CO2 Flow Dynamics of Bladder Injury During Laparoscopy and the Effect of the Content of the Abdominal Viscera During Injury – Experimental Study

1 Department of Obstetrics and Gynecology, Kafkas University School of Medicine, Kars, Turkey
2 Department of Surgery, Kafkas University Faculty of Veterinary Medicine, Kars, Turkey
3 Department of Urology, Kafkas University School of Medicine, Kars, Turkey

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article

Abstract

Background. Despite the well-known and easily recognizable signs of bladder injury during laparoscopy, some injuries remain unnoticed. Intra-operative diagnosis of a urinary bladder injury provides the opportunity to repair and prevent later complications involving the formation of fistula, infection, ascites and impairment of renal function. Small and unrecognized bladder injuries increase the chance of morbidity and permanent organ dysfunctions.

Objectives. The aim of the study was to evaluate the CO2 flow dynamics of bladder injury occurring during laparoscopy and the effect of the content of the abdominal viscera during injury.

Material and Methods. The study involved eight male New Zealand rabbits. Following urinary catheterization of the rabbits with an 8-gauge urinary catheter connected securely to a urinary drainage bag, pneumoperitoneum was created at a pressure level of 12 mm Hg. The experiment consisted of three phases. After the observational phase (Phase 1), the anterior wall of the urinary bladder was perforated with the tip of a 21 G needle (Phase 2) and methylene blue was administrated to evaluate the CO2 flow dynamics (Phase 3).

Results. The amount of CO2 consumption and accumulation in the urinary drainage bags differed significantly among the three phases of the experiment (p < 0.05). There was no CO2 consumption or accumulation in the urinary drainage bags during Phase 1. The amount of CO2 consumption and accumulation in the urinary drainage bags during Phase 2 was significantly higher than during Phase 3.

Conclusions. Urinary catheterization helps in the diagnosis of small or unnoticed urinary bladder injuries occurring during laparoscopy. CO2 flow and consumption is lower if the viscosity of the content overlying the injury site is higher (Adv Clin Exp Med 2015, 24, 5, 775–781).

Key words: animal experimentation, catheterization, intraoperative complications, laparoscopy, urinary bladder.
of fistula, infection, ascites and impairment of renal function. Small and unrecognized bladder injuries increase the chances of morbidity and permanent organ dysfunctions. Although unexplained hematuria, fever and flank pain in the post-operative period help in forming an early post-operative diagnosis, in most cases the suffering patient has to undergo another surgical procedure.

Gomez et al. suggested searching for the presence of clear fluid in the operative field, visible bladder laceration and the gas distension of the urinary drainage bag as intra-operative signs of iatrogenic bladder injuries [14]. However, despite well-known and easily recognizable signs some bladder injuries remain unnoticed intra-operatively.

The aim of this experimental study was to evaluate the CO$_2$ flow dynamics of bladder injury occurring during laparoscopy. In addition, the effect of the content of the abdominal viscera during injury was studied.

Material and Methods

The study was carried out in the Surgery Department of Kafkas University Faculty of Veterinary Medicine between August 4th and 10th, 2011. Prior to the experiment, the study was approved by the Animal Ethics Committee of Kafkas University (26/2011 HADYEK). Kafkas University Faculty of Veterinary Medicine provided the participating animals.

The Sample and the Preparations for the Experiment

Two-year-old male New Zealand rabbits (n = 8) weighting between 3220 and 3540 g were used in the experiment. Male rabbits were chosen because urinary bladder catheterization was easier and their longer urethras were considered to be more protective against gas leakage.

All the rabbits were fed as usual before the experiments. However, oral feeding and hydration were stopped 8 h prior to the anticipated procedures. All operations were performed between 4 and 8 PM by the same surgical team.

Anesthesia and Intra-Operative Settings

One hour before the initiation of anesthesia, a single dose of one quarter of a 1000 mg cefazoline sodium ampule was administered intramuscularly. Anesthesia was initiated by intramuscular administration of 70 mg/kg xylazin HCl (Rompun® 2% 50 mL Bayer) and 80 mg/kg ketamin HCl (Ketasol 10% inj, 10 mL vial Richter Pharma), and maintained with sevofluarane 2–2.5% mixed with dry air.

