

## Circulating MiR-10b, MiR-1 and MiR-30a Expression Profiles in Lung Cancer: Possible Correlation with Clinico-pathologic Characteristics and Lung Cancer Detection

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Circulating microRNAs have been recognized as promising biomarkers for the detection of lung cancer. The objective of this study was to evaluate miR-10b, miR-1 and, miR-30a in the plasma samples of lung cancer patients to confirm any possible relevance in the early detection of lung cancer. Plasma samples from 47 non-small-cell lung cancer patients and 41 cancer-free subjects were evaluated for selected microRNAs using the real-time PCR method. To evaluate the tobacco smoking effects on microRNAs expression, the studied groups were categorized into two subgroups: never-smokers and smokers. MiR-1/miR-30a expression levels were significantly reduced in lung cancer, while the miR-10b level was significantly elevated. We found that smoking had significant effects on the levels of circulating microRNAs in the smokers of the cancer-free group (a significant up-regulation of miR-10b and significant down-regulation of miR-1/miR-30a), and lung cancer patients (a significant elevation of miR-10b). Receiver operating characteristic curve analysis showed that miR-10b with an area under the curve of 0.861, and miR-1/miR-30a with values of 0.905 and 0.889 for the same parameter, could distinguish non-small-cell lung cancer patients from cancer-free subjects. Our findings demonstrated significant differences in the expression of microRNAs in lung cancer and the considerable effects of smoking on microRNAs levels. Area under curve analysis showed that miR-10b with 78% sensitivity/78% specificity, miR-1 with 95% sensitivity/80% specificity and miR-30a with 87% sensitivity/83% specificity, might be good (miR-10b/miR-30a) and excellent (miR-1) markers for lung cancer detection.

**Key words:** Clinico-pathologic characteristics, microRNA, carcinoma, non-small-cell lung cancer, plasma

Lung cancer is one of the most obstinate leading causes of cancer death worldwide (1). In 2018,

lung cancer was the most commonly diagnosed malignancy (about 11.6% of all new cases) and the

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most prevalent cause of cancer-related deaths (about 18.4% of all cancer-related deaths)(2). Non-small-cell lung cancer (NSCLC) represents about 80–85% of all lung cancers (3), and based on histological properties may be divided into 3 subgroups with distinct features:1) adenocarcinoma (AC), 2) squamous cell carcinoma (SCC) and 3) large cell carcinoma (LCC) (4). Cigarette smoking has been reported to be the hallmark risk factor (5). In fact, tobacco-associated cancer risk in ex-smokers remains high and equal to that of active smokers (6). Unfortunately, due mainly to the lack of effective diagnostics for early detection (6), many lung tumors are detected at advanced stages, when the 5-year survival rate is barely 15% (7). The National Lung Screening Trial (NLST) for screening of high-risk population through low-dose helical computed tomography(LDCT) demonstrated that an appreciable diminution of 20% in mortality rate may be achieved (8). Considering some disadvantages of LDCT like high false-positive rates, high costs, and potential side effects such as radiation exposure, there is an immediate demand for simpler, non-invasive or semi-invasive, sensitive and reliable methods for pivotal screenings of high-risk individuals, thus, early detection of the disease (4).

MicroRNAs are short non-coding endogenous RNAs (22 nt) that function as gene regulators at post-transcriptional level (9, 10) via binding to the 3' untranslated region (3'UTR) of messenger RNAs (mRNAs) as their targets (7, 11). MicroRNAs are involved in a wide range of physiological and pathological events (12-14). In tumor tissues, patterns of microRNAs expression are consistently different than normal, and some key microRNAs may be traced in such patterns (3). According to the recent findings, there are circulating microRNAs with diagnostic potential for every type of malignancies including lung cancer (6). All tumor cells release microRNAs into the circulation, and it

has been well-established that the level of circulating miRNAs may be altered in cancer (15).

Based on recent findings, microRNAs modulate a wide variety of physiological/cellular events, and deregulation of their expression/function is closely associated with tumorigenesis, angiogenesis, and development, promotion, progression and ultimately metastasis of the malignant cells (16).

