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Serum Transferrin Isoformsin Systemic Lupus Erythematosus (SLE) and Systemic Sclerosis (Ssc)

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Abstract

Objectives: The aim of the study was to determine the serum profile of transferrin isoformsin SLE and SSc as potential biomarkers for the diagnosis and differentiation of diseases.

Methods: The study was carried out in 38 patients with SLE and 43 patients with SSc. Transferrin isoforms were analyzed by capillary electrophoresis method.

Results: The concentration of trisialotransferrin and pentasialotransferrin were significantly lower than that in the controls but tetrasialotransferrin significantly increased, both in SLE and SSc. The level of pentasialotransferrinin SLE was significantly higher than in SSc patients.

Conclusion: The serum profile of transferrin isoforms alters in SLE and SSc. Among the isoforms pentasialotransferrin might be useful as biomarkers of differentiation between diseases.

Keywords: Transferrin isoforms; Systemic lupus erythematosus; Systemic sclerosis

Introduction

The Systemic Lupus Erythematosus (SLE) and Sclerosis (SSc) are a chronic autoimmune diseases attacking connective tissue in particular [1,2].The etiology of SLE and SSc are not clear, but both are accompanied by chronic inflammation. With reference to it the changes in the concentration of acute phase protein such as C - Reactive Protein (CRP), Transferrin (TRF), Haptoglobin (Hp) can be observed. Because of acute phase proteins are mostly the glycoproteins, the alterations in the glycosylation of these proteins can occur [3,4]. The most commonly studied and described change in rheumatic diseases is defect of Immunoglobulin G (IgG) [5,6].There are evidences for the presence of IgG isoforms with different numbers of galactose residues which as a result increases the percentage share of agalactosylisoforms of IgG. The alterations in glycosylation of some glycoproteins such as fibronectin, α1-acid glycoprotein, haptoglobin and transferrin can be also related with the progress of systemic lupus erythematosus [7].To carry out a study we chose transferrin because its structure shows high variability depending on the number of sialic acid residues attached to the carbohydrate chains [8]. On the other hand, protein glycosylation seems to play a major role in the pathogenesis of autoimmune diseases. So, the identification of glycosylation alterations may lead to a better understanding of SLE and SSc pathogenesis, and might permit to evaluation of biological activity of disease. Early diagnosis and treatment play a crucial role in improving the life quality of SLE and SSc patients. Based on evidence that the distribution of transferrin isoforms significantly differs between diseases the purpose of this study was to compare of serum profile of transferrin isoforms in systemic lupus erythematosus and systemic sclerosis.

Materials and Methods

Subjects

The study was carried out on the sera of 62 patients (mean age: 46 years, range: 19-71) admitted to the Department of Rheumatology and Internal Diseases in University Hospital of Bialystok and included 38 patients with Systemic Lupus Erythematosus (SLE) (mean age: 38 years, range: 23-70) and 43 patients with Systemic Sclerosis (SSc) (mean age: 46 years, range: 19-71). The duration of SLE was from 1 year to 21 years and SSc from 3 months to 15 years. The diagnosis was made on the basis of criteria given by the American College of Rheumatology [9,10]. The patients were taking Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) and Disease-Modifying Anti-Rheumatic Drugs (DMARDs) such as methotrexate and sulfasalazine. The sera of 30 healthy subjects were assayed as a control group. Blood samples were collected from each patient once on admission from a peripheral vein after 12h of fasting. The study was in accordance with the Helsinki Declaration and was approved by the Bioethical Committee at the Medical University of Bialystok (No. of approval R-I-002/399/2017). All subjects (healthy and sick) provided informed consent to participate in the studies.

Methods

The determination of Hemoglobin concentration (Hb) and Platelet Count (PLT) were performed on Sysmex XS-800i (Sysmex Corporation, Singapore). The concentration of C-reactive protein and anti-Citrullinated Protein Antibodies (anti-CCP) were determined on the Architect ci8200 (Abbott Laboratories, Abbott Park, IL, USA). Erythrocyte Sedimentation Rate (ESR) was measured by Westergren method on the Sediplus S 2000 (Sarstedt, Germany). The determination of transferrin isoforms was performed by the Capillary Electrophoresis (CE) on a MINICAP electrophoretic

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Table 1: The results of laboratory tests in patients with SLE and SSc and controls.

