Brief Communication

The effect of positive end-expiratory pressure on inflammatory cytokines during laparoscopic cholecystectomy

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ABSTRACT

Objectives: To investigate effects of the positive end-expiratory pressure (PEEP) application of 10 cm H_2O on the plasma levels of cytokines during laparoscopic cholecystectomy.

Methods: A prospective study was conducted on 40 patients who presented to the Department of General Surgery, Medical Faculty, Turgut Özal University, Ankara, Turkey scheduled for laparoscopic cholecystectomy operation during a 10 month period from September 2012 to June 2013. Forty patients scheduled for laparoscopic cholecystectomy operation were randomly divided into 2 groups; ventilation through zero end-expiratory pressure (ZEEP) (0 cm H_2O PEEP) (n=20), and PEEP (10 cm H_2O PEEP) (n=20). All patients were ventilated with 8 ml/kg TV. Levels of interleukin (IL)-6, tumor necrosis factor (TNF)-α, IL 10, and transforming growth factor (TGF)-β1 were measured in the pre- and post-operatively collected samples.

Results: Blood samples of 30 patients' were analyzed for plasma cytokine levels, and 10 were excluded from the study due to hemolysis. Post-operative plasma IL-6 levels were observed to be significantly higher than the pre-operative patients (p=0.035). Post-operative plasma TGF-β1 levels in the PEEP group was found significantly higher compared with the pre-operative group levels (p=0.033). However, there were no significant differences in the pre- and post-operative plasma cytokine levels between the 2 groups.

Conclusion: The application of PEEP of 10 cm H_2O, which has known beneficial effect on respiratory mechanics, does not have any effect on systemic inflammatory response undergoing pneumoperitoneum during laparoscopic cholecystectomy surgery.

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Laparoscopic cholecystectomy is preferred due to its advantages, however pneumoperitoneum leads to reduced compliance of the respiratory system, and decrease in functional residual capacity due to upward displacement of the diaphragm during insufflation.[1] In addition, deterioration in the distribution of gases, progressive atelectasis due to decreased functional residual capacity, and reduction in the production of surfactants are seen during surgery.[2] Positive end-expiratory pressure (PEEP) application seems to be effective on the prevention of atelectases, and it is argued to reduce post-operative complications.[3] The PEEP is applied in patients receiving mechanical ventilation to raise the expiratory pressures above the atmospheric pressures, in order to open the collapsed alveoli and prevent the re-closing of the opened alveoli.[4]

Cytokines are polypeptides produced and released by various cells, regulates immune and inflammatory events, including systematic events against inflammation, growth, and improvement of the cells and injury.[5] It was demonstrated that mechanical ventilation application plays a role in the induction of local and systemic cytokine response in animal and human studies.[6-11] Application of mechanical ventilation with a high TV has shown to cause the release of inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), interleukin (IL) 1-β, and interleukin-6 (IL-6), and accumulation of neutrophils in the lungs.[11] The objective of this study was to investigate the effects of intraoperative PEEP application on inflammatory cytokines by measuring serum levels of TNF-α, IL-6, transforming growth factor-beta 1 (TGF-β1), and IL-10 in the pre- and post-operative periods in the patients undergoing laparoscopic cholecystectomy surgery.

Methods. The study was approved by the institutional review board of Turgut Özal University School of Medicine, Ankara, Turkey. Signed informed consent was obtained from all participants. A prospective study was conducted on 40 patients who presented to the Department of General Surgery, Faculty of Medicine, Turgut Özal University, Ankara, Turkey scheduled for laparoscopic cholecystectomy operation during a 10 month period from September 2012 to June 2013. The patients who were between 18 and 66 years old, without any chronic or acute lung disease, psychiatric and mental defects, and with a physical status of American Society of Anesthesiology I and II were enrolled in the study. Patients having uncontrolled hypertension,

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coronary artery disease, pulmonary disease or heart failure, those receiving immunosuppressive therapy, or having an illness, which may change the immunological state, patients having a body mass index over 35 kg/m^2, scheduled for an operation due to acute cholecystitis, smokers, and those used steroids, nonsteroid anti-inflammatory drugs within the last 48 hours were excluded from the study. Patients were randomly divided into 2 groups; ventilation through ZEEP (0 cm H_2O PEEP) (n=20), and PEEP (10 cm H_2O PEEP) (n=20) using the closed envelope technique.

