# Early laparoscopic cholecystectomy is associated with less risk of complications after the removal of common bile duct stones by endoscopic retrograde cholangiopancreatography

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## ABSTRACT

**Background/Aims:** *Several studies recommend prompt laparoscopic cholecystectomy (LC) following endoscopic retrograde cholangiopancreatography (ERCP) for choledocholithiasis. However, histopathological alterations in the gallbladder during this time interval and the role played by ERCP in causing these changes have not been sufficiently elucidated. To compare early period LCs with delayed LCs following common bile duct stone extraction via ERCP with regard to operation time, hospitalization period, conversion to open cholecystectomy rate, morbidity, mortality, and histopathological alterations in the gallbladder wall.*

**Materials and Methods:** *A total of 85 patients were retrospectively divided into three groups: early period LC group (48-72 h; n=30), moderate period LC group (72 h-6 weeks; n=25), and delayed period LC group (6-8 weeks; n=30).*

**Results:** *The operation time was significantly shorter, and the total number of complication rates and hospital readmission was significantly less frequent in the early period LC group (p<0.05). Ultrasound showed a significantly thicker gallbladder wall (>3 mm) in the moderate and late period LC groups than in the early period LC group (p<0.001). Culture growth was significantly higher, and fibrosis/collagen deposition in the gallbladder wall with injury to the mucosal epithelium was significantly more frequently detected by histopathological examination in the moderate and late period LC groups than in the early period LC group (p<0.05).*

**Conclusion:** *Early period LC following stone extraction by ERCP is associated with shorter operation time, fewer fibrotic changes in the gallbladder, and lower risk for the development of complications. Therefore, LC can be performed safely in the early period after ERCP.*

**Keywords:** *Endoscopic retrograde cholangiopancreatography, endoscopic sphincterotomy, fibrosis, laparoscopic cholecystectomy*

## INTRODUCTION

Laparoscopic cholecystectomy (LC) is the gold standard for treatment of symptomatic cholelithiasis (1-6). Recently, the early time period for LC following stone extraction via endoscopic retrograde cholangiopancreatography (ERCP) has become accepted as superior to the late time period LC in terms of conversion rates, bile duct injury, deep surgical wound infections, length of hospital stay, and cost (7-11). However, histopathological alterations that occur in the gallbladder during this time interval and the role played by ERCP in causing these changes and predicting surgical outcomes have not been sufficiently elucidated (11-13).

Macroscopic changes in the gallbladder, such as adhesion, edema, necrosis, gangrene of the gallbladder, and epithelium injury, occur due to inflammation and fibrosis (13). Fibrosis can be explained as an overgrowth of various tissues and is formed by the excessive deposition of the extracellular matrix (ECM) constituents, including collagen (13,14). Furthermore, fibrosis in the biliary ducts occurs due to chronic inflammatory reactions induced by gallstones, persistent infections, autoimmune reactions, allergic responses, radiation, contrast agents, and tissue injury (14-17). Microscopic assessment of injuries to the epithelium, collagen deposits, polymorphonuclear leukocytes (PMNL), and mononu-

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clear leukocytes (MNL) is important to evaluate the clinical situation (18,19).

The aim of the present study was to compare early LCs following stone extraction via ERCP with delayed LCs with regard to operation time, hospitalization period, conversion rate to open cholecystectomy (OC), morbidity, mortality, and histopathological alterations in the gallbladder.

## MATERIALS AND METHODS

### Patients and ethics

Ethical approval was obtained from the Ethics Committee of Sakarya University (study protocol no. 189.2016). A total of 120 patients with common bile duct stones who underwent LC after removing the stones via ERCP between January 2015 and May 2016 were retrospectively reviewed.

### Methods

A total of 120 patients, treated for choledocholithiasis, were enrolled in this study. All patients underwent LC following gallstone extraction via ERCP. Thirty-five patients were excluded from the study. The remaining 85 patients were divided into the following three groups: Group 1: early period LC (48-72 h) following ERCP (n=30), Group 2: moderate period LC (72 h-6 weeks) following ERCP (n=25), and Group 3: delayed period LC (6-8 weeks) following ERCP (n=30).

Figure 1 shows the flowchart of the study. The three groups were compared statistically in terms of gender, age, body mass index (BMI), operation time, adhesion level (grade), postoperative length of hospital stay, conversion rate to open surgery, biliary duct injury, deep and superficial site infections, and histopathological parameters, such as PMNL and MNL levels, collagen deposition, and damage to the epithelium of the gallbladder. After discharge from the hospital, patients were followed up at 1-week, 1-month, and 6-month intervals.

