

# Relationship between serum levels of miR-204 and clinical features of patients with lumbar disc herniation - an analysis based on 1,589 cases

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**Abstract.** This study aims to investigate the relationship between serum miR-204 and clinical features of patients with lumbar disc herniation (LDH). Clinical data of 1589 LDH patients were retrospectively analyzed. There were 953 patients (group A) with a duration of disease shorter than 3 months, and another 636 patients (group B) with a duration of disease longer than 3 months. A total of 300 healthy volunteers were also selected in the same period. Results showed that there was no significant difference in miR-204 expression between males and females ( $p > 0.05$ ). No significant differences in miR-204 expression level were found among different ages ( $p > 0.05$ ). miR-204 in patients with LHD at or below the fourth lumbar vertebrae was significantly higher than those in patients with LHD above the fourth lumbar vertebrae ( $p < 0.05$ ). miR-204 in 1485 patients with abnormal lumbar spine activity was higher than those in healthy controls ( $p < 0.05$ ). miR-204 expression levels was higher in patients with low back pain than those in patients without low back pain. Among patients with low back pain, miR-204 level in patients with spasm-like waist pain was significantly higher than that in patients with persistent dull low back pain ( $p < 0.05$ ). miR-204 increased with the increase in degree of limited walking ( $p < 0.05$ ). There was no significant difference in miR-204 expression level between patients with bilateral lower limb symptoms and patients with unilateral lower limb symptoms ( $p > 0.05$ ), while miR-204 was significantly higher in patients with lower limb symptoms than that in patients without the symptoms ( $p < 0.05$ ). miR-204 was correlated with

knee tendon reflex, heel tendon reflex and degree of straight leg raise abnormality. miR-204 was significantly decreased in patients with improvement after treatment. miR-204 level was closely related to the clinical characteristics of LDH. miR-204 may potentially serve as a prognostic marker for patients with LDH.

## Introduction

Lumbar disc herniation (1-3) (LDH) is usually caused by the intervertebral disc contents (nucleus pulposus) protruding through its outer membrane (fibrous rings). LDH usually occurs in posterolateral region. As one of the most common diseases, LDH mainly affects patients aged 40-50 years (mean age of 37 years). The incidence in males >35 years is 4.8% (4,5). LDH now has been recognized as a health problem world-wide (6). LDH is the most common diagnosis of lumbar degenerative abnormalities. Typical LDH includes initial low back pain and may eventually develop into persistent sciatica (7). LDH is characterized by the loss of proteoglycan and water content in the nucleus pulposus of the intervertebral disc. Although some genetic determinants have been reported, the etiology of LDH is largely unknown (8). miRNA-204 has differential expression in different types of tumors, and is closely correlated with proliferation, invasion and poor prognosis of tumors (9,10). However, the role of miRNA-204 in LDH is still unclear. In this study, expression of miR-204 in serum was detected by RT-PCR, and relationship between miR-204 expression and clinical features of patients with LDH was investigated.

## Patients and methods

**Clinical data.** Clinical data of 1,589 LDH patients who were diagnosed in Shanghai Sixth People's Hospital East Affiliated to Shanghai University of Medicine and Health Sciences from January 2014 to January 2017 were retrospectively analyzed. Those patients included 869 males and 720 females. There were 953 patients (group A) with a duration of disease shorter than 3 months, and the other 636 patients (group B) were with a duration of disease longer than 3 months. The average age of the patients was  $39.2 \pm 5.8$  years. At the same time, a total of 300 healthy volunteers were also selected, and their average

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Table I. Comparison of clinical data among three groups.

Variables	Healthy control (n=300)	Group A (n=953)	Group B (n=636)	P-value
Sex				
Male/female	169/131	521/432	348/288	0.531
Age (years)	35.6±7.2	35.3±6.2	43.1±5.4	0.889
Duration of disease (days)		45.6±14.3	101.8±11.2	0.003
Smoking	134 (44.7%)	464 (48.7%)	333 (52.4%)	0.437
Drinking	154 (51.3%)	530 (55.6%)	364 (57.2%)	0.853
Lower limb pain		542 (56.9%)	378 (59.4%)	0.524
Lower limb weakness		461 (48.4%)	379 (59.6%)	0.771
Numbness of the lower limbs		91 (9.5%)	179 (28.1%)	0.883
Lumbar activity abnormalities		857 (89.9%)	628 (98.7%)	0.579
Persistent dull back pain		145 (15.2%)	224 (35.2%)	0.512
Spasm-like waist pain		320 (33.6%)	321 (50.5%)	0.442
Straight leg raise positive		322 (33.8%)	322 (50.7%)	0.399
Heel tendon reflex positive		130 (13.6%)	129 (20.3%)	0.723
Knee tendon reflex positive		131 (13.7%)	130 (20.5%)	0.763
Gatism		0 (0%)	5 (0.8%)	0.791

