BENHA MEDICAL JOURNAL bmj

Print ISSN 1110-208X

Online ISSN 2357-0016

Original article

Role of MicroRNA (182) in Differentiation between Benign and Malignant Pleural Effusion

Amira H. Allam [a], Magdy M. Omar [a], Hmasat A. El Noury [b], Abeer M. Rawy [a], Toqa A. Anwar [a], Rehab E. El Sawy [a]

[a] Department of Chest, Faculty of Medicine Benha University, Egypt.

[b] Department of Clinical Pathology, Faculty of Medicine Benha University, Egypt.

Corresponding to: Toqa A. Anwar, Department of Chest, National Heart Institute - Giza, Egypt

Email:

loaiayman.loaiayman@gmail.com

Received: 26 October 2024

Accepted: 29 November 2024

Abstract

Background: There is still a clinical challenge in differentiating between malignant pleural effusion (MPE) and benign pleural effusion (BPE). **Aim:** to evaluate the role of microRNA (182) in differentiation between tuberculous (TPE) and MPE. **Methods:** This cross-sectional study involved 60 patients admitted to the Chest Department, Benha University Hospital. Patients were divided into two groups based on pathological results: Group A (n=45): MPE and Group B: (n=15): TPE. miRNA 182 was measured by qt PCR in both groups and expressed as ΔΔCT and fold change ($2^{-\Delta\Delta Ct}$) **Results**: No statistically significant difference between MPE and TPE regarding ΔΔCT and fold change and the level did not differ significantly between malignant types. Adenosine deaminase (ADA) can significantly differentiate between malignant and TB groups (P<0.001), at cutoff value ≤39 U/L, with 93.33% sensitivity, 100% specificity, 100% PPV and 83.3% NPV. Contrarily, microRNA fold change had 26.67% sensitivity, 40.00% specificity, 57.1% PPV and 15.4% NPV at cutoff value ≤-1.94, ΔΔCT had 80% sensitivity, 60.00% specificity, 85.7% PPV and 50% NPV at cutoff value ≤1.602, both could not differentiate between malignant and TB groups. **Conclusion:** microRNA and ΔΔCT can't distinguish between malignant and TB groups. However, ADA can significantly differentiate between malignant and TB groups at a cutoff value ≤39 U/L. There was a significant positive relationship between ADA on the one hand and LDH and tuberculin skin test on the other hand. However, there was an insignificant correlation between micro-RNA-182 level and LDH or tuberculin skin test.

Keywords: MicroRNA (182); Differentiation; Benign; Malignant Pleural Effusion.

Introduction

In the pleural space, pleural effusion (PE) is defined as an abnormal accumulation of fluid that is the consequence of various types of underlying conditions [1]. Causes of PE may be usefully divided into transudates and exudates based on the protein concentration of pleural fluid [2]. PE can be caused by a variety of conditions, such as congestive heart failure (CHF), cirrhosis, parapneumonic effusions and empyema, pulmonary embolism, TB, collagen vascular diseases, and malignancy [3].

Effusions that are the consequence of cancer cells' direct infiltration of the pleura are referred to as MPE. Paraneoplastic or paramalignant effusions are effusions that are the result of indirect effects of malignancies on the pleural space, including obstruction of mediastinal lymph nodes, bronchial obstruction, pulmonary embolism, superior vena cava syndrome, or decreased oncotic pressure. [4]. The most common histological variety is metastatic adenocarcinoma, as lymphomas or carcinomas of the lung, breast, or ovary are responsible for over 75% of MPEs [5].

Typically, the suspected MPE assessment involves a series of diagnostic examinations, including a pleural biopsy, a thoracocentesis with pleural fluid analysis, and imaging studies, all of which are followed by a thorough medical history and physical examination. [5]. Blind percutaneous pleural biopsy has a decreased sensitivity than image-guided (CT or USG) biopsy; however, it can obtain a yield as high as thoracoscopic parietal pleural biopsy (95%) [6]. During routine cytological testing of MPE, the presence of cancer cells is found only in 60% of cases. The diagnosis is typically made after the use of more invasive techniques, such as thoracoscopy or thoracotomy, as such detection is still insufficient for making clinical decisions [7]. In this case, it would be beneficial to perform an additional marker or group of markers search, as this would allow for a preliminary diagnosis to be made at a much earlier stage. It was suggested that miRNAs could be employed as a factor in the differential diagnosis of MPE and non-MPE [8].

MicroRNAs (miRNAs) are single-stranded, non-coding RNA short nucleotide sequences used as essential post-transcriptional regulators of gene expression. They are detected in both physiological and pathological body fluids, in addition to intracellularly. This group of

molecules is responsible for the regulation of up to 33% of human genes by either directly acting as an oncogene or suppressor gene or by modulating the expression of both oncogenes and suppressor genes. [8]. MiR-182 is a microRNA that is well-studied and is situated on chromosome 7q31-34. It is part of the miR-183 family [9]. The largest number of studies have demonstrated that miR-182-5p targets tumor suppressor genes and increases cell proliferation, and it is a member of the miR-183 cluster. The influence of miR-182-5p on lung cancer cell lines has been evaluated [10].

The purpose of this research was to evaluate the function of microRNA (182) in the differentiation of BPE and MPE.

