

Original Article

Association of miR-372 and miR-137 Expression with Metastasis in Patients with Lung Cancer

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Abstract

Introduction: Identification of metastatic miRNAs and understanding their complex functions provides prognostic and diagnostic bio markers. In this study, the expression of miR-372, fgf-9 gene, miR-137 and cdc42 gene was evaluated in the serum of patients with lung cancer.

Materials and Methods: In the present study, 50 serum samples were collected from healthy individuals and 50 serum samples from people with NSCLC from Masih Daneshvari Hospital in Tehran. Clinicopathological information was collected through questionnaires.

In the molecular study, changes in expression of miR-372, miR-137, fgf-9 and cdc42 genes in healthy individuals and those with lung cancer were evaluated using Real Time PCR.

Results: Expression of miR-372, fgf-9 and cdc42 genes in the first to third stage serum of metastases and expression of miR-137 in serum of the first and second stages of the disease were not significantly different from the normal one. However, in the serum of the fourth stage of the disease, expression of miR-372 and cdc42 gene were significantly increased by 7.3 and 3.4 folds than normal subjects, while in the serum of the fourth stage of the disease, the expression of fgf-9 gene was significantly reduced by 4.46 fold. The expression of miR-137 in the serum of the third and fourth stages of the disease was significantly reduced by 3.2 and 6.8 fold compared to the normal serum.

Conclusion: It is likely that the expression of miR-372, miR-137, fgf-9 and cdc42 genes in human serum could be used to predict the stage of lung cancer metastasis.

Keywords: Lung Cancer; miR-372; fgf-9 Gene; miR-137; cdc42 Gene

1. Introduction

Lung cancer is characterized by high genetic variation and with the lowest number of recurrent mutations which occurs with great frequency [1]. The number of newly diagnosed lung cancer cases and the number of deaths worldwide are responsible for 12.9% of all detected cancers and 19.4% of all deaths from cancer [2]. Lung cancer is one of the most common cancers and the second leading cause of cancer deaths in Iran. The five-year incidence of lung cancer

in Iran was 5.38 per 100,000 people [3]. Smoking is a major risk factor for lung cancer. Smoking causes at least 80% mortality from lung cancer [4]. Histologically speaking, 85% of lung cancers are Non-Small Cell Lung Cancers (NSCLCs) and 15% of lung cancers are Small Cell Lung Cancers (SCLCs) [3]. NSCLCs are divided into subgroups such as lung adenocarcinoma (about 40%), squamous cell carcinoma (25-30%), and large cell carcinoma (10-5%). Therefore,

the most common type of lung cancer is adenocarcinoma [4].

MicroRNAs (miRNAs) are small RNAs (20-22 nucleotides), non-coding and intragenic and controls a large range of biological processes that include apoptosis, evolution, proliferation and differentiation [3, 5]. Additive or decreasing changes in some of the miRNAs that lead to the cancerous process, affect cell growth by interfering with cell cycle regulators. MiRNAs play a key role in the tumor process and control the death of tumor cells in a programmed manner. Through altering their function, the survival of cancer cells can be regulated [6].

MicroRNA-372 is located on region 13 of the long arm of chromosome 19 (19q13.42) and is a member of the miR-371-372 gene family. The miR-372 promotes cellular proliferation, cell cycle, and apoptosis in the tumors of the testicular germ cells, as well as lines of gastric cancer cells. MiR-372 is a prognostic marker for predicting cancer recurrence and survival in patients with non-small cell lung cancer, independent of the stage or type of tissue [7].

The gene encoding MicroRNA-137 (miR-137) is located on the chromosome 1p22. In colorectal cancer, the miR-137 induces cellular G1 blockage and inhibition of invasion through targeting Cell Division Cycle 42 (CDC42). In the adenocarcinoma of the lung, the expression of miR-137 has decreased by more than 20 times in comparison with the normal lung tissue [8]. Fibroblast growth factor 9 (fgf-9) is one of the family members of the fibroblast growth factor (fgf) family that modulates proliferation, differentiation and cellular mobility [9]. The fgf-9 gene is located on the long arm of chromosome 13 (13q12.11) [10]. The fgf family has important implications for fetal growth, tissue repair, and tumorigenicity. The activation of fgf signals, including fgf-9 is associated with the pathogenesis of several cancers. Fgf-9 is expressed in patients with lung adenocarcinoma and the change in the

function of fgf-9 reduces the development and progression of lung adenocarcinoma. The findings indicate that the overall survival time was considerably shorter in patients with a high expression of fgf-9 compared to patients with low expression of fgf-9. The expression of fgf-9 protein was associated with the disease stage and metastasis of the lymph node in patients with lung adenocarcinoma [9].