age was 35.6±7.2 years. Patients in group A were treated with strict conservative treatment, and patients in group B were treated with surgical treatment. All the patients in group A and B were treated for one month. Efficacy was evaluated according to Stauffer-Coventry's (SC) efficacy evaluation criteria (11). The study was approved by the Ethics Committee of Shanghai Jiaotong University Affiliated Sixth People's Hospital (Shanghai, China).

Inclusion criteria: patients diagnosed with LDH (12); patients met the 'LDH diagnosis and treatment guidelines'; patients showed no vertebral spondylolisthesis and spinal stenosis. Exclusion criteria: patients with diabetes mellitus; patients with hypertension; patients with congenital spinal deformities. All the patients or their families signed an informed consent.

**Instruments and reagents.** PCR machine, TaqMan® MicroRNA reverse transcription kit, 100 bp DNA marker, 2x Taq PCR MasterMix (Qiagen, Duesseldorf, Germany), TaqMan® MicroRNA Cells-to-CT™ kit, was all purchased from Thermo Fisher Scientific, Inc. (Waltham, MA, USA). UV-9000S dual-beam UV-visible spectrophotometer was purchased from Shanghai Metash Instruments Co., Ltd. (Shanghai, China). U6 endogenous control primers Guangzhou Shan-Geng-Yuan was purchased from Biotechnology Development Co., Ltd. (Guangzhou, China).

**Extraction of total RNA for the detection of miR-204 expression.** Total miR-204 was extracted from tissue and serum sample according to the methods provided by Thermo Fisher Scientific, Inc. The purity of RNA is expressed at the absorbance value of 260/280 nm, and only the one with ratio between 1.9-2.1 was used. Otherwise, RNA extraction was performed again to get samples with satisfactory quality.

**RT-qPCR reaction conditions.** Total RNA was taken to synthesize complementary deoxyribonucleic acid (cDNA) using the RT Revert Aid First Strand cDNA Synthesis kit and moloney murine leukemia virus (M-MLV) reverse transcriptase (both from Thermo Fisher Scientific, Inc.). The reaction system volume was in total 25 µl, pre-denaturation at 95°C for 5 min, denaturation at 95°C for 30 sec, annealing at 60°C for 45 sec, extension at 72°C for 3 min, with 35 cycles, and then extension at 72°C for 5 min. PCR products were stored at 4°C. Quantitative analysis was carried out using the ABI 7500 fluorescence PCR amplification instrument (Applied Biosystems; Thermo Fisher Scientific, Inc.). Primers used in PCR reaction were listed as follows: U6 forward primer, 5'-CTCGCTTCGGCAGCAC-3'; reverse primer, 5'-AACGCTTCACGAATTTGCGT-3'. miR-204 forward primer, 5'-CTGTCACCTCGAGC TGCTGGAATG-3'; reverse primer, 5'-ACCGTGTCTGGAGTCGGCAATT-3'. Statistical analysis: three replicates were included and the average values were calculated. With U6 as endogenous control, the relative expression level of miR-204 was calculated by 2<sup>-ΔΔC<sub>q</sub></sup> method (13).

**Statistical analysis.** All data were statistically analyzed by SPSS 22.0 [AsiaAnalytics (formerly SPSS China), Shanghai, China]. Measured data were expressed as mean ± standard deviation, and non-parametric KS test was used for comparisons between two groups, and one-way ANOVA test followed by the Least Significant Difference post hoc test was used for comparison among multiple groups. Comparison of enumeration data were performed by χ<sup>2</sup> test. Analysis of the correlation between miR-204 expression and clinical features of intervertebral disc herniation was performed by logistic regression analysis. P<0.05 was considered to indicate a statistically significant difference.

