Original article

Investigating PIK₃R₃ and ATp₂A₁ Genes Expressions in Ventilator-Associated Pneumonia Patients Admitted to the Intensive Care Unit of Masih Daneshvari Hospital in 2016

Hamidreza Jamaati[1], Naghmeh Bahrami[2], Mahya Daustany[3], Payam Tabarsi[4], Behrooz Farzanegan[5], Seyed Mohammadreza Hashemian[1], Abdolreza Mohamadnia*[6]

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_3R_3 and ATp_2A_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_3R_3 expression in patients and healthy groups, respectively. The ATp_2A_1 expression in patients and healthy controls were found 40% and 23.3%, respectively. Comparing the ΔCT obtained for the PIK_3R_3 and ATp_2A_1 genes showed statistically significant differences between the two groups of patients and healthy subjects (p=0.042, p=0.036).

Conclusions: ATp_2A_1 and PIK_3R_3 may be used as biomarkers for early detection of VAP disease. However, further studies are required.

Keywords: ATp_2A_1 gene, PIK_3R_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

1: Chronic Respiratory Diseases Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran.

2: Craniomaxillofacial Research center, Tehran University of Medical Sciences, Tehran, Iran. Oral and Maxillofacial Surgery Department, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran.

3: Department of Biotechnology, Faculty of Sciences, Islamic Azad University, Tehran, Iran.

4: Clinical Tuberculosis and Epidemiology Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran.

5: Tracheal Diseases Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran.

6: Virology Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran. Department of Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

*Corresponding author: Abdolreza Mohamadnia, Tel: +98 9125146410; Fax: +98 021 22431919 E-mail: mohamadnia.ar@gmail.com

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 (PIK_3R_3) and sarcoplasmic reticulum calcium transporting ATPase (ATp_2A_1) 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 Ca^{2+}-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 PIK_3R_3 and $AT_{p2}A_1$ 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 $\Delta\Delta CT$ was calculated. Finally, the folding change was assessed by the equation $2^{-\Delta\Delta CT}$.

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

	Gene		
	PIK$_3$R$_3$	ATp$_2$A$_1$	18S rRNA
Forward primer	GAGAGGGGAATGAAAAGGAGA	GTCTCAGCCAGCCAATCCCT	GTAACCCGTTGAACCCCATT
Reverse primer	ATCATGAATCTCACCCAGACG	AAGGAAATGCATGCGGCCAG	CCATCCAATCGGTAGTAGCG

