AJPS, 2013, Vol. 13, No.1

Anti Cathepsin Antibody In Systemic Lupus Erythematosus

Nahla ghanim Abdul–majeed, Leen K. Mustafa Kamil and Mahmood Raheem Al-Rubaye

Teaching laboratory of medical city, immunology department

Abstract:

Antineutrophil cytoplasmic autoantibodies (ANCA) were found in patients with systemic lupus erythematosus (SLE). Cathepsin G was the major target antigen. However, some ANCA-positive sera did not recognize either of them.The present study was to investigate the unknown target antigens of ANCA (Cathepsin G) in patients with SLE and its relation to the activity of the disease (ds DNA),C1q ,C3and C4.

Seventy four SLE patients referred to immunological department in teaching laboratory\ medical city during period of (1^{st} of march–31^{st} of May) 2011 and 30 apparently healthy individual as a control group were subjected to Antinuclear antibody (ANA), Cathepsin G 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).

The age of SLE patients ranged between (4-71) years with mean age of 32.4. The mean C1q and Cathepsin G levels were 30.14 and 9.7 respectively with significant difference if compare it with the healthy control group**.** A significant result found between mean age of SLE patients and Cathepsin Ab of a P value 0.007.

The Cathepsin G antibody was found in 26 (35%) of SLE patients with a significant P value (0.0001).

The Cathepsin Ab were detected in 18 (43.9%) of a patients with positive ds DNA with no significant P value 0.078. while C1q Ab were detected in 38 (51.4%) of SLE patients and only 18 (47.4%) from them with Cathepsin Ab with statistically significant P value 0.024. Cathepsin Ab found in 19 (42.2%) of SLE patients with low level of C3 and only 6 (21.4%) of normal level of C3 with no significant P value0.076.

This study showed 54(73.0%) of SLE patients had low level of C4 with no significant P value (0.15) and the Cathepsin Ab detected in 22 (40.7%) of them with no statistically significant P value 0.097.

A statistically significant result were found for ANA, C1q,C3,C4 and Cathepsin Ab if we compare the result of SLE patients with control group, while The Cathepsin G antibody was found statistically significant in SLE patients **,**with significant relation to C1q,on other hand no relation found with the activity of the disease (ds DNA),C3 and C4 levels.

Key Word: SLE, Cathepsin G, ds DNA, C1q, C3, C4.

الخلاصة:

وجدت الاجسام المضادة الضادة لسايتوبلازم كريات الدم البيض العدلة نوع P (pANCA) لدى المرضى المصابين بداء الذئب ألاحمراري وكان الكثبسين ج هو المستضد المستهدف، وان نسبة علاقة مضاد ANCA مع مستضاداتها تحت الدراسة.

الغرض من هذه الدراسة هو لمعرفة العلاقة بين مضاد الكثبسين ج وفعالية المرض المتمثلة بالشريط المضاعف للحامض النووي الديوكسي الرايبوزي (dsDNA) ومستوى المتمم الثالث والرابع (C3,C4).

خلال الفترة من الأول من آذار إلى 31 أيارعام2011 تم تحويل 74 مريض مصابين بداء الذئب ألاحمراري إلى قسم المناعة في المختبرات التعليمية في دائرة مدينة الطب وتم اخذ عينات منهم مع 25 عينة من أشخاص أصحاء كمجموعة سيطرة، وتم أجراء فحوصات الاجسام المضادة الضادة للنواة ANA ومضاد الكثبسين ج و c1q باستعمال تقنية الامتزاز المناعي المرتبط بالإنزيم (الاليزا)، وتم فحص الـ dsDNA بواسطة IIF وC3 وC4 بواسطة SRID.

كانت أعمار المرضى تتراوح مابين(4-71) سنة وكان معدل العمر هو 32.4 بينما كان معدل مستوى وC1q المضاد للكثبسين ج هو (30.14 و9.7) على التوالي مع وجود فرق معنوي مقارنة بمجموعة السيطرة. وجد فرق معنوي بين معدل عمر مرضى داء الذئب ألاحمراري ومضاد الكثبسين ج وقيمته (0.007).

وجد مضادالكثبسين ج لدى 26 (35%) من مرضى داء الذئب ألاحمراري مع وجود فرق معنوي قيمنه (0.0001) وقد تم تشخيص مضاد الكثبسين ج لدى 18 (43.9 %) من المرضى الذين لديهم أجسام مضادة للـ dsDNA مع عدم وجود فرق معنوي، بينما الأجسام المضادة لـ C1q وجدت لدى 38 (51.4%) من المرضى ولكن تم تشخيص الكثبسين ج عند 18(47.4%) مع وجود فارق معنوي 0.024 وقد تم تشخيص الكثبسين ج عند 19(42.2%) من مرضى داء الذئب ألاحمراري الذين لديهم انخفاض في مستوى ال C3 بينما كان مستواه طبيعي لدى 6 (21.4%) مع ملاحظة عدم وجود اي فارق معنوي.

