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Orginal Article

The comparison of the mRNA expressions of TGFβ1, bFGF, ıGF-1, NGF and matrix metalloprotease ıı genes in cervical and lumbar disc tissues

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Abstract

Aim: To evaluate and compare the mRNA expression of antioxidant genes between the cervical and lumbar degenerated disc tissues.
Material and Methods: Obtained degenerated Nucleus Pulposus (DNP) materials were divided into two groups, which were cervical DNP (Group I) (n=20) and lumbar DNP (Group II) (n=20). There are 6 men and 14 women in group I with a mean age of 43.8 years and 13 men and 7 women in group II with the mean age of 40.3 years (28 to 54). All cervical DNP materials were obtained between C5 and C6 and all lumbar DNP materials were obtained between L4 and L5 levels. The TGFβ1, bFGF, IGF-1, NGF and Matrix metalloprotease III (MMP III) gene expressions of DNP materials were determined by polymerase chain reaction, Real-time polymerase chain reaction and western blotting techniques.Results: When the results of the two groups were compared by polymerase chain reaction, the expressions of TGFβ1, bFGF, IGF-1, NGF and MMP III was found lower in lumbar DNP group. Also the TGFβ1, bFGF, IGF-1, NGF, and Matrix metalloprotease III genes showed a decrease in gene expression levels when they were analyzed by Real-time polymerase chain reaction.
Conclusion: These data showed that; decrease of the TGFβ1, bFGF, IGF-1, NGF and MMP III genes in the degenerated lumbar disc tissues, may related with the possibility of molecular background of the disease pathogenesis.

Keywords: Degenerated Nucleus Pulposus; mRNA Expression; Cervical; Lumbar.

INTRODUCTION

The intervertebral disc (IVD) is a functional unit of the spine and it allows to movement in bend and twist (1). During this movements it pretends like a dynamic sponge and it absorbs the axial loading forces (2). IVD is formed by the proteoglycan-rich and water-rich nucleus pulposus (NP) who surrounded by collagen-rich annulus fibrosus (AF) (3). The degeneration of this structure leads to chronic low back pain (4). The degeneration of IVD is characterised by degradation of the extracellular matrix and the loss of the water content (5) and it's related with extrinsic, intrinsic and genetic factors (6,7). The degeneration of the IVD may lead to several degenerative pathologies in spine like facet hypertrophy or spinal stenosis (8,9).

Some types of the transforming growth factor (TGF) can play an important role in the anabolic metabolism of IVD and they can upregulate the expression of some metalloproteinases (10). There are several studies about the relation of proliferative responses of TGF, fibroblast growth factor (FGF) and insulin-like growth factor (IGF) (11).Although TGF has induce greater proliferative response, FGF and IGF have similar effect too (11). Beside these reports, numerous studies indicate that; a variety inflammatory mediators cytokines like IL-1∞, TNF-∞, IL-6, IL-1ß, fosfolipaz A2, prostoglandin E2 (PGE2) can play a major role in degeneration processes (12-14). Therefore, the number of the studies to investigate the relation between the degeneration and these mediators are increases (15,16).

The aim of the present study was to evaluate on mRNA expression of TGFβ1, bFGF, IGF-1, NGF and MMP III genes in cervical and lumbar degenerated nucleus pulposus (DNP) tissues and to compare the results between the cervical and lumbar DNP.

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MATERIAL and METHODS

Experimental design

This study was approved by the Ethics Committee of our university,

Obtained DNP materials were divided into two groups, which were cervical DNP (Group I) (n=20) and lumbar DNP (Group II) (n=20).

Surgical procedure: Samples of DNP were collected from forty patients. All patients underwent to simple micro discectomy in same Neurosurgery Clinic. The surgery decision had been confirmed by radiological studies and clinical examination. The written informed consent was signed by all participants before being enrolled into the study. There are 6 men and 14 women in group I with a mean age of 43.8 years (34 to 60) and 13 men and 7 women in group II with the mean age of 40.3 years (28 to 54). All cervical surgeries were made between C5 and C6 and all lumbar surgeries were made between L4 and L5 levels.

