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Serum level of Matrix metalloproteinase-9 in patients with systemic lupus erythematosus.

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Abstract

Background: Matrix metalloproteinase-9 (MMP-9) was involved in inflammation and immune system dysfunctions. Beside immunologic abnormalities, systemic lupus erythematosus (SLE) also presents chronic inflammatory components. Therefore, a role of MMP-9 in SLE pathology might be supposed.

Objective: To investigate the level of matrix metalloproteinase-9 in systemic lupus erythematosus patients and to determine its value in monitoring disease activity.

Patients and methods: Twenty five SLE female patients were included in this study. Ten healthy females where selected as controls. The activity of SLE was evaluated according to SLAM scoring. Using quantitative ELISA Kit provided from R & D system INC. USA, to quantitate the total levels of MMP-9 . These levels were then compared and correlated with the ANA, anti-ds-DNA, lupus nephritis, Raynaud phenomenon, malar rash, photosensitivity, alopecia, and mucosal ulcers.

Results: serum of MMP-9 were found to be significantly higher ($P<0.01$), in SLE patients compared to the control group. Serum MMP-9 show statistical significant correlation with anti-ds DNA, Lupus nephritis, Raynaud phenomenon, malar rash and photosensitivity and it did not-show any statistical significant correlation with ANA, alopecia and mucosal ulcers

Conclusion : The data suggest that MMP-9 could be involved in the pathogenesis of SLE, and serum MMP-9 can be used as marker to monitor disease activity, renal damage, disease progression and amelioration in SLE.

Introduction

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease that exhibits various clinical manifestations, including immune complex depositions in the kidneys and other organs (Hahn, 1993).

The cause of the disease has not yet been defined yet. However, it involves the production of a broad spectrum of autoa-ntibodies against nuclear, cytoplasmic and cell-surface antigens, and different impair-ments of B-and T-cell functions (winche-ster,1996). Proinflammatory cytokines, especially TNF- α and IL-1, were shown to play an important role in the pathogenesis of SLE through direct induction of matrix metalloproteinases (MMPs). Thus MMPs may play a role in the pathogenesis of SLE (Zhang *et al*., 1998).

Matrix metalloproteinases (MMPs) are a family of highly homologous Zn^{2++} dependent enzymes , capable of degrading the extracellular matrix (ECM) and bone marrow. They are present in healthy individuals and have been shown to play an important role in physiological processes such as wound healing, bone resorption and pregnancy. (Brehmer *et al*. 2003).

The potential importance of the many activities of MMPs in inflammatory responses has been suggested by the inhibitory effects of MMPs in several autoimmune diseases. Specific inhibitions

of MMPs in vivo suppresses oedema, pathologic tissue proliferation, and damage to specialized tissue structures in several inflammatory and autoimmune diseases (Wallace *et al*., 1999).

Matrix metalloproteinase-9 (MMP-9) plays an important role in the degradation of type IV collagen, denaturation of collagens types V, VII, X, XII collagens, vitronectin agreecan, galectin-3 and elastin (Zhang *et al*, 1998). Faber-El-Mann *et al*. 2002B, and Matache et al., 2003 in two different studies reported that MMP-9 level was found to be elevated in the serum of SLE patients compared with helathy controls. High MMP-9 activity was found to correlate with the presence of discoid rash, Raynauds phenomenon, pneumonitis, mucosal ulcers and the presence of anti-phospholipid antibodies (APLA).

The aim of this work is to measure the levels of (MMP-9) in sera of systemic lupus erythematosus patients and to determine its value in monitoring disease activity.

Patients and methods

A group of 25 female patients suffering from systemic lupus erythematosus (SLE) participated in this study. Their ages ranged from 12 to 35 years (With a mean age of 24.28 ±6.53).

Ten age matched normal female controls with no family or personal history of SLE were included. Their ages ranged from 15 to 34 years. (with a mean age of 22.1± 5.95). All cases for this study were collected from the out patient clinics of dermatology and medicine in El-Zahraa university hospital. The patients were subjected to complete clinical evaluation thorough history taking and clinical examination which was performed according to the American Rheumatism Association (ARA) criteria. The activity of SLE was evaluated according to the systemic lupus activity measure (SLAM) score.