### Inclusion criteria

Patients >18 y and those with choledocholithiasis who underwent ERCP followed by LC were included in the study.

### Exclusion criteria

Patients <18 y and those with acute cholangitis, severe pancreatitis and post-ERCP pancreatitis, malignancy, or failed ERCP and with immunosuppression, pregnancy, multiple organ failure, or bile duct injuries during ERCP were excluded from the study.

### ERCP procedure

Before the ERCP procedure, all of the patients were evaluated by one or more of the non-invasive techniques demonstrating the biliary system, such as ultrasonography, computed tomography, and magnetic resonance cholangiography. ERCP was performed for therapeutic purposes rather than for diagnostic in all attempts. None of the patients received any antiplatelet agents or anticoagulants before the procedure. Topical pharyngeal anesthesia was administered with 10% lidocaine spray. The ERCP procedure was performed using a sedoanalgesia protocol containing midazolam, fentanyl, and propofol and administered by an anesthetist. Hyoscine N-butylbromide was used to slow intestinal motility. The doses of all medications were titrated according to patient need as well as duration of the procedure. All patients were positioned on the left side as per standardization techniques. The ERCP procedures were performed by using standard video duodenoscopes with a 4.2 mm diameter accessory channel (Fujinon, Japan). Patients with cholangitis received intravenous antibiotics for 3 days and maintained orally afterward. Biliary cannulation was first attempted by using the “wire-guided” cannulation technique. Precut papillotomy, infundibulotomy, or transpancreatic septotomy was used otherwise. Cannulation was followed by endoscopic sphincterotomy (ES) in all the patients. If ES was not large enough for stone extraction, the papilla was dilated with a balloon, selected according to the size of the overlying common bile duct. Stones were extracted by using an extraction balloon and/or a basket. Mechanical lithotripsy was performed when necessary. Complete stone extraction was defined as clearance of biliary stones as confirmed by balloon occluded cholangiography.

### Surgical procedure

Laparoscopic cholecystectomy is performed routinely using a four-trocar approach. After anesthesia, a $CO_2$ pneumoperitoneum was established using a Veress needle and maintained at a pressure of 12 mmHg. A camera was inserted via a 10-mm-port after insufflations with $CO_2$. Other trocars were inserted under direct vision (10-mm-port 2 cm below the Xiphoid process, 5-mm-trocar to the midclavicular line, and 5-mm-trocar to the anterior axillary line). Patients were placed in the left reverse Trendelenburg position. The gallbladder fundus was identified, grasped, and retracted superiorly. The cystic artery and duct were clipped (two distally and one proximally) and cut after gentle and meticulous dissection of Calot's triangle. Then, the gallbladder was dissected away from the liver bed and removed through the epigastric port. After hemostasis, a drain was inserted under the cholecystectomy area in all patients. The trocars were removed under direct visualization, and the trocar incisions were sutured with a 3/0 propylene. If there was no sign of bleeding, the drain was removed on postoperative day 1.

### *Severity of adhesion*

The severity of adhesion was scored as per the following grading scale as suggested by Ercan et al. (18). Grade 1, no adhesions; Grade 2, flimsy adhesions that allow easy dissection; Grade 3, severe adhesions encasing the gallbladder, including fibrosis, that makes dissection difficult; and Grade 4, severe adhesions covering other strictures, such as the duodenum or colon, which do not permit a safe dissection and lead to conversion to OC.

### *Histopathological examination*

Pathological samples were obtained from the corpus, fundus, and neck of the gallbladder and fixed with 10% paraformaldehyde for at least 24 h. After standard tissue processing using the Leica ASP300 Automated Vacuum Tissue Processor, sections of 4 μm from the Leica RM2245 microtome were examined via light microscopy. Samples were stained with hematoxylin and eosin and examined by the same pathologist under a light microscope. Specimens were evaluated to assess inflammation and degree of fibrosis using a modified Verhofstad histopathological scoring system (19) by the same pathologist who was blinded to the patients and groups. The results obtained from this assessment (PMNL, MNL, damage to the mucosal epithelium, and collagen deposition level) were scored on a scale of 0 to 4 and compared with the median average of the scores. Additionally, the total score (PMNL+MNL+injury of the epithelium+collagen deposition scores) was calculated and histopathologically compared in all groups. The total score of histopathological parameters used the grading scale proposed by Verhofstad et al. (19). According to this scale, a total score of grade 0 is the total of the individual scores 0, 1, 2, and 3; a total score of grade 1 is the total of the scores 4, 5, and 6; a total score of grade 2 is the total of the scores 7, 8, and 9; and a total score of grade 3 is the total of the scores 10, 11, and 12.

