The prophylactic effect of different levels of positive end-expiratory pressure on the incidence rate of atelectasis after cardiac surgery: A Randomized Controlled Trial

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Abstract

**Background:** The use of positive end-expiratory pressure (PEEP) can have an important role as one of the ways to prevent and treat atelectasis, but it seems that there is still no consensus about its beneficial level. The aim of this study was to determine the effect of different levels of PEEP on the incidence of atelectasis after heart surgery.

**Methods:** This is a double-blind randomized controlled trial that was adopted from a research project recorded in the Iranian Registry of Clinical Trials. This paper is the result of a research project undertaken at Fatemeh Zahra Hospital (Mazandaran Heart Center) in 2015. 180 patients underwent open heart surgery were selected and were divided randomly into three groups: control, PEEP=8, and PEEP=10 (60 in each group). The patients in the two PEEP8 and PEEP10 intervention groups separately received 8 cm H2O and 10 cm H2O PEEP, respectively, 30 minutes after admission to the ICU for 4 hours and then received 5 cm H2O PEEP until extubation. Atelectasis was examined two hours after the extubation and on the third day after surgery.

**Results:** The incidence rates of atelectasis two hours after extubation on the first day of surgery were 22 (36.7%), 20 (33.3%) and 10 (16.7%) patients in the control, PEEP8 and PEEP10 groups, respectively. The differences were statistically significant among the three groups (p=0.035). The incidence rates of atelectasis on the third day after surgery were 39 (65%), 36 (60%) and 21 (35%) patients in the control, PEEP8 and PEEP10 groups, respectively. The differences were also statistically significant among the three groups (p=0.003).

**Conclusion:** The use of 10 cm H2O PEEP can lead to a reduction in the incidence of atelectasis, intubation time at the ICU and length of ICU and hospital stay. Given that this level of PEEP is effective, this method is recommended to be used in postoperative care of patients.

**Keywords:** Positive End-Expiratory Pressure, Postoperative pulmonary complications, Atelectasis, Cardiac Surgery, Hemodynamic indices, Oxygenation indices.

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Introduction

Pulmonary complication is one of the most important and serious early problems after heart surgery (1, 2), which is a major cause of prolonged hospitalization, increased healthcare costs and mortality (3). Some of the pulmonary complications after heart surgery include atelectasis, pneumonia, pulmonary edema, pleural effusion, acute respiratory distress syndrome (4, 5).

Atelectasis is one of the most common respiratory complications after open-heart surgery. Its prevalence after heart surgeries has been reported Up to 78% of pulmonary

↑ What is “already known” in this topic:
The use of positive end-expiratory pressure is one of the ways to treat atelectasis after cardiac surgery. It is not as a preventive method for the atelectasis. It’s also a controversy about its beneficial level.

→ What this article adds:
The use of 10 cm H2O PEEP can lead to a reduction in the incidence of atelectasis, intubation time at the ICU and length of ICU and hospital stay. Given that this level of PEEP is effective, this method is recommended to be used in postoperative care of patients.
complications (6). The incidence of atelectasis after heart surgery has been reported 75% in an Iranian study (7). Atelectasis is characterized by the collapse of the alveoli, lobules or larger unit respiratory systems that occurs after thoracic surgeries due to reasons such as uneven distribution of ventilation and perfusion due to factors such as anesthesia (8, 9), extracorporeal circulation, sternotomy, analgesics (8, 10), respiratory muscle dysfunction (9), post-operative pain, drainage (11), decreased phrenic nerve activity and diaphragmatic dysfunction (4, 12), which lung volumes are reduced, and atelectasis happens then. The importance of postoperative atelectasis is due to the absence of obvious clinical symptoms, and therefore it does not attract the attention of medical personnel and healthcare staff. In addition, it is progressive and can lead to other pulmonary complications such as nosocomial pneumonia and subsequent increase in the length of hospital stay as well as rising costs for healthcare systems (3, 13).

