Original Article

Predictive Molecular Blood Biomarkers in Non-Small Cell Lung Cancer

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Abstract

Background: Lung cancer is characterized by the uncontrolled growth of cells in the lung tissue. The purpose of the present study was to investigate the expression of MUC1 mRNA and CK19 mRNA biomarkers in patients with non-small-cell lung carcinoma (NSCLC).

Materials and Methods: In this case-control research, thirty samples of cancer blood, thirty samples of cancer tissue, and the same number of healthy samples were prepared. Samples were collected and RNA was extracted, then cDNA was made and gene expression was measured using Real-Time PCR.

Results: Among non-small-cell lung carcinoma patients, the MUC1 mRNA marker was positive for 19 individuals while in the healthy group, it was reported positive in 5 out of 30 individuals. In the patients' group, the CK19 mRNA marker was positive for 16 individuals while in the healthy group, in 6 out of 30 individuals.

Conclusion: The MUC1 mRNA and CK19 mRNA as lung cancer tumor markers were reliable and sensitive; however, further studies are recommended.

Keywords: Non-small-cell lung carcinoma (NSCLC), Marker tumor, CK19-mRNA, MUC1-mRNA

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Introduction

Lung cancer is the most common type of cancer, primarily caused by smoking. Breast cancer and prostate cancer are also relatively common (1). Lung cancer is a disease characterized by the uncontrolled growth of the cell in the lung tissue. If the disease is not treated, this cell growth can be extended beyond

Table 1: Characteristics of primers used in the Real-Time RT-PCR reaction.

Parameters	*MUC1* mRNA	*CK19*-mRNA	18s rRNA
Primer F	GTGCCCCCTAGCAGTACCG	TCCGAACCAAG TTTGAGAC	GTAACCCGTTGAACCCCATT
Length of primer	19	19	20
Primer R	GACGTGCCCCTACAAGTTG G	AATCCACCTCC ACACTGA	CCATCCAATCGGTAGTAGCG
Length of primer	20	18	20
Length of proliferated part	123	222	152
The optimum temperature for Annealing	61.6°C	58.4℃	53.5℃

the lungs and reach surrounding tissues or other organs of the body through metastasis (2). Lung cancer occurs when lung cells have uncontrolled growth. These cells can become tumors and spread to other body organs (3). Lung carcinomas are classified according to the size and shape of malignant cells. Two broad categories are non-small-cell lung carcinoma and small-cell lung carcinoma (4). Among all people with lung cancer, 15% can survive for five years after diagnosis (5).

Nowadays, the challenge in cancer diagnosis is to establish a close and precise relationship between biomarkers and clinical symptoms and have a non-invasive diagnostic method in the early stages of the disease (5, 6). Diagnostic biomarkers identify the presence of infection at an early stage, especially in patients with higher risks. They can help the physician choose the best and most effective treatment methods (7). A tumor marker is a substance in the blood, urine, or some other tissues of the body that its rate can increase in various cases of cancer incidence (8, 9). Cytokeratin 19 (CK19) is a 65 kDa protein produced in insufficient quantities in bronchial epithelial cells branch and abundantly in carcinoma cells (10).

In various carcinomas, changes in cytokeratin expression, such as an increased copy of this gene, can occur at the time of tumor progression, and identifying this marker may help promptly diagnose the disease (11, 12). The expression level of mucin1 (MUC1) rises in neoplastic cells, which probably increases the proliferation of cancer cells and prevents apoptosis and tumor progression (13). Mucins are high molecular weight glycoproteins produced by respiratory, gastrointestinal, and urinary tract epithelial gland cells (13). Mucins play a role in the renewal and separation of epithelial tissue. They are referred to as factors in cell adhesion and connectivity, and signaling (14).

Methods

Specialists selected 30 patients referred to Masih Daneshvari Hospital before any treatment, and 30 healthy subjects voluntarily participated in the present study after medical examination. The control group included individuals with average results of diagnostic tests for lung cancer. An amount of 10 ml of peripheral blood was taken from all present study participants and

Table 2: Real-Time RT-PCR reaction temperatures and times.