Electrocardiography (ECG), oxygen saturation (SpO_2), and non-invasive blood pressure monitoring were performed in patients and anesthesia was induced with 3 mg/kg propofol, one μg/kg fentanyl and 0.6 mg/kg rocuronium. After intubation, considering optimum weights of the patients, volume-controlled mechanical ventilation (Datex-Ohmeda S/5 Avance, Madison, USA) was introduced with 8 ml/kg tidal volume (TV) as the inspirium expiration ratio (I/E) to be 1/2 and 12/minutes. Respiratory rate (RR) was adjusted as the end-tidal CO_2 pressure ($EtCO_2$) to be 35-40 mm Hg. Maintenance of anesthesia was achieved with 2% of sevoflurane in 50% O_2, and 50% N_2O. Heart rate (HR), mean arterial pressure (MAP), SpO2, peak airway pressure (P_{peak}), TV, RR, minute ventilation (MV), and $EtCO_2$ pressure values of the patients were recorded at baseline after intubation with 10 minute intervals during the operation. The PEEP of 10 cm H_20 was applied when the mechanical ventilation was initiated in the PEEP group whereas; PEEP was not applied in the ZEEP group. All patients were administered pneumoperitoneum of 15 mm Hg using CO_2 gas. Patients in both groups with P_{peak} exceeding 35 cm H_20 were planned to be excluded from the study by decreasing the TV or PEEP according to the lung protective strategy during the mechanical ventilation. Totally 2, 8 ml venous blood samples were collected from the patients, one before anesthesia induction, and other after the extubation. Blood samples were centrifuged at 4000 rpm for 10 minutes, and the obtained serums were kept at -800 until the cytokine analysis. The levels of IL 6, TNF-α, IL 10 and TGF-β1 were measured after collecting all the serums.

Once taken in accordance with the manufacturer's recommendations, TGF-β1 (Boster Biological Technology Co., Fremont, California, USA), TNF-α, IL-6, and IL-10 (DIAsource Immuno Assays S.A., Louvain-la-Neuve, Belgium) tests were studied using the ELISA method. Following the testing, Bio Tec ELx800 ELISA was bic hromatically read at the reader as 450 nm, and 490 nm, and the outcomes were calculated.

Statistical analysis was performed using the Statistical Package for the Social Sciences version 15 (SPSS Inc., Chicago, IL, USA). Compliance of the variables with normal distribution was studied using histogram and Kolmogorov-Smirnov test. Descriptive statistics were expressed as mean ± standard deviation for the variables having normal distribution and median (minimum-maximum) for the variables having not normal distribution. Intergroup data were compared using Student's t or Mann-Whitney U test. Chi-square test was used for comparison of the qualitative data. Wilcoxon test was used for comparison of intergroup pre- and post-operative values. Two-way ANOVA and ANCOVA tests were used with correction to define the effect of hypertension on MAP. Effect of hypertension on the change of MAP over time was studied using repeated measurements variance analysis. Since it was seen that sphericity assumption was absent, Greenhouse-Geisser correction was applied. $P<0.05$ was considered as statistically significant.

Results. Plasma cytokine levels of 30 patients were analyzed, and 10 patients were excluded from the study due to hemolysis. Both groups were found to be similar in terms of the demographic features, duration of anesthesia and operation. ($p>0.05$) (**Table 1**). Number of patients having hypertension was significantly higher in the PEEP group compared with the ZEEP group ($p=0.013$) (**Table 1**). No significant difference was found between the 2 groups in terms of intraoperative HR ($p>0.05$). The MAP measured after the intubation was found to be significantly higher in the PEEP group as 103.8 ± 18.9 mm Hg compared with the ZEEP

Table 1 - Demographic and operative properties of patients.

Variables	Group ZEEP (n=15)	Group PEEP (n=15)	*P*-value
Age, year	40.6 ± 11.9	49.2 ± 12.1	0.838
Gender, female/male, n	13/2	13/2	1
Height, kg	76.3 ± 12.8	72.5 ± 13.8	0.947
Weight, cm	165.5 ± 8.5	161 ± 5.3	0.407
ASA I/II	9/6	8/7	0.713
Chronic disorders (n)			
Hypertension	1	7	0.013*
Diabetes mellitus	3	0	0.068
Hypothyroidism	0	1	0.309
Duration of anesthesia (min)	65.3 ± 15.9	55.7 ± 14.1	0.525
Duration of surgery (min)	54 ± 17.2	45 ± 14.4	0.365

Data presented as median (25-75%) and mean ± SD, *$p<0.05$, ZEEP - zero end-expiratory pressure, PEEP - positive end-expiratory pressure, ASA - American Society of Anesthesiology, min - minutes

group (87.3 ± 16.3 mm Hg) (p=0.016). No statistically significant difference was observed between the 2 groups in terms of MAP values at the other times (p>0.05). At the result of Greenhouse-Geisser correction carried out to investigate the effect of chronic hypertension on the MAP values in the groups, no correlation was found between the patients having chronic hypertension and intraoperative MAP values (p=0.325).

