Original Article

Antibodies to selected minor target anti-neutrophil cytoplasmic antigens in systemic lupus patients

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Abstract:

Background: Anti-neutrophil cytoplasmic antibodies (ANCA)minor subsets (elastase , lysozyme , cathepsin G , lactoferrin & BPI)are detected among systemic lupus erythromatus patients causing vasculitis. Systemic lupus erythematosus (SLE) is the prototypic immune complex disease, characterized by excessive autoantibody production, immune complex formation and immunologically mediated tissue injury.

Methods& Patients: A cross-sectional study was conducted on two main groups ,74 patients with SLE and 30 apperantly healthy control volunteers referred to immunology department in teaching laboratories \ medical city during period of (1st of march – 31st of May) 2011. Antinuclear antibody (ANA) ,Cathepsin G, elastase, lactoferrin, lysozyme, bactericidal permeability increasing protein and C1q were detected by enzyme-linked immunosorbent assay (ELISA) technique. While dsDNA was detected by indirect immunofluorescent(IIF) technique and C3,C4 by single radial immunodiffusion (SRID) .

Result: Among patients group the mean levels of Cathepsin G (9.19 IU/ml), Elastase(8.46 IU/ml) , Lysozyme (6.81 IU/ml), Lactoferrin (9.2 IU/ml)and BPI (5.59 IU/ml). Among positive ANA result cathepsin G was detected in high percent (35.1%). In regard to dsDNA positive result elastase Ab was detected in 18 (46.3%) with significant P value 0.00,while Cathepsin G (47.4%) & lactoferrin Ab(31.6%)were significantly found among C1q positive patients .Interestingly lactoferrin Ab associated with low complement C3&C4 levels .

Conclusion: Anti-neutrophil cytoplasmic antibody has a role among SLE patients in which cathepsin G detected in 35.1% among ANA positive patients ,while elastase & cathepsin G,significantly associated with disease activity (positive dsDNA,C1q) ,on other hand lactoferrin had a significant results in association with renal involvement (low C3,C4 levels).

Key word: SLE, cathepsin G, elastase , lysozyme , lactoferrin & BPI.

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Introduction:

SLE is the prototypic immune complex disease, characterized by excessive autoantibody production, immune complex formation and immunologically mediated tissue injury.(1,2). Tissue pathology in all organs reflects a variety of aberrant immunological mechanisms . The inflammatory vascular diseases are particularly intriguing and challenging (3,4). They lead the clinician to consider a broad scope of disorders which can affect individuals across the entire lifespan, and have protean clinical manifestations, and associated with various organ involvement.(5) Vascular disease, can be either as a direct complication of the disease or developing as an accompanying comorbidity impairs significantly the quality of life of patients with SLE and represents the most frequent cause of death in established lupus.(6)

Cytoplasmic constituents of neutrophils may be a cause of formation of specific anti-neutrophil cytoplasmic antibodies (ANCA), which are closely related to the development of systemic vasculitis and glomerulonephritis. ANCA are antibodies directed against enzymes that are found mainly within the azurophil or primary granules of neutrophils. (7,8) Anti-neutrophil cytoplasmic antibodies (ANCA) are serological markers for a significant subset of patients with primary systemic vasculitis. In patients with vasculitis, ANCA targets two major antigens in the primary granules of neutrophils: serine proteinase 3 (PR3) and myeloperoxidase (MPO) . However, human neutrophils contain at least three types of granules, each of which contains a variety of constituent proteins: azurophilic granules [PR3, MPO, bactericidal permeability increasing protein (BPI), elastase (Elast), cathepsin G (Cath G)]; secondary granules [lactoferrin (LF), lysozyme]; and tertiary granules (gelatinase) . Antigens within any of these granules are potential targets for an ANCA response .(9,10)

The development of systemic lupus erythematosus (SLE) vasculitis is of prognostic value. The earlier the vasculitis is treated, the better the prognosis for SLE .Vasculitis have been described in several immunomediated diseases, including SLE, with conflicting results regarding either their prevalence or clinical associations. When assessing disease activity in lupus it is essential to consider the results of immunological tests, although serological tests are useful not only for diagnosis but also for following disease activity. The major role of neutrophils is to phagocytose and destroy infectious

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agents.(9,10) They also limit the growth of some microbes, prior to onset of adaptive (specific) immunological responses. Although neutrophils are essential to host defense, they have also been implicated in the pathology of many chronic inflammatory conditions(8,10).