Blood sampling

Seven milliliters of venous blood were collected and divided into two tube. Three milliliters were collected in heparinized tube. The plasma was separated after centrifugation and stored at -20 ºC for determination of MMP-9.

Four milliliters blood were collected in plain tube and left for 20 min. at 37 ºC. Serum was separated after centrifugation and stored at -20 ºC for ANA and double stranded DNA estimation.

The patients were subjected to:

- Serum level of ANA and double stranded DNA were measured by (ELISA). Commercially available enzyme linked immunosorbent assay method by using their specific kits [B indazymeth ANA, double stranded DNA screen IVD] .
- Assessment of serum MMP-9 level by employing aquantitative sandwich ELISA technique using Quantikine ELISA Kit provided from R & D systems, inc., USA).

Statistical analysis was done.

Data was analyzed by Microsoft Office XP (excel).

Parametric data was expressed as mean ± SD, and non parametric data was expressed as number and percentage of the total.

Comparing the mean ± SD of 2 groups was done using the student's test
Determining the extent that a single observed series of proportions differs from a theoretical or expected distribution was done using the Chi square test

P value > 0.05 is considered non-significant

P value < 0.01 is considered highly significant

Results

This study encompassed 25 patients with an age span of 12-35 years and 10 controls with an age span of 15-34 years. The duration of the disease ranged from 0.33-10 years.

Table 1 and figure 1 represents the results of serum level of metalloproteinase 9 in SLE patients and controls. We obtained a mean serum MMP-9 level of 660.96 ±305.21 ng/ml for SLE patients and

170.70±20.96 ng/ml for control group (P<0.01).

On comparing the serum level of MMP-9 in patients with positive Anti ds DNA and those with negative results, there was a highly statistically significant difference (P<0.01) and on comparing the serum level of MMP-9 in patients with positive ANA, and those with negative results, there was no significant difference (Table 2).

On comparing the serum level of MMP-9 in patients with positive renal affection and those with negative results, there was a highly significant difference (P<0.01) (table 3) .

By comparing the serum level of MMP-9 in patients with positive Raymond's phenomenon and those with negative results, there was a highly significant difference (P <0.01) (table 4). By comparing the serum level of MMP-9 in patients with presence of malar rash and photosensitivity and those with negative results, there was a highly significant difference (P <0.01) (Table 5).

Finally, on comparing serum level of MMP-9 in patients with presence of alopecia and mucosal ulcers and those with negative results, there was no statistically significant difference (P<0.01) (Table 6).

Table 1: Relationship between MMP-9 level in SLE patients and controls

	Mean ng/ml	**SD ng/ml**	**P Value**
Patients	660.96	305.21	0.0000 *
Control	170.70	20.96	

SD = Standard deviation

P vlaue <0.01 significant

Table 2 : Relationship between MMP-9 level and anti ds DNA and ANA in SLE patients

		MMP-9			
		Positive	Negative	Total	P Value
Anti-ds DNA	Positive	14	1	15	0.0000**
	Negative	1	9	10	
ANA	Positive	15	10	25	
	Negative	0	0	0	

** = highly significant at P value <0.01

Table 3: Relationship between MMP-9 level and patients with renal affection in SLE

		MMP-9			
		Positive	Negative	Total	P value
Renal	Positive	13	3	16	0.0038**
	Negative	2	7	9	

** = highly significant at P value < 0.01

Table 4. : Relationship between MMP-9 level and raynaud's phenomenon in SLE patients

		MMP-9			
		Positive	Negative	Total	P value
Raynaud's	Positive	13	1	14	0.0002**
Phenomenon	Negative	2	9	11	

** = highly significant at P value < 0.01

Table 5: Relationship between MMP-9 level and presence of malar rash and photo sensitivity in SLE patients.