أظهرت هذه الدراسة أن 54(73%) من المرضى كان لديهم مستوى الC4 منخفضا مع عدم وجود فرق معنوي عند مقارنتهم بالمجموعة الضابطة مع وجود الأجسام المضادة للكثبسين لدى 22(40.7%) مع عدم وجود فارق معنوي.

Introduction:

We normally have antibodies in our blood that repel invaders into our body, such as virus and bacteria microbes. Antinuclear antibodies (ANAs) are unusual antibodies, detectable in the blood, that have the capability of binding to certain structures within the nucleus of the cells.

The nucleus is the inner rmost core within the body's cells and contains the DNA, the primary genetic material[1,2]. ANAs are found in patients whose immune system may be predisposed to cause inflammation against their own body tissues. The propensity for the immune system to work against its own body is referred to as autoimmunity.

ANAs indicate the possible presence of autoimmunity and provide, therefore, an indication for doctors to consider the possibility of autoimmune illness [1,3].

Antinuclear antibodies (ANA), which occur in a number of different autoimmune disorders including systemic lupus erythematous (SLE).

SLE is a classic autoimmune disease. The immune system is intended as a defense against invading infection (i.e. viruses, bacteria, and parasites) but in lupus this system goes away and results in the formation of molecules such as autoantibodies, immune complexes, and complement which may attack organs of the body. Although lupus is a chronic, potentially life-long condition it is characterized by episodic activity.

In other words, patients unpredictably experience disease flares followed by periods of disease inactivity. Additionally, during a flare, lupus may affect the skin, joints, kidney, brain, lung, heart and gastrointestinal tract although it is unlikely that in any patient all these systems would be involved. In fact, each lupus patient is unique and the severity, frequency, and extent of injury varies from patient to patient [4].

Antibodies to dsDNA are often measured in SLE and are commonly referred to as anti-DNA antibodies. They are very useful in the diagnosis of SLE and assessment of disease activity, and they are associated with lupus nephritis. So anti-DNA antibody testing is very useful in the diagnosis of SLE and is also a useful biomarker of SLE disease activity [13].

Antineutrophil cytoplasmic antibodies (ANCA) is defined by an accumulation of auto-antibodies with specificity against different granulocytic, monocytic and probably endothelial cytoplasmic antigens [5,6]. Cathepsin G belong to a group of intracellular proteases mainly found in lysosomes, especially of the spleen, liver and the kidney.

Cathepsin G is a serine protease and a further pANCA antigen. It participates to a great part in the destruction of osteoid tissue as of its hydrolytic properties. The auto-antibodies against Cathepsin G occur mainly in collagenosis and SLE [7,8].

An intact classical pathway of the complement system is essential for

protection against immune complex diseases. C1q is a central molecule in the first step of the classical complement activation pathway. The globular heads of C1q bind to the Fc regions of immunoglobulins IgM or IgG thus inducing an activation of the other subcomponents of C1,C1r and C1s [9,10].

The presence of Anti-C1q auto antibodies is associated with several autoimmune and renal illnesses. Containing an occurrence of 100% in the hypocomplementaemic vasculitis syndrome. AntiC1q-antibodies act as a diagnostic marker for this disease. They were also described in systemic lupus erythematosus (SLE) and especially in lupus nephritis. It was discovered that up to 60% of patients with SLE and up to 80% of patients with diffuse proliferative lupus nephritis have such antibodies[11].

It is described that in some cases patients with clinical active lupus were found as Anti-ds DNA negative, so Anti-C1q antibodies may serve as an additional tool for rheumatologist to document lupus activity [12].

Materials and Methods:

A cross-sectional study was conducted on two main groups,74 patients with SLE and 30 healthy control volunteers referred to immunological department in teaching laboratory/medical city during period of (1^{st} of march – 31^{st} 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 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.sixty one of them were females and 13 males.

The mean C1q and Cathepsin G levels were 30.14 and 9.7 respectively with significant difference if compare it with the healthy control group, also a significant result was found with C3 and C4 (Table-1). A significant result found between mean age of SLE patients and Cathepsin G Ab of a P value 0.007**.**

Table -1: Show the mean of age, ANA, C1q, C3, C4 and Cathepsin G in SLE patients in comparison to healthy control.

parameters	Type	N	Mean	Sig. (2-tailed)
Age	Patient	74	32.49	.862
	Control	30	31.93	
ANA Index	Patient	74	2.17	.0001
	Control	30	.34	
C1q level	Patient	74	30.14	.006
	Control	30	4.31	
C3 concentration	Patient	74	77.26	.021
	Control	30	102.95	
C4 concentration	Patient	74	15.37	.0001
	Control	30	29.18	
Cathepsin G level	Patient	74	9.75	.001
	Control	30	4.00	