Mir-10b gene is located within the HOXD cluster (ch2q31) (17). Homeobox D10 (HOXD10) inhibits the expression of cell migration and extra cellular matrix remodeling factors ( $\alpha$ 3-integrin/RhoC/MMP14/uPAR). MiR-10b is up-regulated following the binding of Twist to its promoter, leading to a reduced level of HOXD10 (18) and E-cadherin (19), and an increase in kruppel-like factor 4 (KLF4). These cascades induce up-regulation of RhoA/RhoC factors, Rho kinase activation and tumor invasion (16). In NSCLC, miR-10b acts as a carcinoma enhancer by targeting klotho (20), which also occurs to be a prominent therapeutic target (16).

MiR-1 is downregulated in lung cancer. MiR-1 induction considerably reduces the expression of oncogenic targets (MET/Pim-1/ FoxP1/ HDAC4/ Mcl-1) and triggers the activation of caspases 3/7, and Poly (ADP- Ribose) Polymerase 1 (PARP1)(21). In NSCLC, serum miR-1 level is significantly correlated with overall survival of patients, and may be implicated as a non-invasive predictor for cancer prognosis (22). MiR-30a (ch6q.13) (23) has been reported to be down-regulated in metastasis, while it is crucial for maintaining epithelial traits (24). MiR-30a is one of the highly down-regulated microRNAs in metastatic cancers (25) and is declined during TGF- $\beta$ -promoted tumor metastasis, ultimately leading to increased expression levels of invasion/metastasis-associated mesenchymal factors (N-cadherin, Slug, Snail, and Twist) (24, 26).

According to our previous study and computational algorithms, we selected miR-10b as an oncomir along with miR-1 and miR-30a, both of which are involved in lung cancer and have previously been explored in cell lines, tissues and blood (27). The present study explored whether the plasma microRNAs signatures of lung cancer patients had correlation with clinico- pathologic characteristics of patients, and if cigarette smoking had any effects on expression level of microRNAs. We also aimed to investigate whether the selected oncomir and tumor suppressors had any potential to be demonstrated as new markers for early detection of lung cancer.

## Materials and methods

### Study population

NSCLC patients (n=47) and cancer-free individuals (n=41) were selected as subjects, enrolled from Shahid Madani Hospital, Tabriz (2015-2016). Each group was subdivided into two subgroups including smokers (smoking history: at least 5 years, smoking exposure: about more than 20 packs/year) and never-smokers (subjects with no history of past and present smoking, neither active nor passive). Before endoscopic assessment, all

subjects underwent clinical examination; plain chest radiograph; CT scan of the chest, upper abdomen, and brain; fiber-optic bronchoscopy; and bone scan were all conducted. Subjects with the following criteria were included in the research: an age of 40 to 90 years old; having no previous history of other types of cancer prior to participating in this study, as well as no chronic/ acute/ hormonal/ infectious disorders; having no past exposure to carcinogenic agents, including asbestos, and also passive smoking; having no history of any therapeutic procedures (surgery/ chemotherapy/ radiotherapy). Written informed consent was obtained from all subjects. According to the ethical standards (Declaration of Helsinki, 1964), the Ethical/ Scientific Committee of Clinical Research in Tabriz University of Medical Sciences (Tabriz, Iran) authorized the present study (Ethical code: IR.TBZMED.REC.1395.1112).

### Sampling

Blood sample (5-8 ml peripheral venous fasting blood) was obtained from all subjects, and immediately centrifuged, and the plasma was stored at -80°C. To determine the stage of tumor, guidelines of the American Joint Committee on Cancer were used, and histo pathological

**Table 1.** Demographic/clinicopathologic characteristics of all subjects.

Demographic and clinical characteristics		NSCLC patients N=47	Non-cancerous subjects N=41
<b>Gender</b>	male	31(%65)	20 (%48)
	female	16(%35)	21 (%52)
<b>Age</b>	40-59	14 (%29)	13 (%31)
	60≤	33 (%71)	28 (%69)
<b>Smoking History</b>	Non-smoking	9 (%19)	21 (%51)
	smoking	38 (%81)	20 (%49)
<b>Histology</b>	AD	15 (%31)	
	SCC	22 (%46)	
	LC	10 (%23)	
<b>Stage</b>	I	9 (%19)	
	II	23 (%48)	
	III	10 (%21)	
	IV	5 (%12)	

classification was confirmed based on the World Health Organization (WHO) classification. None of the patients had received any therapy before participating in the present study (Table 1).