The P_{peak} values did not exceed 35 cm H_2O in none of the patients who were included in the study and no patient was excluded from the study due to this reason. The P_{peak} values were found to be significantly higher in the PEEP group compared with the ZEEP group after the intubation, and following tenth, twentieth, and thirtieth minutes (p<0.05) (**Table 2**). Dynamic compliance values were found to be significantly higher in the PEEP group than the ZEEP group in the first 50 minutes (p<0.05) (**Table 2**).

Plasma levels of IL-10 were excluded from the analysis as these values were below 1.6 pg/ml, which is the measurable value in all the pre-operative patients, and 27 of the post-operative patients. When all patients were evaluated together for the pre- and post-operative cytokine levels, no significant difference was found in the pre- and post-operative TNF-α and TGF-β1 levels, while post-operative IL-6 levels were significantly higher than the pre-operative values (p=0.035). No significant difference was observed between both groups in terms of the pre- and post-operative plasma levels of IL-6, TNF-α, and TGF-β1 (p>0.05) (**Table 3**). On the intergroup comparison of pre- and post-operative plasma levels of cytokines, no significant difference was found between the levels of IL-6 and TNF-α, while post-operative plasma levels of post-operative TGF-β1 were found to be significantly increased compared with the pre-operative values in the PEEP group (p=0.033) (**Table 3**).

Table 2 - Intraoperative P_{peak} and $C_{dynamic}$ values in both ZEEP and PEEP groups.

Variables	Group ZEEP	Group PEEP	*P*-value
P_{peak}			
After intubation	18.7 ± 4.0	24.2 ± 3.9	0.001†
Tenth min	21.2 ± 4.8	25.5 ± 3.8	0.011*
Twentieth min	23.2 ± 4.1	27.6 ± 3.4	0.004*
Thirtieth min	23.6 ± 2.9	26.3 ± 3.7	0.032*
Fortieth min	24.2 ± 3.5	25.6 ± 4.6	0.397
Fiftieth min	22.7 ± 3.8	26.8 ± 3.7	0.055
Sixtieth min	22.6 ± 4.2	24.5 ± 4.7	0.469
$C_{dynamic}$			
After intubation	28.6 ± 5.9	38.6 ± 11.9	0.009*
Tenth min	25.7 ± 6.0	34.4 ± 9.1	0.004*
Twentieth min	23.0 ± 4.6	29.4 ± 7.1	0.007*
Thirtieth min	21.9 ± 3.5	31.7 ± 7.8	0.001†
Fortieth min	21.1 ± 3.3	35.0 ± 14.3	0.003*
Fiftieth min	22.9 ± 5.0	29.7 ± 7.2	0.036*
Sixtieth min	23.1 ± 4.9	36.1 ± 12.1	0.118

Data presented as mean ± SD, ZEEP - zero end-expiratory pressure, PEEP - positive end-expiratory pressure, min - minute, *P<0.05, †P=0.01

Discussion. In the present study, we measured the serum levels of IL-6, TNF-α, IL-10, and TGF-β1 in patients that underwent pneumoperitoneum due to laparoscopic cholecystectomy in order to investigate the effects of 10 cm H_2O PEEP application on lung inflammation. No significant difference was found between the pre- and post-operative levels of TNF-α, IL-10, and TGF-β1 in all patients, while we found that IL-6 levels were post-operatively increased. We did not observe a significant difference between the groups in terms of the pre- and post-operative cytokine measurements. While no significant change was found at the levels of TNF-α, IL-10, and IL-6, post-operative TGF-β1 levels were found to be higher than the pre-operative levels in patients that underwent PEEP application.

Maracajá-Neto et al[12] demonstrated that the application of 10 cm H_2O PEEP improved the elasticity and resistance of the respiratory system,

Table 3 - Pre-operative and post-operative plasma cytokine levels in both groups ZEEP and PEEP.

