Int J Clin Exp Med 2019;12(8):10380-10386
www.ijcem.com /ISSN:1940-5901/IJCEM0089385

Original Article

Correlation of inflammatory cytokines with radicular pain after lumbar intervertebral disc protrusion

Haiyun Yang, Yunge Zhang, Guiming Sun, Dong Zhao, Yi Ma, Yonghong Hao, Binggang Guan, Qiang Yang

The First Department of Spine Surgery, Tianjin Hospital, Tianjin 300210, China

Received December 4, 2018; Accepted May 7, 2019; Epub August 15, 2019; Published August 30, 2019

Abstract: Objective: The aim of this study was to examine the correlation of inflammatory cytokines with radicular pain after lumbar intervertebral disc protrusion. The degree of radicular pain was quantified and digitized in order to guide clinical drug dose and treatment, and better evaluate curative effects. Methods: A total of 20 patients with lumbar intervertebral disc protrusion, from October 2016 to October 2017, who met inclusion criteria were selected as the observation group. Observation group was further divided into bulging type (Observation Group A, n=6), protrusion type (Observation Group B, n=9), and sequestration type (Observation Group C, n=5), based on results of Magnetic Resonance Imaging (MRI) examinations. A total of 20 normal subjects were collected as the control group. Venous blood was collected after admission, enzyme-linked immunosorbent assay (ELISA) was used to detect expression levels of inflammatory cytokines [interleukin-1 (IL-1), IL-6, IL-18, and tumor necrosis factor-alpha (TNF-α)], and visual analogue scale (VAS) scoring was used to evaluate the degree of radicular pain in each group. Analyses were employed to study the correlation of expression levels of IL-1, IL-6, IL-18, and TNF-α with VAS scores. Results: Compared to those in the control group, expression levels of inflammatory cytokines in Group A, Group B, and Group C were significantly increased ($P<0.05$). Compared to Group A, Group B and Group C had obviously elevated expression levels of inflammatory cytokines ($P<0.05$). Expression levels of inflammatory cytokines in Group C were significantly higher than those in Group B ($P<0.05$). Compared to the control group, VAS scores in Group A, Group B, and Group C were significantly increased ($P<0.05$). Compared to Group A, Group B and Group C were found with obviously elevating VAS scores, while the differences were of statistical significance ($P<0.05$). VAS scores in Group C were significantly higher than that in Group B ($P<0.05$). Expression levels of IL-1, IL-6, IL-18, and TNF-α after lumbar intervertebral disc protrusion were positively correlated with radicular pain. Conclusion: Expression levels of inflammatory cytokines (IL-1, IL-6, IL-18, and TNF-α) in the venous blood of patients with lumbar intervertebral disc protrusion are higher than those in normal people, as the lumbar intervertebral disc protrusion aggravated, and positively correlated with radicular pain. These can be used as indexes to reflect the degree of radicular pain. Quantification and digitization of radicular pain are helpful in guiding the clinical drug dose and treatment, as well as better evaluating curative effects.

Keywords: Lumbar intervertebral disc protrusion, radicular pain, inflammatory cytokines, correlation

Introduction

Lumbar intervertebral disc protrusion is a common clinical disease in orthopedics, mainly characterized by radicular symptoms such as low back pain and paresthesia of the lower limbs. It is implicated with complicated pathological responses, involving a series of related pathological mechanisms. Currently, it is known that inflammation in local intervertebral disc tissue after lumbar intervertebral disc protrusion produces massive inflammatory cytokines, including pro-inflammatory and anti-inflammatory cytokines, to regulate pathological responses after lumbar intervertebral disc protrusion and mediate radicular pain [1]. Inflammatory cytokines produced after lumbar intervertebral disc protrusion consist of pro-inflammatory and anti-inflammatory cytokines. Of these, interleukin-1 (IL-1), IL-6, IL-8, IL-18, and tumor necrosis factor-alpha (TNF-α) are the major pro-inflammatory cytokines that mediate and aggravate inflammation. IL-10 is the main anti-inflammatory factor suppressing inflammation. A current study has shown that [2] highly expressed pro-inflammatory cytokines after occurrence of

lumbar intervertebral disc protrusion are important factors leading to radicular pain. However, correlations of the above inflammatory cytokines with severity of radicular pain that occurs after lumbar disc herniation are not yet known. Therefore, this study aimed to determine the correlation of radicular pain with expression levels of inflammatory cytokines after lumbar intervertebral disc protrusion, to identify the potential as indexes to evaluate degrees of radicular pain.

