

Expression of miR-155 and CEA and VEGF Proteins as Diagnostic Markers in Early Stages of Non-Small Cell Lung Cancer in Peripheral Blood

Seyedeh Afroz Azimi ¹, Hamid Reza Sadegh Nia ², Naghmeh Bahrami ³, Hossein Dargahi ⁴, Hamidreza Jamaati ⁵, Alireza Pasdar ^{1,6}, Mehdi Kazempour Dizaji ⁷, Sadegh Shirian ^{8,9}, Bahareh Zerehsaz ¹⁰, Abdolreza Mohamadnia ⁵

¹ Department of Modern Sciences & Technologies, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran, ² Division of Neurocognitive Sciences, Psychiatry and Behavioral Sciences Research Center, Mashhad University of Medical Sciences, Mashhad, Iran, ³ Craniomaxillofacial Research Center, Tehran University of Medical Sciences, Tehran, Iran, Department of Tissue Engineering and Applied Cell Sciences, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran, ⁴ Management Sciences and Health Economic Department, School of Public Health, Health Information Management Research Center, Tehran University of Medical Sciences, Tehran, Iran, ⁵ Chronic Respiratory Diseases Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran, ⁶ Division of Applied Medicine, Medical School, University of Aberdeen, Forester hill, Aberdeen, UK, ⁷ Department of Biostatistics, Mycobacteriology Research Center, NRITLD, Shahid Beheshti University of Medical Sciences, Iran, ⁸ Department of Pathology, School of Veterinary Medicine, Shahrekord University, Shahrekord, Iran, ⁹ Shiraz Molecular Pathology Research Center, Dr Daneshbod Lab, Shiraz, Iran, ¹⁰ Department of Pharmaceutical Chemistry Faculty of Islamic Azad University of Medical Sciences, Tehran, Iran.

Received: 23 September 2022

Accepted: 1 July 2023

Correspondence to: Mohamadnia A

Address: Chronic Respiratory Diseases Research Center, NRITLD, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Email address: mohamadnia.ar@gmail.com

Background: Cancer is a disease caused by manifestation and abnormal gene expression. Many of the genes that inhibit cancer by the microRNAs. The aim of this study was to investigate the Expression of miR155 gene and CEA and VEGF proteins as Diagnostic Markers in Early Stages of Non-Small Cell Lung Cancer (NSCLC).

Materials and Methods: Fifty pairs of non-small lung cancer specimens of patients from healthy and tumors specimens were collected based on physical examination and diagnosis of an expert, that, in Masih Daneshvari Hospital. 50 healthy volunteers as a control group were volunteered by the physician after examination and filled out the consent form in this study. From all subjects, 6 ml of peripheral blood were taken and examined by Real-Time PCR (RT-PCR) and ELISA.

Results: The expression level of miR-155 in patients was significantly increased compared to the control group ($p < 0.001$). VEGF protein was positive in 34 of the 50 patients and in healthy subjects, 3 persons were positive. The statistical comparison of the amount of positive biomarker was performed in two groups and it was shown that there is a statistically significant difference between these two groups ($P < 0.001$). CEA protein was positive in 38 of the 50 patients and 5 in healthy subjects were positive. The statistical comparison of the amount of positive biomarker was performed in two groups and it was shown that there is a statistically significant difference between these two groups ($P < 0.001$).

Conclusion: This study showed that miR155 gene and CEA and VEGF proteins are relatively good markers for the diagnosis of non-small cell lung cancer patients in Iranian population.

Keywords: Biomarker; miR-155; Carcinoembryonic antigen (CEA); Vascular Endothelial Growth Factor (VEGF); Lung Cancer

