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Serum amyloid a protein level, and its significance in systemic lupus erythematosus patients

Samia M Esmat, MD; Hanan E EL-Sherif, MD **; Somaya Anwar, MD; Sahar Abdel-Atty, MD***; Hanan A Abdel-Reheim, MD******

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Departments of Dermatology*, Rheumatology and Rehabilitation**, Chemical and Clinical Pathology***and Internal Medicine****, Faculty of Medicine, Cairo University, Cairo, Egypt.

khaled1@soficom.com.

Abstract

Objective: To investigate The level of serum amyloid A protein (SAA) in systemic lupus erythematosus (SLE) patients and its importance in disease activity.

Materials and Methods: Forty- two female patients who satisfied four or more of the revised ACR criteria for SLE were included in this study. Fifteen healthy female subjects matched for age were included as controls. Disease activity was assessed by the systemic lupus activity measurement (SLAM) index. Serum amyloid A protein was measured by the particle enhanced nephelometry technique.

Results: Serum amyloid A protein (SAA) levels in SLE patients (95.27±100.62 mg/l) were higher than in the controls (4.08+1.14 mg/l) and the difference was statistically significant. Correlation between the SAA level and some of the disease parameters revealed a statistically significant positive correlation with the SLAM index score, prednisone dose and C-reactive protein. No significant correlation was found with age, disease duration, C3, C4, total leucocytic count, ESR or hemoglobin. Serum AA was significantly higher among patients with lupus

nephritis

Conclusion: Raised levels of SAA may indicate disease activity as well as lupus nephritis in SLE patients but can't be used for monitoring of responses in patients receiving systemic corticosteroid therapy.

Introduction

Serum amyloid A (SAA), is a putative precursor molecule of amyloid-A (AA) protein, formed by proteolytic cleavage by macrophage or polymorph proteinases[1].

It acts as an acute phase reactant, its concentration increases by up to 1000-fold during inflammation, largely owing to cytokine-driven transcriptional upregulation[2].

SAA can be used as a marker of inflammation in some autoimmune disorders such as rheumatoid arthritis, and vasculitis [3] It has a number of immunomodulatory roles. It can induce chemotaxis and adhesion molecule expression and has cytokine-like properties. SAA was found to promote the upregulation of metalloproteinases [4] and to induce collagenase production, providing a means of remodeling the extracellular matrix in areas of inflammation [5].

SAA transiently binds high density lipoproteins-3- (HDL3)to macrophages during an inflammatory response. In this way, it can mediate the delivery of lipids to sites of injury for use in tissue regeneration. However, in chronic inflammatory conditions, persistently high levels of SAA may compromise normal cholesterol transport and contribute to the development of atherosclerosis [6]. Binding sites on the SAA protein for calcium, laminin, and heparin /heparan-sulfate are described as well indicating its ability to affect cell adhesion, migration, proliferation and aggregation[7].

SAA is therefore involved in various physiological and pathological processes, including inflammation, atherosclerosis and thrombosis [8]. The role of elevated levels of SAA over time in predisposision to secondary amyloidosis is debatable[9].

Acute phase serum amyloid A has been reported to be more sensitive than C-reactive protein (CRP) as a marker of disease activity in rheumatoid arthritis [10]. In this study the profile of SAA in systemic lupus erythematosus patients was studied in

relation to clinical manifestations and disease activity.

Methods

A. Patients:

Forty two patients with systemic lupus erythematosus (SLE), attending the Rheumatology and Rehabilitation, internal medicine and dermatology outpatient clinics of Cairo University Hospitals were included in this work. Their ages ranged from 20 to 47 years and the disease duration from 0.25 to 15 years. All patients satisfied 4 or more of the revised American College of Rheumatology (ACR) criteria for classification of SLE [11, 12]. Fifteen healthy female subjects with matched ages served as controls.

B. Clinical assessment:

Full history taking, clinical examination and laboratory investigations were tabulated in accordance to the systemic lupus activity measurement (SLAM) index [13]. This index covers the symptoms that occurred over the previous month and includes 24 clinical and 8 laboratory variables. In addition serum ANA, anti-n-DNA, serum complement “C3 and C4” and C-reactive protein were also assessed.
Patients with obvious bacterial infections and impaired renal functions were excluded as serum amyloid A was reported to increase in these conditions [14], [15].

