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RESEARCH ARTICLE

The diagnostic and predictive values of miRNA-21, miRNA-126, miRNA-513a, and miRNA-98 in patients with non-small cell lung cancer (NSCLC)

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ABSTRACT

Background: The majority of lung cancer cases are non-small cell lung cancer (NSCLC). MicroRNAs (miRNAs) are small non-coding molecules that control target gene(s) expression. **Aims**: Identification of circulating levels of miRNA-21, 126, 513a, and 98 in NSCLC and assessment of their diagnostic and predictive values. **Material and Methods**: In this study, the levels of miRNA-21, 126, 513a, and 98 were evaluated in 19 NSCLC patients and 11 healthy individuals. miRNAs were extracted from patients and healthy control whole blood samples and cDNA was synthesized with the specific primers for each miRNA, real-time quantitative polymerase chain reaction (RT-qPCR) was used to assay the expression pattern of miRNAs. **Results:** The circulating levels of miRNA-21, 126, and 513a were significantly up-regulated in NSCLC patients as compared to the healthy subjects. Of interest, the circulating levels of these miRNAs were about twice higher in stage IV patients than stage III patients. miRNA-513a showed the highest diagnostic accuracy (AUC = 0.942, specificity = 100, and sensitivity = 85.7) and sensitivity was improved when in combination with miRNA-21 (AUC = 0.929, specificity = 90.91, and sensitivity = 92.8). All studied miRNAs showed clear changes during the treatment of NSCLC patients with the platinum regimen. **Conclusions:** miRNA-21, 126, and 513a may be considered as candidate diagnostic circulating biomarkers in NSCLC as well as a potential predictive biomarkers for the response to the platinum regimen. miRNA-513a showed the highest diagnostic accuracy for distinguishing NSCLC patients from healthy controls.

Keywords: Circulating miRNA, diagnostic biomarkers, lung cancer, NSCLC, platinum chemotherapies, predictive biomarkers, RT-qPCR

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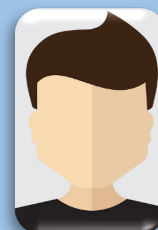

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INTRODUCTION

Non-small cell lung cancer (NSCLC) accounts for ~ 85% of all lung cancer (LC) cases, which also include adenocarcinoma, large cell carcinoma, and squamous cell carcinoma (SCC) (Ma et al., 2015). Five-year survival rates are between 20-30% in patients of NSCLC after surgery. Generally, old persons are more probably develop LC than younger persons, and men are at greater risk than women. The key risk factor for this disease is smoking, as 80% of LC cases in males can be attributed (Liu et al., 2015). In Egypt, data reported by the national population-based cancer registry program of Egypt 2008 to 2011, LC is the sixth common cancer in both sexes, which represents 4.22% of all tumor cases (Ibrahim et al., 2014).

MicroRNAs (miRNAs) are small non-coding RNAs that post-transcriptionally regulate the translation of target genes and influence a series of cellular functions (Such as, proliferation, differentiation, and apoptosis).

They may act as tumor suppressors or oncogenes having regulatory functions on multiple downstream genes with different biological activities and, as a consequence, they offer a more accurate expectation of cell survival than the expression of a single marker or a gene expression profile alone (Calin & Croce, 2006).

miRNA-21 is one of the main miRNAs detected in human carcinomas. It was known to be upregulated in the majority of solid tumors (Volinia et al., 2006). It promotes cell growth, metastasis, and chemotherapy or radiotherapy-resistance of NSCLC cells by targeting tumor suppressor phosphatase and tensin homolog (PTEN) (Liu et al., 2013). Also, miRNA-21 is greatly expressed in the serum of NSCLC patients and low miRNA-21 expression can decrease malignant cell proliferation, migration, and invasion (Zhu et al., 2008; Yang Y et al., 2015; Zhu et al., 2017; Salem et al., 2020).

miRNA-98, a let-7 family member is thought to be associated with many tumors. It may act as a tumor suppressor or oncogene according to the tumor type (Yao et al., 2009). Functionally, miRNA-98 could inhibit malignant cell proliferation, invasion, and epithelial-mesenchymal transition by targeting several important regulators such as Spalt Like Transcription Factor 4 (SALL4), Insulin-like growth factor 1 receptor (IGF1R), and p21-activated kinase 1 (PAK1) (Zhou et al., 2016). Also, it was documented that miRNA-98 promoted chondrocyte apoptosis by decreasing Bcl-2. However, the primary mechanism of miRNA-98 in mediating the malignant phenotypes of NSCLC cells is still vague (Liu et al., 2017)

MicroRNA-126 is located on the 9q34.3 chromosome within the host gene that encoding epidermal growth factor-like domain 7 (EGFL7). It has been suggested to be a suppressor gene due to its dysregulation impact on lung cancer characteristics, including proliferation, migration, and invasion (Nie et al., 2020). MicroRNA-126 regulates these characteristics by targeting EGFL7, CDPK-Related protein Kinase (Crk), and Vascular endothelial growth factor (VEGF) and affects the phosphatidylinositol 3-kinase (PI3K)/AKT signaling pathway (Kim et al., 2014; Zheng et al., 2017).