Under general anesthesia the animals were prepared on the operating table in an approximately 30° head down position. The operative fields were shaved and then cleansed with 10% polyvinylpyrrolidone iodine. A Veress needle was introduced through a 2–3 mm sub-umbilical incision into the abdominal cavity and the cavity was inflated with CO$_2$ until reaching an intra-abdominal pressure level of 12 mm Hg. Following the enlargement of the skin incision to 6 mm, a 5 mm trocar was introduced, and a telescope was introduced through the trocar to visualize the cavity. In order to manipulate the intra-abdominal tissues, a 2 mm trocar was introduced 5 cm lateral to the first one.

When the intra-abdominal pressure had reached the maintenance level of 12 mm Hg, the absence of any gas leakage was confirmed by observing the automatic cessation of the gas inflow. At the end of the experiments the abdominal cavity was deflated and the incision at the abdominal entry site was closed with number 2-0 delayed absorbable sutures.

Experimental Phases

Phase 1

The experiment started following the creation of the pneumoperitoneum. The initial phase was observational and did not include any interventions. Following the secure connection of an 8-gauge urinary catheter to an empty urinary drainage bag, the catheter was introduced into the urinary bladder of the animal and the balloon of the catheter was inflated with 5 mL of saline. The operative setting was not changed for 5 min and the changes in gas flow, intra-abdominal pressure and the volume of the urinary drainage bag were recorded during and at the end of the 5 min.

Phase 2

The anterior wall of the urinary bladder was held with grasping forceps introduced through the 2 mm trocar. The changes in gas flow, intra-abdominal pressure and the volume of gas in the urinary drainage bag were rechecked to ensure the absence of any bladder injury during the grasping process.

The line of the urinary catheter was clamped. A 21-gauge needle was inserted into the abdominal cavity under telescopic view and the urinary bladder was perforated with the tip of the needle (Fig. 1). The needle was then removed from the abdominal cavity and the changes in gas flow, intra-abdominal pressure and the volume of gas in the urinary drainage bag were rechecked. When the gas flow ceased and the intra-abdominal pressure was 12 mm Hg,
the urinary catheter was unclamped. Other than slight movements of the catheter along its longitudinal and transverse axes, the operative setting was maintained unchanged for the next 5 min. The changes in gas flow, intra-abdominal pressure and the volume of the urinary drainage bag were recorded during and at the end of the 5 min.

**Phase 3**
The urinary catheter was clamped and the connected urinary drainage bag was replaced with a new one. Methylene blue diluted with saline solution (50 cc) introduced into the abdominal cavity and the intra-abdominal pressure was maintained at 12 mm Hg. The urinary catheter was unclamped. The line of the urinary catheter and the urinary drainage bag was observed for 5 min. The changes in gas flow, intra-abdominal pressure and the volume of the urinary drainage bag were recorded during and at the end of the 5 min.

**Post-Experimental Care**
The urinary catheter was shortened and secured to prevent migration into the bladder cavity, and was removed on the second post-operative day. A protective collar was placed around the necks of the rabbits in order to prevent them from biting their wounds and the catheter, and to prevent self-injury. The rabbits were kept in separate cages. Oral feeding and hydration with oral analgesics were initiated in the fourth post-operative hour. The protective collars around the rabbits’ necks were removed one week later.

**Statistics**
Statistical analyses were performed using SPSS software, v. 16.0 (SPSS Inc., Chicago, IL, USA).

A comparison of subjects in terms of the amounts of CO₂ consumption at each phase of the experiment and the rabbits’ weights was performed using the Kolmogorov-Smirnov Z test. A comparison of the amounts of CO₂ consumption and the estimated changes in the volume of the urinary drainage bags during each phase was performed using Friedman’s and Wilcoxon’s tests. Correlation analyses of the study parameters were performed using Spearman’s test. A p value of < 0.05 was considered significant.

**Results**
All the rabbits were surviving without notable health problems at the end of the first post-operative month.

In all cases a gas accumulation in the urinary drainage bags was observed. Facilitation of the gas flow into the urinary drainage bags after slight movements of the catheter tips was observed subjectively.

The weights of the rabbits and the volume of CO₂ consumption to create the pneumoperitoneum did not differ among the participating rabbits (p > 0.05). There was no CO₂ consumption or accumulation in the urinary drainage bags during Phase 1 of the experiment. However, CO₂ consumption and accumulation in the urinary drainage bags was observed in all cases during Phases 2 and 3 (Table 1). In addition, methylene blue flowed into the urinary drainage bags during Phase 3 in all cases.

