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Original article

Serum Levels of Sirtuin-1 in Patients with Lung Cancer and its Association with Karnofsky Performance Status

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Abstract. *Background:* Lung cancer is a major cause of cancer-related deaths worldwide. There are conflicting results regarding the role of sirtuin-1 in cancer. This study aimed to evaluate the serum sirtuin-1 levels in patients with lung cancer and its relationship with the quality of life based on Karnofsky performance statue scale (KPS). *Methods:* Serum sirtuin-1 levels were measured in 30 male patients with lung cancer and 50 healthy men. The two groups were matched for age. The difference between the serum levels of sirtuin-1 between the two groups and its relationship with KPS and other clinical parameters were evaluated. Data were analyzed by independent t-test and Pearson correlation, and $P < 0.05$ was considered as significant.
Results: Sirtuin-1 levels were significantly lower in the patients in comparison with healthy subjects ($P < 0.001$). There was also a significant relationship between the serum level of sirtuin-1 with KPS ($P < 0.001$, $r = 0.634$), arterial oxygen saturation ($P < 0.01$, $r = 0.470$), and smoking history ($P < 0.01$, $r = -0.330$). In addition, the serum sirtuin-1 levels were significantly lower in adenocarcinoma than that in squamous cell carcinoma or small cell lung cancer ($p < 0.001$ and $P < 0.05$, respectively). *Conclusion:* The serum levels of sirtuin-1 were lower in patients with lung cancer. In addition, there was a significant correlation between serum levels of sirtuin-1 and KPS, O2 saturation, and smoking history. Further serological and histological studies seem to be necessary due to the existence of conflicting reports regarding sirtuin-1.

Keywords: Lung cancer, Sirtuin-1, Quality of life, Karnofsky performance status.

Background and aim

Lung cancer is one of the major causes of cancer-related deaths worldwide (1). Incidence and mortality in lung cancer are closely linked to the smoking pattern (2). Some of the most common symptoms of lung cancer include cough, hemoptysis, dyspnea, weight loss, and anorexia (3). Lung cancer is generally divided into two types, small and non-small cell type (4). Histological adenocarcinoma is a very common form of lung cancer Incidence and mortality in lung cancer are closely linked to the smoking pattern (4), while squamous cell carcinoma is the common histologic type in Iran (5). Genetic changes associated with lung adenocarcinoma are predominantly KARAS activating mutation and epidermal growth factor receptor (EGFR) (6). There are many risk factors for lung cancer, including smoking, domestic biomass fuels, occupational exposures, ambient air pollution, diet and nutrition, genetic factors, chronic obstructive pulmonary disease (COPD), and other pulmonary conditions (4).

Sirtuins are a nicotinamide adenine dinucleotide-dependent lysine deacetylase that plays a key role in physiological processes and diseases (7). Sirtuin-1 has

been widely studied because of its effects on lifespan extension (8). In cancer, sirtuin-1 has been shown to play different and sometimes contradictory roles (7). Conflicting evidence has revealed that sirtuin-1 acts as a tumor suppressor or tumor promoter (9). Some studies have demonstrated that sirtuin-1 helps repair damaged DNA by affecting the activity of proteins and genes involved in tumor suppressors, such as p53, GATA, and CDH1 (the E-cadherin gene) (10). Some human studies have found that high levels of sirtuin-1 are found in prostate cancer, skin cancer, and acute myeloid leukemia (11-13). On the other hand, new studies have shown a tumor-suppressive role for sirtuin-1. Decreased levels of sirtuin-1 have been reported to be evident in cancers such as prostate cancer, bladder cancer, ovarian cancer, and glioblastoma (10, 14).

The present study aimed to evaluate the serum levels of sirtuin-1 in patients with lung cancer and compare the values with healthy controls. The association between serum levels of sirtuin-1 and quality of life were also assessed in cancer patients based on the Karnofsky performance status scale (KPS).