C. Assessment of serum amyloid A:

- Collection of samples: 5 ml venous samples were withdrawn from patients and controls and stored frozen at –200C.

- Determination of SAA was done by the particle-enhanced immunonephelometry method [16], on the Behring Nephelometer (BN II), Dade Behring Inc., N.Y., U.S.A.

Statistical Methods: Data were processed on a personal computer utilizing the SPSS® 13.0 for Windows® statistical package. Student’s t-test was used when appropriate. Two-tailed analysis with P value less than 0.05 was considered significant. Range, mean, and standard deviations are given. Correlation analysis was performed using Pearson’s correlation.

Results

The general characteristics and clinical features of the studied group are shown in table (1).
Thirty four (80.95%) of the patients were receiving oral steroid therapy. The dose ranged between 5 - 60 mg daily, with a mean of 18.9±12.6 mg /day, for at least 6 months before the time of study. Eight (19.05%) cases were new and hadn't received any treatment yet.
The serum amyloid A level of SLE patients ranged from 2.6- 532 mg/l with a mean of 95.27±100.62 mg/l, while those of the control group ranged from 2.8-5.9 mg/l with a mean of 4.08±1.14; the difference was statistically significant (P=0.0001), table (2).
Serum amyloid level in patients who hasn't received any treatment (mean= 60.34+99.35) were studied. It was significantly higher than controls (P= 0.043) but it was lower than in cases under steroid therapy (regardless their response to treatment) (98.29+ 100.74) and the difference was statistically significant (p=0.039) figure (1).
Correlation between the serum amyloid A level and some of the disease parameters revealed statistically significant positive correlation for the SLAM index score (r=0.377, P=0.02), prednisone dose (in cases under treatment) (r=0.344, P=0.04) and C-reactive protein (r=0.698, P=0.0001) table (3).
Serum amyloid A levels were compared in SLE patients with and without some clinical and laboratory parameters of the disease. The mean serum amyloid A was significantly higher among patients with nephritis (P=0.03), table (4).

Table (1): General characteristics and clinical features of SLE patients:	
Criteria	**SLE patients (N = 42)**
Age (years) **Age of onset** (years) **Disease duration** (years)	25.41±7.32 21.39±5.86 4.31±3.11
Constitutional: Fever Fatigue Weight loss	 25 (59.52%) 31 (73.81%) 29 (69.05%)
Mucocutaneous: Oral ulcers Malar rash / photosensitivity Discoid LE lesions **Alopecia:** Cicatricial Diffuse noncicatricial **Vasculitis:** **Hands:** Palmar erythema Erythema multiforme like lesions	 25 (59.52%) 34 (80.95%) 4 (9.52%) 26 (61.90%) 3 23 8 (19.05%) 34 (80.95%) 34 (80.95%) 21 (50%)
Joint: Arthralgia Arthritis	 38 (90.05%) 21 (50%)
Pulmonary	16 (38.1%)
Raynaud's phenomenon	14 (33.33%)
Carditis	7 (16.67%)
Hypertension	22 (52.38%)
Nephritis	17 (40.48%)
Myalgia/Myositis	8 (19.05%)
CNS affection	19 (45.24%)
Hematological: Leucopenia Thrombocytopenia Hemolytic anemia	 9 (21.43%) 5 (11.9%) -
Autoantibodies: ANA Anti-n-DNA	 40 (95.24%) 28 (66.67%)

Table (2): Comparison between mean serum amyloid-A level of SLE patients and control.			
	SLE patients N= 42	Control group N= 15	P
Mean±SD	95.27±100.62	4.08±1.14	0.0001*
* statistically significan P< 0.05			

