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Serum Expression Levels of Certain miRNAs in Predicting Diagnosis, Prognosis, and Response to Chemotherapy in Malignant Pleural Mesothelioma

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Background: miRNAs are involved in tumor pathogenesis and can therefore be determined in the primary tumor, plasma and serum, and body fluids. As in various cancers, their role in the diagnosis, prognosis, and treatment of patients with malignant pleural mesothelioma (MPM) may be important.

Aims: To analyze the predictive value of miR-16-5p, miR-29c-3p, miR-31-5p, miR-125a-5p, miR-320a, miR-484 and miR-532-5p expressions for diagnosis, prognosis and response to treatment in patients with MPM.

Study Design: Prospective case-control study.

Methods: In the first phase of the study, blood samples were collected from 101 MPM patients before chemotherapy and from 24 healthy donors (HDs). In the second phase, the blood samples were collected from 74 MPM patients who had received chemotherapy when the best overall response and disease recurrence were determined. A quantitative real-time polymerase chain reaction was undertaken to detect the miRNA expression levels. The miRNA expression profiles of MPM patients were compared with those of HDs. The associations between the expression levels of miRNAs and prognosis and response to treatment were then evaluated.

Results: All miRNAs, except miR-31-5p, were expressed differently in MPM relative to that in HDs. The expression level of miR-16-5p decreased when compared with that of HDs, and the expression levels of miR-29c-3p, miR-125a-5p, miR-320a, miR-484, and miR-532-5p increased when compared with that of HDs. The sensitivity and specificity values of miR-29c-3p, miR-125a-5p, miR-320a, miR-484, and miR-532-5p for discriminating MPM from HDs were 85.9% and 59.1%, 95.1% and 62.5%, 87.1% and 79.2%, 82.2% and 58.3%, and 69.3% and 82.6%, respectively. After adjusting for the histological subtype, stage, and treatment, the miR-29c-3p, miR-125a-5p, and miR-484 were associated with longer survival. The miRNA expression levels did not change longitudinally for the determination of chemotherapy response and recurrence.

Conclusion: miRNAs may be useful in diagnosing patients with MPM and provides helpful information in determining the prognosis of patients.

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INTRODUCTION

Malignant pleural mesothelioma (MPM) is a primary malignant tumor of the pleura. The main etiology of MPM is exposure to asbestos or erionite. The incidence of MPM is increasing with the increasing exposure to asbestos in the environment across the world and the increasing consumption of asbestos in the workplace of developing countries.[1-3]

Clinical symptoms in MPM are not specific at the early stages of the disease. Most patients are at an advanced stage of the disease at the time of diagnosis. Therefore, chemotherapy and optimum supportive care are the only treatment options in most cases.[4,5] At this point, early diagnosis is important to determine the prognosis of patients treated with cytotoxic therapy, and determining the proper response to chemotherapy is a critical factor during patient follow-up.

Despite the high risk of MPM in asbestos- or erionite-exposed cohorts, no practical methods have yet been developed for early and differential diagnosis. One of the possible methods for field screening of populations at a high risk of mesothelioma is the determination of disease-specific biological markers in the serum or plasma samples from patients. However, the ideal biological markers for these purposes remain to be determined in MPM.[6] MicroRNAs (miRNAs) are among the promising biomarkers for these purposes owing to their role in MPM pathogenesis.[7,8]

miRNAs, which are noncoding RNA molecules, bind to target mRNAs complementary to their nucleotide sequences and regulate the post-transcriptional gene expression through translational repression or destruction of the mRNA. miRNAs play an essential role in homeostatic processes such as cell proliferation, cell differentiation, and cell death.[9] miRNAs can be determined in the primary tumor, plasma, and serum, hence their role in the diagnosis, prognosis, and response to treatment of patients with MPM, as in various cancers, remains the subject of various studies.[8-11]

In our study, we evaluated the potential benefit of miRNAs; hsa-mir-125a-5p, hsa-mir-320a, hsa-mir-484, and mir-532-3p, which we had determined in our previous study[12], and hsa-miR16-5p[13,14], hsa-miR29c-3p[15], and hsa-miR31-5p[16,17], which we obtained from a review of the relevant literature on the prediction of diagnosis, prognosis, response to chemotherapy, and disease recurrence in patients with MPM.

MATERIALS AND METHODS

Patients and Sample Collection

The present study was prospectively conducted on MPM patients diagnosed between May 2015 and April 2019 at the Department of Chest Diseases, Faculty of Medicine, Eskişehir Osmangazi University. Ethical approval was obtained for the study (17/19.3.18). All participants provided their signed informed consent.

