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Serum Prolidase Activity in Ankylosing Spondylitis and Rheumatoid Arthritis

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Abstract: The aim of the present study was to emphasize the collagen turnover in 2 of the most common chronic inflammatory rheumatic diseases by evaluating serum prolidase activity (SPA) in ankylosing spondylitis (AS) and rheumatoid arthritis (RA). 30 patients who met the modified New York Criteria for the classification of AS, 29 patients who met the 2010 Rheumatoid Arthritis Classification Criteria for the classification of RA, and 31 healthy controls were enrolled in the study. Serum samples of the patients and the controls were collected and SPA was measured by a spectrophotometric method. The comparison of the SPA in these 3 groups was statistically examined. In both patient groups, the SPA was lower than in the control group. SPA in patients with AS was statistically significantly lower than in the control and RA groups ($P < 0.001/P = 0.002$). No statistically significant difference was found between the RA and the control groups ($P = 0.891$). In conclusion, lower SPA is presumably associated with decreased collagen turnover and fibrosis, leading to decreased physical functions in both chronic inflammatory musculoskeletal diseases.

Keywords: ankylosing spondylitis, rheumatoid arthritis, serum prolidase activity

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Introduction

Ankylosing spondylitis (AS) and rheumatoid arthritis (RA) are common and severe chronic inflammatory musculoskeletal diseases with a high burden on society, especially since many patients are affected at a young age, and no cure for the diseases are available.¹ Based on the systemic inflammatory processes, severe alterations in bone turnover are more frequent in patients with rheumatoid arthritis (RA) and ankylosing spondylitis (AS).²

Prolidase is a cytosolic exopeptidase that destroys the imidodipeptide proline or hydroxyproline in the C-terminal. This enzyme is important in the last step of degradation in collagen metabolism and cell growth.³ Bone matrix consists of 90% type I collagen. 25% of type I collagen contains proline and hydroxyproline.⁴

The relationship between prolidase activity and collagen metabolism was defined in previous studies.⁵⁻⁷ Prolidase activity increases when the collagen cycle accelerates.^{8,9} Prolidase, through the regulation of the expression of growth factors and transcription factors, is important in many physiological and pathophysiological processes like wound healing, inflammation, and angiogenesis. SPA has rarely been studied in inflammatory rheumatic diseases.¹⁰

Considering all of this as a backdrop, we sought to measure serum prolidase activity in patients with either AS or RA, and in healthy controls, hypothesizing that SPA should measure differently in patients with inflammatory rheumatic disease than in the control group, because of the underlying abnormal collagen turnover and fibrosis.

Materials and Methods

This prospective, randomized clinical study included 30 patients with AS, 29 patients with RA and 31 healthy controls (HC). The study protocol conforms to the principles of the Declaration of Helsinki and was approved by the institutional ethics review board in Dicle University Hospital. Treatment protocols of patients have not been changed in this study. All patients and controls gave informed consent for the use of their clinical information and serum samples. Past medical history and current medications were recorded, in addition to detailed physical examination in all cases. AS and RA were determined according to the criteria of modified New York¹¹ and 2010 Rheumatoid Arthritis Classification Criteria,¹² respectively.

The age, sex, body mass index, and medications were noted. Blood samples were collected from all subjects. All patients' routine hematological and biochemical parameters were examined. We determined the prolidase levels of serum samples from the patients versus the controls.

The exclusion criteria were the existence of systemic diseases like hypo- or hyperthyroidism, diabetes mellitus and heart failure, history of acute or chronic infections, cerebrovascular disease, alcohol abuse, and the presence of abnormality in the biochemical analysis of blood. Patients who had infectious or endocrine related arthropathy, pregnancy, lactation, or clinically unstable medical illness were excluded. Control subjects consisted of healthy people without any clinical evidence of rheumatic disease or any other systemic disorders.

Measurement of serum prolidase activity

Peripheral venous blood samples were collected from the antecubital vein of patients who had remained supine for at least 15 minutes (min) without discontinuing drug treatment. Aliquots were stored at -80°C to allow analysis. Prolidase activity was determined by a method that determines proline levels produced by prolidase. The supernatant was diluted twofold with physiological serum. 25 microliters of the mixture were preincubated with 75 μL of the preincubation solution (50 mmol/L of a Tris HCl buffer of pH 7.0 containing 1 mmol/L of glutathione, and 50 mmol/L of MnCl_2) at 37°C for 30 min. The reaction mixture, which contained 144 mmol/L of gly-pro at pH 7.8 (100 μL), was incubated with 100 μL of the preincubated sample at 37°C for 5 min. To stop the incubation reaction, 1 mL of glacial acetic acid was added. After adding 300 μL of the Tris HCl buffer of pH 7.8, and 1 mL of ninhydrin solution (3 g/dL ninhydrin was melted in 0.5 mol/L of orthophosphoric acid), the mixture was incubated at 90°C for 20 min and then cooled with ice. Absorbance was then measured at a 515 nm wavelength to determine the proline value by the method proposed by Myara et al.¹³ Intra and interassay coefficients of variations of the assay were lower than 7%.

Statistical analysis

Outcome measures were analyzed using the SPSS package program, and data was shown as the mean \pm



standard deviation. An independent-Samples *t*-test was used to statistically compare the clinical details and results in the groups. The Chi-square test was used for categorical variables. A *P*-value of less than 0.05 was accepted as statistically significant.

Results

The average age of the AS patients (*n* = 30), RA patients (*n* = 29), and healthy controls (*n* = 31) was 40.1 years, 43.1 years, and 39.2 years, respectively. The clinical and laboratory characteristics of the groups are summarized in Table 1.

The demographic data of the patients and healthy controls showed homogeneity, and there were no significant differences in age, body mass index, or female/male ratios between the patients and the healthy controls. No statistically significant difference was found between the groups according to the biochemical parameters.