Materials and Methods

Participants

Thirty male patients with lung cancer were enrolled in this prospective case-control study. The patients were recruited from the pulmonary clinic of Ardabil Imam Khomeini Educational Hospital from Sep. 2018 to Sep. 2019. The inclusion criteria were having cough, chronic dyspnea, and lung mass on a spiral chest CT scan. The diagnosis of cancer was made based on the World Health Organization (WHO) morphological criteria reported by an experienced pathologist after fiber optic bronchoscopic endo-bronchial specimen biopsy. Pathologic reports were histologically classified into adenocarcinoma, squamous cell carcinoma (SCC), and small cell lung cancer (SCLC). Moreover, 50 healthy participants were included as the control group. These participants were matched with the patients in terms of age. Patients with a history of heart failure, diabetes, other malignancies, and renal failure (creatinine > 1.4) were excluded from the study. At the beginning of the study, written informed consent was obtained from all the participants. Demographic information, history of smoking, and spirometry findings from all the participants as well as the dyspnea scale (MMRC), arterial blood oxygen saturation (SpO2), and quality of life with KPS scale were collected from each group. The study protocol was approved by the Ethics Committee of Ardabil University of Medical Sciences, Ardabil, Iran (No. IR.ARUMS.REC.1397.257).

Biochemical measurements

Peripheral blood samples (3-5 mL) were collected in tubes to determine the serum levels of sirtuin-1. Serum sirtuin-1 concentrations were measured using a commercial kit (Crystal day, China) and the electrochemiluminescent method with an Elecsys 2010 Automated Analyzer (Roche Diagnostics). The results are presented as ng/mL.

Statistical Analysis

The results are presented as mean ± standard deviation (SD). Continuous variables were compared using Student's t-test. A comparison between groups was made by ANOVA with the Tukey Kemar post hoc test. Correlation coefficients were assessed using Pearson's correlation test. A value of $P < 0.05$ was considered significant. SPSS version 16.0 and Graph Pad Prism 5software were used for statistical analysis.

Results

Baseline Characteristics of the Sample

A sample of 80, comprising 30 patients with lung cancer and 50 controls, was recruited. The mean age of the group with lung cancer was 66.87 ± 11.73 years, and that of the control group was 63.98 ± 5.70 years ($P = 0.215$) (Table 1). Results showed that BMI values were significantly higher in the control group (27.16 ± 3.44) compared to the group with lung cancer (24.97 ± 3.87, $P < 0.05$).

Analysis of the pulmonary function test revealed that FEV1 values were significantly lower in the group with lung cancer (62.90 ± 21.05) than the control group (90.72 ± 9.27, $P < 0.001$). In addition, FEV1 / FVC values were significantly lower in the group with

Table 1: Baseline characteristics of patients with lung cancer and control subjects

Parameters	Control group	Lung cancer group	P-value
Mean age (year)	63.98 ± 5.70	66.87 ± 11.73	0.215
Body mass index (kg/m^2)	27.16 ± 3.44	24.97 ± 3.87	0.010
Pulmonary function test:			
FEV1 (% of predicted)	90.72 ± 9.27	62.90 ± 21.05	0.000
FVC (% of predicted)	85.66 ± 8.88	80.33 ± 27.33	0.204
FEV1/FVC	86.34 ± 4.23	62.86 ± 11.95	0.000

lung cancer (62.86 ± 11.95) than the control group (86.34 ± 4.23, P < 0.001) (Table 1).

The serum sirtuin-1 levels were significantly lower in lung cancer patients (9.31 ± 2.99) than the control group (12.26 ± 2.68, P < 0.001) (Fig. 1a). In addition, the serum sirtuin-1 levels remained significantly different after adjustment for age and BMI (P < 0.001, Fig. 1b).

