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Original Article

Expression of cytokines IL-1β, IL-6 and TNF-α in intervertebral disc lesions of spinal tuberculosis

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Abstract: Objective: To investigate the gene expression of cytokines IL-1β, IL-6 and TNF-α in intervertebral disc lesions of spinal tuberculosis. Methods: Forty patients with spinal tuberculosis treated in our hospital from May 2014 to May 2016 were included in the spinal tuberculosis group, and other 40 patients with spinal disorders concurrently treated in our hospital were enrolled in the control group. With SPS 9001 kit, the expression levels of cytokines IL-1β, IL-6 and TNF-α of the two groups were examined using the enzyme linked immunosorbent assay (ELISA), and then statistical analyses were performed. Results: The levels of cytokines IL-1β, IL-6 and TNF-α levels among the patients in the spinal tuberculosis group (0.385±0.091 pg/ml, 0.378±0.097 pg/ml, 0.276±0.052) pg/ml, respectively) were significantly higher than those in the control group (0.190±0.066 pg/ml, 0.177±0.058 pg/ml, 0.147±0.043 pg/ml, respectively) ($P<0.05$). Conclusion: The levels of cytokines IL-1β, IL-6 and TNF-α in intervertebral disc lesions of spinal tuberculosis were upregulated, which is worthy of clinical notice.

Keywords: Spinal tuberculosis, intervertebral disc lesions, expression of cytokines IL-1β, IL-6 and TNF-α, clinical significance

Introduction

Tuberculosis is an infection involved in the respiratory tract, which jeopardizes health of the population globally [1]. Infection occurs when there is an immune response induced by the suppression of pathogenic bacteria in some healthy tissues in any part of the spinal column. If tuberculous infection and circulation disorder are major predisposing factors for vertebral lesions, there are generally three concurrent basic pathological changes, namely exudation, proliferation, and necrosis, similar to tuberculosis in other sites. The elderly people with poor nutrition and immune function is a high-risk population. Spinal tuberculosis, a chronic osteoarthrosis with a high prevalence, ranks first among tuberculous osteoarthropathy, mostly manifested as vertebral tuberculosis, but rarely as adnexal tuberculosis. Patients are present with systemic toxicity, such as fatigue, mild fever, and weight loss, which are frequently misdiagnosed as bad colds or other acute infections. The disease tends to be ignored due to its slow development and progression. The infection rate of spine tuberculosis increases with the growing load from the cervical vertebra to the sacrum and coccyx. Tuberculosis is primarily associated with intervertebral disc lesions. Adults are a high-risk population, mainly implicated in thoraco-lumbar segments of the spine.

Under normal circumstances, spinal tuberculosis is secondary to pulmonary tuberculosis (TB). In recent years, TB has become increasingly prevalent with the growing large population, mobility and drug resistance. Three to five percent of active tuberculosis is attributable to osteolytic lesions which mainly affect the spine, with the anterior column most affected. As a result, the stability of the spine is adversely affected by the damaged vertebral body and intervertebral disc attributed to TB, which results in worsening physical condition in the patients. Tubercle bacilli in spinal tuberculosis invade into the cancellous bone through blood transmission. The vast majority of bacterial floras destroy the suppression of the autoimmune system to tubercle bacilli, so only a small amount of remaining bacilli reproduce when the body is under favorable conditions. Gradually,

they form minor lesions which are protected by repair and destruction of the fibrous tissues. In this manner, the potential complicating factors are latent. Mycobacterium tuberculosis (commonly called tubercle bacillus), affected by changes in the immune mechanism and other factors, multiply rapidly, constantly expand the focal zone, forming new focal zones, leading to a variety of clinical manifestations. Occult clinical manifestation of exudative spinal tuberculosis adds difficulty to early diagnosis and treatment of the disease. Patients with spinal tuberculosis vary in clinical manifestations. If the patient has mild symptom, he or she can heal without medication. By contrast, if the patient has severe symptom, the disease aggravates rapidly with the presence of local infection and bone destruction, and develops abscess and dead bone, leading to the affected posterior and lateral convex of the spine and even paraplegia in severe cases.

Up till now, no clear pathogenesis of spinal tuberculosis has been found in clinical practice. Relevant medical research has suggested that the pathogenesis of spinal tuberculosis may be the reason that cellular immunity is affected by the abnormality of cytokines and immune factors [2]. The inflammation of spinal tuberculosis attributed to bone destruction mainly manifests as intervertebral disc erosion. Anti-tuberculosis body phagocytizes tubercle bacilli by exerting immune regulation over macrophage clusters. On one hand, it promotes the production of auto-protective immune mechanism; on the other hand, it can strengthen phagocytosis and digestion of tubercle bacilli by the macrophages, thereby suppressing reproduction and spread of tubercle bacilli [3]. The present study was designed to investigate expression levels of cytokines IL-1β, IL-6 and TNF-α in intervertebral disc lesions of spinal tuberculosis and their clinical implications, as reported as follows.