There were significant differences between AS patients and healthy controls with respect to prolidase activity. SPA was significantly lower in the patients with AS than those of the control subjects (*P* < 0.001). However, even though the SPA in RA patients was lower than that in the healthy controls, no statistically significant difference was seen (*P* = 0.891). We found statistically significant differences between the 2 patient groups. The mean of the serum prolidase levels in the patients with RA were found to be significantly higher than in the group of AS patients (*P* = 0.002). The results were summarized in Table 2.

Table 1. Baseline clinical and laboratory characteristics of the groups.

	AS	HC	RA
Age (years)	40.1 ± 5.71	39.2 ± 5.74	43.1 ± 9.5
<i>P</i> value*		0.539	0.061
Sex (F/M)	12/18	17/14	18/11
<i>P</i> value**		0.246	0.570
BMI (kg/m ²)	24.4 ± 3.8	24.5 ± 3.6	26.5 ± 4.5
<i>P</i> value*		0.983	0.057
ESR (seconds)	25.3 ± 23.3	29 ± 7.9	24.1 ± 11.7
<i>P</i> value*		0.402	0.064
CRP (mg/L)	3.7 ± 11.8	0.8 ± 0.1	1.2 ± 1.4
<i>P</i> value*		0.182	0.208

Notes: *Independent-samples *t* test; **Chi-square test.

Abbreviations: AS, Patients with ankylosing spondylitis; HC, Healthy controls; RA, Patients with rheumatoid arthritis; BMI, Body mass index; ESR, Erythrocyte sedimentation rate; CRP, C-reactive protein.

Table 2. Serum prolidase activity of the groups.

	AS	HC	<i>P</i> value*
SPA (U/L)	548 ± 195.7	912.6 ± 348.6	<0.001**
	RA	HC	
SPA (U/L)	896.4 ± 538.8	912.6 ± 348.6	0.891
	AS	RA	
SPA (U/L)	548 ± 195.7	896.4 ± 538.8	0.002**

Notes: *Independent-samples *t* test; **statistically significant.

Abbreviations: AS, Patients with ankylosing spondylitis; HC, Healthy controls; RA, Patients with rheumatoid arthritis; SPA, Serum prolidase activity.

Discussion

Prolidase has an important role in the cycle of collagen and has not yet been studied in rheumatic diseases in detail. Serum prolidase deficiency has been indicated as a rare etiology of arthritis by Marotte et al.¹⁰ Butbul et al has reported the deficiency of prolidase as associated with systemic lupus erythematosus.¹⁴ We evaluated the SPA in patients with AS and RA. Normal serum prolidase levels are below 1000 U/L. We found the prolidase levels of the healthy controls near this boundary. The SPA in patients with AS and RA were below the limit. This seems to be associated with the disorderliness of collagen turnover.

SPA in osteoarthritis and osteoporosis were studied previously with respect to musculoskeletal diseases.^{9,15,16} Altındag et al found that the SPA was significantly lower in patients with osteoarthritis than in the controls.⁹

Systemic osteoporosis is a common and heterogeneous complication in AS and RA.^{15,16} In Verit's study, it was observed that there was no statistically significant difference in SPA in postmenopausal osteoporotic women when compared with postmenopausal nonosteoporotic and premenopausal nonosteoporotic controls.¹⁷

The production of antibodies to IgG and to type I and type II collagen (CI and CII) was analyzed by enzyme-linked immunospot assays in patients with rheumatoid arthritis (RA) and patients with other inflammatory or degenerative joint diseases.¹⁸ In rheumatic diseases, low SPA shows the ineffective collagen turnover and poor quality of bone, probably leading to lower prolidase activity.¹⁶

RA is associated with the destruction and space narrowing of the joints, while AS is dominated by



bone formation. The causative relationship between inflammation and structural damage in RA is well established, while this relationship is largely unknown but certainly less strong in AS.¹⁹ Although the histological features of the synovial membranes of the peripheral joints in AS are similar to those of RA, intimal cell hypertrophy, fibroblastic proliferation, diffuse histiocyte and lymphocyte infiltration and the presence of fibrin were less marked in the joint tissues of AS than in joint tissues of definite RA. Decreased fibroblastic proliferation and intimal cell hypertrophy were observed in synovial tissues of AS compared with RA.²⁰ Statistically significant lower prolidase activity in AS patients compared with RA patients in our study is compatible with this evidence.

Some studies showed that collagen turnover appears to be positively correlated with degree of exercise.^{15,16} Lower prolidase activity in our study might in part be related to the reduced physical activity levels that these patients tend to exhibit.

In conclusion, lower SPA is presumably associated with the decreased collagen turnover and fibrosis related to decreased physical functions in both of these chronic inflammatory musculoskeletal diseases.

Author Contributions

Conceived and designed the experiments: DU, SE. Analyzed the data: DU, MB. Wrote the first draft of the manuscript: DU, PO. Contributed to the writing of the manuscript: DU, MÇ. Agree with manuscript results and conclusions: DU, KN. Jointly developed the structure and arguments for the paper: DU, HKY, OG. Made critical revisions and approved final version: DU, SE. All authors reviewed and approved of the final manuscript.

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Competing Interests

Author(s) disclose no potential conflicts of interest.

Disclosures and Ethics

As a requirement of publication the authors have provided signed confirmation of their compliance with ethical and legal obligations including but not limited to compliance with ICMJE authorship and competing interests guidelines, that the article is neither under

consideration for publication nor published elsewhere, of their compliance with legal and ethical guidelines concerning human and animal research participants (if applicable), and that permission has been obtained for reproduction of any copyrighted material. This article was subject to blind, independent, expert peer review. The reviewers reported no competing interests.

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