

Clinical importance of SUPAR and Dickkopf-1 level in the etiology of pleural effusion

SUPAR and Dickkopf-1 in pleural effusion

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Abstract

Aim: Two distinct biomarkers, the soluble form of the urokinase-type plasminogen activator receptor (SUPAR) and Dickkopf-1 (DKK-1), play an important role in the mechanisms of inflammation, infection and malignancy. The aim of this study was to evaluate the prognostic value of two different biomarkers in patients with a respiratory disease and investigate the association between combined multiple biomarkers reflecting different pathophysiological processes. **Material and Methods:** We used serum and pleural fluid samples taken from patients diagnosed with pleural effusion by physical and radiological examination. The potential role of DKK-1, a Wnt signalling pathway inhibitor, and SUPAR in pleural effusion occurring due to different etiologies has been examined.

Results: The data show that SUPAR, and DKK-1 levels increased significantly compared to the control groups. It has been shown that SuPAR levels were lower in the pleural fluids and serums of patients with tuberculosis compared to patients with parapneumonic effusions, and that serum SUPAR levels of patients with malignancies were higher compared to patients with tuberculosis and lower compared to parapneumonic patients. It was determined that there was no significant difference in DKK-1 levels in serum and pleural fluids between patients with malignancy, tuberculosis and parapneumonic effusion.

Discussion: Overlapping clinical presentations led to delayed diagnosis and treatment initiation. This results in unnecessary diagnostic procedures. As an indicator, the use of concomitant biomarkers can reverse this situation.

Keywords

SUPAR, Dickkopf-1, Pleural Effusion

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This study was approved by the Ethics Committee of Harran University (Date: 2021-04-26, No: 2021.09.03)

Introduction

Pleural effusion is a pathological condition known as the accumulation of fluid in the pleural space of the lung. The most common causes of this condition are congestive heart failure, pneumonia, pulmonary embolism and various types of cancer [1-3]. Studies show that the mortality rate in patients with non-malignant pleural effusion is between % 25-57 [4]. Pleural effusion, which is commonly seen in the presence of various disease states, has a wide range of differential diagnoses. Further evaluation of pleural effusion by distinguishing transudate and exudate using Light criteria helps determine diagnosis and treatment. However, these criteria are not always sufficient to make a diagnosis and determine the etiology. Therefore, invasive interventions are needed [2, 3]. This information shows that determining the etiology of pleural effusion is not easy.

Dickkopf-1 (DKK-1), which has been found as a new biomarker that causes endothelial cell damage by triggering the release of inflammatory cytokines, has been proven to be closely associated with some malignancies and chronic inflammatory diseases. This biomarker is defined as a secretory protein that can inhibit the Wnt signal transduction pathway [5]. It is well known that the Wnt signaling pathway plays an important role in embryogenesis, organogenesis, and homeostasis [6]. In particular, the discovery of new therapeutic drugs targeting the Wnt signaling pathway is considered promising agents for treating many diseases. DKK-1, defined as a secretory protein that can inhibit the Wnt signal transduction pathway, causes endothelial cell damage by triggering the secretion of inflammatory cytokines. Research shows that this biomarker is closely associated with various malignancies, chronic inflammatory diseases and neurological pathologies. It has been proven that DKK-1, which serves as a new biomarker that causes endothelial cell damage by supporting the release of inflammatory cytokines, is closely associated with various malignancies, chronic inflammatory diseases and neurological pathologies [7-10].

Soluble urokinase plasminogen activator receptor (SUPAR), defined as a different and new inflammatory biomarker, is found at high levels in plasma, serum and urine samples of patients with pneumonia, sepsis, tuberculosis and various cancers. Recent studies have shown that SUPAR levels are also high in pleural fluid [11-15].

As mentioned above, determining the etiology of pleural effusion is not easy or sufficient with current methods. The use of two effective biomarkers such as SuPAR, and DKK-1 to determine the etiology of pleural effusion without the need for invasive interventions is of great importance for scientific research. For this purpose, we aimed to investigate whether changes in serum and pleural fluid SUPAR, and DKK-1 levels can yield the evaluation of the diagnosis of pleural effusion and to evaluate the relationship between pleural effusion conditions that occur due to different etiological reasons and SUPAR, and DKK-1 levels in our study. We sought to examine the prognostic value of two distinct biomarkers in patients with PE and to evaluate combined multiple biomarkers reflecting different pathophysiological processes.

