**Evaluation of Heterozygous Deletion of TP53 Gene in Pleural Fluid Samples: A Case Series of 11 Patients**

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**Abstract**

We described heterozygous deletion of tumor protein 53 (TP53) gene in 11 patients including 2 patients with non-malignant diseases (pneumonia) and 9 patients with malignant diseases [including small cell lung cancer (n = 3), non-small cell lung carcinoma (n = 4), non-Hodgkin's lymphoma (n = 1), and gastric carcinoma (n = 1)]. Chromosomal aberrant status was analyzed by fluorescence in situ hybridization with centromere specific and 17p13.1 locus specific probes. In 3 of 9 cancer patients we did not find malignant pleural effusion with histological examination and/or closed pleural biopsy. Heterozygous deletion of TP53 gene was found to be significantly higher in patients with malignant disease when compared to the patients with benign pleural fluid. As a result, we suggest that heterozygous deletion of TP53 may have indicator value for malignancy; however, further studies are warranted to confirm this suggestion in large patient cohorts.

**Keywords**

Pleural Effusion; Malignancy; TP53 Gene

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**Özet**

Biz, malign hastalığı olmayan 2 hasta (pneumonia) ve malign hastalığı olan 9 hastada (küçük hücreli aksijer karsinomu (n=3), küçük hücreli drp aksijer karsinomu (n=4), non-hodgkin lenfoma (n=1) ve mide kanseri (n=1)) Tümör Protein 53 (TP53) genindeki heterozigot delesyonu tanımladık. Kromozomal aberrant durum, sentromer ve 17p13.1 lokusuna özgü olanlar floresan in situ hybdrizasyon yoluyla analiz edildi. Doku kanres hastason 3’ünde, histolojik değerlendirmeye ve/veya kapali pleval biyopsi ile malign pleval efuzyon tespit etmedik. TP53 geni heterozigot delesyonu malign hastalığı olanlarda benign pleval efuzyonu olanlara kıyasla belirgin olarak yüksek olarak bulundu. Sonuç olarak, TP53 heterozigot delesyonu malignansı için bir belirteç olabileceği one sürmektedir.

**Anahtar Kelimeler**

Plevral Efüzyon; Malgnite; TP53 Gen

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Introduction
Malignant pleural effusion (MPE) is a common complication in patients with advanced cancers, occurring in 15% of cancer-related deaths. MPE is thought to be caused by the hyper permeability of microvascular tissue or invasion of cancer cells into lymphatic vessels. Most pleural effusions (PEs) occur with concomitant tumor. However, in a few patients, multiple cytopathologic examinations of pleural fluid are negative for tumor [1,2], Differential diagnoses of malign and benign pleural effusions remain a challenge. The accuracy of cytological examination of the diagnosis PEs is about 60% and pleural biopsy contributes 7–13% [3]. Video-assisted thoracic surgery is the gold standard procedure in the diagnosis of PEs; however surgical procedures may have several complications. Therefore, avoiding invasive procedures with several serious complications lead clinicians to try to find non-invasive tests such as pleural fluid biomarkers, particularly through the genetic analysis of PEs.

In humans, TP53 gene is located on the short arm of chromosome 17 (17p13.1) [4]. TP53 is the most altered gene in cancer. TP53 mutation was found to be in more than 50% of human cancers. Mutations disabling the TP53 tumor suppressor gene represents the most frequent events in human cancer and typically occur through a double-hit mechanism involving a missense mutation in one allele and a “loss of heterozygosity” deletion encompassing the other [5]. Deletion of TP53 gene has not been reported to date in the diagnosis of malignant pleural effusion. In the present study, we reported numerical chromosomal status in effusion cells derived from 11 patients with malignant and non-malignant diseases by fluorescence in situ hybridization (FISH) with centromere specific and 17p13.1 locus specific probes for chromosomes 17.

Material and Method
The present study was carried out at Süleyman Demirel University Medical Faculty. All patients were male. Eleven pleural effusion specimens were derived from 11 patients including 2 patients with non-malignant and 9 patients with malignant diseases. In 3 of 9 patients with malignancy, the pleural effusion samples showed non-malignant feature. Closed pleural biopsy or medical thoracoscopy performed in these 3 patients indicated there was no malignancy in pleural fluid. In all these malignant cases, metastatic diseases were defined by computed tomography (CT) or magnetic resonance imaging (MRI). Cytological evaluation was also performed in each sample.

Fluorescence in situ hybridization
Fluorescence in situ hybridization targeting TP53 locus was performed for pleural fluid samples. A direct fluorochrome-labeled, dual-color DNA probe cocktail (Cytocell, UK) for P53 -17p13.1 and reference loci (D17Z1) was hybridized to slides of pleural fluid samples. Hybridizations and washings were carried out according to the stringency conditions and the procedures recommended by the manufacturers. Dual-color fluorescent signals were detected and analyzed under epifluorescence microscopy equipped with specific filter sets. At least 200 interphase nuclei were analyzed and scored by independent investigators.