	ESR (mm)/h	Hb (g/L)	PLT (10^3/μL)	CRP (mg/L)	Anti-CCP (U/mL)
Controls (N = 30)	5.5 (4-8)	13.7 (11.3-15.5)	235 (166 - 386)	0.65 (0.30-3.20)	0.5 (0.5-1.3)
SSc (N = 43)	23 (2 -100) P<0.001	12.35 (8.5-14.8) P<0.001	255 (107-561) P=0.051	2.3 (0.2-306.1) P<0.001	0.5 (0.5-230) P=0.079
SLE (N = 19)	30 (10-100) P<0.001	12.3 (8.8-13.6) P<0.001	228 (97-588) P=0.822	3.85 (0.20-56.5) P<0.001	0.5 (0.5 -1.4) P=0.674

SSc: Systemic Sclerosis; SLE: Systemic Lupus Erythematosus; ESR: Erythrocyte Sedimentation Rate; Hb: Hemoglobin; PLT: Platelets Count; CRP: C Reactive Protein; Anti-CCP: Auto Anti bodies to Cyclic Citrullinated Peptide.
Data are median and ranges. The differences between tested groups and controls were estimated by Mann-Whitney U-test.
P<0.05 significant difference

Table 2: The serum concentration of total transferrin and transferrin isoforms in SLE and SSc.

	Total transferrin [g/L]	Disialotransferrin [%]	Trisialotransferrin [%]	Tetrasialotransferrin [%]	Pentasialotransferrin [%]
Control group (N= 30)	2.88 2.27-3.84	0.6 0.3-5.4	3.65 1.6-5.6	78.15 65-84.7	17.2 11.1-32.8
SSc (N = 43)	2.10* 1.04-3.07	0.55 0.2 - 2.1	2.3* 0.1 - 13.6	84.4* 76.5 - 89.6	12.6* 0.7-21.1
SLE (N = 19)	1.73* 0.62-2.46	0.7 0.2 - 1.0	2.0* 0.2 - 4.4	82.8* 77.4 - 86.5	14.5* 11.2-21.6
	P=0.001	P=0.187	P=0.164	P=0.101	P=0.014

SSc: Systemic Sclerosis; SLE: Systemic Lupus Erythematosus
Data are median and ranges. The differences between tested groups and controls were estimated by Mann-Whitney U-test.
*significant differences in comparison with the control group.

system using the MINICAP CDT reagent kit (Sebia, Evry, France). With this technique, charged molecules are separated by their electrophoretic mobility in an alkaline buffer with a specific pH 8.8. The human serum transferrin isoforms are detected as a five major fraction in the following order: asialotransferrin, disialotransferrin, trisialotransferrin, tetrasialotransferrin and pentasialotransferrin.

Statistical analysis

The results were expressed as a median and range. The differences between tested groups and controls and between rheumatic diseases (SLE and SSc) were evaluated using the Mann-Whitney U test. The Sperman's rank correlation coefficient was used to assess the correlation between transferrin isoforms and basic laboratory tests. The differences were considered statistically significant at P<0.05.

Results

Table 1 presents the results of laboratory tests for patients with SLE, SSc and for the control group. The mean levels of CRP and ESR were found to be significantly higher and Hb concentration significantly lower in patients with SLE and SSc than that in controls (P<0.001 for all comparisons), whereas the mean level of PLT and anti-CCP did not differ between rheumatic diseases(SLE and SSc) and control group (P>0.05 for all comparisons).There were no significantly differences between SLE and SSc patients for all examined laboratory tests (P>0.05 for all).The total serum transferrin concentration and distribution of transferrin isoforms in patients with systemic lupus erythematosus and systemic sclerosis are presented in Table 2. The serum transferrin concentration was significantly lower in patients with SLE and SSc when compared with the control group (P<0.001 for both comparisons). There were no differences in disialotransferrin concentration in the study groups (SLE and SSc) when compared to the controls (P=0.054, P=0.691; respectively). The relative concentrations of trisialotransferrin and pentasialotransferrin were significantly lower in SLE patients (P<0.001, P=0.042; respectively) and SSc(P=0.001, P<0.001; respectively) than that in the controls, whereas the relative concentration of tetrasialotransferrin was significantly elevated in both study groups (P<0.001 for both comparisons).The relative concentration of pentasialotransferrin was significantly higher in patients with SLE than in patients with SSc (P=0.014).No significant differences between SSc and SLE were found in disialotransferrin, trisialotransferrin and tetrasialotransferrin (P>0.05 for all comparisons).There were negative correlation between ESR and trisialotransferrin both in SLE and SSc patients (R=-0.346, P=0.025; R=-0.598, P=0.007; respectively). Moreover, the concentration of CRP in systemic sclerosis correlated negatively with trisialotransferrin (R=-0.469, P=0.003). In systemic lupus erythematosus there were positive correlation between hemoglobin concentration and trisialotransferrin(R=0.560, P=0.013).