INTRODUCTION

Cancer is a disease caused by the manifestation and abnormal gene expression. Rapid growth of cancer cells in the initial site with the mechanism of destruction and invasion and indwell in the available space cause appearance of symptoms, and with progression through the blood and lymphatic system and the involvement of other organs (metastasis) causes certain signs and symptoms in the organ that also be involved (1, 2). The cancerous glands in the early stages are limited in a single point and have a topical condition. Then, if they are not detected, they will spread to adjacent tissues and become regionalized and ultimately spread throughout the body and become widespread. If the disease is detected at an earlier stage, it will be to treat the disease easily and the prognosis of the disease will be better. Most of the cancerous diseases in the early stages lack clinical symptoms and can only be known in screening programs (3). There are many factors involved in the occurrence of cancer, including these agents: viruses, bacteria, physical, chemical, genetic and familial factors (4). The behavior of lung tumors also depends on the type of pathology. Lung cancer is known as lung small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) (5). The tumor of the prognostic markers can be measured in both of the serum and tumor tissues (6, 7). Micro-RNAs have a small 18 to 23 nucleotide single-stranded nucleic acid sequences (not translated) and have been shown to play a major role in regulating gene expression by inhibiting target mRNAs. Many of the genes that inhibit cancer and oncogenes are under the strict control of the micro-RNAs. Therefore, any alteration in the expression of these genes regulating micro-RNAs can cause regulatory impairments in genes expression and ultimately lead to cancer, which can be used to detect and prevent micro-RNAs (8-11). Today, there are various methods for evaluating biomarkers in many diseases, one of the most important of which is the Realtime PCR method, which has high sensitivity, and of course, the sensitivity can be increased by repeating sampling from patients (12-18). Excessive expression of

miR-155 in blood samples can be a diagnostic marker for early diagnosis of NSCLC (19). The most important growth factor involved in the process of angiogenesis is vascular endothelial growth factor (VEGF). Vascular endothelial growth factor is a multifunctional cytokine and a homodimer glycoprotein that has antigenic, mitochondrial and endothelial permeability enhancers and is effective in tumor metastasis (20, 21). Various studies have shown that VEGF enhances vascular permeability as a major step in the angiogenesis of tumors (22, 23). Carcinoembryonic antigen (CEA) is a glycoprotein that plays a role in cell adhesion and is naturally produced during embryonic development. This compound is an oncofetal antigen that has been studied. In the studies of association, its high levels are characterized by metastasis and poor prognosis (24). In this study, the biomarker miR-155 and the two selective proteins VEGF and CEA are evaluated in terms of expression in early stages of the disease compared with healthy subjects.

MATERIALS AND METHODS

Sample collection

At first, 50 patients who referred to Masih Daneshvari Hospital were selected based on physical examination and diagnosis a specialist suspected of non-small lung cancer prior to any treatment on them, as well as those with a disease chronic inflammatory or acute infections, were excluded from this study. 50 healthy volunteers as a control group were volunteered by the physician after examination and filled out the consent form in this study. It should be noted that the samples of patients and healthy people include peripheral blood and these individuals are considered as age factors in the same groups.

After being selected, 4 ml of peripheral blood was taken through a conventional bleeding syringe in a glass test tube. Then, this 6 ml of the sample was divided into two parts of 2 ml, the first part of which was 2 ml into a falcon tube containing an anticoagulant, EDTA, and the next 2 ml portion was used for serum extraction and ELISA, they were transferred to the laboratory.

Ethics approval and consent to participate

All blood samples were obtained with patients writing extensively informed consent when they were in hospital and the study was approved by the Masih Daneshvari Hospital ethical committee and conducted in accordance with the ethical guidelines of the Declaration of Helsinki.

RNA extraction, cDNA synthesis and Quantitative Real-Time PCR (qRT-PCR)

Total RNA was extracted using Trizol Reagent (Life Technologies, Invitrogen), and the cDNA was synthesized using the PrimeScript RT Kit (Takara), according to the manufacturer's instructions.

miRNA Quantification by Quantitative Real-Time PCR (qRT-PCR)

miRNA was measured by qRT-PCR method and using U6 miRNA as control. qRT-PCR was performed by SYBR Premix ExTaq Kit (Takara) using a Rotor Gene-QIAGEN device.

The real-time RT-PCR reaction components included (A) 2µL of the template, (B) 4µL of the Master mix, (C) Primer with optimal concentration found in set up tests, (D) Deionized distilled water to reach a final reaction volume of 20µL.

Briefly, the data were obtained as the threshold cycle (Ct) value, and the ΔCt values were determined by subtracting the average GAPDH Ct value from the average target gene Ct value. Relative expression of each gene was calculated by $2^{-\Delta\Delta Ct}$ formula.

To Measure the serum levels of CEA and VEGF by ELISA

Using the Bioassay Technology Laboratory Kit (Cat No. E0080Hu), the serum VEGF protein level was determined according to the kit's instructions. Also, the serum level of CEA antigen was measured by ELISA method using the Can Ag kit.

Statistical analysis

Comparison of the mean of the two groups was done by t-test and comparing the markers positivity in the two groups using the two-sample binomial test using SPSS21 software. The statistical difference was considered significant at $P \leq 0.05$.

