A comparative study between two techniques of subtotal laparoscopic cholecystectomy in goats

[1] M. A. Alkhilani; [2] M. J. Eesa; [2] S. I. Saleh; [3] A. A. Ajeel

[1] Ministry of Agriculture, Iraq.

[2]Department of Surgery and Obstetrics, College of Veterinary Medicine, Baghdad University, Iraq.

[3]Department of Surgery and Obstetrics, College of Veterinary Medicine, Almothana University, Iraq.

E-mail: mohammed2006_eesa@yahoo.com

Accepted on: 12/8/2014

Summary

A comparison between two techniques of subtotal laparoscopic cholecystectomy was studied to find the best technique for treating severe cholecystitis and to study the fate of the remaining part of the gallbladder. All experimental animals had inducing cholecystitis by clipping the cystic duct for five days which was enough for inducing cholecystitis in goats. 24 adult female goats were used in this study. The goats were divided randomly into two equal groups; subtotal laparoscopic cholecystectomy with cystic duct and cystic artery clipping by titanium clips (group A) and laparoscopic subtotal cholecystectomy with cystic duct clipping only (group B). Operations were performed under general anesthesia by using thiopental sodium at a dose of 15 mg/Kg. B.W. intravenously to maintain the inhalation anesthesia by endotracheal tube with a mixture of halothane (1.5- 2.5 %) and oxygen (2-3 %). The intraabdominal pressure with CO_2 was used at a low pressure 8-10 mmHg. The liver function tests included; alkaline phosphates (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total serum bilirubin and C- reactive protein were measured in different intervals; before operation (zero day) and at 1hr, 1st, 3rd, 5th and 7th postoperative day. The liver function tests showed a significant elevation ($P< 0.05$) in alkaline phosphates (ALP) and in the total bilirubin values in group B ($P> 0.05$) comparison with group A and no significant difference in alanine aminotransferase (ALT), aspartate aminotransferase (AST) and C- reactive protein between all experimental groups as well as time intervals. The biopsies were taken at the 3rd, 7th and 15th postoperative day for histopathological of the remaining part of gallbladder which showed that subtotal cholecystectomies could be performed successfully with the two techniques. Degeneration appeared in the remaining part of the gallbladder and offered best in the healing stages.

Keywords: Laparoscopic cholecystectomy, liver, subtotal, goats.

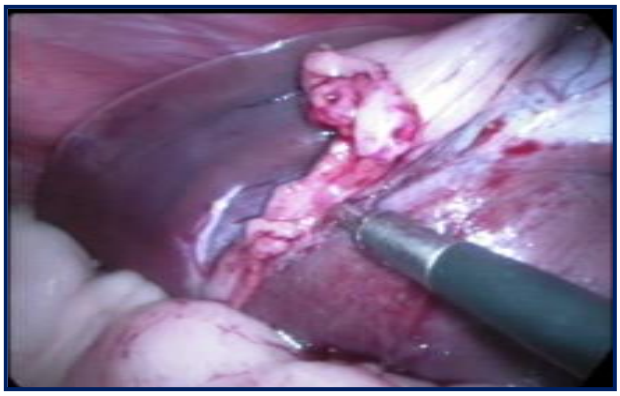
Introduction

When conventional laparoscopic cholecystectomy is not possible, laparoscopic subtotal cholecystectomy is available and makes acceptable alternative to conversion to open cholecystectomy. It avoids the risk of major bile duct injury, and compared with studies of patients undergoing conversion to open cholecystectomy, demonstrates a reduce mortality as well as reduce incidence of wound infections and incision hernia**.** Laparoscopic subtotal cholecystectomy, performed when the cystic duct cannot be identified safely, consists of resecting the anterior wall of the gallbladder and removing all stones (1). Laparoscopic subtotal cholecystectomy introduction can prevent misidentification of the bile duct, whereby injury to the bile duct or other organs is avoided. For these reasons, they attribute the decreased incidences of bile duct injury and conversions to open surgery at their institution, to the introduction of laparoscopic subtotal cholecystectomy (2). Although the laparoscopic subtotal cholecystectomy is commonly performed today, and often substitute for the conversion of conventional laparoscopic cholecystectomy to open cholecystectomy, but the fate of the remaining posterior part of the gallbladder which is attached to the liver is still unknown by researchers; whether is it showed fibrosis or necrosis and may be becomes focus of infection, and no information about its impact on patient's health was found. It was compared two surgical techniques in laparoscopic

