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Investigating PIK_3R_3 and ATp_2A_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_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

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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 ΔΔCT 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.

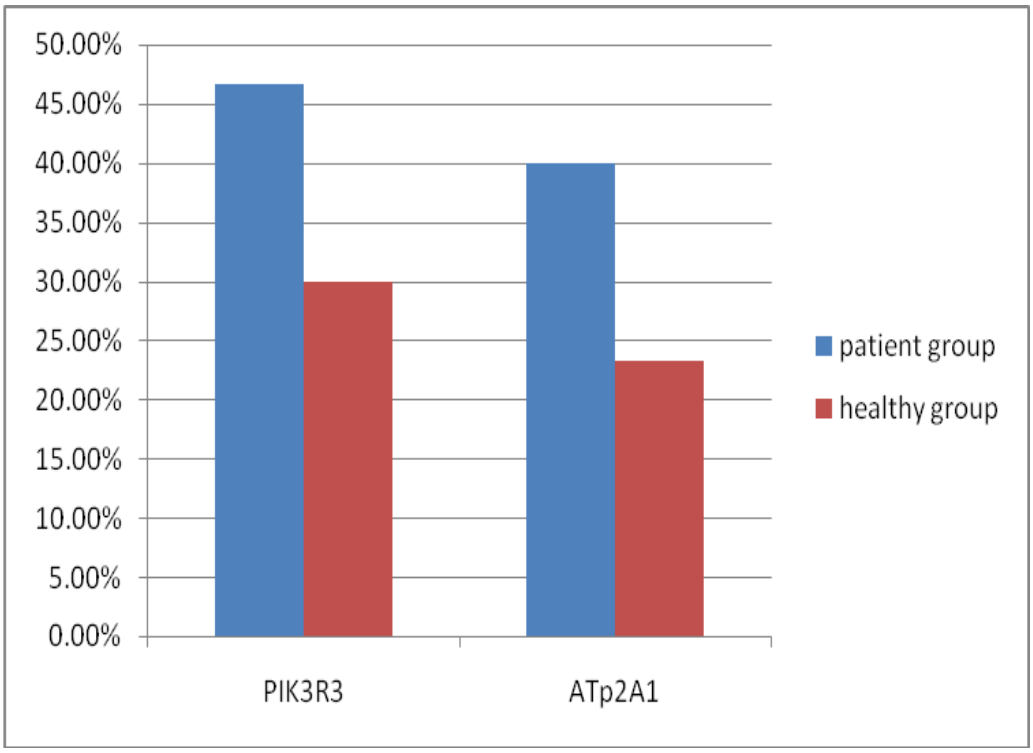

Fig.1. Evaluation of PIK$_3$R$_3$and ATp$_2$A$_1$ mRNA expression in the patient and healthy groups by RT–PCR.

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_3R_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_2A_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_3R_3 and ATp_2A_1 in patients.

Gene	Ratio of gene expression	Folding change gene PIK_3R_3	Folding change gene ATp_2A_1
PIK_3R_3	46.6%	4.25	–
ATp_2A_1	40%	–	3.98