MicroRNA-513 is a subfamily of the miRNA-506-514 cluster with a large diversity in both the number of copies and sequence variations. MicroRNA-513a-3p sensitizes adenocarcinoma cells to chemotherapy by targeting glutathione S-transferase-p1 (GSTP1). Another role of miRNAs that deserves mention is that of sensitizers to radiotherapy. This is of particular importance given that many tumors require combinations of chemotherapy and radiotherapy as optimal modes of treatment. miRNAs may modulate the DNA damage response, thus sensitizing tumor cells to both chemotherapy and radiotherapy (Sarkar, 2014).

SUBJECTS AND METHODS

Subjects

The present study was performed at the Medical Research Institute, Alexandria University. After written informed consent from participants, fresh blood samples were collected from 19 patients with NSCLC admitted to Medical Research Institute Hospital, Ayadi Al-Mostakbal Hospital, and Kasr Al-Ainy Hospital, from October 2018 to October 2019. All patients were diagnosed with NSCLC. Pathologic staging was done according to the TNM classification. Patients with previous radiotherapy or previous chemotherapy were excluded from the study. Table 1 showed the Clinicopathological characteristics of the studied NSCLC patients.

11 subjects with matched age and sex free from any malignancy and not suffering from any chronic health problem were enrolled in the present study after written informed consent as a control group.

The NSCLC patients' group is divided into 2 subgroups according to Clinicopathological characteristics, stage, and treatment protocol. Blood samples were obtained at baseline and after 3 and 6 cycles of chemotherapy. Responses were assessed after 3 cycles, 6 cycles, and concurrent treatment course using Response Evaluation Criteria in Solid Tumors (RECIST) criteria based on the CT scan done every 3 cycles and compared with the baseline CT (Eisenhauer et al., 2009). The study

was conducted following the Declaration of Helsinki and approved by the Ethical Committee of the Medical Research Institute, Alexandria University (IOROH: IORG 0008812).

METHODS

Molecular analysis

Quantitative analysis of miRNA-21, miRNA-98, miRNA-126, and miRNA-513a in the whole blood was performed using a quantitative real-time reverse transcriptase-polymerase chain reaction (qRT-PCR) (Wong & Medrano, 2005). U6-RNA was selected as an internal reference to calculate the relative expression level of these miRNAs.

Total RNA isolation

The total RNA (including miRNAs) was isolated from the whole blood using the miRNeasy Mini Kit (cat. no. 217004) according to the manufacturer's instructions (Qiagen, Germany). The purity of extracted RNA was checked by Nanodrop (the ratio of absorbance at A260/A280 was ≥ 1.7) (Pfaffl, 2004)

Reverse transcription:

MiScript II RT Kit (cat. no. 218160) was used for the reverse transcription of the extracted RNA according to the manufacturer's instructions (Qiagen, Germany). The kit adopts a one-step, single-tube reverse transcription reaction using miScriptHiFlex buffer to promote the conversion of all RNA species (mature miRNA, precursor miRNA, non-coding RNA, and mRNA) into the corresponding cDNA (Ahmed, 2005)

Assessment of circulating miRNAs using PCR

The obtained cDNAs were used for real-time qPCR quantification of mature miRNA-21. miRNA-98, miRNA-126, and miRNA-513a using Primer Assays (forward primers, cat. no. 218073, 218075, and 218076) and the miScript SYBR Green PCR Kit, which contains the miScript Universal Primer (reverse primer) and QuantiTect SYBR Green PCR Master Mix (Qiagen, Germany). The kit was used with miScript PCR Control RUN-6 (Qiagen, Germany).

Quantitative PCR amplification conditions started with an initial denaturation for 15 minutes at 95°C and the then amplification by 55 cycles of qPCR as follows: Denaturation at 95°C for 15 seconds, annealing at 55°C for 30 seconds, and extension at 70°C for 30 seconds. Data were collected using a Rotor-Gene Q-Pure Detection version 2.1.0 (build 9) (Qiagen, USA). The relative expression of miRNAs was quantified relative to that of (RUN-6) reference gene in the same sample by calculating and normalizing the threshold cycles (Ct) values of target miRNAs to that of RUN-6 using the ΔΔCt method (Riedel et al., 2014)

Statistical Analysis

Data are expressed as mean ± SE and median and IQR. Kruskal Wallis, chi-square tests, and the ROC curve were used to analyze the data. SPSS 20.0 was used to perform statistical analysis. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Table 1 describes the Clinicopathological characteristics of NSCLC patients. Regarding the main treatment protocol, 57.89% received induction chemotherapy, then concurrent chemoradiotherapy and the rest of the cases received chemotherapy± palliative radiotherapy. Eight patients representing 42.10 % of the cases were metastatic. Eight cases representing 42.10%were stage IV, while the rest of the cases were stage three patients.