Variable	Pre-operative	Post-operative	*P*-value
IL-6 (pg/ml)			
Group ZEEP	2 (2-22.1)	2 (2-25.5)	0.066
(n=15)	3.3 ± 5.2	5 ± 6.6	
Group PEEP	2 (2-6.1)	2 (2-21.2)	0.144
(n=15)	2.3 ± 1	4.5 ± 5.8	
P-value	0.962	0.716	
TNF-α (pg/ml)			
Group ZEEP	3.9 (2.2-9.6)	3.6 (1.9-8.9)	0.802
(n=15)	4.1 ± 1.9	4.1 ± 1.8	
Group PEEP	3.6 (1.3-5.9)	3.3 (1.1-5)	0.157
(n=15)	3.7 ± 1.1	3.2 ± 1.1	
P-value	0.603	0.664	
TGF-β1 (pg/ml)			
Group ZEEP	5.2 (1-40.2)	6.3 (1.3-76.5)	0.733
(n=15)	12.3 ± 14	13.8 ± 19	
Group PEEP	5.9 (1-55.9)	10.4 (2.1-50.4)	0.033*
(n=15)	9.5 ± 13.3	15.4 ± 13.8	
P-value	0.771	0.300	

Data presented as median (25-75%) and mean ± SD, ZEEP - zero end-expiratory pressure, PEEP - positive end-expiratory pressure, *P<0.05

and had beneficial effects on respiratory mechanics during pneumoperitoneum in the laparoscopic cholecystomy.[7-13] We found similar effects of PEEP application on the lung mechanics in previous studies.

Laparoscopic cholecystectomies have been reported to cause fewer immune response compared with open cholecystectomy surgeries.[14] Similarly in our study, systemic immune response was not much prominent. In the study group, we observed a significant increase only in the post-operative IL-6 levels compared with the pre-operative levels among the measured levels of cytokines.

In an animal trial, Haitsma et al[10] concluded that ventilation strategy with PEEP application of 10 cm H_2O prevented ventilator-induced lung injury by markedly decreasing shift of the cytokines to the alveolar compartment. In an isolated rat lung model Tremblay et al[6] showed that fewer cytokine responses occurred with PEEP application of 10 cm H_2O compared than with groups with PEEP application of 0 cm H_2O. In a study by Meier et al,[7] in order to clarify the effects of PEEP in the systemic and local immune mediator and modulator release in the healthy lungs, they investigated ZEEP and PEEP effects first in isolated rat lungs. According to the results from that study,[7] PEEP might decrease the cytokine release by reducing atelectrauma during the high inspiratory pressure application, as well as an increase in the cytokine release by increasing the pulmonary distension during the low inspiratory pressure application.[7]

In another study[8] investigating whether using low TV and PEEP application have a protective effect from inflammation on the patients undergoing elective surgery, we found similar results for IL-6 as in our study. While the levels of TNF-α, IL-1α, IL-1β, IL-6, macrophage inflammatory protein 1-α and 1-β were not affected by the mechanical ventilation in bronchoalveolar lavage, serum levels of IL-6 and IL-8 were increased with the mechanical ventilation, but no significant difference was found between both groups. Likewise, in our study we found the plasma IL-6 levels were increased in both groups and TGF- levels β1 in the PEEP group, but the difference was not statistically significant.

The TGF-β1 has a central role in the synthesis and degradation of the extracellular matrix collagens, and it is produced by numerous cells, especially by macrophages, fibroblasts, endothelial cells, and platelets.[14] Recent studies have demonstrated the dual effect of TGF-β1 on the regulation cellular response in the apoptosis induced by Fas/Fas ligand (a membrane protein, which main function is to trigger apoptosis). Dhainaut et al[15] concluded that TGF-β1 was not only effective in the late periods, but also in the early periods of ARDS, and potentially might contribute to the development of pulmonary edema. The authors proposed that integrin-induced local activation of TGF-β1 was quite important in the development of pulmonary edema in ARDS, and blockage, or activation of TGF-β1 might be effective in treating this disease.[15] When post-operative levels of TGF-β1were compared with the pre-operative values, we found these values to be significantly higher in the PEEP group. However, although there was a difference between the PEEP and ZEEP groups in terms of the levels of TGF-β1 in the post-operative values, this difference was not statistically significant. Inflammation is an event, which begins and progresses locally, and it seems difficult to detect it in the serum samples.

In conclusion, in this study we found that PEEP application of 10 cm H_2O PEEP has no effect on the serum levels of cytokines compared with the groups in which PEEP was not applied in laparoscopic cholecystectomies with pneumoperitoneum application. Therefore, we believe that given its positive effect on the respiratory functions, PEEP of 10 cm H_2O would be applied in the laparoscopic cholecystectomy operations without worrying about cytokine activation.

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