### MicroRNAs profiling

A comprehensive assessment on aforementioned microRNAs was performed using [www.miRbase.org](http://www.miRbase.org), [www.TargetScan.org](http://www.TargetScan.org), miRTarget-LinkHuman, miRpathDB, and miRmap databases. Targets for each of these microRNAs and their possible involvement in tumorigenesis and progression of cancer were evaluated. The target genes and their related binding site on seed regions of microRNAs were explored via Target Scan (<http://www.targetscan.org/>), and the mature microRNAs' sequences were confirmed according to miRbase data (<http://microrna.sanger.ac.uk/>).

### RNA isolation

For RNA isolation, 1-2 ml yielded plasma was centrifuged (~2,000 rpm for 5 min) in order to obtain cell free starting material. The supernatant (200 µl) was used for total RNA extraction using isolated Exiqon kit (miCUYR™ RNA Isolation Kit-Biofluids, Cat No. #300112 & #300113) according to manufactures' guideline. The concentration, quantity, and purity of RNAs were confirmed using the relative absorbance ratio at A260/A280 and A260/A230 on aspectrophotometer (Nano Drop 2000, Thermo Scientific, Wilmington, DE, USA). The purified RNA was stored at -80°C.

### Reverse transcription (RT-PCR)

For cDNA synthesis, 7 µl template total RNA (5 ng/µl) was used for each sample. According to manufacturer's instruction (miRCURY LNA™ Universal cDNA synthesis kit, Cat No. #203301) cDNA was synthesized in a thermocycler (Eppendorf, Germany).

### Quantitative real-time PCR

Quantitative real-time PCR was carried out in a total volume of 20 µl reaction mixture, and using primers (Exiqon, Denmark. Cat.No.204344, 205637, 205695) in accordance with the manufacturer's

protocol in a Rotor-Gene Q (Qiagen, Germany) instrument. All experiments were conducted in triplicate. To limit artefactual regulation caused by sample normalization, only the values below a minimal threshold ( $C_t \leq 36$ ) were analyzed. Ergo, relative expression of microRNAs was normalized to the expression level of miR-16 (Exiqon, Denmark. Cat. No. 205702) as internal control, and calculated according to the  $2^{-\Delta C_t}$  method (28). MiR-16 as stable internal control was used for data normalization through  $2^{-(C_t \text{ interest gene} - C_t \text{ control})}$  method in human samples (29). Sample size was calculated based on Glenn D method (30).

### Statistical analysis

Statistical analysis of the microRNAs expression between the two groups was conducted based on Mann Whitney, because the distribution of the variables was abnormal (according to Kolmogorov-Smirnov/Shapiro-Wilk test). The difference in microRNAs expression between NSCLC subtypes/stages was estimated through Kruskal Wallis method. The results were analyzed using SPSS v.16 and DATA ASSIST v3.01. The correlation between the prevalence of each microRNA and the demographic/ clinical characteristics (age/ gender/ smoking history/ tumor histology-stage) of NSCLC patients was analyzed with Spearman's and chi-square rank correlation. Moreover, the effects of smoking on microRNAs level were analyzed with Mann Whitney test. Also, receiver-operator characteristic (ROC) was drawn, and the area under the curve (AUC) was measured (based on Youdens index) to determinate the accuracy of each microRNA (AUC: 0.700-0.799 refers to fairly candidate biomarker, 0.800-0.899 refers to good candidate biomarker, 0.900-1.0 refers to excellent candidate biomarker).  $A < 0.05$  was considered statistically significant.