The study was conducted in two phases. In the first phase, the role of miRNAs in the diagnosis of MPM was investigated in a study group of 101 histopathologically diagnosed MPM patients who had not yet received treatment and 24 healthy donors (HDs). The HDs resembled the patient group in terms of age and sex distribution, and none of them had been exposed to asbestos. In the second phase, the role of miRNAs in response to chemotherapy in MPM was investigated. In this phase, the cohort of 101 MPM patients, including those who had received the best supportive care, those who received multimodality therapy, and those who could not be followed up or who died prematurely were excluded. Chemotherapy response was determined in 74 patients who had received only chemotherapy (Figure 1). Patient demographic, clinical, and treatment characteristics were accordingly recorded. The effect of miRNAs on the prognosis of patients in the first phase was also analyzed.

Blood samples were collected from all patients at the time of diagnosis and from HDs. Then, the blood samples were collected at each measurement point of response, at the completion of chemotherapy, and disease recurrence. In the study, the serum sample at the time point when the best overall response was observed during treatment was used. The serum phase of the blood samples was separated by the standard centrifugation method. The serum samples were enumerated and stored at -80 °C in the Biological Bank of Eskişehir Osmangazi University Lung and Pleural Cancers Research and Clinical Center until further analyses.

Platinum-based pemetrexed was used as the chemotherapy regimen.[18] The response was measured after every 2 cycles of chemotherapy. The Modified Response Evaluation Criteria in Solid Tumors (mRECIST) criteria were used to determine the response.[19] If the progressive disease was detected, the patient discontinued the study. If an objective response and a stable disease to treatment

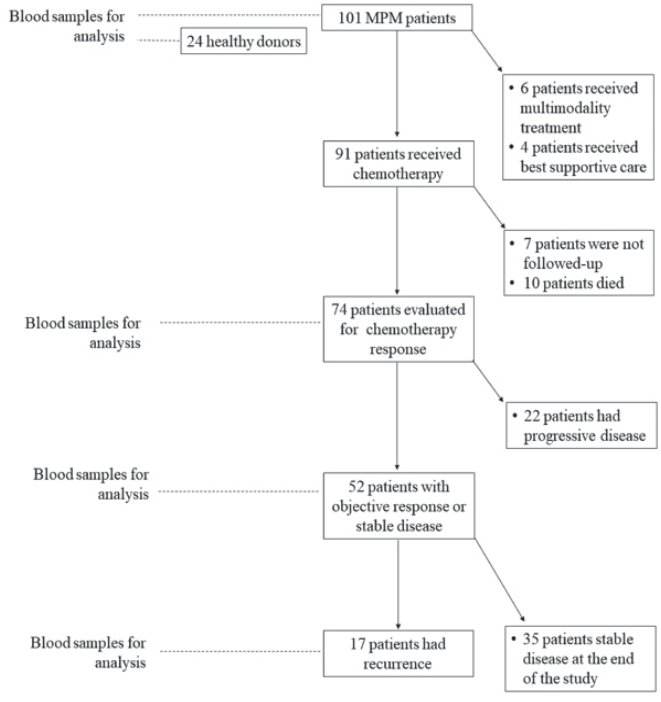

FIG. 1. Flowchart of the study protocol.

were detected, 4-6 cycles of chemotherapy were administered. After the completion of treatment, the patients were followed up regularly every 3 months for the first 6 months, then every 6 months for the next 2 years, and then every year thereafter until disease progression by computed tomography, with PET-CT if and when necessary.

Experimental Protocol

The circular expression levels of miR16-5p, miR31-5p, miR29c-3p, mir-484, mir-125a-5p, mir-320a, and mir-532-5p were determined in the serum samples at each time point (Figure 1).

miRNA Isolation and Quantitation

Total miRNA was isolated from the serum samples of MPM patients using the SanPrep Column microRNA Mini-Prep Kit (Biobasic Inc., Canada) according to the protocol provided by the manufacturer. After isolation, miRNA quantification was performed with the Qubit High Sensitivity RNA Kit (Thermo Fisher Scientific, USA) and the Qubit® 2.0 Fluorometer (Life Technologies, USA).

