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THE FATE OF INTRAPERITONEAL IMPLANTED GALLSTONES IN DOGS

By

Ayman I. Nafeh M.D.[1] ,Mohamed Abbas M.D.[1], Mervat El Damarawy M.D.[2], Laila Abou El Fadi M.D.[3],Oulfat Ham mam M.D.[4], Ahmed Hussein M.D.[5], AIaa Abdel Halim NI.D.[6] and Badawy AL kholy M.D.[7],

Surgical[1] , Intensive care[2] , Bacteriology and Pathology departments Theodor Bilharz Institute , Surgical departments Alexandria[5] and Bani sweif Branch[6] Cairo Universities and Chemical Clinical, Pathology[7] department Cairo University , Egypt.

Introduction: *Little is Known about the natural history of gallstones left behind in the peritoneal cavity during laparoscopic cholecystectomy .*

Aims: *This experimental study was conducted to assess the consequence of implanted intra- abdominal gallstones in dogs.*

Materials and methods: *Eighty four male mongrel dogs were divided into: G1 (n =12), G2 (n=36) and G3 (n =36) Where sterile glass beads with sterile saline , collected bile with different types of gallstones (subgroups) and enriched bile (E.Coli & Staph. aureus) with gallstones were implanted in an omental pouch respectively.*

The gallstones and bile were collected from 50 gallbladders resected from patients with chronic calcular cholecystitis.

Half the number of dogs from each group was re- explored after one week and the rest after six weeks, so as to retrieve the implants with their surrounding tissues for histopathological examination.

Acute inflammatory reaction with different degrees of intensities of polymorphonuclear leucocytes and macrophages were noted in G1 and G2 after one week which completely disappeared six weeks postoperatively. In G3, severe acute inflammatory reaction and abscess formation were observed one week postoperatively, but markedly resolved in animals explored after six weeks without antibiotic coverage.

Conclusions: *This study revealed that intraperitonal lost gallstones cause no serious tissue reaction in dogs. We suggest that lost gallstones during laparoscopic cholecystectomy, unless easy to retrieve, should not prolong the operation time or justify conversion to laparotomy.*

INTRODUCTION

Laparoscopic cholecystectomy has become the treatment of choice for symptomatic cholelithiasis.During this procedure gallbladder perforation , with spillage of bile and gallstones occurs in 13% to 40% (Nathanson et al,1991 and Memon et al ,1997).Little is known about the natural history of gallstones left behind in the peritoneal cavity, but initial opinion thought to be harmless(Najmaldin and Guillou ,1998).

Recently, complications related to these lost gallstones in the form of intra - abdominal abscess (Hornof et al, 1996 and Diez et al,1998), adhesions, gut perforation, and granuloma formations (Warren and Wyatt,1996) necessitating surgical intervention were reported.

However, conversion to open cholecystectomy to retrieve spilled gallstones remain open to debate.

Aim of the work:

This experimental study was undertaken to assess the fate of intraperitoneal implanted gallstones in dogs.

MATERIALS AND METHODS

Animals and experimental groups (table 1):

Eighty four male mongrel dogs weighing 7-11 KG**s** were divided into 3 main groups.

In the control group (G1, n = 12) in which sterile glass beads (3-5 beads per animal) with sterile saline were placed in an omental pouch. In the second group (G2, n = 36) collected bile with 3 – 5 cholesterol (G2 a, n = 12), pigmented (G2 b, n = 12) or mixed (G2 c, n = 12) stones were implanted in an omental pouch respectively.

In the third group (G3, n = 36), bacteria enriched bile combined with 3 – 5 cholesterol (G3 a, n = 12), pigmented (G3 b, n = 12) or mixed (G3 c, n = 12) stones were respectively implanted (Table 1). The stones were provisionaly typed according to their morphological appearances which were verified at the end of the study by stone analysis.

Operative procedure:

The operative procedures were carried out in the animal house at Theodor Bilharz Institute.

Under general anaesthesia with endotracheal intubation, the skin of the upper abdomen of the dog was prepared and draped with sterile towels.

Through a small upper midline incision, the omentum was delivered and an omental pouch was fashioned and closed over the implants with 3/0 prolene suture.

Mass closure of the abdomen using 1/0 prolene, and the skin was closed separately with continuous subcuticular 3/0 prolene suture.

