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Original Article

Up-regulation of soluble P-selectin predicates its prognostic value in patients with ankylosing spondylitis

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Abstract: Ankylosing spondylitis (AS) is a chronic inflammatory disease with a high rate of disability. To find a proper prognosis marker is helpful for the treatment of AS. The purpose of this study was to investigate whether soluble P selectin (SP selectin) exerted effects on the prognosis of AS patients. Firstly, we detected the expression level of SP selectin in 85 AS patients and 60 normal subjects using quantitative real-time-polymerase chain reaction (QRT-PCR) assay. The result demonstrated that SP-selectin was over expressed in AS patients compared with healthy controls and the difference was significant ($P < 0.05$). Chi-square test was used to estimate whether SP selectin was associated with clinicopathologic characteristics. The factors of stages ($P = 0.002$), HLA-B27 ($P = 0.002$), ESR ($P = 0.001$) and C-reactive protein ($P = 0.000$) were considered to be related to the expression of SP selectin, which indicated that SP-selectin might be involved in the development of AS. Besides, the prognosis of AS patients after treatment was explored and analyzed via Cox regression analysis. The analysis suggested that ESR and SP selectin both served as independent prognostic biomarkers for AS (HR = 2.069, 95% CI = 1.049-4.080; HR = 4.562, 95% CI = 1.766-11.784). Taken together, our study revealed that not only the level of SP selectin was upregulated, but also SP selectin could predict the prognosis of AS patients.

Keywords: SP selectin, ankylosing spondylitis, prognosis

Introduction

Ankylosing spondylitis (AS) is a complex multifactor disease characterized by inflammatory back pain which mostly strike young adults [1]. According to statistics, the morbidity rate of AS patients is approximately at 0.2%-1.2% [2]. The incidence of AS exhibits a phenomenon of familial aggregation, considerable geographical and ethnic variation [3]. AS may cause serious disability via affecting the spine and sacroiliac joints and the lesion is irreversible in spine sacroiliac, joint and hip [4, 5]. Until now, the survival situation of AS patients was difficult to control and there was still no effective treatment for them. Therefore, to find out a prognostic biomarker for AS will help for improving the survival rates of the patients.

Soluble P selectin (SP selectin) is a soluble form of the P selectin which is considered as a plasma markers for platelet activation and localizes in the membranes of the α-granules of platelets [6, 7]. Studies have confirmed that the expression of SP selectin is closely related to a variety of thrombotic and cardiovascular diseases [8, 9]. Increased expression of SP selectin has been found in several diseases including inflammatory disease [10-13]. However, the effect of SP selectin on pathogenesis of AS is still not clear.

In this study, we detected the expression of SP-selectin in AS patients and healthy controls and then investigated whether SP-selectin could predict the prognosis of AS patients.

Materials and methods

Sample collection

The study was conducted with 85 AS patients in Luoyang Orthopedic Hospital and approved by the council of ethics of the hospital. The classification of AS patients was based on the modi-

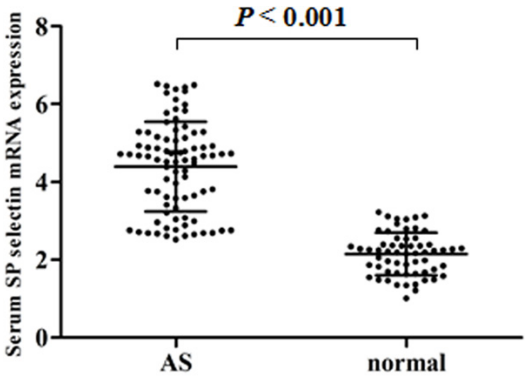

Figure 1. SP selectin was over-expression in AS patients compared with healthy subjects. QRT-PCR was performed to measure the expression levels of SP selectin between patients and healthy controls. A value of $P < 0.05$ was considered to be statistically significant.

fied New York criteria of 1984 [14]. The diagnosed AS patients including 39 males and 46 females aging from 18 to 79 were enrolled. Patients who suffered from heart, brain, liver, kidney diseases or rheumatism were excluded. All the patients hadn't received any form of treatment prior to surgery. Then unified treatments were performed on 85 AS subjects and the assay of curative effect was determined to evaluate the survival situation for AS patients. We considered above 30% improvement of clinical symptoms as effective treatment. 60 healthy people matched with age and gender were obtained as healthy controls. All participators signed informed consent in advance.

The serum samples were extracted from the patients and healthy controls, then put into EDTA blood collection tube immediately, and lastly stored at -80°C for RNA extraction.

Quantitative real-time-polymerase chain reaction (QRT-PCR)

RNA was isolated from serum using RNApure Blood Kit (Tiangen, Beijing, China) according to the manufacturer's instruction. Reverse transcription was performed to synthesize cDNA. Then real-time PCR reaction was carried out to measure the expression of SP selectin in the Applied Biosystems 7900 Fast Real-Time PCR system (Applied Biosystems, Foster, California, USA) with an internal control of GAPDH gene. The expression level of SP selectin was calculated with the $2^{-\Delta\Delta ct}$ method. All the experiments were conducted in triplicate.

Follow-up analysis

A 5-years follow-up was implemented to estimate the prognostic value of SP-selectin in AS patients. The follow-time began from the day that patients received treatment to the time that the condition altered. The clinicopathologic characteristics including the onset age, disease duration, gender, stages, HLA-B27, ESR, and C-reactive protein were recorded ahead of time. The follow-up information was updated every three months and obtained via a telephone or questionnaire.