### *Statistical analysis*

The Kolmogorov-Smirnov test was used to determine whether the distributions of continuous variables were normal. Levene's test was used for the evaluation of the homogeneity of variances. Categorical data were expressed as numbers and percentages. Descriptive statistics for continuous variables were expressed as mean±SD or median ($25^{th}$-$75^{th}$ percentiles), where applicable.

The mean differences among groups were analyzed using a one-way ANOVA, and the Kruskal-Wallis test was applied for comparisons of data with non-normal distribution. When the p-value from the Kruskal-Wallis test statistics was statistically significant, Conover's multiple comparison test was used to determine the group that differed from the others. Categorical data were analyzed using Pearson's chi-square or the likelihood ratio test, when appropriate. When the p-value from the likelihood ratio test was statistically significant, the continuity corrected chi-square test or Fisher's exact test was performed to determine the group that differed from the others.

Data analysis was performed using the Statistical Package for Social Sciences version 17.0 software (SPSS Inc.; Chicago, IL, USA). A p-value <0.05 was considered statistically significant.

## *RESULTS*

### *Perioperative outcomes*

Eighty-five patients were enrolled in the study, with 35 patients excluded for failing to meet the inclusion criteria. The mean ages were 56.2±17.3, 49.8±16.6, and 51.3±13.6 y for the early, moderate, and delayed period LC groups, respectively (p=0.294). A total of 47 female and 38 male patients were included in the study (p=0.736). The mean BMIs were 25.4±4.2, 25.9±4.2, and 26.5±4.3 kg/m$^2$ in the early, moderate, and late period LC groups, respectively (p=0.603). There was no significant difference in preoperative comorbidities, such as hypertension, diabetes mellitus, coronary artery disease, or chronic obstructive pulmonary disease (p>0.05; Table 1).

All common bile duct stones were completely removed by ERCP. Biliary plastic stent (10 French) was inserted in three patients. The biliary stent was inserted in two patients for benign biliary structure that developed secondary to acute pancreatitis and in the remaining one patient for bleeding after sphincterotomy. Prophylactic pancreatic stents (5 Fr) were inserted in nine patients.

In the moderate and late period LC groups, five patients had a history of laparoscopy or laparotomy, but there was no statistically significant difference in preoperative operation history (0.147). The mean operation times were 60 (60-72.5), 85 (65-110), and 85 (60-101.2) min in the early, moderate, and late period LC groups, respectively. Although the operation time for the early period LC group was significantly shorter than that for the moderate and late period LC groups (p=0.003), there was no significant difference between the groups for the hospitalization period (p=0.332; Table 1).

Conversion to OC occurred in one patient of the early, in four patients of the moderate, and in five patients of the late period LC groups (p=0.150). A laparoscopic subtotal cholecystectomy was performed in two patients of the moderate period LC group. The median intraoperative adhesion grade was lower in the early period LC

**Table 1.** Characteristics of the patients

| | Early group (n=30) | Moderate group (n=25) | Delayed group (n=30) | p |
|---|---|---|---|---|
| Age (years) | 56.2±17.3 | 49.8±16.6 | 51.3±13.6 | 0.294† |
| Female | 18 (60.0%) | 14 (56.0%) | 15 (50.0%) | 0.736‡ |
| BMI (kg/m$^2$) | 25.4±4.2 | 25.9±4.2 | 26.5±4.3 | 0.603† |
| HT | 6 (20.0%) | 6 (24.0%) | 9 (30.0%) | 0.665‡ |
| DM | 3 (10.0%) | 2 (8.0%) | 2 (6.7%) | 0.895¶ |
| CAD | 0 (0.0%) | 1 (4.0%) | 3 (10.0%) | 0.113¶ |
| COPD | 3 (10.0%) | 2 (8.0%) | 1 (3.3%) | 0.559¶ |
| Preop laparotomy/ laparoscopy | 2 (6.7%) | 0 (0.0%) | 3 (10.0%) | 0.147¶ |
| Operation time (min) | 60 (60-72.5)[a,b] | 85 (65-110)[a] | 85 (60-101.2)[b] | 0.003$ |
| Intra-op adhesion grade | 1 (0-3) | 2 (1-3) | 2 (1-3) | 0.066$ |
| Hospitalization (days) | 4 (3-7) | 3 (2-9.5) | 3 (2-6) | 0.332$ |