Postoperative course:

The animals were allowed water and milk for the first 24 hours postoperatively, and for normal diet henceforth. None of the animals in this study received antibiotics.

Six animals from G1, G2 and G3 subgroups were re-explored after one week and the remaining after six weeks. The abdominal cavity was examined and the implants with their surrounding tissues were excised and subjected to pathological examination.

Collection of bile and gallstones:

Bile and gallstones were obtained from the excised gallbladders of 50 patients suffering from chronic calcular cholecystitis . These patients were thoroughly evaluated by history taking, clinical examination, laboratory investigations and Abdominal ultrasound.

Cases of acute cholecystitis were excluded to avoid collection of bile with significant infection, all patients were subjected to laparoscopic or open cholecystectomy.

The excised gallbladders containing its bile and gallstones were immediately sent to the bacteriological laboratory in sterile containers and stored at **-** 40°C until used.

Enrichment of the collected bile:

The bile from the stored gallbladders was aspirated with a sterile syringe and poured into a sterile tube .A sample was cultured on blood agar and Mac Conkey's media to confirm its sterility aerobically. Another blood agar plate was cultured and incubated anaerobically in a jar using anaerobic gas pack (**Biomerieux**) to exclude the presence of anaerobes. Culture with positive bile was disgaurded.

A broth was prepared , inoculated with the standard organisms staphylococcus aureus (ATCC 25923) and Escherichia coli (ATCC 25922) separately and incubated overnight at 37°C. The serial dilution was done to reach a concentration of 1×10^6 colony forming units CFV/ML one ml of this concentration was inoculated into 5 cc of sterile bile to obtain the enriched bile (Johnston et al 1994)

Pathological examination:

The recovered specimens of the implants with their surrounding tissues were fixed in 10% buffered formaline solution and pressed into paraffin blocks. Serial sections were done at 4µn thickness and stained with the hematoxyline and Eosin (H.**&** E.) for histopathological examination, and with Masson Trichome for assessment of fibrosis.

Semi-quantitative assessment of the degree of tissue reaction was done using a score from (Zero) to (+ + +) , (Zero) = negative, (+) = mild, (++)= moderate and (+ + +) = marked.

Gallstone analysis:

The implanted stones were collected from the specimens after pathological examination of their surrounding tissues to verify their types.

After grinding of the stone, a portion was extracted with 3 ml absolute alcohol- ether mixture (1/1), 0.5 ml of sulphuric acid -acetic anhydride reagent was added where green colour develops with the presence of cholesterol.

A portion of stone was extracted with few mls of methanol and o.5 ml of diazotized sulphanilic acid was

added, where a violet colour develops with the presence of bile (Ermalinda A.F. 1976).

RESULTS

The results of this study revealed that the dog is a good experimental model it stood well the general anaesthesia as well as the surgical procedures. Only 2 out of 86 dogs died due to anaesthetic causes. None of the animals had wound infection or burst **abdomen** although they did not receive antibiotics.

Gallstone analysis:

Proved the provisional typing according to their macroscopic appearance (Table 1).

Bacteriological examination:

The collected bile was sterile in 32 gallbladders (64%).

Pathological examination:

One week postoperative (table 2):

Macroscopic picture of the received specimens revealed pink indurated and granular appearance of the tissue surrounding the glass beads as well as the stones regardless of their type, size and number. These changes extended in some cases to the **nearby** areas of omentum, visceral and parietal peritoneum. Only one case showed an early abscess formation (G3 a with staphylococcus aureus).

Microscopic picture of these lesions under light microscope in the stained serial sections with H**&** E and Masson Trichome revealed an acute inflammatory reaction mostly neutrophils, angiogenesis, macrophages and with collagen fibrils (Fig. 1 **&** 2).

Six weeks postoperative (Table 3):

Macroscopically, the tissues surrounding the glass beads as well as the stones were grayish white in colour and mildly indurated with smooth inner surface and patchy areas of fibrosis.

Microscopic examination of the surrounding tissues in G1 & G2 revealed reaction **formed** of a cellular connective tissue devoid of inflammatory infiltrate. Specimens of G3 showed mild chronic inflammatory reactions characterized by **infiltrations** with **mononuclear** cells which included macrophages, lymphocytes, rare plasma cells and fibrocytes.

No granuloma or abscess formation was seen in all groups under light microscope (Fig. 3).