Patients and methods

This cross-sectional study included 60 patients admitted at the Chest Department, Benha University Hospital, during the period from 1st of October 2022 to 1st of October 2023. Informed written consent was obtained from the patients. There was an explanation of the study's purpose provided to each patient. The research was given approval by the Research Ethics Committee of the Faculty of Medicine at Benha, University.

Inclusion criteria: admitted patients who suffer from lymphocytic exudative PE with confirmed diagnosis as tuberculous or malignant PE based on pleural biopsy either Abrams pleural biopsy or thoracoscopic biopsy or by other diagnostic means according to diagnostic protocol. **Exclusion criteria:** patients with transudative PE, non-lymphocytic PE, contraindication to pleural biopsy, non-specific pathology results, patients who refused to participate in the study, and those less than 18 years old.

Grouping: Patients were arranged into two groups based on the pathology results: **Group A (n=45)**: patients with MPE and **Group B: (n=15)**: patients with TPE.

The following was applied to all cases that were examined: Detailed history taking, including [Personal history (age, sex, occupation, special habits), past medical history (hypertension, diabetes mellitus, asthma, rheumatoid arthritis, and cardiac disease). **Full clinical examination:** including General and local chest examination. **Routine laboratory investigations:** Complete blood picture (CBC) included hemoglobin (Hb), white blood cells (WBCs), and platelets which were measured using a fully automated hematology analyzer (Sysmex XN-L series, USA). Kidney function tests: the levels of Urea and creatinine were evaluated by using a fully automated chemistry analyzer (Cobas c311, serial number 1480-10). Bleeding profile: Prothrombin time (PT) and

international normalized ratio (INR) were assessed using a semi-automated coagulation analyzer (KC1 Delta, serial number 000163367). The erythrocyte sedimentation rate (ESR) was determined using the appropriate sedimentation rate analyzer. Liver enzymes: alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) levels were assessed using the same chemistry analyzer as for kidney function tests (Cobas c311). **Tuberculin skin test:** The purified protein derivative was administered intradermally to the volar aspect of the forearm in a quantity of 0.1 ml (5 tuberculin units) utilizing the Mantoux method. After 48 to 72 hours, the test was read.

Pleural fluid analysis: Physical examination, which involves [aspect, color, turbidity, and specific gravity], and chemical examination, which includes [proteins, glucose , and lactate dehydrogenase (LDH)]. Light's criteria were utilized to classify effusions as exudates or transudates. Papanicolaou-stained smears were used to cytologically examine for predominant or malignant cells, and hematoxylin and eosin-stained sections of paraffin-embedded cell blocks were performed. **Radiological examination:** plain chest radiography (posteroanterior and lateral views) and computed tomography (CT) scans of the chest. **Pleural biopsy** either thoracoscopic pleural biopsy or closed Abrams pleural biopsy.

MicroRNA:

Before beginning any treatment, PF was obtained through thoracocentesis or pleural biopsy and centrifuged at 3000 rpm/min for 20 minutes. Quantitative real-time PCR (qRT-PCR) was used to detect miRNA-182. The supernatants were immediately frozen and stored at -80°C after being transferred to new microcentrifuge containers (500 µl each). **miRNA extraction:** The miRVana PARIS Kit (Ambion, TX) was used to extract total RNA from pleural fluid supernatant in accordance with the manufacturer's protocol. The NanoDrop 1000 Spectrophotometer was employed to quantify the RNA concentration (NanoDrop Technologies, MA).

Reverse transcription: 5 µl of total RNA (5–10 ng/µl) was reverse-transcribed to cDNA by using AMV reverse transcriptase (TakaRa, Dalian, China) and stem-loop RT primers (Applied Biosystems, Foster, CA). Measurement of the cDNA was conducted on an Applied Biosystems 7900 HT thermocycler using the Taqman miRNA PCR reagent (Applied Biosystems, Foster, CA). PCR conditions: 90°C for 10 minutes, followed by 40 cycles of 90°C for 15 seconds and 60°C for 1 minute. Triplicates of each reaction were used. The CT value is the number of amplification cycles necessary for the fluorescent signal to surpass the background level. This implies that the number of products in the sample is

inversely proportional to the CT levels. The utilization of invariant endogenous controls or reference miRNAs is a frequently employed method for normalizing qPCR data. The fold change in miRNA expression was log2-transformed and expressed as $2^{-\Delta\Delta Ct}$ [11].

$$\Delta CT = (CTmiRNA - CTendogenous\ control)$$

$$\Delta\Delta CT = \Delta CT - \Delta CTof\ the\ control\ group$$

Statistical analysis

SPSS v27 (IBM©, Armonk, NY, USA) was utilized to conduct statistical analysis. To evaluate the normality of the data distribution, the Shapiro-Wilks test and histograms were utilized. The unpaired student t-test was employed to analyze the quantitative parametric data, which were presented as mean and standard deviation (SD). The Mann-Whitney test was used to evaluate quantitative non-parametric data, which were represented as the median and interquartile range (IQR). The Chi-square test or Fisher's exact test was employed to analyze qualitative variables, which were presented as frequency and percentage (%) when appropriate. A two-tailed P value that was less than 0.05 was considered statistically significant. Pearson correlation was employed to estimate the degree of correlation between two quantitative variables. Diagnostic performance was assessed and reported as diagnostic specificity, sensitivity, negative predictive value (NPV) and positive predictive value (PPV) . The ROC curve analysis was employed to evaluate the overall diagnostic performance of each test. The AUC is an index that assesses the overall performance of the test. AUC values greater than 50% indicate acceptable performance, while those around 100% indicate the greatest possible performance.