The statistical analysis of the circulating levels of miRNA-21, miRNA-126, miRNA-98, and miRNA-513a was listed in Table 2 and Figure 1. The circulating levels of miRNA-21, miRNA-126, and miRNA-513a are significantly higher in NSCLC patients than in the healthy control group-(P = 0.012, 0.042, and 0.012, respectively). ROC analysis was performed to evaluate the diagnostic performance of the upregulated miRNA as listed in Table 3. Accordingly, miRNA-21, miRNA-126, and miRNA-513a were efficient as candidate diagnostic biomarkers, and a miRNA-513a showed the highest diagnostic accuracy (AUC = 0.942, 95 % CI 0.77–0.996) for distinguishing NSCLC patients from healthy controls. The combination between every two miRNAs improved the diagnostic efficiency which is illustrated by their better AUC in Table 3 and Figure 2. The highest diagnostic efficiency is achieved using a panel combining miRNA-21& miRNA-513a biomarkers that improved the sensitivity parameter to 92.86.

All studied miRNAs showed clear changes during the treatment of NSCLC patients with the platinum regimen compared to their baseline (the diagnosed patients before any treatment) levels which may potentially predict the response to chemotherapy as presented in Figure 3. Also, it reveals the effect of chemotherapy on the circulating levels of the four studied miRNAs along with the chemotherapy regimen which suggests their proposed role of miRNA-21 and miRNA-513a as a predictive indicator for sensitivity to platinum chemotherapy and also as a therapeutic target that reverses resistance to platinum chemotherapy of NSCLC patients.

Figure 4 showed that stage IV patients (subgroup2-1) have higher (about twice) circulating levels of miRNA-21, miRNA-126, and miRNA-513a than stage III patients. (subgroup2-2) however, these differences are not significant (P= 0.573, 0.142, and 0.662 respectively).

The box plots show the circulating levels of miRNA-21, miRNA-126, miRNA-98, and miRNA-513a. This experiment set was composed of 19 NSCLC patients and 11 healthy controls. Blood samples were obtained and circulating levels evaluated at baseline and after 3 and 6 cycles of chemotherapy. The lines inside the boxes represent the medians. The boxes mark the intervals between the 25th and 75th percentiles, and the whiskers represent the interval between the 5th and 95th percentiles. The dots indicate outliers.

DISCUSSION

There are many unresolved issues in the treatment and diagnosis of NSCLC as the assessment of the risk of recurrence, the selection of an effective chemotherapeutic agent, and efficient tools and approaches for early diagnosis of NSCLC (Florczuk et al., 2017). On the other hand, there is considerable progress was achieved in understanding the molecular mechanisms of lung carcinogenesis and methods of LC detection, which raise the hopes for real chances to develop successful early diagnostic, predictive, and prognostic tools (Florczuk et al., 2017). MicroRNA (miRNA); a short non-coding RNA, is a class of RNAs that play a critical role in gene expression regulation by suppressing mRNAs translation. miRNAs level in tissues and body fluids has emerged as a new diagnostic, prognostic, and/or predictive marker of different diseases including cancers (Mitchell et al., 2008; Chen et al., 2012). It has been shown that miRNAs are more stable in body fluids and prepared tissue relative to mRNA (Mraz et al., 2009; Jung et al., 2010). The ability to collect miRNA non-invasively from whole blood or plasma is a considerable advantage in the practical application of diagnostic and predictive tests over other biomarkers that may require biopsy or resected tissue.

The present study showed significant up-regulation of miRNA-21, miRNA-126, and miRNA-513a circulating levels in NSCLC patients compared to the healthy subjects (P= 0.012, 0.042, and 0.012, respectively) which made them candidate diagnostic biomarkers in NSCLC patients. Also, stage IV patients (subgroup 2-1) circulating levels of the four miRNAs were about double that in stage III patients (subgroup 2-2). Although the difference between the 2 subgroups was not significant, the behavior of the four miRNAs was typical which needs further investigations to find the root causes.

Tumor resistance to chemotherapeutic agents is a great problem in cancer management. The molecular basis of chemotherapeutic agent resistance has developed more and more in the past decades. The finding that malignant cells commonly become resistant while normal cells rarely do assume that specific molecular processes included in malignancies, (e.g., oncogene activation) may play a critical role in drug resistance. Oncogenes or tumor suppressor genes can directly or indirectly impact cancer-cell drug resistance which explains our results that the changes in the miRNAs (oncogenes or suppressor genes) levels during the treatment of NSCLC patients with carboplatin regimen made them potential candidates to predict the response to chemotherapy (d'Amato et al., 2007; Yu, 1998).

MicroRNA-21 is an oncogenic miRNA in human tumors, and it is one of the most miRNAs known to be highly expressed in most solid cancers (Effatpanah et al., 2015; Lv et al., 2016).