Table 1: ***Experimental Groups***

Groups	*Subgroups*	*Numbers*	*Implants*	*Bile*
G 112	------	12	Glass beads with sterile saline	---------
	G 2 a	12	Cholesterol stones	Collected bile
G 2 36	G 2 b	12	Pigment stones	Collected bile
	G 2 c	12	Mixed stones	Collected bile
	G 3 a	12	Cholesterol stones	*Enriched bile
G 3 36	G 3 b	12	Pigment stones	*Enriched bile
	G 3 c	12	Mixed stones	*Enriched bile

* The bile was enriched with Escherichia coli in half of the cases of each Subgroup and Staphylococcus aureus in the remaining half of the cases

Table 2: ***One week postoperative***

Groups	*Numbers*	*Implants*	*Acute inflammation*	*Abscess formation*	*Granuloma formation*	*Adhesions*
G 1	6	Glass beads with sterile saline	+	Zero	Zero	Zero
G 2	18	Gallstones with collected bile	+	Zero	Zero	Zero
G 3	18	Gallstones with bile enriched with staph.	+ + +	+	Zero	Zero
		Gallstones with bile enriched with E. coli	+ + +	Zero	Zero	Zero

Table 3: ***Six weeks postoperative***

Groups	*Numbers*	*Implants*	*Chronic inflammation*	*Abscess formation*	*Granuloma Formation*	*Adhesions*
G 1	6	Glass beads with sterile saline	Zero	Zero	Zero	Zero
G 2	18	Gallstones with collected bile	Zero	Zero	Zero	+
G 3	18	Gallstones with bile enriched with staph.	+	Zero	Zero	+
		Gallstones with bile enriched with E. coli	+	Zero	Zero	+

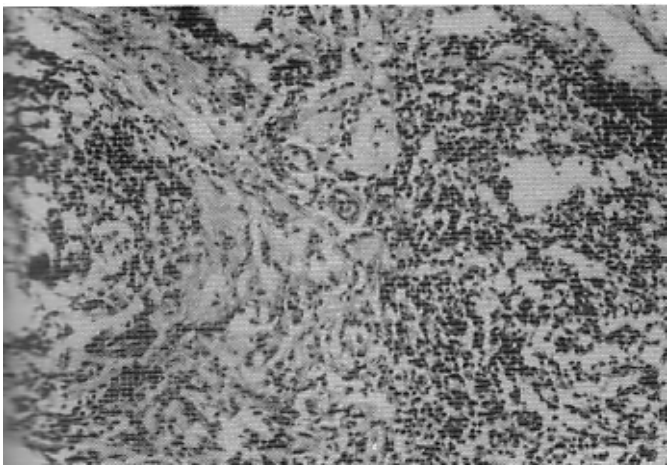

Fig.(1):- Abscess formation in one case with Staph. aureus with cholesterol stone (one week postoperative G3). Masson Trichome ×200

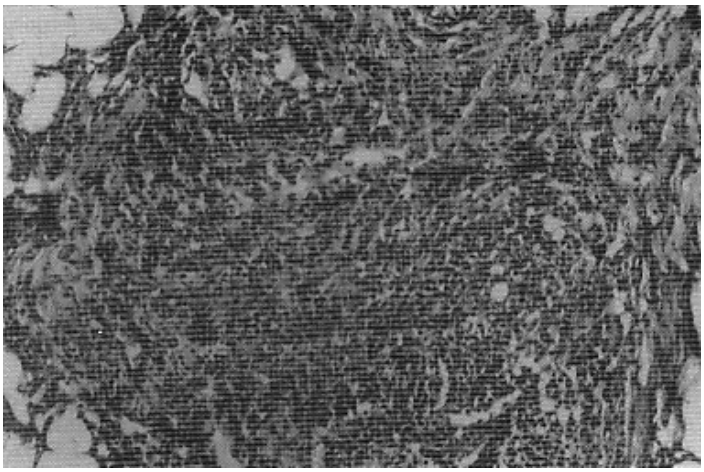

Fig.(2):- Acute inflammatory reaction Staph. aureus with stone (one week postoperative G3). H.& E. ×200