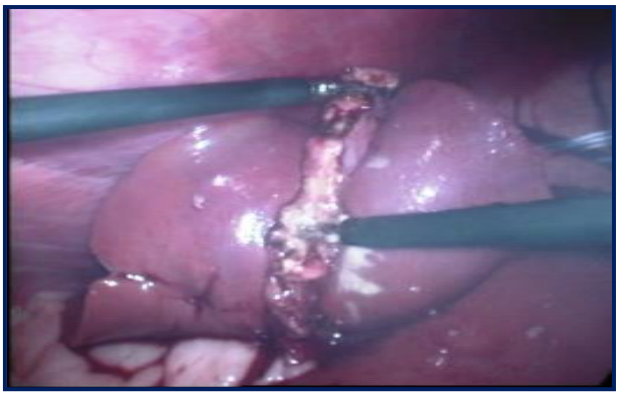
subtotal cholecystectomy which were included clipping of cystic duct and artery in first one and clipping of cystic duct only in the second to find the better technique with best postoperative results. To follow the fate of remaining part of cholecystectomized gallbladder clinically macroscopically, and histopathologically at different periods postoperatively.

Materials and Methods

Twenty- four healthy adult Indagenus female goats, aged between 2.5 to 4 years, and weighed (37 to 45 kg.) were used. The animals were divided randomly into two equal groups; A and B, each group divided into three subgroups according to the period of 3, 7 and 15 days postoperatively to take biopsies for histopathological examination. The animals fasted for 36 hours of food and 12 hours water preoperatively. The treated animals were put under observation postoperatively. In all 24 goats, Cholecystitis was induced experimentally by laparoscopic clipping of cystic duct by titanium clips after identification. At the 5^{th} day post inducing cholecystitis the animals performed laparoscopic subtotal cholecystectomy. Laparoscopic inspection and histopathological examination used as parameters. Thiopentan sodium at a dose of 15 mg/kg B.W. intravenously was used to facilitate introducing endotracheal tube for inhalation anesthesia, which is the method of choice for maintaining anesthesia for prolonged procedure (3). Pneumoperitoneum with CO_2 (8-10 mmHg) was performed in all experimental animals. The subtotal cholecystectomy was begun by clipping the cystic duct and cystic artery in group A, and cystic duct in group B. Then the gall bladder was punctured at the fundus with monopolar electrocautery hook and the suction-irrigation apparatus was used to evacuate the contents. The gallbladder was pulled cranially and laterally, Calot`s triangle and its structure (cystic duct, cystic artery and inferior border of the liver) were put under tension. Subtotal cholecystectomy was performed by leaving the posterior wall of the gall bladder which attached to the liver intact. The incision was performed adjacent to the liver and carried out around to the posterior wall of the gallbladder both incisions were meeting medially (Fig. 1). Any bleeding at the margin borders was coagulated with electrocautery. The mucosa of remnant part of gallbladder was cauterized with spatula to destruct the mucosa and to prevent any secretions (Fig. 2). At the end of operation instruments was removed and abdominal was closed routinely.



Figure, 1: Gall bladder dissection started from the neck upward, in subtotal Cholecystectomy



Figure, 2: mucosal cauterization by hook.

Food and water were free to record the time of returning to eat and drink post operation. Penicillin streptomycin in a dose 20000 I.U., and 10 mg/Kg B.W. respectively, was administered intramuscularly for four days post-operatively. Rectal temperature, respiratory and heart rate were monitored once daily for seven days postoperatively. Skin sutures were removed ten days post-operatively. Biopsies about 1 square cm^2 were taken laparoscopically at the third, seventh and fifteenth day postoperatively. At the same time the abdominal cavity was inspected laparoscopically to watch any pathological changes. The specimens were fixed in 10%

neutral buffered formalin, then routinely processed and embedded in paraffin which were cut at (5-6) microns and stained by hematoxyline and eosin stain (4 and 5) and they were examined under light microscope. The operation periods were depended on the severity of the cholecystitis and other complications occurred during the operations, such as bleeding and visceral injury. Results underwent statistical analysis using Chi Square and analysis of variance (ANOVA). Least significant difference (L.S.D) was also used for the purpose of differentiating between the means of each group and at the different periods. SPSS statistical program was used also (6).

