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MicroRNA-155 expression in exhaled breath condensate of patients with lung cancer

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ABSTRACT

Background: Nowadays, microRNAs (miRNAs) have proved their significant role in the diagnosis and prognosis of various types of malignancy. Accordingly, this study aimed at evaluating the role of miRNA-155 in diagnosis as well as prognosis of lung cancer.
Methods: In this prospective case-control study, exhaled breath condensate (EBC) samples were collected from a group of 15 pathologically-confirmed, chemotherapy/radiotherapy-naïve lung cancer patients, as well as from another control group comprised of 15 patients at high risk for lung cancer development. The expression of miRNA-155 (RQ value) in EBC samples was measured using Quantitative real time Polymerase Chain Reaction (PCR).
Results: There was significant statistical difference between the lung cancer group and the control group as regards RQ value ($p \leq .01$) being higher among the lung cancer group. RQ value among the lung cancer group showed significant statistical difference as regards tumor extension ($p = .004$), and smoking index ($p = .004$). RQ levels correlated significantly with tumor extension ($p = .001$), smoking status ($p = .000$), smoking index ($p = .000$), and family history of lung cancer ($p = .007$), whereas tumor location, histopathological type of the tumor, and sex did not correlate significantly ($p > .05$). The best cut-off value for EBC RQ level was ≥ 3.338 with an overall sensitivity 100%, specificity 100%, positive predictive value 100%, negative predictive value 100%, and overall accuracy 100%.
Conclusion: The expression of miRNA-155 in EBC can be considered as an accurate, non-invasive, promising biomarker for early lung cancer diagnosis and prognosis.

Introduction

Lung cancer is considered as the leading cause of cancer-related deaths worldwide with one out of four cancer-related deaths are caused by lung cancer [1]. In Egypt, lung cancer represents about 8.2% of male cancers [2]. Unfortunately, the majority of lung cancers are diagnosed when the disease is already advanced or metastatic. This late diagnosis ultimately reduces the survival rate of lung cancer [3]. Nowadays, there are several screening protocols for lung cancer yet, they carry the disadvantage of being either invasive, expensive, or provides high false positive rates [4].

Among the various types of cancer-related biomarkers, the small, non-coding ribonucleic acids (RNAs) called microRNAs (miRNAs) are considered the most promising owing to their remarkable stability in biological samples, their cancer-specific nature, and their existence in a variety of body fluids [5]. miRNA-155 functions as a multitask miRNA being involved in numerous biological processes including haematopoiesis, inflammation, immunity besides its role as oncogene [6]. Among the miRNAs, miRNA-155 commonly exerts up-regulation in various solid and hematological malignancies including Hodgkin's lymphoma, some types of Non-Hodgkin's lymphoma, acute myeloid and chronic lymphocytic leukemia, breast, lung, colonic, cervical, pancreatic, thyroid, as well as gastric malignancy [6–18].

The collection of air lining fluids (ALF) in lung cancer is limited by their invasive nature. Accordingly, the non-invasive technique to isolate ALF in exhaled breath condensate (EBC) can serve as a novel and potentially important diagnostic tool in lung cancer detection [19].

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In view of the above, this study was undertaken to evaluate the role of miRNA-155 in EBC samples among lung cancer patients as well as patients at high risk for lung cancer development in an attempt to establish a novel biomarker for early lung cancer detection.

