RESEARCH ARTICLE

Prognostic role of serum HMGB1, endocan, and caveolin level in non-small cell lung cancer

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Lung cancer is the leading cause of cancer-related mortality worldwide for both sexes. The prognosis of the disease is still poor despite improvements in the treatment. Therefore, studies on lung cancer now focus on defining new prognostic markers and new targets in the treatment approach. In this study, we compared the serum levels of Caveolin-1, HMGB1, and Endocan, which can be effective in the carcinogenesis steps, to patients with Non-small cell lung carcinoma (NSCLC) and healthy population. Caveolin-1, HMGB1 and Endocan serum levels were investigated using ELISA method. Caveolin-1 and HMGB1 levels were higher in the patient group than in the control group, whereas the Endocan level was lower in the patient group than in the control group. But there was no statistical significance for the three parameters. In our study, we found no significant difference in terms of Endocan, Caveolin-1 and HMGB1 levels compared to healthy population. The small number of patients is the most important limitation of our study. Nevertheless, we think that the search for a new marker for early diagnosis and treatment of this disease, which is common in the community, should be continued.

Key Words: *Non-small cell lung cancer; Caveolin; HMGB1; Endocan*

INTRODUCTION

Lung cancer is the leading cause of cancer related deaths. Despite improvements in treatment, the prognosis of the disease is poor [1]. Due to the fact that the prognosis is poor, intensive researches continue for early diagnosis and treatment. Non-small cell lung cancer (NSCLC) is histologically divided into several subtypes, among which squamous cell carcinoma (SCC) and Adenocarcinoma (ADC) are the most common. Despite new treatment modalities such as targeted therapies and immunotherapies the overall 5-year survival rate does not reach %20 [2]. Absence of effective screening methods, lack of effective prognostic factors, poor treatment-related predictive factors, and the majority of patients diagnosed at advanced stages may be among the reasons for the poor prognosis of NSCLC. Therefore, identifying novel prognostic factors as biomarkers or novel targets for treatment approaches may be a clinically useful tool for early detection or improved survival of NSCLC. Caveolins are a class of oligomeric structural proteins that are both necessary and sufficient for caveol formation. Interestingly, Caveolin-1 (CAV1) is involved in oncogenic cell transformation, tumorigenesis and metastasis pathogenesis. Caveolae are play an important role in cellular process, including molecule transport, cell adhesion, and signal transduction [3]. CAV1 is an essential structural component of caveolae and functionally regulates the activity of many signaling molecules. This molecules are potentially involved in the development of human cancer [4]. Thus, CAV1 could be a key molecule for growth-related signalling and cancer development. High mobility group box (HMGB) proteins are non-histone nuclear proteins with very different functions in the cell. There is increasing evidence for the role of HMGB1 in cancer progression, angiogenesis, invasion and development of metastases. HMGB1 is a highly conserved structural transcription factor. In the nucleus, HMGB1 plays an important role as a DNA-binding protein to sustain nucleosome structure and as an architectural transcription factor regulating gene expression. However, HMGB1 can be released into the extracellular matrix, where it exerts crucial functions in inflammation and carcinogenesis, such as promotion of angiogenesis, evasion of apoptosis, promotion of tissue invasion and metastasis [5]. Endocan expressed in vascular endothelium, this dermatan sulfate proteoglycan is freely circulating in the bloodstream [6]. Recently, Endocan mRNA levels have been recognized as one of the most important molecular signatures that cause poor prognosis in various types of cancer, including lung cancer [7,8]. Endocan is over-expressed in several carcinoma endothelial cells. Tumour prognosis, metastasis, and angiogenesis were shown to be associated with endocan expression [9]. In our study, the diagnostic role of Caveolin, Hmgb1 and Endocan levels measured in serum for non-small cell lung cancer was investigated.

MATERIAL AND METHOD

Patient selection

In the Medicalpark Gaziantep Hospital Medical Oncology Clinic, between 2014 and 2017, patients diagnosed with histopathologically confirmed NSCLC and healthy volunteers were included in the study. Patient files were scanned and information such as age, gender, routine laboratory tests were obtained retrospectively. Ethics committee approval was obtained before the study.

Obtaining samples

Blood samples remaining from the blood samples of patients for routine checks were collected prospectively directly before the start of first-line systemic chemotherapy. They were centrifuged for 15 min at 1,000g within 1 h of collection. The resulting sera were aliquoted into microtubes and either immediately frozen at -80 C. These samples were taken to the refrigerator at 4°C temperature overnight before measurements were taken. Serum samples were allowed to stand at room temperature for 2 hours before being run by the ELISA method. The samples were then subjected to measurement procedures with mixing using vortex. Caveolin, Hmgb1 and Endocan serum levels were investigated using Elisa method.