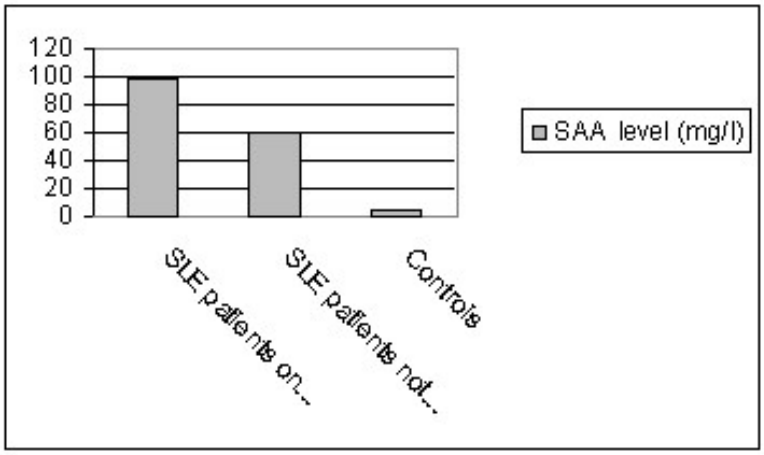
Table (3): Correlation between serum amyloid-A and some of the disease parameters in SLE patients.		
	r	P
Age	-0.221	0.208
Disease duration	-0.093	0.601
SLAM score	0.377	0.02*
ESR	0.297	0.088
HB	-0.273	0.118
TLC	-0.008	0.966
C3	-0.226	0.2
C4	-0.043	0.809
C-reactive protein	0.698	0.0001*
Prednisone dose	0.344	0.04
* statistically significant P< 0.05. ESR=Erythrocyte sedimentation rate, r= Correlation coefficient, HB= Hemoglobin, TLC=Total leucocytic count		

Table (4): Comparison of mean serum amyloid-A according to the presence of some disease parameters in SLE patients.

	Present	Absent	P value
Fever	104.84±117.8	86.28±60.93	0.615
Oral ulcers	114.27±120.09	69.0±38.3	0.215
Raynaud's	105.89±148.02	94.65±72.26	0.766
Arthritis	104.75±131.13	91.02±52.28	0.698
Nephritis	138.18±128.08	66.79±58.94	0.03*
Serositis	106.52±78.39	91.79±117.16	0.679
CNS	91.28±74.39	104.52±121.37	0.708
Anti-n-DNA	79.39±73.28	125.29±128.76	0.195
Discoid LE	106.54±64.67	117.35±101.55	0.164
Malar rash	82.63± 68.43	105.23±97.36	0.561

* statistically significant P< 0.05

Figure 1: Effect of systemic steroids on SAA levels in SLE patients:



Discussion

The mean serum amyloid A in systemic lupus erythematosus patients was significantly higher than the control group. This suggests that SAA might be related to the disease process in SLE. Other workers have reported that SAA levels are greatly elevated in rheumatoid arthritis [8, 17]and correlated significantly with disease activity. In SLE some authors reported high SAA levels in patients with active disease but lower than those seen in RA

patients [18]. Others found that SAA levels in patients with SLE were only modestly raised, even in those with severe active disease, unless significant intercurrent microbial infection was also present [19]. However SLE patients with obvious infections were not included in the current study.

When SAA levels where studied in relation to disease activity parameters in the studied group, SAA showed a significant positive correlation with the SLAM index score, C-reactive protein and insignificantly correlated to the ESR. This significant correlation indicates that disease activity in SLE is associated with raised SAA levels. This finding could suggest that SAA may be useful as a disease activity marker in patients with SLE.
A strong correlation between SAA and C-reactive protein in patients with SLE and other dermatoses was reported by other authors [20]. SAA and C-reactive protein are under the influence of different cytokines; CRP is predominantly stimulated by interleukin-6 (IL-6) [21], while SAA responds preferentially to IL-1 [22], but requires the synergistic action of both cytokines for maximal stimulation [23]. A selective response in the liver of differing degrees of synthesis and degradation may favor one acute phase protein over another in different pathophysiological circumstances [24].

