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Research Article

# Investigation of the Diagnostic and Prognostic Values of Some Specific microRNAs in Meningioma Tumors

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## ABSTRACT

**Objective:** Primary brain tumors are classified as glial or non-glial and benign or malignant. Meningiomas are common benign intracranial tumors. Although the name meningioma refers to a tumor of the lining of the brain called the 'Meninx', it has actually been shown to originate from the spider web-shaped 'arachnoid' membrane (arachnoid cover cells). microRNAs are 18-22 nucleotide long, endogenous, non-protein-coding RNA molecules that negatively regulate gene expression at the post-transcriptional level. In this study, we applied a genome-wide array screen comparing the expression of *miR-145, miR-34a-3p, miR-200a, miR-335, miR-106a-5p, miR-219-5p, miR-375, miR-409-3p miR-197* and *miR-224* in meningiomas.

**Materials and methods:** A total of 40 meningioma patients (13 men, 27 women) and healthy control individuals (12 men, 18 women) aged between 30 and 65 were inclusives in the study. The research was conducted at Gazi University Hospital.

**Results:** In our study, *miR-197* identified as the most highly expressed miRNA in meningiomas compared to other miRNAs. *miR-197, miR-34a, miR-375, miR-219a* and *miR-224* stand out as potential biomarkers in human serum samples of meningiomas patients. Moreover, as per WHO classification *miR-197, miR-34a, miR-375* might be used as potential biomarkes for grade I meningioma while *miR-375* for grade II meningioma.

**Conclusion:** The role of miRNAs in meningiomas is gaining importance each day. Therefore, our study examining the role of miRNAs in meningiomas will shed more light and pave the way for future therapeutic strategy.

**Keywords:** Biomarker, Meningioma, miRNA, *miR-197, miR-34a-3p*

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## INTRODUCTION

Meningiomas can diffuse Central Nervous System (CNS) tumors that account for approximately one-third of all intracranial tumors (Galani V, *et al*., 2017; Dolecek TA, *et al*., 2012). According to the World Health Organization (WHO) classification, the vast majority of meningiomas are grade 1 (typical/benign), less than 10% grade II (atypical/moderate) and III (anaplastic/malignant) tumors (Mei Y, *et al*., 2017). Their incidence increases with age with a Female:Male ratio of 2:1. Furthermore, findings from autopsy and imaging studies show that the prevalence of subclinical meningiomas constitutes roughly 3% of the population (SöylEmEzoğlu F, 2011). It has been implicated in the pathogenesis of many tumors, such as inactivation of tumor suppressor genes or overexpression of oncogenes and specific gene dysfunctions (Amplifikasyonu G, 2009). The most common abnormality identified in meningiomas is deletions in chromosome 22. Loss of chromosomes 1p, 3p, 6q, 10q and 14q were also observed, especially in atypical or malignant meningiomas. It has already been suggested that these deletions can be used as a marker to predict tumor progression (Arslantas A, *et al*., 2022). microRNAs are 18-22 nucleotide long, endogenous, non-protein-coding RNA molecules that negatively regulate gene expression at the post-transcriptional level (McManus MT, *et al*., 2002). In recent years, there has been an increasing research on miRNAs as potential biomarkers for various pathological conditions, including tumors (Tie J, *et al*., 2010). miRNAs can function as oncogenes or tumor suppressors under certain conditions. There are evidences that they play roles in many cellular processes that contribute to tumor formation and development, from proliferation to invasion, from metastasis to angiogenesis (Tie J, *et al*., 2010; Vishnoi A and Rani S, 2017). Many studies analyzing the profile and function of miRNA have yielded important insights into the pathogenesis of different types of tumors, including Glioblastoma Multiforme (GBM), breast cancer, pancreatic cancer, lung cancer, prostate cancer, and colon cancer (Petrescu GE, *et al*., 2019; Mulrane L, *et al*., 2013; Uddin A and Chakraborty S, 2018; Ahmed FE, 2014; Yonemori K, *et al*., 2017). The recent findings indicating that miRNAs are expressed in human blood holds great promise that circulating miRNAs can serve as novel molecular biomarkers for cancer (Yonemori K, *et al*., 2017; Zhang K, *et al*., 2017). In present study, the association between meningioma and gene expression of *miR-145, miR-34a-3p, miR-200a, miR-335, miR-106a-5p, miR-219-5p, miR-375, miR-409-3p miR-197* and *miR-224* were examined in tumor samples obtained from patients with meningioma and healthy individual samples as controls.

