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Research Article

Correlation between the Expression of Inflammatory Factors and the Degree of Intervertebral Disc Degeneration

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Abstract: ***Objective:*** To investigate the relationship between the expression of IL-6, IL-8, IL-15, IFN-a, TNF-a and TRPC6 in the disc tissue of patients with cervical disc degeneration. ***Methods:*** The expression levels of inflammatory factors IL-6, IL-8, IL-15, IFN-α, TNF-α and TRPC6 were analyzed by RT-PCR, and the correlation between inflammatory factors and Pfirrmann grade and inflammatory factors was analyzed. ***Results:*** The mRNA expression levels of IL-6, IL-8, IL-15, TNF - α and TRPC6 were significantly higher in Pfirrmann grade IV-V than in Pfirrmann grade II-III (P<0.05), and IFN-α expression level in IV-V intervertebral disc samples was significantly lower than that in II-III discs (P<0.05); The mRNA expression levels of IL-6, IL-8, IL-15, TNF - α and TRPC6 were positively correlated with pfirmann grading (P<0.05), IFN - α was negatively correlated with pfirmann grading (P<0.05), IL-6, IL-8, IL-15, TNF - α and TRPC6 were positively correlated with each other (P<0.05), IFN - α was negatively correlated with IL-6, IL-8, IL-15, TNF-α and TRPC6 (P<0.05). ***Conclusion:*** IL-6, IL-8, IL-15, IFN-α, TNF- α and TRPC6 are closely related to the degree of cervical disc degeneration.

Key words: Cervical intervertebral disc; Degeneration; Inflammatory factors; Severity; Relevance

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Degenerative disc is easy to lead to degenerative disc herniation, such as lumbar disc herniation, lumbar stenosis and low back pain[1]. These diseases are usually accompanied by acute or chronic refractory low back pain and root pain[2]. Intervertebral disc degeneration is closely related to biological structure and mechanics, blood circulation, molecular and biochemical factors[3-4]. At present, the understanding of the pathophysiological basis of root pain has evolved from the hypothesis of simple mechanical nerve root compression to more complex mechanisms involving mechanical and biochemical mechanisms. It has been reported that cytokines play an important role in the process of intervertebral disc degeneration. The expression levels of interleukin-6 (IL-6), tumor necrosis factor-α(TNF-α) and nerve growth factor (NGF) are related to degenerative disc herniation. Previous studies have confirmed the association between lumbar disc disease and clinical symptoms of cytokine expression, but the relationship between the expression level of inflammatory cytokines in patients with intervertebral disc degeneration and clinical pain mechanism is still controversial.The report is as follows.

1 Material and methods

1.1 General information

A total of 62 patients with cervical disc degeneration who underwent surgical treatment in our hospital from January 2015 to January 2018 were selected. The inclusion criteria were as follows: (1) they met the diagnostic criteria of cervical disc degeneration and had surgical indications; (2) The operation and treatment of cervical disc were performed; (3) The age ranged from 20 to 80 years; (4) Informed consent was obtained from patients. Exclusion criteria: (1) patients with immune system diseases such as ankylosing spondylitis or rheumatoid arthritis; (2) Patients with malignant tumor; (3) Patients with tuberculosis of spine; (4) Patients with systemic infectious diseases. This study was reviewed and approved by the hospital ethics committee. There were 28 males and 34 females with an average age of (51.3 ± 8.7) years and a mean body mass index (BMI) of (22.7 ± 2.4) kg/m2;A total of 86 samples of intervertebral disc tissue were obtained, including C3-422 cases, C4-536 cases, C5-615 cases, C6-713 cases.

1.2 Detection index and method

All patients underwent cervical MRI examination before operation. The severity of intervertebral discs was graded according to Pfirrmann's criteria. According to the structure of intervertebral disc, the boundary between nucleus pulposus and annulus fibrosus, MRI signal and intervertebral disc height, they were divided into grade I to grade V.

RT-PCR: The RNA Extraction Kit (bio service, R1051) was purchased from Beijing Jinshi Baiyou Technology Co., Ltd. after grinding the disc tissue in liquid nitrogen, 1mltr izol was added to every 200 mg tissue and homogenate with a homogenizer. The specific RNA extraction steps were carried out in strict accordance with the instructions. The RNA was then purified by RNeasy minikit (74104, Qiagen) according to the instructions, and then nanodrop (Thermo Fisher) was used for quality control and quantification. The extracted RNA was reverse transcribed into cDNA and RT-PCR was performed. Using GAPDH Gene as internal reference, the expression of inflammatory factors in cervical intervertebral disc samples was calculated by 2 - Δ CT. 2 - Δ CT = ctgadph, the target gene of CT.

1.3 Observation indexes and statistical methods

The correlation between the expression levels of TNF - α, IL-6, NGF and the degree of intervertebral disc degeneration was evaluated by perason method. SPSS 19.0 software was used for statistical analysis. The measurement data in accordance with normal distribution were expressed by means ± standard deviation ($\bar{x} \pm s$) and two independent samples t test was used. The data above two groups were compared by analysis of variance; χ^2 was used for counting data, and the difference was statistically significant (P<0.05).