PCR and RT-qPCR analysis

Total RNA isolation from NP tissues was performed by using TRIzol Reagent (Sigma Chemical Co., St. Louis, MO, USA). Following to DNAse I (RW1 DNAse I from Promega, Madison, WI, USA) treatment, 5 µg of total RNA was used for reverse transcription (RT) reaction which was supplied with oligo dT22 primer and 1 unit of Moloney Murine Leukemia Virus reverse transcriptase (Fermantas, Vilnius, Lithuania). Validation of amplification efficiencies which allows quantitative gene expression level measurement was performed for the all targeted genes and Glyceraldehyde 3-Phosphate Dehydrogenase as well.The TGFβ1, bFGF, IGF-1, NGF, and MMP III gene expression levels were quantified by using the MyGenie96 Thermo Bioneer thermal cycler. The PCR contained 1X Taq DNA polymerase buffer, 1 U of Taq DNA polymerase, 10 µM of each primer, 1.5 mM dNTPmix, 1.5 mM MgCl2, and 1 µL of cDNA template. The amplification was performed under the following conditions: 3 min at 94 °C; 45 sec. at 94 °C, 45 sec at 50-60 °C, and 1 min at 94 °C (30 cycles); 10 min at 72 °C as final extension.

The TGFβ1, bFGF, IGF-1, NGF, and MMP III gene expression levels were also quantified through RT-qPCR by using the real-time PCR analyzer Roche Light cycler (Roche molecular diagnostics, Mannheim, Germany). The RT-qPCR contained 500 nM of forward and reverse primers, 100 nM labeled probe, cDNA (1 µL) and SYBR Green Universal PCR master mix (12.5 µL; Perkin-Elmer Applied Biosystems, California, USA) in 25 µL final reaction volume. The amplification was performed under the following conditions: 2 min at 50 °C; 10 min at 95 °C, followed by 50 cycles of 15 sec at 95 °C, and 1 min at 60 °C.

Recombinant Protein Expression and Western Blotting

E.coli Rosetta harbors pET16b (+) 6XHis plasmid was incubated at 37 °C overnight. Recombinant protein expression was induced by the addition of 0.1 mM IPTG. Protein expression was performed at 37 °C for further 3 hours incubation. The Ni-NTA resin column chromatography was employed for the purification of soluble 6XHis tagged recombinant proteins as instructed by the manufacturer. Following to SDS-PAGE (12%) analysis of the purified recombinant proteins, they were transferred to a polyvinylidene fluoride membrane. Then, the ProteoQwest colorimetric kit with TMB substrate (Sigma), anti-His6 monoclonal mouse antibody, and secondary anti-mouse antibody conjugated with HRP was used for the detection of 6XHis tagged recombinant proteins on membrane. Kaleidoscope prestained ladder was used as molecular mass marker.

RESULTS

As a result, when lumbar DNP (Group II) were compared with cervical DNP (Group I), a decrease in expressions of TGFβ1, bFGF, IGF-1, NGF and Matrix metalloprotease III genes was observed in Group II (Figure 1A, 1B). This phenomenon demonstrates that lomber DNP (Group II) has both decrease expressions of TGFβ1, bFGF, IGF-1, NGF and Matrix metalloprotease III genes.The TGFβ1, bFGF, IGF-1, NGF, and Matrix metalloprotease III genes showed a decrease in gene expression levels when they were analyzed by real-time PCR. (Table 1). When lomber DNP (Group II) were compared with cervical DNP (Group I), in terms of protein expressions; a decrease in TGFβ1, bFGF, IGF-1, NGF and Matrix metalloprotease III genes was observed in Group II (Figure 2).

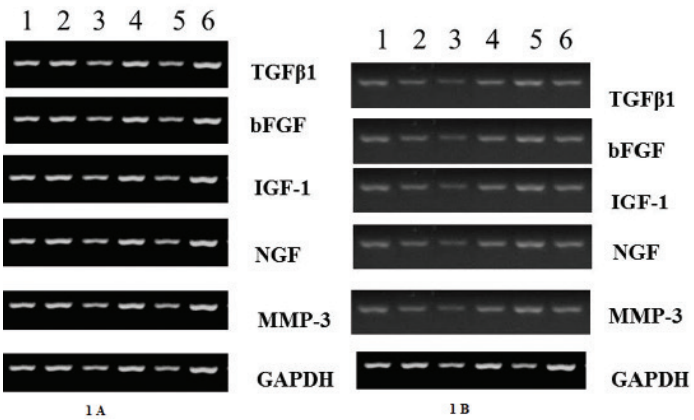

Figure 1. Gene expressions in cervical degenerated nucleus pulposus **(A)** and in lomber degenerated nucleus pulposus **(B).**

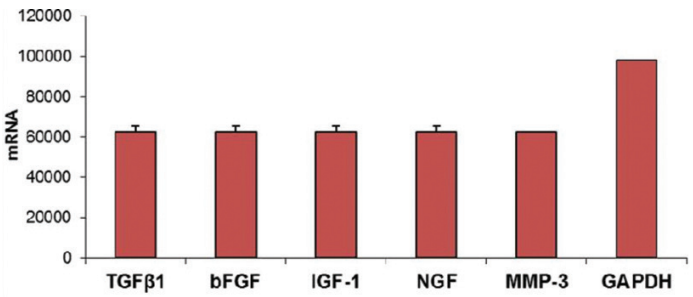

Table 1. Quantitative real-time RT-PCR analysis results for TGFβ1, bFGF, IGF-1, NGF and Matrix metalloprotease III.

