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RESEARCH

The role of interleukin-6 gene in distinction of transudate-exudate in pleural effusions

Plevral efüzyonlarda transude ile eksuda ayrımında interlökin-6 geninin rolü

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Abstract

Purpose: This study aims to appraise *IL-6* gene expression in pleural fluid samples and establish a connection between expression levels and promoter polymorphisms for more accurate pleural effusion classification.

Materials and Methods: A total of 38 adult patients (transudate (19) and exudate (19)) with pleural fluid and 33 healthy controls were included in the study. For the *IL-6* gene expression study, RNA was isolated from transudate and exudate pleural fluids, and expression levels were compared between the two groups. Then, -174G>C (rs1800795), -572G>C (rs1800796) and, -597G>A (rs1800797) polymorphisms were analyzed using LightCycler® 480 II with real-time polymerase chain reaction from genomic DNA of controls and patients.

Results: *IL-6* levels were 7.13-fold more expressed in the exudate group than in the transudate group. No significant difference was found between the transudate-exudate groups in terms of polymorphisms. However, when we compared the transudate-exudate patient groups with the control groups, -174 G>C polymorphism and -597 G>A polymorphism were statistically significant.

Conclusion: Pleural effusion treatment initiates with fluid characterization. In challenging cases, the current parameters are inadequate. Our findings indicate that *IL-6* is a robust biomarker, independently distinguishing exudative and transudative states, surpassing traditional criteria. *IL-6* shows promise for precise pleural effusion characterization, offering insights into pathophysiology and enabling targeted therapeutic interventions.

Keywords: *IL-6* , mRNA expression, pleural effusion, genetic polymorphism

Öz

Amaç: Bu çalışma, plevral sıvı örneklerinde *IL-6* gen ekspresyonunu değerlendirmeyi ve ekspresyon düzeyleri ile promotör polimorfizmleri arasındaki ilişkiyi inceleyerek plevral efüzyon sınıflandırmasında daha doğru sonuçlar elde etmeyi amaçlamaktadır.

Gereç ve Yöntem: Çalışmaya plevral sıvısı bulunan toplam 38 yetişkin hasta (19 transüda, 19 eksüda) ve 33 sağlıklı kontrol grubu dahil edildi. *IL-6* gen ekspresyonu çalışması için, transüda ve eksüda plevral sıvılarından RNA izole edildi ve ekspresyon seviyeleri iki grup arasında karşılaştırıldı. Ardından, kontrol ve hasta gruplarının genomik DNA'larından LightCycler 480 II real-time polimeraz zincir reaksiyonu kullanılarak -174G>C (rs1800795), -572G>C (rs1800796) ve -597G>A (rs1800797) polimorfizmleri analiz edildi.

Bulgular: Eksüda grubunda *IL-6* düzeyleri, transüda grubuna göre 7,13 kat daha fazla ifade edildi. Transüda-eksüda grupları arasında polimorfizmler açısından anlamlı bir fark bulunamadı. Ancak transüda-eksüda hasta grupları kontrol grupları ile karşılaştırıldığında, -174G>C polimorfizmi ve -597G>A polimorfizmi istatistiksel olarak anlamlıydı.

Sonuç: Plevral efüzyon tedavisi sıvı karakterizasyonu ile başlar. Zorlu vakalarda, mevcut parametreler yetersizdir. Bulgularımız, geleneksel kriterlerin ötesine geçerek eksüdatif ve transüdatif durumları bağımsız olarak ayırt eden güçlü bir biyobelirteç olarak *IL-6* 'yı işaret etmektedir. *IL-6* , plevral efüzyonun hassas karakterizasyonu için umut vermekte olup, patofizyoloji hakkında bilgi sağlayarak hedeflenen tedavi yaklaşımlarına olanak tanımaktadır.

Anahtar kelimeler: *IL-6* , mRNA ekspresyonu, plevral efüzyon, genetik polimorfizm

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INTRODUCTION

Although the pleura is considered only a potential space between the lung and chest wall, it plays a critical role in respiratory physiology. Pleura comprises the visceral and parietal pleura. Visceral pleura envelops the lung parenchyma and interlobar fissures. The parietal pleura surrounds the inner surface of the chest wall, mediastinum, and diaphragm[1].