Patients and methods

General data

A total of 20 patients with lumbar intervertebral disc protrusion, hospitalized from October 2016 to October 2017, were collected as the observation group. These 20 patients with lumbar intervertebral disc protrusion were divided into bulging type (Observation Group A, n=6), protrusion type (Observation Group B, n=9), and sequestration type (Observation Group C, n=5), according to Magnetic Resonance Imaging (MRI) results of lumbar intervertebral disc protrusion [3]. In addition, 20 normal subjects were selected as the control group.

Diagnostic criteria

Diagnostic criteria for lumbar intervertebral disc protrusion are shown in **Table 1**.

Inclusion criteria

Inclusion criteria included: (1) Patients that met the above diagnostic criteria for lumbar intervertebral disc protrusion and were accompanied with radicular pain; (2) Patients aged 18-60; (3) Patients that agreed to participate in this study and signed the informed consent; (4) Patients that did not receive other drug treatment and other related treatments in the past 2 weeks; and (5) Patients volunteering to comply with the doctor's advice and strictly cooperate with treatment.

Exclusion criteria

Exclusion criteria included: (1) Patients that did not meet the above inclusion criteria; (2) Pregnant or lactating women; (3) Patients with major internal medicine diseases such as hypertension, diabetes, and heart disease; (4) Patients with history of major diseases like serious primary disease and mental diseases; and (5) Patients with history of lumbar-related diseases, such as lumbar fractures, lumbar surgery, bone tumors, and bone tuberculosis.

Study methods

Venous blood was taken from each patient immediately admission. Blood was centrifuged and the supernatant was collected. Next, levels of IL-1, IL-6, IL-18, and TNF-α in the venous blood of patients were measured using ELISA kits (Boster, Wuhan, Hubei, China), according to the kit instructions.

Visual analogue scale (VAS) scoring [4] was used to evaluate the degree of radicular pain in patients with lumbar intervertebral disc protrusion. Generally, a ruler with 10 cm in length was adopted in VAS scoring, in which "0" points: painless, "10" points: severe pain, and 1 cm in the middle: 1 point of pain degree. Patients selected the appropriate pain scores according to their own pain degree. Specific scoring standards are shown in **Table 2**.

Observation indexes

VAS scores in each group were observed. Expressions of inflammatory cytokines (IL-1, IL-6, IL-18, and TNF-α) in the venous blood in each group were measured. Correlation of expression levels of inflammatory cytokines (IL-1, IL-6, IL-18, and TNF-α) with the degree of radicular pain in each group were analyzed.

Statistical methods

Statistical Product and Service Solutions (SPSS) 20.0 software was used for statistical analyses. Data are expressed as mean ± standard deviation and *t*-test was employed for data meeting normal distribution and homogeneity of variance. Pearson's correlation analysis was applied to examine correlation. $P<0.05$ suggests that differences are statistically significant.

Results

Comparison of general data

In this study, 20 patients with lumbar intervertebral disc protrusion were enrolled, including 6 cases of bulging type (Observation Group A), 9 cases of protrusion type (Observation Group B),

Table 1. Diagnostic criteria for lumbar intervertebral disc protrusion

No.	Symptom description
1	Lumbar injury or chronic strain
2	Radiating pain, numbness and paresthesia of bilateral or unilateral hip(s) or lower limb(s), and pain may be increased due to increased abdominal pressure
3	Lumbar scoliosis, changes in physiological curvature, limited lumbar mobility, and paraspinous tenderness, percussion pain or radiating pain and numbness of bilateral or unilateral lower limb(s) when percussion
4	Positive in Lasegue test and Bragard test
5	Lumbar degeneration based on X-ray examination
6	Location and extent of protruded intervertebral disk can be confirmed by computed tomography (CT)/MRI examination

Table 2. VAS scoring

Score (point)	Description
0	Painless
1-3	Mild pain
4-6	Moderate pain
7-10	Severe pain

and 5 cases of sequestration type (Observation Group C). The basic information of individuals from each group are shown in **Table 3**. Based on comparison, there were no differences in gender and age among all groups (P>0.05) and the results were comparable.

Expression levels of inflammatory cytokines in venous blood in each group

As shown in **Table 4**, the lowest expression levels of various inflammatory cytokines in the venous blood were found in the control group, while expression levels of inflammatory cytokines in venous blood in Group C were the highest. Compared to the control group, expression levels of inflammatory cytokines were significantly increased in Group A, Group B, and Group C (P<0.05). Compared to those in Group A, expression levels of inflammatory cytokines in Group B and Group C were significantly increased (P<0.05). Compared to Group B, expression levels of inflammatory cytokines in Group C were significantly elevated and differences were of statistical significance (P<0.05).