Materials and methods

This prospective case-control study was conducted in collaboration between the Medical Biochemistry Department and the Chest Diseases Department at Ain Shams University in the period between October 2015 and April 2016. The study included 30 patients subdivided into two study groups; the first group included 15 patients with recent pathologically-confirmed lung cancer in whom no adjunctive chemotherapy or radiotherapy was received, while the second group included 15 control patients at high risk for lung cancer development including heavy smokers (smoking 20 or more cigarettes per day, or 20 or more pack-years), patients exposed to a second hand smoke [1], patients with chronic obstructive pulmonary disease (COPD) [20], patients with long-term exposure to other lung irritants e.g., radon gas, asbestos, arsenic, chromium, nickel and tar [21], as well as patients with positive family history of lung cancer [22]. Patients with any form of cancer other than lung cancer, patients with lung cancer who received previously or were currently receiving adjunctive chemotherapy or radiotherapy, patients with inflammatory disorders as well as patients with immune disorders were excluded. Demographic as well as clinical data were collected from patients. Verbal consent was obtained from all patients and the study was approved by the ethical committee of the Faculty of Medicine, Ain Shams University.

Exhaled breath condensate collection

Samples of EBC were collected at the pulmonary function laboratory of the Chest Department, Ain shams University Hospitals. The samples were obtained in the liquid state during oral tidal breathing for 10 min while the patient was seated using commercially available condenser EcoScreen™ (Jaeger, Hoechberg, Germany). Sample collection was performed according to the international guidelines [23]. Each patient provided EBC sample for biochemical measurements and all samples were labeled with unique identifier to protect subject confidentiality.

MiRNA-155 qRT-PCR assay

Extraction of miRNA-155 from EBC samples was done at the Medical Biochemistry Department using miRNeasy Mini Kit, Qiagen, Germany (catalog number: 217004), according to the producer's protocol. Measurement of RNA purity and concentration were determined by spectrophotometric measurement of absorbance at 260 nm for RNA detection and 280 nm for protein detection.

The relative quotient (RQ) value of miRNA-155 was calculated. This RQ value signifies the ratio of miRNA-155 expression in the collected EBC samples.

Statistical analysis

Statistical analysis was done using Statistical Package for Social Sciences software (SPSS for Windows, version 20.0; SPSS Inc, Chicago, IL). Descriptive statistics were presented as mean ± standard deviation (SD), median, range as well as percentage and frequency. Test for normality was done using the Shapiro-Wilk test. Chi-square test was used for comparison between qualitative variables. Mann–Whitney rank sum *U* test and Kruskal–Wallis test were used for comparison between non-normally distributed non-parametric quantitative variables. Receiver Operating Characteristics (ROC) curve was used to discriminate positive from negative results and to determine the threshold value for optimal sensitivity and specificity. Statistical significance was set at $p < .05$.

Results

A total of 30 patients participated in this study, the mean (SD) age of the lung cancer group was 71.33 (6.28) years, while the mean (SD) age of the second group of patients at high risk for lung cancer development was 45.8 (7.16) years. The basic characteristics of all studied patients are shown in Table 1. There was significant statistical difference as regards EBC miRNA-155 expression (RQ value) between the lung cancer group and the control group ($p \leq .01$) being higher among the lung cancer group (Table 1).

In lung cancer group, the comparison between different variables as regards RQ value showed significant statistical differences as regards tumor extension ($p = .004$), and smoking index ($p = .004$) where metastatic lung cancer patients as well as heavy smokers had higher RQ levels in comparison to non-metastatic lung cancer patients and light smokers, respectively (Table 2).

As shown in Table 3; EBC RQ levels in studied patients correlated significantly with tumor extension ($p = .001$), smoking status ($p = .000$), smoking index ($p = .000$), and family history of lung cancer ($p = .007$), whereas the tumor location, histopathological type of the tumor, and sex did not show significant correlation ($p > .05$).

ROC curve was done to determine the best cut-off value for EBC RQ value that discriminate the lung cancer group from the control group. Accordingly, the best cut-off value was ≥3.338 with an overall sensitivity of 100%, specificity 100%, positive predictive value 100%, negative predictive value 100%, and overall accuracy 100% (Table 4 and Fig. 1).

