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Full Length Research Paper

Serum cytokines, a diagnostic tool for herniated lumbar disc type

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Inflammatory mediators such as cytokines have been suggested to be important in the pathophysiology of disc herniation. However, few studies have been ever conducted for evaluating the serum levels of these cytokines. This study aimed at assessing the agreement of serum and operation-field cytokines in diagnosis of herniated lumbar disc type. In this study, 43 patients with lumbar disc herniation were recruited in Tabriz Imam Reza Hospital during a 12-month period. According to the type of herniation, the patients were categorized in two groups: with excursion or sequestration (group A) and with bulging (group B) of disc, with 22 and 21 cases, respectively. The level of interleukin (IL)-1α, IL-6 and tumor necrosis factor (TNF)-α was determined in nucleus pulposus (NP) and serum of the patients by employing enzyme-linked immunosorbent assay (ELISA) method. Agreement rate between the two readings was determined. There was full agreement between the serum and NP readings for all the studied parameters. The optimal cut-off points for serum IL-1α, IL-6 and TNFα were ≤0.25, ≤0.05 and ≤0.7 pg/ml, respectively, for discrimination between the extrusion and bulging discs. Serum levels of IL-1α, IL-6 and TNFα may be applicable for preoperative diagnosis of the type of the herniated lumbar disc, especially TNFα, and IL-6 parameters which have a high sensitivity and specificity for differentiation between bulging and extrusion or sequestration discus.

Key words: Intervertebral disk displacement, IL-1α, IL-6, TNFα, serum.

INTRODUCTION

Disc herniation is one of the most common health-medical problems all over the world. In United States, the occurrence rate has been reported 1 in 32. Annually, 8/4 million people around America suffers from discus hernia (American Surgeon's Editorial, 2004). Lumbar disc herniation is one of the most prevalent disc herniation between the vertebra in spine and can occur at any age with two patterns (bulging, extrusion or sequestration) (Roberts et al., 2006). It seems that the symptoms of extrusion and sequestration lumbar disc are more severe than the bulging discs (Aithala et al., 2010). In most cases, pain is the main symptom, although the mechanism of pain and root irritation has not been clarified yet clearly (Olmarker and Rydevik, 1991; Rydevik et al., 1984; Rydevik, 1990). Meanwhile, the role of cytokine has been considered (Kelly, 1956; Marshall et al., 1973; McCarron et al., 1987; Saal et al., 1990; Saal, 1995).

Defining the relation of cytokine and growth factors with vertebral disc has been of interest to various researchers in two recent decades and they got some conflicting results (Le Maitre et al., 2007; Haro et al., 1996; Levine et al., 1984; Ohtori et al., 2011; Nagashima et al., 2009). The aim of this study was to examine the difference of

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Abbreviations: IL, Interleukin; **TNF,** tumor necrosis factor; **NP,** nucleus pulposus; **ELISA,** enzyme-linked immunosorbent assay; **ROC,** Receiver-operating characteristic.

cytokines types and levels in extrusion or sequestration and bulging discus hernia in both nucleus pulposus (NP) and serum samples.

MATERIALS AND METHODS

In this analytical research which was performed in the Neurosurgery Department of Tabriz Imam Reza hospital from 2009 up to 2010, 43 patients with disc herniation were studied in two groups by magnetic resonance imaging (MRI) assessment: 22 cases in extrusion or sequestration group and 21 cases in Bulging group. Patients with previous surgery history and any infection were excluded from study. Pre- inflammatory cytokines [interleukin (IL)-6, IL-1α and tumor necrosis factor (TNFα)] were measured in NP and serum of patients and compared in two groups. Sampling was performed by method which was described by Nygaard et al. (1997).

Before surgery, blood samples of patients were taken and serum samples kept in freezer. In the operation room, after complete homeostasis and preventing the entrance of blood to the surgery field, annulus was cut and nucleus pulposus was out. These samples were frozen directly in liquid nitrogen within 2 min and subsequently at -7°C. The samples were cut into small pieces with the help of a knife, re-suspended in frozen NaCl, and again placed in ice bath. The pieces then were grinded by homogenizer and were ultra-centrifuged for 15 min and the supernatant was used to examine the IL-6 and IL-1α, TNFα by Bender Medsystems commercial kits using the enzyme-linked immunosorbent assay (ELISA) method. Serum samples were also assessed by same kits and method. The laboratory had no information about the clinical condition of patient and the type of hernia and also the surgeon was not reported about the test results.

Statistical analysis

The collected information is represented as average, standard deviation (mean ± SD) and percentage. Differences between groups were analyzed using independent sample T-test and Mann-Whitney U-test. Correlation assessment was analyzed by Spearman's correlation coefficient. For determination of cut-off points, receiver-operating characteristic (ROC) curve were used. McNemar test was used for difference between correlated proportions. Difference were considered to be significant when P≤0.05. The employed application is SPSS version 15.