Results

This research was conducted on 60 patients (45 MPE, 15 TPE). Regarding the baseline characteristics, the age of the studied patients ranged from 32 to 70 years with a mean of 48.7 ± 12.5 years. There were 36 (60%) males and 24 (40%) females, 36 (60%) were smokers and 24 (40%) nonsmokers. Age was significantly lower in group B than in group A (38.2 ± 3.3 Vs 52.4 ± 12.34, P <0.001). 21 (46.67%) in group A were smokers, 24 (53.33%) were nonsmokers while in group B all patients were smokers (P <0.001).

Group B showed a significantly lower INR than group A (P<0.001, 0.037). Group B expressed significantly higher WBCs, neutrophils, ESR, and ALT (P<0.05). The clinical presentation of both groups was significantly different, with all patients in both groups presenting with SOB. Stitching chest pain was present in all patients in group B, whereas it was present in

only six (13.33%) in group A. Compressing chest pain, Loss of appetite, Difficult swallowing, Dry cough, Face & neck edema and Loss of weight were present in group A {9 (20%), 3 (6.67%),9 (20%), 9 (20%), 3 (6.67%), 18 (40%)} with no patients in group B presented by these symptoms. On the other hand fever and productive cough were present in group B only (6 (40%), 9 (60%)) (P<0.001), **Table 1.**

Massive effusions were more common in group A in which serosanguinous free effusions were more common, while yellowish loculated effusions were more common in group B (P<0.05). There was an insignificant difference between both groups regarding other baseline criteria, **Table 1.**

LDH and ADA levels were substantially higher in group B than in group A concerning the pleural aspiration parameters (P=0.007, <0.001). Group B demonstrated a substantially lower glucose level than group A (P<0.001). There was an insignificant difference between both groups regarding protein level, MicroRNA (fold change), and ΔΔCT, **table 2**. The final diagnosis in group A (malignant group) was as follows; 3(6.67%) patients had grade I lung adenocarcinoma, 15 (33.33%) grade II lung adenocarcinoma, 6 (13.33%) patients had metastatic adenocarcinoma, 3 (6.67%) metastatic grade II adenocarcinoma, 15 (33.33%) patients had mesothelioma, and 3 (6.67%) patients had small cell carcinoma. The mean ΔΔCT in patients with grade I lung adenocarcinoma was 3.03 ± 0.1, grade II lung adenocarcinoma 0.83 ± 0.92, in patients with metastatic grade I adenocarcinoma was 1.41 ± 0.08, metastatic grade II adenocarcinoma 1.16 ± 0.01, in patients with mesothelioma was 0.33 ± 1.19, in small cell carcinoma was 3.63 ± 0.04 and in patients with TB was 1.38 ± 1.2. The mean of small cell carcinoma was the highest, followed by Grade I lung adenocarcinoma, and Mesothelioma had the lowest mean. However, the difference was statistically insignificant (P = 0.091), **table 3**.

The tuberculin skin test and the ADA level exhibited a significant positive correlation, while the ADA level and glucose level demonstrated a significant negative correlation. There was a significant positive relationship between the ADA level and the Tuberculin skin test, while a significant negative relationship was observed between the ADA level and glucose. There was an insignificant correlation between ADA and Lymphocytes, protein, and Micro RNA expression. There was an insignificant correlation between Micro RNA expression and pleural aspirate parameters or tuberculin test, **table 4.**

ADA can significantly differentiate between malignant and TB groups

(P<0.001), at cutoff value ≤39 U/L, with 93.33% sensitivity, 100% specificity, 100% PPV and 83.3% NPV with AUC of 1.00, microRNA (fold change) had 26.67% sensitivity, 40.00% specificity, 57.1% PPV and 15.4% NPV at cutoff value ≤-1.94 in differentiating between malignant and TB effusions (P=0.953), $\Delta\Delta C_T$ had 80% sensitivity, 60.00% specificity, 85.7% PPV and 50% NPV at cutoff value ≤1.602, but results were statistically insignificant (P=.091), **table 5, Figure 1**