The concentration and volume of the pleural fluid in the lungs are stable. However, pleural fluids occur when there is an extreme change in body homeostasis. This situation shows that there is a good control system in the fluid occurrence mechanism[2].

The potential gap where fluid presents is called the pleural cavity. Left and right pleural spaces are completely separated from each other by the mediastinum[3]. This pathological condition is a common problem in pulmonology, although the incidence varies according to etiology, 90% of all pleural effusions (PE) are associated with congestive heart failure, malignant processes, and pneumonia[4].

PE is one of the most important causes of pulmonary mortality and morbidity[5]. The main issues related to PEs are the distinction between exudates and transudates and the accurate determination of the effusion etiology[6]. The pleural fluid is classified into two groups as transudate and exudate according to Light criteria. Using the classical Light criteria while distinguishing transudates from exudates, it is useful to understand the pathogenic mechanisms that result in PE[7,8]. Understanding the correct pathogenic mechanisms plays an important role in the characterization of effusion. Determination of effusion type is the most important step in avoiding unnecessary surgical procedures.

After thoracentesis was defined in 1852, the separation of the pleural fluid as transudate-exudate began. Previously, the protein level of the pleural fluid above 3,0 g/dL was defined as exudate[9]. The underlying etiology must be well known to understand the physiopathology of effusion fluid[10]. Although it is not difficult to establish the etiological diagnosis in transudates, it is not possible to make a definite distinction in approximately 20% of patients with exudate fluid, despite the invasive and non-invasive tests performed to diagnose the exudate[11,12]. The literature review showed that many potential biomarkers have been studied as alternatives to the Light criteria. However, most of these studies have focused on enzymatic activity level or enzyme concentration[13].

In some cases, although the etiology of the disease is the same, the characteristics of the fluid appear to be different. The response of *IL-6* produced by monocytes and macrophages in the pleura varies according to the etiology of the disease and plays an important role in the classification of effusions[14]. In the literature, only *IL-6* levels of serum and effusion fluid have been investigated in PEs and compared with the etiology of diseases[15]. The role of cytokine cells in the differentiation of PEs has also been shown in some studies in recent years[16]. Cytokines secreted by cells are relatively low molecular weight proteins in response to a variety of different stimuli and act as mediators of the organism to various infections and inflammatory and immunological stimuli[17].

Pleural effusion is a buildup of fluid in the space between the lungs and the chest wall. Various underlying conditions can cause this fluid, and differentiating between two main types of effusion, transudative and exudative, is crucial for proper diagnosis and treatment. Differentiating between transudative and exudative effusions traditionally relies on methods like the Light criteria, but these methods can have limitations. Researchers have implicated the *IL-6* gene in various inflammatory processes. In this study, we also suggest a potential role for *IL-6* expression in distinguishing between transudative and exudative effusions.

Determining the nature of the fluid is a crucial initial step to protect the patient from unnecessary surgical interventions. This study is noteworthy for being the first investigation into the interleukin-6 (*IL-6*) gene expression in pleural fluid. Particularly in ambiguous cases, it is demonstrated that *IL-6* can be utilized as a definitive diagnostic tool on its own. This underscores the significance of our research in offering valuable insights for precise differential diagnosis, especially in uncertain clinical scenarios.

This study aimed to investigate the expression of the *IL-6* gene in pleural effusions and assess its potential as a biomarker for differentiating transudative from exudative effusions. In this study, expression values of *IL-6* gene and promoter polymorphisms were analyzed from effusion fluids and blood samples of the patients. The effects of the results were investigated on transudate-exudate fluids. Also,

blood samples were obtained from both patients and healthy controls, and the promoter polymorphisms (-172G>C, -572G>C, -597G>A) of the *IL-6* gene were evaluated.

In the hypothesis of our study, we aimed to save patients from unnecessary surgical procedures and facilitate rapid diagnosis and treatment by determining the *IL-6* gene as a parameter in the differential diagnosis of transudate-exudate. The purpose of this study was to demonstrate that *IL-6* gene polymorphisms and expression contribute to determining the type of pleural fluid (transudate or exudate) in patients with pleural effusion. Our findings revealed that the expression of the *IL-6* gene was significantly higher in exudate fluids, particularly in cases where the nature of the fluid was uncertain. Hence, this parameter alone could be adequate for determining the type of pleural fluid.