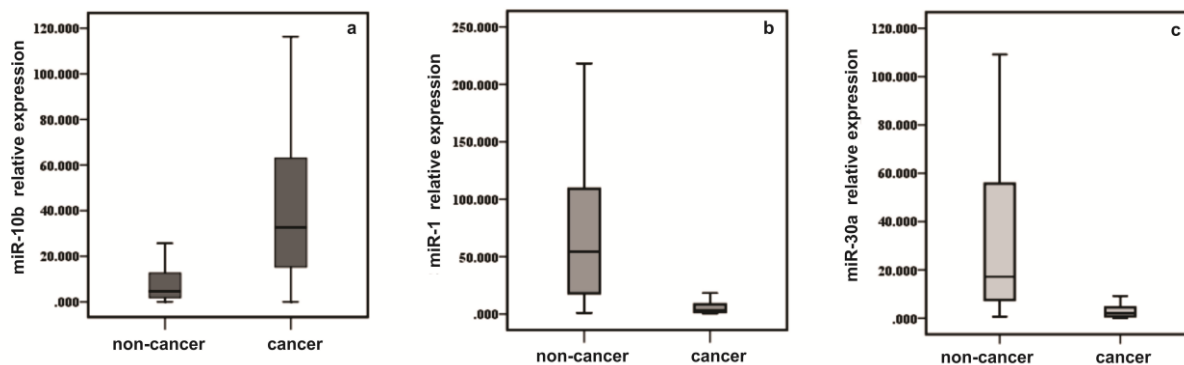
## Results

### MicroRNAs profiling in plasma

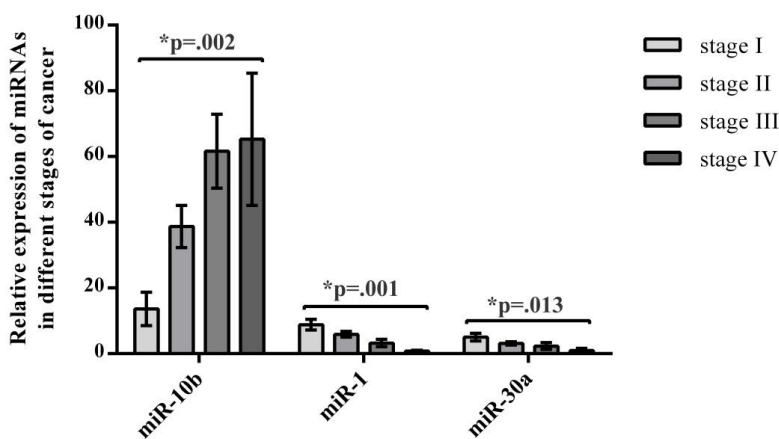
Real-time PCR was applied to evaluate the

expression levels of microRNAs in the two groups (Fig. 1) and in the clinical stages of NSCLC (Fig. 2). Based on the  $\Delta CT$  values, distances between all subjects were calculated for hierarchical clustering (Fig. 3). Accordingly, the correlations between the

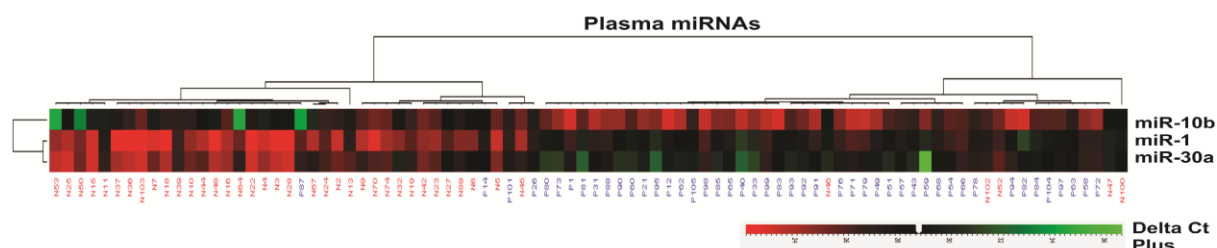
expression level of microRNAs and demographic/ clinico-pathologic characteristics of cancer patients were evaluated. Moreover, the effects of cigarette smoking on microRNAs expression were analyzed (Fig. 4).



**Fig. 1. MicroRNAs expression in NSCLC patients and non-cancerous group.** Expression of miR-10b (a), miR-1 (b) and miR-30a (c) in NSCLC patients and non-cancerous groups was compared ( $P < 0.05$ ).



