Investigating PIK\textsubscript{3}R\textsubscript{3} and ATp\textsubscript{2}A\textsubscript{1} Genes Expressions in Ventilator-Associated Pneumonia Patients Admitted to the Intensive Care Unit of Masih Daneshvari Hospital in 2016

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Abstract

Background: Infectious diseases such as ventilator- associated pneumonia (VAP) are one of the serious problems in intensive care units (ICU) of hospitals. To date, there has been no appropriate clinical and diagnostic marker for early detection of this disease. In this study, expression of PIK\textsubscript{3}R\textsubscript{3} and ATp\textsubscript{2}A\textsubscript{1} genes in patients with VAP were assessed to be used as biomarkers to identify and confirm the disease.

Methods: This study was conducted by using peripheral blood samples of 60 individuals, including 30 patients with VAP and 30 healthy volunteers. First, the peripheral blood samples were taken and then RNA was extracted and converted into cDNA. Finally, the assessment of genes was performed by Real-time PCR.

Results: In peripheral blood samples, 46.6% and 30% were positive for PIK\textsubscript{3}R\textsubscript{3} expression in patients and healthy groups, respectively. The ATp\textsubscript{2}A\textsubscript{1} expression in patients and healthy controls were found 40% and 23.3%, respectively. Comparing the ∆CT obtained for the PIK\textsubscript{3}R\textsubscript{3} and ATp\textsubscript{2}A\textsubscript{1} genes showed statistically significant differences between the two groups of patients and healthy subjects (p=0.042, p=0.036).

Conclusions: ATp\textsubscript{2}A\textsubscript{1} and PIK\textsubscript{3}R\textsubscript{3} may be used as biomarkers for early detection of VAP disease. However, further studies are required.

Keywords: ATp\textsubscript{2}A\textsubscript{1} gene, PIK\textsubscript{3}R\textsubscript{3} gene, Ventilator- associated pneumonia (VAP).

Introduction

Ventilator-associated pneumonia (VAP) is a kind of pneumonia appeared among patients admitted to the intensive care unit (ICU) of hospitals about 48 to 72 hours after admission (1). In fact, hospital-acquired infections in the ICU of hospitals result in more than 30% of hospital-acquired infections (2). Ventilator-associated pneumonia is one type of pneumonia...
occurred in the patients with an artificial airway
(3, 4).

In patients with mechanical ventilation, the
risk of VAP increases between 1 to 3% daily,
and there is disagreement among experts
regarding the diagnosis, including both
microbiological and clinical diagnosis, and
treatment approaches (5).

In the past, it was believed that the pathogens
enter the respiratory tract through mechanical
ventilation and related equipment, resulting in
pneumonia. However, it was later shown that
VAP occurs through the bacteria existing in
airways and upper digestive tracts and material
aspirated to the lower airways (6-8).

Previous studies suggest that predisposing
factors such as age, burns, and the severity of
underlying diseases may impact the risk of
ventilator-associated pneumonia (9).

Gram-negative enteric bacilli, pseudomonas
and Staphylococcus aureus, are considered as
three main bacterial causes of VAP (10, 11).
Also, genetic predisposition is imperative in
patients hospitalized in ICUs. Some of these
patients are more susceptible to infectious
diseases, and some may not be affected (12). It
seems the expression of some genes may make
the patients more vulnerable to these infections.
Investigation of this can introduce the specific
biomarkers for susceptibility of the patients to
VAP (12).

Phosphatidylinositol 3-kinase regulatory
subunit (PIK3R3) and sarcoplasmic reticulum
calcium transporting ATPase (ATP2A1) genes
are two candidates as VAP biomarkers. These
encode proteins which are involved in
stimulating chemotaxis, cell migration, and
intracellular signaling and play important roles
in immune system functions against bacteria
(12-16).

It was shown lipopolysaccharide (LPS) in
individuals with infectious diseases cause heart
muscle dysfunction and the sarco/endoplasmic
reticulum Ca²⁺-ATPase, are associated with the
heart relief (17).

In this study, the Real-time PCR as a high
sensitivity method for detecting biomarkers was
employed (18). The use of diagnostic biomarkers
is under investigation and identifying specific
biomarkers for a disease is challenging and
requires other complementary studies (18, 19).

This study aims to investigate the expression
of PIK3R3 and ATP2A1 genes in peripheral blood
cells of patients with VAP. Determination of
these two genes expression in ICU patients may
be useful in the early stages because of their
reduction in response to LPS stimulation.

Materials and Methods
Sampling and Real-time PCR
In this study, two groups, namely patients with
VAP and control groups, were designated.
Groups of patients with VAP were identified and
selected by ICU experts in Masih Daneshvari
Hospital, Tehran, Iran. The control group
revealed no VAP symptom after clinical
examinations. The consent forms were also
completed by both groups, and the participants
have been consciously included in the study.

First, 1.5 ml peripheral blood sample was
taken from each person in both groups and was
used for RNA extraction. RNA extraction was
performed by RNA Blood Minikit (Qiagen.
Germany, Cat. NO52304). Using NanoDrop, the
extracted RNA quality was examined and were
immediately entered into the cDNA synthesis
process. The cDNA synthesis was performed by
Viva 2-sTep RT PCR kit (Cat no. RTPL12,
Vivantis Technologies, Malaysia). The quality
of the synthesized cDNA was checked by the
NanoDrop. Then, the cDNAs were kept at -80 °C
and used for Real-time RT-PCR (Cinna Green
qPCR Mix kit, Cat. No: MM2041 SinaColon,
Iran). The samples were examined in triplicate.

The required primers were designed using the
Allel ID7 software. Specifications of the primers
are shown in Table 1.