The measurements were performed in duplicate for each sample. All concentrations/absorbance graph curves of these tests and calculations related to the results were made using the program of Biotek_ELx808 (Winooski, Vermont, USA).

Caveolin levels were quantitatively measured in accordance with the manufacturer's instructions with the SunRed brand kit, Catalog Number 201-12-1728 (Shanghai Sunred Biological Technology Co., Ltd. Shanghai/China). Analysis was performed using double-antibody sandwich enzyme

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immunoassay technique. The sensitivity of the test was 0.313 ng/mL and the detection range was 0.5-90 ng/mL. Intra-assay and inter-assay presision variation coefficients were 8.7% and 6.3%, respectively.

HMGB1 levels were also assessed in a manually performed with commercially available quantitative sandwich-enzyme-linked immunosorbent assay (ELISA) kit, following the manufacturer's instructions (Rel Assay Diagnostics® Mega Tıp Ltd, Turkey). The minimum detectable level of Human HMGB1 was 0.06 ng/mL and detection range was 1-32 ng/mL. The intra- and inter-assay coefficients of variations were 7.2% and 8.4% respectively.

Serum endocan levels were assessed with commercially available quantitative sandwich-enzyme-linked immunosorbent assay (ELISA) kit, following the manufacturer's instructions (Rel Assay Diagnostics® Mega Tıp Ltd, Turkey). The minimum detectable level of human serum endocan was 2.56 ng/mL and detection range was 75-2400 ng/mL. The intra- and inter-assay coefficients of variations were 5.38% and 4.13% respectively.

Statistical analysis

Statistical analyzes were performed using SPSS for Windows 15.0 software. The normal distribution suitability of the interchanges was examined using visual (histogram and probability plots) and analytical directions (Kolmogorov-Smirnov/Shapiro-Wilk tests). In the Kolmogorov-Simirnov test, cases where the p value was above 0.05 were found as normal distribution. When normal distribution was not determined, the patient and control group were compared using the Mann-Whitney U test.

RESULTS

A total of 42 participants were enrolled in the study, 19 (45.2%) patients and 23 (54.8%) healthy volunteers. The mean age of the patients was 63.2 ± 9.5 (Range 43-82). Two of the patients (10.5%) were female and 17 (89.5%) were male patients.

Caveolin was found in the patient group as 48.3 ± 84.1 (Range 5.5-250) ng/ml, Hmgb1 3.15 ± 3.14 (Range 0.08-12.18) ng/ml and Endocan 250.7 ± 180.5 (Range 36.2-789.6) ng/L. In the control group, caveolin was determined as 26.4 ± 38.9 (Range 3.46-176.8) ng/ml, Hmgb1 2.98 ± 2.79 (Range 0.39-8.83) ng/ml and Endocan 301.2 ± 174.6 (Range 109.8-722.6) ng/L.

Since caveolin levels did not show a normal distribution, the patient and control group were compared using the Mann-Whitney U test. There was no statistically significant difference between the patient and control groups in terms of caveolin levels (p=0.471).

Since Hmgb1 levels did not show a normal distribution, the patient and control group were compared using the Mann-Whitney U test. No statistically significant difference was found between the patient and control group in terms of Hmgb1 levels

(p=0.919).

Since Endocan showed no normal distribution, the patient and control group were compared using the Mann-Whitney U test. No statistically significant difference was found between patient and control group in terms of Endocan levels (p=0.283). (Table 1)

DISCUSSION

HMGB1, a proinflammatory cytokine, has a key role in many pathological processes such as inflammation, cell migration and tissue regeneration [10]. HMGB1 can also be involved in many aspects of development of malignant processes, such as cell proliferation, cell migration, and tumor angiogenesis [11]. Recent studies suggest that downregulation of HMGB1 in NSCLC may alter disease progression and may increase sensitivity to chemotherapy drugs through the inhibition of HMGB1-mediated cell autophagy and cell apoptosis [12]. Based on this information, it can be concluded that there is a relationship between serum and tissue HMGB1 levels and NSCLC progression.