VAS scores in each group

According to **Table 5** and **Figure 1**, VAS scores were the lowest in the control group and the highest in Group C. Compared to the control group, VAS scores in Group A, B, and C were clearly increased (P<0.05). Compared to Group A, Group B, Group C had evidently elevated VAS scores (P<0.05). Compared to Group B, VAS scores in Group C were clearly increased (P< 0.05).

Correlation of inflammatory cytokines with VAS scores of radicular pain

Correlation of inflammatory cytokines with VAS scores was further analyzed. Of note, R^2 value in the analysis of correlation between IL-1 in the venous blood in patients with lumbar intervertebral disc protrusion and VAS scores was 0.5730, and IL-1 was significantly positively related to radicular pain (P<0.05) (**Figure 2A**).

Table 3. General data in each group

Group	Male (n)	Female (n)	Average age (years old)
Control group	11	9	45.7 ± 10.67
Observation Group A	3	3	39.1 ± 12.27
Observation Group B	6	3	44.1 ± 11.77
Observation Group C	3	2	41.1 ± 12.93

The R^2 value in the analysis of relationship between IL-6 in the venous blood in patients with lumbar intervertebral disc protrusion and VAS scores was 0.3337, and IL-6 was significantly positively correlated with radicular pain (P<0.05) (**Figure 2B**). The R^2 value in the analysis of correlation between IL-18 in the venous blood in patients with lumbar intervertebral disc protrusion and VAS scores was 0.5927. It revealed a significantly positive relationship between IL-18 and radicular pain (P<0.05) (**Figure 2C**). The R^2 value in the analysis of correlation between TNF-α in the venous blood in patients with lumbar intervertebral disc protrusion and VAS scores was 0.2449. TNF-α had a statistically positive correlation with radicular pain (P<0.05) (**Figure 2D**).

Trends of inflammatory cytokines and VAS scores

According to **Figures 3** and **4**, average expression levels of inflammatory cytokines (IL-1, IL-6, IL-18, and TNF-α) observed at 3, 7, 14, and 21 days after admission showed decreasing trends in the observation group. Additionally, average VAS scores of patients in the observation group showed a downward tendency. This reducing trend of each inflammatory factor in the observation group was consistent with the declining of VAS scores.

Discussion

Lumbar intervertebral disc protrusion induces very complex pathological responses. Inflammation is an important pathological process. It is one of the essential pathological mechanisms leading to radicular pain of lumbar intervertebral disc protrusion. A current study reported that [5] many inflammatory cytokines produced after the inflammatory reaction of lumbar intervertebral disc protrusion can infiltrate and stimulate the nerve root through a series of biochemical reactions, thereby resulting in low back pain and other radicular symptoms. After the occurrence of lumbar interver-

Table 4. Expression levels of inflammatory cytokines in each group ($\bar{x}$ ± s, pg/mL)

Group	IL-1	IL-6	IL-18	TNF-α
Control group	5.66 ± 1.33	3.78 ± 2.56	3.91 ± 2.19	5.01 ± 2.34
Observation Group A	22.98 ± 5.66*	31.31 ± 4.23*	29.99 ± 5.12*	33.19 ± 4.34*
Observation Group B	38.11 ± 6.77$^{*,\#}$	48.99 ± 4.31$^{*,\#}$	41.22 ± 5.91$^{*,\#}$	47.19 ± 4.78$^{*,\#}$
Observation Group C	50.11 ± 6.63$^{*,\#,\Delta}$	59.16 ± 5.43$^{*,\#,\Delta}$	56.77 ± 4.98$^{*,\#,\Delta}$	60.11 ± 6.66$^{*,\#,\Delta}$

Note: In comparison with the Control group, $^{*}P<0.05$, in comparison with Observation Group A, $^{\#}P<0.05$, in comparison with Observation Group C, $^{\Delta}P<0.05$.

Table 5. VAS scores in each group [$\bar{x}$ ± s, point(s)]

Group	VAS score
Control group	0.26 ± 0.01
Observation Group A	6.89 ± 1.21*
Observation Group B	7.39 ± 1.56$^{*,\#}$
Observation Group C	8.10 ± 1.21$^{*,\#,\Delta}$

Note: In comparison with the Control group, $^{*}P<0.05$, in comparison with Observation Group A, $^{\#}P<0.05$, in comparison with Observation Group C, $^{\Delta}P<0.05$.