## MATERIALS AND METHODS

### *Study population*

A total of 40 patients who applied to the Department of Neurosurgery and diagnosed with meningioma from brain tumors and 30 healthy volunteers were included in the study. Healthy group were selected from those who applied to Gazi hospital's check-up center and whose blood sample came to the central laboratory, and those who met our admission and exclusion criteria. Some of the serums obtained from the routine blood of these people were reserved for the study. Likewise, some of the serums obtained from the blood samples taken from meningioma patients at the time of diagnosis were separated. All separated serums were stored at -80 degrees until analysis.

For the qRT-PCR assay, a fixed constant of synthetic cel-miR-39 (Genepharm, China) was added to 100 µl serum sample as the internal control. Total RNA isolation from samples was performed using the QIAGEN miRNeasy Serum/Plasma Advanced (Cat.no:217204) kit. Overall, total RNA was obtained from 200 µl serum in a yield of approximately 75-100 µng. The cDNA synthesis was made from the RNA samples using the kit (Cat.

No:339340, miRCURY LNA RT Kit, Qiagen). Quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR) assay was performed by BioMark HD system (Fluidigm, South San Francisco, CA, USA) according to the manufacturer's instructions. All reactions, was performed in triplicate, including controls without template RNA. The expression levels of selected miRNAs were normalized to cel-miR-39 (Zhang K, *et al*., 2017).

### *Statistical analysis*

For the statistical evaluation, the conformity of the variables to the normal distribution was examined with the Shapiro-Wilk test. Normally distributed t-test was used to compare the groups. The data which were not normally distributed, Mann-Whitney U test for comparison analyzes between two independent groups, Kruskal Wallis test for comparison analyzes between three groups, post hoc test: Mann-Whitney U test with Bonferroni correction descriptive statistics are expressed as Mean ± SE and Mean ± SD. One-Way Anova:Post-hoc:Tukey HSD Test, tamhane t2 test, Dunnett test, Kruskal Wallis H test, Post hoc:Dunn Sidak tests were used to compare the expression levels of miRNAs. Statistical significance was accepted as $p<0.05$. IBM SPSS package program version 22 was used in the evaluation of the data.

## RESULTS

A total of 40 patients who applied to the Department of Neurosurgery and diagnosed with meningioma from brain tumors and 30 healthy volunteers were included in the study. Having 40 meningioma patients with a mean age of 48.77 ± 13.09 and 30 healthy controls with a mean age of 44.34 ± 15.04 years. A total of 70 participants were included in this study *(Table 1)*.

**Table 1: Age and gender data of groups**

| Groups | Age | Sex | |
|---|---|---|---|
| | | Male | Female |
| | (Mean ± SD) | (Mean ± SD) | (Mean ± SD) |
| Meningiom | 48.77 ± 13.9 | 46.92 ± 10.9 (n:13) | 49.81 ± 11.4 (n:27) |
| Healthy | 44.34 ± 15.4 | 47.72 ± 12.7 (n:12) | 40.16 ± 16.7 (n:18) |
| p-value | p>0.05 | p>0.05 | p>0.05 |

A multiphase study was designed to identify markedly altered miRNAs in serum that could distinguish meningioma from healthy controls and serve as potential biomarkers for meningioma. The study was conducted to compare the expression of human miRNAs in pooled serum samples from 40 pre-operative meningioma participants with those from 30 healthy controls for initial screening. The serum fold change of the preoperative group compared with the healthy control group is shown in *Figure 1*.