Materials and methods

Participants

Forty patients with spinal tuberculosis treated in our hospital from May 2014 to May 2016 were enrolled in our study. All the eligible patients showed clinical manifestations of lumber and back pain, restricted activities, had spinal tuberculosis as demonstrated by medical history, preoperative radiographic data, as well as postoperative pathological examination. All the patients were required to provide with informed written consents. The patients who had unclear consciousness, associated severe hepatorenal diseases and mental illness were excluded. In addition, 40 patients with benign spinal disorders concurrently treated in our hospital were included as controls. This study was approved by the Hospital Ethics Committee.

Specimen collection and storage

Spinal tuberculosis group: During the operation, the tuberculose lesions were removed from the patient's body. The intervertebral disc tissues were taken out as lesion specimens, including necrotic endplate, nucleus pulposus and anulus fibrosus. The specimens were placed on the sterile gauze, by which the blood was pipetted. Finally, after rinse, they were quickly stored in the refrigerator at -80°C.

Control group: The normal intervertebral disc tissues from which blowout fracture and vertebral compressed fracture had been resected were taken out of the body of the patient via posterior and intervertebral accesses, including necrotic endplate, nucleus pulposus and anulus fibrosus. The specimens were processed as previously described.

Study methods and measure outcomes: With the SPS 9001 kit (Beijing Zhongshan, China), the levels of cytokines interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor alpha-α (TNF-α) were detected following the instructions of the kit.

Procedures are described below: first, contrast solution: the standard powder of the control group was diluted with 1 ml of DDW; second, 10x lotion: the lotion was diluted to 1x with DDW; third, substrate: A, and B reagents in equal volume were evenly mixed for 15 min for future use, then 100 μL was added into per well; fourth, standard solution: the standard solution was diluted with the diluent attached in the kit. With the initial concentration of 500 pg/ml, the solution was diluted twice every gradient, with a total of 8 times; fifth, sample dilution: the serum to be tested was diluted 10 times with DDW; sixth, detection of the concentrations of the inflammatory cytokines in serum to be tested: testing of the optical density (OD)

Table 1. General data of the two groups

Group	Case	Gender		Age (year)
		Male	Female	
ST	40	23 (57.5%)	17 (42.5%)	38.2±10.6
Control	40	21 (52.5%)	19 (47.5%)	38.1±13.0
χ^2		2.71		1.886
P		0.13		0.52

Note: ST denotes spinal tuberculosis.

Table 2. Cytokine IL-1β levels in patients of the two groups ($\bar{x}$±s)

Group	Case	IL-1β (pg/ml)	t	P
ST	40	0.385±0.091	10.971	0.012
Control	40	0.190±0.066		

Note: ST denotes spinal tuberculosis.

values of the samples in each group was followed by the instructions. The procedures were as follows: the standard solution and the samples to be tested were bound to the coated specific antibody and then reacted for 2 h away from light at room temperature. The samples were washed with lotion 3 times; HRP-conjugated secondary antibody was added and then the mixture was reacted for 2 h away from light at room temperature, followed by three times of washing. Finally, the OD values at 450 nm and 570 nm of the samples were measured using a spectrometer. The standard curves were drawn according to the concentrations of standard solution after gradient dilution; the concentrations of samples were calculated and the differences among the groups were analyzed.

Statistical analysis

Statistical analysis was performed using the SPSS software, version, 20.0. Categorical data were compared using a chi-square test. Measurement data were expressed as standard deviation ($\bar{x}$±s), and the differences between the two groups were compared with the use of the t-test. An alpha level of 0.05 was considered statistically significant.

Results

General data of the two groups

The general data at baseline were similar among the patients in the two groups, and the differences were not statistically significant (P>0.05, **Table 1**).

Comparison of IL-1β levels between the two groups

The cytokine IL-1β levels in patients with spinal tuberculosis were significantly higher than those in the control group (P<0.05, **Table 2** and **Figure 1A**).

Comparison of IL-6 levels between the two groups

The IL-6 levels in patients with spinal tuberculosis were significantly higher than those in the control group (P<0.05, **Table 3** and **Figure 1B**).

Comparison of TNF-α between the two groups

The *TNF-α* levels in patients with spinal tuberculosis were significantly higher than those in the control group (P<0.05, **Table 4** and **Figure 1C**).