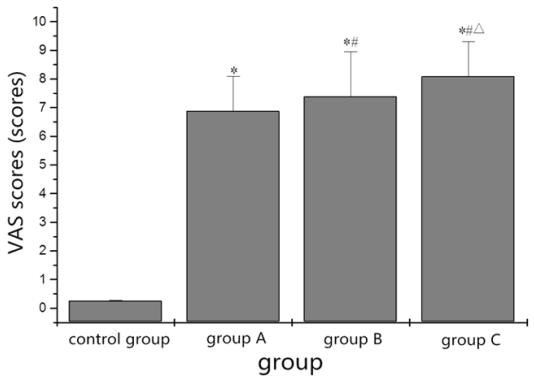

Figure 1. VAS scores in each group. Note: In comparison with the Control group, $^{*}P<0.05$, in comparison with Observation Group A, $^{\#}P<0.05$, in comparison with Observation Group C, $^{\Delta}P<0.05$.

tebral disc protrusion, a variety of factors, such as ischemia, mechanical compression, ion imbalance, and release of oxygen free radicals, can activate various intracellular inflammation-related receptors and cellular pathways, causing extracellular release of various downstream pro-inflammatory cytokines, including IL-1, IL-6, IL-18, and TNF-α, and incidence of inflammation. In particular, inflammatory cytokines play important roles in promoting, maintaining inflammation and mediating nerve root inflammation in prominent intervertebral disc tissues [6, 7]. These inflammatory cytokines contribute to different biochemical roles in stimulating nerve root and pain receptors, leading to occurrence of radicular pain, facilitating amplification of radicular pain, and reflecting changes in pain [8].

IL-1 is an important member of pro-inflammatory cytokines. IL-1 plays a pro-inflammatory role by regulating prostaglandin expression. An animal experiment confirmed that [9] with increase of IL-1 expression, prostaglandin level was also increased. The degree of pain reflex was positively correlated with its expression. In addition, another study indicated [10] that IL-1 was highly expressed in degenerative lumbar intervertebral disc tissues and can induce secretion of massive phospholipase A2 and prostaglandin E2 in intervertebral disc tissues, thus mediating and aggravating inflammation. Therefore, IL-1 is considered as an inducer of radicular pain after lumbar intervertebral disc protrusion. In addition to IL-1, IL-6 is also a key factor in occurrence of radicular pain after lumbar intervertebral disc protrusion. A study showed [11] that, in rat models of radicular pain, pain inhibitors significantly reduced expression of IL-6 in tissues and suppressed the degree of the pain, indicating that IL-6 exerts vital function to give rise to radicular pain. Moreover, an animal experiment [12] showed that IL-1, IL-6, and TNF-α may lead to significant hyperalgesia, with the degree of hyperalgesia positively correlated with the dose of IL-6. These findings indicate that there are close relationships of pain with IL-1, IL-6, and TNF-α. An important pro-inflammatory factor, TNF-α is extensively existed and highly expressed in degenerative intervertebral disc tissues, considered as one of the important factors causing disc degeneration and radicular pain [5, 13, 14]. Some scholars have found that [13] TNF-α in epidural fat of lumbar intervertebral disc protrusion patients with radicular pain was significantly higher than that in epidural fat of those without radicular pain. At the same time, these inflammatory cytokines are closely interrelated and interact on each other. High expression of TNF-α can

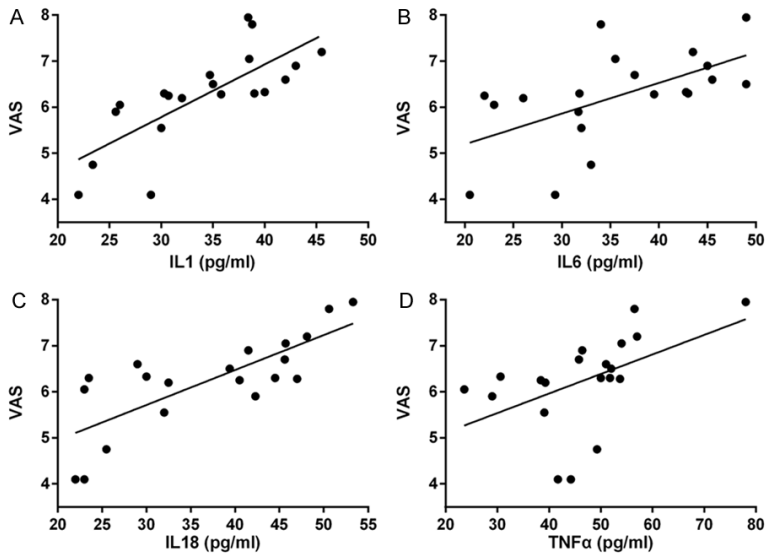

Figure 2. Scatter diagram of correlation. A. IL-1 and VAS. B. IL-6 and VAS. C. IL-18 and VAS. D. TNF-α and VAS.