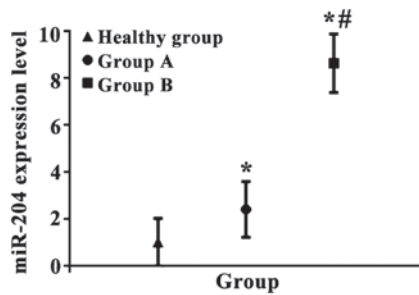


Figure 1. miR-204 expression level in group A, group B and healthy group. \*P<0.05 compared with healthy group; #P<0.05 compared with group A.

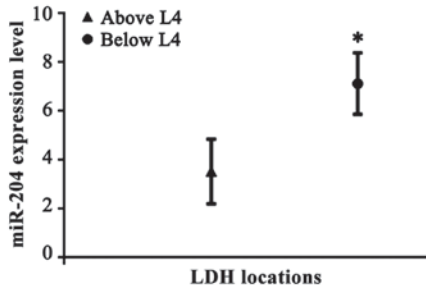


Figure 2. Expression level of miR-204 in patients with lesions below lumbar L4 and above lumbar L4. \*P<0.05 compared with lesions above lumbar L4.

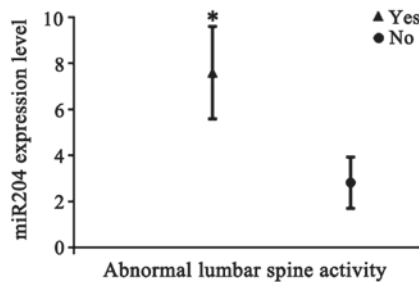


Figure 3. Effects of lumbar spine activity on miR-204 expression. \*P<0.05 compared with healthy control without lumbar spine activity.

**Results**

*General information.* No significant differences in sex and age were found among three groups (P>0.05). LDH at or below L4 is the dominant type, and accounted for 66% (1,049 cases) of all the patients. The average duration of disease was 45.6±14.3 days in group A and 101.8±11.2 days in group B. Patients in group A and B usually showed lower extremity pain and abnormal lumbar motion. Among 1,589 patients, 1,343 cases (84.5%) were positive in straight leg raise test, 539 cases (33.9%) were positive in heel tendon reflex, and 543 (34.2%) cases were positive in knee tendon reflex. Only 5 patients (0.3%) showed gatism, and all of them were in group B (Table I). Significant differences in medical history between 2 groups were found (P<0.05).

*There was no significant difference in miR-204 expression level between male group and female group (p>0.05). No significant differences in miR-204 expression level were found among patients in different age groups (P>0.05). Expression*

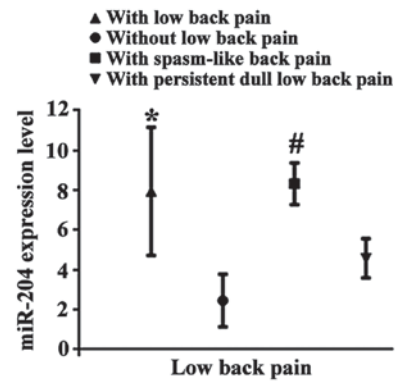


Figure 4. Effects of lumbar back pain on miR-204 expression. \*P<0.05 compared with those without low back pain; #P<0.05 compared with persistent dull low back pain.

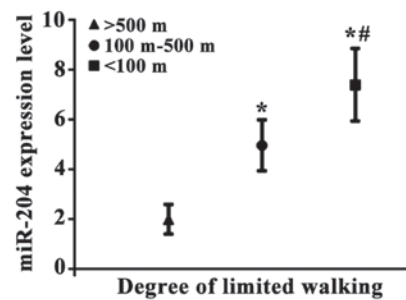


Figure 5. Effects of the degree of limited walking on expression level of miR-204. \*P<0.05 compared with walking for more than 500 m; #P<0.05 compared with walking for 100-500 m.

level of miR-204 was significantly higher in group B than that in group A (8.653±1.251 vs. 2.432±1.184), and it was significantly higher in group A than that in the control group (2.432±1.184 vs. 1.032±1.015; P<0.05) (Fig. 1).

