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Analysis of miRNA-199-5p expression levels in serum samples of patients with lumbar disc degeneration

Fatma Tuba Akdeniz[1*], Zerrin Barut[2]

[1] Department of Medical Biology, Yeditepe University, Faculty of Medicine, İstanbul, Turkey
[2] Department of Basic Medical Sciences, Faculty of Dentistry, Antalya Bilim University, Antalya Turkey

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ABSTRACT

Lumbar disc degeneration is a condition caused by damage to the disc due to various causes, which results in disc material coming out of the disc space. MicroRNAs are small, non-coding RNAs that play a role in the regulation of gene expression by binding to mRNA. MiRNA-199 has previously been studied in the context of intervertebral disc degeneration, and its role in the disease has been reported. The purpose of this study was to look into the role of miRNA 199 in Lumbar Disc Degeneration. This study included 26 patients with Lumbar Disc Degeneration who were admitted to the Neurosurgery Clinic at Yeditepe University Hospital and 26 completely healthy volunteer controls. After isolating microRNA from control and patient sera, was converted into cDNA, concentration measurements were taken, and PCR was used to analyze miRNA-199 expression. miRNA-199-5p expression levels were found to be statistically significantly higher in patients than in controls (P = 0.024). miRNA-199-5p Delta CT levels were also evaluated by ROC analysis (p = 0.014). miRNA-199-5p may be a candidate for a biomarker believed to play a role in disease prognosis in patients with Lumbar Disc Degeneration.

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Introduction

Lumbar Disc Degeneration (LDD) involves wear and tear to the lumbar intervertebral disc, and it is most commonly seen in the L3–L4 and L4–S1 vertebrae (1). Degeneration of the disc causes it to bulge out of its socket. This is known as bulging or loss of disc space. In addition, advanced degeneration causes the disc to lose water and compress the nerves, resulting in pain (1). LDD is a pathological condition that causes low back pain and can occur as a result of endogenous and exogenous factors (2). Age, genetic, mechanical and physiologic factors may cause disc degeneration (3).

LDD is a significant cause of low back pain and herniated discs. Disc damage has been reported to be caused by DNA damage, an inappropriate response to damage, oxidative stress and cellular aging. This damage leads to degeneration in the extracellular matrix (ECM) of the disc. Furthermore, oxidative stress accelerates disc degeneration by affecting cell aging, inflammation, autophagy and DNA methylation (4).

Studies have evaluated the levels of antioxidant mRNA in cases of Intervertebral Disc Herniation (IDH) (5). It has also been reported that oxidative stress increases apoptosis in various components of the disc and accelerates the formation of intervertebral disc degeneration by inducing apoptosis in the endplate chondrocytes, nucleus pulposus cells and annulus fibrosus cells of the disc (6).

According to a previous report, disc degeneration makes the disc vulnerable due to a loss of elasticity and firmness (7). Inhibition of apoptosis has been mentioned in relation to the treatment of lumbar disc herniation (8). Moreover the relationship between mechanical stress and disc degeneration -due to aging and genetic factors- has been reported to be associated with disc collapse (9. We chose to focus on miRNA-199, given its known role in the development of degenerative diseases.

MicroRNAs (miRNAs) are non-coding RNAs that were discovered in 1993. miRNAs regulate the expression of target genes by binding to the untranslated regions (UTRs) of messenger RNA's (mRNA's) (10). They are approximately 22 nucleotides long. They are gene-regulatory molecules at the post-translational level. In addition to their role in biological functions such as proliferation, migration, invasion, apoptosis and autophagy, they have recently been studied in relation to the development of cancer, diabetes and neurological diseases (11, 12). In rheumatoid arthritis, miRNA-199-3p has been shown to induce apoptosis (13).