Real-time step	Temperature	Duration
Initial activation	95ºC	10 min
	40 cycles	
Denaturation	59ºC	15s
Annealing	56-60ºC	60s
Extension	72ºC	20s

entered into falcon tubes containing EDTA anticoagulant and transferred to the laboratory. RNA was extracted from samples immediately after transferring to the lab. RNA extraction using RNeasy Midi Kit*:* This step was performed by (Qiagen Cat no.75144) RNeasy Midi Kit. The red blood cells (if present) were firstly lysed using Lysis Buffer, and then centrifuging was done, and the obtained cell mass was entered into extraction stages after twice washing with PBS. Finally, the total mRNA obtained from each column was dissolved in the preservative buffer of provided kit and prepared for the next stage.

In the next stage, cDNA was made using RTPL12. Cat no) Viva 2-steps RT-PCR Kit) and according to the kit's protocol. Specific primers for each marker were designed through AlleleID6 software and ordered to make (Table1).

EvaGreen is a fluorescence color that has been used in the kit produced by Cynacolon Company (Cat No.BT11101). The components needed for reaction have been provided in the master mix of the equipment. The members of RT-PCR real-time response included the following cases:

a. Pattern Sequence to the extent of 2 µl
b. Master mix at 4 µl
c. The primer according to the most suitable concentration found in the initial setup tests
d. Deionized distilled water to the extent in which the final reaction volume reaches 20 µL

The temperatures and reaction times were adjusted according to the kit's instructions(table2). The study was approved by the Masih Daneshvari Hospital ethical committee and conducted following the ethical guidelines of the Declaration of Helsinki (IR.SBMU.NRITLD.REC.1394.115).

Results

The statistical population of the present study included two groups of patient individuals and healthy individuals. According to the calculated sample size, some 30 subjects were selected for each group. According to the results obtained from the t-test, there was no significant difference between these two groups in terms of mean age. Therefore, it can be argued that the age factor had no conflicting effect on understudy groups (p-value = 0.432).

In the present study, 18srRNA was used as a reference gene. The expression level of the reference gene of the study (18srRNA) can be relatively calculated from the measured ct value of each sample.

The obtained results indicated no significant difference between the control and experimental groups, confirming the accuracy of selecting this marker as the reference gene of the present study (p-value = 0.292).

Analyzing expression level of understudy markers (CK19-mRNA and MUC1 mRNA)

After extracting the reaction results of Real-time RT-PCR, the number of subjects in each of the main groups with a positive impact on markers' expression was identified. Among 30 patients with non-small-cell lung carcinoma, the MUC1 mRNA

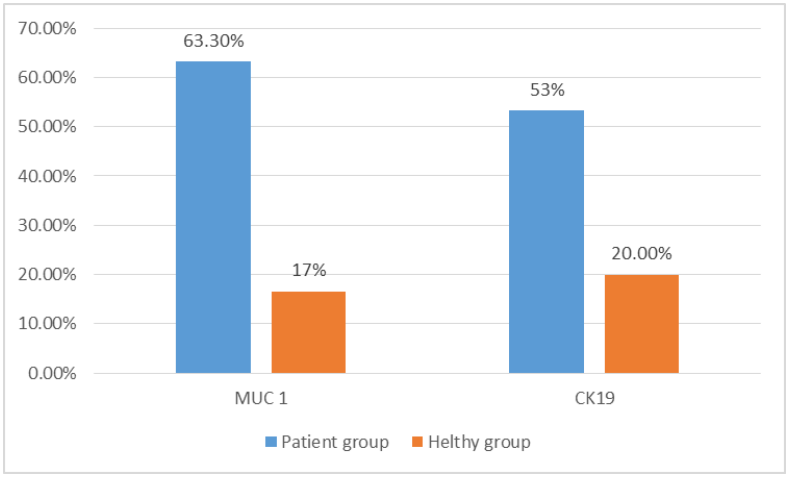

Figure 1. Percentage of positive cases of CK19-mRNA and MUC1 mRNA markers in normal and patient samples.