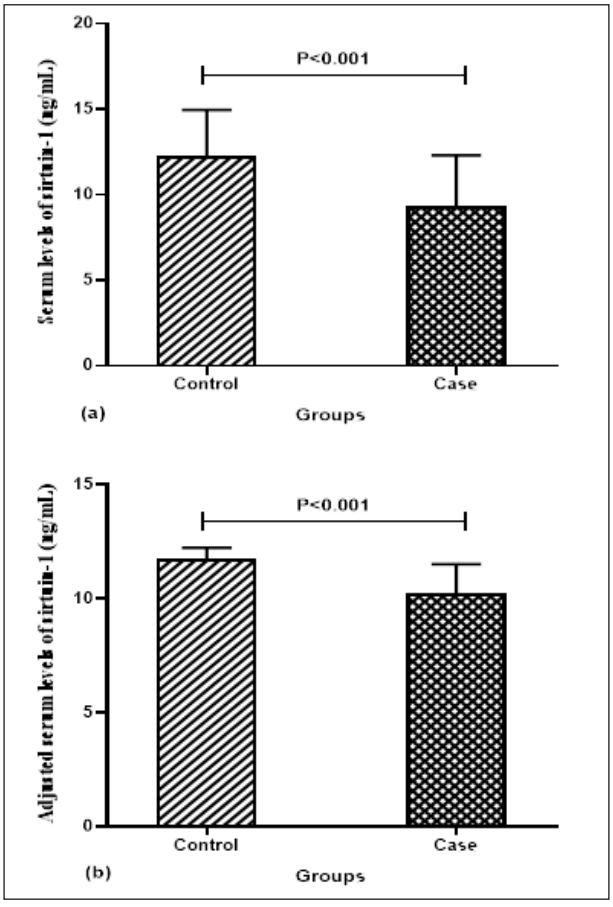

Figure 1: The mean ± SD of serum levels of (a): Sirtuin-1 and (b): adjusted Sirtuin-1 for the age and BMI in the study groups.

Pathologic results revealed that adenocarcinoma was observed in 5 (16.66%), SCC in 14 (46.67%), and SCLC in 11 (36.67%) patients. The serum levels of sirtuin-1 in patients based on pathological results are presented in Figure 2. Evidently, in patients with adenocarcinoma, serum levels of sirtuin-1 were significantly lower than those of the SCC and SCLC groups (P < 0.05 and P < 0.001, respectively). There was no significant difference between the SCC and SCLC groups with respect to the serum levels of sirtuin-1 (Figure 2).

Based on KPS, the serum levels of sirtuin-1 in the group with <60 showed a significant decrease compared to 61-80 and 81-100 groups (P < 0.01 and P < 0.001, respectively) (Figure 3).

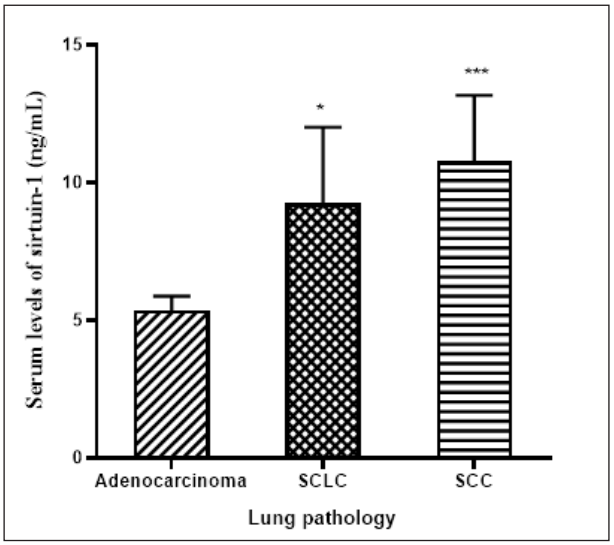

Figure 2: The mean ± SD of serum levels of Sirtuin-1 in the study groups based on pathological diagnosis. SCLC: small-cell lung cancer, SCC: squamous cell carcinoma. Adenocarcinoma Vs. other groups: *; P <0.05, ***; P <0.001.

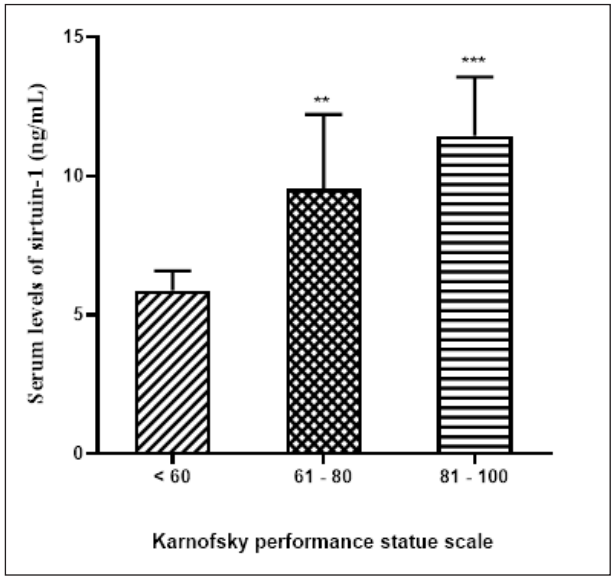

Figure 3: The mean ± SD of serum levels of Sirtuin-1 in the study groups based on karnofsky performance statue scale.

Relationship between Serum Levels of Sirtuin-1 and the Study of Parameters

The results showed a significant correlation between sirtuin-1 levels and KPS (r = 0.634, $P < 0.001$; Fig. 4a), O_2 saturation (r = 0.470, $P < 0.01$; Fig. 4b), and smoking history (r = -0.330, $P < 0.01$; Fig. c). On the other hand, there was no association between sirtuin-1 and FEV1, FVC, BMI, and MMRC (Table 2).