**Fig. 2. MicroRNAs expression in different clinical stages of NSCLC.** Relative expression (Mean $\pm$ SE) of miR-10b, miR-1 and miR-30a in four stages of the NSCLC was compared ( $P < 0.05$ ).



**Fig. 3. Cluster analysis and heat map of microRNAs expression.** Differences between all studied subjects and assays were considered for hierarchical clustering and heat map according to the  $\Delta CT$  values using Pearson's Correlation. In the present graph, rows represent selected microRNAs, and columns represent all subjects. Furthermore, red indicates an increase with  $\Delta CT$ s below the middle level, while green represents a decrease with  $\Delta CT$ s above the middle level ( $P < 0.05$ ).

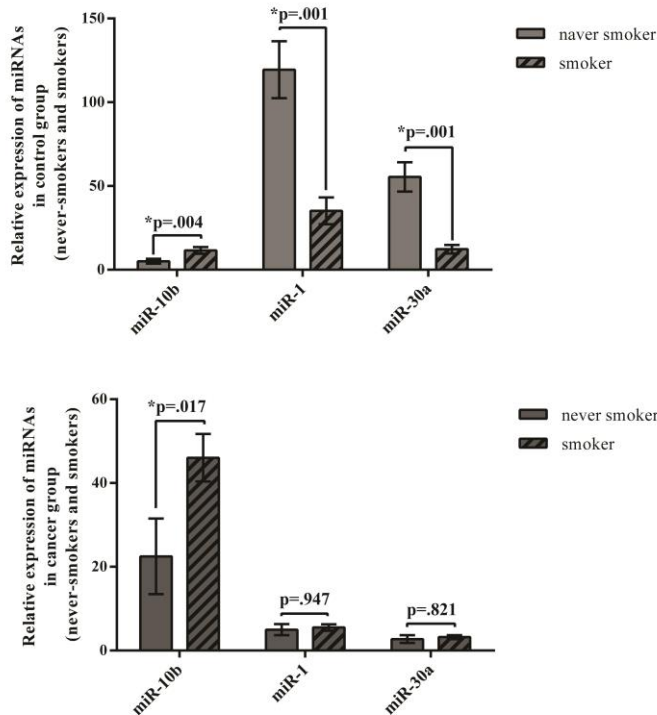


Fig. 4. The effects of cigarette smoking on microRNAs expression in the cancer-free subjects and cancer patients. Significant effects of cigarette smoking on microRNAs expression in the smoker and never smoker individuals of the cancer-free group (a) and lung cancer patients (b) were evaluated (P<0.05).

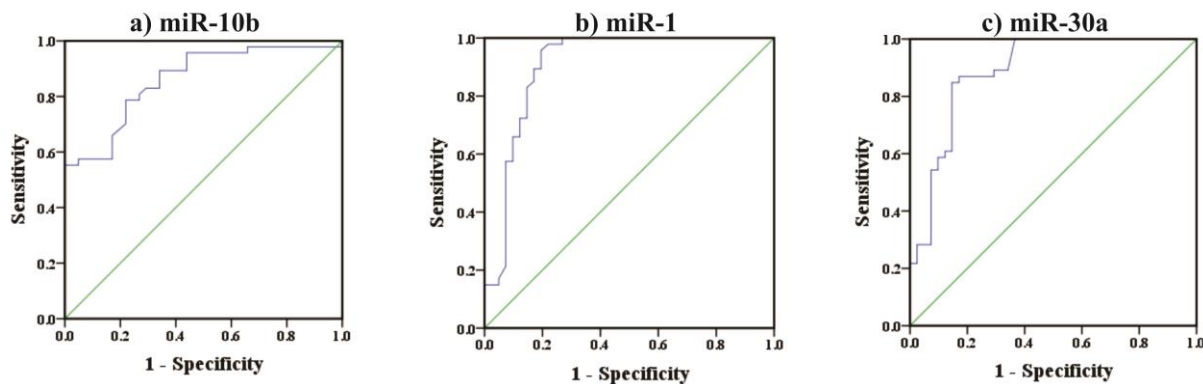


Fig. 5. Diagnostic value of microRNAs. MiR-10b produced 0.861 AUC value (%95 CI: 0.785-0.937, P <0.001), miR-1 with 0.905 AUC (%95 CI: 0.832-0.978, P <0.001) and miR-30a with 0.889 AUC (%95 CI: 0.817-0.961, P <0.001).