RESULTS

22 patients (51/2%) had an extrusion or sequestration disc (group A), while 21 patients (48.8%) had a bulging disc (group B) herniation. In all cases, MRI and computed tomographic (CT) results were in accordance with the final diagnosis. The amount of serum TNFα in group A was 2.57 ± 2.39 ρg/ml, and that in group B was 0.20 ± 0.36 ρg/ml. The average of serum TNFα in group A was noticeably higher (p<0.001). The amount of nucleus pulposus TNFα in group A was 4.34 ± 5/43 ρg/ml, and in group B was 0.21 ± 0.33ρg/ml. The average of nucleus pulposus TNFα in group A was noticeably higher (p<0.001).

The amount of serum IL-1α in group A was 1.40 ± 1.36 ρg/ml, while that in group B was 0.17± 0.27 ρg/ml. The average of serum IL-1α in group A was noticeably higher (p<0.001). The amount of nucleus pulposus IL-1α in group A was 1.45 ± 1.18 ρg/ml, and in group B was 0.20 ± 0.29ρg/ml. The average of nucleus pulposus IL-1α in group A was noticeably higher (p<0.001). The amount of serum IL-6 in group A was 6.23 ± 12.27 ρg/ml, and in group B was 0.18 ± 0.57 ρg/ml. The average of serum IL-6 in group A was noticeably higher (p<0.001). In addition, the amount of nucleus pulposus IL-6 in group A was 28/10 ± 84/74 ρg/ml, and in group B was 0.27±0.88 ρg/ml. The average of nucleus pulposus IL-6 in group A was noticeably higher (p<0.001). The correlation between nucleus pulposus and serum variants in all patients was as follows: There was a noticeable and strong positive correlation between the serum and nucleus pulposus TNFα, IL-1α and IL-6 quantities in all patients (respectively p<0.001, rho=0.827, p<0.001, rho=0.903 and p<0.001, rho=0.905). There was also a noticeable and strong positive correlation between the serum and nucleus pulposus TNFα, IL-1α and IL-6 quantities in group A patients. Furthermore, there was a noticeable and strong positive correlation between the serum and nucleus pulposus IL-1αand IL-6 quantities in group B patients but there was no noticeable correlation between the serum and nucleus pulposus TNFα quantities in this group (p<0.001, rho=0.296).

Cut-off points in these cytokines to estimating extrusion or sequestration by using ROC curve (Figure 1), was as follows:

a) Serum TNFα: The best cut-off point≥0.7 ρg/ml with 83% sensitivity and 90% specificity.
b) Nucleus pulposus TNFα: The best cut-off point ≥0.9 ρg/ml with 77% sensitivity and 95% specificity.
c) Serum IL-1α: The best cut-off point ≥0.25 ρg/ml with 77% sensitivity and 76% specificity.
d) Nucleus pulposus IL-1α: The best cut-off point ≥0.35 ρg/ml with 82% sensitivity and 81% specificity.
e) Serum IL-6: The best cut-off point ≥0.05 ρg/ml with 95% sensitivity and 81% specificity.
f) Nucleus pulposus IL-6: the best cut-off point ≥0.05 ρg/ml with 95% sensitivity and 81% specificity.

The results of examining the agreement of serum and nucleus pulposus parameters showed that there was complete agreement between TNFα, IL-1α and IL-6 parameters in serum and nucleus pulposus in both two groups. In comparison, there was no noticeable statistical difference between the average of TNFα, IL-1 and Il-6 in serum and nucleus pulposus in the two groups.

DISCUSSION

In this research, the level of TNFα, IL-1α, IL-6 in NP and serum of patients with disc herniation and their difference in two types of hernia: bulging and extrusion or sequestration discs was studied. Based on the results,