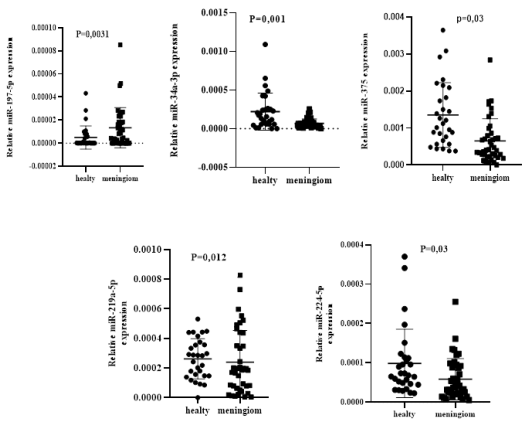


**Figure 1: The relative expression levels of 5 identified miRNAs in serum from pre-operative (pre-op) meningioma patients and healthy controls**

The expression levels of the 10 miRNAs in the 40 pre-operative serum samples were stratified using 3 types of clinicopathological parameters (Age, sex and WHO tumor grade). When classified according to tumor grade, *miR-197*-5p, *miR-34a-3p* and *miR-375* showed significant differences associated with pathological grades *(Figure 2)*.

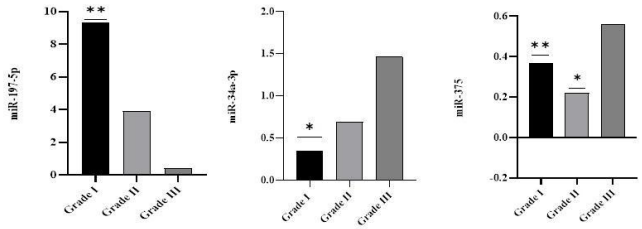


**Figure 2: The relative expression of *miR-197-5p, miR-34a*-5p and *miR-375* in different tumor grades**

Receiver Operating Characteristic (ROC) curve analyzes were performed to evaluate the diagnostic accuracy of miRNAs in the serum between meningioma patients and normal subjects. The Area Under the Curve (AUCs) and 95% Confidence Intervals (CI) for *miR-145, miR-34a-3p, miR-200a, miR-335, miR-106a-5p, miR-219-5p, miR-375, miR-409-3p miR-197* and *miR-224* is shown in *Figures 3 and 4*.

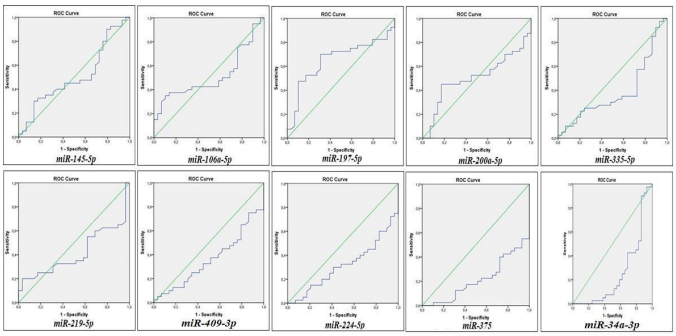


**Figure 3: The Receiver Operating Characteristic (ROC) curves of the identified miRNAs**

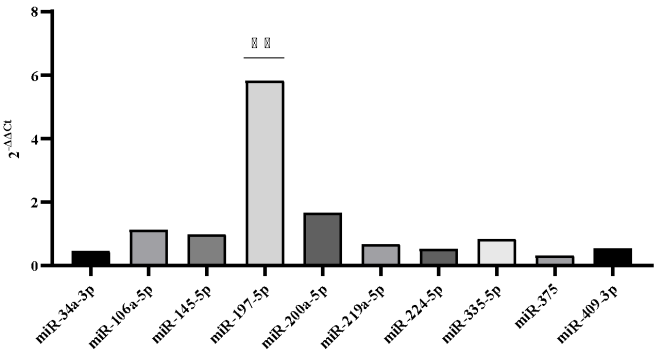


**Figure 4: Fold change expression results of miRNAs in meningioma**

## DISCUSSION

microRNAs play important roles in post-transcriptional gene regulation. It is known to regulate many pathways involved in cell proliferation, morphogenesis and differentiation (Vishnoi A and Rani S, 2017). Saydam O, *et al*., examined the relationship between different types of miRNAs and meningiomas and found that it altered miRNA levels in different types of

