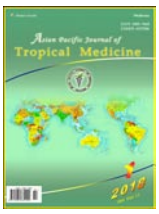

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Expression of fibronectin and MMP-3 and its significance in the patients with ankylosing spondylitis

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ABSTRACT

Objective: To observe the expression and significance of fibronectin and metalloproteinase-3 (MMP-3) in patients with ankylosing spondylitis (AS). **Methods:** A total of 30 AS patients in our hospital and 30 healthy volunteers were selected in our study. Fibronectin and MMP-3 were measured and compared between these two groups. The AS group received sulfasalazine 2 g daily for 3 months. Bath ankylosing spondylitis disease activity index, bath ankylosing spondylitis functional index, bath ankylosing spondylitis metrology index, erythrocyte sedimentation rate and C-reactive protein were compared before and after treatment. Pearson's linear-correlation analysis was used to determine relationships between parameters. **Results:** Totally 28 patients in the AS group completed the study. Fibronectin and MMP-3 in peripheral blood of AS patients were evidently higher than that in the normal control group (P<0.05). After treated by sulfasalazine, the level of expressing Fibronectin and MMP-3 significantly decreased compared with baseline values (P<0.05). Pearson's linear-correlation analysis showed that serum fibronectin and MMP-3 level had a positive correlation with bath ankylosing spondylitis disease activity index global assessment, spine pain, night pain, general pain, erythrocyte sedimentation rate and C-reactive protein (P<0.05). **Conclusions:** The expression of fibronectin and MMP-3 in AS patients were significantly higher than that in the normal control group, and they all decreased significantly after treatment. It indicated that both fibronectin and MMP-3 were correlated closely with the onset of AS.

1. Introduction

Ankylosing spondylitis (AS) is a chronic, inflammatory and rheumatological disease involving the axial skeleton, sacroiliac joint, and periphery joints, and its etiology remains obscure[1]. The clinical manifestations of AS mainly include pain, stiffness, spinal mobility limitation, and chest expansion, which has serious impacts on work and quality of life[2]. The prevalence of AS is 1.67% in Asia and 0.2%-0.4% in China[3,4]. Two thirds of these patients will progress into partial or full stiffening of spine several years after onset[5]. Fibronectin and metalloproteinase-3 (MMP-3) are main factors in the inflammation and fibrosis pathology in AS[6,7]. And the aim of this study is to observe the expression of fibronectin and MMP-3 in AS patients and explore related significance.

2. Materials and methods

2.1. Diagnostic criteria

Thirty consecutive patients were diagnosed with AS according to

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the modified New York classification criteria and CT examination that showed sacroiliitis level was below[8].

2.2. Inclusion criteria

The patients inclusion criteria were listed as follows: (1) between 16 to 69 years old, (2) conformed AS diagnosis according to diagnostic criteria[8]; (3) had not received sulfasalazine, methotrexate, thalidomide and glucocorticoid for at least four weeks prior participation of the study; (4) voluntarily signed the informed consent form. This study was approved by the Ethical Committee of Shaoxing Central Hospital.

2.3. Exclusion criteria

Patients were excluded as follows: (1) had other rheumatoid diseases that require treatment with sulfasalazine, methotrexate, thalidomide, leflunomide and glucocorticoid; (2) women in pregnancy or lactation stage; (3) patients who were hypersensitive to the testing drug; (4) complicated with serious primary diseases of heart, lung, liver, kidney system, or had mental diseases.

2.4. Drop-out criteria

The patients (1) did not conform to inclusion criteria during the research process; (2) did not take medicines according to the research; (3) had evident side effects and cannot recover spontaneously after another 1 week of treatment.

2.5. Patients

A total of 30 confirmed AS patients that visited out-patient or in-patient Department of Rheumatology of Shaoxing Central Hospital from May 2015 to March 2017 were enrolled in this study, and they were diagnosed according to the above mentioned AS diagnostic criteria. And 30 healthy volunteers were also enrolled as the normal control group in this research.