**MiR-10b was overexpressed in NSCLC**

Significant increase in miR-10b expression in plasma of the NSCLC patients was observed (P <0.001) (Fig. 1a). Also, miR-10b was found to show a significant up-regulation in four clinical stages of NSCLC (P=0.002) (Fig. 2). No significant correlation between miR-10b overexpression and demographic/ clinical characteristics of the cancer

group was found. However, we observed a moderate positive correlation between miR-10b level and clinical stages of NSCLC patients (P <0.001, R=+0.54).

**MiR-1 was down-regulated in NSCLC**

When expression of miR-1 in the NSCLC group was compared to the cancer-free samples, a considerable decrease of miR-1 (P<0.001) was

observed in patients (Fig. 1b). Down-regulation of miR-1 was also found with significant differences in four clinical stages of NSCLC ( $P = 0.001$ ) (Fig. 2). Furthermore, we noticed a moderate negative correlation between miR-1 expression and the clinical stages of NSCLC ( $P < 0.001$ ,  $R = -0.596$ ).

#### **MiR-30a was down-regulated in NSCLC**

Evaluation of miR-30a expression in NSCLC cases demonstrated lower levels of this microRNA in comparison with the cancer-free group ( $P < 0.001$ ) (Fig. 1c). Down-regulation of miR-30a was also detected with significant difference in four clinical stages of NSCLC ( $P = 0.013$ ) (Fig. 2). We also found a moderate negative correlation between miR-30a expression and the clinical stages of NSCLC ( $P < 0.001$ ,  $R = -0.538$ ).

#### **Evaluation of the effects of smoking on microRNA expression**

The effects of smoking on expression of microRNAs in the cancer-free (Fig. 4a) and cancer groups (Fig. 4b) was analyzed. Our study found significant effects of smoking on microRNAs expression in the smokers in comparison to with that of never-smoker individuals in the cancer-free group, including: a considerable elevation of miR-10b ( $P = 0.004$ ) and significant down-regulation of miR-1 ( $P < 0.001$ ) and miR-30a ( $P < 0.001$ ) in the smokers of the control group. Evaluation of the effects of cigarette smoking on microRNAs level in lung cancer patients found a significant up-regulation of miR-10b ( $P = 0.017$ ) in smokers.

#### **ROC curve analysis**

ROC analysis (with %95 CI) of miR-10b and miR-1/miR-30a indicated a 0.861 AUC (0.785-0.937,  $P < 0.001$ ) for miR-10b, and higher AUCs of 0.905 (0.832-0.978,  $P < 0.001$ ) and 0.889 (0.817-0.961,  $P < 0.001$ ) for miR-1 and miR-30a, respectively. Taking the optimum cut-off into account, miR-10b showed a sensitivity and specificity of both 78 percent (Fig. 5a), and was deemed a promising circulating microRNA for diagnosis of NSCLC. MiR-1 with 95% sensitivity

and 80% specificity (Fig. 5b), and miR-30a with 87% sensitivity and 83% specificity (Fig. 5c) could also be good (miR-30a) and an excellent (miR-1) markers for detection of NSCLC, respectively.

#### **Discussion**

Current routine diagnostics for lung cancer include sputum cytology, chest imaging and bronchoscopy assessments, that can discover only 15-20% of lung cancer cases prior to metastasis. We suggested a new approach for future clinical application, not only as a complementary diagnostic investigation for conventional diagnostics, but also for monitoring of prognosis and response to therapy through blood sampling with minimal invasiveness, low cost and time-saving characteristics.