meningiomas, with increased expression in some and decreased expression in others. They found an increase in the expression levels of *miR-335, miR-98 and miR-181a,* and a decrease in the expression levels of *miRNA-200a, miRNA-373* and *miRNA-575* in different meningioma cases. Moreover, *miRNA-200a* inhibited meningioma growth during *in vitro* studies (Saydam O, *et al*., 2009). Various miRNAs (including *miR-106a-5p, miR-219-5p*, *miR-375*, *miR-197*, *miR-224* and *miR-409-3p*) are known to be dysregulated in meningioma patients (Zhi F, *et al*., 2016).

In previous studies, downregulation of *miR-34a-3p* in high-grade meningiomas has been reported (Werner TV, *et al*., 2017; El-Gewely MR, *et al*., 2016). *miR-34a-3p* targets the *Bcl-2* gene in HBL-52 cells. It has been suggested that high expression of *Bcl-2* may contribute to increased cell proliferation and decreased apoptosis of tumor cells (Ludwig N, *et al*., 2015). Overexpression of *miR-34a-3p* in meningioma inhibits cell proliferation and induces apoptosis. Therefore, it is thought that *miR-34a-3p* can be used as an anticancer drug in meningiomas (Misso G, *et al*., 2014). In our study, similar results were obtained which complements previous studies on the *miR-34a-3p* expression in meningiomas. We found that *miR-34a-3p* expression was lower in the meningioma patient group compared to the healthy control group ($p<0.001$).

*miR-145* expression was seen in atypical and anaplastic tumours as cross-checked to benign meningiomas (Yao Z, *et al*., 2017). Moreover, meningioma cells with high *miR-145* levels had impaired migratory and invasive potential *in vitro* and *in vivo.* Polymerase Chain Reaction (PCR) array studies of *miR-145* overexpressing cells suggested that Collagen type V alpha (*COL5A1*) expression was down regulated as a consequence of *miR-145* over expression. Accordingly, *COL5A1* expression was significantly upregulated in atypical and anaplastic meningiomas (Wilisch-Neumann A, *et al*., 2014).

*miR-197* has been extensively studied in the carcinogenesis progression of cancers through a variety of mechanisms, including apoptosis, proliferation, angiogenesis, metastasis, drug resistance and tumor suppressor, and has also been implicated in the prognosis of cancers (Wang DD, *et al*., 2016; Dai W, *et al*., 2014; Ghafouri-Fard S, *et al*., 2021). El-Gewely MR, *et al*., found that *miR-197* was lowly expressed in meningioma cells, while *IGFBP5* was highly expressed (El-Gewely MR, *et al*., 2016). They demonstrated that *miR-197* inhibits the pro-apoptotic *IGFBP5* gene by binding to conserved binding sites in its 3′ Untranslated Region (3′UTR) (El-Gewely MR, *et al*., 2016). In a study conducted by Zhi F, *et al*., 2017 regarding meningiomas, it was found that *miR-197* expression was lower than in healthy controls. In our study, meningioma patients showed higher expression of *miR-197* than controls ($p=0.003$) which is contrary to the study of Zhi F, *et al*., 2017. The expression of *miR-197* in our study may differ from previous studies for reasons such as sample size, study method, gender, race and environmental factors.

*miR-375-5p* is highly expressed in meningiomas (Zhi F, *et al*., 2017). Therewithal, it is thought that *miR-375-5p* may be an important biomarker in meningioma patients pre- and post-operatively (Zhi F, *et al*., 2017). In our study, *miR-375* expression was found to be lower in meningioma patients compared to controls ($p=0.003$). We think that our results would be an important milestone for future studies, since no studies of *miR-375* related to meningioma have been found in the literature till date.