Table 2: Pearson correlation analysis of study parameters with Sirtuin-1

	Sirtuin-1	
	r	**P value**
Age	-0.078	0.680
BMI	-0.105	0.582
FEV1 (% predicted)	0.076	0.688
FVC (% predicted)	0.257	0.171
FEV1/FVC	-0.344	0.063
mMRC	-0.021	0.913

FEV1: forced expiratory volume in 1 second, FVC: forced volume capacity, mMRC: modified medical research council.

Discussion

The results of the present study revealed that the serum levels of sirtuin-1 were lower in patients with lung cancer than the healthy controls. In addition, based on pathological results, the decrease in serum sirtuin-1 level was more pronounced in patients with adenocarcinoma compared to other groups. In addition, there was a significant positive association between the KPS and the serum sirtuin-1 levels.

There is already much controversy about the role of sirtuin-1 in cancer. Overexpression of sirtuin-1 has been reported in some types of human cancers such as acute myeloid leukemia, prostate cancer, and non-melanoma skin cancer (11, 12). These findings suggest a tumorigenic role for sirtuin-1. On the other hand, recent studies have reported reduced levels of sirtuin-1 in some cancers, such as breast cancer, bladder cancer, glioblastoma, ovarian cancer, and prostate cancer (14). Indeed, the results of these observations indicate the probable role of tumor suppressor for sirtuin-1. We found that serum levels of sirtuin-1 were significantly decreased in patients with lung cancer compared to healthy controls. Although these results did not indi-

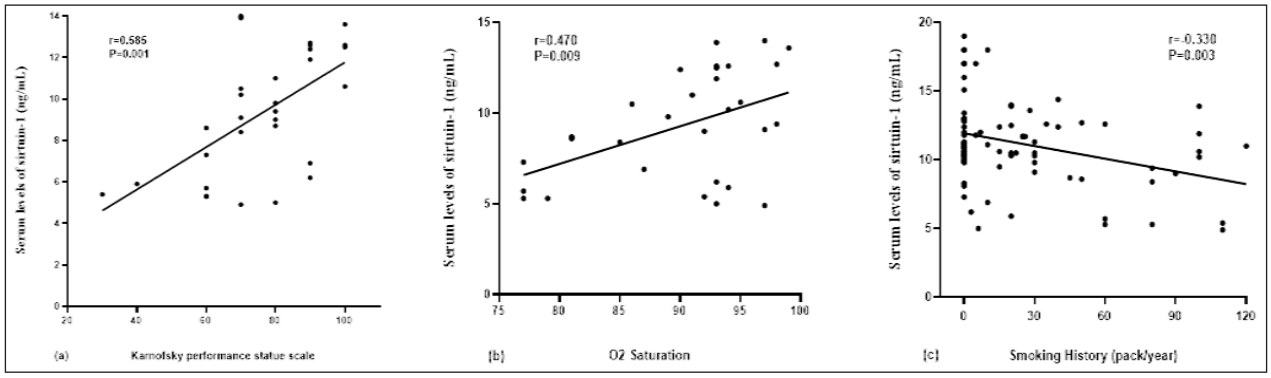

Figure 4: Pearson correlation analysis of (a): karnofsky performance status scale and Sirtuin-1 serum levels, (b): O_2 saturation and Sirtuin-1 serum levels, and (c): smoking history and Sirtuin-1 serum levels.

cate the potential role of sirtuin-1 in lung cancer, it may, at least in part, be indicative of its role in lung cancer.

Contradictory results regarding sirtuin-1 in various cancers and studies may be due to several factors. Studies on tumor cell lines and tissues have reported that increased expression of sirtuin-1 results in the inactivation of tumor suppressor proteins (15). This can cause angiogenesis, cell growth, and resistance to chemotherapy. Nevertheless, studies on transgenic mice have shown the role of tumor suppressor for sirtuin-1. Findings from sirtuin-1 transgenic mice have demonstrated that mice were resistant to liver cancer following reduced inflammation and DNA damage (16). The development of tumors such as lymphomas, carcinomas, sarcomas, and teratoma has also been reported in sirtuin-deficient mice (14). Interestingly, to date, no transgenic mice have shown evidence of the oncogenic role of sirtuin-1.