Table 1. Clinicopathological characteristics of NSCLC patients.

Parameters	Description	No	Percentages %
Age	›60	5	26.31%
	≤60	14	73.68%
Gender	Female	2	10.53%
	Male	17	89.47%
Stage	IV	8	42.10%
	III	11	57.89%
LN (lymph node invasion)	Yes	6	31.57%
	NO	13	68.42%
Metastasis	YES	8	42.10%
	NO	11	57.89%
The main treatment protocol	Chemotherapy± palliative radiotherapy	8	42.1 %
	Induction chemotherapy + concurrent chemoradiotherapy	11	57.89 %

Table 2. The changes in the circulating level of miRNAs in NSCLC patients.

	Control (n = 11)	Patients before treatment (n = 14)	After 3 cycles of chemotherapy (n = 10)	After chemotherapy (n = 15)	H(p)
			miRNA-21		
Min. – Max.	0.19 – 1.85	0.06 – 410.30	0.06 – 380.70	0.30 – 837.26	13.353*
Mean ± SE.	1.0 ± 0.19	51.01 ± 31.21	82.14 ± 38.20	122.0 ± 67.55	(0.004*)
Median (IQR)	0.74 (0.56–1.66)	7.14(1.9–10.1)	17.16(4.2–127.5)	16.11 (5.6–54.3)	
$P_{Control}$		0.012*	0.004*	0.001*	
Sig. bet. groups		p_1=0.568, p_2=0.366, p_3=0.807			
			miRNA-126		
Min. – Max.	0.37 – 3.09	0.25 – 12.02	0.11 – 28.86	0.0 – 1.62	23.568*
Mean ± SE.	1.0 ± 0.25	3.06 ± 0.79	6.16 ± 2.75	0.40 ± 0.15	(<0.001*)
Median (IQR)	0.76 (0.42–1.11)	2.08 (1.71–3.52)	2.32 (1.38–6.63)	0.06(0.01–0.77)	
$P_{Control}$		0.042*	0.042*	0.065	
Sig. bet. groups		p_1=0.870, p_2<0.001*,p_3<0.001*			
			miRNA-513a		
Min. – Max.	0.20 – 3.20	0.27 – 228.17	0.50 – 1791.54	40.56– 4790.6	30.075*
Mean ± SE.	1.0 ± 0.29	40.25 ± 20.77	241.66 ± 175.16	457.47 ± 311.08	(<0.001*)
Median (IQR)	0.60 (0.34–1.12)	10.44 (6.3–13.6)	28.0 (4.4–220.9)	104.6 (61.1–247.6)	
$P_{Control}$		0.012*	0.001*	<0.001*	
Sig. bet. groups		p_1=0.340, p_2=0.002*,p_3=0.071			
			miRNA-98		
Min. – Max.	0.30 – 2.22	0.30 – 9.87	0.0 – 4.42	0.02 – 14.03	2.29
Mean ± SE.	1.0 ± 0.18	1.98 ± 0.66	1.18 ± 0.45	3.48 ± 1.33	0.515
Median (IQR)	0.98 (0.58–1.14)	1.19 (0.72–2.13)	0.65 (0.31–1.42)	0.51 (0.06–6.69)	

H: H for Kruskal Wallis test, Pairwise comparison bet. every 2 groups were done using Post Hoc Test (Dunn's for multiple comparisons test), $P_{Control}$: p-value for the comparison between control and each group, p_1: p-value for the comparison between diagnosed Patients before treatment and C3, p_2: p-value for the comparison between diagnosed Patients before treatment and after chemotherapy, p_3: p-value for the comparison between C3 and after chemotherapy, *: Statistically significant at $p < 0.05$.

Table 3. Agreement (sensitivity, specificity) for circulating individual miRNAs and their combinations measured by qRT-PCR assay.

	AUC	p	95% C.I		Cut off	Sensitivity	Specificity	PPV	NPV
			LL	UL					
miRNA-21	0.864	0.002*	0.705	1	1.71	78.57	81.82	84.6	75
miRNA-126	0.792	0.014*	0.592	0.992	1.23	85.71	81.82	85.7	81.8
miRNA-513a	0.942	<0.0001	0.77	0.996	2.4	85.71	100	100	84.6
miRNA-21& miRNA-126	0.864	0.002*	0.705	1.000		71.43	90.91	90.9	71.4
miRNA-21& miRNA-513a	0.929	<0.001*	0.794	1.000		92.86	90.91	92.9	90.9
miRNA-126& miRNA-513a	0.922	<0.001*	0.787	1.000		85.71	90.91	92.3	83.3

AUC: Area Under a Curve, p value: Probability value, CI: Confidence Intervals, NPV: Negative predictive value, PPV: Positive predictive value, UL: Upper Limit, *: Statistically significant at $p < 0.05$, LL: Lower Limit, p: p-value for the comparison between healthy control and diagnosed Patients before treatment, #Cut off was choose according to Youden index.