cDNA Synthesis

cDNA synthesis was performed following the kit protocol with the miRNA cDNA Synthesis Kit with Poly (A) Polymerase Tailing (AbmGood, Canada). A total of 10 µl of the reaction volume was prepared with 10 ng miRNA, 1X Poly (A) polymerase reaction buffer, 1.5 mM ATP, 2.5 mM $MnCl_2$, 0.5 U Poly (A) polymerase yeast, and RNase-free water. The reaction mix was incubated at 37 °C for 30 min, and a 2 µl of miRNA Oligo (dt) adapter was added; the resultant mixture was incubated at 65 °C for 5 min and then cooled. Then, 500 µM of dNTP, 1X of RT buffer, 200 U of OneScript RTase, and RNAase-free water were added to the reaction mix, followed by vortexing and incubating at 42 °C for 15 min. The reaction was stopped by a 10-min incubation at 70 °C, followed by rapid cooling. The incubation steps were performed with the MiniAmp ™ Plus Thermal Cycler (Applied Biosystems, Waltham, MA, USA) device. The isolated cDNAs were used in the qRT-PCR reaction.

Quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR)

qRT-PCR was performed on the StepOne™ Real-Time PCR System (Applied Biosystems, Carlsbad, CA) with the RT-PCR mix composed of 5 µl of the BrightGreen miRNA qPCR Master Mix (Applied Biological Materials Inc, Canada), 0.5 µl of the Forward and Reverse Primer, 2 µl of cDNA, and 2 µl of nuclease-free water as per the following RT-PCR conditions: 95 °C for 10 min and 40 cycles of 95 °C for 10 s, 63 °C for 15 s, and 72 °C for 20 s.

miRNA RT-PCR primers of U6-2 (Cat. No. MPH0001), hsa-mir-16-5p (Cat. No. MPH02234), hsa-mir-29c-3p (Cat. No. MPH02392), hsa-mir-31-5p (Cat. No. MPH02458), hsa-mir-125a-5p (Cat. No. MPH01080), hsa-mir-320a (Cat. No. MPH01422), hsa-mir-484 (Cat. No. MPH01715), and hsa-mir-532-5p (Cat. No. MPH01804) were purchased from the Applied Biological Materials (Richmond, BC, Canada).

Calculation of miRNA Expression

The comparative threshold cycle (Ct) values of the reactions were obtained by the StepOne Software v2.3 for endogenous control U6, hsa-mir-16-5p, hsa-mir-29c-3p, hsa-mir-31-5p, hsa-mir-125a-5p, hsa-mir-320a, hsa-mir-484, and hsa-mir-532-5p primers. The ΔCt values of the samples were determined by normalization with the Ct values of endogenous control U6.

The relative miRNA gene expressions of patient and HDs samples were analyzed by the comparative Ct (2-ΔΔCt) method using the Microsoft Excel program.

Statistical Evaluation

Data were analyzed using the SPSS program SPSS version 15.0 for Windows (SPSS Inc., Chicago, IL, USA). Continuous data were expressed as the mean ± standart deviation (SD), median, and minimum-maximum (min-max) values. Normality tests (Shapiro-Wilk) and graphs were created for continuous data. The t-test was used when variables showed a normal distribution and the Mann-Whitney U test when they did not. The miRNAs ΔCt values of MPM patients at each time point were compared using the Wilcoxon test. Categorical variables were expressed in terms of frequency and percentage. Chi-square tests or exact tests, if necessary, were applied for comparisons.

Receiver operator characteristics curve analyses (ROC) were performed to determine the patient and the control groups for each biomarker (MedCalc Statistical Software 19.0.1, Belgium). The biomarker cut-off value was calculated in the ROC analysis, which yielded a significance value of $p < 0.05$ for the area under the curve (AUC) value. The values of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR+), and negative likelihood ratio (LR-) of biomarkers whose cut-off value was calculated for differentiating between the patient and control group.

Survival time was determined by subtracting the date of pathological diagnosis from the date of patient death. The median survival time and 95% confidence intervals (CI) were calculated using the Kaplan-Meier method to determine survival probability. The log-rank test was used to determine the influence of biomarkers on survival.

In determining the effect of each biomarker on prognosis, adjustment was made according to histopathology, stage, and effects of treatment. Hazard ratios and 95% CI were calculated by multivariate Cox regression analysis to determine the good and poor prognoses of the biomarkers at the calculated cut-off values. $p < 0.05$ was considered to indicate the statistical significance level.