Results and Discussions

In inducing cholecystitis the macroscopic and microscopic result showed that five days period was enough for inducing mild cholecystitis. The mean operation time was 67 min. The treated animals had returned to eat and drink within a short time post recovery from anesthesia and with normal activity. This indicated less postoperative pain. No significant changes occurred in physiological activities post operations. Two animals one of each group (8.3%) had shown pain few days postoperatively. This might be due to the adhesion occurring between gallbladder, adjacent omentum and viscera. Pneumoperitoneum with CO_2 was successfully produced in experimental goats with minimal complications such as that which occurred at the beginning of the experiment that few cases showed pneumo-omentum at the onset of the insufflation. Pneumo-omentum at the onset of the insufflation coincided with research (6) who found that when Veress needle placed blindly, there is a potential for its misplacement in the subcutaneous space, vascular space, viscera, omentum and mesentery or retroperitoneal. These complications could occur in humans as mentioned by (7) who found that the overall incidence of operative complications was 22 from 350 (6.29%), among them three of pneumo-omentum were related to inappropriate CO_2 insufflation. The low intra-abdominal pressure used in present study (8-10 mm Hg) was enough for a good manipulation of the instruments in the abdominal cavity and allowed a good operation field and visualization. These results were consistent with what was reported by some researches (8), This was consistent with findings of (9) who said that the low-pressure technique could be employed in the majority of patients subjected to laparoscopic cholecystectomy and reducing the pressure of the pneumoperitoneum to (7 mmHg) tended to produce lower incidence of postoperative shoulder-tip pain in human. It may also prevent mortality due to CO2 embolism (10). At the 3rd postoperative day, mild adhesions observed between the omentum and the site of the operation, while at the 7th post-operative day five animals (three from group A and two from group B) had showed severe adhesions (16.6%). Mostly the adhesions were circumscribed to the operation site and did not occur in other regions in the abdominal cavity. The incidence of adhesions occurred in this study was low in comparison with the rate of occurrence in open surgery and this result comes in agreement with result of adhesion in laparoscopic surgery (11). These adhesions may be a result of the liver bed damage in dissection of cystic duct, cystic artery and omentum attached to the inflamed gallbladder. The histopathological findings were as following according to the period of biopsy: At the 3rd postoperative day group (A) were characterized by large necrotized area, with neutrophils aggregation around blood vessels and between muscle fibers (Fig. 3). Other sections showed fibrin network with inflammatory cells infiltration, dilated blood vessels with proteineus material in their lumen. While group (B) showed neutrophils infiltration in the stroma, as well as neutrophils aggregation in dilated blood vessel (Fig. 4). Neutrophils infiltration appeared in their lumen, around them, and around mucosal gland. There were network of fibrin deposition with neutrophils infiltration and fragmentation of muscle fibers (Fig. 5). These findings showed presence of necrotic area and proteineus materials in the blood vessels lumen in group A, which was avascular due to the cystic artery clipping. While there were congested blood vessels and a network of