Material and Methods

Study population and experimental design

Pleural fluid obtained by thoracentesis from patients over the age of 18 and malignancy, tuberculosis, and parapneumonic effusion with pleural effusion detected by physical examination and radiological findings and venous blood taken simultaneously between August 2021-March 2022. Pleural fluid samples were characterized as exudate or transudate using Light's criteria. The samples obtained were divided into 4 groups: control, malignancy, tuberculosis and parapneumonic effusion.

Measurement of serum and pleural fluid DKK-1 and SuPAR Level

DKK-1 (Human Dickkopf-like Protein 1, Elisa Kit, SunRedBio, Shanghai, Chinese) and SUPAR (Human suPAR Elisa Kit, SunRedBio, Shanghai, Chinese) levels in serum and pleural fluid samples taken from the participants were determined using ELISA kits in accordance with the manufacturer's instructions. Data analysis results in serum and pleural fluid are given in units of ng/ml or pg/ml.

Statistical analysis

Results are presented as mean \pm standard deviation. Statistical analysis of the results was performed using Student's t-test for comparison of pairs and between group comparisons of qualitative and quantitative variables were performed by using the Fisher exact test and Mann-Whitney U-test, respectively. One-way ANOVA was used for parametric variables and Kruskal-Wallis analysis for nonparametric variables (GraphPad Prism 8.0, USA). The p-value is considered statistically significant when it is less than 0.05.

Ethical Approval

This study was approved by the Ethics Committee of Harran University (Date: 2021-04-26, No: 2021.09.03).

Results

SUPAR levels of serum and pleural fluid

It was determined that SUPAR levels in serum samples of patients with malignancy, tuberculosis and parapneumonic effusion were significantly increased compared to control groups (Table 1). At the same time, the study showed that the serum SUPAR levels of patients with malignancy were higher compared to patients with tuberculosis, while the serum SUPAR levels of patients with parapneumonic effusion were found to be significantly higher than those of patients with malignancy and tuberculosis (Figure 1a). It was determined that SUPAR levels in the pleural fluids of patients with malignancy, tuberculosis and parapneumonic effusion increased significantly compared to control groups. At the same time, the study showed that the SUPAR levels in the pleural fluids of patients with tuberculosis and parapneumonic effusion did not change compared to patients with malignancy, while the SUPAR levels in the pleural fluids of patients with parapneumonic effusion were found to be significantly higher compared to patients with tuberculosis (Figure 1b).

DKK-1 levels of serum and pleural fluid

It was determined that DKK-1 levels in the serum samples of patients with malignancy and tuberculosis did not change compared to the control groups, but DKK-1 levels in the serum

Table 1. Baseline characteristics of the samples and of the patients.

Parameter	Control n=8	Malignancy n=11	Tuberculosis n=11	Parapneumonic Effusion n=11	P value	
SuPAR	Serum	6,00±0,15	12,90±2,96*	10,13±2,17*,#	15,12±3,20*,#,\$	<0,05
	pleural fluid	6,32±0,40	26,34±4,39	24,46±3,53*	32,45±6,46*,\$	<0,05
	serum/pleural fluid ratio	0,95±0,04	0,50±0,13*	0,43±0,12*	0,47±0,08*	<0,05
DKK-1	Serum	335,97±59,37	837,39±586,89	579,99±364,90	782,36±356,30*	<0,05
	pleural fluid	367,20±57,15	841,64±477,28*	729,45±448,48*	1313,08±1103,84*	<0,05
	serum/pleural fluid ratio	0,95±0,30	1,23±1,07	0,87±0,42	0,79±0,45	<0,05

*p<0.05 indicates a statistically significant difference compared to the control group, #p<0.05 indicates a statistically significant difference compared to the malignancy group, and \$p<0.05 indicates a statistically significant difference compared to the tuberculosis group