MATERIALS AND METHODS

Sample

In this study, patients who had not undergone a previous surgical procedure such as thoracentesis or tube thoracostomy and patients whose pleural fluid was drained by thoracentesis for the first time were included. Thirty-eight patients with pleural effusion (19 transudates, 19 exudates) who were admitted to the Thoracic Surgery Department at Erciyes University Faculty of Medicine with malignancy, chest pain, embolism, dyspnea or complaints of unknown cause were included in the study. Patients with comorbid mental disorders and chronic physical diseases (obesity, autoimmune disorders, endocrine disorders, etc.) were excluded. Healthy volunteers over 18 years of age without any chronic or acute physical illness were included as a control group. They voluntarily participated in the study and their confidentiality was protected. Informed consent was obtained from all participants accepted to the study and throughout the study, all patients were given protocol numbers and their confidentiality was taken into consideration.

Procedure

We obtained an ethics committee certificate for this study from the Erciyes University Ethics Committee with the meeting decision dated 27.05.2017 and numbered 2017/262. Erciyes University Ethics Committee approved this project according to the Declaration of Helsinki revised in 2000. In the differentiation of transudate-exudate of pleural fluid, Light criteria were used. Pleural fluid and 2ml blood samples with EDTA were obtained from 38 patients (19 exudates, 19 transudates) admitted to the Thoracic Surgery Department. 33 healthy volunteers without infection and systemic diseases were included as the control group. The setup of the study is as shown in the flow diagram below (figure 1); we used blood samples for *IL-6* promoter polymorphisms and pleural fluid for *IL-6* gene expression studies. PEs and blood samples were taken under sterile conditions and RNA and DNA were isolated in Medical Genetics Department at Erciyes University Faculty of Medicine.

rs1800795, rs1800796 and rs1800797 SNP genotyping

38 patients with pleural effusion and 33 healthy controls were included in the study. Total DNA was extracted from blood samples using High Pure PCR Template Preparation (Roche Diagnostics, Vienna, Austria) according to the manufacturer's instructions. Polymorphisms were compared in terms of the three strongest promoter polymorphisms of the *IL-6* gene (-172G>C, -572G>C, -597G>A) in patients with PE and healthy individuals. -172G>C (rs1800795), -572G>C (rs1800796), and -597G>A (rs1800797) promoter region genotypes were identified by using real-time LightCycler® FastStart DNA Master HybProbe (Roche Diagnostics, Vienna, Austria). These promoter changes were determined by fluorescent using specific probes that hybridized at the annealing step of the amplicon PCR cycle. For the -172G>C SNP, the melting temperature was 54.92 ^{o}C for the C allele and 61.76 ^{o}C for the G allele. For -572G>C SNP, the melting temperature was 63.59 ^{o}C for the C allele and 69.65 ^{o}C for the G allele. For -597G>A SNP, the melting temperature was 60.20 ^{o}C for the A allele and 50.75 ^{o}C for the G allele.

IL-6 gene expression

RNA was isolated from the pleural fluid. First, PEs were centrifuged at +4 ^{o}C at 2000 rpm for 15 minutes and the supernatant was discarded. RNAs were isolated by trizol method (GENEzol™ Reagent) by washing the pellet with 1% PBS (Phosphate Buffered Saline) and stored at -80 ^{o}C. All RNA samples were found to have an A260:A280 ratio between 1.8 and 2.0 with spectrophotometer (BioSpec-nano/Shimadzu) and a total RNA content of more than 100 ng/µl. High Fidelity cDNA Synthesis Kit