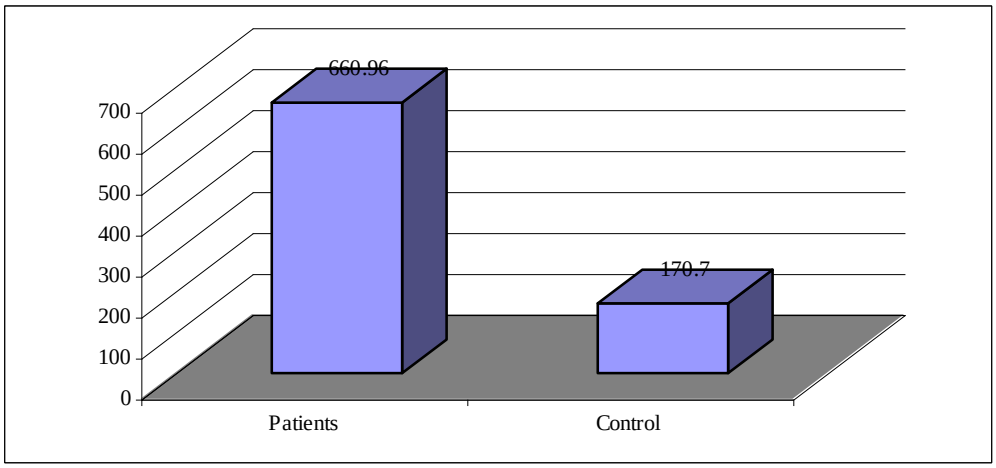
		MMP-9			
		Positive	Negative	Total	P value
Malar	Positive	13	1	14	0.0002**
	Negative	2	9	11	
Photo	Positive	13	1	14	0.0002**
	Negative	2	9	11	

Table 6: Relationship between MMP-9 level and presence of alopecia and mucosal ulcers in SLE patients.

		MMP-9			
		Positive	Negative	Total	P value
Alopecia	Positive	8	2	10	0.0956 (NS)
	Negative	7	8	15	
Mucosal ulcers	Positive	5	2	7	0.4670 (NS)
	Negative	10	8	18	

NS= non significant

Fig MMP-9 among patients and control groups



Discussion

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the increased production of autoantibodies and by T-cell dysfunction associated with general clinical manifestations (Faber – Elmann *et al*. 2002 A).

Matrix metalloproteinase-9 (MMP-9) was involved in inflammation and immune system dysfunctions. Beside immunologic abnormalities, SLE also presents chronic inflammatory components. Therefore, a role of MMP-9 in SLE pathology was supposed (Matache *et al*., 2003).

In the present study, serum level of MMP-9 was investigated to determine its value in monitoring SLE disease activity. We investigated the level of MMP-9 in the sera of 25 SLE patients compared with 10 healthy controls. The serum level of MMP-9 was significantly higher in all studied SLE cases when compared with the control level ($P<0.01$). These results are in

agreement with that of Liu et al, 2004 and Matache *et al* 2003 who reported that MMP-9 level in sera of SLE patients is significantly higher than those in normal control

There was significant correlation of serum MMP-9 with anti-ds DNA antibodies. These results were not in agreement with Makowski and Ramsby 2003 who found that serum MMP-9 , inversely correlates with anti-ds DNA antibodies, which are a specific marker for SLE, so it may be important in monitoring disease activity during antibody deposition in tissues. There was no significant correlation of serum MMP-9 with anti-nulcear antibodies.

There was significant correlation between serum MMP-9 and the presence of lupus nephritis. These results were not in agreement with Faber-Elmann *et al*. (2002 B) and Liu *et al*. (2004) who found no correlation between serum MMP-9 and renal involvement. Mc Millan *et al*. 1996 demonstrated that MMP-9 is produced by glomerular epithelial cells.

There was significant correlation between serum MMP-9 and the presence of raynaud phenomenon. These result were in agreement with Faber-Elmann *et al* (2002) B who found a correlation between high serum MMP-9 and raynaud phenomenon.