Table1: Baseline characteristics of the studied groups

		Group A (Malignant group) (n=45)	Group B (TB group) (n=15)	P value	Total(N 60)
Age (years)	**Mean± SD**	52.4 ± 12.34	38.2 ± 3.3	<0.001*	48.7 ± 12.5
	Range	32 - 70	35 – 43		32 - 70
Sex	**Male**	24 (53.33%)	12 (80%)	0.078	36 (60%)
	Female	21 (46.67%)	3 (20%)		24 (40%)
Smoking	**Smokers**	21 (46.67%)	15 (100%)	<0.001*	36 (60%)
	Non-smokers	24 (53.33%)	0 (0%)		24 (40%)
Smoking index (Si)	**Mean± SD**	296.7 ± 346.56	346 ± 76.23	0.589	314.2 ± 302.18
	Range	0 - 900	240 – 450		0 - 900
	Median (IQR)	0 (0 – 560)	340 (300-400)		
Comorbidities	**None**	21 (46.67%)	9 (60%)	---	
	HTN	12 (26.67%)	0 (0%)		
	DM	15 (33.33%)	0 (0%)		
	IHD	3 (6.67%)	0 (0%)		
	Cancer colon	3 (6.67%)	0 (0%)		
	Cancer prostate	3 (6.67%)	0 (0%)		
	Heart failure	3 (6.67%)	0 (0%)		
	HCV	0 (0%)	6 (40%)		
	HIV	0 (0%)	3 (20%)		
Symptoms	**SOB**	45 (100%)	15 (100%)	<0.001*	60 (100%)
	Compressing chest pain	9 (20%)	0 (0%)		9 (15%)
	Loss of appetite	3 (6.67%)	0 (0%)		3 (5%)
	Difficult swallowing	9 (20%)	0 (0%)		9 (15%)
	Dry cough	9 (20%)	0 (0%)		9 (15%)
	Face & neck edema	3 (6.67%)	0 (0%)		3 (5%)
	Loss of weight	18 (40%)	0 (0%)		18 (30%)
	Stitching chest pain	6 (13.33%)	15 (100%)		21 (35%)
	Fever	0 (0%)	6 (40%)		6 (10%)
	Productive cough	0 (0%)	9 (60%)		9 (15%)
Side of effusion	**Right**	33 (73.33%)	9 (60%)	0.329	42 (70%)
	Left	12 (26.67%)	6 (40%)		18 (30%)
Amount of effusion	**Massive**	36 (80%)	3 (20%)	<0.001*	39 (65%)
	Moderate	6 (13.33%)	6 (40%)		12 (20%)
	Moderate to massive	3 (6.67%)	3 (20%)		6 (10%)
	Mild to moderate	0 (0%)	3 (20%)		3 (5%)

Type of effusion	**Free**	45 (100%)	6 (40%)	<0.001*	51 (85%)
	Loculated	0 (0%)	9 (60%)		9 (15%)
Physical appearance of effusion	**Serosanguinous**	40 (88.89%)	0 (0%)	<0.001*	40 (66.67%)
	Hemorrhagic	5 (11.11%)	0 (0%)		5 (8.33%)
	Yellow turbid	0 (0%)	15 (100%)		15 (25%)
		Laboratory investigations			
Hb (g/dL)	**Mean± SD**	10.6 ± 2.8	11.8 ± 2.74	0.131	10.9 ± 2.83
	Range	6.8 - 16	7.6 - 14.6		6.8 - 16
RBCs (*10^{12} /L)	**Mean± SD**	3.7 ± 1.38	3.9 ± 1.19	0.630	3.77 ± 1.34
	Range	1.3 - 5.2	1.9 - 4.9		1.3 - 5.2
Platelets (*10^9/L)	**Mean± SD**	238.9 ± 118.2	275.6 ± 62.61	0.257	249.7 ± 107.94
	Range	58 - 420	190 – 361		58 - 420
WBCs (*10^3/µL)	**Mean± SD**	6.3 ± 2.88	9.42 ± 4.34	0.001*	7.1 ± 3.57
	Range	1.7 - 11	3.4 – 14.3		1.7 - 14.3
Lymphocytes (*10^9/L)	**Mean± SD**	2.9 ± 1.96	1.92 ± 0.73	0.058	2.7 ± 1.8
	Range	0.4 - 7	0.34 – 2.85		0.34 - 7
Neutrophils (*10^9/L)	**Mean± SD**	3.9 ± 2.21	5.35 ± 2.21	0.031*	4.3 ± 2.3
	Range	1.2 - 10	1.8 – 7.2		1.2 - 10
ESR (mm/hr)	**Mean± SD**	25.2 ± 27.36	118.2 ± 17.9	<0.001*	48.3 ± 48.19
	Range	0 - 100	100 – 145		0 - 145
	Median (IQR)	15 (5 – 35)	115 (101 – 130)		17 (10-100)
INR	**Mean± SD**	1.15 ± 0.22	1.02 ± 0.08	0.037*	1.12 ±0.2
	Range	0.9 - 1.7	0.9 - 1.1		0.9-1.7
Serum creatinine (mg/dL)	**Mean± SD**	1.1 ± 0.32	1.2 ± 0.39	0.356	1.11 ± 0.34
	Range	0.6 - 1.7	0.9 - 1.9		0.6 - 1.9
Urea (mg/dL)	**Mean± SD**	29.2 ± 8.59	30.2 ± 10.44	0.713	29.56 ± 9.04
	Range	21 - 54	22 – 50		21 - 54
ALT (U/L)	**Mean± SD**	21.5 ± 2.02	23.6 ± 4.66	**0.016***	22.05 ± 3.01
	Range	19 - 25	19 – 30		19 - 30
AST (U/L)	**Mean± SD**	19.3 ± 6.69	18.2 ± 2.88	0.529	19.2 ± 5.93
	Range	11 - 33	15 – 22		11 - 33

HTN: hypertension, DM: diabetes mellitus, IHD: ischemic heart disease, HCV: hepatitis C virus, HIV: human immunodeficiency virus, SOB: shortness of breath, Hb: hemoglobin, RBCs: red blood cells, WBCs: white blood cells, ESR: erythrocyte sedimentation rate, ALT: alanine aminotransferase, AST: aspartate aminotransferase, *: statistically significant as p value <0.05.