As the evidence points out, circulating microRNAs are very stable, and resistant to blood RNase even in severe conditions (22). However, under pathological conditions, intracellular microRNAs are released into the circulation dominantly from tumor cells via tumor-derived microvesicles/exosomes (31). Despite the few recent studies, the clinical importance of miR-10b in the plasma samples of NSCLC has not yet been clearly explained. Huang et al. and Pan et al. in their studies found that in NSCLC cells, miR-10b increased cell proliferation and inhibited apoptosis by targeting Klotho protein (20, 32). Liu et al. found that miR-10b acted as an oncomir by positively targeting *KLF4* and consequently promoting proliferation and invasion of A549 cell line (NSCLC) (16). Furthermore, Huang et al. showed that in miR-10b-silenced NSCLC cells, apoptosis-inducing factors like Fas/FasL, Bax and caspase3 were up-regulated and apoptosis-inhibiting molecules including Bcl-2 and PCNA were down-regulated (20). The present study in agreement with the aforementioned studies was able to demonstrate the overexpression of miR-10b in NSCLC. Also, up-regulated miR-10b was positively associated with the clinical stages of

NSCLC. The present study was also in agreement with our previous study, which had reported up-regulation of miR-10b in BAL cell fraction and sputum cells of NSCLC patients, and a positive correlation between miR-10b up-regulation and the clinical stages of cancer (27). These results complemented the recent study which reported high miR-10b expression in tissue samples of NSCLC patients, which in fact was correlated with advanced pathological stage of NSCLC patients (32). However, this study did not find any correlation between miR-10b level and demographic/clinical pathological characteristics of patients. Consistent with the present findings, an elevation in miR-10b level was discovered in NSCLC patients' plasma samples, which could be applied as a novel diagnostic biomarker in the evaluation of lymph node-positive cases (33). Another research group found a significantly positive correlation between miR-10b and the TNM stages. It was suggested that miR-10b level, as a prognostic biomarker, might be a notable candidate in development of additional/alternative therapies for NSCLC (19, 34). Our analysis showed that elevated levels of miR-10b may be represented as a diagnostic signature to help with detection of NSCLC patients.

MiR-1 was down-regulated in lung cancer. Nasser et al. reported that miR-1 by repression of *Mcl-1* function induced apoptosis in lung cancer. Hence, miR-1 affected the tumorigenic potential of cancer cells by reducing the expression levels of oncogenes like *MET*, *FoxP1*, *Pim-1*, and *HDAC4*. Cessation of miR-1 expression facilitates metastasis (21). MiR-1 regulates tumor growth and metastasis in lung cancer through down-regulation of *MET* oncogene (35). Hence, induction of miR-1 expression in A549 cells might potentiate the sensitivity of these cells to doxorubicin by means of facilitating the activation of caspase 3/7, PARP-1, and depletion of Mcl-1 (21). The present study found that miR-1 level was significantly reduced in NSCLC patients. Moreover, down-regulated miR-1

expression was negatively correlated with the advanced clinical stages of NSCLC. These results were in agreement with our previous study which had reported a down-regulation of miR-1 in BAL cell fraction and sputum cells of NSCLC patients, and found a negative correlation between low levels of miR-1 and the clinical stages of cancer (27). Besides, findings of the present study were in agreement with Hu et al.'s findings. They found 11 serum microRNAs with more than a 5-fold change among subjects with longer and shorter survival rates. According to their findings, miR-1 level was considerably associated with cancer patients' survival (22).

More recently, miR-30 down-regulation was reported in lung cancer (36, 37). Kumarswamy et al. reported a significant decrease of miR-30a expression in resected tissue of lung cancer patients in comparison with the corresponding normal samples. They found that miR-30a by targeting *Snai1* induced suppression of epithelial mesenchymal transition, invasion and metastasis (24). Consequently, the reduced level of miR-30a in SCC patients (38) could distinguish SCC patients from normal individuals (39). Boeri et al. in their study revealed that miR-30a along with another 9 top altered microRNAs were spotted in lung cancer tissues which had formerly been detected with computed tomography (CT) (40). Another study suggested that miR-30a level significantly decreased in the plasma of NSCLC patients, which was in agreement with our previous study (27). Also, the down-regulated miR-30a was negatively associated with the advanced clinical stages of NSCLC, that was in agreement with Cheng et al.'s study. They reported that in the early breast tumors, miR-30a levels were significantly correlated with poor clinical characteristics (like advanced stage/lymph node metastasis) (41).