RESULTS

The study population included 50 healthy people and 50 NSCLC patients. These two groups corresponded in terms of age variables. T-test was compared with mean age and did not show a significant difference in mean age (Table 1).

Table 1. Average age comparison: Comparison of mean age in patients with NSCLC and healthy subjects using t-test

Group	Age (years)		
	Age Range	Average	SD
Patients (50 persons)	25-48	33.95	10.16
Healthy (50 persons)	24-45	32.85	12.28
		P=0.434	

After collecting the results of the Real-Time PCR, individuals who had positive outcomes for the biomarker expression in both the healthy subjects and the patient group were identified.

First, Ct was determined as described above, and the relative difference of miR-155 biomarker expression with the $\Delta\Delta Ct$ method was done for biomarkers.

miR-155 biomarker, it was shown that after expression of Ct, the expression levels of miR-155 in patients were significantly increased compared with the control group ($p < 0.001$), and the number of primary biomarker copies in patients is 1.74 times more than normal people (Figure 1).

The serum protein level of VEGF was calculated by ELISA technique. The mean serum level of the biomarker was higher in patients than healthy subjects. The rate of the biomarker expression was reported positive in 34(68%) patients. On the other hand, the expression of this biomarker was reported positive in 3(6%) healthy subjects. Finally, the statistical comparison of the biomarker positivity in the patients and healthy subjects was done by two-sample binomial test, which showed a statistically significant difference between the two groups ($P < 0.001$).

The serum level of CEA protein was also calculated by ELISA technique. The mean serum level of this biomarker was higher in patients than in healthy subjects. The rate of expression of this biomarker was reported in 38(76%) patients' positive. On the other hand, the biomarker expression was reported positive in 5 (10%) healthy.

Finally, the statistical comparison of the biomarker positivity in the patients and healthy subjects was done by two-sample binomial test, which showed a statistically significant difference between the two groups ($P < 0.001$) (Figure 2).

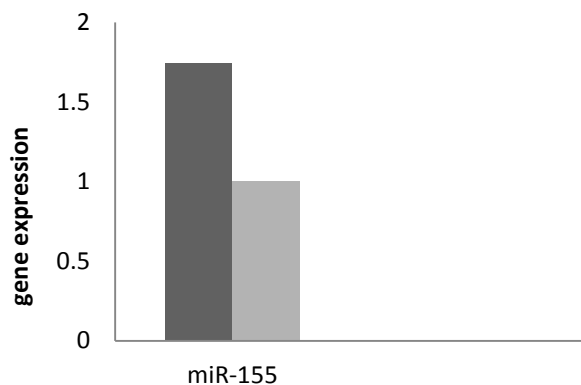


Figure 1. miR-155 expression in patients and healthy subjects

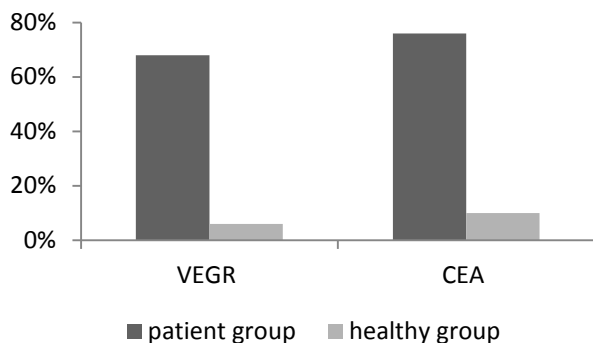


Figure 2. Serum levels of CEA and VEGF in patients and healthy people

DISCUSSION

Lung cancer is one of the main causes of mortality among people. Lung cancer occurs when lung cells begin to grow without control and become tumorous. Lung tissue usually affects cancer in 10 to 30 years before the symptoms are detected. Sometimes lung cancer can spread to other parts of the body and can be cured if it be diagnosed soon (25).

The behavior lung tumors also depend on the type of pathology. Lung cancer is recognized as both small cell carcinoma and non-small cell carcinoma (25).

One of the challenges facing today's cancer patients is how to deal with lung cancer. While treatment may be

effective in the early stages, the majority of patients (77%) are not identified until the progression of the cancer and advanced stages. Treatment for lung cancer depends on the type of cancer, the extent of cancerous tissue and the patient's condition. Unlike some therapeutic successes, the combination of drugs and new methods is not as effective in improving the patient's status and longevity (26, 27).