Researchers have become interested in miRNA-199 in recent years. In a study of lung cancer, overexpression of miRNA-199 was associated with decreased tumour cell proliferation and was found to act as a tumour suppressor gene (14). miRNA-199 has also been reported to play a role in cell growth and differentiation (15). miRNA-199 plays a vital role in the maintenance of normal homeostasis and the regulation of disease pathogenesis. Additionally, miRNA-199 has been found to be highly expressed in breast, colon and testicular tissue, have low expression in the thymus, liver and kidney, and show almost no expression in the brain (12, 16).

miRNAs play a role in the development of several di-

* Corresponding author. Email: tuba.akdeniz@yeditepe.edu.tr

seases, including LDD. They have also been reported to be involved in various disc degeneration processes (17). This study sought to examine the levels of miRNA-199-5p in patients with LDD. It also aimed to compare the expression levels of miRNA-199-5p in patient serums and control group serums to determine how they affect disease prognosis.

Materials and Methods

miRNA isolation, cDNA synthesis

This study included 26 serum samples of patients with Lumbar Disc Degeneration. The patient group consisted of patients who applied to Yeditepe University Hospital Neurosurgery Clinic. The patients did not any chronic disease and had not undergone surgery. The control group consisted of 26 serum samples obtained from completely healthy volunteer controls. The patients included in the study were between 21 and 59 years old. Serum samples were separated from the blood taken from the peripheral blood samples of patients and controls by centrifugation (4000 rpm for 15 minutes). The miRNAs were isolated from serum samples, according to the kit procedure, using the miReasy Kit *(Cat. No./ID: 217184- Qiagen Strasse 1, 40724 Hilden, Germany)*, and converted to cDNA using the miRCURY LNA RT Kit *(Cat. No./ID: 339340- Qiagen Strasse 1, 40724 Hilden, Germany)*. Following sample concentration measurements, dilutions were made, and expression levels of microRNA 199-5p *(miRCURY 199-5, YP00204494 Lot: 201803080050-4-Qiagen- Qiagen Strasse 1, 40724 Hilden, Germany)* were determined by PCR in Rotor-Gene (Rotor-Gene Q-Qiagen) using the miRCURY LNA SYBR Green PCR *Kit (Cat. No./ID: 339346-Qiagen Science, Maryland 20874, USA)*. Housekeeping assay, RNU6-(lot:20800469-1- *Qiagen Strasse 1, 40724 Hilden, Germany*) was used as an internal control.
Patients were evaluated according to the Oswestry and VAS scales:
The % values obtained from the Oswestry assessment are classified as follows:
0%-20%: Low back pain is not a significant problem in the patient's life.
20%-40%: Low back pain slightly limits the patient's daily life.
40%-60%: Low back pain severely limits the patient's daily life.
60%-80%: The patient's daily life is completely restricted due to low back pain.
80%-100%: Bedridden patient (or symptoms exaggerated) (35).

Statistical analysis

The IBM SPSS Statistic 22 (IBM Corp., Armonk, NY, USA) program was used for statistical analyses. The p-values less than ($p<0.05$) were considered significant. The Mann-Whitney U test was utilized for comparisons between two groups for parameters that did not show normal distribution in the evaluation of miRNA-199-5p expression levels. ROC analysis was performed to determine miRNA-199-5p serum levels in the patient and control groups and to reveal the diagnostic value of miRNA-199-5p. The MedCalc Program was used for this.

Results

Demographic data

The mean ages of the patients and controls included in the study were 40.66 and 40.53 years, respectively. According to the age group, there was no difference between the control and patient groups. There were 7 male and 19 female patients. The majority of the patients in the patient and control groups were female.

