Levels of Pentraxin 3 and relationship with disease activity in patients with Ankylosing Spondylitis

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ABSTRACT

Objective: Ankylosing spondylitis is a chronic inflammatory disease of the sacroiliac joint and vertebral column. Pentraxin (PTX) 3 is an acute phase protein known to be associated with chronic inflammation. This study was performed to test the hypothesis that serum PTX3 levels might be elevated as a marker of inflammation in patients with ankylosing spondylitis. Material and methods: A total of 73 patients older than 20 years (48 males, 25 females, mean age 32.30 ± 6.40 years) were included. The ankylosing spondilitis group consisted of 46 patients (18 females, 28 males, mean age 33.30 ± 6.12 years) diagnosed with ankylosing spondilitis by the Modified New York Criteria, and the control group consisted of 27 healthy individuals (7 females, 20 males, mean age 30.59 ± 6.62 years). Groups were compared by demographic, anthropometric, biochemical data, and by serum PTX3 levels. The ankylosing spondilitis group was also divided into 2 subgroups (active or remission) by disease activity according to the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and compared by serum PTX3 levels. PTX3 was measured with the enzyme linked immunosorbent method. Results: PTX3 levels were higher in the ankylosing spondylitis group compared to the control group (0.29 ± 0.83 ng/mL vs. 0.09 ± 0.06 ng/mL, p=0.009). Levels of serum PTX3 were similar in groups with active and remitted ankylosing spondilitis (0.34 ± 0.99 ng/mL vs 0.37 ± 1.15 ng/mL, p>0.05). No correlation was determined between PTX3 and disease activity (p>0.05). Conclusion: These results are supportive of the hypothesis that levels of serum PTX 3 might be elevated in association with inflammation in patients with ankylosing spondylitis; however, results also demonstrate that there is no significant correlation with disease activity

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Keywords: Ankylosing spondylitis; Inflammation; Pentraxin 3

INTRODUCTION

Ankylosing spondylitis, the prototype disease in the spectrum of spondyloarthropathies, is a chronic inflammatory rheumatic disorder that primarily affects the axial skeleton, sacroiliac joints and entheses[1]. Inflammatory markers including c-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) have been demonstrated to be elevated in association with disease activity[2].

Pentraxin (PTX) 3 is a member of a complex superfamily of multifunctional proteins characterized with cyclic multimeric structure[3]. PTX family, known as acute phase reactants consist of CRP and serum amyloid protein known as short pentraxins, and PTX3 known as long pentraxin; and are involved in natural immunity and inflammation[4,5]. PTX3 has been reported to be elevated in relationship with inflammation in atherosclerotic cardiovascular diseases[6-9], and rheumatological diseases including systemic lupus erythematosus, rheumatoid arthritis and small vessel vasculitis[10-12].

This study was performed to test the hypothesis that serum PTX3 levels might be elevated as a marker of inflammation in patients with ankylosing spondylitis. On this purpose, serum PTX3 levels were compared in patients with ankylosing spondilitis and healthy controls and the relation was evaluated between PTX3 levels and disease activity. Additionally, patients with ankylosing spondilitis were divided in 2 subgroups as active and remission and serum PTX3 levels were compared.

MATERIALS AND METHODS

This study was performed with patients diagnosed

with ankylosing spondilitis by the Modified New York criteria at the Department of Internal Medicine and Rheumatology Clinics of Goztepe Training and Research Hospital of Istanbul Medeniyet University[13] and healthy controls referring for check-up purposes. Ethics committee approval (Dated 17.05.2012, Numbered 11/) and written informed consent of participants were obtained prior to the performance of any study procedures. Principles of the Declaration of Helsinki were followed throught the study.

STUDY INCLUSION CRITERIA FOR THE PATIENT GROUP

A diagnosis of ankylosing spondylitis by the Modified New York criteria.

STUDY INCLUSION CRITERIA FOR THE CONTROL GROUP

No history of medical diseases, presentation to the outpatient's clinic for check-up purposes, and receiving no medications.