Therefore, diagnosis is timely and important in its early stages. One of the new methods for the detection of tumors is molecular methods. The goal of molecular diagnostic methods is to identify patients with cancer and the highest probability of positive response to targeted therapies. Following this, patients can be evaluated on a regular basis (28, 29).

The marker tumor can be considered as a molecule that determines the probability of cancer, or provides information about the possibility and behavior of cancer, such as metastasis and its spread, and the likelihood of a recurrence and reversal of cancer (30, 31)

The tumor of the prognostic markers can be measured both of the serum and tumor tissues. Serum markers have the ability to detect the spread of tumor mass or hidden metastases by examining their concentration. The most commonly used tissue markers in this regard are likely to be molecules that can show the spread of the tumor by increasing cell proliferation or spreading metastasis in the tissue (6).

Mature micro-RNAs be created with sequences approximately 21 to 24 nucleotides. Micro-RNAs can play their regulatory role by controlling the translation level in two ways. If these micro-RNAs are not fully complementary to the RNA sequence by the binding to it will inhibit translation. If they are completely complementary to the target sequence, they will break it. It has now been found that the profile of the expression of micro-RNAs varies between the normal and tumor tissues, and with some of them, the tumor tissue can be purified from the normal tissue (32, 33).

Micro-RNAs can play a role in various mechanisms for cancer. There are several methods for evaluating these markers, one of the most important of which is PCR, which

has a high sensitivity and, of course, can increase the sensitivity by repeating the sample.

The miR-155 is likely to play a major role in releasing from immune surveillance and will cause escape the immune system (34).

Vascular endothelial growth factor (VEGF) is a multifunctional cytokine and a homodimer glycoprotein with a molecular weight of 23-45 kDa, which has angiogenic, mutagenic and endothelial cell permeability enhancer and it is effective in the tumor metastasis process (20).

Various studies have shown that VEGF enhances vascular permeability as a major step in the angiogenesis of tumors (35).

CEA is a glycoprotein that plays a role in cell adhesion and is naturally produced during embryonic development, but production after birth is limited to gut epithelial tissue, so it does not exist in the blood of healthy adults. CEA expression is re-expressed in people with severe cancer and plays an important role in the development of epithelial and mucus cancers (36).

In the study of Mohammadnia et al., the mean CEA marker level was higher in the patients group than the control group, and this difference was statistically significant ($P < 0.001$) and the sensitivity of this test was 36.6%. Based on the findings of this study, CK19 mRNA is a specific marker in the peripheral blood for the diagnosis of lung cancer, and it has also been shown that CEA can be a specific marker for early diagnosis of lung cancer in peripheral blood (37). In this study, The CEA marker was also evaluated by ELISA method and the results showed that the serum level of this marker was higher in patients than healthy subjects and there is a significant difference between them.

Oyama et al. reported high levels of embryonic cardio myocyte antigen in the serum level of patients with non-small cell cancers, and high rates, especially in NSCLC-type cancer than the other types (38). We have acquired similar results in our study.

Xue et al. in 2016 showed that MiR-21 and MiR-155 contributed to the progression of non-small cell carcinoma, which was similar to the results of this study and the present study, and showed that the expression of MiR-155 in patients has been more than healthy subjects (39).

Yang et al. showed an increased expression of MiR-21 and MiR-155 in NSCLC-type cancer patients, which is consistent with the results of the recent study (40).

CONCLUSION

In conclusion, the results of CEA and VEGF and MiR-155 markers in non-small cell lung cancer patients in Iranian population showed a significant increase in these important cancer markers and could be a major contributor to cancer progression. Unfortunately, the proprietary protocol, which is not a specific marker for lung cancer detection, has yet to be found. Therefore, more studies are recommended in patients with different populations and with a higher statistical society.

Acknowledgment

This research is part of a PhD dissertation on molecular medicine that was conducted in Mashhad University of Medical Sciences, Masih Daneshvari Hospital, Shahid Beheshti University of Medical Sciences. It is also part of the efforts of the professors and colleagues of Mashhad Medical Sciences Universities and Masih Daneshvari Hospital of Shahid Beheshti University of Medical Sciences is sincerely thanked.