fibrin in group B, this is a sign of further progress in healing in group B than of in group A. Histopathological findings in group (A) at the 7th day reviewed necrotization of the epithelial layer of mucosa with neutrophils infiltration in the sub epithelial layer and congested blood vessels with neutrophils infiltration in their lumen and around them (Fig. 6). The blood vessels were seen with hemolytic red blood cells and neutrophils infiltration between muscle fibers. There was also granulation tissue with adipose tissue infiltration and necrosis of muscular layer with fibrin deposition around blood vessel was seen, and dilated blood vessels contained proteineus material in the lumen (Fig. 7). Proliferation of granulation tissue was found around mucosal glands which were surrounded by sever necrosis and they converted into structureless substance. The histopathological examination in group (B) at the 7th postoperative day showed atrophy of the epithelial mucosa which become rounded and neutrophils infiltration in the subepithelial layer and there was fibrin deposition in the stroma (Fig. 8 and 9). Dilated mucosal glands with inflammatory cells infiltrated in the connective tissue were found around glands. There was neutrophils aggregation in necrotized stroma. The epithelial layer became rounded and lined by cuboids or simple cells with inflammatory cells infiltration in the lamina propria. There was necrosis of mucosal gland, granulation tissue proliferation and fibrin deposition. The presence of differences in the histopathological picture was clear at the seventh day between group A and B, through the existence of progress in healing in group B which was confirmed by the appearance of the connective tissue infiltration, despite of the presence of inflammatory cells in the lamina propria, granulation tissue infiltration, atrophy of the mucosal layer, and necrosis in the mucous glands. However, the histopathological changes in group A including the presence of severe necrosis, which has reached the muscle layer with the presence of hemolytic red blood cells and proteineus materials in the lumen of blood vessels and fibrin has emerged only now after seven days of surgery, while the fibrin was present in group B on the third day postoperatively. At the 15th postoperative day in group A, there was granulation tissue characterized by presence of blood vessels with neutrophils infiltration in their lumen and fibroblast proliferation which was perpendicular arranged around blood vessels (Fig. 10). Necrotization of epithelial mucosa was seen, with neutrophils infiltration in sub epithelial layer. At the 15th day the lesions in group (B) were characterized by severe proliferation of fibrous connective tissue, which was highly cellular with mononuclear cell infiltration. The fibrous connective tissue was surrounding necrotized mucosal gland. There was mature fibrous connective tissue in the stroma, characterized by regular fibers direction with less fibroblast cells and more collagen deposition (Fig. 11). The healing appeared in advance phases in group B at the fifteenth day postoperatively and this was evident from the proliferation of connective tissue, which was characterized by regular collagen fibers deposition and less fibroblast cells, compared with more necrosis, less fibrous connective tissue deposition and continued granulation tissue appearing in group A. These findings show that the survival of blood supply to the remaining piece of the gallbladder helped in shortening the healing time in group B compared with group A. The importance of blood supply to the inflammatory area is supported by (12) who viewed that the circulatory disturbances could play a role in the pathogenesis of acute acalculous cholecystitis. The monocyte–macrophage system and neutrophils granulocyte activation are highly important in the inflammatory process. And the neutrophils granulocytes represent the earliest immune cell flow to the site of inflammation, and the terminal phase of tissue repair is fibrosis in the liver bed, which was well demonstrated by the histological investigation (13). The presence of granulation tissue formation and fibrous connective tissue in group B at the 7th day is supported by (14)who viewed that in case of blood supply presence, the expensive tissue defect became filled with granulation tissue, and in such injuries the repaires filled by connective tissue replacement. While the

presence of proteineus material may be due to interruption of blood supply in group A which led to red blood cells hemolysis and resulting in severe necrosis. Liver enzymes showed a significant (P< 0.05) decline in the mean of the values of alkaline phosphatase (ALP) in group A for all time periods, compared with group B (Table, 1). In another way the liver injury which occurred during the cystic duct and artery dissection may be the cause of this increase in ALP titers. There were no significant differences in the mean values of the AST between A and B and in different periods (Table, 2). And there was no significant difference at the level of (P> 0.05) in the mean values of ALT between the different groups A and B and in different periods also (Table, 3). The results also showed a decline in the values of the mean of total bilirubin on the 5th and 7th day significantly in the level of (P > 0.05) compared with other time periods in groups A and B (Table, 4).The results showed no significant differences (P > 0.05) in C-reactive protein between different groups of different intervals in the number of negative and positive samples (Table, 5).

Table, 1: Alkaline phosphates (U/L) (ALP)

Time Group	Zero day	1 hrs.	1st. day	3rd. day	5th. day	7th. Day
A	49.7 ± 4.6 b	46.7 ±5.2 b	52.5 ± 4.2 b	55.3 ± 4.9 a	51.2 ± 4.1 a	49.1 ± 4.2 b
B	70.7 ± 9.6 a	69.6 ± 8.8 a	65.7 ± 5.7 a	66.8 ± 6.2 a	63.1 ± 6.8 a	67.5 ± 8.3 b

Different small letters vertically refers to the existence of significant differences (P< 0.05).

Table, 2: Aspartate aminotransferase (U/L) (GOT) (AST)

Time Group	Zero day	1hrs.	1st.day	3rd. day	5th. day	7th.day
A	128.0±11.25	136.66± 15.7	135.4±17.32	124.4± 12.03	125.6±11.77	128.66±14.08
B	117.25± 2.8	127.25±17.72	121.08±13.37	122.16±11.92	116.83±11.33	120.5±13.28

There are no significant differences in the mean values between the different groups A, B and in the different periods.