Another factor influencing the contradictory results of sirtuin-1 in cancer may be related to the different functions of sirtuin-1 in different tissues. On this basis, it has been shown that increased sirtuin-1 expression is coincident with poor clinical outcomes in gastric cancer, whereas in ovarian cancer, it is associated with better survival of patients (17, 18). Therefore, it seems that sirtuin-1 is involved in different signaling pathways in carcinogenesis.

The exact mechanism of sirtuin-1 in exerting inhibitory effects on cancer progression is unclear. In a study by Latifkar et al., they showed a link between sirtuin-1 and lysosomes' function in breast cancer, concluding that reduction of sirtuin-1 levels results in the production of a secretome series containing exosomes and lysosomal hydrolase that affect cell survival (19). In lung cancer, inhibition of sirtuin-1 expression in a manner dependent on SUMOyaltion causes cancer metastasis in the hypoxic condition (20). In a study by Sun et al., it was reported that under hypoxic conditions, various factors such as NF-ϰB and hypoxic-inducible factor-1α (HIF-1α) are activated and can affect cancer metastasis. Sirtuin-1 has been shown to affect lung cancer metastasis by modulating these factors (20). Consistent with these findings, it has been shown in ovarian cancer that hypoxia results in epithelial-mesenchymal transition (EMT) as a result of decreased expression of sirtuin-1 (21). The accumulation of proinflammatory factors in lung cancer metastasis has also been reported. The anti-inflammatory effects of sirtuin-1 have been identified in many studies (22). Therefore, it is thought that by reducing sirtuin-1, a variety of pathways such as NF-kB and activator protein 1 (AP-1) are activated and can be effective in accumulating inflammatory factors and exacerbating lung cancer status (23, 24). In addition, in breast cancer, sirtuin-1 can modulate EMT by suppressing the transforming growth factor beta (TGF-β) pathway through deacetylating Smad4 (25).

We also showed a significant positive correlation between the KPS and serum levels of sirtuin-1 in patients with lung cancer. Some studies report an increased level of sirtuin-1 in the tissue sample of patients with lung cancer (26). Although our results were not consistent with findings from previous studies on the association of KPS with serum levels of sirtuin-1 (27, 28), this may be due to differences in sirtuin-1 levels in cancerous tissue and serum samples, a point which requires further research.

The results of the pulmonary function test (PFT) revealed that FEV1 and FEV1/FVC values in patients with lung cancer were significantly lower than in healthy control groups. The results of the PFT in the present study were consistent with the results of previous studies (22, 29). The findings also indicated that there was a significant positive correlation between O2 saturation and serum level of sirtuin-1 in patients with lung cancer. Previous studies have reported lower levels of sirtuin-1 protein in chronic diseases such as asthma and COPD compared to healthy controls (22). It has also been demonstrated that serum sirtuin-1 levels are highly correlated with airway obstruction and have a strong negative association with smoking, which suggests the possible role of oxidative stress in reducing sirtuin-1 serum levels (22). We did not investigate the possible role of oxidative stress and inflammatory factors with serum levels of sirtuin-1 in this study; still, it can be concluded that low serum levels of sirtuin-1 in patients with lung cancer and a significant negative association with smoking history, at least in part, reflects the possible role of inflammatory factors and oxidative stress in cancer condition, which merits further studies.

There was no similar study regarding the measurement of serum sirtuin-1 level in patients with lung cancer. Still, our study had some limitations. First, the subjects included were all male; further studies are required to assess the serum levels of sirtuin-1 in both sexes. Second, given the contradictory results in relation to the association of sirtuin-1 in patients with cancer, both serological and pathological examination of sirtuin-1 may provide a more accurate assessment. Finally, since a medium sample size was used, further studies with a larger sample size may be needed, especially regarding the association of sirtuin-1 with the pathological type of lung cancer.

Conclusion

In summary, the results of the present study revealed that the serum levels of sirtuin-1 were lower in patients with lung cancer compared to healthy controls. There was also a significant relationship between serum sirtuin-1 level with the quality of life based on KPS and O2 saturation in these patients. Since contradictory results on the role of sirtuin-1 in patients with cancer have been reported, and previous studies have mainly focused on changes in sirtuin-1 levels in cancer tissue samples, concurrent serological and pathological studies seem to be necessary.

Acknowledgment

This is a report of a database from the study entitled "Evaluation of serum levels of sirtuin-1 in lung cancer" registered in the Research Committee of Ardabil University of Medical Sciences.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article".

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