REFERENCES

1. Azizi F, Hatami H, Janghorbani MJ. Epidemiology and control of common diseases in Iran. Tehran: Eshtiagh Publications. 2000:602-16.
2. Sprod LK, Mohile SG, Demark-Wahnefried W, Janelsins MC, Peppone LJ, Morrow GR, et al. Exercise and Cancer Treatment Symptoms in 408 Newly Diagnosed Older Cancer Patients. *J Geriatr Oncol* 2012;3(2):90-7.
3. National Lung Screening Trial Research Team; Aberle DR, Adams AM, Berg CD, Black WC, Clapp JD, et al. Reduced

- lung-cancer mortality with low-dose computed tomographic screening. *N Engl J Med* 2011;365(5):395-409.
4. Kang SM, Sung HJ, Ahn JM, Park JY, Lee SY, Park CS, et al. The Haptoglobin β chain as a supportive biomarker for human lung cancers. *Mol Biosyst* 2011;7(4):1167-75.
 5. Kwak EL, Bang YJ, Camidge DR, Shaw AT, Solomon B, Maki RG, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. *N Engl J Med* 2010;363(18):1693-703.
 6. Duffy MJ. Role of tumor markers in patients with solid cancers: A critical review. *Eur J Intern Med* 2007;18(3):175-84.
 7. Lønning PE. Study of suboptimum treatment response: lessons from breast cancer. *Lancet Oncol* 2003;4(3):177-85.
 8. Dickinson BT, Kisiel J, Ahlquist DA, Grady WM. Molecular markers for colorectal cancer screening. *Gut* 2015;64(9):1485-94.
 9. Mattie MD, Benz CC, Bowers J, Sensinger K, Wong L, Scott GK, et al. Optimized high-throughput microRNA expression profiling provides novel biomarker assessment of clinical prostate and breast cancer biopsies. *Mol Cancer* 2006;5:24.
 10. Kazempour Dizaji M, Farzanegan B, Bahrami N, Khoshnam Z, Fathi M, Dargahi H, et al. Expression of miRNA1, miRNA133, miRNA191, and miRNA24, as Good Biomarkers, in Non-Small Cell Lung Cancer Using Real-Time PCR Method. *Asian Pac J Cancer Prev* 2022;23(5):1565-70.
 11. Shahi A, Bahrami N, Tabatabaei RR, Dizaji MK, Jamaati H, Mohamadnia A. Analysis of Blood and Tissue miR-191, miR-22, and EGFR mRNA as Novel Biomarkers for Breast Cancer Diagnosis. *International Journal of Cancer Management* 2022;15(1): e117612.
 12. Harquail J, Benzina S, Robichaud GA. MicroRNAs and breast cancer malignancy: an overview of miRNA-regulated cancer processes leading to metastasis. *Cancer Biomark* 2012;11(6):269-80.
 13. Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell* 2005;120(1):15-20.
 14. Ambros V. MicroRNA pathways in flies and worms: growth, death, fat, stress, and timing. *Cell* 2003;113(6):673-6.
 15. Karimi R, Mohamadnia A, Hosseini F, Bahrami N, Jamaati H. Evaluation of Chemokines and Cytokines as Biomarkers for Disease Severity in COVID-19-Infected Patients. *Jundishapur Journal of Microbiology* 2024;17(1): e143611.
 16. Bahrami N, Jamaati H, Farzanegan B, Nejad RA, Mohammadi F, Naghdi M, et al. Special Real-time PCR and Conventional PCR for Detection of Rhino-Orbital-Cerebral Mucormycosis. *Journal of Cellular & Molecular Anesthesia* 2024;9(1): e145366.
 17. Jamaati H, Bahrami N, Tabarsi P, Khosravi A, Kiani A, Abedini A, et al. Multi-Gene Expression in Anthracosis of the Lungs as One of the Risk Factors for Non-Small Cell Lung Cancer. *Asian Pac J Cancer Prev* 2017;18(11):3129-33.
 18. Najafi Z, Mohamadnia A, Ahmadi R, Mahmoudi M, Bahrami N, Khosravi A, et al. Proteomic and genomic biomarkers for Non-Small Cell Lung Cancer: Peroxiredoxin, Haptoglobin, and Alpha-1 antitrypsin. *Cancer Med* 2020;9(11):3974-82.
 19. Gao F, Chang J, Wang H, Zhang G. Potential diagnostic value of miR-155 in serum from lung adenocarcinoma patients. *Oncol Rep* 2014;31(1):351-7.
 20. Cines DB, Pollak ES, Buck CA, Loscalzo J, Zimmerman GA, McEver RP, et al. Endothelial cells in physiology and in the pathophysiology of vascular disorders. *Blood* 1998;91(10):3527-61.
 21. Verstraete M. Endothelial cell-mediated coagulation, anticoagulation and fibrinolysis. 1995.
 22. Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. *Nature* 2000;407(6801):249-57.
 23. Gupta K, Zhang J. Angiogenesis: a curse or cure? *Postgrad Med J* 2005;81(954):236-42.
 24. Bast RC Jr, Ravdin P, Hayes DF, Bates S, Fritsche H Jr, Jessup JM, et al. 2000 update of recommendations for the use of tumor markers in breast and colorectal cancer: clinical practice guidelines of the American Society of Clinical Oncology. *J Clin Oncol* 2001;19(6):1865-78.
 25. Eckardt JR, Bentsion DL, Lipatov ON, Polyakov IS, Mackintosh FR, Karlin DA, et al. Phase II study of picoplatin as second-line therapy for patients with small-cell lung cancer. *J Clin Oncol* 2009;27(12):2046-51.
 26. Mauer AM, Cohen EE, Ma PC, Kozloff MF, Schwartzberg L, Coates AI, et al. A phase II study of ABT-751 in patients with advanced non-small cell lung cancer. *J Thorac Oncol* 2008;3(6):631-6.