Discussion

Over the past years, lung cancer ranked second common cancer among both males and females and is responsible for one out of four cancer-related mortality [24,25]. Moreover, the majority of lung cancers are diagnosed after the disease become advanced or metastatic [26]. All of the above mentioned facts mandates the establishment of new modalities for early lung cancer diagnosis as well as accurate prognosis. Accordingly, this study was

Table 1
Basic characteristics of studied patient.

Variable	Lung Cancer (n. 15)	Control (n. 15)	p
Age, mean ± SD (Median)	71.33 ± 6.28 (71)	45.80 ± 7.16 (44)	≤.01
Sex, Male/Female (Number)	13/2	10/5	.195
Smoking status, Number			
Smoker	14	9	.031
Non-smoker	1	6	
Smoking index, Number			
Heavy Smoker	11	6	.065
Light Smoker	3	3	
Family history for lung cancer, Number			
Positive	4	2	.361
Negative	11	13	
Alcohol intake, Number			
Alcoholic	7	4	.256
Non-alcoholic	8	11	
EBC miRNA-155 expression, Mean Rank (Median)	22.13 (29.35)	8.87 (0.89)	≤.01

EBC, exhaled breath condensate; miRNA, microRNA; RNA, ribonucleic acid.

Table 2
Comparison between different variables as regards EBC miRNA-155 expression in lung cancer group.

Variables		EBC miRNA-155 expression		X^2 (or) U^*	p
		N	Mean Rank		
Histopathological type	Adenocarcinoma	10	7.1	3.255	.354
	Squamous cell carcinoma	3	10		
	Large cell carcinoma	1	5		
	Small cell lung cancer	1	14		
Tumor location	Peripheral	9	8.22	0.056	.814
	Central	6	7.67		
Tumor extension	Non-metastatic	4	2.5	8.25	.004
	Metastatic	11	10		
Smoking status	Smoker	14	8.5	2.625	.105
	Non-smoker	1	1		
Smoking index	Heavy smoker	11	10	8.25	.004
	Light smoker	3	2.5		

*Chi-Square for Mann-Whitney Test.
EBC, exhaled breath condensate; miRNA, microRNA; RNA, ribonucleic acid.

Table 3
Correlation between miRNA-155 expression and different variables among study groups.

		Group	Sex	Smoking status	Smoking index	Histo-pathological type	Tumor location	Metastatic extension	Family history of lung cancer	EBC miRNA-155 expression
Group	Correlation Coefficient	1.000	0.236	0.394	0.336	.	.	.	0.167	−0.766
	Sig	.	0.208	0.031	0.069	.	.	.	0.379	0.000
	N	30	30	30	30	15	15	15	30	30
Sex	Correlation Coefficient	0.236	1.000	0.068	0.313	0.081	−0.320	−0.207	−0.118	−0.114
	Sig	0.208	.	0.720	0.092	0.773	0.245	0.459	0.534	0.549
	N	30	30	30	30	15	15	15	30	30
Smoking status	Correlation Coefficient	0.394	0.068	1.000	0.631	−0.185	−0.218	−0.443	0.276	−0.606
	Sig	0.031	0.720	.	0.000	0.509	0.435	0.098	0.140	0.000
	N	30	30	30	30	15	15	15	30	30
Smoking index	Correlation Coefficient	0.336	0.313	0.631	1.000	−0.146	0.123	−1.000	0.437	−0.672
	Sig	0.069	0.092	0.000	.	0.603	0.662	0.000	0.016	0.000
	N	30	30	30	30	15	15	15	30	30
Histopathological type	Correlation Coefficient	.	0.081	−0.185	−0.146	1.000	−0.075	0.146	−0.522	0.329
	Sig	.	0.773	0.509	0.603	.	0.789	0.603	0.046	0.231
	N	15	15	15	15	15	15	15	15	15
Tumor location	Correlation Coefficient	.	−0.320	−0.218	0.123	−0.075	1.000	−0.123	0.185	−0.063
	Sig	.	0.245	0.435	0.662	0.789	.	0.662	0.510	0.824
	N	15	15	15	15	15	15	15	15	15
Metastatic extension	Correlation Coefficient	.	−0.207	−0.443	−1.000	0.146	−0.123	1.000	−0.364	0.768
	Sig	.	0.459	0.098	0.000	0.603	0.662	.	0.183	0.001
	N	15	15	15	15	15	15	15	15	15
Family history of lung cancer	Correlation Coefficient	0.167	−0.118	0.276	0.437	−0.522	0.185	−0.364	1.000	−0.481
	Sig	0.379	0.534	0.140	0.016	0.046	0.510	0.183	.	0.007
	N	30	30	30	30	15	15	15	30	30
EBC miRNA-155 expression	Correlation Coefficient	−0.766	−0.114	−0.606	−0.672	0.329	−0.063	0.768	−0.481	1.000
	Sig	0.000	0.549	0.000	0.000	0.231	0.824	0.001	0.007	.
	N	30	30	30	30	15	15	15	30	30