Table 2: Tuberculin skin test, Pleural fluid analysis and Final diagnosis of the studied groups

		Group A (Malignant group) (n=45)	Group B (TB group) (n=15)	P value
Tuberculin skin test	**No reaction**	21 (46.67%)	-------	-----
	Up to1mm	12 (26.67%)	--------	
	1-2mm	6 (13.33%)	-------	
	2-3 mm	6 (13.33%)	--------	
	Mean± SD	-------	23.6 ± 7.87	
	Range	--------	15 – 35	
Protein (g/dL)	**Mean± SD**	3.4 ± 0.29	3.5 ± 0.27	0.162
	Range	3 - 3.88	3.14 - 3.8	
LDH (U/L)	**Mean± SD**	921.3 ± 129.38	1020.4 ± 77.82	0.007*
	Range	700 - 1060	900 – 1118	
Glucose (mg/dL)	**Mean± SD**	69.1 ± 9.14	37.4 ± 7.34	<0.001*
	Range	60 - 96	30 – 50	
ADA (U/L)	**Mean± SD**	25.7 ± 8.07	79.4 ± 26.7	<0.001*
	Range	12 - 40	45 – 120	
MicroRNA (fold expression)	**Mean± SD**	-0.9 ± 3.67	-1.3 ± 3.07	0.707
	Range	-11.18 - 1.98	-5.65 – 2	
ΔΔCт	**Mean± SD**	0.8 ± 1.53	1.4 ± 1.21	0.177
	Range	-2.24 - 3.63	0 - 2.77	

LDH: lactate dehydrogenase, ADA: adenosine deaminase, TB: tuberculosis. : statistically significant as p-value <0.05.

Table 3: ΔΔCT regarding the final diagnosis

	ΔΔCT	
	Mean± SD	**Range**
Grade I lung adenocarcinoma	3.03 ± 0.1	2.91 - 3.1
Grade II lung adenocarcinoma	0.83 ± 0.92	-0.78 - 1.6
Metastatic grade I adenocarcinoma	1.41 ± 0.08	1.34 - 1.49
Metastatic grade II adenocarcinoma	1.16 ± 0.01	1.15 - 1.17
Mesothelioma	0.33 ± 1.19	-1.13 - 2.02
Small cell carcinoma	3.63 ± 0.04	3.59 - 3.66
TB	1.38 ± 1.2	0 - 2.77
P value	0.091	

Table 4 : Correlation between ADA and Micro RNA Level with other variables

	ADA		Micro RNA (fold expression)	
	r	P	r	P
Lymphocytes	-0.233	0.072	0.136	0.298
LDH	0.399	**0.001***	0.005	0.966
Protein	0.188	0.150	0.161	0.218
Glucose	-0.706	**< 0.001***	0.113	0.390
Tuberculin skin test	0.936	**< 0.001***	-0.0463	0.724
Micro RNA level	-0.144	0.269		

Table 5: Diagnostic accuracy for differentiation between malignant and TB groups

	Cutoff	Sensitivity	Specificity	PPV	NPV	AUC	P value
MicroRNA (fold expression)	≤-1.94	26.67	40.00	57.1	15.4	0.507	0.953
ΔΔCT	≤1.602	80.00	60.00	85.7	50.0	0.640	0.091
ADA (U/L)	≤39	93.33	100.00	100.00	83.3	1.00	**<0.001***

