Concentration and microheterogeneity of acute-phase glycoproteins in patients with Systemic Lupus Erythematosus

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Abstract

Purpose: **To determinate glycosylation of selected acute-phase glycoproteins (AGP, ACT, CP) and serum concentration of this proteins in Systemic Lupus Erythematosus (SLE) patients.**

Patients and methods: **The study was carried out on 35 patients with active SLE and 15 healthy volunteers. The immunological measurements were performed at first day of hospitalisation, before receiving treatment. The concentration of CRP, AGP, ACT and CP were evaluated by electroimmunoassay using anti-AGP, anti-ACT, anti-CP antibodies. CRP levels were determined by radial immunodiffusion with anti-CRP antibodies. The microheterogeneity of the acute phase proteins was assessed by agarose affinity electrophoresis using Con A as a ligand, as was described by Bøg-Hansen.**

Results: **Between SLE patients and control group statistically significant differences ($p<0.01$) were observed in serum concentration of all investigated parameters. There were no significant differences in serum acute-phase proteins levels with regards to patient's age, sex and disease activity. The reactivity coefficients: AGP-RC, ACT-RC, CP-RC in SLE patients were similar to the healthy group. The precipitate curves were similar in both groups. The main difference was in the area of the precipitant, which was bigger in the SLE patients.**

Conclusions: **Configuration of analysis serum concentration and heterogeneity of acute-phase proteins is one of important diagnostic tests in SLE.**

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Abbreviations: SLE – Systemic Lupus Erythemetosus, APP – acute-phase proteins, AGP – α1-acid glycoproteins, ACT – antichymotripsin, CP – ceruloplasmin, CRP – C-reactive protein, RC – reactivity coefficient, Con A – concanavalin A, IL-interleukin.

Introduction

The concentration and microheterogeneity of acute-phase proteins (APP) differs in acute and chronic types of inflammation [1,2]. The qualitative changes of some acute-phase glycoproteins are referred as a major microheterogeneity. Affinity electrophoresis with a lectin, concanavalin A (Con A) as a ligand has been successfully used to determine acute-phase glycoproteins microheterogeneity. The concentration and microheterogeneity of acute-phase proteins can be used in early diagnosis, management and prognosis of chronic inflammatory stages.

Systemic Lupus Erythematosus (SLE) is a chronic autoimmunological inflammation and is the most clinically and serologically diverse of the autoimmune connective tissue diseases; it may affects any organ of the body and displays a broad spectrum of clinical and immunological manifestations. In other chronic inflammatory disease, like in rheumatoid arthritis, ancylosing spondylitis, polymyalgia rheumatica and Crohn's disease a significantly decreased proportion of acute phase proteins reacting with Con A were observed.

In the present study, we evaluated the determination of concentration and microheterogeneity of α1-acid glycoprotein (AGP), antichymtripsin (ACT), ceruloplasmin (CP) and C-reactive protein (CRP) in early diagnosing of SLE. In order to get a better insight into acute-phase proteins network regulation in Systemic Lupus Erythematosus, we analysed levels of this proteins in the sera from 35 SLE patients.

Table 1. **Characteristics of SLE patients**

Patients number	35	
Sex (M:F)	3:32	
Median age in years (range)	34.7 (21–50)	
Median duration of disease in years (range)	6.9 (0.5-28.0)	
Clinical manifestations	Arthritis	13
	Skin manifestation	20
	Serositis	7
	Fever	15
Positive antinuclear antibodies (ANA)	35	
Haematological symptoms	Anaemia	25
	Leukopenia	27
	Thrombocytopenia	17

Table 2. **Medium serum levels of acute phase proteins and medium reactivity coefficient in the SLE patients and healthy individuals**

	SLE patients	Healthy individuals
Median serum levels of AGP (ng/ml)	1234.0 (967.7-1615.7)*	623.7 (432.1-678.9)
RC-AGP	1.29 (0.976-1.361)	1.02 (0.876-1.298)
Median serum levels of ACT(ng/ml)	669.0 (578.0-781.0)*	221.6 (132.8-265.8)
RC- ACT	3.98 (3.12- 4.54)	4.25 (4.01-4.87)
Median serum levels of CP (ng/ml)	538.0 (481.0-589.0)*	267.7 (198.4-311.7)
RC- CP	1.298 (0.87-2.43)	1.4 (0.76-2.2)
Median serum levels of CRP (ng/ml)	21.7 (11.6-41.0)*	7.1 (2.9-13.5)

* p value statistically significant

Patients and methods

The study included 35 patients with active SLE. They had been hospitalised in The Department of Rheumatology and Internal Diseases, Medical University in Białystok from March 2001 to June 2003. All patients fulfilled The American Rheumatism Association revised criteria for classification of SLE [3]. The activity of the SLE was graded at the time of blood sampling, based on SLEDAI criteria [4]. The clinical profile of the patients is presented in the *Tab. 1*.