STUDY EXCLUSION CRITERIA FOR THE PATIENT AND CONTROL GROUPS

Presence of acute infection, other rheumatological diseases in addition to ankylosing spondilitis, inflammatory diseases (inflammatory bowel disease etc.), severe hepatic, renal and cardiac disease, malignancy.

STUDY DESIGN

Demographic features, concomitant diseases, smoking and alcohol consumption habits, disease duration and localization, medications, family history, peripheral joint involvement and extraarticular signs were evaluated. Anthropometric and biochemical data were recorded. Detailed physical and locomotor system examinations were performed. Groups were compared by their demographic, anthropometric, biochemical data and serum PTX3 levels. Patients with ankylosing spondylitis were also divided into 2 subgroups (active and remission) and serum PTX3 levels were compared. A correlation analysis was performed to determine the relationship between PTX3 and disease activity.

CLINICAL EVALUATION METHODS IN PATIENTS WITH ANKYLOSING SPONDILITIS

Disease activity was evaluated with Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), functional state was evaluated with Bath Ankylosing Spondylitis Functional Index (BASFI), spinal mobility was evaluated with Bath Ankylosing Spondylitis Metrology Index (BASMI)[14], and ESR and CRP were evaluaetd as laboratory markers of inflammation.

ANTHROPOMETRIC MEASUREMENTS

Weight, waist circumference and height were measured by the same person using standard measurement devices. Body mass index (BMI) was calculated by dividing the weight in kilograms with square of height in meters (kg/m^2). Blood pressure was measured after at least 10 minutes of rest and at sitting position, using mercury syphygmomanometers evaluating the Korotkoff Phase I and Phase V voices in both arms. A second measurement was performed in the arm with higher blood pressure. At least 3 minutes of rest was permitted in between the measurements and arythmetic mean of systolic and diastolic pressures were calculated.

BIOCHEMICAL FEATURES

Biochemical daha (fasting plasma glucose, urea, creatinin, alanin aminotransferase), complete blood count, ESR and CRP values of the patients were recorded from their medical records. Venous blood samples were obtained following 8-12 hours of fasting to determine serum PTX3 levels. These blood samples were centrifuged and plasma were separated within a maxium of 60 minutes at 4000 rpm for 10 minutes. All samples were stored at -80C until the analysis. Plasma PTX3 measurement was performed at the biochemistry laboratory of the hospital with a commercial kit using the Enzyme-Linked ImmunoSorbent Assay (ELISA) method (Aviscera Bioscience Inc., USA). Intra and inter-assay coefficient of variations (CVs) of ELISA kit of PTX3 were 4-6% and 8-10%, respectively. Analytic sensitivity of the test was 0.02 ng/mL.

STATISTICAL ANALYSIS

Number Cruncher Statistical System (NCSS) 2007 & Power Analysis and Sample Size (PASS) 2008 Statistical Software (Utah, USA) was used to performed statistical analysis. Descriptice statistical methods (mean, standard deviation, median, frequency, rate) were performed in addition to Student t test in two group comparisons of quantitative parameters with normal distribution, and Mann Whitney U test in two group comparisons of parameters without normal distribution. One way analysis of variance (ANOVA) test was used in the comparison of three or more groups with normal distribution and Tukey Honestly Significant Difference (HSD) test was performed to determine the group

with difference; comparison of three or more groups without normal distribution was performed with the Kruskal Wallis test and Mann Whithey U test was performed to determine the group with difference. Pearson Chi-Square test, Fisher's Exact test and Yates Continuity Correction test were used to evaluate qualitative data. Spearmen's correlation analysis was used to determine the relationship between the parameters. P values of <0.05 were considered significant.

RESULTS

A total of 73 subjects (48 males, 25 females, mean age 32.30 ± 6.40 years) were included in the study. The ankylosing spondilitis group consisted of 46 patients (18 females, 28 males, mean age 33.30 ± 6.12 years), and control group consisted of 27 healthy individuals (7 females, 20 males, mean age 30.59 ± 6.62 years). The ankylosing spondilitis group was divided into subgroups by the BASDAI scoring. Twenty six patients with a BASDAI score of ≥ 4 constituted the active disease group and 20 patients with a BASDAI score of < 4 constituted the remission group.