Table, 3: Alanine aminotransferase U/L (ALT) (GPT)

Time Group	Zero day	1hrs.	1st.day	3rd. day	5th. day	7th.day
A	53.3 ± 2.3	53.3 ± 205	53.0 ±2.7	53.4 ± 3.1	53.2 ±2.3	51.1 ±2.1
B	50.8 ± 2.8	51.3 ± 2.6	53.2 ±2.9	57.2 ± 3.3	53.2 ±2.0	50.8 ±2.2

There are no significant differences in the mean values between the different groups A, B in the different periods.

Table, 4: Total bilirubin (mg/dl)

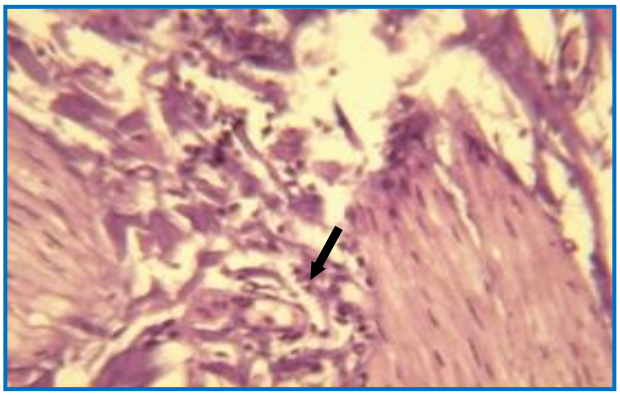
Time Group	Zero day	1hrs.	1st.day	3rd. day	5th. day	7th.day
A	0.023±0.0004 A b	0.021 ±0.0003 A b	0.021±0.0004 A b	0.018 ±0.004 B b	0.018 ±0.004 B b	0.022±0.004 B a
B	0.029 ± 0.005 A a	0.033 ± 0.007 A a	0.039 ±0.007 A a	0.034 ± 0.005 A a	0.028 ±0.005 B a	0.022±0.002 B a

*** Different small letters vertically refers to the existence of significant differences (P< 0.05).**
*** Similar small letters vertically refers to the absence of significant differences (P>0.05).**
*** Different capital letters horizontally refers to the existence of significant differences.**

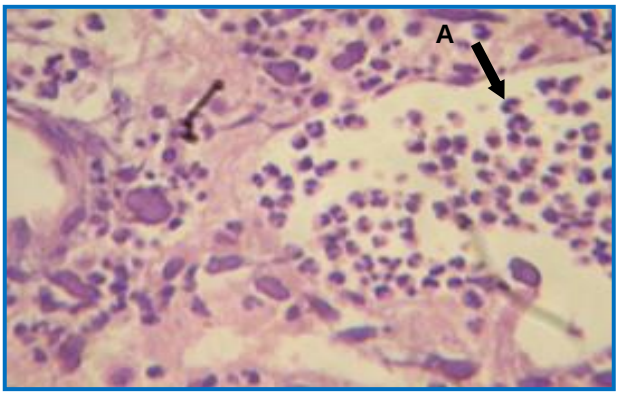
Table, 5: C - reactive protein

Time Group	Total No.	Zero day +	Zero day -	1hrs. +	1hrs. -	1st.day +	1st.day -	3rd. day +	3rd. day -	5th. Day +	5th. Day -	7th.day +	7th.day -
A	12	4	8	9	3	10	2	6	6	4	8	2	10
B	12	3	9	6	6	11	1	7	5	3	9	2	10

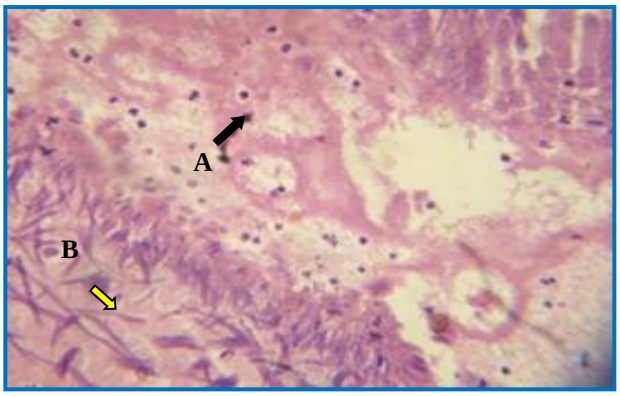
There is absence of differences in the mean values between the different groups and periods.