To prevent and treat pulmonary complications after heart surgery, various methods have been proposed, including active respiratory physiotherapy such as deep breathing along with coughing, incentive spirometry, frequent change of position in bed and faster ambulation of patients, as well as passive methods such as intermittent positive-pressure breathing (IPPB), positive end-expiratory pressure (PEEP) and continuous positive airway pressure (CPAP) (14). Given the simultaneous use of all active respiratory physiotherapy procedures, the high incidence rate of atelectasis after heart surgery is still reported in patients (7, 15). One of these methods that are utilized less as a preventive operation is the use of PEEP in patients undergoing mechanical ventilation in the intensive care unit or during recovery. PEEP is applied along with an increase in respiratory factors, lung volumes and improved gas and alveolar exchanges (16, 17), and is recommended for the main treatment of collapse created in patients under anesthesia with healthy lungs (18, 19), atelectasis treatment and improved arterial oxygenation (20, 21). Some studies on the benefits of PEEP different levels reported that elevated values could lead to a reduction in ventilator duration and increased survival odds ratio (22).

A study on the adverse effects of high levels (PEEP>10 cm of water) reported an increase in ventilation duration, prolonged intubation (23) and increased survival odds ratio (22). In our study, the only significant difference in relation to the incidence rate of atelectasis (28), using the following formula and considering a minimal difference of 25% between the two groups, α=0.05, β=0.20, p1=0.50 in the control group, and p2=0.25 in the experimental group.

\[
\frac{n}{2} = \frac{[1.96 + 0.84] \sqrt{(1.96^2 + 0.84^2) (0.50 \times 0.50 + 0.25 \times 0.75)]}{1.25 - 0.25} = 55
\]

Considering a dropout rate of 30%, the sample size increased to 77 individuals, which was then further rounded up to 80 (per group) to enhance the accuracy. According to our CONSORT diagram (Fig. 1), after the dropout, each group ended up to contain 60 samples.

After legal procedures and obtaining necessary approvals from the authorities and ethics committees at Mazandaran University of Medical Sciences, Iran, referring to Mazandaran Heart Center, the patients were selected. Using accessible sampling and a random-number table patients were randomly assigned into three groups: control (PEEP=5), PEEP=8 and PEEP=10. Researcher reviewed the list of patients waiting for heart surgery, interviewed the patients and explained about this survey. Consent was taken from the patients after having inclusion criteria.

Inclusion criteria were non-emergency open heart surgery (coronary artery bypass grafting, heart valve replacement, combined with sternotomy and cardiopulmonary bypass techniques), age range of 18-65 years, lack of ejection fraction less than 30% in the preoperative angiography sheet due to its negative effects on the patient’s breathing pattern, no history of chronic lung disease and

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any damage to the lungs, no previous history of open heart or lung surgery, no history of rib fractures and chest tube, no history of head or nasal trauma, neurologic disease history and frequent sinus infections.

Exclusion criteria included history of chemotherapy, the use of immunosuppressants during three months prior to surgery, arterial systolic pressure less than 90 mmHg despite fluid intake, arterial PH less than 7.30, arterial carbon dioxide pressure over 50 mmHg, arterial oxygen saturation less than 80% despite receiving supplemental oxygen, blood hemoglobin less than 7 g/dl after surgery, serum creatinine over 3.5 mg/dl and body mass index (BMI) over 40 kg/m² due to increased risk of developing postoperative respiratory complications, as well as postoperative hemodynamic instability (systolic blood pressure below 80 mmHg), aortic clamp time over 150 minutes, cardiopulmonary bypass time over 240 minutes, use of intra- and postoperative intra-aortic balloon pump (IABP), intubated for more than 24 hours, retransmission to the operating room, require ventilation - therapeutic protocol and hypotension after intervention up to 10 mmHg of baseline blood pressure. The participants were allocated in three groups of PEEP8, PEEP10, and control using Excel’s RANDBETWEEN function. Each group was identified by a letter: A to PEEP8, B to PEEP10, and C to control group. 180 envelopes were sequentially numbered from 1 to 180 for three groups. Each opaque and sealed envelope contained a letter (A, B, or C) randomly selected using RANDBETWEEN function of Excel. This procedure was carried out by someone not involved in the project. The first eligible patient was designated as number one, and the envelope numbered one was then opened, and the patient was allocated to one of the three groups based on the letter contained in the envelope. The data were statistically analyzed. 240 patients were included by randomization method.
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The researcher collected the required information and followed up the patients in all three groups. Since the patients and radiologists, who interpreted the chest X-ray, were unaware of the type of intervention and grouping, the study was considered as double-blind trial. Data were analyzed using SPSS version 20 software through descriptive statistic such as frequency, mean, standard deviation and analytical statistics, ANOVA, Chi-square, McNemar’s and repeated measures ANOVA tests. A p-value <0.05 was considered significant in this study.