Friedman’s test showed that the amount of CO₂ consumption and accumulation in the urinary drainage bags (Table 2) was significantly different among the three phases of the experiment (p < 0.05). Wilcoxon’s test was used to compare the amount of CO₂ consumption and accumulation in the urinary drainage bags of each phase with the others. All the values were significantly different at each phase (p < 0.05). CO₂ consumption and accumulation in the urinary drainage bags during Phase 2 was significantly higher than during Phase 3. There was no CO₂ consumption and accumulation in the urinary drainage bags during Phase 1.

The weight of the rabbits correlated with the amount of CO₂ consumption (r = 0.846, p = 0.008) and the accumulated CO₂ in the urinary bags (r = 0.909, p = 0.002) in Phase 2. However, in Phase 3, weight did not correlate with the amount of CO₂ consumption (r = 0.698, p = 0.54) or the accumulated CO₂ in the urinary bags (r = 0.479, p = 0.230). In addition, the amount of CO₂ consumption in Phase 2 correlated with the amount of urinary drainage bag CO₂ accumulation in
Phase 2 (r = 969, p < 0.0001); and CO₂ consumption in Phase 3 correlated with the amount of urinary drainage bag CO₂ accumulation in Phase 3 (r = 943, p < 0.0001).

**Discussion**

**Principal Findings**

This experimental study demonstrated that a complete penetrating injury of the urinary bladder (even as small as the tip of a 21-gauge needle) during laparoscopic surgery causes diffusion of CO₂ into the cavity of the urinary bladder. Thus, transurethral insertion of a urinary catheter tightly connected to a urinary drainage bag helps to diagnose an occult urinary bladder injury through observation of an inflated urinary drainage bag. The immediate passage of intra-abdominally administered methylene blue into the urinary drainage bag makes the diagnosis certain. However, a change in the content of the abdominal viscera from gas to liquid (methylene blue) decreased both CO₂ consumption and accumulation in the urinary drainage bags.

**Strengths of the Study**

As far as the authors know, this is the first study demonstrating the CO₂ consumption and accumulation patterns of a bladder injury occurring during laparoscopy in an experimental animal model. In addition, the study also demonstrated the importance of the characteristics of the content of the abdominal viscera during injury. The findings of this study suggested that liquid covering the injured site diminished the gas flow into the urinary bladder. Thus, a slower movement of the gas and liquid mixture from the abdominal cavity into the bladder and the urinary drainage bags caused less decrease of the intra-abdominal pressure and in turn less CO₂ consumption and accumulation in the urinary drainage bags. Moreover, it was reasonable to think that the

Table 1. Comparisons of selected parameters of the eight rabbits that participated in the experiment. The values are presented as mean ± standard deviation or percent (%)

<table>
<thead>
<tr>
<th></th>
<th>Mean ± standard deviation</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight of the rabbits (g)</td>
<td>3306.20 ± 105.55</td>
<td>0.287</td>
</tr>
<tr>
<td>Initial CO₂ used to maintain the abdominal pressure of 12 mm Hg (mL)</td>
<td>837.50 ± 17678</td>
<td>0.876</td>
</tr>
<tr>
<td>CO₂ use in Phase 1† of the experiment (mL)</td>
<td>0</td>
<td>n/a</td>
</tr>
<tr>
<td>CO₂ use in Phase 2‡‡ of the experiment (mL)</td>
<td>1550.00 ± 213.81</td>
<td>0.844</td>
</tr>
<tr>
<td>CO₂ use in Phase 3‡‡‡ of the experiment (mL)</td>
<td>962.50 ± 176.78</td>
<td>0.918</td>
</tr>
<tr>
<td>Increase in estimated urinary bag volume in Phase 1 (mL)</td>
<td>0</td>
<td>n/a</td>
</tr>
<tr>
<td>Increase in estimated urinary bag volume in Phase 2 (mL)</td>
<td>1331.25 ± 188.86</td>
<td>0.870</td>
</tr>
<tr>
<td>Increase in estimated urinary bag volume in Phase 3 (mL)</td>
<td>768.75 ± 128.00</td>
<td>0.892</td>
</tr>
<tr>
<td>Presence of methylene blue in the urinary catheter or bag (%)</td>
<td>100</td>
<td>n/a</td>
</tr>
</tbody>
</table>

* Kolmogorov-Smirnov Z test, † Phase 1 – 5-min interval following the placement of the urinary catheter, ‡‡ Phase 2 – 5-min interval following the perforation of the urinary bladder with the tip of a 21-gauge needle, ‡‡‡ Phase 3 – 5-min interval following the intra-abdominal administration of methylene blue.