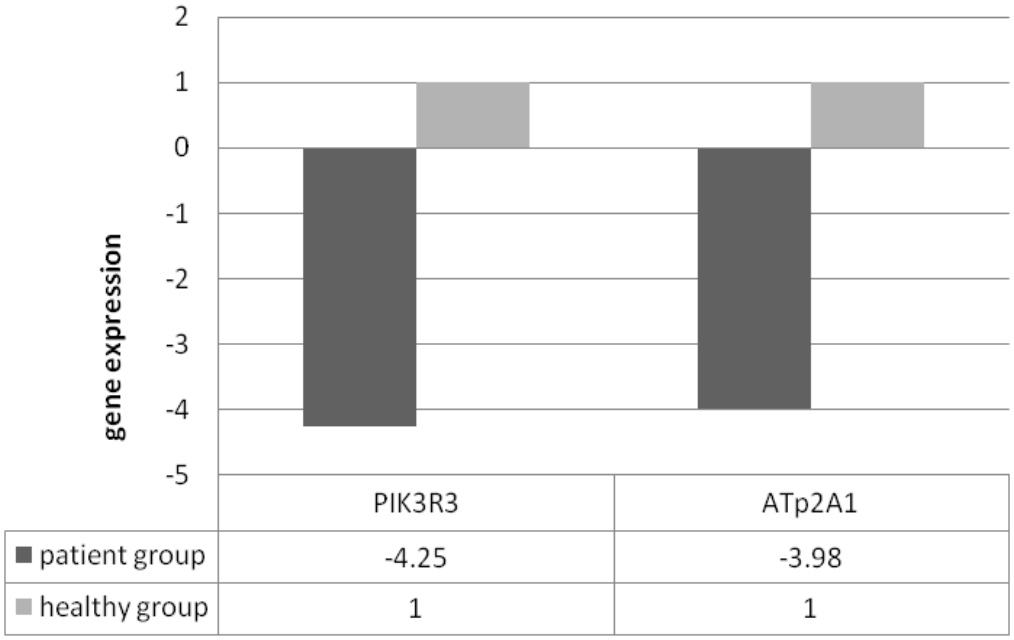

Fig. 2. The difference in expression of PIK_3R_3 and ATp_2A_1 mRNA expression in the patient and healthy groups

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_3R_3 and ATp_2A_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.

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References

1. Gastmeier P, Geffers C. Prevention of ventilator-associated pneumonia: analysis of studies published since 2004. Journal of Hospital Infection. 2007;67(1):1-8.

2. Ghazvini K, Ghanaat J, Malek Jm, Yazdan Pm, Lrani N. Incidence of nosocomial pneumonia and bacterial agents causing this infection in intensive care unit in Ghaem educational hospital in Mashhad. Journal of Ilam University of Medical Sciences. 2005; 13(4):55-61.

3. Augustyn B. Ventilator-associated pneumonia risk factors and prevention. Critical care nurse. 2007;27(4):32-9.

4. Fahimi F, Ghafari S, Jamaati H, Baniasadi S, Tabarsi P, Najafi A, et al. Continuous versus intermittent administration of piperacillin–tazobactam in intensive care unit patients with ventilator-associated pneumonia. Indian journal of critical care medicine: peer-reviewed, official publication of Indian Society of Critical Care Medicine. 2012;16(3):141.

5. Apostolopoulou E, Bakakos P, Katostaras T, Gregorakos L. Incidence and risk factors for ventilator-associated pneumonia in 4 multidisciplinary intensive care units in Athens, Greece. Respiratory care. 2003;48(7):681-8.

6. Hess DR, Kallstrom TJ, Mottram CD, Myers TR, Sorenson HM, Vines DL. Care of the ventilator circuit and its relation to ventilator-associated pneumonia. Respiratory care. 2003;48(9):869-79.

7. Kollef MH. The prevention of ventilator-associated pneumonia. New England Journal of Medicine. 1999;340(8):627-34.

8. Jamaati Hr, Dokouhaki P, Ahmadzadeh Z, Taheri Sa, Bigdeli M, Izadi S, et al. The effects of air pollution on acute respiratory conditions. Respirology. 2003;8(2):213-30.

9. Silva Júnior JMd, Rezende E, Guimarães T, Campos EVd, Magno LA, Consorti L, et al. Epidemiological and microbiological analysis of ventilator-associated pneumonia patients in a public teaching hospital. Brazilian journal of infectious diseases. 2007;11(5):482-8.

10. Boostani V, Dehghan F, Karmostaji A, Zolghadri N, Shafii A. Incidence of Hospital-Acquired Bacterial Pneumonia and Its Resistance Profiles in Patients Admitted to Intensive Care Unit. Global Journal of Health Science. 2016;9(3):73.

11. Jamaati HR, Malekmohammad M, Hashemian MR, Nayebi M, Basharzad N. Ventilator-Associated Pneumonia: Evaluation of Etiology, Microbiology and Resistance Patterns in a Tertiary Respiratory Center. Tanaffos. 2010;9(1):21-7.

12. Swanson JM, Wood GC, Xu L, Tang LE, Meibohm B, Homayouni R, et al. Developing a gene expression model for predicting ventilator-associated pneumonia in trauma patients: a pilot study. PloS one. 2012;7(8):e42065.