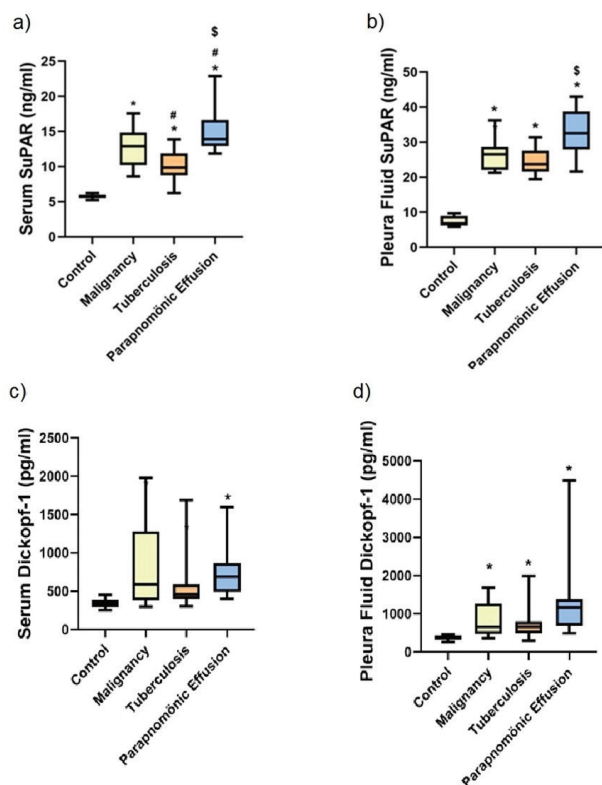


Figure 1. Box-plots showing comparison of serum (a) and pleural fluid (b) SUPAR levels and serum (c) and pleural fluid (d) Dickkopf-1 levels from clinical specimens

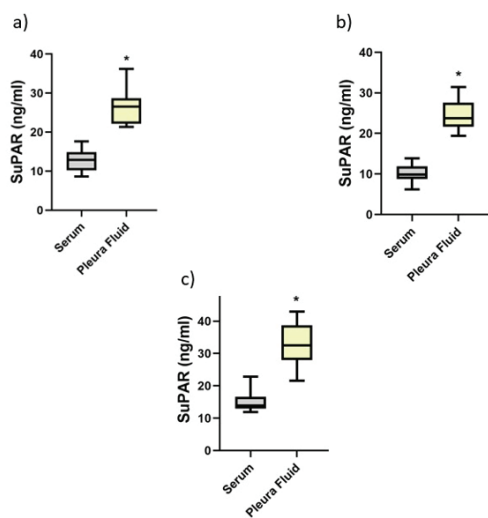


Figure 2. Comparison of serum and pleura fluid SUPAR levels from clinical samples with malignancy (a), tuberculosis (b) and parapneumonic effusion (c)

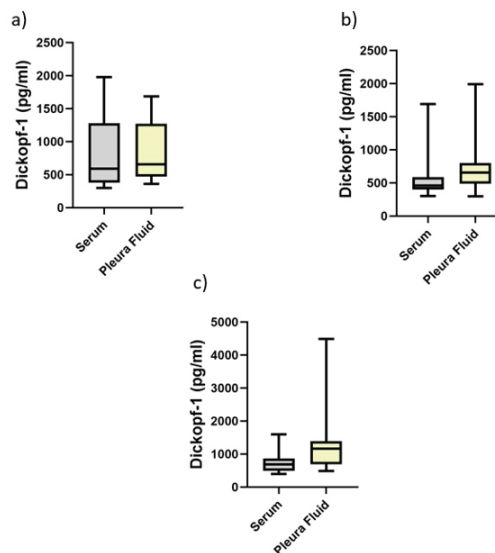


Figure 3. Comparison of serum and pleura fluid DKK-1 levels from clinical samples with malignancy (a), tuberculosis (b) and parapneumonic effusion (c)

samples of patients with parapneumonic effusion increased significantly compared to the control groups. However, the study found that there was no significant difference in serum DKK-1 levels between patients with malignancy, tuberculosis and parapneumonic effusion. (Figure 1c). It was determined that DKK-1 levels in the pleural fluid of patients with malignancy, tuberculosis and parapneumonic effusion were significantly increased compared to control groups. However, the study found that there was no significant difference DKK-1 levels in pleura fluid between patients with malignancy, tuberculosis and parapneumonic effusion. (Figure 1d).

SuPAR levels of patients with malignancy, tuberculosis and parapneumonic effusion in the pleural fluids and serum samples SUPAR levels in the pleural fluids of patients with malignancy, tuberculosis and parapneumonic effusion were found to be significantly higher than the SUPAR levels in serum samples (Figure 2).

DKK-1 levels of patients with malignancy, tuberculosis and parapneumonic effusion in the pleural fluids and serum samples DKK-1 levels in the patients with malignancy, tuberculosis and parapneumonic effusion were not significantly changed between serum and pleura fluid samples (Figure 3).

