Relationship between tumor necrosis factor β gene polymorphism and acute respiratory distress syndrome after operations for esophageal carcinoma

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[Abstract] Background and Objective: A single nucleotide polymorphism of the tumor necrosis factor β (TNF-β) gene affected the level of tumor necrosis factor α and was associated with prognosis of acute respiratory distress syndrome (ARDS). This study was to investigate the association between the TNF-β and ARDS after operation for esophageal carcinoma. Methods: Thirty-four patients with and 116 patients without ARDS after radical resection for thoracotomy esophageal carcinoma were recruited in the Fourth Hospital of Hebei Medical University from January 2005 to June 2007. Peripheral blood samples were collected and DNA extracted, TNF-β genotype was determined by restriction fragment length polymorphism (RFLP). Results: There was no significant difference between the two groups in the TNF-β genotype and allele frequency (P>0.05). The time of mechanical ventilation was shorter and that of staying in the intensive care unit was longer for ARDS patients with the 1/2 genotype in the TNF-β than for those with other genotypes (both P<0.05). The frequency of the 1/1 genotype and 1 allele in the TNF-β was significantly higher in the group of surviving patients with ARDS than in the group of death patients. The odd ratios for mortality of two groups were 18.5 and 11.2, respectively. Conclusions: TNF-β did not appear to be a contributing factor influencing the morbidity of the patients with ARDS after operation for esophageal carcinoma, however, it might affect the development and prognosis of ARDS.

Key words: acute respiratory distress syndrome, tumor necrosis factor β, gene polymorphism, esophageal cancer

Surgical resection is the most effective treatment for esophageal carcinoma. With the development of surgical and intensive care techniques, the scope of surgical intervention is expanding. As a result, postoperative complications, especially the life-threatening ones, are remarkably changed. For instance, the incidence and mortality of anastomotic stoma leakage, chylothorax and empyema are significantly reduced. However, complications such as acute respiratory distress syndrome (ARDS) are increasing. The specific mechanisms of ARDS remain unknown, and the investigation of mechanisms and treatment modalities of ARDS is a major issue of intensive care medicine.

In clinical practice, after radical resection of esophageal carcinoma, some patients may present ARDS, others will not; some ARDS patients will recover after appropriate therapy, while others may not. The causes of these differences are unknown, and are likely to be explained by the individual differences which, reflected from the genetic level, is genetic polymorphism. In recent years, some researchers proposed that the genesis and development of a disease is influenced by genetic factors, and gene polymorphism usually determines the susceptibility and prognosis of an individual to diseases. Currently, gene clone research on the complex diseases such as ARDS is in the preliminary stage. It is proposed that the polymorphism of tumor necrosis factor-β (TNF-β) is an important factor influencing ARDS susceptibility and prognosis. The present study aimed to analyze the association of TNF-β polymorphisms with the occurrence and prognosis of postoperative ARDS in esophageal carcinoma patients, further explore the pathogenesis of ARDS, determine risk population and look for treatment target, thereby paving the way for the gene therapy of this disorder.

Data and Methods

Study population

The diagnosis of ARDS was based on the criteria recommended by American College of Chest Physicians/Society...
of Critical Care Medicine (ACCP/SCCM) and European and American ARDS Society. A total of 34 patients with ARDS after radical resection of esophageal carcinoma treated in Intensive Care Unit (ICU) of the Fourth Affiliated Hospital of Hebei Medical University from January 2005 to June 2007 were recruited. There were 24 men and 10 women aged 49–71 years, with a median of 60. Clinical data were collected, including disease history, duration of mechanical ventilation (receiving assistant ventilation), and duration of ICU stay. Death cases referred to ICU death cases. A total of 116 patients without ARDS after radical resection of esophageal carcinoma were selected as controls, including 88 men and 28 women aged 36–74 years, with a median of 62. Informed consents were obtained from the patient or their family members.

The patients with cardiac function of grades II, III and IV (New York Heart Association criteria) and those with lung function of grades III and IV (classification of lung functions) were excluded. The operations were uneventful, without intraoperative massive hemorrhage. The durations of operations were within 4 h. Patients were given oxygen therapy, antimicrobial therapy and parenteral nutrition support after operation.

Methods

**Specimen collecting and storage** Fasting venous blood (2 mL) was drawn from the median cubital vein of each patient in the morning, put in the EDTA anticoagulation tube and stored at -80°C.

**Whole blood genome DNA extraction** Blood genome DNA extraction kit (0.5–5 mL) produced by Beijing TIANGEN was used for DNA extraction.