CDC42 is a member of the Rho GTPase family and acts as an important molecular switch [11]. The CDC42 gene is located on the short arm of chromosome 1 (1p36.12) [12].

Cdc42 is mainly involved in the formation of actin, cellular migration, migration and cell growth. The researchers found that Cdc42 was over-expressed in several types of human cancers; the excessive expression of Cdc42 was linked to carcinogenesis and the progression of many human tumors. The findings showed that Cdc42 was overexpressed in patients with primary lung cancer [11].

MiR-372 binds directly to its target gene, the fgf-9 gene. MiR-372 enhances proliferation and invasion of lung squamous cells by inhibiting fgf-9. Fgf-9 is highly expressed in patients with lung adenocarcinoma, and this aberrant expression of fgf-9 may inhibit lung adenocarcinoma progression. [13]

Decreased expression of Cdc42, Cdk6, cyclin D1, p-ERK1/2 and p-Rb occurs due to increased miR-137 expression. miR-137 is involved in the carcinogenesis of lung cells by inhibiting the expression of two Cdc42 and Cdk6 genes. [14]

NSCLC is the leading cause of death from cancer worldwide, and the overall 5-year survival rate for lung cancer is only about 10-15%. The main reason for this low five-year survival is that the disease is diagnosed in three quarters of the patients late, so the disease progresses and surgery is not possible. In the past 10 years, research on prognostic and prognostic bio markers has shown improvements in the early screening

of lung cancer. Therefore, considering the availability and recognition of the expression of miRNAs and genes in serum samples in numerous studies, and because, the serum is useful for biomarker-related studies, in this study, expression of miR-372, fgf-9 gene, miR-137 and cdc42 gene was evaluated in the serum of patients with lung cancer.

2. Materials and Methods

The present study was descriptive-analytic and random sampling was performed. After obtaining satisfaction from the patients, the expression of miR-372, fgf-9, miR-137 and cdc42 gene in the serum of healthy individuals and people with lung cancer was quantitatively evaluated. The sample consisted of 50 serum samples of healthy subjects and 50 serum samples of patients with NSCLC that were collected from Masih Daneshvari Hospital in Tehran. Patient samples were collected from people who were in the first to fourth stage of cancer.

2.1 Questionnaire Design

To record personal and clinicopathologic data, first, consent was obtained from people to enter this study, and then a questionnaire was designed. In the questionnaire, the patient's information included the name, date of referral, age, sex, occupation, smoking (type and duration), duration of the disease, location, type of tumor, tumor stage, family history of the patient, history of other illness, primary tumor and the tumor after treatment was collected.

2.2 Blood Sampling

Blood samples were taken from patients and controls and transferred to the EDTA (Ethylenediaminetetraacetic acid) anticoagulant tubes.

2.3 Isolation of the Plasma

Given the need for plasma to measure MicroRNA, blood plasma was isolated by

refrigerating centrifuge (HANIL-South Korea) at 4 ° C for 15 minutes at a radius of 1900 rpm and deposited in free RNase free microbes. They were kept at 80 ° C (JAL-Iran) freezer until the tests were carried out.

2.4 Extraction of miRNA from Plasma

To detect gene expression, miRNA should be extracted from the plasma. The RNA was extracted using a Qiagen-Germany extraction kit, according to the kit protocol.

2.5 Quantitative and Qualitative Research of the Extracted RNA

After the extraction of RNA through spectrophotometry (USA, Thermofisher) and agarose gel electrophoresis (Iran-Padideh Nojen pars), its quantity and quality was investigated.

2.6 Stages of Agarose Gel Electrophoresis

First, 0.25 g of agarose powder (Germany -MERCK) was weighed and with heat was dissolved in a volume of 25 ml of Tris-Acetate-EDTA 1X (TAE 1X) buffer. The gel was transferred to the electrophoresis container and the shoulder was inserted into it. Then the TAE 1X buffer was added. The resulting mixture of RNA extraction was transferred to the well and a voltage of 0-1 volts per 1 cm length of gel was established between (91 - 111 volts). Finally, a gel doc device (gel documentation system) was used to view the gel (Germany -NANOLYTIC).

2.7 Preparing RNA Template

The Poly A polymerase enzyme adds to the end of the nucleotide sequence of miRNA, the poly A tail, and makes the sequence longer and more stable. To prepare the RNA template, 200 ng of miRNA was mixed with 0.5 µl of enzyme, 1 µl of ATP, 1 µl of buffer, and placed in thermocycler (China-BIOEREE) for 30 minutes at 37 ° C.