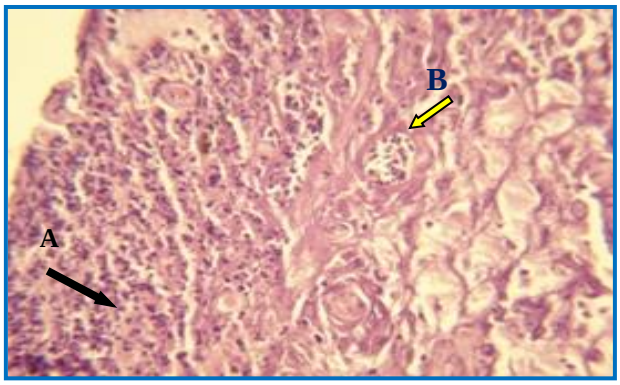
Figure, 3: There are neutrophils infiltration between muscle fibers. Group A, three days post operation, (H and E 40X).



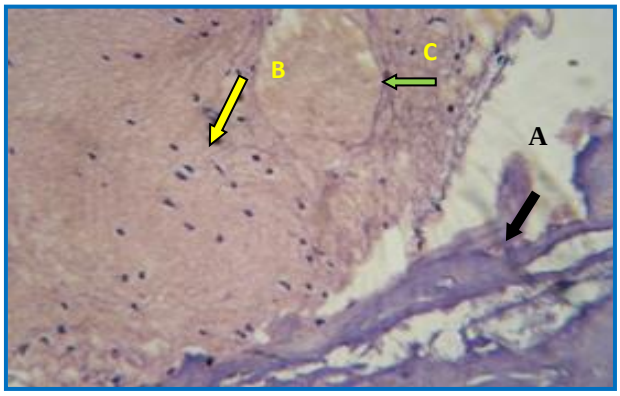
Figure, 4: Show neutrophils aggregation in dilated blood vessel (A). Group B, three days post operation (H and E40X)



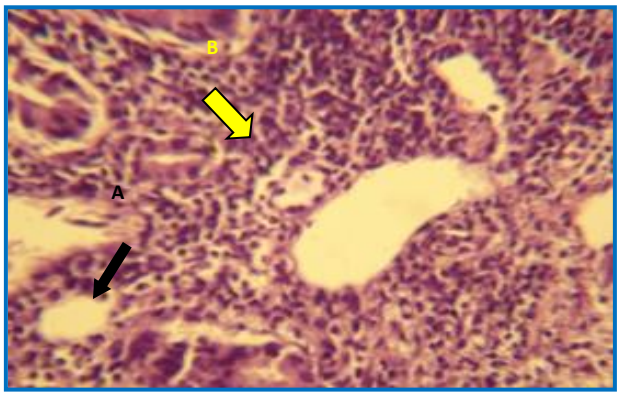
Figure, 5: Group B, three days postoperation there is fibrin network deposition with neutrophils infiltration (A), fragmented muscle fibers (B). (H and E 40X).



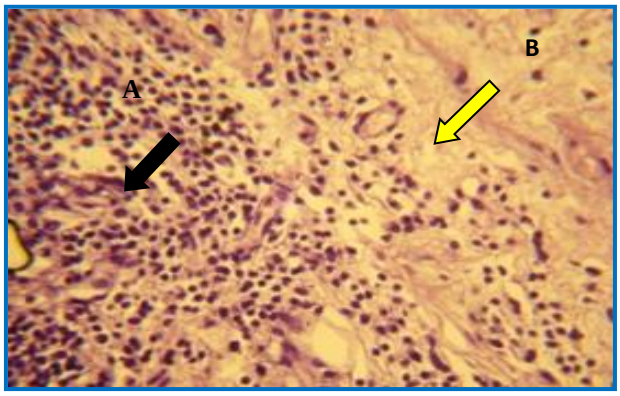
Figure, 6: There is necrotization of the epithelial layer of mucosa with neutrophils infiltration in subepithelial layer (A) and congested blood vessel with neutrophils in their lumen (B). Group A, post seven days (H and E40X)



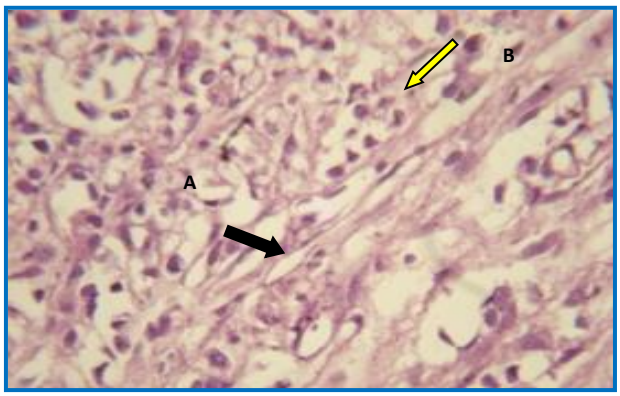
Figure, 7: Shows necrotic area (A) with fibrin deposition (B) and dilated blood vessel contain proteineus material (C). Group A, seven days post operation (H and E 40 X).