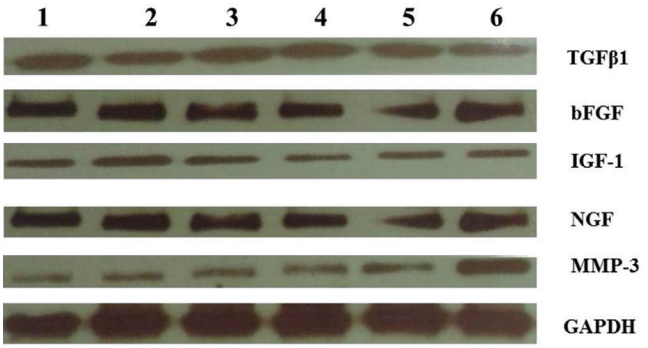

Figure 2. Protein expressions of TGFβ1, bFGF, IGF-1, NGF and Matrix metalloprotease III.

DISCUSSION

Degenerative disc disorder is one of the most common and important disorders in general and neurosurgical practices. Although the exact pathogenesis is not well known, there are several hypotheses about the associations between the pathological or aging changes and genetic background (17,18). The IVD regulate its activity via anabolic or catabolic substances (19,20). While the TGF-β, IGF, and the Bone Morphogenetic Proteins are anabolic regulators, MMPs and cytokines are catabolic regulators (20,21), MMPs and cytokines are the catabolic regulators (22-24). The IVD degeneration can be result by the imbalance of between these two regulators. Kang et al showed that in their study; in DNP; nitric oxide, interleukins, prostaglandins and different MMPs are produced spontaneously in increased amount (14)

There are several studies about the proliferative effect of the TGF-β. Walsh et al. reported that; the consecutively four injections of TGF-β (once per week), could induce to a stimulatory effect in the mouse caudal lumbar disc (25). But controversially Wallach et al reported that; the TGF-β1 gene transduction result with the inhibition of PG synthesis (26). But it should be kept in mind the intradiscal application of TGF might stimulate angiogenesis and as a result of it might be stimulate the nerve ingrowth too, therefore, the intradiscal TGF injection may exacerbate the symptoms of disc degeneration like pain (27).

In Park et al study controversy to our study, they didn't find any difference between the lumbar and cervical discs in term of gene expression patterns in degenerated intervertebral disc tissue, but they investigated unlike us the mRNA expression of alkaline phosphatase, Sox9, aggrecan, type I collagen, type II collagen and osteocalcin. Also they didn't find any statistical differences in terms of, mRNA expressions for all matrix-associated genes between the lumbar and cervical degenerated disc tissues after TGF-β1 treatment (28).

Because of the back pain is more common than the cervical pain the studies usually focus on lumbar disc pathologies rather than cervical discs (29-31). Further there are some well-known anatomical and structural differences between the lumbar and cervical discs (32), but we cannot argue this for the gene expressions. In our study we tried to investigate this opinion by the evaluation of the gene expression differences between the cervical and lumbar degenerated discs. Because of this, we aimed to reveal the mRNA expression of TGFβ1, bFGF, IGF-1, NGF and MMP III genes in disc tissue of cervical and lumbar discs. Our results revealed that the significant decrease in expressions of TGFβ1, bFGF, IGF-1, NGF and MMP III genes was observed in lumbar DNP when we compared with cervical DNP. Scott et al. reported that; richest zone collagen content of the NP is cervical area and the lowest collagen content of the NP was in lumbar area (33,34) this situation may be related by the cervical discs have much greater cells number or cervical discs have higher gene expression. This situation may the reason of our results.

Our study has several limitations; first of all we haven't any control group so the results didn't compare with healthy groups. Secondly, several pro-inflammatory cytokines could be used for the evaluation and compare the degenerated lumbar and cervical disc tissues.

CONCLUSION

In conclusion, we showed decrease in expressions of TGFβ1, bFGF, IGF-1, NGF and MMP III genes in lumbar discs, which may contribute to disease pathogenesis.

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