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 PIK$_3$R$_3$ and ATp$_2$A$_1$ gene expression
In peripheral blood samples, 46.6 % of patients (14/30) were positive for PIK$_3$R$_3$ markers. Among the healthy individuals, nine out of 30 cases have positive PIK$_3$R$_3$ biomarkers. Moreover, 40% of patients (12/30) have the positive ATp$_2$A$_1$ marker, and seven out of 30 healthy individuals have positive ATp$_2$A$_1$ biomarkers (Figure 1). The experiments were performed with three replications.
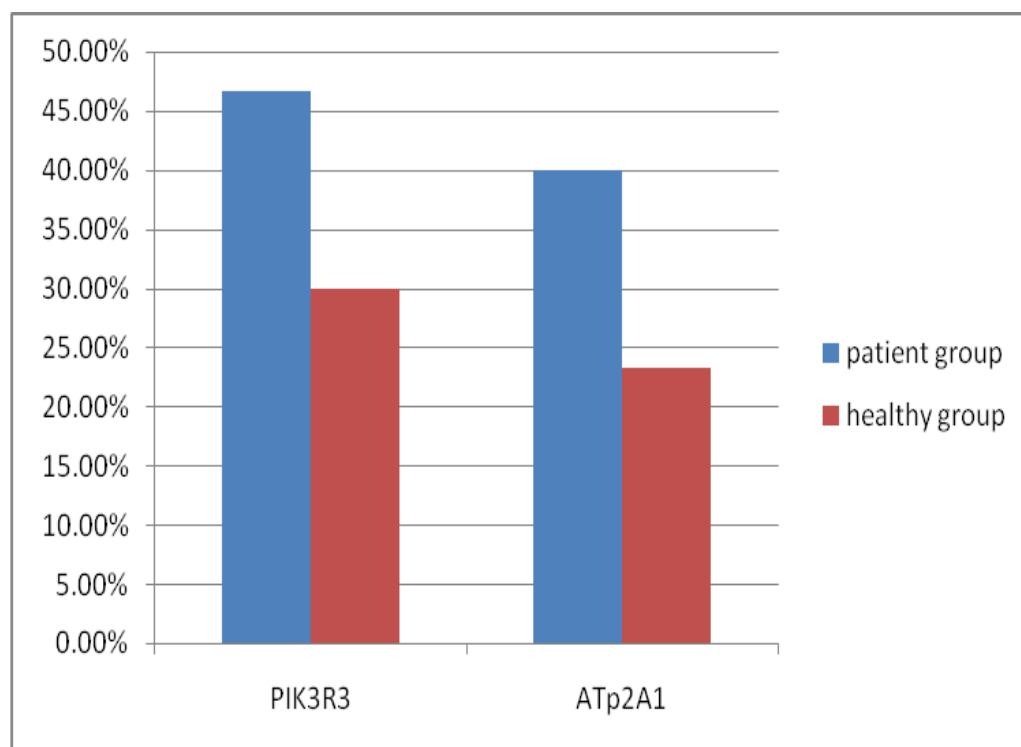
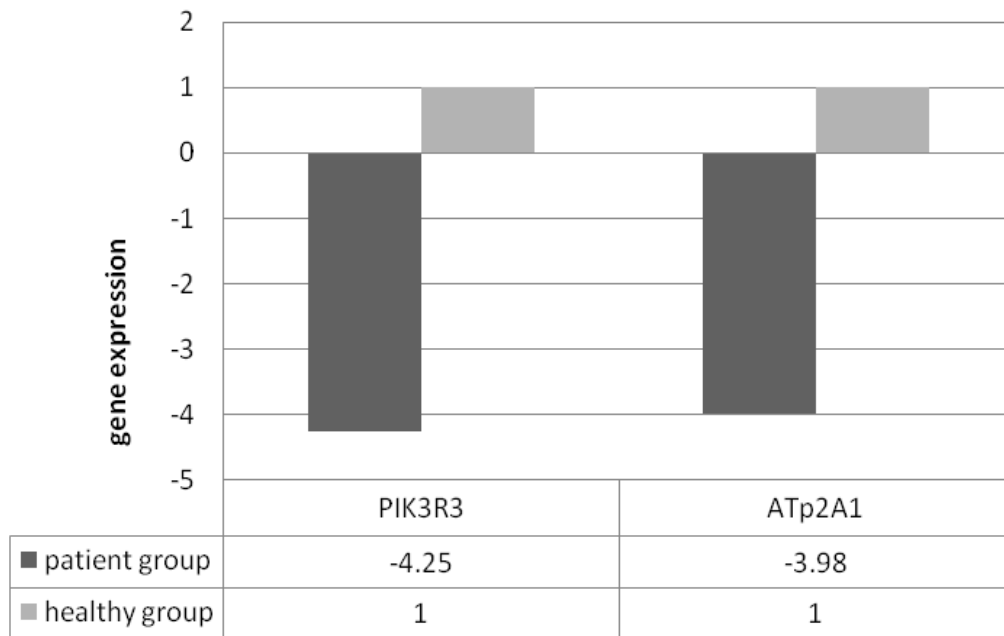**Fig.1.** Evaluation of PIK$_3$R$_3$and ATp$_2$A$_1$ mRNA expression in the patient and healthy groups by RT–PCR.

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 PIK$_3$R$_3$ gene showed statistically significant differences between the two groups of patients and healthy subjects (p=0.042). Comparing the ΔCT obtained for the ATp$_2$A$_1$ 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 PIK$_3$R$_3$ and ATp$_2$A$_1$ in patients.

Gene	Ratio of gene expression	Folding change gene PIK$_3$R$_3$	Folding change gene ATp$_2$A$_1$
PIK$_3$R$_3$	46.6%	4.25	–
ATp$_2$A$_1$	40%	–	3.98

	PIK3R3	ATp2A1
■ patient group	-4.25	-3.98
■ healthy group	1	1

Fig. 2. The difference in expression of PIK$_3$R$_3$ and ATp$_2$A$_1$ 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 PIK$_3$R$_3$ and ATp$_2$A$_1$ 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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