Discussion

Spinal tuberculosis is a chronic osteoarthrosis with high morbidity. In the onset period, tubercle bacilli, under the influence of the changes in the immune mechanism and other factors, reproduce in a rapid manner, expand their lesion zones, and produce new lesion zones, resulting in a wide range of clinical manifestations. Clinically, the pathogenesis of spinal tuberculosis remains unclear. Related medical studies have suggested that the pathogenesis of spinal tuberculosis may be the abnormal cellular immunity caused by cytokines and immune factors [2]. However, few studies have been involved in the expression of inflammatory factors in tuberculosis foci. The present study explored the levels of cytokines IL-1β, IL-6 and TNF-α in the intervertebral disc lesions of spinal tuberculosis and their clinical value.

IL-1β, a type of robust pro-inflammatory cytokine, plays an inflammatory role by stimulating and regulating other inflammatory cytokines [4]. IL-1β activates macrophages and monocytes, affecting the inflammatory infiltration in the intervertebral discs of spinal tuberculosis [5]. Matrix metalloproteinases (MMP) are a type of enzymes promoting the degradation of intervertebral discs and cartilage [6-8]. IL-1β may upregulate the MMP levels, promote the

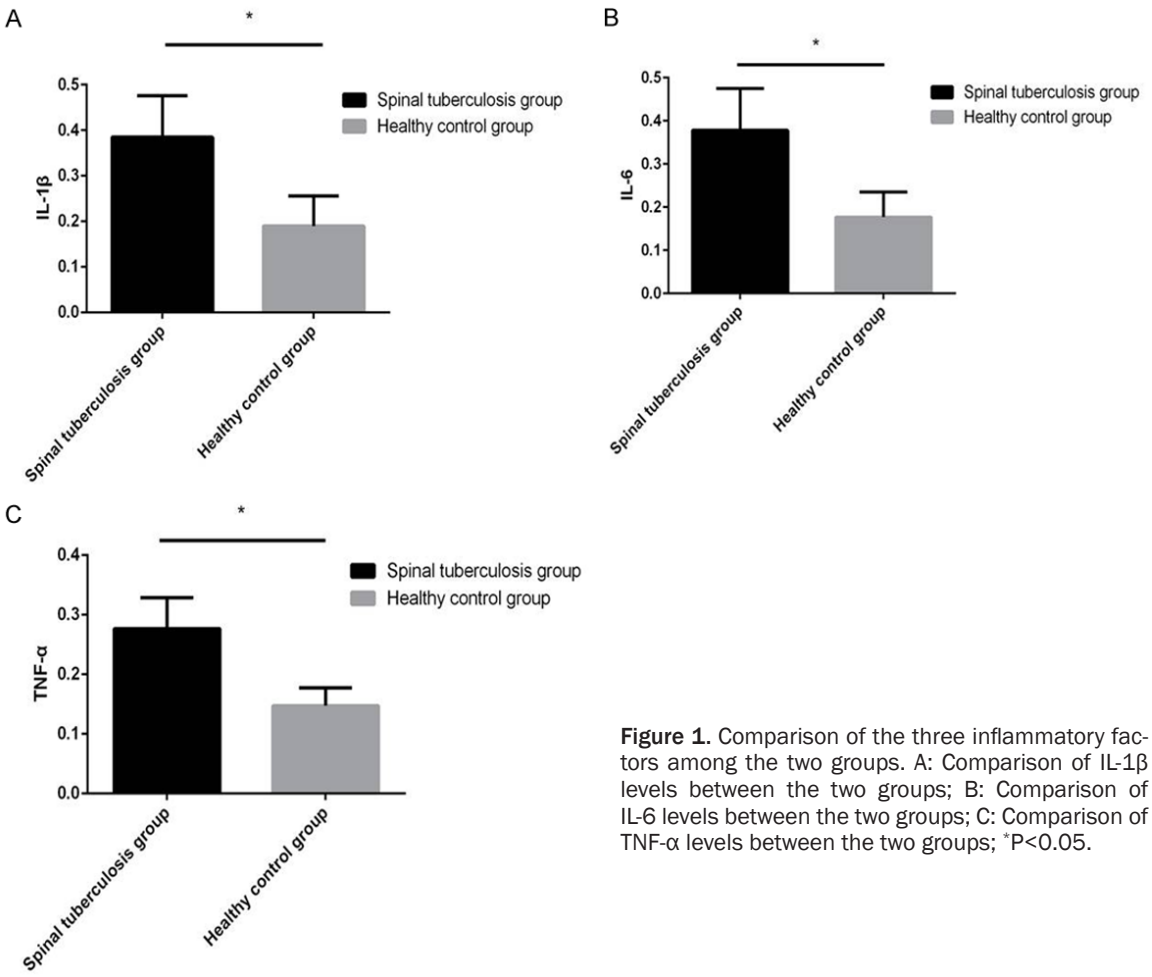

Figure 1. Comparison of the three inflammatory factors among the two groups. A: Comparison of IL-1β levels between the two groups; B: Comparison of IL-6 levels between the two groups; C: Comparison of TNF-α levels between the two groups; *P<0.05.