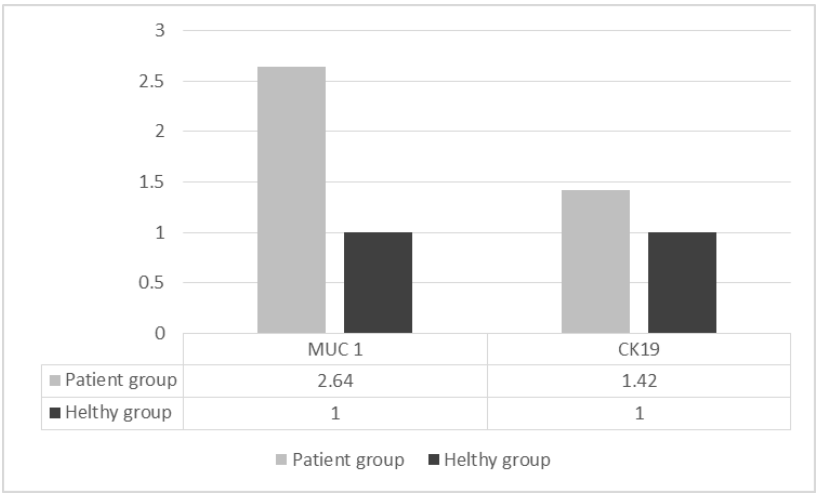

Figure 2. Differences in the expression of CK19-mRNA and MUC1 mRNA markers in normal and patient samples.

marker was positive for 19 individuals. In the healthy group, this number was 5 out of 30. Statistical comparison for positivity of this marker in patients and healthy groups using a two-sample binomial test showed a significant difference between the two groups (P value=0.031).

CK19-mRNA marker was positive for 16 out of 30 subjects in the patient's group. The value was 6 out of 30 subjects in the healthy group. Statistical comparison for positivity of this marker in patients and healthy groups using a two-sample binomial test showed a significant difference between the two groups (P value=0.001).

Investigating the difference between markers' expression in two understudy groups:

Relative levels of markers' expression differentiation between patients and healthy individuals were measured. For this purpose, the ΔΔct method was used for both the CK19-mRNA and MUC1 mRNA. However, the value of ΔΔct for CK19-mRNA was calculated to be equal to -0.5, which mathematically indicated that the average number of initial copies of this marker in patients is 1.42 times more than healthy subjects. Meanwhile, the value of ΔΔct for MUC1 -mRNA was calculated to equal 1.4, which mathematically indicated that the average number of initial copies of this marker in patients is 2.64 times more than healthy subjects.

Discussion

In the present study, 30 patients with an age range of 30-66 years old and a mean age of 45 years and 30 healthy individuals with an age range of 29-66 years old and a mean age of 48 years were investigated. The purpose was to evaluate the early diagnosis of lung cancer through CK19 and MUC1 genes using the Real-time PCR method.

Among various methods, tumor biomarkers that are produced with tumor tissue from the body in response to the presence or progression of cancer and found to a large extent in blood, urine, and tumor tissue compared with healthy people can be considered as appropriate criteria for early diagnosis of disease (2).

Investigation of tumor expression of CK19 and MUC1markers in various cancers indicates the vital role of these genes in the incidence and development of tumors (15).

A study found that expressions of CK8, CK7, Ck18, and CK19 have been observed in over 90% of breast carcinomas (16). This study included 575 breast cancer patients with a range of 22 to 102 years old, and the expression of CK19 was observed in 539 cases, i.e., more than 90% of patients (93.7%). The value indicated increased expression resulting from the present study (16).

In a study conducted by L. Yan et al. (2015), the expression value for all MRNA, KS1/4, and LUNX genes was positive in 6.6% of peripheral blood of NSCLC patients. LUNX, CK19, and KC1/4 had a higher level of expression in NSCLC patients than in individuals with benign lung disease, and the result follows the results of the present study (17).

In this regard, a study was conducted in 2011, and the expression of the MUC1 gene was carried out using the immunohistochemistry technique. This analysis showed that MUC1 increased expression in 87% of patients with lung adenocarcinoma and 39% of patients with squamous cell carcinoma. In the study, the MUC1 gene was also increased in expression for patients compared with healthy individuals (18, 19).

Conclusion

In this study, the expression of CK19 and MUC1 biomarkers increased in cancer patients compared to healthy individuals in the early stages. Cancer patients are more prone to infections than other people. Pneumonia is one of the most common infections in this group of people due to a weakened immune system and exposure to resistant bacteria. And is one of the main factors in their hospitalization in the ICU or hospitalization of cancer patients for a long time for treatment, the possibility of exposure to resistant bacteria, and sometimes this factor increases the mortality rate in these patients. Therefore, biomarkers' diagnosis of cancer patients in the early stages can be beneficial. Additional studies are recommended, of course.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

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