Clinical features of the groups are demonstrated in Table I. Age and sex demographics were similar between the groups. BMI (p=0.004), alanine aminotransferase (ALT) (p=0.041), white blood cell count (p=0.001), thrombocyte (p=0.001), ESR (p=0.001) and CRP (p=0.001) were higher, whereas hemoglobin levels were lower (p=0.006) in the ankylosing spondilitis group compared to the control group. Serum PTX3 levels were significantly higher in the ankylosing spondilitis group compared to the control group (0.29 ± 0.83 ng/mL vs. 0.09 ± 0.06 ng/mL, p=0.006) (Figure 1).

Serum PTX3 levels were higher in the groups with active and remission disease compared to the controls (0.34 ± 0.99 ng/mL and 0.37 ± 1.15 ng/mL vs. 0.09 ± 0.06, respectively, p= 0.041). Levels of serum PTX3 were similar in groups with active and remitted ankylosing spondilitis.

Clinical and treatment features of patients in active and remitted ankylosing spondylitis are presented in Table II. Mean rates of lumbalgia (p=0.026), hip pain (p=0.006), entesitis (p=0.004), morning stiffness (p=0.002), BASDAI (p=0.001) and BASFI (p=0.002) were higher in the active ankylosing spondylitis group compared to ankylosing spondylitis patients in remission. There was one patient without any treatment in both active and remitted ankylosing spondylitis groups. Mean treatment duration was 7.27±5.17 years in active ankylosing spondylitis group and 5.50±5.03 in remitted ankylosing spondylitis group (p=0.212).

TABLE I. CLINICAL FEATURES OF STUDY GROUPS

	Overall (n=73)	Ankylosing spondilitis group (n=46)	Control group (n=27)	P value
Age (year)	32.30±6.40	33.30±6.12	30.59±6.62	0.081
Sex (female/male), n(%)	25 (34.2)/48 (65.8)	18 (39.1)/28 (60.9)	7 (25.9)/20 (74.1)	0.372
Body mass index (kg/m^2)	25.07±3.29	25.84±3.48	23.76±2.50	**0.004**
Smoking, n(%)	35 (47.9)	23 (50.0)	12 (44.4)	0.829
Alcohol, n(%)	4 (5.5)	4 (8.7)	0 (0)	0.290
Glucose (mg/dL)	88.00±10.71	88.76±12.25	86.70±7.44	0.375
Creatinin (mg/dL)	0.85±0.16	0.84±0.16	0.87±0.16	0.390
ALT (U/L)	19.66±10.32	21.28±11.89	16.89±6.13	**0.041**
Hemoglobin (g/dL)	13.42±1.66	13.01±1.68	14.10±1.40	**0.006**
Leukocyte (10^3/mm^3)	8.00±2.31	8.61±2.50	6.96±1.50	**0.001**
Thrombocyte (10^3/mm^3)	254.93±61.13	275.65±62.76	219.63±38.44	**0.001**
Sedimentation (mm/hour)	29.85±23.05	39.54±23.74	13.34±6.87	**0.001**
CRP (mg/dL)	1.42±2.93	1.93±3.47	0.56±1.30	**0.001**
Pentraxin 3 (ng/mL)	0.22±0.67	0.29±0.83	0.09±0.06	**0.009**

ALT: Alanin aminotransferase, CRP: c-reactive protein. Data are expressed as mean±SD unless otherwise is indicated, Significant p-values are highlighted in **bold**