**TNF-β gene polymorphism detection** Polymorphism region of TNF-β intron 1 was amplified by polymerase chain reaction (PCR). Primers of PCR were designed according to reference 4 and synthesized by Shanghai Sangon Biological Engineering Technology and Services Co., Ltd. Sequences of Primers 1 and 2 were 5’-CCGTGCTTCTGCTTGGACTA-3’ and 5’-AGAGGCTGATGCTTTGGACTA-3’, respectively. PCR reaction kit was provided by Promega Company. PCR reaction system (25 µL) contained 2 µL template DNA, 1 µL primers of each one, 12.5 µL Taq enzyme and 8.5 µL deionized water. The reaction conditions were as follows: pre-denaturation at 95°C for 3 min, denaturation at 95°C for 1 min, annealing at 65°C for 1 min, extension at 74°C for 1 min, and final extension at 74°C for 6 min, totally 30 cycles. The products (5 µL) were bathed with restriction endonuclease Nco I (Promega Company) in 37°C water for 4 h. Digested products were performed with 2% agarose gel electrophoresis with voltage of 100 V for 30 min. Electrophoresis products of allele TNF-β1 were 586 bp and 196 bp, and that of TNF-β2 was 782 bp.

**Statistical analysis** SPSS10.0 software was used for statistical analysis. Data were expressed as mean ± standard deviation (SD). Chi-square test was used for Hardy-Weinberg equilibrium analysis and comparison of allele frequencies. Impact of genotypes on the duration of mechanical ventilation and ICU stay was analyzed with rank-sum test. A value of P<0.05 was recognized as significant.

Results

**General conditions**

The in-hospital mortality of the ARDS patients was 23.53% (8/34); the 116 controls were all alive.

**TNF-β genotype**

Three genotypes (GG, GA and AA) were identified by endonuclease digestion and electrophoresis. Homozygote TNF-β 1/1 of genotype GG was cut into two fragments of 586 bp and 196 bp; homozygote TNF-β 2/2 of genotype AA was indivisible with fragment length of 782 bp; heterozygote TNF-β 1/2 of genotype GA was cut into two fragments of 782 bp, 586 bp, and 196 bp (Fig. 1).

![Figure 1 TNFβ genotypes](image)

Homozygotes in lane 1, 4 (GG, 196 bp, 586 bp) and lane 2 (AA, 782 bp); heterozygote in lane 3, 5, 6 and 7 (GA, 196 bp, 586 bp, 782 bp); DNA marker in lane 8.

**Distribution of TNF-β genotype**

Genotype distributions of ARDS and control groups were in consistent with Hardy-Weinberg equilibrium ($\chi^2=1.059, 1.39; P>0.05$), indicating that the sample was a good representative of the population.

There was no significant difference in the TNF-β allele and genotype distribution between the two groups ($P>0.05$, Table 1).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>TNFβ genotypes and allele frequency in controls and patients with acute respiratory distress syndrome (ARDS) (number %)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group</strong></td>
<td><strong>Number</strong></td>
</tr>
<tr>
<td></td>
<td>1/1</td>
</tr>
<tr>
<td>Control</td>
<td>116</td>
</tr>
<tr>
<td>ARDS</td>
<td>34</td>
</tr>
<tr>
<td>$\chi^2$</td>
<td></td>
</tr>
<tr>
<td>$P$</td>
<td></td>
</tr>
</tbody>
</table>
might not be the determinant of ARDS occurrence after radical ventilation and time of staying in intensive care unit (ICU) for the patients with ARDS

<table>
<thead>
<tr>
<th>Geno-</th>
<th>Number</th>
<th>T-MV (d, mean±SD)</th>
<th>Mean rank</th>
<th>T-ICU (d, mean±SD)</th>
<th>Mean rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>type</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/1</td>
<td>10</td>
<td>14.80±0.5224</td>
<td>26.500</td>
<td>20.60±8.154</td>
<td>26.700</td>
</tr>
<tr>
<td>2/2</td>
<td>10</td>
<td>12.40±1.6847</td>
<td>20.100</td>
<td>14.60±15.529</td>
<td>19.300</td>
</tr>
<tr>
<td>1/2</td>
<td>14</td>
<td>2.28±1.638</td>
<td>9.210</td>
<td>5.42±1.989</td>
<td>9.640</td>
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<tr>
<td>P</td>
<td></td>
<td>0.000</td>
<td></td>
<td>0.000</td>
<td></td>
</tr>
</tbody>
</table>

T-MV, time of mechanical ventilation; T-ICU, time of staying in ICU; SD, Standard deviation.