The control group consisted of 15 healthy individuals (4 males, 11 females) with mean age of 42.3 years. There were no significant differences in age and sex between the patients and the controls.

Serum AGP, ACT, CP levels were measured by electroimmunoassay using anti-AGP, anti-ACT, anti-CP antibodies [5]. CRP levels were determined by radial immunodiffusion with anti-CRP antibodies.

The microheterogeneity of the acute-phase proteins was assessed by agarose affinity electrophoresis using Con A as a ligand, as was described by Bøg-Hansen [6]. 50 μM Con A was included in the first dimension gel. Electrophoresis was carried out for 60-70 minutes, at 20 V/cm. Two gel adjacent of the first dimension gel, one containing specific antibodies, and the other containing methyl-mannosid, were cast. Electrophoresis of second dimension was carried out for 18 hours at 1.5 V/cm. The gel was dried and stained by Commasie brillant blue. This method reveals 4 microheterogeneous variants of AGP, ACT and CP. Variant 0 is nonreactive with ConA, variant 1st is slightly reactive, variant 2nd – strongly reactive, 3rd – very strongly reactive, often precipitated in the first dimension. The area under the precipitate curves was determinated planimetry and the reactivity coefficient (RC) was calculated according to formula:

$$RC = \frac{\text{Sum of Con reactive variants}}{\text{Sum of Con nonreactive variants}}$$

All blood samples were collected from each patient before receiving treatment. Serum samples were stored at -80°C until assayed.

Statystical analysis

Data were presented as mean ± 1 SD or median (range), depending on distribution provided by Shapiro test. Data for concentration in serum acute-phase proteins from healthy individuals and patients with SLE were analysed using Student's T-test. In the case of skeweew distribution we used Mann-Whitney's test. Differences between group were regarded as statistically significant at $p<0.05$.

Results

α1-acid glycoprotein (AGP)

Serum levels of AGP in SLE patients with are shown in *Tab. 2*. AGP level in all patients with SLE were very high and were significantly higher than in healthy population ($p<0.001$). There were no significant differences in serum AGP levels with regards to patients age, sex and disease activity ($p=0.52$). The median reactivity coefficient (AGP-RC) was 1.29 (1.361-0.9760). AGP-RC was similar like in healthy individuals ($p=0.321$). The precipitate curves were similar in both groups. The main difference was in the area of the precipitant, which was bigger in the SLE patients (*Fig. 1*).

Antichymotripsin (ACT)

The median levels of ACT in sera patients with SLE were 669.0 ng/ml (578.0-781.0 ng/ml) and were significantly higher compared with those in healthy individuals ($p<0.001$). There were no significant differences in serum AGP levels with regards to patients age, sex and disease activity ($p=0.312$).

The median reactivity coefficient (ACT-RC) in SLE patients was 3.98 (3.12-4.54) and was similar to ACT-RC in healthy group ($p=0.123$) (*Fig. 2*). The precipitate curves were similar in both groups. The main difference was in the area of the precipitant, which was bigger in the SLE patients. The picture was corresponded to the AGP curves.

Figure 1. **Affinity electrophoresis, using concanavalin A of AGP in the healthy group (1) and SLE patient (2)**

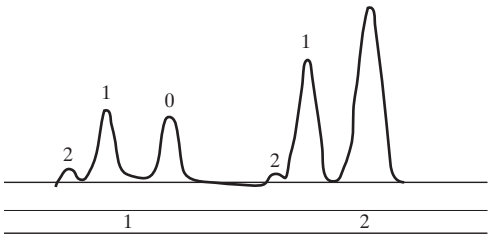
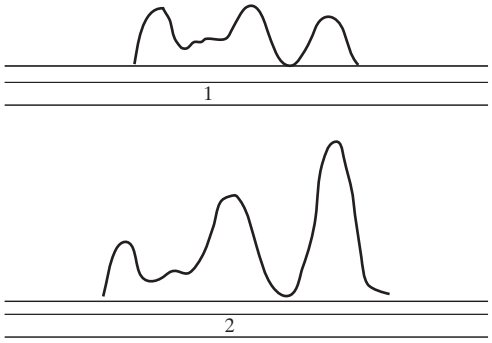