EBC, exhaled breath condensate; miRNA, microRNA; RNA, ribonucleic acid.

Table 4
Predictive performance of EBC miRNA-155 among studied patients.

RQ for EBC miRNA-155	Sensitivity	Specificity	PPV	NPV	p
Positive if ≥3.338	100%	100%	100%	100%	.000

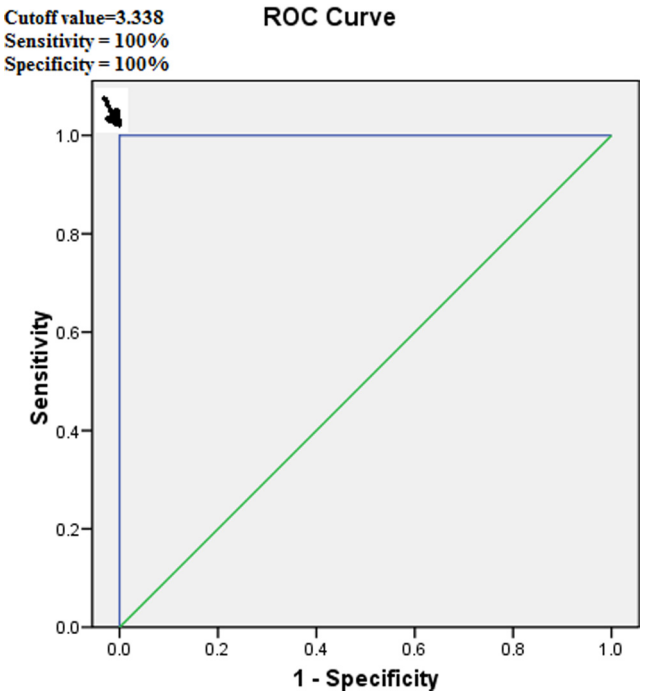

Fig. 1. ROC curve for lung cancer prediction using EBC miRNA-155.