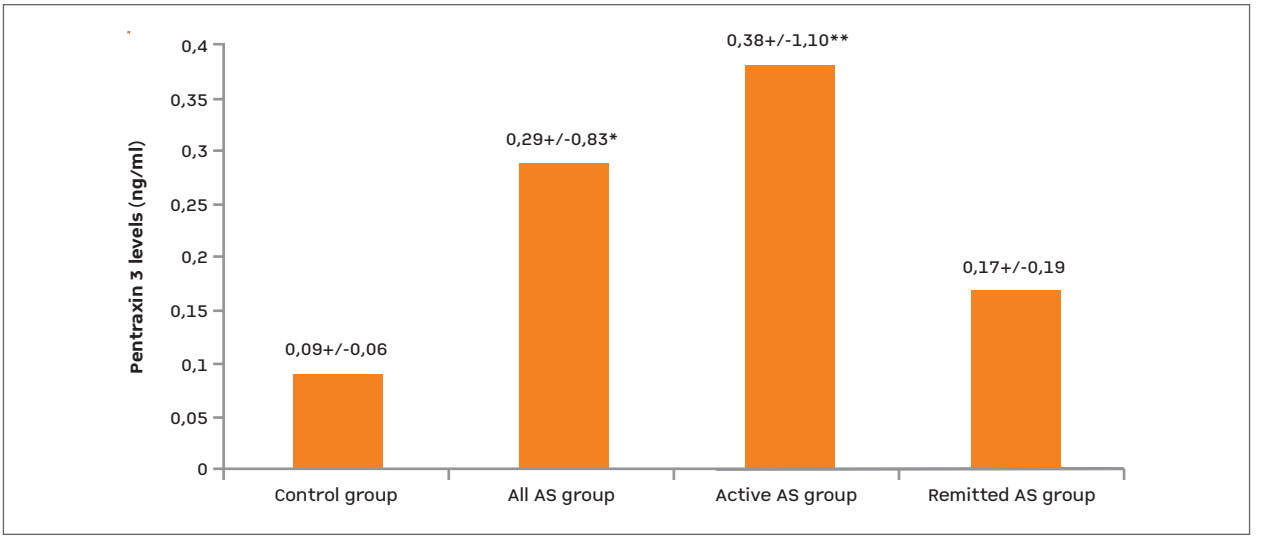

FIGURE 1. Pentraxin 3 levels in study groups

AS: ankylosing spondilitis; *:p=0.09, AII AS group vs. control group, **: p=0.030, Active AS group vs. Remitted AS group. Data are expressed as mean±SD, Kruskal Wallis Test is used.

No significant correlation was determined between PTX3 and the parameters of age, BMI, ESR, CRP, BASDAI, BASMI and BASFI in ankylosing spondilitis group (both active and remisson subgroups) and controls.

DISCUSSION

Results of this study demonstrated that serum PTX3 was higher in patients with ankylosing spondilitis in association with inflammation compared to healthy controls; however this elevation did not correlate significantly with disease activity.

PTX3 is known as an endogenous modulator of inflammatory response and has been reported to be elevated in inflammatory rheumatological diseases in association with disease activity[15,16]. Studies have demonstrated that PTX3 levels were higher and associated with disease activity in patients with systemic lupus erythematosus compared to controls[10], and that elevated PTX3 levels modulated the inflammatory process involved in the cardiovascular disease process in patients with rheumatoid arthritis[17,18]. Levels of PTX3 have been reported to be elevated in patients with small vessel vasculitis in comparison to healthy controls as an acute phase reactant; however, no correlation was determined between PTX3 and CRP levels[12]. Studies have reported that levels of ESR, CRP and interleukin 6 are elevated in association with disease activity in patients with chronic inflammatory disease[19]. This study was performed to test the hypothesis that serum PTX3 levels might be elevated as a marker of chronic inflammation in patients with ankylosing spondylitis. Serum levels of PTX3 were therefore compared in patients with ankylosing spondilitis and healthy controls and the relation was evaluated between PTX3 levels and disease activity. Additionally, patients with ankylosing spondilitis were divided in 2 subgroups as active and remission and serum PTX3 levels were compared. Consequently, serum PTX3 levels were higher in patients with ankylosing spondilitis compared to healthy controls; however, no significant relationship was determined with disease activity. Additionally, no significant difference was determined in serum PTX3 levels of patients with active or remitted disease. On the other hand, there was not significantly correlation between PTX3 levels and axial vs. peripheral disease. These results suggest that PTX3 levels are elevated in patients with ankylosing spondylitis in association with chronic inflammation, but cannot be used as a marker of disease activity.