(Roche Diagnostics GmbH, Mannheim, Germany) was used for first-strand cDNA synthesis. The process was carried out in accordance with the manufacturer's instructions. cDNA was amplified for 10 minutes at 29 °C, 60 minutes at 48 °C, and 5 minutes at 85 °C. The quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analyses for *IL-6* and beta-actin (ACTB) genes were conducted using a Roche LightCycler 480 instrument. Real Time ready catalog assays, which are short FAM-labeled hydrolysis probes containing locked nucleic acid were used for RT-PCR reactions (Roche Diagnostics GmbH, Mannheim, Germany). Real Time ready Catalog (Roche Diagnostic GmbH, Mannheim, Germany) primer-probe kits for each gene were used in the study. Expression levels of genes were determined using relative quantification RT-PCR with a LightCycler 480 II system (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's instructions with a pre-incubation step at 95 °C for 10 minutes, followed by 45 cycles at 95 °C for 10 seconds, 60 °C for 30 seconds, and 72 °C for 1 second. Semiquantitative PCR reactions were run in duplicate. Beta-actin (ACTB) was selected as the reference gene for normalization purposes. The alterations in gene expression levels between the case and control groups were assessed through the $2^{-\Delta\Delta Ct}$ method of relative quantification.

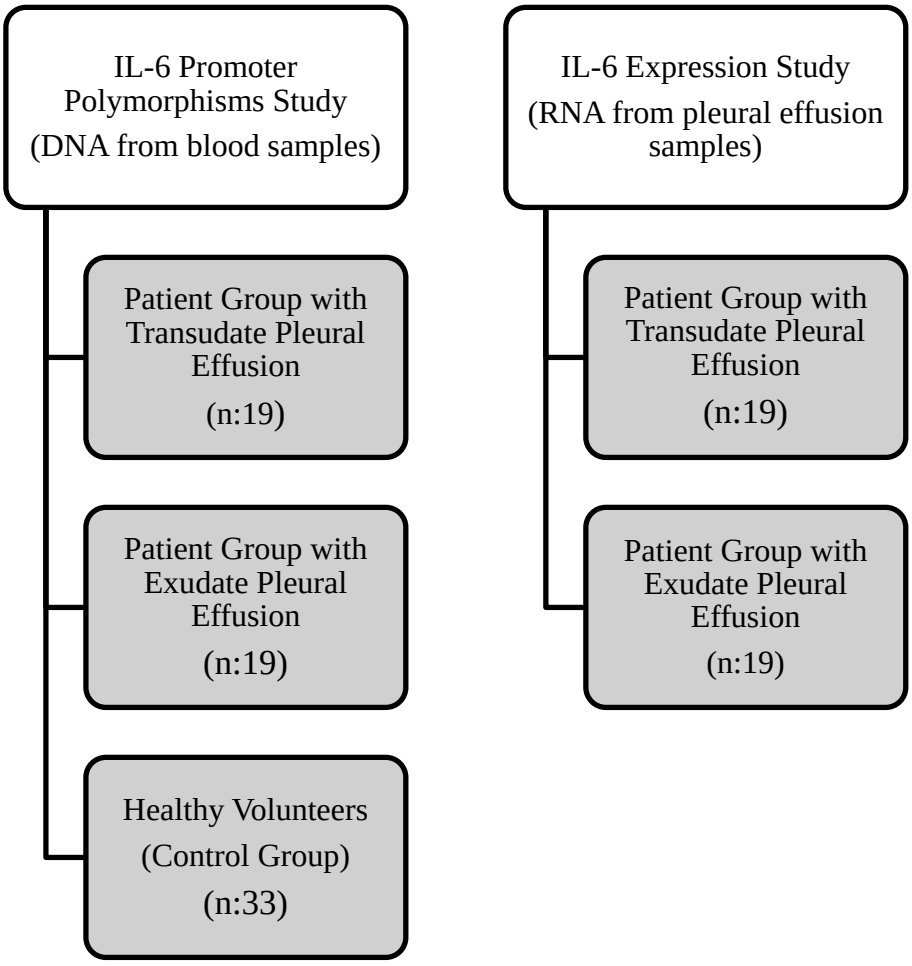

Figure 1. The flow diagram illustrates how we used the samples collected from patients and healthy volunteers included in the study in the method.