The 18S rRNA gene was selected as the
reference gene and evaluated in both groups.

By calculating the CT difference between
the interest gene and reference gene, ΔCT was
obtained. Then, the ΔCT difference between the
samples of patients and healthy subjects was
measured, and ΔΔCT was calculated. Finally,
the folding change was assessed by the equation
2⁻⁰ΔΔCT.
PIK3R3 and ATP2A1 in Ventilator-Associated Pneumonia

Table 1. Specifications and sequences of primers used in Real-time RT-PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>PIK3R3 Forward primer</th>
<th>ATP2A1 Forward primer</th>
<th>18S rRNA Forward primer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GAGAOGGGGAATGAAAAGGAGA</td>
<td>GTCTCAGCCAGCCAATCCCT</td>
<td>GTAACCCGTTGAACCCCATTT</td>
</tr>
<tr>
<td>Reverse</td>
<td>ATCATGAATCTCACCCAGACG</td>
<td>AAGGAAATOCATGCGGCCAG</td>
<td>CCATCAATCGGTAGTAGCG</td>
</tr>
</tbody>
</table>

Statistical Methods
Results were analyzed by using the SPSS software Version 20 about 5% Type I error and 20% Type II error. The data mean and standard deviation were calculated for both groups, and the t-test was performed. The relationship between gene expressions was analyzed using Chi-square test.

Results
There were 21 men and nine women in patient’s group and 22 men and eight women in healthy groups.

In patients with VAP and healthy individuals, the mean age was 56±5.91 and 50.52±8.84, respectively with no significant difference (P=0.598).

Regarding the gender, statistical analysis also showed no significant difference between these two groups (p=0.698).

Expression of 18S rRNA as a reference gene
In this study, the 18 S rRNA gene was selected as the reference gene and evaluated in both groups by Real-time PCR. The average CT was statistically analyzed for patients and control groups, and it was shown that there is no significant difference between the two groups (p= 0.680), indicating that the gene can be used as a reference gene.

Analysis of PIK3R3 and ATP2A1 gene expression
In peripheral blood samples, 46.6% of patients (14/30) were positive for PIK3R3 markers. Among the healthy individuals, nine out of 30 cases have positive PIK3R3 biomarkers. Moreover, 40% of patients (12/30) have the positive ATP2A1 marker, and seven out of 30 healthy individuals have positive ATP2A1 biomarkers (Figure 1). The experiments were performed with three replications.

Fig.1. Evaluation of PIK3R3 and ATP2A1 mRNA expression in the patient and healthy groups by RT–PCR.
The difference between gene expression in peripheral blood of patients and healthy individuals

To analysis of the results, the ΔΔCt method was used, and the folding change was calculated by the equation $2^{-\Delta\Delta CT}$. Comparison of gene expression in patients with VAP was shown in Table 2.

Comparing the ΔCT obtained for the PIK3R3 gene showed statistically significant differences between the two groups of patients and healthy subjects ($p=0.042$). Comparing the ΔCT obtained for the ATP2A1 gene showed statistically significant differences between the two groups of patients and healthy subjects ($p=0.036$) (Figure 2).

Table 2. Folding change of expression for PIK3R3 and ATP2A1 in patients.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Ratio of gene expression</th>
<th>Folding change gene PIK3R3</th>
<th>Folding change gene ATP2A1</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIK3R3</td>
<td>46.6%</td>
<td>4.25</td>
<td>–</td>
</tr>
<tr>
<td>ATP2A1</td>
<td>40%</td>
<td>–</td>
<td>3.98</td>
</tr>
</tbody>
</table>

Fig. 2. The difference in expression of PIK3R3 and ATP2A1 mRNA expression in the patient and healthy groups

Discussion

Ventilator-associated pneumonia is one of the most severe and frequent infections in the ICUs (5). According to the reports, this disease can increase mortality up to 30% (20). In this regard, patients' weaning from mechanical ventilation and the duration of its use should be noted (15). According to some studies, the use of silver-coated endotracheal tubes in ventilation devices, compared with conventional tubes, can delay early-onset VAP (10). Also, there are some strategies which are useful in the prevention of VAP such as hands and mouth hygiene, staff training, putting the open side of the tube at a proper position (21-23).

Regarding the statistics, the patients admitted to ICU, especially hospitalized patients with underlying conditions and invasive procedure in their treatment, are mostly at risk of nosocomial infections (24, 25).

Previous studies suggest that there are genetic factors which can affect the risk of nosocomial infections (19, 26, 27). Therefore, evaluating and examining genes or biomarkers by using peripheral blood can be one of the preventive strategies of infectious diseases in the ICU at different stages. It is similar to studying biomarkers employed in cancers (18). For example, it was showed the
LUNX biomarker is a specific marker for lung cancer cells in peripheral blood and pleural fluid (28). A biological sample such as peripheral blood is mostly investigated for diseases such as cancers. However, with advances in molecular and genomic sciences, such examination in other diseases is also growing (29). Moreover, changes in the pattern of gene expression can also be used in early diagnoses of nosocomial infections such as VAP (27, 29, 30).

McDunn et al. defined 85 genes which can be useful in detecting patients with VAP before clinical diagnosis. The data showed that the difference in the expression of some genes could be efficient in the diagnosis of disease before the appearance of clinical symptoms of infection (27).

This study aimed to facilitate identifying and diagnosis of VAP in the early stages using PIK3R3 and ATP2A1 genes as biomarkers in peripheral blood. It should be noted that the use of biomarkers for infectious diseases such as VAP is in the early stages of development; therefore, further studies are recommended to obtain stronger results in the future through detecting other biomarkers.

Acknowledgment
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References