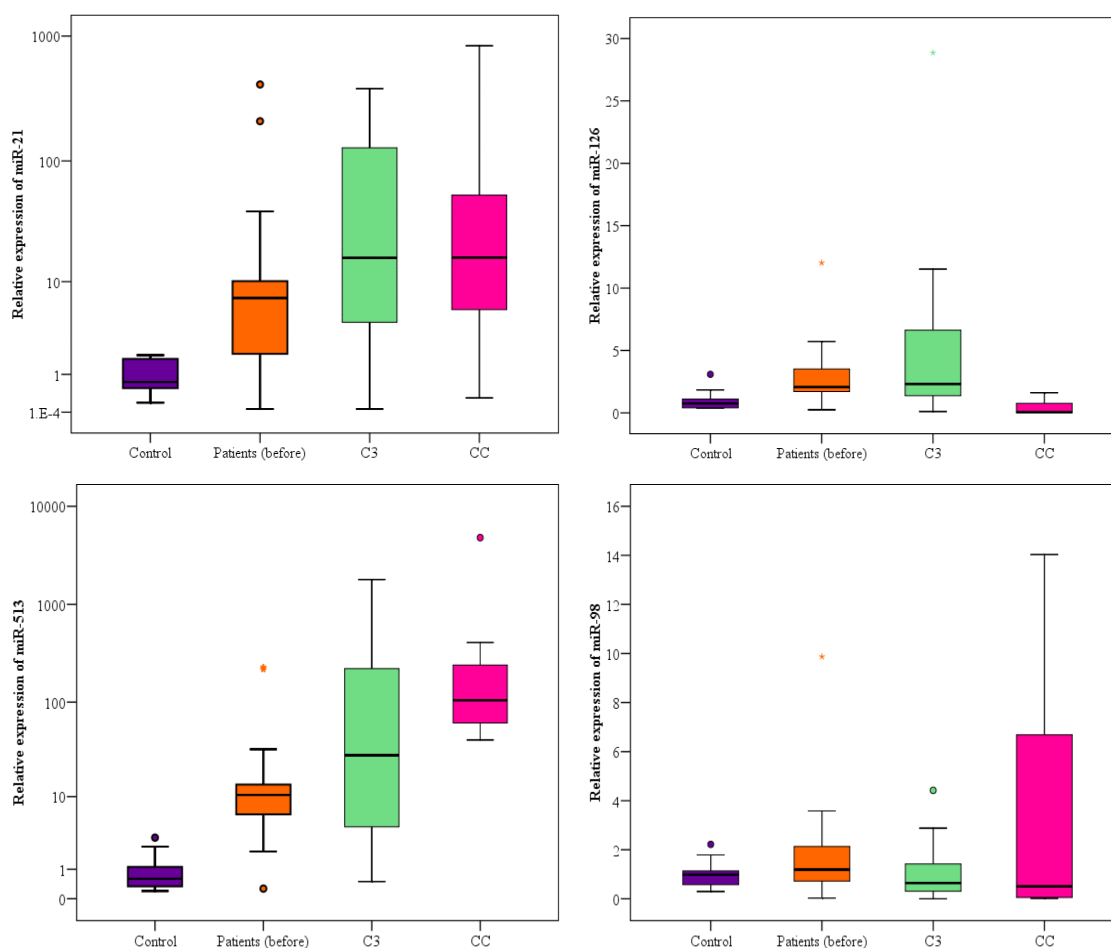

Figure 1. The graphical representation of the changes in the circulating level of miRNAs in NSCLC patients

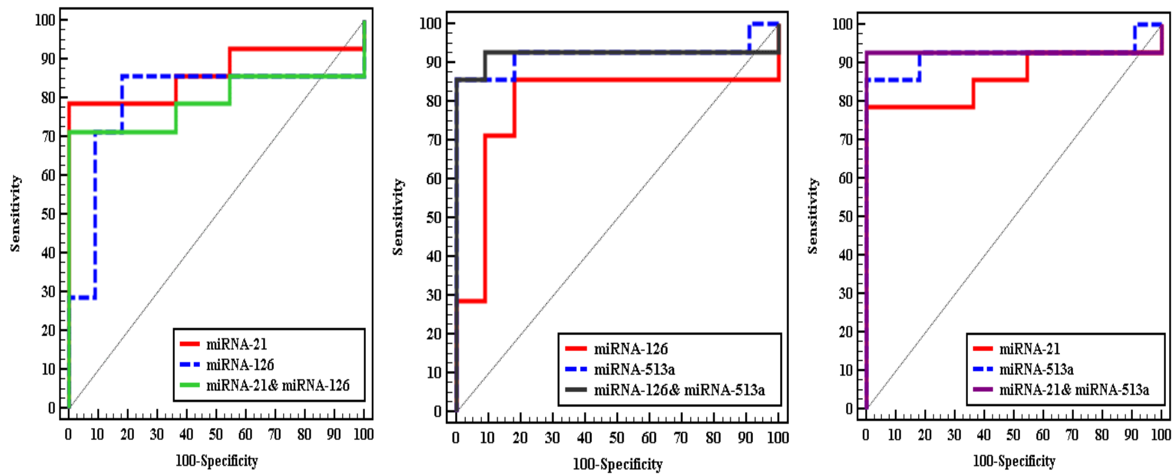

Figure 2. ROC curve for circulating individual miRNAs and their combinations measured by qRT-PCR assay.