Results

After excluding 23 patients from the control group, 18 from PEEP8 group and 17 from PEEP10 group, 60 patients were finally analyzed in each group. Comparison of demographic variables among the groups indicates that the data for age, sex, body mass index and smoking were not significantly different (Table 1).

In addition, there was no statistically significant difference among the three groups for variables associated with surgery including type of surgery, number of chest tubes, number of grafted vessels, duration of anesthesia, duration of aortic clamping and duration of cardiopulmonary bypass pump (Table 2).

The chest x-ray results showed significant difference among the three groups for incidence of atelectasis two hours after extubation (p<0.05). There was a difference among the three groups for incidence of atelectasis among the three groups for variables associated with surgery including type of surgery, number of chest tubes, number of grafted vessels, duration of anesthesia, duration of aortic clamping and duration of cardiopulmonary bypass pump (Table 2).

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Table 1. Comparison of demographic variables among patients undergoing heart surgery in three groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Groups</th>
<th>Test and P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year), (mean ± SD)</td>
<td>Control (n=60)</td>
<td>PEEP = 5 (n=60)</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>35 (58.33)</td>
<td>34 (56.73)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>25 (41.67)</td>
<td>26 (43.37)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>Yes, n (%)</td>
<td>6 (10)</td>
</tr>
<tr>
<td>No, n (%)</td>
<td>54 (90)</td>
<td>55 (91.73)</td>
</tr>
<tr>
<td>BMI (kg/m²), (mean ± SD)</td>
<td>27.29±5.54</td>
<td>26.38±4.62</td>
</tr>
</tbody>
</table>

Table 2. Comparison of variables associated with surgery among patients undergoing heart surgery in three groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Groups</th>
<th>Test and P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of surgery</td>
<td>Control (n=60)</td>
<td>PEEP = 8 (n=60)</td>
</tr>
<tr>
<td>Vessels, n (%)</td>
<td>48 (80)</td>
<td>47 (78.33)</td>
</tr>
<tr>
<td>Valves, n (%)</td>
<td>8 (13.33)</td>
<td>8 (13.33)</td>
</tr>
<tr>
<td>Vessels and valves, n (%)</td>
<td>4 (6.77)</td>
<td>5 (8.33)</td>
</tr>
<tr>
<td>Number of chest tube</td>
<td>1, n (%)</td>
<td>8 (13.33)</td>
</tr>
<tr>
<td>2, n (%)</td>
<td>34 (56.77)</td>
<td>33 (55)</td>
</tr>
<tr>
<td>3, n (%)</td>
<td>18 (30)</td>
<td>20 (33.33)</td>
</tr>
<tr>
<td>Number of grafted vessels</td>
<td>1, n (%)</td>
<td>31.7</td>
</tr>
<tr>
<td>2, n (%)</td>
<td>31.7</td>
<td>32.3</td>
</tr>
<tr>
<td>≥3, n (%)</td>
<td>31.7</td>
<td>32.3</td>
</tr>
<tr>
<td>Anesthesia time (h), (mean ± SD)</td>
<td>4.55±0.89</td>
<td>4.61±0.85</td>
</tr>
<tr>
<td>Aortic clamp time (min), (mean ± SD)</td>
<td>56.90±22.34</td>
<td>57.28±22.13</td>
</tr>
<tr>
<td>Cardiopulmonary bypass time (min), (mean ± SD)</td>
<td>79.02±21.3</td>
<td>80.28±24.3</td>
</tr>
</tbody>
</table>

NS: Not Significant
33.3%) and the intervention group of PEEP10 (10 patients, 16.7%) (p= 0.035), but there was no statistically significant difference between the control group (22 patients, 36.7%) and PEEP8 (20 patients, 33.3%) (p= 0.70). On the third day after surgery, the incidence rate of atelectasis had statistically significant difference between the intervention group of PEEP10 (21 patients, 35%) and the control group (39 patients, 65%) (p= 0.001), as well as between the intervention groups of PEEP10 and PEEP8, 36 patients (60%) (p= 0.02). However, there was no statistically significant difference between the PEEP8 group and the control group (p= 0.79) (Table 3).