Post-hoc power analysis was performed using the G*Power demo program. For the distinction between benign and malignant diseases, the post-hoc power (1-beta error) of the study was calculated to be 96.8% when the effect size was 0.80, the alpha error was 0.05, the sample size of patients with MPM was 101, and the sample size of HDs was 24. For discrimination between the chemotherapy response groups, the post-hoc power (1-beta error) of the study was calculated to be 90.8% when the effect size was

0.40, the alpha error was 0.05, and the sample size of patients who received chemotherapy was 72.

RESULTS

The mean age ± SD (min-max) of the 101 patients included in the first phase of the study was 64.34 ± 10.60 (36-88) years. Of these, 64 (63.3%) were men and 37 (36.7%) were women. Seventy-four (73.3%) patients had epithelioid, 18 (17.8%) had mixed, and 9 (8.9%) had sarcomatoid cell type disease. Twenty-four (23.8%) patients were in stage I-II and 77 (76.2%) were in stage III-IV. The median Karnofsky performance score was 90 (min-max: 60-100).

Expression Values of the miRNAs at the Time of Diagnosis

Comparison of the miRNA-ΔCt values of MPM and HDs and fold change (FC) is shown in Table 1. All miRNAs, except miR-31-5p, were expressed differently in MPM than in HD. It was found that the expression level of miR-16-5p decreased when compared with that of HD, and the expression levels of miR-29c-3p, miR-125a-5p, miR-320a, miR-484, and miR-532-5p increased when compared with that of HD.

The AUC and predictive values of miRNAs with ROC analysis in distinguishing MPM from HD were determined. The ROC curves of the mentioned miRNAs are depicted in Figure 2. miR-29c-3p, miR-125a-5p, miR-320a, miR-484, and miR-532-5p were found to be useful in distinguishing MPM from HD. The cut-off and AUC values of miR-16-5p and miR-31-5p in MPM and HD were not significantly different (>3.45 and 0.597; ≤5.40 and 0.566, respectively).

The sensitivity, specificity, PPV, NPV, LR+, and LR− values of significant miRNAs for distinguishing MPM from HDs are shown in Table 2.

The Effect of miRNAs on the Prognosis of MPM

The median survival time of patients according to the miRNA ΔCt values is shown in Table 3. Patients with a lower expression level of miR-29c-3p, miR-125a-5p, and miR-484 showed longer survival than patients with higher expression levels.

After adjusting for stage, histological subtype, and treatment variables, the miR-29c-3p, miR-125a-5p, miR-484, and treatment were found to be effective for survival, whereas only miR-125a-5p and treatment were found to be effective in multivariate analysis (Table 4).