Statistical analysis

We used G*Power version 3.1.9.2 software to determine the necessary sample size. The analysis revealed that a sample size of 70 subjects was adequate to ascertain the significance of the correlation, with a Cohen's effect size of d= 0.350, α type I error of 5%, and a power of 80%. Data analysis was performed with SPSS® 16.0 for Windows Student Version software and GraphPad Prism 5.0 program. Histogram, Q-Q plots were examined and Shapiro-WILK's test was performed to assess the normality of the data. The outliers were excluded from the statistical tests. We used Pearson chi-squared analysis and the Mann-Whitney U test to compare genotype differences. The Kruskal-Wallis H test was used to compare expression levels between genotypes. Data were expressed as frequencies and percentages, median and interquartile range. Statistical significance was considered at $p<0.05$ (two-tailed).

RESULTS

In total of 71 subjects, 38 patients with pleural effusion and 33 healthy subjects were included in this study. The patient groups were 19 with transudates and 19 with exudates. The mean age of 27 males and 11 females in the pleural effusion group was 63.37±7.78 years, and the healthy group was 43.7±9.7 years. In our study, the prevalence of transudate was 54%, and exudate was 46% in male patients. In female patients, this ratio was 50% transudate and 50% exudate. There was no significant difference in fluid distribution between male and female patients. The high level of *IL-6* gene expression in the exudate group was found to be a positive correlation according to age ($p=0.0001$).

IL-6 promoter region rs1800795, rs1800796 and rs1800797 SNP Genotyping Results

DNAs were isolated from blood samples of patients with PE and healthy control group. Real-Time PCR was used to amplify target points which are the G>C (rs1800795) change at the -174 position of the *IL-6* gene, the G>C change at position -572 (rs1800796), and finally the G>A change at position -597 (rs1800796). The melting curve was used for analysis.

The rs1800795 and rs1800796 polymorphisms in the promoter region of the *IL-6* gene contained GG, GC, or CC genotypes. The rs1800797 polymorphism in the promoter region of the *IL-6* gene contained GG, GA, or AA genotypes. rs1800795, 1800796, and rs1800797 SNP genotypes and alleles distribution are given in Table 1. rs1800795 and rs1800797 polymorphisms were found statistically significant between the patient and control groups ($p<0.05$). The rs1800796 polymorphism was not statistically significant between the patient and the control groups.

Table 1. Genotype and allele frequencies of -174G>C (rs1800795), -572G>C (rs1800796), -597G>A (rs1800797) polymorphisms of *IL-6* gene between cases and controls

		dbSNP frequency* (%)	Controls n(%)	Transudate n(%)	Exudate n(%)	P value
-174G>C rs1800795	GG	77.6	20 (61)	13(68)	9(47)	0.01
	GC	18.6	13(39)	4(21)	4(21)	
	CC	0.48	0	2(11)	6(32)	
-572G>C rs1800796	GG	54.8	27 (82)	13(72)	15(83)	0.529
	GC	27.6	6(18)	6(33)	4(17)	
	CC	17.6	0	0	0	
-597G>A rs1800797	GG	77	29(88)	13(72)	11(61)	0.018
	GA	18.4	4(12)	4(22)	8(39)	
	AA	4.6	0	2(11)	0	

*1000 Genomes Project Phase 3 (http://www.ensembl.org/Homo_sapiens/Variation)

The frequencies of -174 G>C (rs1800795), -572 G>C (rs1800796) and -597 G>A (rs1800797) variants in the general population and in our study. General population data were obtained from the Ensembl Genome Browser database. Statistically significant value was accepted as $p<0.05$.

The frequency of C allele in -174G>C polymorphism was higher in our transudate-exudate patient group

compared to the control group. However, the frequency of A allele in the -597G>A polymorphism was higher in the patient group ($p<0.05$).

In our study, distinctions in *IL-6* expression levels between transudate and exudate pleural fluids were ascertained. In the mRNA expression analysis of the patient cohort, the relative quantification of *IL-6* and ACTB genes revealed a statistically significant elevation in the exudate group compared to patients with transudates ($p\leq0.001$) (Figure 2). Furthermore, when considering the mean age of the subjects, a statistically significant difference in expression values was observed, with exudate group expression levels being markedly higher than those of the transudate group ($p <0.001$).