BMI: Body mass index; HT: Hypertension; DM: Diabetes mellitus; CAD: Coronary artery disease; COPD: Chronic obstructive pulmonary disease; Preop: Preoperative
†One-Way ANOVA; ‡Pearson's Chi-square test; ¶Likelihood Ratio test; $Kruskal Wallis test; [a]Group I vs Group II (p<0.05); [b]Group I vs Group III (p<0.01)

**Table 2.** Perioperative features and comparison of the groups according to the interventions

| | Early group (n=30) | Moderate group (n=25) | Delayed group (n=30) | p |
|---|---|---|---|---|
| Conversion to open cholecystectomy | 1 (3.3%) | 4 (16.0%) | 5 (16.7%) | 0.150† |
| Laparoscopic subtotal cholecystectomy | 0 (0.0%) | 2 (8.0%) | 0 (0.0%) | 0.082† |
| Biliary tract damage | 0 (0.0%) | 2 (8.0%) | 2 (6.7%) | 0.163† |
| Superficial wound infection | 1 (3.3%) | 1 (4.0%) | 3 (10.0%) | 0.506† |
| Deep wound infection | 0 (0.0%) | 2 (8.0%) | 3 (10.0%) | 0.101† |
| Incisional hernia | 0 (0.0%) | 1 (4.0%) | 1 (3.3%) | 0.410† |
| Reproduction in culture | 0 (0.0%)[a] | 3 (12.0%) | 5 (16.7%)[a] | 0.022† |
| Preoperative gallbladder USG | | | | |
| Effusion | 0 (0.0%) | 2 (8.0%) | 2 (6.7%) | 0.180† |
| Increased wall thickness | 7 (25.0%)[a,b] | 17 (68.0%)[b] | 23 (76.7%)[a] | <0.001‡ |
| Hydropic gallbladder | 6 (21.4%) | 10 (40.0%) | 13 (43.3%) | 0.177† |
| Post-op ERCP | 2 (6.7%) | 6 (24.0%) | 5 (16.7%) | 0.179† |
| Hospital readmission | 0 (0.0%)[b] | 4 (16.0%)[b] | 4 (13.3%) | 0.024† |
| Number of total complications | 0.10±0.30[a,b] [3] | 1.00±1.47[b] [25] | 0.93±1.59s [28] | 0.008¶ |
| Complication | | | | 0.012† |
| Absent | 27 (90.0)[a,b] | 14 (56.0)[b] | 19 (63.3)[a] | |
| Present | 3 (10.0)[a,b] | 11 (44.0)[b] | 11 (36.7)[a] | |

†Likelihood Ratio test; ‡Pearson's Chi-square test; ¶Kruskal Wallis test; [a]Group I vs Group III (p<0.05); [b]Group I vs Group II (p<0.05); OC: Open cholecystectomy; SC: Subtotal cholecystectomy; USG: Ultrasonography

group than in the moderate and late period LC groups (grade 1=0-3, grade 2=1-3, and grade 2=1-3, respectively), but there was no significant difference (p=0.066). The multi-ERCP history was significantly higher in patients of the moderate and late period LC groups than in those of the early period LC group (p=0.003; Table 2). Ultrasound showed a significantly thicker gallbladder wall (>3 mm) in the moderate and late period LC groups than in the early period LC group (p<0.001; Table 2).

### ***Morbidity, mortality, and complications***

Mortality did not occur. However, a lower incidence of biliary tract injuries, deep and superficial wound infections, incisional hernias, and bacterial culture growth was observed in the early period LC group than in the moderate or delayed period LC groups. The frequency distributions of biliary tract injuries were 0 (0.0%), 2 (8.0%), and 2 (6.7%) in the early, moderate, and late period LC groups, respectively (p=0.163). The distributions of superficial wound infections were 1 (3.3%), 1 (4.0%), and 3 (10.0%) in the early, moderate, and late period LC groups, respectively (p=0.506). The distributions of deep wound infection were 0 (0.0%), 2 (8.0%), and 3 (10.0%) in the early, moderate, and late period LC groups, respectively (p=0.101). There was no significant difference among the three groups related to incisional hernia (p=0.410; Table 2). Hemorrhage, need for reoperation, acute myocardial infarction or coronary injury, cerebrovascular disease, or pulmonary emboli did not occur.