According to Table 4, the incidence rate of atelectasis in the two times studied in each of the three groups showed that the incidence rate of atelectasis in the control group on the third day after surgery (n= 39, 65%), with one case improvement and 18 new cases, was significantly different based on McNemar's test compared with the time of two hours after extubation (n= 22, 36.6%), (p<0.001). In addition, the incidence rate of atelectasis in the PEEP8 group on the third day after surgery (n= 36, 60%), with four cases improvement and 20 new cases, was significantly different compared with a time of two hours after extubation (n= 10, 16.6%) (p<0.001) (Table 4).

Tukey post hoc test revealed statistically significant difference for the mean (SD) duration of intubation in the ICU between the intervention group of PEEP10 and the control group (p<0.001), as well as between the intervention group of PEEP10 and the intervention group of PEEP8 (p<0.001). However, no significant difference was observed between the intervention group of PEEP8 and the control group (p=0.97). The mean (SD) duration of stay in ICU had statistically significant difference between the intervention group of PEEP10 and the control group (p<0.001); but no significant difference was found between the intervention group of PEEP8 and the control group (p=0.36). Moreover, considering the duration of hospitalization, there was a statistical significant difference between the intervention group of PEEP10 and the control group (p<0.001), as well as between the intervention group of PEEP10 and the intervention group of PEEP8 (p<0.001). Nevertheless, no significant difference was found between the intervention group of PEEP8 and the control group (p=0.91). The data have been shown in Table 5.

**Discussion**

The results of this study demonstrated that the prophylactic use of 10 cm H2O PEEP in patients with mechanical

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**Table 3. Comparison of atelectasis incidence rate during two hours and on the third day after surgery between three groups**

<table>
<thead>
<tr>
<th>Time after extubation</th>
<th>Groups</th>
<th>Atelectasis Test and P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 hours after extubation</td>
<td>Control, n (%)</td>
<td>22(36.66) 60(100)</td>
</tr>
<tr>
<td></td>
<td>PEEP8, n (%)</td>
<td>20(33.33) 60(100)</td>
</tr>
<tr>
<td></td>
<td>PEEP10, n (%)</td>
<td>10(16.66) 60(100)</td>
</tr>
<tr>
<td>Total, n (%)</td>
<td>52(88.88) 60(100)</td>
<td>16.3=x²</td>
</tr>
</tbody>
</table>

**Table 4. Comparison of atelectasis incidence rate between two hours after extubation and on the third day after surgery in each group**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Atelectasis incidence (3 day after surgery)</th>
<th>Total</th>
<th>McNemar's test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Yes, n (%)</td>
<td>21(35)</td>
<td>60(100)</td>
</tr>
<tr>
<td></td>
<td>No, n (%)</td>
<td>1(1.66)</td>
<td>60(100)</td>
</tr>
<tr>
<td>2 hours after extubation</td>
<td>Yes, n (%)</td>
<td>16(26.66)</td>
<td>60(100)</td>
</tr>
<tr>
<td></td>
<td>No, n (%)</td>
<td>20(33.33)</td>
<td>60(100)</td>
</tr>
<tr>
<td>PEEP8</td>
<td>Yes, n (%)</td>
<td>1(1.66)</td>
<td>20(33.33)</td>
</tr>
<tr>
<td></td>
<td>No, n (%)</td>
<td>21(35)</td>
<td>60(100)</td>
</tr>
<tr>
<td>Total, n (%)</td>
<td>36(60)</td>
<td>60(100)</td>
<td>6.70=x²</td>
</tr>
<tr>
<td>2 hours after extubation</td>
<td>Yes, n (%)</td>
<td>2(3.33)</td>
<td>60(100)</td>
</tr>
<tr>
<td></td>
<td>No, n (%)</td>
<td>19(31.66)</td>
<td>60(100)</td>
</tr>
<tr>
<td>Total, n (%)</td>
<td>21(35)</td>
<td>60(100)</td>
<td>16.3=x²</td>
</tr>
</tbody>
</table>