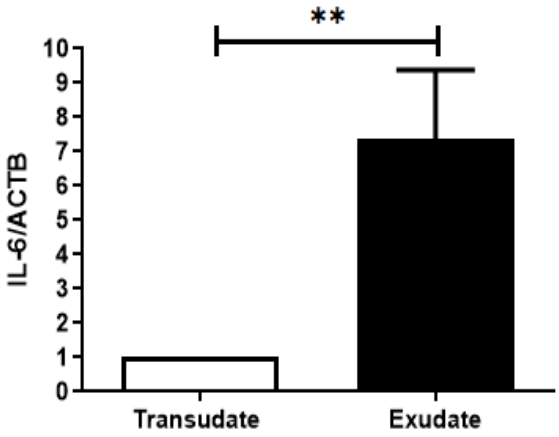

Figure 2. The *IL-6* mRNA expression comparison between transudate and exudate groups. Results of $2^{-\Delta\Delta}$ CT by relative quantification of *IL-6* gene with ACTB gene.

DISCUSSION

It is reported that approximately 1.5 million new PE cases are detected in the USA every year, resulting from more than 60 different disease processes[18]. PEs are caused by the imbalance between excessive pleural fluid development and pleural fluid absorption[19]. Heart failure, pneumonia and malignant neoplasm are the most common conditions that cause effusions[20].

While evaluating our study group's etiology statistically, we separated them as malignant (13/38, 34.2%) and non-malignant (25/38, 65.8%). The malignant group was lung cancer (7/13, 18.4%), breast cancer (2/13, 5.3%), liver cancer (2/13, 5.3%), stomach cancer (1/13, 2%), and prostate cancer (1/13, 2.6%). The non-malignant group was congestive heart failure (13/25, 34.2%), trauma (2/25, 5.3%), and patients with effusion who were admitted to the clinic because of various reasons (10/25, 26.3%).

Although Sertogulları et al. reported that gender is a significant factor in etiology, there was no significant difference regarding gender in many studies. In this study, no statistically significant difference was found between genders in both transudates and exudates [21]. A statistically significant difference was found between the mean age (63.37) and the distribution of the transudate and exudate ($p <0.0001$). According to this distribution, the prevalence of exudate was higher in the group above the mean age (n:16, 84.2%) than the transudate (n:3, 15.8%). This indicates that age plays an important role in the classification of transudate-exudate.

In a meta-analysis study of 1148 patients, Heffner et al. showed that Light criteria were highly sensitive in determining exudates. In rare cases, especially in patients with left ventricular failure due to congestive heart disease, although diuretic therapy has been applied, it has been determined that protein content in transudate fluid has increased and acted like pseudo exudate. This situation shows that Light

criteria have high sensitivity, but low specificity [22]. In other words, the pleural fluid that accumulates in traumas is not always considered as exudate or transudate in heart failure. In our study, there are also transudate-exudate differences in the pleural fluid with the expected etiology of the disease. This suggests that each patient has individual differences.

In particular, *IL-6* plays an important role in the initiation of the acute phase and other systemic responses during inflammation[23]. In this study, a statistically significant difference was found between *IL-6* gene expression data in transudate and exudate samples ($p \leq 0.001$). Exudates were expressed 7.13-fold more than transudes. According to these results, it has been shown that the *IL-6* gene can be used as a biomarker in transudate-exudate classification.

This work is the first study examining the differences in expression of the *IL-6* gene between the PEs as transudates and exudates, and the differences between promoter polymorphisms (-174G>C, -572G>C, -597G>A) in patients with effusion fluid and healthy controls. Additionally, promoter single nucleotide polymorphisms (SNPs) are important markers in demonstrating the expression of the gene functionally because of their effect on gene transcription. These polymorphisms have been used as a functional variant to investigate the role of high levels of *IL-6* in many common diseases, and this confirms the key role of *IL-6* in human health and disease.

Terry et al. reported that considering the overall effect of the -174G>C polymorphism in population-based studies, C allele would indicate a lower expression level than G allele, because haplotypes with the lowest expression level of -174G comprise about 5% of the population[24].