*Effects of different LDH locations on miR-204 expression.* LDH at or below L4 is the dominant type in group A and B, and accounted for 66% (1,049 cases) of all the patients. Expression level of miR-204 was significantly higher in those patients than in the patients with LDH above L4 (7.135±1.254 vs. 3.546±1.328; P<0.05) (Fig. 2).

*Effects of lumbar spine activity and low back pain on miR-204 expression.* Expression levels of miR-204 in 1,485 patients with abnormal lumbar spine activity were higher than these in healthy controls (7.624±2.006 vs. 2.843±1.115; P<0.05) (Fig. 3). Patients with low back pain accounted for 84.1% (1,336 cases) of all the patients in this study, and expression level of miR-204 was higher in those patients than that in patients without low back pain (7.946±3.211 vs. 2.465±1.323). Among patients with low back pain, miR-204 expression levels in patients with spasm-like back pain were significantly higher than those in patients with persistent dull low back pain (8.324±1.045 vs. 4.589±0.987; P<0.05) (Fig. 4).

*Effects of the degree of limited walking on expression level of miR-204.* Patients with limited walking (486 cases, 30.6%) were all accompanied by lumbar pain. Among the patients,

Table II. Results of specific experiments and miR-204 expression levels.

Test	Positive		Negative		P-value
Knee tendon reflex test	644 (40.5%)	7.052±2.041	945 (59.5%)	2.654±1.322	0.015
Heel tendon reflex test	259 (16.3%)	6.875±1.846	1.330 (83.7%)	1.742±1.023	0.023
Straight leg raise test	261 (16.4%)	7.425±2.144	1.328 (83.6%)	3.522±2.001	0.034

Table III. Correlation between miR-204 expression and clinical characteristics of LDH.

Factors	Univariate analysis	
	HR (95% CI)	P-value
miRNA-204 (patients vs. control)	1.648 (1.122-2.896)	0.010
Sex (male vs. female)	0.821 (0.277-2.424)	0.779
Lower limb symptoms (yes vs. no)	2.132 (1.288-2.715)	0.014
Low back pain (yes vs. no)	0.821 (0.357-1.847)	0.673
Abnormal lumbar spine activity (yes vs. no)	3.053 (1.282-7.323)	0.011
Limited walking (yes vs. no)	2.123 (1.557-4.575)	0.011
Location (above L4 vs. below L4)	2.121 (1.231-3.625)	0.025

LDH, lumbar disc herniation.

96 patients could walk longer than 500 m, 255 patients between 100-500 m, and 135 cases below 100 m. With the increase in degree of limited walking, expression level of miR-204 also increased (2.021±0.589 vs. 4.985±1.025 vs. 7.425±1.456; P<0.05) (Fig. 5).

*Effects of lower limb symptoms on miR-204 expression level.* In this study, expression levels of miR-204 in patients with bilateral lower limb symptoms (446 cases) were not significantly different from those in patients with unilateral lower limb symptoms (546 cases) (7.662±1.258 vs. 6.588±1.322; P>0.05), while miR-204 expression level was significantly higher in patients with lower limb symptoms (992 cases) than that in patients without these symptoms (597 cases) (7.248±1.224 vs. 3.587±1.142; P<0.05) (Fig. 6).

Expression levels of miR-204 in straight leg raise positive, heel tendon reflex positive, and knee tendon reflex positive patients were significantly higher than those in negative patients (Table II).

*Comparison of expression levels of miR-204 before and after treatment.* Compared with the pretreatment levels, expression levels of miR-204 were significantly decreased in the 1,436 patients (90.4%) after treatment (5.898±1.214 vs. 1.898±1.211; P<0.05) (Fig. 7).

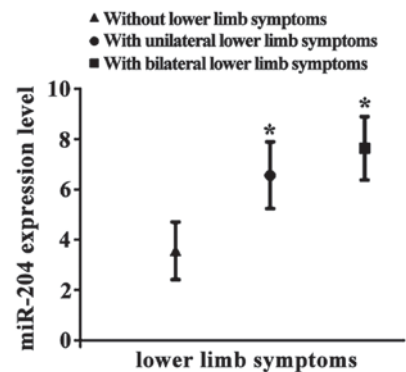


Figure 6. Effects of lower limb symptoms on miR-204 expression level. \*P<0.05 compared with those without lower limb symptoms.