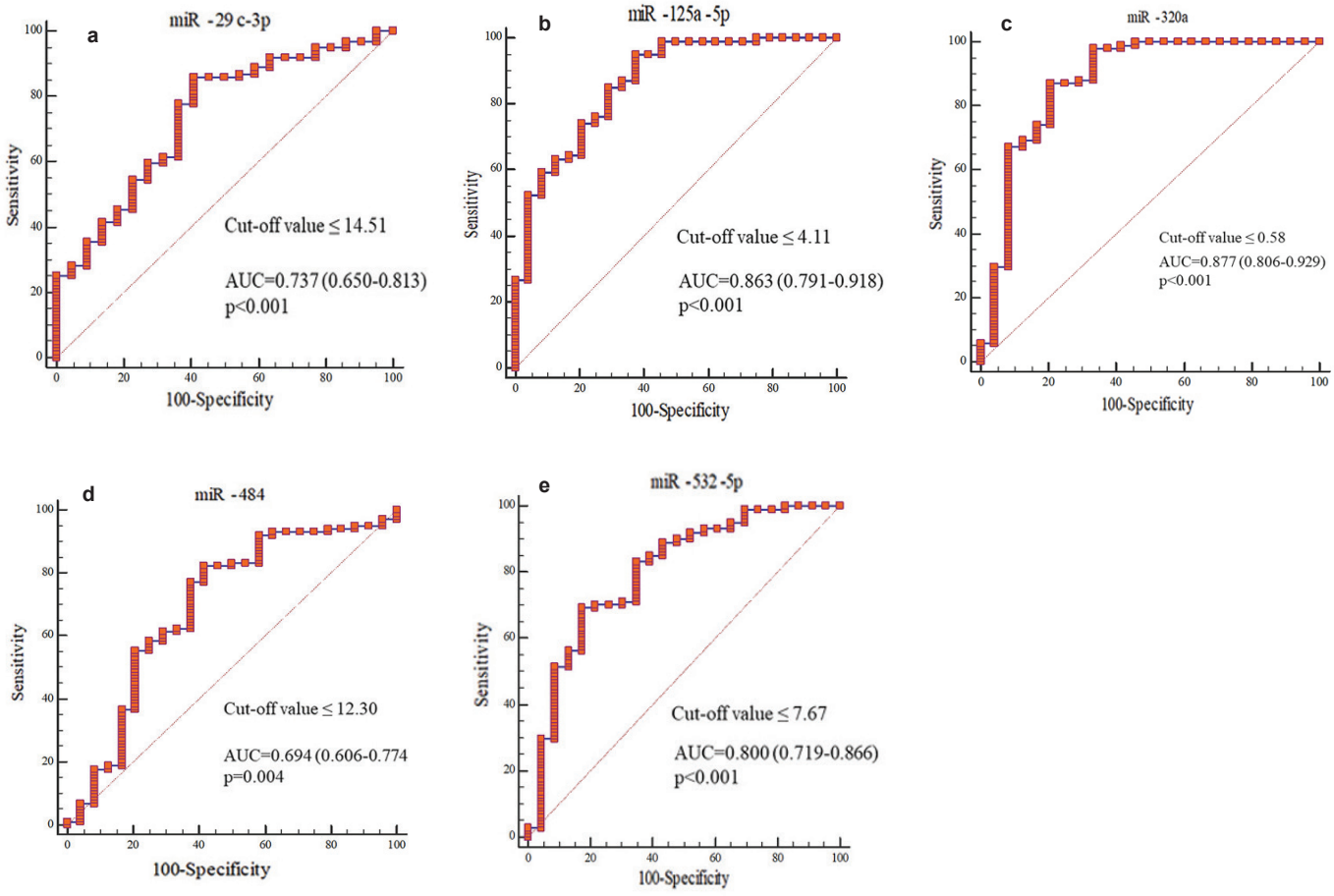

FIG. 2. ROC curves of miR-29c-3p (a), miR-125a-5p (b), miR-320 (c), miR-484 (d), and miR-532-5p (e), which are important miRNAs in distinguishing malignant pleural mesothelioma from healthy donors.

TABLE 1. The Comparison of miRNA ΔCt Values of Malignant Pleural Mesothelioma and Healthy Donors

	MPM (n = 101) Mean ΔCT values ± SD (min.-max.)	HD (n = 24) Mean ΔCT values ± SD (min.-max.)	FC	p
miR-16-5p	1.76 ± 4.90 (-9.18 - 14.16)	-0.71 ± 5.19 (-13.36 - 7.09)	0.18	0.030
miR-29c-3p	8.59 ± 5.26 (-2.17 - 17.73)	12.96 ± 4.26 (4.68 - 18.25)	20.68	0.001
miR-31-5p	3.69 ± 5.22 (-7.48 - 15.20)	4.54 ± 5.38 (-8.08 - 10.51)	1.80	0.479
miR-125a-5p	-4.92 ± 5.27 (-15.45 - 6.76)	3.09 ± 4.86 (-8.61 - 9.29)	257.78	<0.001
miR-320a	-4.60 ± 4.77 (-15.68 - 6.07)	4.27 ± 5.89 (-11.25 - 10.30)	467.88	<0.001
miR-484	7.56 ± 4.39 (-4.01 - 19.15)	10.63 ± 4.96 (-2.24 - 15.82)	8.40	0.003
miR-532-5p	5.59 ± 5.78 (-5.78 - 19.59)	12.57 ± 6.54 (-4.20 - 21.89)	126.24	<0.001

MPM: Malignant pleural mesothelioma; HD: Healthy donors; FC: fold change (2^-ΔΔCT).

TABLE 2. Sensitivity, Specificity, PPV, NPV, LR+, and LR- Values of Significant miRNAs to Differentiate Malignant Pleural Mesothelioma From the Healthy Donors

miRNA	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR+	LR-
miR-29c-3p	85.9 77.4-92.0	59.1 36.4 - 79.3	89.8 84.5 - 93.5	49.9 35.7 - 64.1	1.90 1.1 - 3.2	0.38 0.2 - 0.6
miR-125a-5p	95.1 88.8 - 98.4	62.5 40.6 - 81.2	91.4 86.4 - 94.7	75.2 54.9 - 88.3	2.53 1.5 - 4.3	0.079 0.03 - 0.2
miR-320a	87.1 79.0 - 93.0	79.2 57.8 - 92.9	94.6 88.9 - 97.5	59.3 45.8 - 71.6	3.56 1.6 - 7.8	0.33 0.2 - 0.5
miR-484	82.2 73.3 - 89.1	58.3 36.6 - 77.9	89.2 83.7 - 93.1	43.8 31.2 - 57.2	1.85 1.1 - 3.0	0.39 0.2 - 0.6
miR-532-5p	69.3 59.3 - 78.1	82.6 61.2- 95.0	94.4 87.4 - 97.6	39.0 31.2 - 47.5	3.99 1.6 - 9.8	0.37 0.3 - 0.5

PPV: Positive predictive value; NPV: negative predictive value; LR+: positive likelihood ratio; LR-: negative likelihood ratio.