Table 2. Comparison of the parameters at different phases of the study. The values are presented as mean ± standard deviation

<table>
<thead>
<tr>
<th>Phase 1‡</th>
<th>Phase 2‡‡</th>
<th>Phase 3‡‡‡</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO₂ use (mL)</td>
<td>0古城</td>
<td>1550.00 ± 213.81古城</td>
<td>962.50 ± 176.78古城</td>
</tr>
<tr>
<td>Estimated urinary bag volume increase (mL)</td>
<td>0古城</td>
<td>1331.25 ± 188.86古城</td>
<td>768.75 ± 128.00古城</td>
</tr>
</tbody>
</table>

* Friedman and Wilcoxon signed ranks test were used in comparison of three and two groups, respectively, † Phase 1 – 5-min interval following the placement of the urinary catheter, ‡‡ Phase 2 – 5-min interval following the perforation of the urinary bladder with the tip of a 21-gauge needle, ‡‡‡ Phase 3 – 5-min interval following the intra-abdominal administration of methylene blue. a – Phase 1 vs. Phase 2, p = 0.011. b – Phase 1 vs. Phase 3, p = 0.011. c – Phase 2 vs. Phase 3, p = 0.012. d – Phase 1 vs. Phase 2, p = 0.012. e – Phase 1 vs. Phase 3, p = 0.011. f – Phase 2 vs. Phase 3, p = 0.012.
contents of the abdominal viscera during a laparoscopic surgical procedure, including tissue debris, serum, shaped elements of the blood and clot particles, might completely occlude small perforations. Thus, interaction with contents having a higher viscosity would obscure a small perforation.

Although gas distension of the urinary drainage bag has been reported as a diagnostic symptom in the diagnosis of the iatrogenic penetrating injuries during laparoscopy [14], that statement was based on the observations encountered during some operations and on expert opinions.

Limitations of the Study

Placement of a urinary catheter may identify a urinary bladder injury which affects all the layers of the bladder wall. However, delayed burns resulting from incomplete abrasions, lacerations and cautery burns would still be missed intra-operatively. In addition, it is almost impossible to find the exact site of the injury in when the injury is very small. In the current study, although the bladder perforations were made by the authors, the exact site of the perforations was impossible to re-visualize in four (50%) of the cases. Intra-abdominal administration of methylene blue also seems unhelpful for this purpose.

Although the amounts of CO₂ consumption and accumulation in the urinary drainage bag are presented for every phase of the study, the reader should notice that the values were mostly estimated. The CO₂ consumption values were read from the insufflator device, which only showed one decimal, and the accumulated CO₂ in the urinary drainage bags was estimated by the appearance of the bags and the measurement scale printed on them.

In this study the perforations were situated on the anterior wall of the urinary bladders; it can’t be certain that the findings will be the same for perforations located on different sites of the urinary bladder and urinary tract.

Although it is reasonable to think that the placement of urinary catheters with larger diameters in human subjects will cause higher amounts of CO₂ consumption and accumulation in urinary drainage bags, the evidence presented here is based on the use of 8-gauge catheters in rabbits. A larger wall area of a hollow organ may conduct the elevated intra-abdominal pressure over a larger area and may cause easier blockage of the catheter’s flow. In addition, the tissue content and thickness of the urinary bladders of different species may result in different reactions during a perforation. Thus, the study should be reproduced in human subjects where the urinary bladders were accidently perforated.

Comparison with Previous Studies

If injuries occurring during laparoscopy are not recognized early and managed appropriately, mortality and morbidity rates increase [15]. In addition, failure to make an intra-operative diagnosis causes more severe health problems and a greater medico-legal liability, even if the injury is diagnosed post-operatively.

Most of the life threatening injuries occurring during laparoscopy involve the vascular and gastrointestinal systems, and more than one-third of the injuries occur during the insertion of the Veress needle or the primary trocar [16]. In contrast to vascular and gastrointestinal injuries, urological injuries mostly occur during gynecological surgery; the rate of bladder injuries during laparoscopic hysterectomies ranges from 0.02% to 8.3% [17].