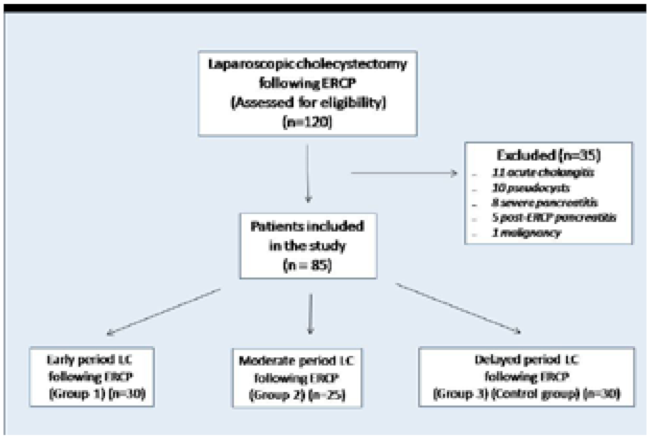


**Figure 1.** Study flowchart

Overall, a higher incidence of complications and higher complication rates were observed in the moderate and late period LC groups than in the early period LC group. Additionally, a statistically significant difference was detected in the delayed period LC group for the rate of at least one complication (p=0.012). The moderate and delayed period LC groups were also associated with a significantly higher incidence of culture growth (p=0.022) and hospital readmission (p=0.024) than the early period LC group, respectively (Table 2).

### ***Histopathological results***

The specimens were evaluated to assess inflammation and degree of fibrosis using a modified Verhofstad histopathological scoring system. The median PMNL and MNL infiltration scores were 1 (0-2) and 1 (1-2), respectively, in the early period LC group; 1 (0-2) and 1 (0-2), respectively, in the moderate period LC group; and 1 (0-2) and 1 (1-2), respectively, in the delayed period LC group, with no significant difference among the three groups (p>0.05).

The score related to damage to the mucosal epithelium was significantly lower in the early period LC group [1 (0-1)] than in the moderate [2 (1-2)] and late period LC groups [2 (1-2.25); p=0.002]. The median collagen deposition in the gallbladder wall was also significantly higher in the moder-

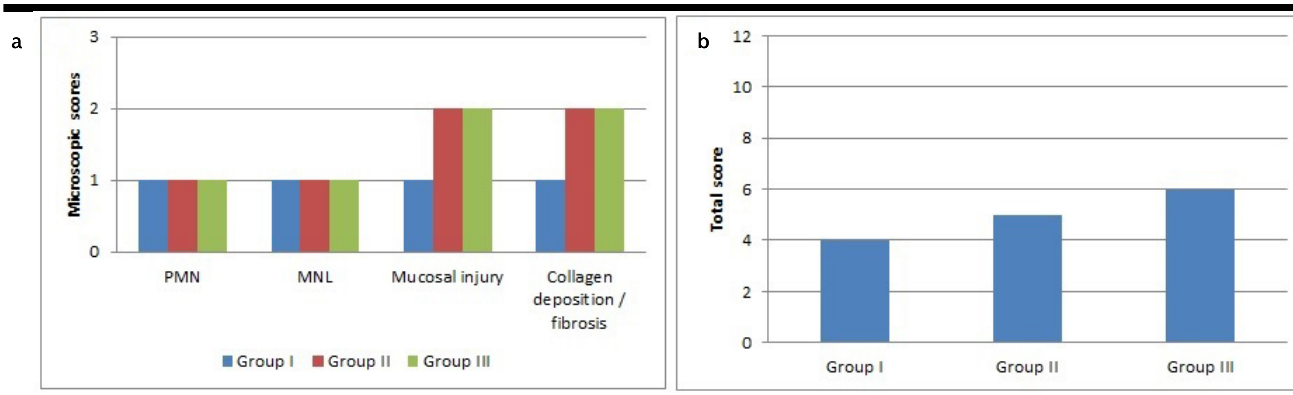


**Figure 2.** Distributions of microscopic alterations (a) and total scores in groups (b)

ate and late period LC groups than in the early period LC group (p=0.031). Additionally, the median total score (including PMNL and MNL infiltration, injury to the mucosal epithelium, and fibrosis/collagen deposition) was significantly lower in the early period LC group [4 (2.75-6)] than in the moderate [5 (2.5-8.5)] and late period LC groups [6 (4-8); p=0.036; Figure 2a and 2b; Table 3].