TABLE 3. The comparison of median survival time of malignant pleural mesothelioma according to the miRNA ΔCt Values

miRNA	Median survival time (months)	p
miR-16-5p		
> 3.45	15.0 ± 2.9 (9.4 - 20.6)	0.708
≤ 3.45	15.0 ± 1.6 (11.8 - 18.2)	
miR-29c-3p		
> 14.51	20.0 ± 4.43 (11.3 - 28.7)	0.035
≤ 14.51	15.0 ± 1.5 (12.0 - 18.0)	
miR-31-5p		
> 5.40	15.0 ± 1.5 (12.0 - 18.0)	0.270
≤ 5.40	14.0 ± 1.4 (11.3 - 16.7)	
miR-125a-5p		
> 4.11	30.0 ± 9.8 (10.8 - 49.2)	0.008
≤ 4.11	12.0 ± 1.2 (9.6 - 14.4)	
miR-320a		
> 0.58	15.0 ± 4.0 (7.1 - 22.9)	0.109
≤ 0.58	13.0 ± 1.3 (10.5 - 15.5)	
miR-484		
> 12.30	20.0 ± 3.4 (13.4 - 26.6)	0.047
≤ 12.30	14.0 ± 1.5 (11.0 - 17.0)	
miR-532-5p		
> 7.67	13.0 ± 1.1 (10.8 - 15.2)	0.785
≤ 7.67	15.0 ± 1.0 (13.0 - 17.1)	

TABLE 4. Hazard Ratio and 95% CI of miRs Associated with the Prognosis in the Adjusted Model

Variables	HR 95%CI	p
Cell type		
Epithelioid	1	
Non-epithelioid	1.430 (0.895-2.284)	0.135
Stage		
I-II	1	
III-IV	0.999 (0.582-1.715)	0.997
Treatment		
Yes	1	
No	3.105 (1.836-5.251)	<0.001
miR-29c-3p		
High#	1	
Low	2.299 (1.148-4.605)	0.019
miR-125a-5p		
High#	1	
Low	5.659 (2.067-15.493)	0.001
miR-484		
High#	1	
Low	2.527 (1.383-4.619)	0.003

HR: Hazard ratio; CI: confidence interval, #According to the cut-off values.

The Relationship of miRNAs to Radiological Response in Patients with MPM Receiving Chemotherapy

Of the 74 patients whose response to chemotherapy could be assessed, 44 (59.5%) were women and 30 were men. The mean age of the patients was 64.0 ± 9.4 (36-81) years. A total of 55 patients (74.3%) had epithelioid, 12 (16.2%) patients had mixed, and 7 (9.5%) patients had sarcomatoid cell type disease. Sixteen patients (21.6%) were at stage I-II and 58 (78.4%) were at stage III-IV.

The radiological response rates of patients to chemotherapy were as follows: 22 (29.7%) progressive disease, 27 (36.5%) stable disease, 24 (32.4%) partial response, and 1 (1.4%) complete response. The miRNA ΔCт values at pretreatment and at the time of best overall radiological response were compared (Table 5).

Consistent with the radiological response, no differences were noted in the expression levels of miRNAs between the pretreatment and chemotherapy response measurements.

The ΔCт values of miRNAs in the serum samples collected at the end of treatment and during the disease recurrence were not significantly different. The ΔCт values of miR-16-5p, miR-29c-3p, miR-31-5p, miR-125a-5p, miR-320a, miR-484, and miR-532-5p