2.6. Treatment

Patients in the AS group received sulfasalazine 2 g per day. The treatment continues for 3 months. And the healthy volunteers in the normal control group received no medicine.

2.7. Detection of fibronectin and MMP-3

Fibronectin and MMP-3 in the venous blood of AS patients were measured by ELISA method before and after the research respectively. The fibronectin and MMP-3 reagent kits were bought from Nanjing Jiancheng Bioengineering Institute. And the specific operation processes were conduceted according to the instructions. Fibronectin and MMP-3 in the venous blood of healthy volunteers patients were measured only once before the research.

2.8. Clinical data collection

Bath ankylosing spondylitis disease activity index (BASDAI), bath ankylosing spondylitis functional index, bath ankylosing spondylitis metrology index, patient's global assessment, spinal pain, general pain and night pain were used for scoring (1-10) patients with AS. Physical examination, blood and urine tests, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) measurement were observed before and after the treatment in AS group.

2.9. Statistical analysis

The measurement data were expressed by mean±standard deviation (mean±SD). Statistical comparisons were conducted by *t*-test. Pearson's linear-correlation analysis was used to determine relationships between parameters. Statistical analysis was carried out using SPSS version 13.0 software. Statistical significance was considered at $P<0.05$.

3.Results

3.1. General materials

In AS group, 1 patient withdrew from the research because of poor response, and 1 patient withdrew from the study for increasing liver enzymes. Finally 28 cases finished the research, including 21 male and 7 female with a mean age of (33.50±10.82) years. The mean duration of illness was (12.68±6.12) years, with HLA-B27 positive in 23 cases and negative in 5 cases. And all 30 healthy volunteers in the normal control group finished blood measuring in this study.

3.2. Changes of fibronectin and MMP-3 levels in venous blood of AS group

Fibronectin and MMP-3 in venous blood of AS patients were significant higher than that in the normal control group ($P<0.05$, Table 1). Three months after treated with sulfasalazine, the level of fibronectin and MMP-3 in the AS group were significant lower than that before treatment ($P<0.05$, Table 1).

Table 1

Detection of fibronectin and MMP-3 in peripheral blood of AS patients at baseline (mean±SD).

Group	Cases (n)	Time (mon)	Fibronectin (ng/mL)	MMP-3 (ng/mL)
Normal	30	0	652.81±170.14	209.23±191.51
AS before treatment	28	0	967.06±241.05*#	316.40±174.20*#
AS after treatment	28	3	823.13±270.23	280.32±172.02

Note: *P<0.05, compared with normal control group at 0 month; #P<0.05 compared with 3 months after treatment.

3.3. Lab examination

Levels of ESR and CRP in AS group were significantly decreased compared to that before treatment (P<0.05, Table 2).

Table 2

Changes of ESR and CRP of AS patients related to baseline (mean±SD).

Group	Cases (n)	Time (mon)	ESR (mm/h)	CRP (mg/dL)
AS	28	0	20.37±12.33	17.28±13.02
AS	28	3	14.11±13.51*	12.14±13.21*

Note: *P<0.05, compared with before treatment.

3.4. Changes of clinical data of AS patients compared to baseline

After treated by sulfasalazine for 3 months, scores of BASDAI, global assessment, spine pain, night pain and general pain in AS group were significantly improved compared with that before treatment (P<0.05). And scores of bath ankylosing spondylitis functional index and bath ankylosing spondylitis metrology index in the AS group were of no evident change compared with that before treatment (P>0.05, Table 3).

3.5. Correlation detection

Pearson's linear-correlation analysis showed that serum fibronectin level had a positive correlation with BASDAI, global assessment, spine pain, night pain, general pain, ESR and CRP (r=0.635, 0.562, 0.431, 0.470, 0.536, 0.528, 0.469, P<0.05); serum MMP-3 level had a positive correlation with BASDAI, global assessment, spine pain, night pain, general pain, ESR and CRP (r=0.521, 0.507, 0.463, 0.514, 0.476, 0.531, 0.502, P<0.05).