In a functional study, Chavez et al. confirmed the presence of SNP haplotypes in the *IL-6* promoter. In addition, the -174G>C polymorphism showed an important factor in reducing endotoxin-induced *IL-6* release. They reported that the polymorphisms at position -597 and -174 were associated with *IL-6* levels in endotoxin-stimulated cells and that polymorphism at position -572 did not affect *IL-6* levels in leukocytes[25]. They pointed out the importance of understanding the function of genetic variants before starting community-based association studies. While the study of Terry et al. confirms our findings, Chavez and Fishman reported a higher level of *IL-6* for G alleles.

Sharma et al. could not find -174G>C and -597G>A polymorphisms related to high *IL-6* levels and thrombotic risk[26].

In the study of Boeta-Lopez et al., as correlated with our study; in subjects with plasma *IL-6* levels compared, they found a significantly higher level of *IL-6* in patients with -597A allele than those with G allele[27]. In our study, a statistically significant difference was found control group and patient group for -597G>A polymorphism.

The study of Peng et al. reported that the -174G>C, -572G>C and -597G>A *IL-6* promoters could be a tumor marker for the treatment of cancer. They showed that -174G>C is associated with significantly higher cancer risk in Asians and Caucasians. In particular, they showed that -597G>A was significantly associated with increased risk of lung cancer. In addition, the presence of A allele was associated with significantly higher cancer risk in the Caucasus, but it was reported that the same situation was not the case in Asians[28].

In conclusion, in our study, it was shown that the expression level of *IL-6* is an important marker in transudate and exudate classification. In accordance with the literature, in our patients -174G>C and -597 G>A linkage disequilibrium was shown.

When the polymorphisms compared to the expression data; in accordance with the literature, in our study, it was seen that the expression levels were higher in the -174C and -597A alleles which were seen as mutant alleles.

In our polymorphism study, patients with transudate and exudate pleural effusion and healthy volunteers as a control group were compared in terms of polymorphisms and no statistically significant difference was found. However, when patients with effusion (both transudate and exudate) were compared with healthy volunteers, -174CC and -597AA were more common in the patient group, especially in the group with high *IL-6* gene expression values. One of the remarkable results of our study was that the *IL-6* gene expression level was high in two of our patients (they were evaluated as transudate by the clinic), whose classification according to the Light's criteria were challenging, and both patients had the mutant allele -174CC/-597AA genotype. However, this situation may not be remarkably accurate for correlation, since two different tissues were studied.

According to our findings, the higher frequency of -174CC and -597AA in the patient group shows the risk of pleural effusion. In addition, when the literature is analyzed, it is observed that the frequency of these alleles varies according to the population. Therefore, cohort studies are recommended for further evaluation

There were some limitations to our study. First, the heterogeneity of the patient groups limits our ability to obtain reliable results when comparing polymorphisms between transudate and exudate. The fact that not all potentially important promoter polymorphisms were studied is also a limitation. In order to make these data more reliable, more patients should be studied and worked with more specific groups of patients.

In conclusion, in our study, we found that the levels of the *IL-6* gene play a crucial role in classifying pleural effusion. The fact that the expression level was 7.13-fold higher in exudates demonstrated its role as a significant marker for interstitial fluids. However, although the fluid was transudate in two of our patients, *IL-6* gene expression data were higher than the transudate group, which strengthened this hypothesis. Nevertheless, in future studies, *IL-6* protein will accelerate the diagnosis, protect patients from unnecessary surgical procedures and be a target for neo-adjuvant treatments.