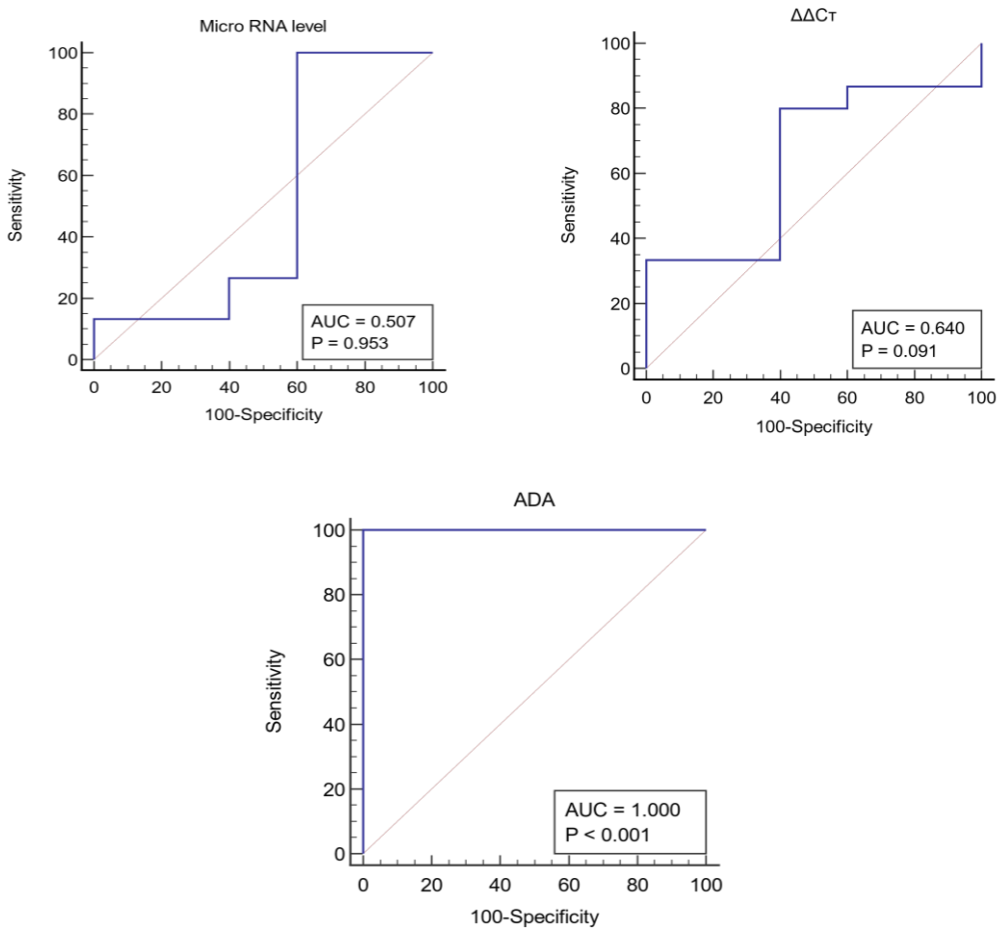

Figure 1: ROC curve analysis of MicroRNA, ADA and ΔΔCT for differentiation between malignant and TB groups

Discussion

A clinical challenge persists in the differential diagnosis of BPE and MPE. The next step is to obtain cytology, which is usually carried out through pleural fluid aspiration or pleural biopsy. Recent years have demonstrated that certain miRNAs are essential for the development of MPE. With additional evidence, the miR-182-5p identified may serve as potential diagnostic biomarkers for MPE [12,13]. The aim of this study was to assess its efficacy in distinguishing MPE from TPE.

In the present study, both groups presented an insignificant difference in terms of gender. However, age was significantly lower in group B (TB group). Other studies gave conflicting results where Zhu et al. [12] agreed

with the present study while Tamiya et al. [14] found insignificant differences between both studied groups regarding age.

In the present study, the laboratory findings revealed that the TB group had significantly higher WBCs, ESR, and ALT than the malignant group, while the TB group had a significantly lower INR than the malignant group. In alignment with the present study, Wei et al. [15] revealed that there was a significant difference between the two groups regarding WBCs and CRP where it was higher in BPE than the MPE group (p<0.001)**.** Additionally, Liam et al. [16] demonstrated that patients with TPE had significantly higher ESR levels than those with MPE, reinforcing the diagnostic utility of ESR despite its overlap between conditions**.** Tuberculosis can lead to alterations in liver function, which may enhance the synthesis of coagulation factors, resulting in a lower INR. The inflammation created by TPE may also affect coagulation pathways, leading to increased clotting activity [17,18].

Considering pleural aspiration parameters, LDH and ADA were significantly higher in the TB group compared to the Malignant group (P=0.007, <0.001). Glucose was significantly lower TB group (P<0.001). This coincides with Wei et al., study [15] which revealed that ADA and LDH levels were increased with a statistical significance in BPE compared to MPE patients (P< 0.001). Krishnan et al. [19] demonstrated that LDH levels were lower in patients with lung malignancies compared to those without malignancies, but the results were statistically insignificant (P = 0.517). Depending on the ROC curve, an ADA cut-off value of less than 16.5 U/L can be used to diagnose malignant effusions, with a sensitivity of 91.5% and 65% and a specificity of 92.5% and 81.4%, respectively. The P value is less than 0.0001.

In this study, there was an insignificant difference between the MPE group and the TPE group regarding protein, microRNA (fold change), and $\Delta\Delta C_T$. Zhu et al.,[12] Bao et al. [13] and Tamiya et al. [14] Abd-El-Fattah et al., [21] showed different results than our study where miRNA 182 was significantly higher in the MPE than in the BPE samples. Our findings differ from those of previous investigations, which may be explained by the fact that they utilized multiple miRNAs, as a single miRNA is insufficiently sensitive and specific [21].

The final diagnosis of the studied groups was as follows; 3 (6.67%) patients had grade I lung adenocarcinoma, 15 (33.33%) patients had grade II lung adenocarcinoma, 6 (13.33%) patients had metastatic adenocarcinoma, 3 (6.67%) patients had metastatic grade II adenocarcinoma, 15 (33.33%) patients had mesothelioma, and 3 (6.67%) patients had small cell carcinoma in

the malignant group. All patients in the TB group were confirmed as TB cases. Agalioti, et al., mentioned that small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) are the two primary forms of lung cancer that cause MPE. NSCLC, which includes subtypes such as squamous cell carcinoma, adenocarcinoma, and large cell carcinoma, is responsible for approximately 80-85% of all lung cancer cases. [22]. There were insignificant differences among the different pathological types of lung cancers regarding the mean $\Delta\Delta C_T$ values in our study. However, the highest values were shown in small cell carcinoma followed by grade I adenocarcinoma, and the lowest value was shown in mesothelioma. Song et al.,[23] highlighted that miRNA expression profiles were generally similar among different grades of lung adenocarcinoma. Frydrychowicz et al. [24] indicated that while certain miRNAs like miR-155 and let-7a-2 were aligned with poor survival outcomes in lung adenocarcinoma, their expression levels did not significantly differ between early-stage tumors (such as grade 1) and more advanced stages (grade 2)

Gee et al. [25] while trying to evaluate the molecular distinctions between mesothelioma and lung adenocarcinoma, he employed microRNA microarrays to identify patterns in the most differentially expressed microRNAs. Malignant pleura mesothelioma (MPM) was found to be down-regulated in comparison to lung adenocarcinoma in terms of specific miRNAs, particularly those belonging to the miR-200 family. However, the clustering analysis indicated an imperfect separation between the two cancers, suggesting that their miRNA expression profiles are not markedly different despite the downregulation of specific miRNAs in MPM

The level of ADA and the tuberculin skin test demonstrated a significant positive relationship, whereas the level of ADA and glucose exhibited a significant negative correlation. There was an insignificant correlation between ADA and Lymphocytes, protein, and Micro RNA expression. ADA is often elevated in TPE due to immune response, and LDH, which increases due to cell damage or death [26].