2.8 Synthesis of cDNA

Following preparation of the RNA template, the next steps were performed for complementary DNA (cDNA) synthesis using the cDNA synthesis kit (Thermo Scientific):

5 µl of RNA Template (100 ng constant for each sample) was added to a new 0.2 µl microtube; then, 7 µl of water and gene-specific primer were added to all samples. The specimens were placed in a thermocycler for 5 min at 65 ° C (70 Lid). Subsequently, the samples were removed from the thermocycler and µl2 dNTP, 4 µl reaction buffer, 1 µl RT and 1 µl RNAase inhibitor were added to each sample. The specimens were placed in a thermocycler according to schedule (42 °C (60 min), and Lid 45 °C).

2.9 Primers' Design

The primers used were designed by means of the NCBI site and the Allele ID software. The sequence of primers is shown in Table 1.

Table 1. Primers Used in Real Time PCR

| Primer name | Sequence | Amplicon |
|-------------|-------------------------------|----------|
| fgf-9 F | 5'-CTTTGGCTTAGAATATCCTTA-3' | 132 bp |
| fgf-9 R | 5'-AGTGACCACCTGGGTCAGTCC-3' | 132 bp |
| U6 F | 5'-GCTTCGGCAGCACATATAC-3' | 137 bp |
| U6 r | 5'-ATTCCGTTTCTGGGAGGG-3' | 137 bp |
| miR-372 F | 5'-GCCCACAAAGTCTGCGACAT-3' | 121 bp |
| miR-372 R | 5'-CCAGTGCAGGGTCCGAGGT-3' | 121 bp |
| cdc42 F | 5'-GATGGTCTGTTGGTAAA-3' | 127 bp |
| cdc42 R | 5'-GAGTATATGGTTCTCCACC-3' | 127 bp |
| miR-137 F | 5'-GCGCGCTTATTGCTTAAGAATAC-3' | 112 bp |
| miR-137 R | 5'-GTGCAGGGTCCGAGGT-3' | 112 bp |

2.10 Evaluation of Primer Performance and Real Time PCR Reaction

The efficiency of the designed primers was determined and the standard curve for each of them was plotted. To do so, five dilutions were first made from cDNAs, and then the Real Time PCR (Polymerase chain reaction) (BIONEER-South Korea) was repeated twice for each dilution, with each of the primers separately. At the end, the standard curve for each primer was plotted

based on the values of Ct obtained versus the dilutions used. Using the gradient curve (slope) obtained and the relationship of $E = 10^{(-1/\text{slope})} - 1$, the reaction efficiency (E) was calculated for each primer. Measurement of the expression of miR-372, fgf-9, miR-137 and cdc42 gene was repeated by propagation by Real Time PCR based on standard and relative methods. The Real Time PCR reaction was performed using the Exicycler™ 96 bioneer (South Korea) fixed-type device and the Eva Green color. Samples were prepared according to the protocol of Kit Universal RT miRNA qPCR made by Exiqon-Denmark to the final volume of 20 µl and the replication of miRNAs, genes and reference gene with the same thermal program. Each cycle consisted of four incubation stages of 95 ° C for 5 minutes, 95 ° C for 30 seconds, 60 ° C for 30 seconds, and 72 ° C for 30 seconds. After the reaction, the expression rate was measured using $\Delta\Delta\text{ct}$ method and statistical analysis was performed with REST software.

2.11 Statistical Method

2.11.1 The Studied Variables

Since the comparison between the healthy and metastatic groups was done, the $2^{-\Delta\Delta\text{CT}}$ formula (Livak formula) was used to calculate the differences in expression of miR-137, miR-372, fgf-9 and cdc42. The reference gene in this study was the U6 gene. REST software was used to calculate $2^{-\Delta\Delta\text{CT}}$.

2.11.2 Methods and Tools of Data Analysis

Data were analyzed using SPSS 22 software and Excel. Descriptive and demographic variables were described using frequency indices, frequency percentages and graphs. In the inferential findings section and testing the hypotheses, binomial test was used to compare the accuracy of the survey method.