The Cathepsin G antibody was found in a level above 10 U\ ml which is considered positive in 26 (35%) of SLE patients, while all healthy control group was negative with a significant P value (0.001), (table -2)

Table -2: Anti-Cathepsin G distribution among study groups
P value =0.001*

			Cathepsin G		
	subjects		**+ve**	**-ve**	**Total**
Type	**Patient**	Count	26*	48	74
		% within Type	35.1%	64.9%	100.0%
	Control	Count	0	30	30
		% within Type	.0%	100.0%	100.0%
Total		Count	26	78	104
		% within Type	25.0%	75.0%	100.0%

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 Cathepsin Ab were detected in 18 (43.9%) with no significant P value 0.078, (table-3).while C1q Abs were detected in 38 (51.4%) of SLE patients and only 18 (47.4%) from them with Cathepsin Ab with statistically significant P value 0 .024 (table-4).

A statistically significant result found between C1q and C3, C4 level with a P value of 0.003 and 0.002 respectively, also a significant result found between C3 and C4 level of P value 0.0001

Table -3: The relation of the presence of Cathepsin Ab with the activity of the disease (ds DNA +ve)
* Pvalue =0.078.

		Cathepsin G		**Total**
		+ ve	**- ve**	
ds DNA	+ve No.	18*	23	41
	%	43.9%	56.1%	100%
	-ve No.	8	25	33
	%	24.2%	75.8%	100%
Total	No.	26	48	74
	%	35.1%	64.9%	100%

Table - 4: The relation of the presence of Cathepsin Ab with the C1q Antibody.

***** P value =0.024

			Cathepsin G		
			+ve	**-ve**	**Total**
C1q	+ve	Count	18*	20	38
		% within C1q	47.4%	52.6%	100.0%
	-ve	Count	8	28	36
		% within C1q	22.2%	77.8%	100.0%
Total		Count	26	48	74
		% within C1q	35.1%	64.9%	100.0%

This study showed that 45(60.8%) of 74 SLE patients represented with level of C3 below normal, 28 with normal level and only 1 with elevated C3 level with no statistically significant P value 0 .45 (table-5). Cathepsin Ab found in 19 (42.2%)of SLE patients with low level of C3 and only 6 (21.4%) with normal level of C3 with no significant P value0.076 (table-5).

Table-5: The relation of the presence of Cathepsin Ab with the levels of C3 (normal, increase and decrease)

P value =0.076*

Normal value for C3 (84 -193) mg\dl

			Cathepsin G		
			+ve	-ve	**Total**
C3 concentration	**Decrease**	Count	19	26	45
		% within C3	42.2%	57.8%	100.0%
	Normal	Count	6	22	28
		% within C3	21.4%	78.6%	100.0%
	Increase	Count	1	0	1
		% within C3	100.0%	.0%	100.0%
Total		Count	26	48	74
		% within C3	35.1%	64.9%	100.0%

(Table-6) showed 54(73.0%) of SLE patients had low level of C4 with no significant P value (0 .15) and the Cathepsin Ab detected in 22 (40.7%) of them with no statistically significant P value 0.097.

Table-6: The relation of the presence of Cathepsin Ab with the levels of C4 (normal and decrease)

*P value =0.097

Normal value for C4 (20-40) mg\dl

			Cathepsin G		
			+ve	-ve	Total
C4 concentration	**Decrease**	Count	22	32	54
		% within C4	40.7%	59.3%	100.0%
	Normal	Count	4	16	20
		% within C4	20.0%	80.0%	100.0%
Total		Count	26	48	74
		% within C4	35.1%	64.9%	100.0%

Discussion:

Systemic lupus erythematosus can be complicated by vascular inflammation that mainly involves the small vessels such as venules, arterioles and capillaries [14].

A pathogenetic role for various autoantibodies in the development of vasculitis has been postulated.

Antibodies to neutrophil cytoplasmic antigens (ANCAs) have been extensively studied as markers for systemic vasculitis.

The target antigens for p-ANCAs in these disorders are usually unclear although several were detected such as myeloperoxidase [14] lactoferrin (LF), Cathepsin G (CG) [15].

Interestingly, different antigenic specificities of ANCA have been associated with distinct clinical subsets i.e. anti-elastase and/or anti-LF with central nervous system in SLE. Also, a recent study by (16) correlated anti-CG with active renal lesions.

The mean C1q and Cathepsin G levels were 30.14 and 9.7 respectively with significant difference if compare it with the healthy control group , this could be related to an enzyme known as Cathepsin G which regulates the ability of immune cells known as neutrophils to secrete hemicals 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. "Cathepsin G affects a very early step in this kind of immune response, so inhibiting it has attractive potential for developers of therapeutics.