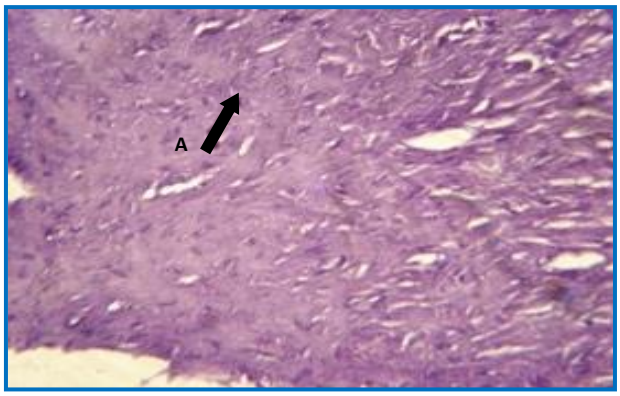
Figure, 8: Shows atrophy of epithelial mucosa which become rounded (A), with neutrophils infiltration in subepithelial layer (B). Group B, seven days post operation (H and E 40 X).



Figure, 9: There is neutrophils infiltration in subepithelial layer (A), and fibrin deposition in Stroma (B). Group B, seven days post operation (H and E 20 X).



Figure, 10: Shows granulation tissue (A) with neutrophils in blood vessel lumen (B). Group A, fifteen days post operation (H and E 40 X).



Figure, 11: There is mature fibrous connective tissue in the stroma (A). 11: Group B, fifteen days post operation, (H and E 20X).

Similar results of increase in ALP titers were recorded (15), as consequences hepatocyte pathology; cholecystitis should be regarded as an important additional cause of reactive hepatitis. These findings also agree with research (16) that showed gall stones and cholecystitis had abnormal liver histology, and the most sensitive indicator of clinically important liver pathology was a raised serum alkaline phosphates value, and liver changes associated with cholecystitis. Also they support the inference that reactive hepatitis might be the result of a recent episode of common bile duct obstruction in human. As a common disease, cholecystitis should be regarded as an important additional cause, so an obstructive pattern with raised alkaline phosphates values was the most frequent abnormality. Elevations of AST and ALT activities are associated with hepatocellular damage from any cause (17). While other (18) reported that ALT enzyme is not present in large enough amount in liver cells of horse, ruminant and pig. Increasing in total bilirubin could be a result of many causes, that hemolytic disease and hepatobiliary disease are the most common causes of hyperbilirubinemia (17). Bile salts that are reabsorbed by the enterohepatic circulation increase plasma bilirubin, which under normal levels, is excreted in the urine. However, when bile ducts are damaged, bilirubin accumulates in blood and can be used as an indicator of hepatic disease (19). Although there were differences in bilirubin values among all groups in this study, but these values were in the normal range. In a study on Nubian goats, (20) found that the values of total bilirubin were o.55 to 1.86 in different experimental groups treated with chemotherapy. This explains that the bilirubin values in present study were less than those of other study (20), which proved that they were in the normal range. This also is supported by others (17) whose test result of 3.1 mg/dl was significantly elevated over values typical for his experimental animals, and it was interpreted to represent a real elevation.

They found the same result about adhesions that the laparoscopic cholecystectomy reduced the rate of adhesion formation when compared with the open technique (21). This was supported by other studies (22) proved conventional cholecystectomy had seven times more adhesions than laparoscopic cholecystectomy, and far distant adhesions developed also. This observation is agree with others (22) who

found that liver bed adhesions developed in six of twenty laparoscopic cholecystectomies dogs, in which far adhesions developed only at the trocar site beyond the xyphoid and at the 5mm trocar sites, and no other far adhesion was detected.

The operation time agreed with researchers (23) mentioned that the median operating time for laparoscopic subtotal cholecystectomy was 92.5 min. (range, 50-140 min). And this result is consistent with researches (24), who recorded the median operation time of laparoscopic subtotal cholecystectomy as 65.5± 15.2 minute.