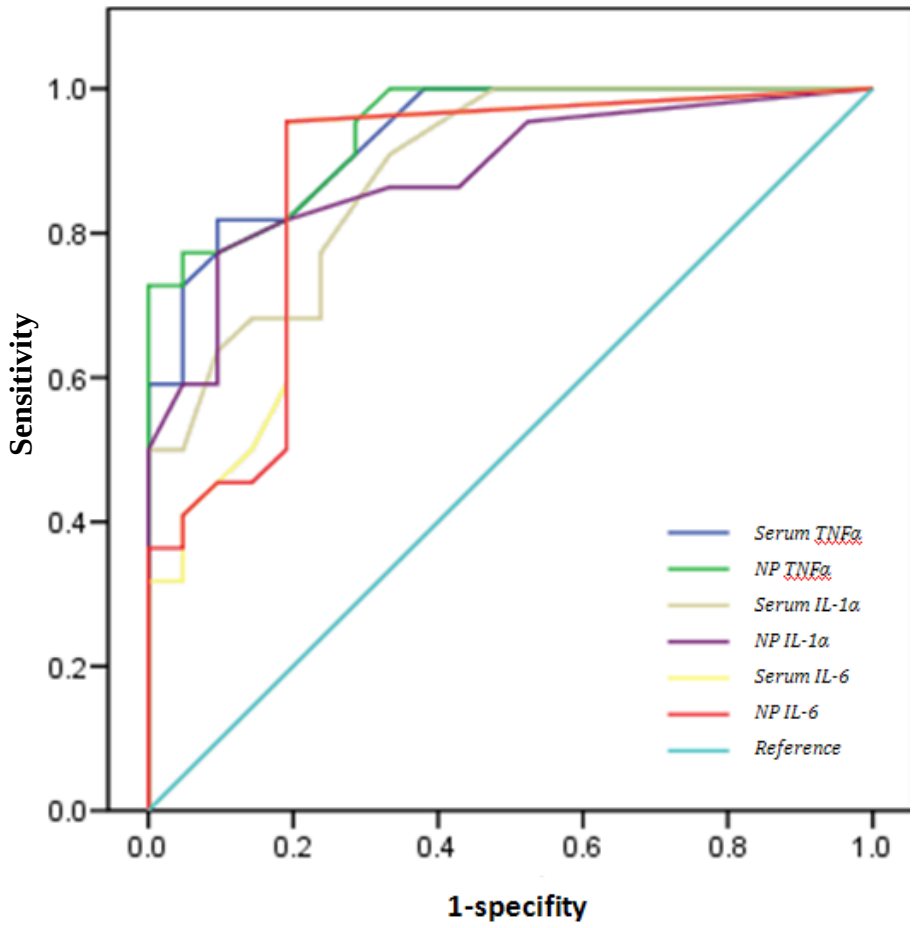

Figure 1. Serum and NP cytokines ROC curve for determination of extruded disc.

the levels of these cytokines in serum and nucleus pulposus in extrusion or sequestration discus was considerably high. Several studies showed different results.

Nygaard et al. (1997) represented that there is no considerable difference between the level of cytokines like TNFα, IL-6 in normal and herniated discs. However, the levels of LTB4 and TXB2 in disc herniation are considerably higher and it is concluded that the several kinds of discs have different inflammatory processes (Nygaard, et al., 1997). In another research, Kang et al. (1996) represented that IL-6 level in hernia discus is considerably higher than normal discus, but there is no difference between TNFα, IL-6 levels in the two groups. Study on 30 hernia patients showed that the IL-6 level topically increases in these patients (Specchia et al., 2002). A study on 77 patients with perforated, extrusion and sequestration discs also showed that the IL-1 level is topically higher in perforated patients, while there is no difference in TNFα and IL-6 levels in three groups (Takahashi et al., 1996). In addition, Genevay et al. (2008) represented that TNFα level in hernia patients is considerably higher. A study showed that elevated CSF IL-6 concentration could indicate pathological nerve damage or degeneration of lumbar radiculopathy (Ohtori et al., 2011).

A real time-PCR study showed that IL-1 β and TNFα cytokines are produced by human degenerated intervertebral cells and IL-1β is expressed at higher levels in degenerated discs (Le Maitre et al., 2007). Another study also showed that the concentration of TNFα, IL-1 β and IL-6 cytokines in colony-stimulating factor (CSF) can indicate certain pathological aspects of cervical myelopathy or lumbar radiculopathy (Nagashima et al., 2009). Other studies showed that cytokines like IL-1 β and IL-6 have high concentrations in the herniated discs associated with myelopathy (Demircan et al., 2007). In an Immunohistochemical study, IL-4, IL-6, IL-12 and IFNγ levels were modest in surgical degenerated and higher in herniated discs (Shamji et al., 2010). A study on 39 patients with disc herniation and sciatica showed that serum and CSF level of TNFα, IL-1, IL-6, IL-8, and IFN-γ were normal except IL-8 (Brisby et al., 2002).

As earlier mentioned, the results of various studies on this field are different and sometimes incoherent. However, we represented that the cytokines levels in

extrusion or sequestration discs in nucleus pulposus and serum are higher than bulging discs. This is the first study that compares cytokines levels in serum and nucleus pulposus in bulging and extrusion or sequestration herniated discs. It is demonstrated that releasing the substances from inner section of discus (NP) in perforated cases can cause local inflammation and evoke the leukocytes (Byröd et al., 2000; Olmarker et al., 1996; Yabuki et al., 1998). These phenomena increase the vessels permeability and cause the elevation of cytokines in blood (Brisby et al., 2002). Simultaneous elevation of nucleus pulposus and serum cytokines levels (with high correlation and agreement) in our study confirms this fact.

One of the other advantages of this study is the definition the cut-off points of cytokines for serum and nucleus pulposus in bulging and extrusion or sequestration discus .Therefore, using TNFα, IL-6 parameters have a high sensitivity and specificity for differentiation between bulging and extrusion or sequestration discus. By considering the high compatibility of serum and nucleus pulposus levels, serum measurements can be used as a simple and non-invasive method to diagnose and estimate the patient condition. However, we need more study to examine the exact role of these inflammation parameters in discus hernia pathogenesis.

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