There was significant correlation of serum MMP-9 with malar rash and photosensitivity. These results were in agreement with Faber-Elmann etal. (2002) B who found strong correlation between high serum MMP-9 and presence of malar rash and photosensitivity.

There was no significant correlation of serum MMP-9 with presence of alopecia and mucosal ulcers. These results were not in agreement with Faber-Elmann *et al*. (2002) B who found strong correlation between serum MMP-9 and presence of mucosal ulcers .

The origin of the elevated MMP-9 in sera of SLE patients is not known. MMP-9 has been shown to be secreted by peripheral blood cells such as T-cells, neutrophils and macrophages (Matache *et al*. 2003).

It has also been demonstrated that MMP-9 is produced by glomerular epithelial (McMillan *et al*, 1996) and mesangial cells (Lovett *et al*, 1992) as well as by pleuntic cells. Moreover, the association between cytot-oxic treatment, which represents the severity of SLE- related organ impairment, and high serum MMP-9 in the sera support the notion that the diseased organs are the source of MMP-9 activity in SLE patients (Faber-Elmann *et al*. 2002B).

TNF - α and IL-1 were shown to play an important role in the pathogenesis of SLE (Segal *et al*. 1997). It has been shown in several systems that these cytokines induce MMP-9 production (Guedez *et al*, 1996) and thus the inducation of the latter MMPs in part of the pathogenic effect of these cytokines in SLE. MMPs of both T cells and macrophages facilitate secretion of TNF α by cleavage of membrane-bound form (Gearing *et al*. 1994).

Thus, these examples demonstrate the mutual regulatory effects of MMP on the pro-inflammatory cytokines and vice versa.

We conclude that MMP-9 might play a role in the pathogenesis of SLE, and the measurement of plasma/serum activity levels of this metalloproteinase may provide important information when monitoring patients treated with drug that interfere with MMP-9 activity.

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قياس مستوي الأنزيم البروتيني الفلزي-9 في مصل الدم في مرضى الذئبة الحمراء

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يشارك الأنزيم البروتيني الفلزي -9 في حدوث الالتهابات واضطراب الجهاز المناعي . و بجانب الاضطرابات المناعية التي تحدث في مرض الذئبة الحمراء فإنه يوجد مكونات التهابية ولهذا يمكن إيجاد دور للإنزيم البروتيني الفلزي-9 في حدوث مرض الذئبة الحمراء .

وفي هذا البحث تم قياس مستوي الانزيم البروتيني الفلزي -9 في مصل الدم لخمسة وعشرين مريضة بالذئبة الحمراء وايضاً لعشرة متطوعات موائمات للمريضات في العمر والسن وقد تم تصنيف النشاط المرضي للمريضات إكلينيكيا وبمساعدة عدد من الاختبارات المعملية مثل الأجسام المضادة لنواة الخلية من نوع (anti ds DNA) .

ثم تمت عمل مقارنة بين مستوي الانزيم في مصل الدم وبعض المؤشرات الإكلينيكية المصاحبة لمرض الذئبة الحمراء مثل احمرار الوجنة والحساسية وقرح الغشاء المخاطي والثعلبة وداء رينو والتهابات الكلي .

وقد وجد أن الارتفاع في مستوي الانزيم البروتيني الفلزي-9 في مرضى الذئبة الحمراء ذو دلالة إحصائية وأيضاً هناك علاقة ذو دلالة إحصائية بين هذا الانزيم والاجسام المضادة لنواة الخلية من نوع (anti ds DNA) وأيضاً مع الأعراض الاتية مثل التهاب الكلي ، داء رينو ، احمرار الوجه ، الحساسية الضوئية ونخلص من هذا البحث إلي أن مستوي الانزيم البروتيني الفلزي-9 في مصل الدم عند مرضى الذئبة الحمراء له دور في منشأ مرض الذئبة الحمراء ويمكن استخدامه كدالة لمتابعة أو قياس درجة النشاط المرضي لهذا المرض.