Methods:

A cross-sectional study was conducted on two main groups ,74 patients with SLE and 30 healthy control volunteers referred to immunologyl department in teaching laboratories\ medical city during period of (1st of march – 31st of may) 2011. From each individual 5ml of blood was collected and divided into several 0.5aliquot and all frozen at -20ċ till used . Antinuclear antibody (ANA) ,Cathepsin G, elastase, lactoferrin, lysozyme, bactericidal permeability increasing protein and C1q were detected by enzyme-linked immunosorbent assay (ELISA) technique. While dsDNA was detected by indirect immunofluorescent(IIF) technique and C3,C4 by single radial immunodiffusion (SRID) .

Results:

The age of SLE patients ranged between (4-71) years with mean age of 32.4 years . Sixty one of them were females and 13 males. The mean levels of Cathepsin G were (9.19 IU/ml), Elastase(8.46 IU/ml) , Lysozyme (6.81 IU/ml), Lactoferrin (9.2 IU/ml)and BPI (5.59 IU/ml).The concentration of these ANCA subtypes above 10 IU\ml were considered positive .Table (1) show that ANA positive patients were associated with cathepsin (35.1%) positive followed with Elastase (32.4%).

Table (1) The distribution of the different ANCA subtypes among study groups

	Cathepsin G		Elastase		Lysozyme		Lactoferrin		BPI		Total
	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	
Patient ANA +ve	26 35.1%	48 64.9%	24 32.4%	50 67.6%	21 28.4%	53 71.6%	14 18.9%	60 81.1	10 12.7%	64 86.3%	74
control	0 0%	30 100%	0 0%	30 100%	0 0%	30 100%	0 0%	30 100%	0 0%	30 100%	30
Total	26	78	24	80	21	83	14	90	10	94	104

In this study from those of 74 patients with SLE only 41(55.4%) were in active state i.e. ds DNA positive and the Elastase Abs were detected in 19 (46.3%) with significant P value 0.004 as shown intable (2).while C1q Abs were detected in 38 (51.4%)of SLE patients (p value0.006) and only 18 (47.4%) of them had Cathepsin G Ab with statistically significant (P value 0 .024)and 12 (31.6%) had lactoferrin Abs with significant P value(0 .004) ,table (2).

Table (2) the relation between different ANCA subtypes and the disease activity (ds DNA +ve, C1q)

	Cathepsin G		Elastase		Lysozyme		Lactoferrin		BPI		Total
	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	
DNA +ve	18 43.9%	23 56.1%	19* 46.3%	22 53.7%	15 36.6%	26 63.4%	7 17.1%	34 82.9%	7 14.6%	35 85.4%	
DNA-ve	8 24.2%	23 75.8%	5 15.2%	28 84.8%	6 18.2%	27 81.8%	7 21.2%	26 78.8%	4 12.5%	28 87.5%	74
C1q +ve	18* 47.4%	20 52.6%	16 42.1%	22 57.9%	14 36.8%	24 63.2%	12* 31.6%	26 68.4%	7 16.3%	31 83.8%	
C1q -ve	8 22.2%	28 77.8%	8 22.2%	28 77.8%	7 19.4%	29 80.6%	2 5.6%	34 94.4%	4 11.1%	32 88.9%	74
control	0 0%	30 100%	0 0%	30 100%	0 0%	30 100%	0 0%	30 100%	0 0%	30 100%	30
Total	26	78	24	80	21	83	14	90	10	94	104

P-value DNA with cathepsin G 0.078, Elastase 0.004, Lysozyme 0.081,Lactoferrin 0.651, BPI 0.792 .