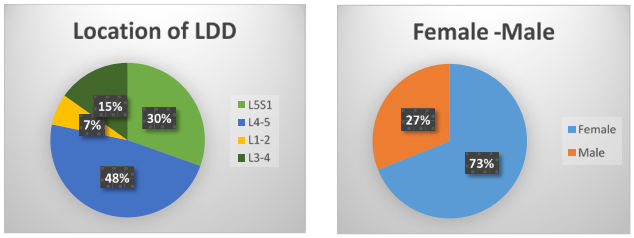
In the study, 48% of the patients had L4-L5 localized disc degeneration, followed by 30% with L5-S1 localized disc degeneration (Figure 1A). Seventy-three percent of the patients in the study were women (Figure 1B). According to the Oswestry quality of life scale, 45% of the patients had severely restricted life activities (Figure 2A), and 74% had a VAS pain rating scale value greater than 50. (Figure 2B).

There was no correlation between the ages of patients with Oswestry values greater than 40% and those with values less than 40% ($p>0.05$).

There was no correlation between age and miRNA-199 expression levels ($p>0.05$).

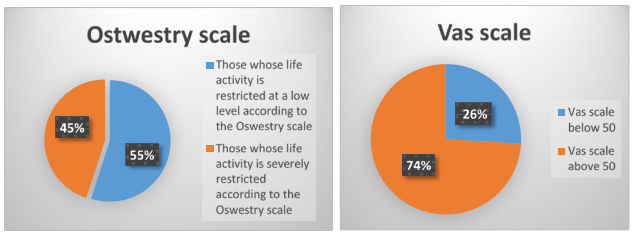
miRNA-199-5p expression level

The level of miRNA-199-5p expression in the patient group was found to be statistically significantly higher than in the control group. The Mann-Whitney U statistical



A) Location of LDD *B) Ratio of Female-Male in patient group*

Figure 1. A and B: Demographic data of patients with in Lumbar Disc Degeneration.



A) Oswestry scale - life activity level *B) Vas scale*

Figure 2. A and B: Pain scale with in Lumbar Disc Degeneration.

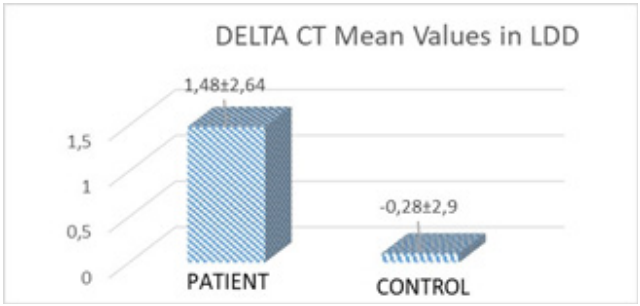
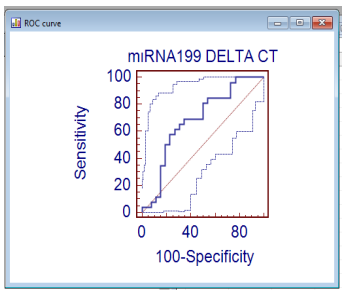


Figure 3. mRNA-199-5p expression levels in patients and control with LDD.

Table 1. Comparison of delta CT Values by Groups.

		N	Mean	S.D.	Mean Rank	Sum of Ranks	p
miRNA199-5p delta CT	Patient	26	1.48	2.64	31.23	812.00	0.024*
	Control	26	-0.28	2.99	21.77	566.00	



Sensitivity: 69,2
Specificity: 65,4
Criterion : >0,05

Variable	miRNA199_DELTA_CT miRNA199 DELTA CT
Classification variable	DATA
Positive group	
DATA	= 1
Sample size	26
Negative group	
DATA	= 0
Sample size	26
Disease prevalence (%)	unknown
Area under the ROC curve (AUC)	0,682
Standard Error	0,0742
95% Confidence Interval	0,538 to 0,804
z statistic	2,452
Significance level P (Area=0.5)	0,0142

Figure 4. ROC analysis of mRNA-199-5p expression in patients with LDD (mRNA199-5p.delta CT values).

analysis test was used to evaluate the delta CT levels of patients and controls that did not have a normal distribution, and the p-value was found to be (p = 0.024). *(*Figure 3, Table 1*)*.