13. Deane JA, Fruman DA. Phosphoinositide 3-kinase: diverse roles in immune cell activation. Annu Rev Immunol. 2004; 22:563-98.
14. Rückle T, Schwarz MK, Rommel C. PI3Kγ inhibition: towards an'aspirin of the 21st century'? Nature reviews Drug discovery. 2006;5(11):903-18.
15. Ferrandi C, Ardissone V, Ferro P, Rückle T, Zaratin P, Ammannati E, et al. Phosphoinositide 3-kinase γ inhibition plays a crucial role in early steps of inflammation by blocking neutrophil recruitment. Journal of Pharmacology and Experimental Therapeutics. 2007;322(3):923-30.
16. Sasaki T, Suzuki A, Sasaki J, Penninger JM. Phosphoinositide 3-Kinases in Inunimity: Lessons from Knockout Mice. Journal of biochemistry. 2002;131(4):495-501.
17. Heitner SB, Hollenberg SM. The cardiac force-frequency relationship and frequency-dependent acceleration of relaxation are impaired in lipopolysaccharide-treated rats: is the phospholamban-SERCA axis a therapeutic target? Critical Care. 2009;13(2):1.
18. Karimi S, Mohamadnia A, Nadji SA, Yadegarazari R, Khosravi A, Bahrami N, et al. Expression of two basic mRNA biomarkers in peripheral blood of patients with non-small cell lung cancer detected by real-time rt-PCR, individually and simultaneously. Iranian biomedical journal. 2015;19(1):17.
19. Mohamadnia A, Karimi S, Yadegar Azari R, Naji SA, Khosravi A, Bahrami N, et al. Expression Of CK19 Gene In Patients With Lung Cancer And Its Comparison With Carcinoembryonic Antigen In Peripheral Blood. Journal of Payavard Salamat. 2016;9(5):459-68.
20. Gosselink R, Clerckx B, Robbeets C, Vanhullebusch T, Vanpee G, Segers J. Physiotherapy in the intensive care unit. Neth J Crit Care. 2011;15(2):66-75.
21. Sole ML, Byers JF, Ludy JE, Zhang Y, Banta CM, Brummel K. A multisite survey of suctioning techniques and airway management practices. American Journal of Critical Care. 2003;12(3):220-30.
22. Jamaati HR, Shadmehr MB, Aloosh O, Radmand G, Mohajerani SA, Hashemian SM. Evaluation of plethysmography for diagnosis of postintubation tracheal stenosis. Asian Cardiovascular and Thoracic Annals. 2013;21(2):181-6.
23. Hashemian SM-R, Digaleh H. A Prospective Randomized Study Comparing Mini-surgical Percutaneous Dilatational Tracheostomy with Surgical and Classical Percutaneous Tracheostomy: A New Method Beyond Contraindications. Medicine. 2015;94(47):e2015.
24. Diouf E, Beye M, Diop NM, Kane O, Seydi A, Ndiaye P, et al. [Nosocomial ventilator-associated pneumonia in a tropical intensive care unit]. Dakar medical. 2005;51(2):81-8.
25. Velayati AA, Mehrabi Y, Radmand G, Maboudi AAK, Jamaati HR, Shahbazi A, et al. Modification of Acute Physiology and Chronic Health Evaluation II score through recalibration of risk prediction model in critical care patients of a respiratory disease referral center. International journal of critical illness and injury science. 2013;3(1):40.
26. Jamaati H, Bahrami N, Abniki M, Tabarsi P, Farzanegan B, Doroudinia A, et al. Real-time RT-PCR Detection of HCN4 and ADAM8 genes in ventilator-associated pneumonia patients Hospitalized in intensive care unit. Journal of Cellular and Molecular Anesthesia. 2016;1(4):163-7.
27. McDunn JE, Husain KD, Polpitiya AD, Burykin A, Ruan J, Li Q, et al. Plasticity of the systemic inflammatory response to acute infection during critical illness: development of the riboleukogram. PloS one. 2008;3(2):e1564.
28. Cheng M, Chen Y, Yu X, Tian Z, Wei H. Diagnostic utility of LunX mRNA in peripheral blood and pleural fluid in patients with primary non-small cell lung cancer. BMC cancer. 2008;8(1):1.
29. Bahrami N GM, Jamaati HR, Mohamadnia A, Dargahi H, Kazempour dizaji M, Khosravi A, Heshmatnia J, Vahabi P, Bahrami NA. Expression of two essential mRNA biomarker in the peripheral

blood as possible biomarkers for diagnosis of non-small cell lung carcinoma. Minerva Pneumologica. 2016 55(3):31-6.

30. Cobb JP, Moore EE, Hayden DL, Minei JP, Cuschieri J, Yang J, et al. Validation of the riboleukogram to detect ventilator-associated pneumonia after severe injury. Annals of surgery. 2009;250(4):531.