Statistical analysis

SPSS 20.0 software (SPSS Inc., Chicago, USA) was used to operate the statistical analyses. Differences of SP selectin expression at mRNA level between two groups were compared by students' t test. The association of SP selectin expression and clinicopathologic characteristics were analyzed through χ^2 test. Prognostic value of SP selectin for AS was assessed via Cox regression analysis.

Results

Expression of SP selectin in AS patients and healthy controls

QRT-PCR assay was taken to evaluate the expression of SP selectin at mRNA level in 85 AS patients and 60 healthy controls. The result demonstrated that SP selectin expression in AS patients was significantly higher than that in healthy individuals (4.389 ng/ml vs. 2.149 ng/ml, $P < 0.001$), as displayed in **Figure 1**.

Association between SP selectin and clinicopathologic characteristics

In the study, AS patients were divided into two groups: high SP selectin expression group (SP selectin-high, n = 47) and low SP selectin expression group (SP selectin-low, n = 38). As displayed in **Table 1**, SP selectin expression of AS patients was influenced by the stages ($P = 0.002$), HLA-B27 ($P = 0.002$), ESR ($P = 0.001$), and C-reactive protein ($P = 0.000$), while it has no relationship with the onset age ($P = 0.643$), gender ($P = 0.530$), and disease duration ($P = 0.685$).

Prognostic role of SP selectin for AS patients

Among the 85 patients, 65 patients were defined as the elevated SP selectin expression

Table 1. Association of SP selectin expression and clinicopathologic characteristics in AS patients

Pathological parameters	Cases	SP selectin expression		*P*
		High expression	Low expression	
Onset age				0.643
≤ 16	28	17	11	
16-25	32	18	14	
> 25	25	12	13	
Gender				0.530
male	39	23	16	
female	46	24	22	
Disease duration (years)				0.685
≤ 2	20	12	8	
2-10	42	24	18	
> 10	23	11	12	
Stages				0.002
I+II	59	26	33	
III+IV	26	21	5	
HLA-B27				0.002
positive	75	46	29	
negative	10	1	9	
ESR (mm/hr)				0.001
> 30	41	30	11	
< 30	44	17	27	
C-reactive protein				0.000
positive	48	38	10	
negative	37	9	28	

Table 2. Relationship of SP selectin and therapeutic effects

Groups	Cases	Cases of effectiveness	*P*
Elevated SP selectin	65	36	0.019
Normal SP selectin	20	17	

Table 3. Prognostic value of SP selectin and clinicopathologic characteristics according to Cox regression analysis

Pathological parameters		HR	95% CI	*P*
SP selectin	Low expression	-	-	-
	High expression	4.562	1.766-11.784	0.002
ESR	< 30	-	-	-
	> 30	2.069	1.049-4.080	0.036

group (higher than the upper limit of healthy people) and 20 patients belonged to the normal SP selectin expression level (lower than the upper limit of healthy subjects), then the follow-up was conducted with two groups. 6 months later, AS patients with high SP selectin level exhibited worse therapeutic effect compared with those with normal SP selectin level (P = 0.019) (**Table 2**). Hence, we speculated that higher expression of SP selectin contributed to the deterioration of AS patients.

Multivariate analysis according to Cox regression analysis showed that ESR (HR = 2.069, 95% CI = 1.049-4.080, P = 0.036), and SP selectin expression (HR = 4.562, 95% CI = 1.766-11.784, P = 0.002) were independent prognostic markers for AS (**Table 3**).

Discussion

AS is a chronic inflammatory rheumatic disease with a prevalence ranging from 0.1% to 0.9% [15, 16]. The diagnosis time is often late. The time interval between symptom onset and diagnosis is about 5 to 8 years [17]. Besides, the new bone formation in the progress of AS could cause deformity and disability, which seriously affects people's lives. In addition to early diagnosis, to improve the survival situation of AS patients is also important for curing the disease. Until now, the studies have explored many prognostic markers for AS and genes were the mostly studied ones.

SP selectin, a member of P selectin family, is known as an adhesion molecular which is confirmed to play an important role in inflammatory foci [18]. AS was reported to be associated with microvascular dysfunction such as atherosclerosis [19]. However, the relationship between elevated circulating cell adhesion molecules (CAMs) including platelet activation markers such as

P-selectin and the pathogenesis of atherosclerosis had been shown, the link between SP selectin and AS was still obscure [20, 21].

In the present study, we detected the expression of SP selectin in AS patients and controls. Namely, the expression of SP selectin was higher in AS patients than in healthy controls which hinted SP selectin might affect the occurrence of AS. To further verify whether SP selectin participate the development of AS, the association between SP selectin and clinicopathologic characteristics was analyzed. The outcome proved that the expression of SP selectin was influenced by the stages, HLA-B27, ESR, and C-reactive protein.

Another interesting finding was that the level of SP selectin could influence overall survival of AS patients in our study. Besides, the prognostic value of SP selectin had been reported in several malignancies such as acute coronary syndrome, colorectal cancer, chronic congestive heart failure, presumed myocardial ischemia, severe trauma and rheumatoid arthritis in previous studies [13, 22-25]. So we performed a Cox regression analysis to estimate the prognostic value of SP selectin in AS. As a result, ESR and SP selectin were considered as impact factors in the prognosis of AS and they might also be an independent prognostic marker.

In summary, SP selectin is over expressed in AS patients and it may be involved in the development of AS. As respect to its prognostic value, our findings provided some evidences for inferring that it acts as a prognostic marker. However, how the SP selectin functions in the development of AS and whether it is a prognostic biomarker still needed to be further investigated.

Disclosure of conflict of interest

None.

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