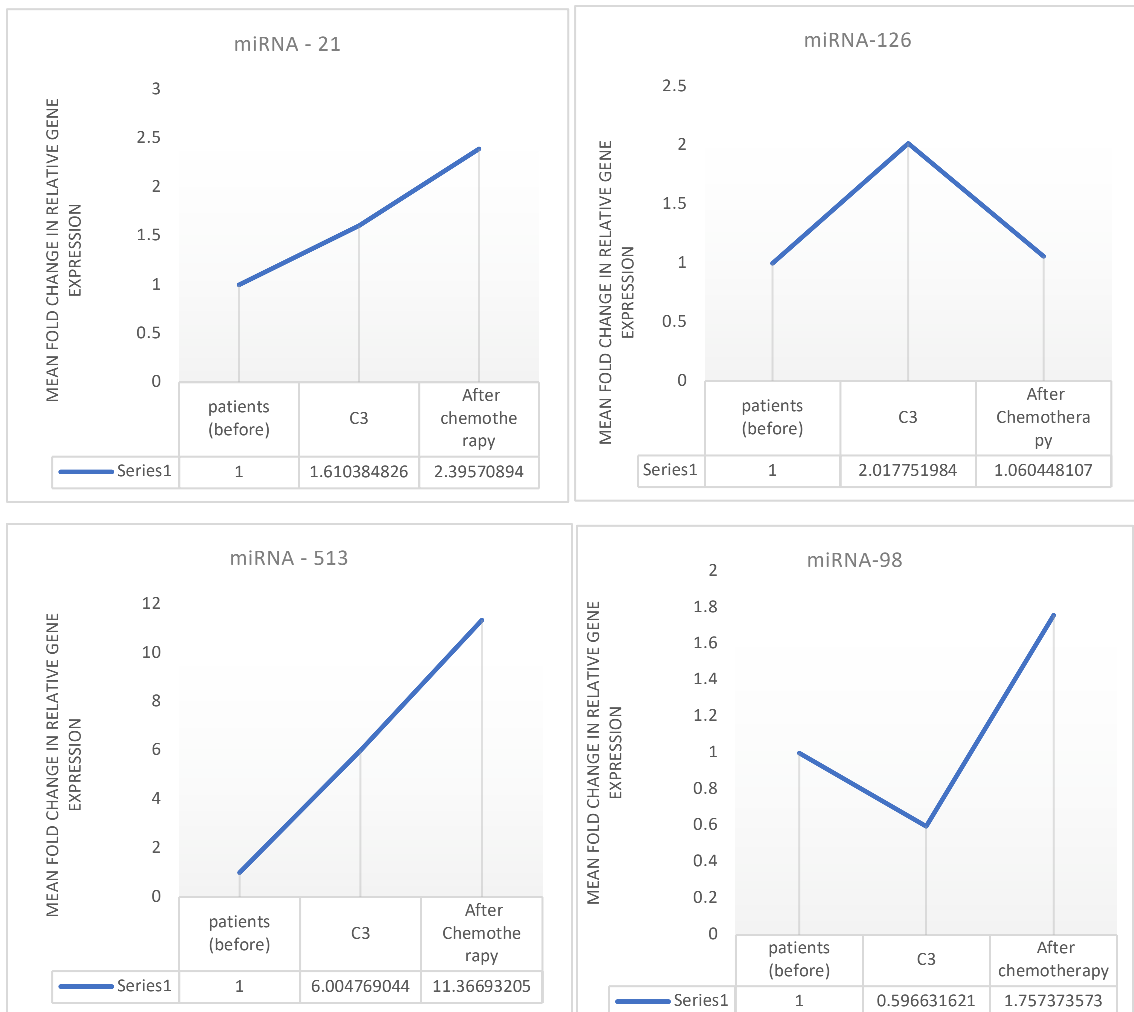

Figure 3. The proposed effect of chemotherapy on circulating miRNAs level.

