Frequency of Anti Lactoferrin Antibodies in Patients With Systemic Lupus Erythematosus and Rheumatoid Arthritis

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ABSTRACT:

BACKGROUND:

Lactoferrin is a multifunctional iron-binding protein present in several mucosal secretions as well as in secondary granules of polymorphonuclear leukocytes.Anti-Lactoferrin antibodies, which belong to antineutrophil cytoplasmic antibodies have been described in several immunomediated diseases, including Systemic Lupus Erythematosus and in patients with Rheumatoid Arthritis and others, with conflicting results regarding either their prevalence or clinical associations.

OBJECTIVE:

Detection of anti-Lactoferrin antibodies in Systemic Lupus Erythematosis and Rheumatoid Arthritis patients and studying its association to disease activity in comparison to healthy controls.

PATIENT& METHODS :

The study involved 74 Systemic Lupus Erythematosis patients, 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 30 apparently healthy individual. Antinuclear antibody, complement C1q, rheumatoid factor and lactoferrin antibody were detected by enzyme-linked immunosorbent assay technique While double stranded DNA was detected by indirect immunofluorescent technique and complements (C3, C4) by single radial immune diffusion.

RESULTS:

Anti-lactoferrin Ab was detected in 14(18.9%) SLE patients, 4 (8.9%) Rheumatoid arthritis patients. Both C3&C4 levels were decreased significantly in Systemic lupus with positive anti lactoferrin Ab level in comparison to healthy controls(p value 0.024). In this regard Circulating immune complex was positive in 38(51.3%) systemic lupus patients only 12(31.5%) had positive lactoferrin antibody level, (p value 0.004).double stranded DNA was detected in 41(55.4%) Systemic lupus patients only 7(17.0%) of them had positive lactoferrin Ab level. Anti - Lactoferrin Abs showed neither a significant correlation with Rheumatoid factor IgG& IgM (P value 0.159) nor with rheumatoid factor IgA (P value 0. 857).

CONCLUSION:

Anti-lactoferrin antibodies could be detected in patients with Rheumatoid Arthritis and more often in patients with Systemic Lupus with significant correlation to decrease complements levels in comparison to healthy control.

KEY WORDS: anti lactoferrin antibodies, systemic lupus, rheumatoid arthritis.

INTRODUCTION:

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. They are detected in a number of autoimmune disorders against, established target antigens like Proteinase 3 (PR3), Myeloperoxidase (MPO), Bactericidal permeability-increasing protein (BPI) ,Lactoferrin (LF), Lysozyme (LZ), Elastase (EL), Cathepsin G (CG), and Azurocidin(AZ).[1]

ANCA are serological marker for certain primary small vessels vasculitides that can further activate tumor necrosis factor alpha(TNFα) primed neutrophils to degranulate ,releasing proteinases and reactivate oxygen species to cause small vessel wall damge.[2] 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. Anti-LF antibodies have experimentally been shown to increase both the magnitude and duration of

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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. Inflammation of small-sized blood vessels is a hallmark of systemic lupus erythematosus and Rheumatoid arthritis is the most common inflammatory form of arthritis, LF antibodies levels were measured as they have possible role in pathogenesis and in active disease expression .[3]

Systemic lupus erythematosus (SLE) is a chronic inflammatory disease that has protean manifestations and follows a relapsing and remitting coursei,SLE is characterized by the appearance of autoantibodies against nuclear antigens and the involvement of multiple organ systems, including the kidneys. The precise immunological events that trigger the onset of clinical manifestations of SLE are not yet well understoodod. More than 90% of cases of SLE occur in women, frequently starting at childbearing age.There is no serologic test that reliably measures disease activity in SLE.The 'gold standard' is the anti-dsDNA antibody test, which has been used as a marker of disease activity by clinicians in SLE for over 35 years .More recently autoantibody assays have been developed that show greater promise in gauging SLE disease activity, specifically anti-nucleosome and anti-C1q antibodies (especially with renal disease activity) [4] .

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 initial symptoms include painful swelling of the metacarpophalangeal joints with morning stiffness of the joints. Reliable and earliest possible diagnosis is dispensable to keep the disease under control with suitable therapy and to avoid irreversible joint damage. Rheumatoid factor (RF or RhF) is an autoantibody 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 .In patients with rheumatoid arthritis (RA), the reported prevalence of ANCAs has ranged from 20 to 50%, and these predominantly show a pANCA pattern [5].

This study aimed to detect lactoferrin antibodies in SLE & RA and its association with diseases activity.

MATERIAL AND METHODS:

A cross-sectional study was conducted on three main groups ,74 patients with SLE ,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. From each individual 5ml of venous blood was collected and divided into several 0.5aliquot and all frozen at -20ċ till used. Antinuclear antibody (ANA), Anti Lactoferrin Ab, Rf and C1q were detected by enzyme-linked immunosorbent assay (ELISA) technique company (IMMUCHEM-France) .While dsDNA was detected by indirect immunofluorescent (IIF) technique company(Euroimmun – Germany), and C3,C4 by single radial immunodiffusion (SRID)company(LTA – Italy).