Observations made that linked the earliest stages of inflammation to neutrophils, which are a kind of immune system fire starter. They arrive first at sites of injury, infection or irritation and secrete chemicals that bring in secondary waves of other immune attack cells.

Anti-Cathepsin G was evident in the presence or absence of ANCA and it correlates with disease activity while other target antigens were not predictive of disease exacerbation.

In the present study, there was a significant difference in the serum level of anti-Cathepsin G in patients with SLE compared to healthy group.

In support to our findings DeBandt (16) found a highly striking incidence of anti-Cathepsin G that reached 62%. The higher incidence of anti-Cathepsin G in the latter study may be attributed to the selection of patients (all patients had lupus nephritis).

Thus, the presence of anti-Cathepsin G may be related to renal affection but the patients' number was quite small to verify this finding that needs further investigation.

The exact mechanism by which ANCA and its subsets (Cathepsin G) may contribute to granulocyte mediated vascular damage is multiple. Perinuclear ANCA has been shown to induce an increased release of reactive oxygen species and granule contents by granulocytes [14].

Another pathogenetic mechanism 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 [14].

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 [15] .In conclusion, the prevalence of ANCA in different rheumatic disorders (RA and SLE) indicates severe disease with increased inflammatory activity. The antibodies directed against target antigens may act as a marker for disease subsets. [16]

Antibodies to dsDNA are often measured in SLE and are commonly referred to as anti-DNA antibodies .They are very useful in the diagnosis of SLE and assessment of disease activity, and they are associated with lupus nephritis.

The ds DNA positive and the Cathepsin Ab were detected in 18 (43.9%) with no significant because In most cases, the auto antigens are non-renal and become renal targets because of the physiological properties of the high flow, high-pressure perm- selective filtration function of the glomerulus. Circulating auto antigen can deposit in glomeruli as part of circulating immune complexes (e.g. Cathepsin, C1q) or become a planted target antigen by their physico-chemical properties that predispose to their glomerular fixation[17].

A potentially unique model of deposition of a 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 (including Cathepsin G) is targeted by ANCA. These ANCA-activated neutrophils have altered flow characteristics resulting in their lodging in small vessels, particularly glomeruli, resulting in renal injury.

Inflammatory renal disease in the context of autoimmunity occurs because the kidney is targeted by effector responses.

The effectors of autoimmunity in the kidney are many, but most often disease is initiated either by antibody deposition or infiltration of immune cells. Once antibodies are deposited, their exposed Fc (fragment crystalline) regions activate and recruit inflammatory cells, and initiate complement activation.

This process leads to further cellular infiltration, and secretion of inflammatory mediators by both infiltrating and endogenous cells. Infiltrating cells, which include neutrophils, T-cells and macrophages, and platelets also secrete soluble mediators and directly interact with renal cells and each other to perpetuate the disease process.

The kidneys may become affected by antibody-mediated mechanisms where the auto antigen resides outside the kidney. Deposition of resulting immune-complexes within the kidneys subsequently triggers tissue damaging events (e.g. lupus nephritis), or the antigen and antibodies are neither derived nor deposited within the kidneys. However, the interaction of antibodies with the antigens, or with antigen-bearing cells, causes the disease (e.g. ANCA vasculitis and glomerulo-nephritis, suggest that molecules on the lymphocyte surface that bind Cathepsin G

recognize not only the active site of Cathepsin G but also other sites of this molecule, but the active site has an important function in this binding because stimulation of lymphocytes requires the binding of proteolytically active Cathepsin G.)[18].

Complement activation is usually assessed by determining the levels of individual complement components such as C3 and C4. Classic pathway activation is indicated by low levels of C3 and C4. Alternate pathway activation is indicated by low levels of C3 but normal C4.

There is a significant association between low complement levels and lupus nephropathy[19].

It was observed that elastase, and Cathepsin G was capable of splitting C3 and C5 into smaller fragments electrophoretically similar to C3a and C5a. This was especially critical in the evaluation of C4 which is heterogeneous in humans and thus has slightly variable mobilities. Other Complement chronic inflammatory diseases e.g. SLE, Chronic rheumatoid arthritis are similar in several ways. They--both involve the development of inflammatory disease.

Despite the fact that the involved tissues in both cases have been shown to contain cells which synthesize many complement components are at reduced levels. This suggests that complement may be utilized in both of these disease .Immune complexes are commonly found in SLE patients and are thought to initiate the complement cascade and contribute to the progression of the disease.

In SLE C3 is the most consistently depressed complement component and complement cleavage is believed to occur primarily through the classical pathway with the alternative activating pathway in an amplifying role [20].

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