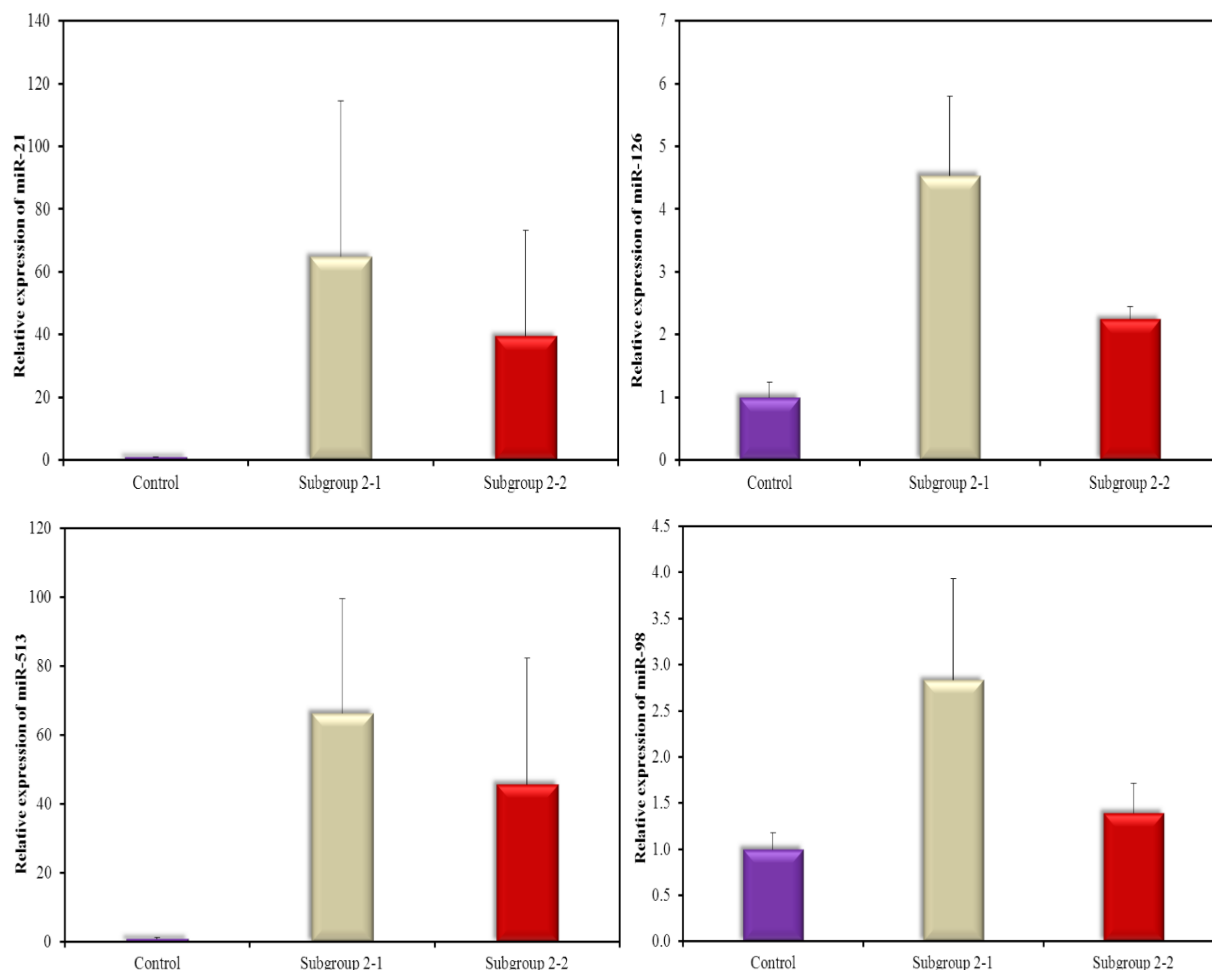

Figure 4. The comparison of circulating miRNAs means fold change between Subgroup 2-1 and Subgroup 2-2 before any treatment.

It promotes cell growth, metastasis, and chemotherapy or radiotherapy-resistance of NSCLC cells by targeting tumor suppressor PTEN (Liu et al., 2013). Also, miRNA-21 is greatly expressed in the serum of NSCLC patients and that low miRNA-21 expression can decrease malignant cell proliferation, migration, and invasion (Zhu S. et al., 2008; Yang Y. et al., 2015; Zhu H. et al., 2017).

The present findings indicated that the blood level of miRNA-21 was significantly up-regulated in the NSCLC patients at baseline by about 51-fold control value. Also, data showed that using a cut-off value of 1.71 to discriminate patients from healthy controls, produces sensitivity of 78.57% and 81.82% specificity with AUC equal to 0.864 which validates the suitability of miRNA-21 as a potential biomarker for the diagnosis of NSCLC and these results were consistent with Abu-Duhier et al. (2018). In line with this suggestion, Markou et al. (2008) and Liu et al. (2013) investigated the level of miRNA-21 expression in NSCLC cell lines (A549, SPC-A1, and H2170) and human tissues, respectively. Both showed consistent results of significant up-regulation of the relative expression of miRNA-21in NSCLC cell lines and patients compared to normal control groups (Markou et al., 2008; Liu et al., 2013). The results indicated further up-regulation of the circulating miRNA-21 during carboplatin chemotherapy regimen after three and six cycles. On the other hand, numerous data proved that upregulation of miR-21 could inhibit the sensitivity of NSCLC to chemotherapy and radiotherapy and this revealed that miRNA-21 can be used as a predictive marker to platinum chemotherapy in agreement to Wei et al. (2011) who suggested that the blood miRNA-21 is considered a predictive indicator for sensitivity to platinum chemotherapy of NSCLC patients.