3. Results

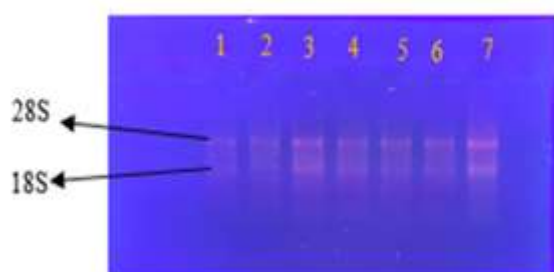
Demographic and clinical data of patients were extracted from the information recorded in the electronic system of cancer department of the hospital. (Table 2)

Table 2. How to record patient and clinicopathological information

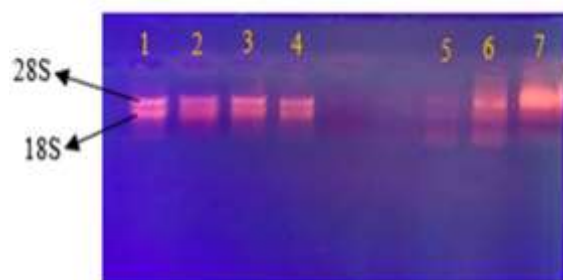
| Specifications | Variable | Frequency (%) |
|---------------------------------|---------------|------------------|
| Number of patients | person | 50 person |
| Age | 51-60 | 20% (10) |
| | 61-70 | 46% (23) |
| | 71-80 | 30% (15) |
| | Up to 80 | 4% (2) |
| Sex | Man | 35 persons (70%) |
| | Woman | 15persons (30%) |
| Tobacco use (type and duration) | consumer | 36persons (72%) |
| | Not taking | 14persons (28%) |
| Patient's family history | has it | 22persons (44%) |
| | does not have | 28persons (56%) |

3.1 The Result of Extracting RNAs and Examining their Quality

After serum isolation and extraction of RNA, the agarose gel electrophoresis was used to check the quality of the extracted RNAs. The presence of the 18S and 28S ribosomal bands associated with a number of samples in Figure 1 shows that the extracted RNAs have good quality.



a) miR-372 and the fgf-9 gene



b) miR-137 and the cdc42 gene

Figure 1. Agarose gel electrophoresis to evaluate the quality of the extracted RNA No. 1-6: Examples, No. 7: Marker.

3.2 Examining the Quantity of Extracted RNAs

An absorbance ratio of 260 to 280 was calculated by a Nanodrop (spectrophotometer) and the concentration of RNA was achieved. The absorption ratio of 260 to 280 determines the purity of the extracted nucleic acid. In the present study, the absorption ratio from 260 to 280 was between 1.8 and 2 and the quality of extracted RNAs was confirmed.

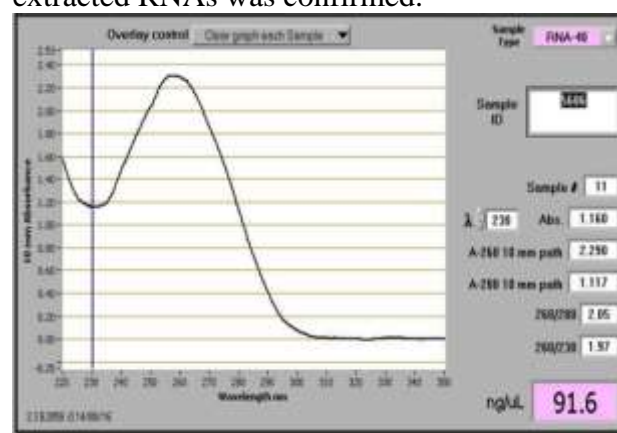
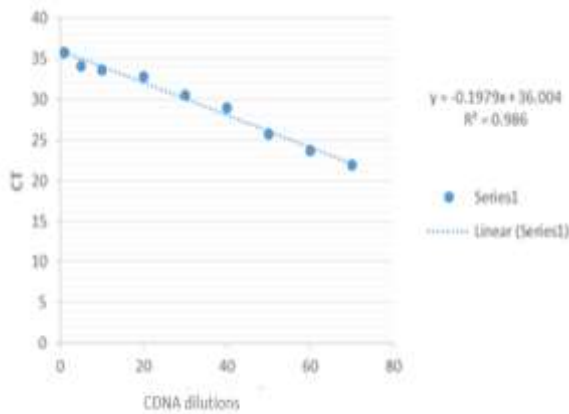


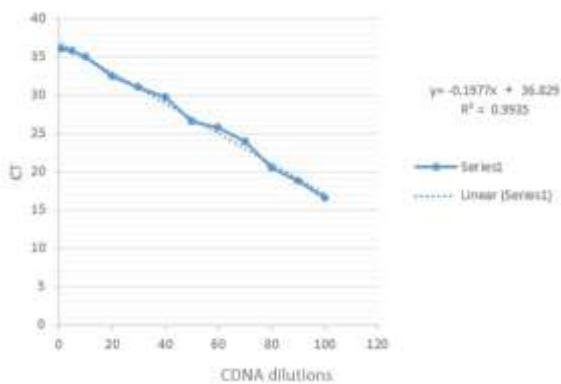
Figure2. Using Nanodrop to Study Extracted RNA Quantity