The failure to detect a significant correlation between the SAA level and the ESR may be attributed to the fact that ESR is only an indirect measure of disease activity and reflects mainly fibrinogen levels. Furthermore, changes in the ESR occur slowly and are influenced by factors such as anemia, the size and shape of red blood cells, lipid levels, and hypergammaglobulinemia [8]. In this study no correlation could be detected between SAA levels and the presence of arthritis. When compared to ESR or C-reactive protein SAA is considered the best marker available for the assessment of inflammatory joint disease in rheumatoid arthritis and ankylosing spondylitis [4, 8, 25]. However according to our findings, this seems not to be the case for joint inflammation associated with SLE.
Another interesting point of view is the described role of plasma SAA as a precursor of Amyloid A (AA) protein in secondary amyloidosis an exceptionally rare complication in SLE [19]. The acute phase response involves a major rearrangement of plasma protein synthesis by the liver through increased production of some proteins and reduced levels of others [24]. It is thought that the function of this reaction is to confine the source of inflammation and limit autolytic damage by phagocytic cells [26]. However, in chronic disease, the continued presence of these proteins may exacerbate the inflammatory process, directly result in deposition of amyloid fibrils that contribute to tissue damage [18]. SAA is a serum precusor of amyloid A protein, the fibrillar component in reactive amyloid deposits [27]. Compared to rheumatoid arthritis,

secondary amyloidosis is considered a rare complication of SLE [19]. However in one study it was detected in 7% of SLE patients namely, those with long standing disease or those with long course of immunosuppresive therapy [28].

High levels of SAA were found to correspond with the incidence of reactive systemic amyloidosis in SLE and other inflammatory diseases [19]. Autoantibodies to amyloid A protein were demonstrated in one third of SLE cases but their presence was not significantly associated with the development of secondary amyloidosis [29]. Recent studies showed that high concentration of SAA is not sufficient for the development of amyloidosis and that genetic susceptibility through polymorphism of the SAA gene is an important back ground of amyloidogenesis [30, 31]. Those susceptible patients with high risk alleles (SAA 1.5) may be liable to develop reactive amyloidosis [32].

The present study implied a positively significant correlation of SAA levels in SLE patients under treatment, with the dose of prednisone they were receiving. This might be due to the fact that more severe cases required higher doses of prednisone. On the other hand corticosteroid hormones including dexamethasone, corticosterone, hydrocortisone, and aldosterone may be involved in the upregulation of SAA mRNA expression and thereby increase SAA production [33]. They may affect the synthesis of this protein through altering the production of several cytokines as IL-1, IL-6 [34, 35]. Therefore if elevation of SAA levels is implicated in in the development of amyloidosis, the propriety of using corticosteroid treatment to the patients at risk should be considered [31].

In this study patients who hasn't received treatment had significantly higher levels of SAA compared to normal controls. These levels were signifcantly lower when compared to those who received treatment regardless their response to treatment. This would support the explanation of the positive correlation between the SAA level and prednisone dose received, is secondary to the stimulatory effect of systemic steroid therapy on SAA. This finiding may partially negate its value in monitoring the response to treatment.
In lupus, profound activation of cytokine production and the acute phase response have been reported to be associated with a markedly increased risk for the development of atherosclerosis[36]. Moreover, extrahepatic production of the SAA (SAA1 and SAA2 protein isoforms) in a number of atherosclerotic lesions including endothelial cells, cultured smooth muscle cells and monocyte-macrophage cell lines has been reported [33]. In a previous study, women with longer duration and a higher cumulative dose of of prednisone use as well as those with prior coronary events were more likely to have carotid atheromatous plaques [37]. High levels

of SAA in cases receiving high doses of systemic corticosteroid may be behind the high incidence of atherosclerosis in those cases [33].

As regards to the clinical manifestations, in our study the mean SAA level was significantly higher among patients with lupus nephritis. A significant relationship between elevated levels of SAA and renal disease was reported [38] to be due to the presence of inflammation, as evidenced by increased levels of specific cytokines [35].

Conclusions:

We could therefore conclude that elevated SAA level is a marker that reflects disease activity in SLE patients especially in cases with nephritis. Since SAA level correlates positively with corticosteroid doses received, it cannot be used for monitoring the response of treatment in patients receiving this medication.

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