In the present study, there was an insignificant correlation between micro-RNA level and other variables including lymphocytes, LDH, protein, glucose, and tuberculin skin test. Similarly, Khalifa et al. [27] found that while there were positive correlations between some miRNAs and LDH levels, these did not reach statistical significance for all members. Specifically, a significant positive correlation was noted for miR18a with LDH ($p = 0.003$), while other correlations, including those involving miR19b-1, were not statistically significant despite being observed**.**

Gui and Xiao [28] performed a systematic review about the role of various biomarkers, including ADA and miRNAs, and noted that while some studies found associations between specific miRNAs and disease states, the overall correlation between circulating miRNA levels and ADA was often weak or insignificant. This emphasizes the need for further research to clarify the relationship between these biomarkers across different diseases.

In the present study, ADA with AUC of 1.00 can significantly differentiate between malignant and TB groups ($P<0.001$), at cutoff value ≤39 U/L below that level is considered malignant, with 93.33% sensitivity, 100% specificity, 100% PPV and 83.3% NPV. In parallel to the present study, Fei et al. [29] reported that utilizing multiple indicators, including serum ADA and effusion ADA ratios, resulted in an AUC of 0.919 for distinguishing TPE from non-TPE, with sensitivity and specificity rates of 90.3% and 94.5%, respectively. This suggests that while ADA alone is a potent marker, its diagnostic efficacy increases when used alongside other clinical parameters, reinforcing its role in the differential diagnosis of PE.

In this study, microRNA had 26.67% sensitivity, 40.00% specificity, 57.1% PPV and 15.4% NPV at cutoff value ≤-1.94. ΔΔCT had 80% sensitivity, 60.00% specificity, 85.7% PPV and 50% NPV at a cutoff value ≤1.602. However, both microRNA and ΔΔCT cannot differentiate between malignant and TB groups. This was contrasting Balatti et al. [30] study and Tamiya et al.,[14]. where the AUCs of miR (82) and miR-210 for the diagnosis of Ad-MPE were 0.87 and 0.81, respectively. The combined AUC for these miRNAs was 0.88. They asserted that those miRNAs can perform a beneficial role in the less-invasive screening of PE for diagnostic evaluations.

Conclusion

MicroRNA (fold change) and ΔΔCT cannot differentiate between malignant and TB pleural effusions. However, ADA can significantly differentiate between MPE at a cutoff value ≤39 U/L. While there was a significant positive relationship between ADA on one hand and LDH and tuberculin skin test on the other hand, there was an insignificant correlation between micro-RNA-182 level and LDH or tuberculin skin test.

References

1. **Jany, B.; Welte, T.** Pleural Effusion in Adults-Etiology, Diagnosis, and Treatment. Dtsch. Aerzteblatt Int. 2019, 116, 377–386.
2. **Stephen J Chapman, Robert JO Davies, Mark Woodhead B:** Pleural effusions Clin Med 2004;4:207–10 https://doi.org/10.7861/clinmedicine.4-3-207
3. **Light RW.** Clinical practice. Pleural effusion. N Engl J Med. 2002;346:1971-7.