Table 3 TNFβ genotypes and allele frequency in patients with ARDS

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Genotype frequency [number(%)]</th>
<th>Allele frequency [number(%)]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1/2+2/2</td>
<td>1/1</td>
</tr>
<tr>
<td>Survivals</td>
<td>26</td>
<td>22(84.6)</td>
<td>4(15.4)</td>
</tr>
<tr>
<td>Deaths</td>
<td>8</td>
<td>2(25.0)</td>
<td>6(75.0)</td>
</tr>
<tr>
<td>χ²</td>
<td>9.872</td>
<td></td>
<td>11.769</td>
</tr>
<tr>
<td>P</td>
<td>0.003</td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>OR</td>
<td>16.5</td>
<td></td>
<td>11.2</td>
</tr>
</tbody>
</table>

Discussion

ARDS is a severe complication after radical resection of esophageal carcinoma with a mortality of more than 50%. Studies have demonstrated that the pathogenesis and prognosis of ARDS was associated with genetic background, and polymorphisms of TNF gene was related to the susceptibility of individuals to ARDS.5,4 TNF gene has polymorphisms which manifest as base pair variations of the TNF-α and TNF-β genes. It is currently recognized that the primary polymorphism point of TNF-β is located in Nco I site of the first intron, that is, +252(G/A) allele.8 Our results showed that genotypes and allele distributions of TNF-β gene were not significantly different between ARDS and control groups, indicating that the polymorphisms of TNF-β might not be the determinant of ARDS occurrence after radical resection of esophageal carcinoma, which was consistent with the study by Qiu et al.9

The present study also showed that ARDS patients with TNF-β12 genotype had relatively shorter durations of mechanical ventilation and ICU stay. In ARDS group, those carrying TNF-β1/1 genotype had 16.5-fold higher risk of death than those carrying other genotypes, and the relative risk of death for those carrying allele 1 was 11.2, indicating that the polymorphisms of TNF-β gene influence the prognosis of ARDS, and allele 2 is a protective gene. The result is consistent with that conducted by Qiu et al.,9 where the proportion of allele 1 was significantly lower in survival ARDS patients than in died ARDS patients (47.1% vs. 80.0%), and the relative risk of death for those carrying allele 1 was 4.5. This is likely due to the regulatory effect of TNF-β on TNF-α expression and its relationships with the pathogenesis and prognosis of septic shock.10,11 Many studies5,12 showed that TNF-α played an important role in the genesis and development of ARDS and was one of the pro-inflammatory cytokines released in the early phase of inflammatory response and ARDS, and its serum level was associated with the genesis and development of ARDS.

However, Stüber et al.7 found that two of the 17 patients with sepsis who carried TNF-β2/2 survived, while 12 of 19 patients with TNF-β1/2 survived (mortality: 88% vs. 37%), indicating that TNF-β2 allele was a risk allele. Wang et al.12 also had consistent results, while TNF-β1/2 polymorphism was not associated with ARDS mortality in the study by Gong et al.26

It is still controversial whether the genesis, development and prognosis of ARDS are associated with gene polymorphisms. This is probably due to following aspects. First, it is related with sample size, inclusion criteria and selection of controls.14 In most investigations, the causes of ARDS are heterogeneous, including all kinds of pulmonary and extrapulmonary factors, and basic conditions of the patients are different. Meanwhile, the control group is usually healthy individuals or other patients who are not susceptible to ARDS, these factors might confound polymorphisms of ARDS markedly. Moreover, the comparability of genetic polymorphism is low between different ethnic populations and geographic areas. Thus, the association of one genetic polymorphism with ARDS in one area should be validated in other areas. Our patients were from the same region, aged primarily between 50 and 70. Most of them had smoking history, weight loss before operation, shared common underlying disease and predisposing factor-selective resection of esophageal carcinoma. This predisposing factor is similar in functioning time and degree. Therefore, the patients selected in our study are ideal for the investigation of ARDS genetic polymorphism. However, the sample size is relatively small and further investigation with larger sample size is mandatory. Second, ARDS is a process influenced by multiple genes and is affected by other cytokines and genes. Third, although ARDS is partly influenced by genetic factors, non-genetic factors also play an important role, including timing of ICU admission, whether appropriate treatment is used and whether postoperative complications occur.

In summary, our study showed that the polymorphism of
TNF-β was to some extent associated with the prognosis of ARDS after radical resection of esophageal carcinoma. The results are valuable for further exploring the mechanisms of ARDS, identifying susceptible patients, determining clinical outcome, seeking new treatment target and future immunotherapy and gene therapy of ARDS. But for such a complex disorder, the study of genetic immunity is currently in preliminary stage.

References