2 Results

2.1 Comparison of inflammatory factors among different Pfirrmann grades

In 86 cases of cervical intervertebral disc samples, 16 cases were classified as grade II, 28 cases as grade III, 31 cases as grade IV and 11 cases as grade v. the mRNA expression levels of IL-6, IL-8, IL-15, TNF - α and TRPC6 in Pfirrmann grade IV-V were significantly higher than those in grade II-III (P < 0.05), and the expression level of IFN - α in grade IV-V was significantly lower than that in grade II-III (P < 0.05).

2.2 Correlation analysis between inflammatory factors and Pfirrmann classification

The mRNA expression levels of inflammatory factors IL-6, IL-8, IL-15, TNF - α and TRPC6 were positively correlated with Pfirrmann grading (P < 0.05), while the expression of IFN - α was negatively correlated with Pfirrmann grading (P < 0.05).

2.3 Correlation analysis of different inflammatory factors

IL-6 was positively correlated with IL-8, IL-15, tnf- α and TRPC6 (P<0.05), IL-8 was positively correlated with IL-15, tnf- α and TRPC6 (P<0.05), IL-15 was positively correlated with TNF-α and TRPC6 (P<0.05), TNF-α and TRPC6 were positively correlated (P<0.05), IFN α was negatively correlated with IL-6, IL-8, IL-15, TNF- α and TRPC6 (P<0.05).

3 Discussion

The results showed that IL-8 was the highest mRNA expression gene in cervical intervertebral disc samples, and the mRNA expression levels of IL-6, IL-

8, IL-15, TNF - α and TRPC6 in PFIR rmann grade IV-V were significantly higher than those in grade II-III.Recent studies have found that proteoglycan content and tissue osmotic pressure decrease in the process of intervertebral disc degeneration. IL-8 is a chemokine, belonging to the CXC subfamily. Especially in the acute phase, IL-8 is secreted by a variety of cell types in response to inflammatory stimuli.It is known that IL-8 can induce hyperalgesia through the local production of sympathetic amines that sensitize nociceptors, thus leading to pain.In cervical intervertebral discs, oxidative / nitrite stress and injury caused by mechanical loading may be an important reason for the increase of IL-8 level. Therefore, the specific mechanism of cervical disc pain may be closely related to the expression level of IL-8.

In addition to IL-8, IL-6 is also an inflammatory cytokines with many biological effects. After tissue injury, it can not only promote monocyte differentiation into macrophages, but also activate a variety of intracellular signal transduction pathways by binding to non signal membrane bound IL-6 receptor and then interacting with membrane proteins. IL-6 is considered to be involved in the pathogenesis of spinal cord neuropathic pain, especially in the pain stimulation of symptomatic radiculopathy and peripheral nerve injury. Previous studies have shown that lumbar radicular pain caused by disc herniation is associated with the increase of IL-6. This study also proved that IL-6 is closely related to Pfirrmann classification of cervical disc, which indicates that IL-6 has an important effect on the degeneration of the disc.

TRPC6 is a new target in the study of pain and inflammation. The expression of TRPC6 contributes to mechanical hyperalgesia. In cartilage and intervertebral disc, TRPC6 can regulate cell phenotype stability and cell aging in vitro. The expression of TRPC6 is closely related to fibrosis. Our results also suggest that TRPC6 may be involved in the fibrosis related mechanism of cervical disc degeneration. Studies have shown that TNF-α is a joint factor involved in the initiation of the inflammatory cascade of intervertebral disc, and IL-15 can regulate the proliferation and activation of T and B cells in inflammatory immune response. IFN-α is an important regulator of the immune system. IFN-α is negatively correlated with the degree of cervical disc lesions, indicating that IFN-α is significantly inhibited in the process of pathological changes. Comprehensive analysis showed that IL-6 was positively correlated with IL-8, IL-15, TNF - α and TRPC6, while IFN - α and IL-6 were negatively correlated with IL-8, IL-15, TNF-α and TRPC6. The results showed that inflammation, pain receptor and immune regulation were involved in the process of intervertebral disc degeneration.

In conclusion, inflammatory cytokines are related to the pathogenesis of pain and the progression of degenerative disc in patients with degenerative disc herniation. With the increase of disease degree, the expression levels of TNF - α and IL-6 gradually increased, and the expression of NGF gradually decreased. At the same time, the expression level of TNF - α in patients with low back pain was significantly higher than that in patients with leg pain.

References

[1] Xu C. Study on the role and mechanism of exosomes from mesenchymal stem cells in promoting the repair of intervertebral disc degeneration [D].Second Military Medical University,2017.

[2] Liu SH. Expression and significance of phospholipid transporter in human degenerative disc [D].University of South China,2017.

[3] Xia Y. Expression and significance of nuclear factor E2 related factor 2 in human degenerative disc [D]. University of South China, 2016.

[4] Li W. Study on the mechanism of IL-10 and TGF- on the inflammatory response to canine disc degeneration [D]. Second Military Medical University, 2015.