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دراسة مقارنة لتقنيتين لإستئصال الغدة الصفراء منظاريا في المعز

[1]ماجد عبد الرحمن غضبان و [2]محمد جواد عيسى و[2]سروة ابراهيم صالح و [3]علي عباس عجيل

[1] وزارة الزراعة، العراق.

[2] فرع الجراحة والتوليد، كلية الطب البيطري، جامعة بغداد، العراق.

[3] فرع الجراحة والتوليد، كلية الطب البيطري، جامعة المثنى، العراق.

E-mail: mohammed2006_eesa@yahoo.com

الخلاصة

أجريت الدراسة لمقارنة تقنيتين مختلفتين للازالة الجزئية للصفراء منظاريا للوصول الى التقنية المثلى في عمليات الازالة المنظارية الجزئية للصفراء لعلاج حالات الالتهابات الشديدة التي تصاب بها ولمعرفة مصير الجزء المتبقي من الغدة الصفراء. استعملت في الدراسة 24 من اناث المعز البالغة, والتي قسمت عشوائيا الى مجموعتين متساويتين، تم احداث التهاب الصفراء في جميع حيوانات التجربة بربط قناة الصفراء بمشبك وتركها لخمسة ايام، اجريت للمجموعة الاولى ازالة جزئية منظاريا مع ربط قناة وشريان المرارة بمشابك التيتانيوم. أما المجموعة الثانية فأجريت لها عملية ازالة جزئية منظاريا مع الابقاء على شريان الصفراء وربط القناة فقط بمشابك التيتانيوم. اجريت جميع العمليات الجراحية تحت التخدير العام باستعمال ثايوبنتال الصوديوم وبجرعة 15 ملغم لكل كيلوغرام من وزن الجسم للمساعدة في ادخال انبوب الرغامي لادامة التخدير العام باستعمال مزيج من الهالوثين بنسبة 1.5- 2.5 % والاوكسجين بنسبة 2- 3 %. استعمل غاز ثاني اوكسيد الكربون لنفخ البطن في المعز وتحت ضغط منخفض(8- 10 ملم زئبق). جرت متابعة الفعاليات الفسلجية والاعراض السريرية والتي تضمنت: سرعة القلب والتنفس, ودرجة الحرارة، والعودة الى الطعام والماء في فترات متزامنة مع توقيتات اخذ عينات الدم لقياس انزيمات الكبد والتي شملت ال ALP, AST, ALT, C-reactive protein، ونسبة البليروبين الكلي ابتداء من قبل العملية، والساعة الاولى بعد العملية, واليوم الاول والثالث والخامس والسابع بعد العملية الجراحية. الفعاليات الفسلجية والملاحظات السريرية بينت نجاح عمليات ازالة الصفراء منظاريا في المعز من خلال عدم ظهور اختلافات مهمة في هذه الفعاليات قبل وبعد العمليات الجراحية لحيوانات التجربة. كما ان الازالة المنظارية الجزئية لكيس الصفراء قد تمت بنجاح ايضا مع عدم ظهوراختلاطات معنوية. واظهرت نتائج فحوصات انزيمات الكبد زيادة في مستوى ال ALP, والبليروبين الكلي في مجموعة B مقارنة مع مجموعة A . ولم يظهر اختلاف معنوي في مستويات ال AST , ALT, C- reactive protein بين المجموعتين، وفي التوقيتات المتعددة اعلاه ولكل المجموعات ايضا. اخذت عينات من الجزء المتبقي من الصفراء في الايام: الثالث والسابع والخامس عشر بعد العملية الجراحية لاجراء الدراسة النسجية المرضية. أظهرت نتائج الدراسة النسجية المرضية للجزء المتبقي من الصفراء امكانية اجراء الجراحة المنظارية الجزئية بالتقنيتين المذكورتين اعلاه على الرغم من تفوق مجموعة B من خلال قلة النخر والسرعة الاكبر في التئام ألجزء المتبقي مما يبين اهمية استمرار التجهيز الدموي في مراحل الالتئام، حيث قل التنكس الكلي وقلل من امكانية تحوله الى مركز للانتان والاختلاطات الغير مرغوب بها.

الكلمات المفتاحية:أزالة الصفراء منظارياً ، الكبد ، الأستئصال الجزئي ، المعز.