TABLE 5. ΔCт Values of miRNA Before Treatment and During the Response Measurement According to the Radiological Response

miRNA	Progressive disease (n = 22)	Stable disease (n = 27)	Objective response* (n = 25)
miR-16-5p			
Before treatment	2.16 ± 1.09	0.91 ± 0.83	1.33 ± 1.16
Response measurement	3.37 ± 1.12	2.06 ± 0.91	0.33 ± 1.32
p	0.270	0.268	0.386
miR-29c-3p			
Before treatment	9.36 ± 1.06	7.20 ± 1.00	6.99 ± 1.01
Response measurement	9.31 ± 1.07	7.73 ± 0.83	6.57 ± 0.99
p	0.944	0.627	0.549
miR-31-5p			
Before treatment	4.20 ± 1.16	2.48 ± 0.90	2.63 ± 1.10
Response measurement	4.80 ± 1.21	2.05 ± 0.99	2.51 ± 1.17
p	0.501	0.693	0.899
miR-125a-5p			
Before treatment	-3.79 ± 1.30	-6.07 ± 0.96	-6.55 ± 0.99
Response measurement	-3.27 ± 1.27	-6.17 ± 1.05	-6.41 ± 1.36
p	0.477	0.922	0.860
miR-320a			
Before treatment	-3.71 ± 1.19	-5.59 ± 0.86	-5.92 ± 0.94
Response measurement	-2.95 ± 1.12	-5.72 ± 0.96	-5.55 ± 1.07
p	0.297	0.900	0.667
miR-484			
Before treatment	7.59 ± 1.04	8.52 ± 0.83	6.24 ± 0.98
Response measurement	8.83 ± 0.77	7.21 ± 0.90	5.44 ± 1.32
p	0.257	0.224	0.546
miR-532-5p			
Before treatment	7.96 ± 1.24	4.28 ± 1.07	3.85 ± 1.05
Response measurement	6.92 ± 1.40	4.32 ± 1.02	3.43 ± 1.53
p	0.496	0.972	0.739

*Objective response means partial + complete response.

at the end of treatment and during disease recurrence were 1.60 ± 083 and 1.23 ± 0.95 (p = 0.737), 6.22 ± 1.21 and 16.33 ± 1.17 (p = 0.941), 1.11 ± 1.15 and 1.76 ± 1.14 (p = 0.615), −7.32 ± 1.07 and -6.87 ± 1.03 (p = 0.585), -6.74 ± 0.96 and -6.38 ±1.02 (p = 0.675), 6.75 ± 0.98 and 7.74 ± 0.84 (p = 0.381), 2.83 ± 1.06 and 4.09 ± 1.01 (p = 0.285), respectively.

DISCUSSION

In this study, miR-29c-3p, miR-125a-5p, miR-320a, miR-484, and miR-532-5p showed a significant difference between MPM and HDs; particularly, the AUC values of miR-125a-5p, miR-320a, and miR-532-5p were > 0.800. After adjusting for histological subtype, stage, and treatment, miR-29c-3p, miR-125a-5p, and miR-484 were found to be associated with longer survival. However, the serum levels of miRNAs did not change during the longitudinal follow-up of chemotherapy response and the detection of relapse.

miRNA analysis and the miRNA expression changes in tumor tissue samples for the diagnosis of MPM have been the subject of several studies. In these studies, a large number of miRNAs were found to exhibit different expression levels in the mesothelioma tumor tissue when compared with HDs and subjects with benign pleural pathology.[12,13,17,20] However, circulating miRNAs appear to be promising biomarkers for MPM diagnosis, prognosis analysis, and treatment follow-up as they are relatively easy to analyze, repeatable when needed, and inexpensive.[7,8,21] In this regard, the determination of circulating miRNAs remains a hot topic for MPM.

A comprehensive study revealed that the expression levels of miR-1281, miR-32-3p, and miR-197-3p were significantly increased in patients with MPM when compared with that in healthy or asbestos-exposed individuals, suggesting that these 3 markers may be helpful in diagnosis.[22] In another study, miR-101, miR-25, miR-26b, miR-335, and miR-433 showed increased expression levels in the serum of 14 patients with MPM when compared with that in 10 patients with benign pleural disease, whereas their miR-191 and miR-223 expression levels were decreased.[23] Kirschner et al.[24] demonstrated that the AUC value of increased expression of miR-625-3p in the serum was 0.820 to discriminate MPM between healthy asbestos-exposed and unexposed individuals.

In this study, the expression levels of miR-29c-3p, miR-125a-5p, miR-320a, miR-484, and miR-532-5p were found to be significantly higher in MPM patients when compared with that in HDs. These miRNAs were found to behave like oncogenic miRNAs, although the expression level of miR-16-5p was tumor-suppressive. Our results demonstrated that miR-125a-5p had a high sensitivity and a reliable LR- value, definitely indicating further invasive procedure whenever there was an increase in the expression and indicating that it has a reliable value to exclude MPM when there is no increase in expression.