3.3 Evaluation of the efficiency of primers

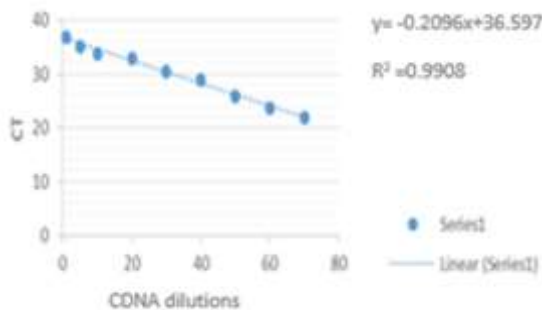
In this study, using the cDNA dilution method, the efficiency of primers used was calculated and shown in Figure 3.



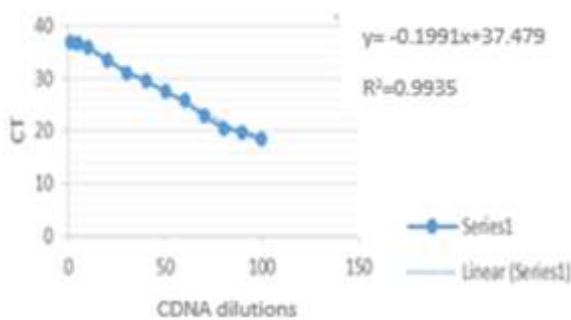
a) The efficiency of the fgf-9 gene specific primer: 98%



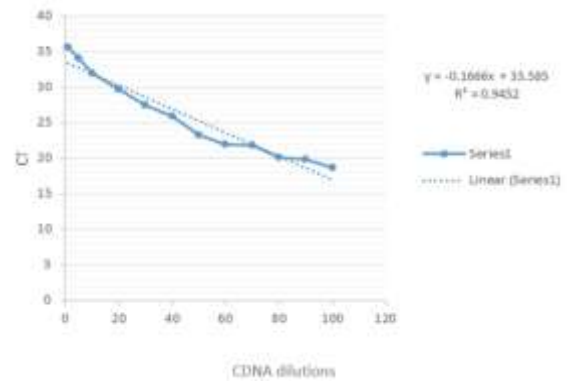
b) The efficiency of the miR-372 specific primer: 99%



c) The efficiency of the cdc42 gene specific primer: 99%



d) The efficiency of the miR-137 specific primer: 99%



e) The efficiency of the U6 gene specific primer: 94%

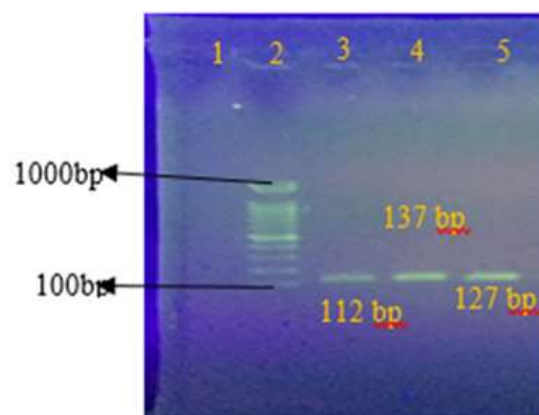
Figure 3. Evaluation of the efficiency of specific primers used for

a) fgf-9 gene, b) miR-372, c) cdc42 gene, d) miR-137 and e) U6 gene.

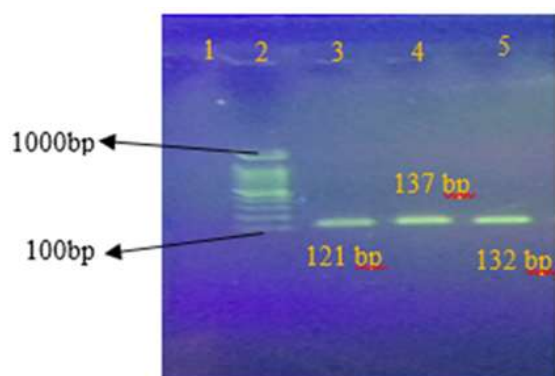
According to Figure 3, the primer efficiency for fgf-9, miR-372, cdc42 and miR-137 genes are 98%, 99%, 99% and 99%, respectively.