Case Series
The key features of these cases are summarized in Table 1. The mean age was found as 75.3 years. Of the 9 malignant patients, 3 patients have small cell cancer, 4 patients have non-small cell cancer, 1 patient has non-Hodgkin’s lymphoma, and 1 patient has gastric cancer. The diagnosis of 2 patients with benign disease was pneumonia. There were lower levels of heterozygous deletion in TP53 gene in patients with benign disease (cases 10-11) and patients with malign disease non-malignant pleural fluid (cases 1-3) when compared to patients with malignant pleural fluid (cases 4-9). TP53 gene deletion ratio in patients with benign pleural effusions was detected at less than 10% while the ratio in patients with malign effusion was found over 10% (table 1, figure 1).

Table 1. The Heterozygous Deletion of PS3 and Patients Characteristics

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Origin/ Histology/ Stage</th>
<th>Pleural Fluid</th>
<th>Heterozygous Deletion of PS3 Gene (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>59</td>
<td>Small cell cancer, Limited stage</td>
<td>Non-malignant</td>
<td>3.6</td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>Small cell cancer, Advanced stage</td>
<td>Non-malignant</td>
<td>1.2</td>
</tr>
<tr>
<td>3</td>
<td>61</td>
<td>Non-small cell cancer, adenocarcinoma, stage IIIB</td>
<td>Non-malignant</td>
<td>5.9</td>
</tr>
<tr>
<td>4</td>
<td>73</td>
<td>Gastric adenocarcinoma, stage IV</td>
<td>Malignant</td>
<td>9.2</td>
</tr>
<tr>
<td>5</td>
<td>66</td>
<td>Small cell cancer, Advanced stage</td>
<td>Malignant</td>
<td>17.2</td>
</tr>
<tr>
<td>6</td>
<td>58</td>
<td>Non-small cell cancer, adenocarcinoma, stage IV</td>
<td>Malignant</td>
<td>36.2</td>
</tr>
<tr>
<td>7</td>
<td>67</td>
<td>Non-small cell cancer, NOS, stage IV</td>
<td>Malignant</td>
<td>25.2</td>
</tr>
<tr>
<td>8</td>
<td>79</td>
<td>Non-Hodgkin’s Lymphoma</td>
<td>Malignant</td>
<td>15.3</td>
</tr>
<tr>
<td>9</td>
<td>86</td>
<td>Non-small cell cancer, adenocarcinoma, stage IV</td>
<td>Malignant</td>
<td>2.1</td>
</tr>
<tr>
<td>10</td>
<td>56</td>
<td>Pneumonia</td>
<td>Non-malignant</td>
<td>0.65</td>
</tr>
<tr>
<td>11</td>
<td>88</td>
<td>Pneumonia</td>
<td>Non-malignant</td>
<td>1.35</td>
</tr>
</tbody>
</table>

Discussion
Cancer is one of the main causes of exudative PEs. Many of the biochemical markers in pleural fluid are currently being investigated and/or utilized in the clinic. However, the differential diagnosis is still difficult because the mechanism of PE formation

![Figure 1. Interphase fluorescence in situ hybridization (FISH) results. FISH using P53 (TP53) gene deletion probe, reveals Green(G); chromosome D17Z1 locus, Red(R); chromosome 17p13.1 (PS3 gene). Blue; DAPI. Normal nuclei: 2G/2R, Nuclei with PS3 deletion: 2G/1Rv]
is multifactorial and not completely understood. Furthermore, detecting malignant and non-malignant effusion is still a challenging issue; biological behavior and differentiation of each malignant cell are different. For this purpose, genetic studies of pleural effusion have been reported [6]. But the detection of TP53 gene heterozygous deletion in pleural fluid has not been reported to date. Therefore in the present paper, we hypothesized that heterozygous deletion of TP53 gene could be a beneficial candidate in the differential diagnosis of MPEs.

We found higher levels of heterozygous deletion of TP53 in PEs of malignant origin than in PEs of benign origin. However, malignant patients with benign pleural effusion had TP53 gene deletion similar to PEs of benign origin (table 1). Cora et al. found that there is an association between chromosomes aneuploidies and pleural effusion cell status [6]. The FISH method was used in this study. In another genetic study of pleural fluid, p16 gene homozygous deletion in pleural effusion was found to be strongly related to malignant status and higher metastatic potential [7]. The same method was used for genetic testing in this study.

Fluorescence in situ hybridization is a cytogenetic technique that uses fluorescent probes that bind to only those parts of the chromosome with a high degree of sequence complementarity. Its role seems to increase in detecting the characterization of chromosomal rearrangements and marker chromosomes, the detection of micro deletions, and the prenatal diagnosis of common aneuploidies [8-10]. By using an interphase-FISH technique, specific genetic aberrations have been reported in effusion samples of patients with cancer [11,12]. The FISH technique, as seen in our series, may be a safe method in the differential diagnosis of malignant and benign pleural effusion. PS3 is the most altered gene in cancer. More than 50% of human cancers are afflicted with a TP53 mutation. The previous studies suggested that the use of p53-antibodies has potential diagnostic value for several cancers [13,14]. But TP53 mutation has not been evaluated in body fluid samples for cancer diagnosis. For this reason our results may be important. However, the number of cases is quite small and it is not a homogeneous group. Despite these limitations, we believe that future genetic studies may be planned in the light of the present report.

**Competing interests**

The authors declare that they have no competing interests.

**References**


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