**Table 5. Comparison of mean duration of intubation, duration of stay in ICU and duration of hospitalization the three groups**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Groups</th>
<th>Control (n=60)</th>
<th>PEEP = 8 (n=60)</th>
<th>PEEP = 10 (n=60)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of intubation in ICU (hour), (mean ± SD)</td>
<td>10.03±2.48</td>
<td>10.12±2.22</td>
<td>7.94±1.92</td>
<td>F=18.48</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Length of stay in ICU (day), (mean ± SD)</td>
<td>4.53±0.99</td>
<td>4.30±0.97</td>
<td>3.48±0.81</td>
<td>F=20.93</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Length of stay in hospital (day), (mean ± SD)</td>
<td>8.37±1.2</td>
<td>8.28±1.07</td>
<td>7.02±1.04</td>
<td>F=27.74</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>
ventilation in the ICU after heart surgery can lead to a significant reduction in the incidence rate of atelectasis after extubation on the first and third days after surgery. It seems that using this level of PEEP prevents the collapse of the airways, high functional residual capacity (FRC), improved alveolar performance with increased pressure and volume of the alveoli (29) as well as elevated pulmonary compliance(30). However, 8 cm H2O PEEP and 5 cm H2O PEEP had no significant effect in reducing the incidence rate of atelectasis. In a similar study on the effect of 5 cm H2O PEEP and 10 cm H2O PEEP after coronary artery bypass surgery, a significant difference was observed between the two groups in terms of atelectasis incidence. Thus, some degree of atelectasis was found among the intervention (26.7%) and the control (56.7%) groups that the level of 10 cm H2O PEEP reduced the incidence rate of atelectasis, which is in line with the present study. Compared with our study, in the mentioned study the patients in the intervention group did not receive constant PEEP, but the rate varied between 5 to 10 cm of water until tracheal extubation depending on the patient's condition. In addition, the patients in the control group received PEEP less than 5 cm of water. However, in our study, all patients in the control group received at least 5 cm H2O PEEP; this could be the reasons for the high percentages in both intervention and control groups in the above study compared with the present study (28).

In another study on the effect of PEEP on respiratory function after heart surgery in three groups, the patients experienced three levels of PEEP including 0, 5 and 10-15 cm of water after heart surgery. The results showed that the PEEP levels of less than 10 cm H2O had no effect on the reopening of the lungs with atelectasis, which is in line with the above study. The difference of the current research with the above study design is the use of various levels and periods of PEEP. Thus, the PEEP in the mentioned study was applied 3 to 5 hours after admission in the ICU until extubation, and the patients did not receive PEEP greater than 10 cm of water within the constant time so that the patients were given PEEP for 15 minutes and again after 20 minutes of pause. In the present study, the patients received 8 or 10 cm H2O PEEP for a maximum of 4 hours continuously, which the time remained constant for all samples (31). A prospective clinical trial that was carried out on non-hypoxic patients connected to mechanical ventilation with normal chest X-ray showed that 0, 5 or 8 cm H2O PEEP levels had no effect on the incidence of atelectasis; it is in line with the present study. The results of the study showed that the levels of 5 to 8 cm H2O PEEP improved hypoxia and reduced the incidence rate of ventilator-associated pneumonia in the patients. Unlike the present study the patients did not undergo heart surgery, but the patients in the general and trauma intensive care units were studied (32).

PEEP has a specific effect on improving pulmonary compliance that is effective in preventing atelectasis (30). It seems that the best special effects of PEEP are applied via the ventilator when using endotracheal tube in the ICU, not after extubation and with non-invasive methods; because according to some studies on cardiac surgical patients, the use of similar methods of positive pressure ventilation via face mask such as continuous positive airway pressure (CPAP) and inspiratory resistance-positive expiratory pressure (IR-PEEP), which are non-invasive ventilation, did not show clinical effects (33, 34).