Table 3. IL-6 levels of the two groups ($\bar{x}\pm s$)

Group	Case	IL-6 (pg/ml)	t	P
ST	40	0.378±0.097	9.541	0.023
Control	40	0.177±0.058		

Note: ST denotes spinal tuberculosis.

Table 4. TNF-α levels of the two groups ($\bar{x}\pm s$)

Group	Case	TNF-α (pg/ml)	t	P
ST	40	0.276±0.052	12.091	0.009
Control	40	0.147±0.043		

Note: ST denotes spinal tuberculosis.

degradation of intervertebral disc tissues, and improve inflammatory reactions and the presence of the inflammatory process [9]. The results of this study showed that cytokine IL-1β levels were significantly higher among the patients with spinal tuberculosis than controls (P<0.05), suggesting that in intervertebral discs of spinal tuberculosis, IL-1β might be the cytokine triggering the inflammatory process, which is prone to resulting in abnormality in intervertebral discs. Studies have showed that IL-6 was mainly secreted by monocytes and myeloma cells, which may be associated with osteoporosis [10-12]. The present study revealed that the IL-6 levels were significantly higher among the patients with spinal tuberculosis than normal controls. Clinically, spinal tuberculosis is mainly manifested as erosive bone disease, which indicates a close association of osteoporosis with the intervertebral disc lesions of spinal tuberculosis. IL-6 may impact the suppression of intervertebral discs on catabolic enzymes. A small amount of diverse protein synthesis is present in the nucleus pulposus of normal vertebral disc tissues. Proteoglycans tends to be lost in the presence of IL-6, thereby inhibiting the synthesis of fibroblasts [13]. The inflammatory process of intervertebral disc developed with the changes in

inflammatory cytokines. IL-6 changed the pathology of intervertebral disc and induced autoimmune mechanism by regulating the changes in inflammatory cytokines and immune cytokines [14]. The results of this study show that the cytokine IL-6 levels in patients with spinal tuberculosis were significantly higher than those in controls ($P<0.05$), suggesting that IL-6 inflammatory mediators may play a crucial role in the presence of spinal tuberculosis in the intervertebral disc.

Studies have demonstrated that TNF-α is present in the intervertebral disc of spinal tuberculosis [15-17]. TNF-α is a cytokine which has various biological activity and makes immune regulation over leucocytes and connective tissues. On one hand, stimulation to the cells by TNF-α leads to the production of protease and prostaglandin; on the other hand, it also exerts pro-inflammatory effects by promoting vascular permeability. What's more, it also has an absorption function as it is a robust inducer of bone resorption. Osteoclasts can release a variety of protease and lactic acid while osteoblasts can secrete and synthesize bone matrixes to help bone remodeling and formation [16-19]. In the process of bone growth and formation, osteoclasts and osteoblasts cooperatively fully develop their actions. Studies have found that TNF-α can provide a pathway which is a favorable precondition for absorption and differentiation of osteoclasts [20, 21]. Through the pathway, TNF-α induces osteoblasts to express macrophage colony-stimulating factor and NF-kB receptor activator ligands, thereby suppressing lesions of spinal tuberculosis. The results of immunohistochemical staining showed that the expression levels of TNF-α positive cells were higher than that among the patients with spinal tuberculosis lesions than those with the normal intervertebral discs. This suggests that TNF-α might affect the damages attributed to the cancellous bone at the spinal tuberculosis lesions, resulting in degeneration/necrosis of intervertebral discs and disappearance of intervertebral space. It also validates that TNF-α can directly impact the peripheral blood and tissues, lymphatic vessels and nerves, block the diffusion of nutrition in intervertebral disc between vertebral bodies. Consequently, inflammatory cells were produced and TNF-α regeneration enhanced. The results of this study shows that cytokine TNF-α levels were significantly higher among patients with spinal tuberculosis than those in the control groups ($P<0.05$), which implicates that TNF-α might destroy cancellous bone of tuberculosis via the abovementioned pathway. TNF-α inflammatory mediator may play a critical role in the intervertebral disc lesions of spinal tuberculosis, but additional studies are still required to explore its mechanism by relevant medical researchers.

In conclusion, an upregulation in the levels of cytokines IL-1β, IL-6 and TNF-α in the intervertebral disc lesions of spinal tuberculosis may contribute to an increase in intervertebral disc infiltration, which warrants further clinical studies.

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Disclosure of conflict of interest

None.

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