3.4 Investigate and confirm the synthesis of cDNA and performing conventional PCR

The specificity of the primers used in the present study was confirmed using conventional PCR (China-BIOERE) PCR method. Figure 4 shows the primer specificity associated with a number of samples.



Column 1: Negative control, Column 2: Marker 100 bp
Column 3: The miR-137 PCR product, Column 4: PCR product for U6, Column 5: The cdc42 PCR product.
a) PCR product for miR-137, U6 and cdc42 genes



Column 1: Negative control, Column 2: Marker 100 bp
Column 3: The miR-372 PCR product, Column 4: PCR product for U6, Column 5: The fgf-9 PCR product.
b) PCR product for miR-372, U6 and fgf-9 genes

Figure 4. Conventional PCR with specific primers.

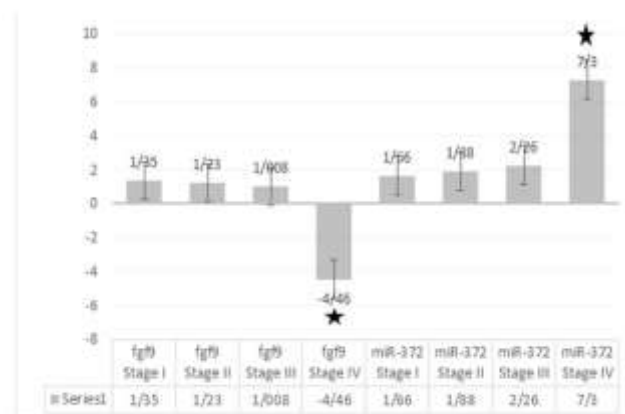
The PCR product was applied on agarose gel. The presence of a single band for each gene on the gel indicated that no nonspecific band was present and the primer used was bound to the target gene.

3.5 Results of Expression of miR-372, fgf-9, miR-137 and cdc42 Gene Using Real Time PCR

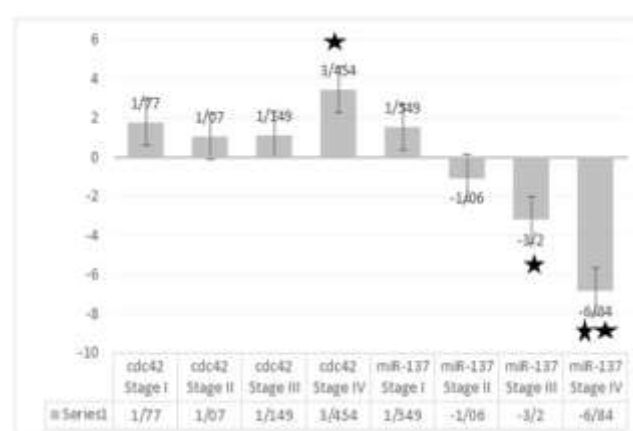
After confirming the creation of the cDNA and performing conventional PCR and observing the genes that showed primer specificity, from Quantitative Real-Time PCR (Q-RT PCR) method was used and expression of miRNAs and genes was investigated. This test was performed three times for each sample. REST software was used to analyze the results.

3.6 Results of Expressing miRNAs and Genes Examined According to the Stage of Cancer

The expression levels of miR-372, fgf-9, miR-137 and cdc42 gene are shown in Figure 4 with respect to the cancer stage.



a) Expression of miR-372 and fgf-9 gene.



b) Expression of miR-137 and cdc42 gene.

Figure 5. The expression of a) miR-372, fgf-9 gene, b) miR-137 and cdc42 gene according to the cancer stage.

According to Diagrams 3 and 5, the expression of miR-372, fgf-9 gene and cdc42 gene in serum samples from the first to third stage of the disease and also, the expression of miR137 in serum of patients with first and second stage of disease were not significantly different from that of normal serum samples. However, the expression of miR-372 in serum samples of the fourth stage of the disease (metastasis stage) with a significant level of $P < 0.05$ ($P=0.022$) was significantly increased by 7.3 times the normal serum samples. The level of expression of miR-137 in the serum of patients with the third and fourth stage (metastasis stage) of the disease with significant level $P < 0.05$ ($P=0.042$) and $P < 0.01$ ($P=0.003$) and had a significant decrease of 3.2 and 6.8, compared to the normal serum samples. The expression of

fgf-9 target gene in serum samples of the fourth stage of the disease (metastasis stage) with a significant level (($P < 0.05$) ($P=0.024$)), had a significant reduction of 46.4 folds compared to normal serum samples. In the serum samples of the fourth stage of the disease, which is the phase of metastasis, the level of expression of cdc42 gene was 3.4 times higher than normal serum samples (($P < 0.05$) ($P=0.032$)).