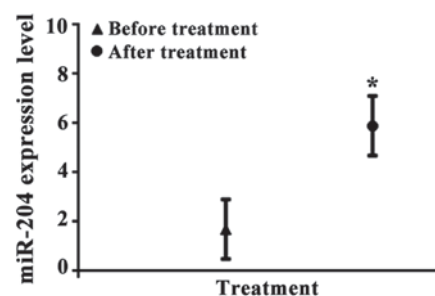


Figure 7. Comparison of expression levels of miR-204 before and after treatment. \*P<0.05 compared with those before treatment.

Logistics regression showed that miR-204 expression was closely correlated with clinical characteristics of LDH patients (Table III).

**Discussion**

LDH is the most common cause of lumbar nerve root disease. LDH is also one of the most common orthopedic diseases that can cause low back pain and sciatica (14). The pathogenesis of LDH is complex and varied, and disc degeneration continues after two years of onset (15). The pathogenesis of LDH is still unclear (16). This study aimed to detect the expression of miR-204 in serum derived from patients with LDH, so as to explore the correlation between miR-204 expression and the occurrence of LDH.

Logistics regression analysis showed that expression of miR-204 was closely correlated with the location of LDH (r=0.4967, P=0.001). It has been reported that miR-204 regulates a variety of cell development processes that plays an important role in several types of cancer, such as renal cell carcinoma (17), gastric cancer (18), and gliomas (19). Studies

have shown that miR-204 is abnormally expressed in these cancers, and its level is closely related to tumor progression and pathological grading (20,21). However, the expression level of miR-204 in LDH and its involvement in the pathogenesis of LDH is still unclear. In our study, miR-204 levels were elevated in serum in patients with LDH compared with healthy control, and miR-204 levels were also increased with the prolonged duration of the disease.

To further validate the relationship between miR-204 levels and clinical characteristics of patients with LDH, expression levels of miR-204 in LDH patients with different clinical symptoms were statistically analyzed. Results showed that clinical symptoms of patients with LDH and miR-204 expression levels were also closely correlated. Location of LDH, abnormal lumbar spine activity, lower limb symptoms and limited walking were all correlated with the expression level of miR-204. Studies have shown that other miRNAs are also abnormally expressed in LDH. Jing and Jiang (22) showed that miR-93 was significantly downregulated in degenerative nucleus pulposus, and its level was associated with intervertebral disc degeneration and overexpression of miR-93-stimulated type II collagen expression in nucleus pulposus cells. Yu *et al* (23) showed that miR-10b was overexpressed in human degenerative nucleus pulposus, and its expression level was positively correlated with the degree of intervertebral disc degeneration. However, their involvement in LDH remains to be studied.

This study showed that expression level of miR-204 was significantly decreased after treatment ( $P < 0.05$ ), and miR-204 expression level may serve as a prognostic marker for LDH patients. The differential expression of miR-204 in patients with and without surgical operations was not studied, however, this may be the focus of our future studies. Pearson and Lurie systematically analyzed the clinical data of patients with LDH to identify the features associated with the therapeutic effect (24). Surprisingly, patients with surgical treatment showed better outcomes than patients without surgical treatment. However, it is difficult to select a suitable treatment with high safety and efficiency, low incidence of complications and low risk (25). Low risk may reduce the efficiency. Therefore, the possible outcomes and risks should be fully considered by the physicians and patients.

In conclusion, the expression level of miR-204 is closely related to the clinical characteristics of patients with LDH, and miR-204 expression level may serve as a prognostic marker for patients with LDH.

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#### Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

#### Authors' contributions

JP wrote the manuscript. JP and YL were responsible for treating patient. YaZ and YuZ performed the experiments of RT-qPCR and analyzed the data. All authors read and approved the final study.

#### Ethics approval and consent to participate

The study was approved by the Ethics Committee of Shanghai Jiaotong University Affiliated Sixth People's Hospital (Shanghai, China). Signed informed consents were obtained from the patients or the guardians.

#### Patient of consent for publication

Not applicable.

#### Competing interests

The authors declare that they have no competing interests.

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