig.	N.Sig.		N.Sig.		Sig.*		N.Sig.	

*P value significant (0.047) < 0.05

The positive results of anti-Elastase Abs 6 (18.8%),anti-Lactoferin Abs 3(9.4%), anti-Cathapsin G Abs 8(25%) and anti-Lysozyme Abs 6 (18.8%) found had non significant association with RF IgA in study groups (p values 0.742, 0.857, 0.692, 0.742 respectively). Table (5)

Table(5)The comparison of anti-Elastase Ab,anti-Lactoferin Ab,anti-Cathapsin G Ab and anti-Lysozyme Ab with RF IgA in rheumatoid arthritis patients and healthy control.

Study groups		Anti-Elastase Ab		Anti-Lactoferin Ab		Anti-Cathapsin G Ab		Anti-Lysozyme Ab	
		+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve
Patient group with RF IgA NO. 45	+ve No.32	6 18.8%	26 81.3%	3 9.4%	29 90.6%	8 25%	24 75%	6 18.8%	26 81.3%
	-ve NO.13	3 23.1%	10 76.9%	1 7.7%	12 92.3%	4 30.8%	9 69.2%	3 21.4%	10 18.6%
Control group with RF IgA NO. 25	+ve NO. 0	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%
	-ve NO.25	0 0%	25 100%	2 8%	23 92%	1 4%	24 96%	0 0%	25 100%
χ^2 test	P value	0.742		0.857		0.692		0.742	
	Sig.	N.S.		N.S.		N.S		N.S	

The mean concentration of Elastase Abs7.08± 3.28 (P-value: 0.001), , Cathapsin G Abs9.20± 8.80(P-value: 0.003) and Lysozyme Ab 8.1±3.90(P-value: 0.002) respectively in rheumatoid arthritis patients with significant difference if compare it with the healthy control group ,while the mean of anti-Lactoferrin Abs 5.02± 6.28(P-value: 0.496) in rheumatoid arthritis patients without significant difference when compare it with the healthy control group. Figure (1).

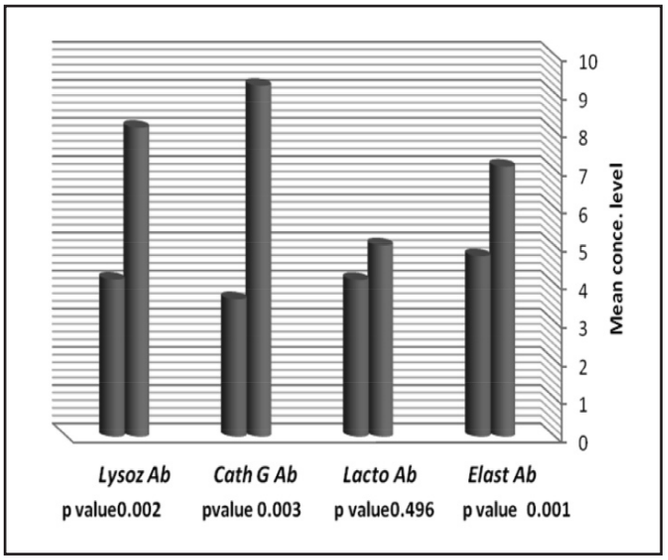

Figure (1) Show the mean concentration of Elastase Ab, Lactoferin Ab, Ab,anti-Cathapsin G Ab and anti-Lysozyme Ab levels in rheumatoid arthritis patients in comparison healthy control.

Discussion:

Rheumatoid arthritis (RA) are autoimmune disorder that are characterized by the production of autoantibodies against a variety of antigens. It is possible that more than one process could contribute for different diseases manifestations. One of these may be vascular injury. Antibodies to neutrophil cytoplasmic antigens (ANCAs) have been extensively studied as markers for systemic vasculitis.(12) ANCAs interact with their target antigens on cytokine-primed neutrophils, causing neutrophil activation via several signaling pathways that lead to interaction with the endothelium, degranulation, cytokine production, and tissue damage. Given the presence of autoantibodies, the assistance of autoreactive T-helper cells and B cells appears to be required for disease to develop (13,14). 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). RA patients the prevalence of RF based on the results of ELISA technique varies between (70-80)%.In this study it was 100% (45 /45) of RA patients were positive for Rf which is autoantibody most relevant in rheumatoid arthritis that contribute to the disease process, with highly significant P value (0.0001)which is in agreement with Bukhari. MA etal 2002 & Swedler W etal 2007 (15,16) . Regarding RF IgG as well as RF IgM were positive 68.9% arthritis patients Whilst RF IgA were positive in 71.1% patients and non of the healthy group had positive level for any of the isotypes with highly significant P value (0.0001) for all isotypes (IgG,IgM,IgA) this finding is in consistence with .Swedler .W & Halldorsdottir .HD &2007 (16 ,17).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 (18). In the present study, the rate of Elastase Abs and Cathapsin G Abs in RA patients in relation to RF screen were 25% and 26.7% with a significant P value(0.017,0.019) respectivelly and it is in accordance with Raptis et al & Mustila et al. (9,19) who showed that positivity of Elastase Abs and Abs were associated with clinical and laboratory findings indicating increased inflammatory activity because its(Elastase and Cathapsin G) were belong to a class of enzymes known as proteases which are a new class of cytokines that may play a significant role in chronic inflammation and joint destruction. Furthermore there is a elevation in serum level of Lysozyme Abs(25%) in RA patients in relation to RF screen with a significant P value(0.017) and these increased of lysozyme level probably reflects an increased secretory activity by monocytes/macrophages in RA. This interpretation is in concordance with previous studies in which the elevation of serum level of lysozyme can be assumed to derive from monocytes/macrophages. Thus indicating peripheral blood monocyte activation in RA measured as increased monocyte cell surface expression of β2-integrins and as increased adhesion.also the Tumour necrosis factor-α (TNF-α) stimulates lysozyme production by monocytes and macrophages and the release of lysozyme by neutrophils . Furthermore, elevated levels of TNF-α in serum have been measured in RA . The increased secretion of lysozyme into the peripheral blood could thus be mediated by TNF-α.(11) Surprisingly. there was no significant correlationfound between RF screen, IgG , IgM &IgA isotypes and anti-lactoferrin Ab.In addition mean concentraton of lactoferrin Ab in rheumatoid patients was not significantly higher than that for health controls (p value 0.496) in accordance to a study done by Nässberger. L.etal 2004 .While disagrees with a study done by Chikazawa H 2000 etal, that showed a significant differences in lactoferin antibody levels between RA and healthy controls and conceded it as markers of inflammation . (20, 21). In other hand there was no a significant correlation found between RF IgG , RF IgM isotypes and Elastase Abs ,Lysozyme Abs but the mean concentration of Elastase Abs and Lysozyme Abs in rheumatoid patients were significantly higher than that for health control(p value 0.001, p value 0.002) respectively which agree with study done by Gouni-Berthold I et al and Torsteinsdo ttir, L et al. that showed a significant differences between RA and healthy controls in Elastase Abs and Lysozyme Abs leveles

and conceded it as markers of inflammation . (10,11).While Cathapsin G Abs has been shown significant correlation with RF IgG , RF IgM isotypes.In addition mean concentraton of Cathapsin Ab in rheumatoid patients was significantly higher in RA patients than that for health controls (p value 0.003) these finding is contributed to p-ANCA (Cathapsin G)that shown to induce an increased release of reactive oxygen species and granule contents by granulocytes (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 (21). On the other side of this study there was no correlation between RF IgA isotype and Elastase Abs, Cathipsin Abs and Lysozyme Abs (P value 0.692) which is in agreement with Swedler W et al (18).Furthermore RF screen had a significant relation with Anti -Elastase Ab,Anti- Cathapsin Ab and Anti-Lysozyme but no correlation with RF isotypes(exept Anti-Cathapsin G) as mention above, This result may be consistent with understanding that those isotypes comprise members of the same family of autoantibodies that rise against the same antigen and within the same immune response.(22,23.24)

Conclusion:

Elastase Ab, Cathapsin G Ab and Lysozyme Ab were found as inflammatory markers more than Lactoferrin Ab in Rheumatoid arthritis patients comparing to healthy control and the Cathapsin G Ab has a significant correlation to RF IgG, and RF IgM isotypes while RF IgA had no relation with Perinuclear Anti-Neutopholic Cytoplasmic Antibodies(P-ANCA).

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