ROC analysis was used to evaluate the delta CT values of patients and controls, and statistical significance was highlighted (p = 0.014) (Figure 4).

To assess the utility of miRNA-199-5p as a biomarker in LDD, ROC curve analysis was used. When delta C*T* values were used, the specificity and sensitivity of miRNA-199 -5p were 64.3% and 67.9%, respectively (95% CI = 0.535-0.804, AUC = 0.682, p = 0.014).

Discussion

According to existing research, genetic risk factors play a significant role in degenerative disc disease (DDD) (18, 19). Many miRNA studies have been conducted on lumbar disc degeneration (20, 21). miRNA-199-5p has been reported to accelerate the apoptosis of nucleus pulposus cells in intervertebral disc degeneration (22). Studies have assessed the relationship between miRNA-125 and apoptosis regulation in cases of LDD (23). miRNA-583 expression has been linked to degenerative nucleus pulposus tissue in intervertebral disc degeneration (24). Apoptosis has also been shown to increase in various regions of the disc during disc degeneration. Research has identified apoptosis, inflammation and degradation of the ECM as hallmarks of disc space narrowing (6, 25).

In a study conducted with a large number of miRNAs, miRNA-665 was found to increase the apoptosis of nucleus pulposus cells and promote degradation mediated by miRNA-665. In the same study, several miRNAs were reported to trigger inflammation associated with disc degeneration and apoptosis around the disc (17, 26). Another study investigated miRNA-199 in patients with uterine leiomyomas. In these patients, miRNA-199 expression was shown to be down-regulated, and its proliferation-inhibitory and apoptosis-enhancing properties were highlighted (27).

A study reported that miRNA-199 increases apoptosis in different diseases, such as acute myeloid leukemia (AML) (28). Studies have also shown a link between oxidative stress and disc herniation, which is associated with apoptosis (8, 29).

Lumbar disc disease is characterized as a degenerative condition and miRNA-199 is notably overexpressed in the context of degenerative diseases. In this study, the miRNA-199-5p expression level in serum samples of patients with LDD was found to be higher than in serum samples of the control group. This finding aligns with previous research that associates elevated miRNA-199 expression levels with tissue degeneration in the disk region and suggests a link between miRNA-199 and LDD prognosis.

According to one study, preoperative disc degeneration was generally asymmetric and found to be slightly more prevalent in elderly patients (30). Various publications have also noted that disc degeneration increases with age (31, 32). The age range of the patients included in this study was 21 to 59 years.

According to a 2013 study, disc space narrowing increased with age and was more common in women (33). By comparison, A 2021 study revealed that age-related disc degeneration began later in men. The same study reported that reductions in disc height due to degeneration were the same in both sexes at an advanced age (34).

In the present study, 73% of the patients were women and 27% were men. The high proportion of female patients in the group, which was selected randomly aligns with the trends noted in previous studies.

Studies of LDD have indicated that degeneration is predominantly occurs in the L4–L5 vertebrae. In the current study, 48% of the participants had degeneration localized in the L4–L5 vertebrae, a finding that aligns with existing literature on the common locations of degeneration. The limitation of our study is the small number of sample size. For this reason, prospective, multicentric and large case series studies should be conducted in the near future. Our results are in line with those of a previous study that showed miRNA-199 to be involved in the process associated with disc degeneration. Therefore, the high expression of miRNA-199 in sera from LDD patients suggests that it may serve as a potential biomarker for LDD diagnosis.

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Interest conflict

The Authors report no conflicts of interest concerning the materials or methods used in this study or the findings reported in this article.

Authors Contributions

Study conception and design: Fatma Tuba Akdeniz, Zerrin Barut. Data collection: Fatma Tuba Akdeniz. Analysis and interpretation of results: Fatma Tuba Akdeniz, Zerrin Barut. Draft article preparation: Fatma Tuba Akdeniz. Supervision: Fatma Tuba Akdeniz, Zerrin Barut. All authors reviewed the results and approved the final version of the article.

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