In the present study, we used a systematic approach through evaluation of circulating microRNAs signatures in NSCLC. The developed



plasma-based microRNA yielded a sensitivity of 78% and a specificity of 78% for miR-10b. In 2011, Roth et al. discovered that elevated levels of circulating miR-10b with an AUC of 0.899 might be considered as a novel diagnostic tool with minimal invasiveness in lung cancer (33). Lu et al. were able to successfully distinguish normal and oral cancer plasma samples based on miR-10b level, which yielded an AUC of 0.87 (42). In total agreement with the mentioned findings, the present study found that over-expression of miR-10b in NSCLC plasma was reasonably accurate for detection of NSCLC. Furthermore, the present results indicated that miR-1 and miR-30a with AUCs of 0.905 and 0.889, respectively, could help discern NSCLC patients from cancer-free subjects. These results were partially consistent with our previous study which had reported that miR-10b and miR-1 could be excellent and fair candidates to discriminate NSCLC patients from cancer-free subjects, respectively (27).

The present study was only designed for evaluation of microRNAs in patients with NSCLC, and not in its subtypes. There were 47 patients in total: 15 adeno, 22 squamous, and 10 large cell carcinoma. According to sample size and statistical tests, it was not sensible to divide these subtypes and draw a ROC curve for each one, separately.

In the present study, evaluation of the effects of cigarette smoking on microRNAs expression in both smokers and never-smokers in cancer patients and cancer-free group showed significant differences of miR-10b, miR-1 and miR-30a in the cancer-free group and miR-10b in the cancer group. According to previous studies, alteration in microRNAs levels were correlated with smoking and human cancer (5, 43, 44). There are several pathways involved in lung cancer that may affect the tumor suppressor genes as well as miR-30a and miR-1. Moreover, the heterogeneity of human samples, in contrast to the cell lines, could affect the gene expression. Until now, there has not been

any reported study on miR-1 and miR-30a expression in NSCLC patients to be implicated in our selected microRNAs.

Recent clinical studies about NSCLC mentioned above, have suggested sets of differently expressed microRNAs as promising circulating biomarkers for monitoring prognosis and diagnosis of lung cancer. Because of the high heterogeneity in human subjects, the data showed a wide range of distribution especially in small size studies. In addition, RNase activity of blood might affect the microRNAs (3-7, 12, 15, 22, 31, 33, 40).

This study suggested that miR-10b, miR-1 and miR-30a, could be considered as helpful diagnostic signatures in NSCLC patients. This classifier is fairly new and could have important molecular pathologic applications. However, there still remains an important question concerned with these signatures, which is concerned with the tissue origin of the circulating microRNAs. There are some ambiguous questions about circulating microRNAs. The first, whether the studied microRNAs can be emanated from the tumor cells or maybe from the host reactions to tumorigenesis. Second, whether the signatures of the evaluated microRNAs play any role in the lung cancer development (40). However, according to the mentioned studies, it was notable that our selected microRNAs were involved in lung cancer. Also, another important regard is how to agree on a valid universal signature. It was surprising that results from different studies often varied. However, there are complicated reasons for this occurrence, which may have arisen from the differences in population intrinsic genetic heterogeneity, pathologic sample subtypes, different sample size, collection and processing of samples, technology platforms, bioinformatic approaches and data analysis, different experiences and expertise of the researchers.

In summary, our study showed that the alteration in microRNAs expression in plasma

could help distinguish the NSCLC patients from healthy subjects. The small size of the patients was the main limitation of this study. While our findings were consistent with the previous studies, exploration of more samples could be helpful in improvement of our molecular approach. At the moment, there is not a comprehensive method for screening of high risk population or early detection of lung cancer patients, which can complement the other existing traditional diagnostic tools. Considering the noninvasive nature of plasma sampling and due to its reproducibility and easy detection of microRNAs, blood-based microRNAs may be recognized as novel helpful signatures in lung cancer early detection.

It is notable that the present study is the first report in Iran about assessment of miR-10b, miR-1 and miR-30a expression profiles in blood samples of lung cancer patients, and evaluation of the effects of tobacco smoking on microRNAs level. Until now, the role of miR-30a has only been evaluated in other malignancies like breast cancer. Hence, there are few studies about this particular type of microRNA, and the combined effects of these microRNAs in systemic circulation of patients with lung malignancies.

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#### Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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