Ankylosing spondylitis treatment may have an effect on PTX3 levels. Nevertheless the similarity between treatment features of both active ankylosing spondylitis group and the group in remission, suggests that there is no significant impact of treatment on PTX 3 levels.

TABLE II. CLINICAL FEATURES OF PATIENTS WITH ACTIVE AS AND REMITTED AS

	AS Total (n=46)	Active AS group (n=26)	Remitted AS group (n=20)	P value
Lumbalgia (n,%)	34 (73.9)	23 (88.5)	11 (45.0)	**0.026**
Hip pain (n,%)	37 (80.4)	25 (96.2)	12 (60.0)	**0.006**
Peripheral arthritis (n,%)	8 (17.4)	7 (26.9)	1 (5.0)	0.113
Entesitis (n,%)	26 (56.5)	20 (76.9)	6 (30.0)	**0.004**
Uveitis (n,%)	4 (8.7)	3 (11.5)	1 (5.0)	0.622
Family history (n,%)	5 (10.9)	4 (15.4)	1 (5.0)	0.369
Morning stiffness (hour)	1.04±0.94	1.35±0.90	0.62±0.85	**0.002**
BASDAI	4.58±2.08	6.06±1.15	2.65±1.24	**0.001**
BASFI	2.32±2.89	3.52±3.25	0.75±1.18	**0.002**
BASMI	6.54±2.17	7.08±2.54	5.85±1.31	0.083
DMARD (n,%)	16 (34.8)	11 (42.3)	5 (25.0)	0.363
NSAID (n,%)	35 (76.1)	21 (80.8)	14 (70.0)	0.494
Steroid (n,%)	1 (2.2)	1 (3.8)	0 (0)	1.000
NSAID+DMARD (n,%)	15 (32.6)	10 (38.5)	5 (25.0)	0.517
NSAID+DMARD+Steroid (n,%)	1 (2.2)	1 (3.8)	0 (0)	1.000
Anti TNF- (n,%)	17 (37.0)	9 (34.6)	8 (40.0)	0.947
NSAID+ Anti TNF- (n,%)	8 (17.4)	5 (19.2)	3 (15.0)	1.000
NSAID+DMARD+ Anti TNF-α (n,%)	1 (2.2)	0 (0)	1 (5.0)	0.435

AS: Ankylosing Spondylitis, **BASDAI:** Bath Ankylosing Spondylitis Disease Activity Index, **BASFI:** Bath Ankylosing Spondylitis Functional Index, **BASMI:** Bath Ankylosing Spondylitis Metrology Index, **DMARD:** Disease-Modifying Antirheumatic Drug, **NSAID:** Nonsteroidal Anti-inflammatory Drug, **TNF:** Tumor necrosis factor. Data are expressed as mean±SD unless otherwise is indicated, Significant p-values are highlighted in **bold**

Levels of ESR and CRP were higher in our patients with ankylosing spondilitis compared to controls; however, these levels did not differ significantly in patients with active or remitted ankylosing spondilitis. These results are supportive of the idea that ESR and CRP levels might be elevated in association with systemic inflammation in patients with ankylosing spondylitis, although they do not demonstrate a relationship with disease activity.

This is the first study investigating serum PTX3 level and its relationship with disease activity in patients with ankylosing spondilitis to our knowledge. However, the rather low number of study patient and controls is a major limitation of the study. Additionally, it would had been useful to include Ankylosing Spondylitis Disease Activity Score (ASDAS) as a marker of disease activity in addition to BASDAI.

Consequently, results of this study demonstrated that levels of serum PTX 3 might be elevated in association with chronic inflammation in patients with ankylosing spondylitis; but PTX3 cannot be used as a marker of disease activity.

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