miRNA-98 is upregulated and functions as an oncogene in gastric cancer, (Yao et al., 2009) colon cancer (Pathak et al., 2015), and SCLC (Du et al., 2009). The dual roles of miRNA-98 are probably due to targeting different genes in different types of cancers. The results of the present study revealed non-significant up-regulation of circulating miRNA-98 level in whole blood samples of NSCLC at baseline compared to the control group by about 1.98-fold. In contrast to our finding, Yang et al. (2015), and Zhou et al. (2017) showed that miRNA-98 was significantly down-regulated in human NSCLC tissue/cells compared with the normal tissues and cells by qRT-PCR. This discrepancy may be due to the nature of samples used as we used blood while the other studies used cancerous tissues. After three cycles of chemotherapy, circulating miRNA-98 was down-regulated to be 0.59-fold relative to the baseline value. On the other hand, after the sixth cycle, it was up-regulated to be 1.75-fold compared to the baseline level.

Regarding circulating miRNA-126; the results indicated significant up-regulation in NSCLC patients at baseline by about 3-fold compared to the control group which is statistically significant (H (p)= 23.568 (<0.001*)) and revealed miRNA-126 diagnostic value. The results indicated that the best cut-off value to discriminate healthy controls from patients was 1.23 with a sensitivity of 85.71%, 81.82 % specificity, and AUC 0.792. These results revealed its potential as a diagnostic biomarker. On the other hand, Previous studies approved that miR-126 was a promising positive factor for overall survival for NSCLC patients and its prognostic value (Zheng et al., 2017).

In contrast to the present data, Kim et al., (2014) evaluated expression levels of miRNA-126 in 72 NSCLCs and 30 benign lung tissues by qRT-PCR and found that miR-126 expression was significantly downregulated in NSCLCs compared with the benign control group (Kim et al. 2014). Kim's results were consistent with Zheng et al., (2017) and Tafsiri et al., (2016), who implemented their experiments on either cell lines or human tissues (Zheng et al., 2017; Tafsiri et al., 2016). However, the discrepancy with our results could be explained by the type of samples as we assayed the circulating level, not tissue levels. Also, our results showed that circulating miRNA-126 mean fold change up-regulated to 2 folds after 3 cycles of carboplatin and back to control level after the chemotherapy regimen.

In our study, the baseline level of miRNA-513a was markedly up-regulated in NSCLC patients compared to the control level by about 40-fold. The diagnostic value of miRNA-513a was supported by its AUC 0.942, sensitivity of 85.71%, and 100 % specificity using a cut off value of 2.4 to discriminate patients from healthy controls. On the other hand, the effect of the platins on the miRNA-513a level was assessed by monitoring the relative expression to that in the diagnosed patients before any treatment (baseline). Our results showed that circulating miRNA-513a level up-regulated to six folds after three cycles of carboplatin and to 11.3 folds after the chemotherapy regimen. This difference was statistically significant (p = 0.001).

Other studies demonstrated that miR-513a-3p can sensitize human lung adenocarcinoma cells to platinums by targeting Glutathione S-transferase P1 (GSTP) (Marengo et al., 2019; Zhang X. et al., 2012). Results including ours showed that the circulating miRNA-513 may be a potential novel biomarker for the diagnosis of NSCLC as miRNA-513a is significantly elevated in the blood of NSCLC patients. Its expression pattern and other findings reflect its sensitivity to platinum chemotherapy for NSCLC, suggesting that circulating miRNA513 may be a predictive indicator for sensitivity to platinum chemotherapy (Zhang X. et al., 2012). The limitations of the study are the small sample size and the small number of miRNAs.

CONCLUSION

We can conclude that miRNA-21, miRNA-126, and miRNA-513a could be candidate diagnostic circulating biomarkers in NSCLC. Also, miRNA-21 and miRNA-513a could be potential predictive biomarkers for the response to the platinum regimen. These conclusions have a great impact on the unsolved issues of clinical decision-making either diagnosis or selection of specific chemotherapy with a higher probability of being effective.

miRNA-513a showed the highest diagnostic accuracy (AUC = 0.942, specificity=100, sensitivity = 85.7, and 95 % CI 0.77 –0.996) for distinguishing NSCLC patients from healthy controls and sensitivity was improved when combining it with miRNA-21 (AUC = 0.929, specificity = 90.91, sensitivity = 92.86, and 95 % CI 0.794 –1.000).

Although the response to a given therapeutic agent can vary personally between patients with similar clinical profiles and tumor morphology with the complicated genetic nature of LC, which is very clear in our results, still the miRNA-21 and miRNA-513a can add value in response prediction as a potential predictive biomarker.

CONFLICTS OF INTEREST

All authors declare no conflicts of interest.

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