27. Horner M, Ries L, Krapcho M, Neyman N, Aminou R, Howlander N, et al. SEER Cancer Statistics Review, 1975-2006, National Cancer Institute. Bethesda, MD. 2009.
28. Wistuba II, Gelovani JG, Jacoby JJ, Davis SE, Herbst RS. Methodological and practical challenges for personalized cancer therapies. *Nat Rev Clin Oncol* 2011;8(3):135-41.
29. Cho JY. Molecular diagnosis for personalized target therapy in gastric cancer. *J Gastric Cancer* 2013;13(3):129-35.
30. D'Alessandro R, Roselli M, Ferroni P, Mariotti S, Spila A, Aloe S, et al. Serum tissue polypeptide specific antigen (TPS): a complementary tumor marker to CA 15-3 in the management of breast cancer. *Breast Cancer Res Treat* 2001;68(1):9-19.
31. Hwa HL, Kuo WH, Chang LY, Wang MY, Tung TH, Chang KJ, et al. Prediction of breast cancer and lymph node metastatic status with tumour markers using logistic regression models. *J Eval Clin Pract* 2008;14(2):275-80.
32. Almeida MI, Reis RM, Calin GA. MicroRNA history: discovery, recent applications, and next frontiers. *Mutat Res* 2011;717(1-2):1-8.
33. Zhang XW, Yang HY, Fan P, Yang L, Chen GY. Detection of micrometastasis in peripheral blood by multi-sampling in patients with colorectal cancer. *World J Gastroenterol* 2005;11(3):436-8.
34. Hamanaka R, Yokose T, Sakuma Y, Tsuboi M, Ito H, Nakayama H, et al. Prognostic impact of vascular invasion and standardization of its evaluation in stage I non-small cell lung cancer. *Diagn Pathol* 2015;10:17.
35. Locker GY, Hamilton S, Harris J, Jessup JM, Kemeny N, Macdonald JS, et al. ASCO 2006 update of recommendations for the use of tumor markers in gastrointestinal cancer. *J Clin Oncol* 2006;24(33):5313-27.
36. Ordoñez C, Screaton RA, Ilantzis C, Stanners CP. Human carcinoembryonic antigen functions as a general inhibitor of anoikis. *Cancer Res* 2000;60(13):3419-24.
37. Mohamadnia A, Karimi S, Yadegar Azari R, Naji SA, Khosravi A, Bahrami N, et al. Expression of CK19 gene in patients with lung cancer and Its comparison with carcinoembryonic antigen in peripheral blood. *Payavard Salamat* 2016;9(5):459-68.
38. Oyama T, Kawamoto T, Matsuno K, Osaki T, Matsumoto A, Isse T, et al. A case-case study comparing the usefulness of serum trace elements (Cu, Zn and Se) and tumor markers (CEA, SCC and SLX) in non-small cell lung cancer patients. *Anticancer Res* 2003;23(1B):605-12.
39. Xue X, Liu Y, Wang Y, Meng M, Wang K, Zang X, et al. MiR-21 and MiR-155 promote non-small cell lung cancer progression by downregulating SOCS1, SOCS6, and PTEN. *Oncotarget* 2016;7(51):84508-19.
40. Yang M, Shen H, Qiu C, Ni Y, Wang L, Dong W, et al. High expression of miR-21 and miR-155 predicts recurrence and unfavourable survival in non-small cell lung cancer. *Eur J Cancer* 2013;49(3):604-15.