Statistical Analysis

The student T test and chi-square 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 74 SLE patients 61(82.4%) of them were females and 13(17.6%) males their age ranged between (16-51) years with mean age of 32.49±.14.78years. A control matched group include 30 apparently healthy individual 8(32%) males, 22 (68%) females with mean age of 30.80±13.1 years.

Anti nuclear antibodies(ANA) were detected (Titer≥1.2) in all SLE patients but not in healthy controls with a highly significant (P value -0.0001),.anti anti lactoferrin antibody level was more than 10 U/ml in14(18.9%) SLE patients but not in healthy controls with significant (p value-0.01) as in Table (1).

Table 1: ANA& Anti-Lactoferrin Ab in SLE Patients In Comparison to Healthy Control.

Study groups	ANA Index				Anti- Lactoferrin Ab			
	Positive ≥1.2		Negative <1.2		Positive (≥10 U/ml)		Negative (<10 U/ml)	
	No.	%	No.	%	No.	%	No.	%
SLE patients	74	100	0	0	14	18.9	60	81.1
Healthy control	0	0	30	100	0	0	30	100
Total	74		30		14		90	
P-value	0.0001*				0.01*			

*significant P value: < 0.05

As a marker of SLE disease activity ,decreased complements level C3(28.9%) & C4 (25.9%) in association to positive anti lactoferrin antibodies (≥10 U/ml) showed significant p-values 0.024,0.011) respectively as in table(2)

Anti dsDNA levels &circulating immune complex (CIq) were positive in (17.1% 12%,.) SLE patients respectively in association to positive Anti lactoferrin Ab(≥10 U/ml) with a significant(p value 0.004) for CIq .

Table 2: C3 & C4 Levels in SLE Patients in Association to Anti- Latoferrin Antibodies.

			Anti-Lactoferrin IgG		P value
			+ve	-ve	
C3 level	Decrease	No	13	32	0.024*
		%	28.9	71.1	
	Normal	No	1	28	
		%	3.6	96.4	
C4 level	Decrease	No	14	40	0.011*
		%	25.9	74.1	
	Normal	No	0	20	
		%	0	100	
Total C3& C4 level	Decrease		27	72	-
	Normal		1	48	

*significant P value: < 0.05)
** C3 NV. (91-156)mg/dl
*** C4 NV. (20-50) mg/d

Table 3: Detection of Anti dsDNA & C1q in SLE Patients in Association to lactoferrin Ab

SLE patients			Anti-Lactoferrin IgG +ve	Anti-Lactoferrin IgG -ve	P value
Anti ds DNA	+ve	No.	7	34	0.651
		%	17.1	82.9	
	-ve	No.	7	26	
		%	21.2	78.8	
	Total		14	60	
C1q	+ve	No.	12	26	0.004 *
		%	31.6	68.4	
	-ve	No.	2	34	
		%	5.6	94.4	
	Total		14	60	

*signficaat P value < 0.05
**CIq NV. Up to 25U/ml ,
** Anti dsDNA Titer ≥10 (+ve)

RA patients includes 45 patients 17 (37.7%) males, 28 (62.3% females age ranged between (18-66) years with mean age of (36.08±14.71) years.. 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 value0.0001) ;However The anti lactoferrin antibody was found more than 10 U/ml in 4(8.9%) rheumatiod arthritis patients but not in healthy control with no significant(p value 0.093) as in table (4).

Table 4: Rheumatoid factor screen& lactoferrin antibodies in rheumatoid patients in comparison to healthy controls.

Study groups	RF screen				Anti- Lactoferrin			
	Positive (≥25 U/ml)		Negative (<25 U/ml)		Positive (≥10 U/ml)		Negative (<10 U/ml)	
	No.	%	No.	%	No.	%	No.	%
Rheumatic arthritis No.45	45	100	0	0	4	8.9	41	91.1
Healthy control No. 30	0	0	30	100	0	0	30	100
Total	45		30		4		71	
P-value	0.0001*				0.0933			

*P value significant < 0.05

Regarding to RF isotypes the positive results of rheumatic factor IgG, IgM, and IgA levels comparing to positive results of anti-Lactoferrin in rheumatiod arthritis patients, were for RF IgG 31 (68%) and RF IgM 31 (68%) ,4(12.9%) of both of them had anti-lactoferrin level more than 10 U/ml with no significant (p value 0.159) .While in the patients who had positive RF IgA 32 (71.1%) only 3(9.4%) their serum anti-lactoferrin concentration more than 10 U/ml, statistically (P value 0.857) not significant ,table(5).

Table 5: Rheumatoid factor isotypes in rheumatoid patients in comparison to lactoferrin antibodies level.