There was no significant difference between patients with biliary injury and those without injury regarding median PMNL infiltration score, damage to the mucosal epithelium, and collagen deposition (p>0.05). Moreover, patients with biliary injury had significantly higher median MNL infiltration score (p=0.014) and total scores (p=0.017).

There was no significant difference between patients with deep surgical site infection and those without regarding median PMNL infiltration score, damage to the mucosal epithelium, total score, or collagen deposition (p>0.05). However, patients with deep surgical site infection had higher median MNL scores, and the difference was significant (p=0.046).

In the early period LC group, there were well-developed glandular branches in the epithelium, and the gallbladder cells had a high columnar appearance. No collagen deposition was detected in the subepithelial area, and the lymphocyte count in the lamina propria was within the normal ranges (Figure 3). In the delayed period LC group (Group 3), atrophy and intensive inflammatory cell infil-

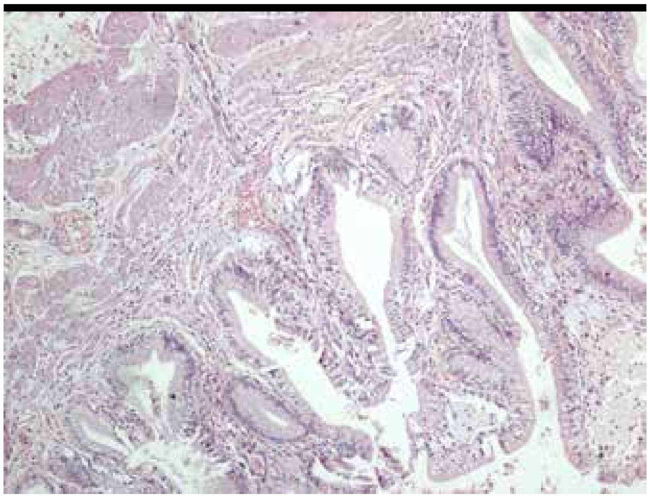

**Figure 3.** Absence of collagen deposition and lymphocytes in the lamina propria in the early period LC group (hematoxylin and eosin; H&E×100)

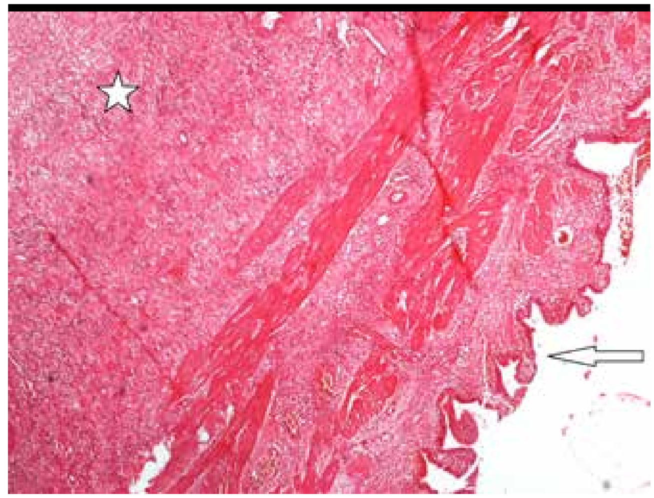

**Figure 4.** Widespread collagen deposition in the delayed period LC group (arrow: gallbladder epithelium, star: submucosal collagen deposition; H&E×40)

**Table 3.** Histopathological alterations according to the groups

| | Early group (n=30) | Moderate group (n=25) | Delayed group (n=30) | p |
|---|---|---|---|---|
| PMNL infiltration | 1 (0-2) | 1 (0-2) | 1 (0-2) | 0.585† |
| MNL infiltration | 1 (1-2) | 1 (0-2) | 1 (1-2) | 0.350† |
| Injury on the epithelium | 1 (0-1)a,b | 2 (1-2)a | 2 (1-2.25)b | 0.002† |
| Collagen deposition/fibrosis | 1 (0-2)a,b | 2 (1-3)a | 2 (1-3)b | 0.031† |
| Total score | 4 (2.75-6)b | 5 (2.5-8.5) | 6 (4-8)b | 0.036† |
| Grade 0 | 13 (43.3) | 9 (36.0) | 5 (16.7) | 0.074$ |
| Grade 1 | 13 (43.3) | 5 (20.0) | 12 (40.0) | 0.157$ |
| Grade 2 | 4 (13.3) | 7 (28.0) | 8 (26.7) | 0.335$ |
| Grade 3 | 0 (0.0)a,b | 4 (16.0)a | 5 (16.7)b | 0.015‡ |