4. **Rice TW, Rodriguez RM, Barnette R, Light RW.** Prevalence and characteristics of pleural effusions in superior vena cava syndrome. Respirology 2006; 11: 299–305.
5. **John E. HEFFNER** Diagnosis and management of malignant pleural effusions Respirology (2008) 13, 5–20
6. **Pairman, L., Beckert, L. E. L., Dagger, M. & Maze, M. J.** 2022. Evaluation of pleural fluid cytology for the diagnosis of malignant pleural effusion: a retrospective cohort study. *Intern Med J,* 52**,** 1154-1159.
7. **Antonangelo, L., Sales, R. K., Corá, A. P., Acencio, M. M., Teixeira, L. R. & Vargas, F. S.** 2015. Pleural fluid tumour markers in malignant pleural effusion with inconclusive cytologic results. Curr Oncol, 22, e336-41.
8. **Wojczakowski, W., Kobylarek, D., Lindner, J., Limphaibool, N. & Kaczmarek, M.** 2019. MicroRNAs–novel biomarkers for malignant pleural effusions. Clin Chest Med, 23, 133-40.
9. **Avila MA, Dufour JF, Gerbes AL, Zoulim F, Bataller R, Burra P, Cortez-Pinto H, Gao B, Gilmore I, Mathurin P, Moreno C, Poznyak V, Schnabl B, Szabo G, Thiele M, Thursz MR.** Recent advances in alcohol-related liver disease (ALD): summary of a Gut round table meeting. Gut. 2020 Apr;69(4):764-780.
10. **Wang, M., Wang, Y., Zang, W., Wang, H., Chu, H., Li, P., et al.** 2014. Downregulation of microRNA-182 inhibits cell growth and invasion by targeting programmed cell death 4 in human lung adenocarcinoma cells. Tumour Biol, 35, 39-46.
11. **Qureshi, R. & Sacan, A**. 2013. A novel method for the normalization of microRNA RT-PCR data. BMC Med Genomics, 6 Suppl 1, S14.
12. **Zhu, L.-R., Yuan, R.-X., Xia, X.-B., Wang, Y., Zhu, Y.-M., Fi, L., et al.** 2022. Assessment of a panel of miRNAs in serum and pleural fluid for the differential diagnosis of malignant and benign pleural effusion. Cancer Biomarkers, 33, 71-82.
13. **Bao, Q., Xu, Y., Ding, M. & Chen, P.** 2020. Identification of differentially expressed miRNAs in differentiating benign from malignant pleural effusion. Hereditas, 157, 6.
14. **Tamiya H, Mitani A, Saito A, Ishimori T, Saito M, Isago H, et al.** Exosomal MicroRNA Expression Profiling in Patients with Lung Adenocarcinoma-associated Malignant Pleural Effusion. Anticancer Res. 2018;38:6707-14.
15. **Wei T-T, Zhang J-F, Cheng Z, Jiang L, Li J-Y, Zhou L.** Development and validation of a machine learning model for differential diagnosis of malignant pleural effusion using routine laboratory data. Therapeutic Advances in Respiratory Disease. 2023;17:17534666231208632.
16. **Liam C, Lim K, Wong C.** Differences in pleural fluid characteristics, white cell count and biochemistry of tuberculous and malignant pleural effusions. The Medical Journal of Malaysia. 2000;55:21-8.
17. **Dhiman, R. K., Saraswat, V. A., Rajekar, H., Reddy, C. & Chawla, Y. K.** 2012. A guide to the management of tuberculosis in patients with chronic liver disease. *Journal of clinical and experimental hepatology,* 2**,** 260-70.
18. **Sekaggya, C., Nalwanga, D., Von Braun, A., Nakijoba, R., Kambugu, A., Fehr, J., et al. 2016.** Challenges in achieving a target international normalized ratio for deep vein thrombosis among HIV-infected patients with tuberculosis: a case series. BMC hematology, 16, 1-5.
19. **Krishnan, V. G., Kunoor, A., Keechilath, P. & Mehta, A. A. 2021.** Diagnostic utility of pleural fluid carcinoembryonic antigen in patients

with exudative pleural effusion. Lung India, 38, 139-43.

20. **Li, C., Kazzaz, F. I., Scoon, J. M., Estrada-Y-Martin, R. M. & Cherian, S. V. 2022.** Lymphocyte predominant exudative pleural effusions: a narrative review. Shanghai Chest, 6.
21. **Abd-El-Fattah AA, Sadik NAH, Shaker OG, Aboulftouh ML.** Differential microRNAs expression in serum of patients with lung cancer, pulmonary tuberculosis, and pneumonia. Cell biochemistry and biophysics. 2013;67:875-84.
22. **Agalioti, T., Giannou, A. D. & Stathopoulos, G. T.** 2015. Pleural involvement in lung cancer. Journal of thoracic disease, 7, 1021.
23. **Song, Y., Kelava, L. & Kiss, I.** 2023. MiRNAs in Lung Adenocarcinoma: Role, Diagnosis, Prognosis, and Therapy. International Journal of Molecular Sciences, 24, 13302.
24. **Frydrychowicz, M., Kuszel, Ł., Dworacki, G. et al.** MicroRNA in lung cancer—a novel potential way for early diagnosis and therapy. J Appl Genetics 64, 459–477 (2023). https://doi.org/10.1007/s13353-023-00750-2
25. **Gee Gretchen V., Koestler Devin C., Christensen Brock C., Sugarbaker David J., Ugolini Donatella, Ivaldi Giovanni P., Resnick Murray B., Houseman E. Andres, Kelsey Karl T., Carmen J:** Downregulated microRNAs in the differential diagnosis of malignant pleural mesothelioma 2010,IJC ,127;12
26. **Li Y, Chen Z, Yang P, Duan H, He J, Gong L,** et al. Differentiating between tuberculous and non-tuberculous pleural effusions using the pleural fluid ratio of 10× adenosine deaminase/lactate dehydrogenase. J Thorac Dis. 2023;15:2627-35.
27. **Khalifa MM, Zaki NE, Nazier AA, Moussa MA, Haleem RA, Rabie MA, et al.** Prognostic significance of microRNA 17–92 cluster expression in Egyptian chronic lymphocytic leukemia patients. Journal of the Egyptian National Cancer Institute. 2021;33:37.
28. **Gui, X. & Xiao, H.** 2014. Diagnosis of tuberculosis pleurisy with adenosine deaminase (ADA): a systematic review and meta-analysis. Int J Clin Exp Med, 7, 3126-35.
29. **Fei G, Yijun M, Weijiang J, Huimin C, Fang L.** Biomarkers for distinguishing tuberculous pleural effusion from non-tuberculosis effusion: a retrospective study. BMC Infectious Diseases. 2023;23:771.
30. **Balatti, V., Maniero, S., Ferracin, M., Veronese, A., Negrini, M., Ferrocci, G., et al.** 2011. MicroRNAs Dysregulation in Human Malignant Pleural Mesothelioma. Journal of Thoracic Oncology, 6, 844-51.

To cite this article: Amira H. Allam, Magdy M. Omar, Hmasat A. El Noury, Abeer M. Rawy , Toqa A. Anwar , Rehab E. El Sawy. Role of MicroRNA (182) in Differentiation between Benign and Malignant Pleural Effusion. BMFJ 2025;42(4):403-418.