Figure 2. **Affinity electophoresis, using concanavalin A of ACT in the healthy group (1) and SLE patients (2)**



Ceruloplasmin (CP)

The median levels of CP in sera patients with SLE were 538.0 ng/ml (481.0-589.00 ng/ml). They were almost two times higher than in control group ($p<0.001$). There were no significant differences in serum CP levels with regards to patients age, sex and disease activity ($p=0.52$).

The median reactivity coefficient (CP-RC) in SLE patients was 1.298 (0.87-2.43) and was similar to CP-RC in healthy group ($p=0.123$). The precipitate curves were similar in both groups. The main difference was in the area of the precipitant, which was bigger in the SLE patients. The picture was corresponded to the AGP and ACT curves.

C-reactive protein

The median levels of CRP in sera patients with SLE were 21.7 ng/ml (11.6-41.0 ng/ml). It was almost three times higher than in control group ($p<0.001$).

Discussion

Values of concentrations of acute-phase protein and decrease values of reactivity coefficient were previously found in chronic inflammatory processes. This parameter can be also useful in diagnostic and management of SLE [7]. Changes observed in sera of SLE patients were similar to healthy individuals. Reactivity coefficients of AGP, ACT and CP were normal, compared with results using sera control group. SLE activity did not alter the reactivity with Con A in sera SLE patients. Although concentrations of AGP, ACT, CP increase significantly. There was one exception – C-reactive protein. Generally levels of serum CRP were normal or only slightly higher than in control group. All these above featured changes are very typical for SLE. Some authors suggested that elevated levels of CRP might serve as a marker for infection in SLE [8]. However, the usefulness of this measure has been controversy.

Therefore, it seems likely that the reactivity of proteins with Con A depends on regulatory mechanisms of inflammatory reaction than on the disease itself. Still unexplained is a lack of change and normal value of RC in active SLE. Unchanged glycosylation pattern in this disease may be explained by the very low stimulation of hepatic synthesis of proteins. The expression of this is very low C-reactive protein level. In the other hand levels of AGP, ACT, CP were very high. This observation suggests that glycosylation is differently regulated than synthesis of the acute-phase proteins. The main role in the regulatory process has probably cytokines.

Baumann suggested that there are two main groups of cytokines, which influence on synthesis and action of acute-phase proteins [10]. Type I cytokines (IL-1α, β and TNF-α) stimulate the synthesis of such proteins like CRP, Serum Amyloid A, AGP. Macrophages and monocytes release these cytokines. They can also decrease synthesis of other proteins like CP, ACT. Type II cytokines (interleukin-6, interleukin-11, leukemia inhibitory factor) stimulate the second group of acute-phase proteins, like fibrinogen, ACT, CP [11].

Quantification of the serum IL-10 level showed increased levels in SLE and RA patients as compared to healthy controls. Serum IL-6 level was found to be elevated in SLE patients. IL-10 has a potent immunosuppressive activity, IL-10 did not correlate with APP either in SLE patients. However, the elevation of IL-10 serum levels in SLE and the correlation between IL-10 and IL-6 in SLE may suggest that IL-10 may play a central role in regulation of synthesis acute-phase proteins [12].

It has been reported that the antibodies to modified CRP were present in some autoimmunological disease. These antibodies represent a novel group of autoantibodies, first described in one patient with SLE and later in patients suffering from toxic oil syndrome (TOS). The occurrence of antibodies to CRP and to other acute-phase proteins in a larger group of patients with SLE and other autoimmune diseases were demonstrated [13]. Patients with SLE have a high incidence of antibodies to acute-phase proteins, preferentially to CRP.

The diagnosis and management of SLE is still predominately based on clinical parameters. Nevertheless, specific laboratory diagnostic test may often be critically helpful. Configuration of analysis serum concentration and heterogeneity of acute-phase proteins is one of important diagnostic test in SLE.

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