3.7 Patient information

Demographic and clinical data of patients were extracted from the information recorded in the electronic system of cancer department of the hospital. Of the 50 patients with lung cancer, 35 were male (70%) and 15 were female (30%). Of these, 36 (72%) had tobacco use, and 14 (28%) had no smoking. Of the 50 patients studied, in terms of having a history of cancer among family members, 28 patients (56%) had a history of cancer among family members were 22 patients (44%) of cancer in the family had. another remarkable point is that of these, 23 (46%) were in the range of 61-70 years of age, suggesting lung cancer is more likely to be aging.

4. Discussion

Changes that lead to lung cancer include growth signals, insensitivity to growth inhibitory signals and escape from apoptosis, unlimited reproduction power, angiogenesis, tissue invasion and metastasis [15]. Lung cancer is responsible for more deaths than breast cancer and clones and prostate cancer [5].

Studies have confirmed the steady presence of miRNAs in human body fluids including sputum, urine, milk, cerebrospinal fluid, semen, and serum and plasma. Use of extracellular miRNAs in body fluids is less invasive compared to tumor tissue biopsy. Since the present study aimed to reach the diagnostic goal, in the serum of people with cancer, where microRNAs, as well as genes have been altered (reduced or increased),

early diagnosis of cancer can be of great help in the future of the disease.

Using real time PCR, the expression of miR-372, the target gene fgf-9, miR-137 and the target gene of cdc42 were evaluated.

Studies have shown that miR-372-3p was found significantly overexpressed in both lung squamous cell carcinoma (LSCC) tissues and cell lines, whereas FGF9 mRNA was found underexpressed in LSCC tissues. The decrease in FGF9 gene expression is due to the binding of MiR-372-3p to the 3'UTR mRNA portion of the FGF9 gene. 3'UTR is the untranslated region of the FGF9 gene. Decreased miR-372 expression increases FGF9 gene expression in LSCC and decreases invasion and mitosis and cancer cell growth in LSCC. Inhibition of FGF9 gene expression by miR-372 increases the proliferation of cancer cells and also increases their invasive property in LSCC.[13] In the present study, miR-372($P < 0.05$ ($P=0.022$)) directly binds to fgf-9 ($P < 0.05$ ($P=0.024$)) 3'UTR and thus reduces the expression of fgf-9 and increases the process of metastasis.

Expression levels of miR-137 in NSCLC cell lines or tissue were significantly lower than in a normal human lung cell line or adjacent normal tissues. The data also show that upregulation of miR-137 inhibited the proliferation of NSCLC cells, whereas silencing of miR-137 promoted the proliferation of NSCLC. In addition, Transforming Growth Factor Alpha (TGFA) has been identified as a direct target gene of miR-137 in NSCLC cells. Finally, knockdown of TGFA led to the suppression of NSCLC cell proliferation. Overall, findings indicated that miR-137 served as a tumor suppressor in NSCLC and its suppressive effect is mediated by repressing TGFA expression [16]. Cdc42 ($P < 0.05$ ($P=0.724$), $P < 0.05$ ($P=0.032$)) as a target for miR-137 ($P < 0.05$ ($P=0.042$), $P < 0.01$ ($P=0.003$)), reduces expression of miR-137 in serum of third and fourth stage

of disease and eventually leads to disease progress.

The findings showed that miR-372 expression in ovarian carcinoma was significantly less than normal ovarian tissue and benign tumors. That excessive expression of miR-372 has an inhibitory effect on cell proliferation and causes apoptosis. Therefore, miR-372 plays an important role in inhibiting ovarian tumor growth and is a significant target in the treatment of ovarian cancer. The high expression level of ATAD2 (ATPase family, AAA domain containing 2) was correlated with malignancy in ovarian carcinoma.

Decreased ATAD2 gene expression inhibits cell proliferation and metastasis in ovarian cancer [17]. In the present study, miR-372 expression was significantly increased ($P < 0.05$, (0.022)) in serum of stage IV disease, which resulted in increased metastasis. MiR-372 induces metastasis by inhibiting fgf-9 gene expression ($P < 0.05$ ($P = 0.024$)).