Cancer development is a highly complicated dynamic process. In recent years, many studies have shown an association between HMGB1 and cancer development [13]. However, HMGB1 is not yet clear importance in the diagnosis and prognosis of cancer. In a study by Xia et al., it was observed that the levels of HMGB1 detected by immunohistochemistry, RT-PCR and Western blot techniques were higher when compared with healthy population in NSCLC patients [14]. It was also noted in this study that HMGB1 levels were significantly higher in patients diagnosed with NSCLC to provide a diagnosis. In addition, researchers have found that HMGB1 levels correlate with the stage of the disease. As a result, they suggested that HMGB1 had a key role in the progression of NSCLC. In this study, it is stated that three methods (RT-PCR, ELISA and immunohistochemistry) have similar sensitivity in measuring HMGB1 levels. In our study, we measured HMGB1 levels with ELISA method. We found higher levels of HMGB1 in NSCLC patients but there was no statistically significant difference between patient and control group. Endocan is endothelial dermatan sulphate released from lung and kidney tissue [6]. Endocan interacts with growth factors such as hepatocyte growth factor, thus stimulating epithelial growth. For this reason, endocan is thought to be an effective molecule in the continuation of tumorigenesis [15]. The association between endocan overexpression and poor survival in lung cancer has been demonstrated [16]. In study conducted by Grigoriu et al., endocan mRNA increase was detected in tumor tissue endothelial cells of NSCLC patients [17]. Based on the study, researchers have suggested that endocan levels increase in response to endothelial cell activation in the tumor cell and claim that endocan may be a marker of rapid tumor growth. In this study, a significant increase in endocan levels was found in a group of patients with previously untreated NSCLC compared with healthy controls. High endocan levels and presence of metastasis and decreased survival were correlated. There was also a correlation between lymph node involvement and endocan levels, although not statistically significant. Researchers have attributed this to the fact that the number of patients is low. At the end of this study, endocan levels claimed to be markers for the extension of the disease or the elevated endocan levels after treatment may be a marker for recurrence. Interestingly, our results demonstrated lower Endocan serum levels in patients group than healthy group. But, no statistically significant difference was found between these groups. Caveolin upregulation has been shown in many types of cancer [18]. In these studies caveolin-1 is said to be aggressive, such as tumor cell invasion and migration [19]. In addition, high caveolin levels in lung and prostate cancer have been associated with poor prognosis. In two separate studies by the authors, it has been shown that caveolin regulates endothelial adhesion in lung cancer cells by ROS-dependent mechanism. As a result of this study, it was stated that caveolin has a critical role in tumor progression [20,21]. Many different types of cancer, such as the lung, breast, prostate, and pancreas, have been shown to correlate with metastasis in caveolin levels. In lung cancer, caveolin has been shown to contribute to the development of metastasis by increasing anoikis resistance and increasing invasion and migration [22]. Lung cancer is the most frequent cancer in the world and the leading cause of the cancer related deaths. Significant progress has been made in the diagnosis and treatment of lung cancer in recent years. However, information on the pathogenesis of the disease is still limited. Moreover, there is no effective method to provide early recognition of the disease. It is clear that early recognition in NSCLC is very important in reducing the mortality of the disease. Therefore, the search for an ideal marker for early diagnosis is ongoing.

One of the problems encountered in the treatment of NSCLC is lack of predictive treatment-related markers. In the evaluation of the efficacy of antiangiogenic agents used in treatment in recent years, appropriate predictive marker presence will be helpful in directing treatment.

In this study, HMGB1, Endocan and Caveolin levels which we thought were

TABLE 1

Comparison of patient and control groups in the level of Caveolin, Hmgb1 and Endocan

	Patients *n=19* (45.2%)	Healthy volunteers *n=23* (54.8%)	p Value
Caveolin, ng/ml (min-max $\bar{x}$ ±Sd)	5.5-250 (48.3 ± 84.1)	3.46-176.8 (26.4 ± 38.9)	0.471
Hmgb1, ng/ml (min-max $\bar{x}$ ±Sd)	0.08-12.18 (3.15 ± 3.14)	0.39-8.83 (2.98 ± 2.79)	0.919
Endocan, ng/L (min-max $\bar{x}$ ±Sd)	36.2-789.6 (250.7 ± 180.5)	109.8-722.6 (301.2 ± 174.6)	0.283

n=number of individuals

The Mann-Whitney U test was used for the analysis

important in the development and progression of NSCLC were compared with the healthy population but no statistically significant difference was detected. This was may be due to the small number of patients in our study. It would also be useful to compare different measurement techniques such as ELISA and RT-PCR. In our study, only serum HMGB1, Caveolin and Endocan levels were measured but not measured in the tumor tissue. This is another situation that limits our work. However, we believe that significant results can be achieved with studies involving many patients at different stages and different assay technics.

CONCLUSION

There are many studies in the literature about HMGB1, Endocan and Caveolin levels in NSCLC. In these studies, different measurement techniques were used. In some of the studies, the measurement was performed in the tissues obtained from the tumor tissue while in the other part the serum levels were measured. The number of patients in these studies is limited. In our study, HMGB1 and Caveolin levels were found to be high in patients with NSCLC compared with the healthy population in accordance with the literature, but the Endocan levels were lower in the patient group contrary to the literature. As a result of our study, we think that it is necessary to validate the measurement method of these three proteins which are thought to play a role in different stages of carcinogenesis. Nevertheless, we think that the search for a new marker for early diagnosis and treatment of this disease, which is common in the community, should be continued**.**

ETHICAL APPROVAL

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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