undertaken to evaluate the expression of miRNA-155 in EBC samples of patients with lung cancer as well as patients at high risk for lung cancer development using quantitative real time PCR technique. The choice of EBC was based upon its non-invasive yet accurate nature. Moreover, the expression of miRNA-155 in EBC is related directly to the lungs rather than the expression of miRNA-155 in the blood or any other non-pulmonary samples which might extend the scope of the underlying disease to include the whole body rather than the lungs solely. EBC was used in previous studies to evaluate other tumor-related biomarkers but to the best of our knowledge, this study is the first to address the role of miRNA-155 expression in EBC samples of patients with lung cancer. In this study, the expression of miRNA-155 in EBC samples was significantly higher among lung cancer patients in comparison to patients at high risk for lung cancer. This up-regulation of miRNA-155 in lung cancer patients supports its pivotal role as an oncogene. Such finding comes hand in hand with the results in other studies that declared miRNA-155 was one of the miRNAs that exhibit over expression in lung cancer [27]. In lung cancer group; the RQ level in EBC was significantly higher among metastatic compared to localized lung cancer patients. Similarly, heavy smokers showed significantly higher EBC RQ levels compared to light smokers. Yet, RQ level did not differ significantly as regards the histopathological type of lung cancer, the tumor location, nor the smoking status of lung cancer patients. Although smoking plays a fundamental role in lung cancer development [28] yet, the finding in the present study that RQ levels did not differ significantly as regards the smoking status of lung cancer patients must be viewed in terms that non-smokers represented only one patient and this limited number might make such result questionable. Our study also confirmed that the EBC RQ levels correlated significantly with the tumor extension, the smoking status, the smoking index, as well as the family history of lung cancer. Such correlation between RQ levels and both smoking status and smoking index reflects their impact in the pathogenesis of lung cancer. Thus, our findings suggest that higher RQ levels in EBC were attained in metastatic, heavy smoker patients with positive family history of lung cancer. Accordingly, miRNA-155 expression can serve not just as a diagnostic biomarker but as a prognostic one as well. In concordance to our results as well, Wang et al., [29] provided evidence that miRNA-155 predicted the prognosis and lymphatic invasion of non-small cell lung cancer (NSCLC), Lv et al. [30] also reported that the high expression of miRNA-155 in lung cancer correlated with poor prognosis. Other studies; Mizuno et al. [31], Sochor et al. [32], Gao et al. [33], Geng et al. [34] also confirmed that miRNA-155 is one of the best characterized miRNAs that shows significant up-regulation in lung cancer tissues, as well as in plasma and sputum samples, and was thus regarded to as a promising biomarker for the diagnosis and poor prognosis of NSCLC. Similarly, Raponi et al., [13] reported that in NSCLC, miRNA-155 served as an oncogene and was linked to poor prognosis. In another study, Yanaihara et al. [16] reported significant correlation between miRNA-155 expression levels and lung cancer progression, an additional valuable result in this study was the lack of any significant correlation with any other clinical or pathological factors. Similarly, the results of our study showed that neither the tumor location, the histopathological type of the lung cancer, nor the sex correlated significantly with EBC RQ levels. Yet, it is important to mention that a recent large scale study reported that miRNA-155 did not prove to have any prognostic or predictive impact [35].

In the present study, lung cancer patients were significantly older compared to those at high risk for lung cancer. This was not surprising especially with data in other studies suggesting that there is a long lag period between the exposure to risk factors potentiating lung cancer and the onset of the disease itself with high predominance of lung cancer among elder patients aging above 60 years old [36]. Moreover, our study, similar to other previous studies, reported higher incidence of lung cancer among males compared to females [37–39].

When ROC curve was done to determine the best cut-off value for EBC RQ value that discriminate the lung cancer group from the control group, the best cut-off value was $\geq$3.338 with an overall sensitivity of 100%, specificity 100%, positive predictive value 100%, negative predictive value 100%, and an overall accuracy 100%. This high positivity rate documents the pivotal role of EBC miRNA-155 in lung cancer. Thus, this cut-off value might raise the hope for non-invasive early diagnosis of lung cancer yet, it would have been extremely beneficial in this study to include normal healthy individuals to establish another cut-off value to discriminate healthy individuals from those at high risk of lung cancer development.

An important limitation in this study is the limited total number of patients included as well as patients with individual subtypes of lung cancer.

Conclusion

This study provides evidence that miRNA-155 was expressed in EBC. This simple, non-invasive yet, accurate method of sampling can provide a promising tool for lung cancer research. The high expression of miRNA-155 in lung cancer can serve as a potential biomarker for early lung detection as well as for prognosis. Further large scale studies are needed to evaluate the role of miR-155 in various histopathological subtypes and stages of lung cancer and to further evaluate the role of the dysregulated expression of miRNA-155 as a future targeted therapy for lung cancer.

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Conflicting Interest

No conflicting of interest.

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