Steroid receptor coactivator 3 (SRC3) is a target gene for miR-137 and miR-137 inhibits cancer cell proliferation in NSCLC by affecting part of that gene. Numerous studies have shown that in lung cancer cells, miR-137 expression significantly suppresses the cell cycle at G1 stage and also decreases the growth of cancer cells by decreasing the expression of CDC42 and CDK6 genes [18]. In the present study, miR-137 expression ($P < 0.05$ ($P = 0.042$), $P < 0.01$ ($P = 0.003$)) is reduced in the third and fourth stages of cancer, which increases the expression of cdc42 gene ($P < 0.05$ ($P = 0.724$), $P < 0.05$ ($P = 0.032$)) and proliferation of lung cancer cells and eventually progresses to the disease.

The profile of five miRNA expressions, including miR-21, miR-143, miR-155, miR-210 and miR-372 in bronchoalveolar samples from people with NSCLC and control group and showed that these miRNAs play a role in tumorigenesis and growth. Therefore, using the evaluation of

the expression profile of these five miRNAs in bronchoalveolar and sputum fluids, non-small cell lung cancer can be detected in a timely manner [19]. According to the results of this study, the expression of miR-372 in the fourth stage of the disease and in patients with metastatic lung cancer was significantly increased ($P < 0.05$ ($P = 0.022$)). MiR-372 binds directly to fgf-9 3'UTR ($P < 0.05$ ($P = 0.024$)) and thus leads to decreased fgf-9 gene expression and increased metastasis.

MiR-137 disrupts the spread and migration of breast cancer cells by targeting the expression of an estrogen-related receptor alpha (ERR α) nuclear receptor. In the present study, a significant reduction $P < 0.01$ ($P = 0.003$) in the expression of miR-137 in the fourth stage of the disease caused a significant increase in the expression of the cdc42 gene $P < 0.05$ ($P = 0.032$) and, as a result, increased metastasis [20].

Exogenous expression of miR-372 reduced expression of p62 and increased the migration and mobility of squamous cancer cells in the head and neck [21]. The A549 cells of the lung adenocarcinoma, which expressed excess miR-372, were faster and were expanded with a high percentage of cells in the S-cycle of the cell, and their ability to migrate and attack extracellular matrix increases [22]. In the present study, expression of miR-372 in the fourth stage of lung cancer is significantly increased ($P < 0.05$ ($P = 0.022$)).

Consequently, suppresses the expression of the fgf-9 gene ($P < 0.05$ ($P = 0.024$)) and promotes disease and metastasis.

The ability of invasive lung cancer cells to be significantly increased by three miRNAs including hsa-miR-137, hsa-miR-182 and hsa-miR-372. They also showed that five miRNAs including hsa-let-7a, hsa-miR-221, hsa-miR-137, hsa-miR-182 and hsa-miR-372 were linked to the survival and recurrence of cancer in patients with NSCLC [23]. Based on the results of our study, the expression of miR-137 expression in the third and fourth stage of

the disease was significantly decreased ($P < 0.05$ ($P=0.042$), $P < 0.01$ ($P=0.003$)) and the expression of miR-372 ($P < 0.05$ ($P=0.022$)) in the fourth stage of the disease increases significantly and enhances the process of metastasis.

In the present study, the results showed that in the third stage, miR-137 expression $P < 0.01$ ($P=0.003$) was decreased and this level is increased in the fourth stage, which increases the expression of the cdc42 gene $P < 0.05$ ($P=0.032$) and cell proliferation. This process can lead to disease progression. Also, miR-372 ($P < 0.05$ ($P=0.022$)) directly attaches to fgf-9 3'UTR ($P < 0.05$ ($P=0.024$)) and thus reduces the expression of fgf-9. The expression of miR-372 increases the proliferation and invasion of lung cancer cells by inhibiting fgf-9. Therefore, it may be possible to check the expression of miR-372 and the fgf-9 gene to predict the metastasis of lung cancer. Demographic data of patients showed a significant relationship between sex and smoking with lung cancer. There is also no significant relationship between the incidence of lung cancer among family members and the incidence of lung cancer. Considering the fact that in the present study, 46% of patients are aged between 61 and 70, it is concluded that the disease develops more often in the aging period.

5. Conclusion

Based on the present study, with regard to the benefits of serum, including access and easy use of it, as well as the benefits of miRNAs, which includes stability and easy detection of serum, it is suggested that miRNAs in the serum should be used for early diagnosis and screening of lung cancer. This study's data also showed that in the stage of metastasis, expression of miR-372, miR137, fgf-9 gene and cdc42 gene was significantly changed ($P < 0.05$) and it is likely that they can be a good candidate to check whether the treatment has been effective or if the tumor's tissue towards metastasis is in progress. To prove

this, research needs to be done on a larger number of samples.

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Conflict of interest

The authors declare no conflict of interest.

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