Positive RF > 20 U/ml	Anti Lactoferrin IgG		Total No.	P value
	Positive >10 U/ml	Negative < 10 U/ml		
RF IgG No.	4	27	31	0.159
%	12.9	87.1		
RF IgM No.	4	27	31	0.159
%	12.9	87.1		
RF IgA No.	3	29	32	0.857
%	9.4	90.6		

The last table (6) explains the association between lactoferrin antibodies mean concentration in SLE patients 11.81±19.47 U/ml & RA patients 5.02±6.28 U/ml in comparison to healthy control 3.41±0.93 U/ml ,3.11±1.4 U/ml respectively with a significant (p value 0.023) for SLE patients.

Table 6: Anti lactoferrin Ab level in SLE & RA patients in comparison to healthy control.

categories	Lactoferrin Ab Mean conc. U/ml	SD**	T-Test	Df***	p. value
SLE	11.81	8.47	2.31	77	0.023*
Control group	3.41	0.93			
RA	5.02	4.28	1.63	73	0.106
Control group	3.11	1.40			

*P value significant < 0.05
**SD= Standard deviation
***Df = degree of freedom

DISCUSSION:

Systemic lupus erythematosus (SLE) &Rheumatoid arthritis (RA) are autoimmune disorders that are characterized by the production of auto antibodies 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.[6]

Antinuclear antibody ANA testing is very useful in establishing a diagnosis of systemic lupus erythematosus .In this study ANA positive in all patients with SLE, as for lactoferrin antibodies(LF Abs) they were elevated above normal level(10 U/ml) in 14 (18.9%) which was significantly higher than that in normal individuals (p value0.01) this agrees with studies, done previously Sinico RA etal 2000 [2] & Reveille J D 2004[4] that showed presence of LF Abs in patients with SLE is well recognized based on molecular science that have shown that lactoferrin is multitasking protein ,exhibit antibacterial, antiviral, anti inflammatory activity and that anti-LF Abs binding to LF block the anti-inflammatory activity of it, which implies a deterioration in host defense against microorganisms and inflammation [2,4].

A decrease complement (C3 and C4) levels is a hallmark involved in the pathogenesis of SLE for that they were measured in sera of SLE patients in comparison to elevated lactoferrin antibodies levels and showed a significant decrease in both complements levels p values(0.024 and 0.011) respectively. Recent studies Van Timmeren MM etal 2009 [7] & Gou SJ..etal 2013 [8] suggest a previously unsuspected but crucial role for alternative pathway complement activation in ANCA disease pathogenesis. and in vitro studies indicates that ANCA are causally involved in disease pathogenesis mainly through activation of neutrophils resulting in endothelial cell injury.

ANCA, neutrophils and complement activation causing an inflammatory amplification loop that may explain the severe leukocytoclastic inflammation that is typical for ANCA-associated diseases.[7,8]

Surprisingly, There was no significant correlation between LF-Abs positivity and SLE disease activity in regard to (dsDNA). It is hypothesized that the release of LF by neutrophils chemotactically attracted to DNA-anti-DNA complexes may act as a feedback loop to modulate the inflammatory response in SLE this in accordance with Kavanaugh,A.2002[9]. While disagree with a study that demonstrate that anti-LF are frequently present in patients affected by SLE& are associated with some clinical manifestations and other auto antibodies including dsDNA (P value 0.004) Caccova.D ,eatal. 2005 [1]

In current study, circulating immune complex levels that activate complement via the alternative pathway ,showed differences between, active SLE and inactive SLE in regard to lactoferrin Abs positivity (p value 0.004).This positive correlation are in agree with - Kettritz. R 2008[10] that found Circulating auto antigens can deposit in glomeruli as part of circulating immune complexes (e.g lactoferrin ,C1q) or become a planted target. A potentially unique model of deposition of non renal antigen in the kidney is seen in anti neutrophil cytoplasmic antibody (ANCA) associated small vessel vasculitis, where target auto antigens originating in neutrophil cytoplasmic granules and expressed in the cell membrane is targeted by ANCA. These ANCA –activated neutrophils have altered flow characteristics resulting in their loading in small vessels, particularly glomeruli, resulting in renal injury. [10]

For 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(11) & Swedler W etal 2007 [12].

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 this finding is in consistence with .Swedler .W & Halldorsdottir .HD &2007 [12,13].

In this study no significant correlation was found between RF IgG , IgM &IgA isotypes and anti-lactoferrin Abs. In addition mean concentraton of lactoferrin Abs in rheumatoid patients was not significantly higher than that for health controls (p value 0.106) in accordance to a study done by Nässberger. L.etal 2004 [14] .While disagrees with a study done by Chikazawa H 2000 etal [15] **,** that showed a significant differences in lactoferrin antibody levels between RA and healthy controls and conceded it as markers of inflammation .

Unlike RA, SLE patients LF Abs mean level was significantly higher than healthy control (p value 0.023) in coordinate with Caccavo .D 2005 etal (1) &Chikazawa H 2000 etal [15] that found significant correlation between lactoferrin Abs in SLE in comparison to healthy controls. The presence of these antibodies is simply a reflection of B cell hyperactivity in SLE. & that anti-LF antibodies bind to LF and block the anti-inflammatory activity of LF, which implies deterioration in host defense against microorganisms and inflammation. [16]

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