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REFERENCES

1. Zielinska-Krawczyk M, Krenke R, Grabczak EM, Light RW. Pleural manometry–historical background, rationale for use and methods of measurement. Respir Med. 2018;136:21-8.
2. Miserocchi G, Venturoli D, Negrini D, Del Fabbro M. Model of pleural fluid turnover. J Appl Physiol. 1993;75:1798-1806.
3. Porcel JM, Light RW. Pleural effusions. Dis Mon. 2013;59:29-57.
4. Maldonado F, Lentz RJ, Light RW. Diagnostic approach to pleural diseases: new tricks for an old trade. F1000Res. 2017;6:1135.
5. Krishna R, Rudrappa M. Pleural Effusion. Treasure Island, FL; StatPearls Publishing; 2018.
6. Feller-Kopman D, Light R. Pleural Disease N Engl J Med. 2018;378:740-51.
7. Rosse C, Gaddum-Rosse P. Hollinshead's Textbook of Anatomy. Baltimore, Lippincott Williams & Wilkins.; 1997.
8. Tse HTK, Gossett DR, Moon YS, et al. Quantitative Diagnosis of Malignant Pleural Effusions by Single-Cell Mechanophenotyping. Sci Transl Med. 2013;5:212ra163.
9. HEFFNER JE. Diagnosis and management of malignant pleural effusions. Respirology. 2007;13:5-20.
10. Bouros D, Pneumatikos I, Tzouvelekis A. Pleural ınvolvement in systemic autoimmune disorders. Respiration. 2008;75:361-71.
11. Karkhanis VS, Joshi JM. Pleural effusion: diagnosis, treatment, and management. Emerg Med. 2012;4:31-52.
12. Porcel JM, Light RW. Diagnostic approach to pleural effusion in adults. Am Fam Physician. 2006;73:1211-20.
13. Antas P, Borchert J, Ponte C, Lima J, Georg I, Bastos M et al. Interleukin-6 and -27 as potential novel biomarkers for human pleural tuberculosis regardless of the immunological status. Microbes Infect. 2024;26:105238.
14. Kumar H, Kawai T, Akira S. Pathogen Recognition by the Innate Immune System. Int Rev Immunol. 2011;30:16-34.
15. Zamzam MA, Abd El-Aziz AA, El Wahsh RA, Sonbol AA, Abu El Nour SM. Role of interleukin-6 in diagnosis of pleural effusion. Egypt J Chest Dis Tuberc. 2016;65:173-7.
16. Shu CC, Wang JY, Hsu CL et al. Diagnostic role of inflammatory and anti-inflammatory cytokines and effector molecules of cytotoxic T lymphocytes in tuberculous pleural effusion. Respirology. 2015;20:147-54.
17. Yensey Ç, Aktoğu S, Kalenc S, Onur F. Proinflammatory cytokines: are they usefull in differential diagnosis of pleural effusions? Ege Tıp Dergisi. 2006;45:19-24.
18. Yalcin NG, Choong CKC, Eizenberg N. Anatomy and pathophysiology of the pleura and pleural space. Thorac Surg Clin. 2013;23:1-10.
19. Diaz-Guzman E, Dweik RA. Diagnosis and management of pleural effusions: a practical approach. Compr Ther. 2007;33:237-46.
20. Sertoğullarından B, Dallı A, Ekin S. How should approach to pleural effusion? J Chest Dis Crit Care Med. 2015;2:50-5.
21. Heffner JE, Brown LK, Barbieri CA. Diagnostic value of tests that discriminate between exudative and

transudative pleural effusions. Primary Study Investigators. Chest. 1997;111:970-80.
22. Ferrari SL, Ahn-Luong L, Garnero P, Humphries SE, Greenspan SL. Two promoter polymorphisms regulating interleukin-6 gene expression are associated with circulating levels of c-reactive protein and markers of bone resorption in postmenopausal women. J Clin Endocrinol Metab. 2003;88:255-9.
23. Terry CF, Loukaci V, Green FR. Cooperative influence of genetic polymorphisms on interleukin 6 transcriptional regulation. J Biol Chem. 2000;275:18138-44.
24. Rivera-Chavez FA, Peters-Hybki DL, Barber RC, O'Keefe GE. Interleukin-6 promoter haplotypes and interleukin-6 cytokine responses. Shock. 2003;20:218-23.
25. Sharma A, Singh K, Biswas A, et al. Impact of interleukin 6 promoter polymorphisms (−174 G>C, −572 G >C and −597 G >A) on plasma *IL-6* levels and their influence on the development of DVT: a study from India. Hematology. 2018;23:833-8.
26. Boeta-Lopez K, Duran J, Elizondo D, Gonzales E, Rentfro A, Schwarzbach AE et al. Association of interleukin-6 polymorphisms with obesity or metabolic traits in young Mexican-Americans. Obes Sci Pract. 2018;4:85-96.
27. Peng X, Shi J, Sun W, Ruan X, Guo Y, Zhao L et al. Genetic polymorphisms of *IL-6* promoter in cancer susceptibility and prognosis: a meta-analysis. Oncotarget. 2018;9:12351-64.