PMNL: Polymorph nuclear leucocytes; MNL: Mononuclear leukocyte
†Kruskal Wallis test, $Pearson's Chi-square test, ‡Likelihood Ratio test, aGroup I vs Group II (p<0.05); bGroup I vs Group III (p<0.01)

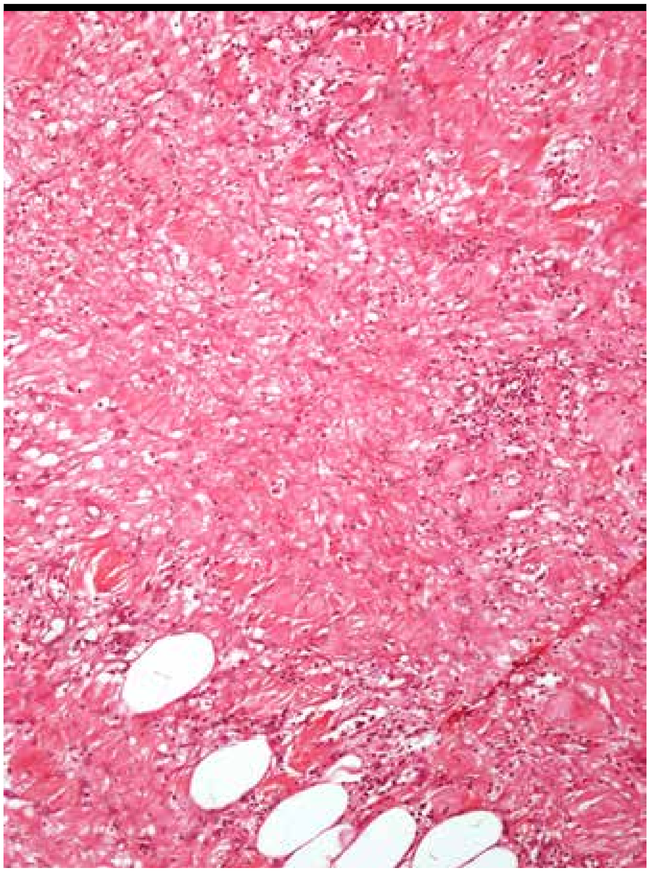

**Figure 5.** Close view of submucosal collagen deposition (H&E×100)

tration were detected in the epithelium of the gallbladder. Furthermore, widespread collagen deposition was observed in its subepithelial layer (Figures 4 and 5).

## DISCUSSION

Several studies recommend prompt LC following ERCP for choledocholithiasis and biliary pancreatitis with the aim of decreasing the risk of recurrent pancreatitis, morbidity, and other biliary complications (recurrent common bile duct stones and acute cholecystitis) (1-3). Nevertheless, recurrent biliary complications occur in up to 20% of patients during the interval between these operations (3,4). Most studies have reported that the early period LC following ERCP is optimal, is cost effective, and reduces morbidity because a delayed LC is challenging due to fibrotic changes, adhesions, and scarring of the gallbladder that occur over time (4-6). Lau et al. (7) and Reinders et al. (8) recommended early elective LC after ERCP for common bile duct stones because early period LC is safer and reduces recurrent biliary-related events. Additionally, Sahu et al. (9) reported a favorable outcome and less expense for early versus delayed LC following ERCP. Salman et al. (10) suggested early LC after ERCP for choledocholithiasis because delayed LC was associated with a longer operation time and postoperative hospital stay, more frequent conversion to open surgery, and postoperative complications. The results of these studies indicated that early LC is superior to late LC. Furthermore, Coppola et al. (11) suggested LC 'as soon as possible' after ERCP, and Costi et al. (12) reported that most patients (79%) undergo LC within 5 to 10 days after ERCP, with times ranging from 1 to 49 days.

In the present study, our findings were similar to those of the above-mentioned literature. When comparing demographic and clinical characteristics, such as mean age, gender, BMI, and comorbidities, there were no statistically significant differences; therefore, the outcome assessment was almost standard for all groups. However, the mean operation time was significantly shorter in the early period LC group than in the moderate and delayed period LC groups. Furthermore, a higher intraoperative adhesion grade and higher rates of conversion to OC, subtotal cholecystectomy, biliary tract damage, superficial wound infection, deep wound infection, development of incisional hernia, and requirement of postoperative ERCP were more frequently observed in the moderate and delayed period LC groups than in the early period LC group. Moreover, the total number of complications and complication rates, hospital readmission, and culture growth was significantly higher in the moderate and delayed period LC groups than in the early period LC group.