Another study investigated the effects of PEEP in patients after coronary artery bypass graft surgery at three levels of 0, 5 and 10 cm H2O. These levels had no effect on the incidence of atelectasis (35), contrary to the present findings. In this study, the incidence of atelectasis was measured on the fifth day after surgery; the sample size in their three groups was 44 people. In the present study, the incidence of atelectasis was measured on the first and third days and the sample size was 180; this seems to be the reason for the differences in the results. It also appears that the mean duration of cardiopulmonary bypass in above study was much longer than the present study; prolongation of bypass duration is directly related to the incidence of pulmonary complications after heart surgery, including atelectasis (6).

Comparison of the atelectasis findings in each group between two hours after extubation and on the third day after surgery indicated that the incidence of 18 new cases of atelectasis in group PEEP5; 20 new cases of atelectasis in group PEEP8 on the third day after surgery than two hours after extubation was statistically significant. It seems that 8 cm H2O PEEP was not effective for 4 hours and did not significantly differ from the control group receiving 5 cm H2O PEEP. The duration of 8 cm H2O PEEP should also be increased to be effective.

The present study demonstrated that the duration of mechanical ventilation in the ICU had significantly decreased in patients who received 10 cm H2O PEEP compared to the control group and the group received 8 cm H2O PEEP. This decline might be due to better levels of arterial oxygenation in ICU after heart surgery in early times by applying 10 cm H2O PEEP. Many studies have confirmed that high levels of PEEP can improve arterial oxygenation after heart surgery (22, 23, 25).

Based on the results of a study on the influence of 5 cm H2O PEEP and 8 cm H2O PEEP after heart surgery, duration of intubation in the ICU had no differences between the two groups (25). This result is consistent with the present study and the duration was reduced only in the 10 cm H2O PEEP group. Another study on PEEP following coronary artery bypass grafting reported that levels over 10 cm H2O PEEP increased ventilation duration (23), contrary to our results. This difference seems to be due to the fact that the average age is an important factor in the delayed extubation and in an increase of connectivity to ventilator after heart surgery (36). In the mentioned study, the average age in the group with high levels of PEEP was equal to 65±8 years; but in the present study, age over 65 years was one of the exclusion criteria.

In the current study, there were significant differences between the incidence rate of atelectasis after extubation on the first day and on the third day, duration of intubation, length of ICU stay and hospitalization in PEEP10 group compared with the control and the PEEP8 groups. Considering the history of cardiac surgery and the advent
of subsequent novel care methods, we can also see the incidence of atelectasis as common pulmonary complications. Although numerous research have been done on the effect of PEEP in patients with severe acute respiratory syndrome, its effects on respiratory function in patients after heart surgery has been less published. Researchers still have not reached a clear consensus on the effective level and duration of its use after heart surgery as a preventive action.

Based on current literature, no study investigated the three levels of PEEP as a preventive measure to avoid atelectasis incidence after heart surgery. Therefore, there are needs for further studies in this area. Future studies on the duration of PEEP more than four hours is recommended.

Limitations of this study included failure to control pain and anxiety in the patients as confounding variables until the third day after surgery, inability to use identified heart surgeon and anesthesiologist, failure to recruit a specified physiotherapist due to their different skills in physiotherapy; These limitations were outside the control of the reseacher.

Conclusion
The results revealed that the use of 10 cm H2O PEEP as a preventive measure had a significant impact in decreasing the incidence of atelectasis, and reducing the duration of intubation in the ICU in the early period after heart surgery. Considering the advantages of PEEP at this level including ease of use, cost-effectiveness, lack of physical fatigue, no need for patient cooperation, easy implementation and low risk, it is recommended to use this method in conjunction with other conventional techniques for preventing respiratory complications after heart surgery.

Acknowledgements
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Ethics Statement
This study was registered in the Iranian Registry of Clinical Trials (IRCT201507307494N14), and obtained the code of ethics committee (IR.MAZUMS.REC.94-1855).

Conflict of Interests
The authors declare that they have no competing interests.

References
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