Discussion

The pathogenesis of respiratory disorders is multifactorial. Many pathophysiological alterations have been described in inflammation, infection and malignancy. Various clinical

conditions result in pleural effusions, which is associated with increased morbidity and mortality. Pleural effusion is a pathological condition known as the accumulation of fluid in the pleural space of the lung [1]. The most common causes of this condition are congestive heart failure, pneumonia, pulmonary embolism and various types of cancer. Further evaluation of pleural effusion by distinguishing transudate and exudate using criteria helps determine diagnosis and treatment. However, these criteria are not always sufficient to make a diagnosis and determine the etiology. In addition, invasive interventions are needed [2, 3]. As a biological response, the effects of biomarkers are observable and quantifiable biological changes in cellular components, processes, structures, or functions within the development and progression of diseases. They, therefore, can be assessed as indicators of a physiological or pathological biological process. In various human body fluid samples, biomarker effects can be objectively measured. Some biomarker tests have been evaluated for lung diseases. Multiple uses of biomarkers can contribute significant additive effects to give rise to diagnosis and treatment. We examined whether changes in serum and pleural fluid SUPAR, and DKK-1 levels could yield the evaluation of the diagnosis of pleural effusion and the relationship between pleural effusion conditions that occur due to different etiological reasons and SUPAR, and DKK-1 levels. It has been found that pleural SUPAR levels of patients with malignant pleural effusion are increased in the literature [16-18]. Similarly, it was found that SUPAR levels increased in the pleural fluid of patients with parapneumonic effusion [19]. Additionally, a recent study showed that serum SUPAR levels are associated with the severity of pneumonia and mortality rates [20-24]. Consistent with literature studies, in our study, it was determined that SUPAR levels in the pleural fluids of patients with malignancy, tuberculosis and parapneumonic effusion were significantly higher than the SUPAR levels in serum samples.

However, SUPAR levels in serum samples of patients with malignancy, tuberculosis and parapneumonic effusion were found to be significantly increased compared to control groups. At the same time, the study showed that the serum SUPAR levels of patients with malignancy were higher compared to patients with tuberculosis, while the serum SUPAR levels of patients with parapneumonic effusion were found to be significantly higher than those of patients with malignancy and tuberculosis. Similarly, SUPAR levels in the pleural fluids of patients with malignancy, tuberculosis and parapneumonic effusion were found to be significantly increased compared to control groups. At the same time, the study showed that the SUPAR levels in the pleural fluids of patients with tuberculosis and parapneumonic effusion did not change compared to patients with malignancy, while the SUPAR levels in the pleural fluids of patients with parapneumonic effusion were found to be significantly higher compared to patients with tuberculosis.

It was determined that DKK-1 levels in the serum samples of patients with malignancy and tuberculosis did not change compared to the control groups, but DKK-1 levels in the serum samples of patients with parapneumonic effusion increased significantly compared to the control groups. However, the study found that there was no significant difference in serum DKK-

1 levels between patients with malignancy, tuberculosis and parapneumonic effusion. It was determined that DKK-1 levels in the pleural fluid of patients with malignancy, tuberculosis and parapneumonic effusion were significantly increased compared to control groups. However, the study found that there was no significant difference DKK-1 levels in pleura fluid between patients with malignancy, tuberculosis and parapneumonic effusion.

The results of our study will contribute to the literature in terms of identifying new biomarkers called SuPAR, and DKK-1 in determining the causes of fluid in the pleural space and providing a different perspective on the treatment course of the disease.

Conclusion

Considering that biomarkers are not specific and common in predicting diseases, we proposed that the use of biomarker combinations can lead to better clinical diagnostic criteria for pulmonary diseases. Whether more than one biomarker is connected to pulmonary diseases independently is unknown. Due to the correlation between many biomarker levels, measuring one biomarker may not always replace the relationship of another biomarker with respiratory disease. Our data indicates that employing multiple biomarkers may result in a more precise assessment of individual biomarker associations. According to our findings, more investigation is needed to fully explore both SUPAR, and DKK-1's potential as a respiratory illness therapeutic agent. The study cannot be interpreted to suggest a direct benefit of combining biomarkers.

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Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and Human Rights Statement

All procedures performed in this study 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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Conflict of Interest

The authors declare that there is no conflict of interest.

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