There is evidence that *miR-219-5p*, which can individuate meningioma patients from healthy group with high sensitivity, can discriminate pre- and post-operatively from meningioma patients, can help monitor the effect of surgical resection in clinical practice (Zhi F, *et al*., 2017). The widespread use of this miRNA panel is believed to have significant potential as a conbined diagnostic and monitoring biomarker for meningioma (Zhi F, *et al*., 2017; Zhi F, *et al*., 2013). Similar results were obtained for the *miR-219a-5p* in our study. It was observed that *miR-219a-5p* was expressed more in the meningioma patient group than in the healthy control group ($p=0.0012$).

In a study investigating *miR-224* expression and its relationship with histological grading and postoperative recurrence in patients with meningioma, it was found that IOMM-Lee and CH157 inhibited *miR-224* expression in meningioma cells, and that *miR-224* inhibition increased apoptosis and suppressed cell proliferation (Wang M, *et al*., 2015). In addition, this study identified *ERG2* as a new target of *miR-224* and indicated that the *miR-224-ERG2* axis played a critical role in regulating the apoptosis and proliferation of meningioma cells (Wang M, *et al*., 2015). Considering the grades, *miR-224* in grade I was found to be lower and therefore it is thought to be an important biomarker when separated by grades (Zhi F, *et al*., 2017). Wang M, *et al*., 2015 studied *miRNA-224* in meningioma and found significantly higher *miRNA-224* expression in meningioma and a positive correlation between tumor grade and *miRNA-224* expression in tissues as well as compared to normal tissues was found. Patients with low *miRNA-224* expression had a significantly longer disease course and lower recurrence rate compared to other cases of meningiomas. In our study, the expression of meningioma patiens was found to be lower than healthy controls ($p=0.03$) indicating that our results are consistent with the literature and expression of *miR-224* has been found to be lower in grade I.

In summary, we tried to demonstrate that miRNAs can be an important potential biomarker target for meningiomas in our present study. In particular, *miR-197* was expressed more than other miRNAs in this group of patients. Therefore, it may be a potential biomarker target in the pre-operative process. *miR-197, miR-34a, miR-375, miR-219a* and *miR-224* stand out as potential biomarkers in meningiomas in human serum samples. It also strengthens the possibility that *miR-197, miR-34a, miR-375* in grade I and *miR-375* in grade II could be used as a potential biomarker in our meningioma.

## CONCLUSION

There is no known biomarker for the diagnosis and evaluation of the prognosis of meningioma disease yet. In the last decade, it has been shown that epigenetic factors play important roles in tumor pathogenesis. In particular, non-coding RNA molecules such as miRNAs may be potential new therapeutic targets in meningiomas. In addition, due to the potential use of miRNAs, tumor diagnosis can be made easily in the early period non-invasively, and pre-surgical planning can be made in terms of prognosis, and some of the intraoperative difficulties (waiting for biopsy results, etc.) will be eliminated. In our study, *miR-197, miR-219a* expressions were higher in meningioma patients compared to controls, and *miR-34a, miR-224* and *miR-375* expressions were lower in controls. We observed that the expressions of *miR-335, miR-409, miR-106* and *miR-145* did not show any significant difference. However, *miR-197* was found to be the most highly expressed miRNA in meningiomas compared to other miRNAs. *miR-197, miR-34a, miR-375, miR-219a* and *miR-224* stand out as potential biomarker targets in human serum samples of meningiomas patients. In addition, according to the WHO classification, *miR-197, miR-34a, miR-375* grade I and *miR-375* grade II also strengthened the possibilities that they could be used as a potential biomarker for meningiomas. However, there is a need for further studies on these miRNAs with more comprehensive inclusion of patients.

## DECLARATIONS

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### *Author contributions*

All authors contributed to the study conception and design. All authors

read and approved the final manuscript.

### *Data availability*

Data are available upon reasonable request.

### *Ethics approval*

The study was approved by the Clinical Research Ethics Committee of Gazi University, Ankara, Turkey.

### *Consent to participate*

Informed consent was obtained from all individual participants included in the study.

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