In a study conducted by Jelovsek et al., only two out of 5 cystotomies (40%) were recognized during total laparoscopic hysterectomy procedures [18] and the authors suggested the use of a routine cystoscopy with intravenous indigo carmine to diagnose bladder and ureter injuries. However, in a previous study the use of routine cystoscopy was cost-saving only if the rate of ureteral injury exceeded 2% for laparoscopic-assisted vaginal hysterectomies [19]. In addition, cystoscopy is time consuming, and many gynecologists are not familiar with it. In the current study, although the cystotomies were performed intentionally, four of them (50%) could not be re-visualized. However, gas consumption and accumulation in the urinary drainage bags was demonstrated in all cases.

The presence of clear fluid in the operating field was considered a probable sign of urinary bladder injury [14]. However, urinary catheterization along with elevated intra-abdominal pressure may obscure the injury during laparoscopy, by pushing the produced urine into the urinary drainage bag (similar to the passage of methylene blue into the urinary drainage bags observed in the present study). In addition, leakage of urine into the abdominal cavity contaminated with blood or irrigation solution may easily be missed.

Direct inspection of the bladder walls [14], the use of methylene blue or indigo carmine diluted with 200–300 mL of sterile normal saline instilled through a Foley catheter, intentional cystotomy to inspect the internal bladder in case of doubt [20] and cystoscopy [21, 22] have been suggested for intra-operative diagnosis of urinary bladder injuries. However, they all consume time and increase costs. In addition, many surgeons do not use them in cases where there are no doubts, but unnoticed injuries are always cases where there were no doubts.
A previously published experiment evaluated the role of a rectally placed urinary catheter to diagnose iatrogenic descending colon injuries and the dynamics of CO₂ consumption and accumulation in drainage bags [23]. In that study, CO₂ consumption and accumulation in drainage bags following the perforation of the descending colon of rabbits were lower than the amounts measured in this study. However, in that study the intestines were not prepared preoperatively, and the stool content of the large bowel might have decreased the amount of CO₂ transferred from the abdominal cavity into the drainage bags. In addition, urinary catheters are prepared to fit the urethra, not the rectum, and 5 of the catheters became obstructed in that study.

In another study, methylene blue was used as a marker to detect gastric perforations of 1.2 mm and greater with or without air insufflation, and air extravasation was demonstrated with perforations of 2.0 mm or larger [24]. In the present study the specific diameters of the injuries were not measured, but a 21-gauge needle with an outer diameter of 0.8 mm was used to create the urinary bladder perforations. Although cystoscopic examinations were not performed to determine extravasation of methylene blue or air into the bladder viscera, both the gas and methylene blue were observed in the urinary drainage bags.

Possible Clinical Implications of the Study

A previous study concluded that gas distension of the urinary drainage bag was one of the signs of urinary tract injury [25] and that in the usual operative settings gas distension could not be explained without an injury. However, various publications have found that gas distension of urinary bags was not present in all cases of bladder injuries occurring during laparoscopy, even in the presence of urinary catheterization [26–28]. Thus, intra-operative conditions and variations may affect the passage of gas into the urinary bladder and drainage bags. The present study suggests that CO₂ gas is transferred into the bladder through an injury with a diameter of 0.8 mm and that the content of the abdominal viscera affects the gas passage into the bladder.

In light of these findings the authors suggest the use of a urinary catheter in all laparoscopies involving the lower abdomen, checking urinary drainage bags for distension and visualizing the contents. However, before checking the urinary drainage bags it is essential to thoroughly clear the operating field and the intra-pelvic area in order to remove any visible or invisible substances with high viscosity. Despite these precautions it is not known whether an inability to demonstrate distension of the drainage bags guarantees the integrity of the bladder wall. Until more evidence accumulates in the medical literature, it is better to follow up all patients who have undergone laparoscopy for an adequate period of time.

The authors concluded that urinary catheterization helps in the diagnosis of small or unnoticed urinary bladder injuries occurring during laparoscopy in rabbits. CO₂ flow from the intra-abdominal space into the drainage bags is less if the viscosity of the content overlying the injury site is higher.

References


Address for correspondence:
Kahraman Ülker
Kafkas Üniversitesi Tip Fakultesi
Kadin Hastalıkları ve Doğum Anabilim Dali
36000 Kars
Turkey
Tel.: +90 50 55 70 05 74
E-mail: kahramanulker@hotmail.com

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