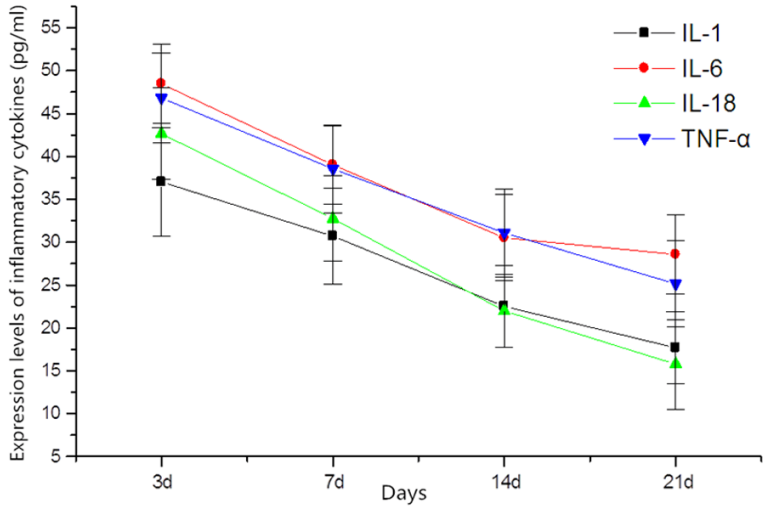

Figure 3. Line chart of change trends of expression levels of inflammatory cytokines.

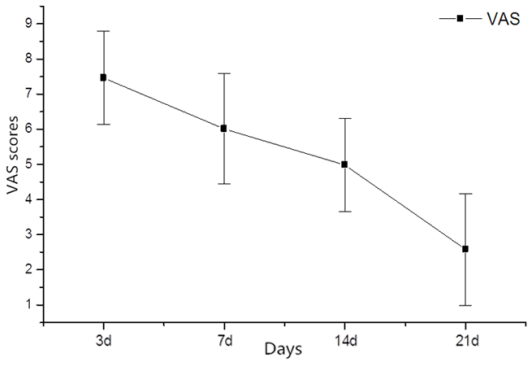

Figure 4. Line chart of change trends of VAS scores.

increase secretion of IL-1 and IL-6, further producing cytokines and inflammatory mediators by stimulating other cells in the periphery, exacerbating inflammatory responses, promoting the production of nerve growth factor, and acting directly on pain receptors, thereby mediating radicular pain and amplifying the degree of pain [15, 16]. IL-18 is an important downstream component of inflammatory responses. After inflammation occurs, many kinds of cytokines [17] and reactive oxygen [18] enter into the cells, along with imbalance of ions inside and outside the cells, which causes aggregation of inflammasomes [such as Nod-like receptor pyrin domain-containing protein 3 (NLRP3)] in the body [19, 20]. This results in the activation of inactive Caspase1 precursor to obtain the function of enzyme digestion and cutting inactive IL-18 precursors into mature factor, thereby pro-inflammatory IL-18 is produced and released outside the cells to mediate inflammation. Results of this present study showed that after occurrence of lumbar intervertebral disc protrusion, expression levels of inflammatory cytokines in the peripheral venous blood of patients were increased, indicating that there is an inflammatory response in patients with lumbar intervertebral disc protrusion. This confirms the finding from the clinical point of view. Moreover, the degree of radicular pain in patients with lumbar intervertebral disc protrusion was aggravated with the degree of lumbar intervertebral disc protrusion and increases of inflammatory cytokines in the body. This suggests correlation between levels of inflammatory cytokines and degree of radicular pain after lumbar intervertebral disc protrusion. Results of statistical analyses showed that inflammatory cytokines

(IL-1, IL-6, IL-18, and TNF-α) in lumbar intervertebral disc protrusion were positively associated with radicular pain.

In conclusion, expressions of inflammatory cytokines (IL-1, IL-6, IL-18, and TNF-α) in the peripheral blood of patients with lumbar intervertebral disc protrusion are correlated with the degree of radicular pain, which can serve as indicators of radicular pain in the clinical practice.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Qiang Yang, The First Department of Spine Surgery, Tianjin Hospital, No. 406 Jiefang South Road, Hexi District, Tianjin 300210, China. Tel: +86-022-60911000; Fax: +86-022-60910608; E-mail: wenyuadingsd@163.com

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