Discussion

The systemic lupus erythematosus and systemic sclerosis are auto immune disorders affecting multiple organ systems. The pathogenesis of these diseases are often unknown, so the clinical course of these diseases is varied and difficult to predict. Moreover, it has been shown that SLE and SSc are chronic inflammatory diseases with the alterations in the concentration and glycosylation of plasma acute-phase proteins [4,7]. The diagnosis of SLE and SSc is still predominately based on the clinical parameters [9,10]. However the specific laboratory diagnostic test may often be critically helpful. In the present study, we evaluated and compared the profile of transferrin isoforms in systemic lupus erythematosus and systemic sclerosis. We have noticed the differences in total transferrin concentration and the shifts in the glycosylation profile of transferrin isoforms in systemic sclerosis and systemic lupus erythematosus. We showed the reduction in mean serum concentration of total transferrin in patients with systemic lupus erythematosus and systemic sclerosis. A decreased level of transferrin, as a negative acute-phase protein, in these rheumatic diseases is mainly due to the accompanyingchronic

inflammatory process. But the major achievement we obtained in our study was the shift in the profile of transferrin isoforms in SLE and SSc. To separate the transferrin isoforms we used capillary electrophoresis technique, which is highly correlated with the HPLC consider as a reference method [11]. This technique allows to separate transferring for five individual fractions according to their sialylation level asialotransferrin, disialotransferrin, trisialotransferrin, tetrasialotransferrin and pentasialotransferrin. First of all, we have not detected the asialotransferrin in patients and in controls. Second, we did not observe differences in disialotransferring concentration. Thus, disialotransferrin does not have a practical significance in diagnosing of SLE and SSc. But we observed the shifts in other transferrin isoforms. The study revealed a significant decrease in trisialotransferrin and pentasialotransferrin and significant increase intetrasialotransferrin in patients with SLE and SSc. It's first study concerning the specific isoforms of transferrin in these rheumatic diseases. In earlier study we only demonstrated an increase in relative concentration of carbohydrate-deficient isoforms of transferrin in patients with rheumatoid arthritis and systemic sclerosis[4].Due to the facts that Carbohydrate-Deficient Transferrin(CDT) is a sum of asialo, monosialo and disialotransferin, and there were not changes in disialotransferrin levels and asialotransferrin was not visible in these patients, we suggest that only monosialotransferrin might be responsible for changes in CDT concentration. Because of relative concentration of pentasialotransferrin was significantly higher in patients with SLE than that in SSc patients, the determination of this isoform may be useful to differentiate SLE from SSc. Next, we analyze the correlation between basic laboratory tests and transferrin isoforms. The ESR levels correlate negatively with trisialotransferrin concentration both in SLE and SSc patients. Also in SSc patients, CRP concentration correlated negatively with trisialotransferrin. These relationships may confirm the assumption that decrease of trisialotransferrin is probably associated with active immune response. Moreover, the decreased concentration of hemoglobin was parallel with decrease of trisialotransferrin in SLE patients. It's probably associated with anemia occurring in chronically ill persons.

Conclusion

The current study showed the changed serum profile of transferrin isoforms in SLE and SSc patients. Additionally, we can suggest a potential role of pentasialotransferrinin differentiation of rheumatic diseases. Our results indicate that the determination of transferrin isoforms may be potential biomarkers for diagnosis and differentiation SLE and SSc.

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