P-value C1q with cathepsin G 0.024, Elastase 0.068, Lysozyme 0.097,Lactoferrin 0.004, BPI 0.526 .

*P-value ≤0.05 significant

This study showed that 45(60.8%) of SLE patients had C3 levels below normal ,28 with normal levels and only 1 with elevated C3 level with no statistically significant (P value 0 .45) , Cathepsin Abs were detected in 19 (42.2%)of SLE patients that had low level of C3 and only 6 (21.4%) had normal level of C3 with no significant(P value0.076) table (3).
Table (3) showed 54(73.0%) of SLE patients had low level of C4 with no significant P value(0 .15) and the lactoferrin Abs were detected in 14(25.9%) of them with statistically significant (P value 0.011) .

Table (3) the relation of the presence of different ANCA subtypes with the levels of C3&C4 (normal, decrease and elevated)

	Cathepsin G		Elastase		Lysozyme		Lactoferrin		BPI		Total
	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	
S. C3 level Low	19 42.2%	26 57.8%	17 37.8%	28 62.2%	13 28.9%	32 71.1%	13* 28.9%	32 71.1%	8 15.6%	38 84.4%	
Normal	6 21.4%	22 78.6%	6 21.4%	22 78.6%	7 25 %	21 75%	7 21.2%	1 3.6%	3 11.1%	24 88.9%	
elevated	1 100%	0 0%	1 100%	0 0%	1 100%	0 0%	0 0%	1 100%	0 0%	1 100%	74
S. C4 level Low	22 40.7%	32 59.3%	19 35.2%	35 64.8%	17 31.5%	37 68.5%	14* 25.9%	40 74.1%	8 14.8%	46 85.2%	
Normal	4 20%	16 80%	5 25%	15 75%	4 20%	16 80.6%	0 0%	20 100%	2 10.5%	17 89.5%	74
control	0 0%	30 100%	0 0%	30 100%	0 0%	30 100%	0 0%	30 100%	0 0%	30 100%	30
Total	26	78	24	80	21	83	14	90	10	94	104

P-value of C3 with cathepsin G 0.076, Elastase 0.121, Lysozyme 0.261,Lactoferrin 0.024, BPI 0.801 .
P-value of C4 cathepsin G 0.097, Elastase 0.406, Lysozyme 0.331,Lactoferrin 0.011, BPI 0.640 .
Normal value for C3 (84 -193) mg\dl
Normal value for C4 (20-40) mg\dl
Table (4) showed a significant correlation between elastase with cathepsin G and lysozyme, while cathepsin G with elastase, lysozyme& BPI. On other hand BPI mostly correlated with cathepsin G & lysozyme.

Table (4) the correlations of different ANCA subtypes between them and with ANA,C1q, C3&C4

		ANA	C1q	C3	C4	Elastase	Cathepsin G	Lactoferrin	Lysozyme	BPI
Elastase	r	0.134	0.178	-0.124-	-0.248*	1	0.389**	0.173	0.599**	0.297*
	P	0.256	0.130	0.294	0.033		0.001	0.140	0.000	0.010
Cathepsin G	r	0.139	0.015	-0.023-	-0.224-	.0389**	1	0.077	0.357**	0.401**
	P	0.237	0.897	0.844	0.056	0.001		0.515	0.002	0.000
Lactoferrin	r	-0.091-	0.172	-0.223-	-0.235-*	0.173	0.077	1	0.174	0.120
	P	0.443	0.142	0.057	0.044	0.140	0.515		0.139	0.311
Lysozyme	r	0.289*	0.081	-0.077-	-0.168-	0.599**	0.357**	0.174	1	0.540**
	P	0.012	0.494	0.512	0.151	0.000	0.002	0.139		0.000
BPIScore	r	0.388**	0.133	-0.207-	-0.059-	0.297*	0.401**	0.120	0.540**	1
	P	0.001	0.258	0.077	0.620	0.010	0.000	0.311	0.000	

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

Discussion :