A number of inflammation processes that follow flesh injuries can restore tissue integrity and facilitate wound healing (13). When the epithelial and/or endothelial cells are damaged, they release inflammatory mediators, and the inflammation process, including vasoconstriction, hemostatic plug, thrombus, and formation of the provisional ECM, begins (13). Early responders to inflammation, such as PMNLs and monocytes, which are a type of MNL that generally transform into macrophages at the site of an injury, produce cytokines and chemokines in 2 to 3 days, and eliminate tissue debris and dead cells. PMNLs and MNLs produce cytokines, growth factors, and chemokines that lead to the activation of macrophages and fibroblasts (which later transform into myofibroblasts) (14). Four days after the injury, the most proliferative cells are the fibroblasts that produce a collagen-based matrix, and wound contraction is stimulated by the myofibroblasts (15). Finally, fibrosis occurs due to persistent infections, chemical agents, contrast agents, radiation, and tissue injuries caused by myofibroblasts that produce an excess deposition of EMC including collagen (15-17,19).

Polymorphonuclear leukocytes and MNL infiltration, collagen deposition, and damage to the epithelium are histopathological markers that indicate inflammation and fibrosis at the injury site. Damage to the epithelium indicates injury as irritation develops in the mucosa over time (16,17). The level of collagen deposition is an easy, simple, and repeatable indicator that demonstrates the level of fibrosis, and there is a small difference between the pathologists' views on the effect of collagen deposition (15,17). In our study, PMNL and MNL infiltration results were similar in all groups, and there were no statistically significant differences in terms of these parameters. Therefore, all three groups were almost homogenized or randomized in terms of the inflammation level. However, collagen deposition and damage to the mucosal epithelium were detected more in the moderate and delayed period LC groups than in the early period LC group. These results indicate that over a period of time, inflammation and the use of a contrast agent may increase the formation of fibrosis after ERCP.

The use of a contrast agent during ERCP is necessary to assess the anatomy of the biliary tree, the formation of stones, and the existence of malignant tumors (5,18,19). Indeed, the ERCP process itself can cause damage to the biliary system, and procedures, such as papillary cannulation, sphincterotomy, or balloon dilatation, can lead to post-ERCP pancreatitis, bleeding, and perforation (20). Interestingly, the contrast agent may also directly trigger periportal inflammation and fibrosis over time (21). Acute pancreatitis occurs at a rate of 5% to 7% after ERCP with EST (21,22). A systematic review and meta-analysis by Tse et al. (22), including 12 randomized controlled trials (3450 patients), reported that a guidewire-assisted cannulation technique significantly reduces post-ERCP pancreatitis compared with contrast-assisted cannulation. Therefore, patients with post-ERCP pancreatitis associated with contrast agents may have impaired surgical outcomes. Salman et al. (10) reported that early period LC following ERCP (within 72 h) has better clinical outcomes, probably due to the inflammatory processes. Furthermore, Ghnnam et al. (23), comparing early LC versus delayed LC following ERCP, showed that delayed LC involves a longer operation time due to scarring and fibrosis in the biliary tree and Calot's triangle. Additionally, the use of a contrast agent during ERCP may escalate the inflammatory process due to bacterial colonization that may eventually result in the formation of fibrosis and adhesion at Calot's triangle (24). Schiphorst et al. supported this mechanism of bacterial colonization, reporting that bacteria are isolated from the bile in 60% of patients who underwent ERCP with EST (25). In the present study, bacterial culture growth was found to be higher in the delayed period LC group than in the early period LC group.

Our study has some limitations. First, this is a retrospective study that comprises a small sample size. Second, there is not enough information about the amount of contrast agent and contrast passage to the gallbladder during ERCP. Finally, to the best of our knowledge, it is the first study to evaluate the histopathological alterations to the gallbladder that develop over time following ERCP.

In conclusion, early period LC following stone extraction by ERCP is associated with low incidence of fibrosis. It can be performed safely in 48 to 72 h. In contrast, late LC surgery can be more difficult to perform and brings a higher probability of complications and hospital readmission due to fibrotic and inflammatory alterations.

***Ethics Committee Approval:*** Ethics committee approval was received for this study from the Ethics Committee of Sakarya University (Study Protocol Number: 189.2016).

***Informed Consent:*** Written informed consent was obtained from the patients who participated in this study.

***Peer-review:*** Externally peer-reviewed.

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