Santarelli et al.[25] analyzed the expression of miR-126 in the serum samples of 44 MPM patients, 196 asbestos-exposed healthy workers, and 50 healthy individuals. They found that the sensitivity of the decrease in miR-126 expression to discriminate MPM from asbestos-exposed workers was 60% and the specificity was 75% and that the sensitivity to discriminate MPM from healthy individuals was 73% and the specificity was 74%. The sensitivity of the miR-126-3p level in the plasma to discriminate MPM patients from healthy individuals was 59% and the specificity was 72% (AUC 0.614).[21] In another study, the sensitivity and specificity for miR-103 were 83% and 71% in distinguishing MPM patients from asbestos-exposed individuals and 78% and 76% from healthy individuals.[26] MiR-548a-3p and miR-20a were examined in 60 cases with MPM, 20 asbestos-exposed individuals, and 20 healthy subjects. The sensitivity and specificity of high expression levels of these miRNAs for MPM were reported to be 100% and 87%, respectively.[27]

miRNAs may act as tumor suppressors or oncogenes and affect the ultimate survival outcome.[7,11] A high expression of miR-16 was reported in the tumor tissue and plasma, indicating a tumor-suppressive effect, while a high expression of miRNA-486 in the tumor tissues was associated with a longer life expectancy.[13] A recent study revealed that the increase in the expression of miR-31 behaved as an oncogene in the tumor tissues, implying a poor prognosis in MPM with the sarcomatoid subtype.[16] De Santi et al.[28] performed a study on tumor and normal pleural samples in an MPM series of 96 cases to identify the novel pathways in miRNA expression, diagnosis, prognosis, and treatment and identified 63 miRNAs that exhibited statistically significant expression. They found that the expression of let-7c-5p and miR-151a-5p could help determine prognosis.[28]

Increased hsa-miR-29c expression acted as an independent prognostic factor associated with prolonged survival after cytoreduction in a surgical series.[15] Kirschner et al.[17] reported that the miR score, consisting of the expression levels of miR-21-5p, miR-23a-3p, miR-30e-5p, miR-221-3p, miR-222-3p, and miR-31-5p in the tumor tissues, was associated with prolonged survival (20 months) in patients undergoing surgical treatment. A past study determined prognosis-related miRNA in patients with MPM and found that deficiency of miR-99a, let-7, and miR-125b was associated with poor prognosis in the tumor tissue samples obtained from 30 MPM patients who were not candidates for surgery.[29]

In our study, miR-29c-3p, miR-125a-5p, and miR-484 were found to be significantly associated with a more prolonged survival when they showed decreased expression. According to previous studies, the lack of an association at the level of miR-31 maybe because we did not discriminate by the histopathological subtype in our study.[16] We believe that the lack of a prognostic association with miR-16 in our study may be because we used serum samples while these past studies used tissue samples. Thus, tumor heterogeneity may also play a role.

It was found that resistance to chemotherapy increased when the mRNA expression of the tumor suppressors decreased in the patients. On the contrary, increased expression of a miRNA behaved like a tumor suppressor-induced chemotherapy-induced apoptosis. One study thereby indicated that the expression of miR-

15a, miR-16, and miR-34a was downregulated in MPM cells with an acquired drug resistance. Moreover, transfection with miR-15a or miR-16 mimics abrogated resistance to cisplatin, gemcitabine, or vinorelbine, whereas miR-34a only abrogated resistance to cisplatin and vinorelbine.[30]

In our study, we did not observe any changes in the miRNA levels during treatment monitoring and recurrence. There may be several reasons affecting the results of our study at this stage, as detailed here: The changes in the miRNA expression in the serum or tissue during tumor progression or regression may be affected by chemotherapy. Another issue is that the expected change in the miRNA levels in the tumor due to treatment may not be reflected in the serum. As no study has yet investigated the effects of miRNAs on the subject, further evaluation was not possible at this stage.

Consequently, miR-29c-3p, miR-125a-5p, miR-320a, miR-484, and miR-532-5p, especially miR-125a-5p, may help diagnose MPM patients with high sensitivity, specifically without the need for advanced invasive procedures. In addition, miR-29c-3p, miR-125a-5p, and miR-484 provide helpful information to determine the prognosis of patients. However, further studies are warranted to monitor the treatment and determine recurrence.

Ethics Committee Approval: The study was approved by the Eskişehir Osmangazi University Ethical Committee (17/19.3.18).

Informed Consent: All participants provided their signed informed consent.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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