Although not all manifestations of SLE can be attributed to immune complexes, these complexes do appear to have a central role in the pathology and immunopathology of SLE (11).Coexistence of anti-neutrophil cytoplasmic antibodies (ANCA) and antinuclear antibodies (ANA) in systemic lupus erythematosus (SLE) patients is an interesting phenomenon (12 ,13). Neutrophil specific granules are susceptible to release their contents extracellularly and have an important role in initiating inflammation.(14). Vasculitis may manifest in as high as 56% of lupus patients throughout their life,(15)

the presence of antineutrophil cytoplasmic autoantibodies, mainly associated with primary systemic vasculitis,.either through direct compromise of the vascular wall by pathogens, or through antigen-induced autoimmune and inflammatory processes (15,16)

In the current study cathepsin G detected among 35.1% of ANA positive patients this agree with study done by Scientists at Washington University School of Medicine in St. Louis have found a new role for this enzyme that may make it a target for anti-inflammatory treatments.(17,18)

The finding by researchers shows that cathepsin G regulates the ability of the 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 that is a result of cytokine over activity can lead to tissue damage, causing pain, limited mobility. (19)

The relation to dsDNA anti-Elastase was reported to present itself as a significant value (p-value 0.004). Elastase is known to stimulate endothelial tissue factor, which in turn could be relevant to the development of vascular inflammation .On the other hand, Anti-Elastase may play a role in maintaining inflammation of the vascular wall by inducing both degranulation and respiratory burst of primed granulocytes (19,20) . Released reactive oxygen species can penetrate cell membranes and induce the release of altered DNA, which may subsequently result in the formation of anti-DNA antibodies this result agreed with a study done by Falk RJ,Terell .(20)

C1q mostly correlated with lactoferrin and cathepsin G and found to be involved in numerous biological functions like modulation of the inflammatory response, iron metabolism, recruitment of polymorphonuclear leukocytes and antimicrobial defense. Human lactoferrin inhibits activation of the classical complement pathway through inhibition of formation of the classical C3 convertase .This finding agreed with a study done by Kijlstra,A. (21)

Immune complexes were thought to bind to the C1q. Positive results for 'immune complexes' were found in patients with SLE and correlated with the presence of active disease and the presence of hypocomplementemia and elevated titres of anti-DNA antibodies .(22)

Patients with SLE had a significant association of Lactoferrin with low level of C3&C4 and that agreed with Manderson study which could be explaned by the role of complement in the pathogenesis of SLE is paradoxical. On one hand,the complement components appear to mediate autoantibody-initiated tissue damage. On the other hand, the complement system appears to have protective features as hereditary deficiencies of some complement components are associated with an increased risk for SLE.(22) and the hypotheses for this linking is the association between complement deficiencies and SLE could be explained by several mechanisms, including impaired clearance of immune complexes and impaired handling of apoptotic cells,

aberrant tolerance induction or changes in cytokine regulation. Complement deficiency can lead to impaired handling of immune complexes in SLE, supporting the hypothesis that a defect in handling immune complexes formed between antibodies and selfantigens.(22)

A significant P-value found between Elastase and both cathepsin G&lysozyme of 0.001and 0.000 respectivly, while cathepsin G with, BPI, Elastase and lysozyme.

Lysozyme with cathepsin G,elastase and BPI .This could be explaned that human neutrophils utilize a variety of destructive enzymes during the process of phagocytosis. These include proteinases, phosphatases, glycosidases, nucleases, and oxidases. The major enzymes have been determined to be elastase, cathepsin G, myeloperoxidase, and lysozyme, while minor proteins include collagenase, lactoferrin, alkaline phosphatase, and a myriad of other proteins (20).

Conclusion:

Anti-neutrophil cytoplasmic antibody has a role among SLE patients in which cathepsin G detected in 35.1% among ANA positive patients ,while elastase & cathepsin G,significantly associated with disease activity (positive dsDNA,C1q) ,on other hand lactoferrin had a significant results in association with renal involvement (low C3,C4 levels).

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