4. Discussion

AS is a chronic inflammatory disease that affects 1% of the general population. As one of the most severe types of spondyloarthropathy disease, AS normally affects the spinal vertebrae and sacroiliac joints, which can cause debilitating pain and loss of mobility. A total of 90% of patients with AS are HLA-B27 positive. Traditional therapies include nonsteroidal anti-inflammatory drugs or adrenal cortical hormones, but they can only alleviate the symptoms and cannot improve symptoms[9,10]. Clinically, sulfasalazine, methotrexate and leflunomide were used to treat peripheral arthritis of AS effectively, but failed in therapeutic course. Sulfasalazine continues to be frequently prescribed as one kind of nonbiologic disease-modifying antirheumatic drugs to treat AS, despite of an unclear efficacy profile[11,12]. Some studies have suggested that sulfasalazine can improve peripheral joint disease[13-15], and it is also effective in treating the spinal symptoms in patients without associated peripheral joint disease[16,17]. In our study, sulfasalazine was used to treat the patients and the results showed that scores of BASDAI, global assessment, spine pain, night pain and general pain were significantly improved (P<0.05) compared with baseline values. It showed that sulfasalazine could largely achieve a therapeutic purpose for AS patients.

Fibrosis is important in the pathology change of AS and it can lead to bone destruction and joint deformation[18]. Fibrosis is a chronic and progressive process characterized by an excessive deposition of extracellular matrix components, such as collagen and fibronectins. It is believed to follow chronic tissue inflammation and ultimately leads to organ scarring and subsequent loss of function. Excessive collagen is a biological marker of fibrosis, and its expression is regulated by many kinds of cytokines[19]. The increase of extracellular matrix in the tissue, with fibronectin and collagen being the major components, can ultimately lead to development of fibrosis. Fibronectin has been shown to co-localize with aggregations of fibroblasts[20]. Matrix MMP-3 can degrade proteoglycan, collagen type III, IV, IX, XI and other protein substrates in extracellular matrix[21]. In this research, we aimed to investigate the expression of collagen and MMP-3 and its significance in AS patients.

Our study showed that the serum level of both fibronectin and MMP-3 in AS patients were significantly higher than that in normal control group (P<0.05). The possible reason is that the

Table 3

Changes of clinical data of AS patients compared to baseline (mean±SD).

Treatment	BASDAI	BASFI	BASMI	Global assessment	Spinal pain	General pain	Night pain
Before	4.80±0.31	1.42±1.01	2. 02±1.31	4.93±1.84	4.12±2.53	4.21±2.13	4.01±2.10
After	3.66±1.22*	1.27±0.63	1.91±1.16	3.72±1.70*	3.02±2.50*	3.23±2.03*	2.66±2.04*

Note: BASFI: bath ankylosing spondylitis functional index; BASMI: bath ankylosing spondylitis metrology index; *P<0.05, compared with before treatment.

local or system inflammation activated fibroblasts and synovial cells produced more fibronectin and MMP-3. After treatment, the expression of fibronectin and MMP-3 were both significantly decreased, indicating that the fibronectin and MMP-3 level could reflect the disease activity of AS. What's more, both serum fibronectin and MMP-3 level had a positive correlation with BASDAI, global assessment, spine pain, night pain, general pain, ESR and CRP, showing that serum fibronectin and MMP-3 correlated with the onset of AS. It showed fibronectin and MMP-3 played an important regulatory role in the fibrosis pathogenesis of AS.

In conclusion, our study showed that the expression of fibronectin and MMP-3 in AS patients were significantly higher than that in the normal control group, and they all decreased significantly after treatment. It indicates that both fibronectin and MMP-3 are correlated closely with the onset of AS.

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