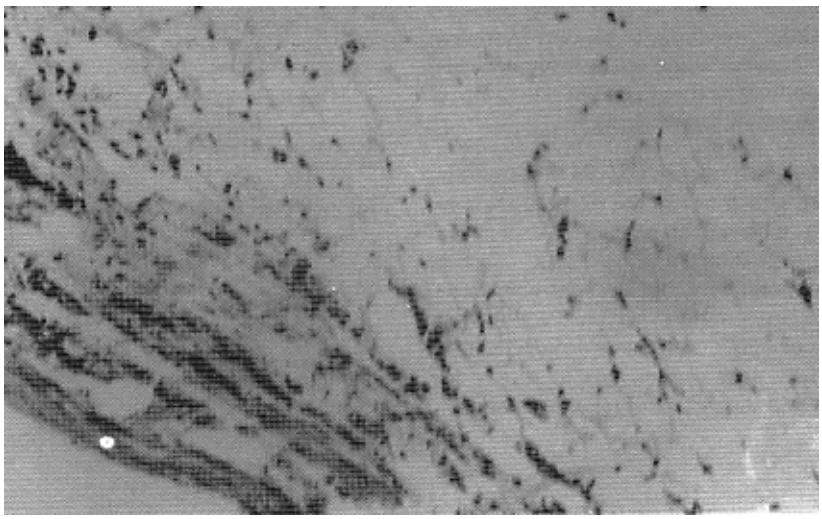

Fig.(3):- Mild Inflammation (six weeks postoperative). Masson Trichome ×200

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DISCUSSION

Laparoscopic cholecytectomy has become the treatment of choice for calcular gallbladder disease. During this procedure perforation of the gallbladder with spillage of stones into the peritoneal cavity occurs frequently (40%) (McDonald et al, 1997). With improved instrumentation and increased experience the incidence of this complication is decreasing. Dropped stones may be difficult to retrieve completely, particularly those lost beyond the field of operation, e.g. in the right subhepatic space.

Managing of intraperitoneally retained gallstones is controversial, as their natural course is not well known. Many authors have reported considerable **morbidity** (Witton et al, 1993;Warren and Wyatt, 1996; **Memon** et al, 1997; Patterson and Nagy,1997 and Zamir et al, 1999) and this was the reason to undertake this experimental study.

In the present study various types, numbers and sizes of stones in combination with collected or enriched bile were placed intraperitoneally to mimic the clinical situation.

No remarkable pathological differences were noted between the tissue reaction to glass beads and different types of stones whether with sterile or enriched bile six weeks postoperatively.

These results support the **suggestion** that in the absence of a significant trauma, the peritoneal cavity is remarkably resistant to the presence of a single noxious agent (McEntee et al, 1990) However, in our study there was tissue reaction of variable severity in all specimens obtained one week after implantation, reaching to abscess formation in only one animal (cholesterol stone with staph. enriched bile). This severe reaction may be due to its big size (2 cm. in diameter) and its irritating mamillated surface.

On the other hand, Johnston et al in 1994 revealed that bile in combination with gallstones in the peritoneal cavity of rats was associated with an increased risk of intra-abdominal adhesion formation and possible abscess formation 4 weeks postoperatively, but no intra-abdominal lesions were detected in animals which received an intraperitoneal injection of saline, sterile bile or infected bile. The difference between the two studies may be due to the different animal species and the timing of re- exploration.

Conclusion:

Intraperitoneal implanted gallstones with sterile or infected bile remain inert for six weeks and cause no serious tissue reaction in dogs.

However, in the clinical situation it is mandatory to avoid spillage of gallstones during dissection and delivery of the gallbladder through the abdominal wall by enlarging the incision of the port if needed.

Every effort should be made to remove intraperitoneal dropped gallstones (especially those of large size). Abdominal wash with saline, prophylactic systemic course of antibiotic and ultrasonographic follow up may be needed, but routine conversion to laparotomy is not justifiable.

REFERENCES

1. Rattner DW, Ellman L, Warshaw AL: Portal vein thrombosis after elective splenectomy: An underappreciated, potentially lethal syndrome. Arch. Surg. 1993; 128: 565-570.

2. Diez J, Arozamena C, Gutierrez L , Bracco J , Mon A, Sanchez Almeyra R,Secchi M. Lost stones during laparoscopic cholecystectomy.HPB Surg 1998;11(2):105-8;discuss 108-9.

3. Ermalinda A.F. 1976. analysis of calculi in fundamentals of CL. Chem.
   ch : 18 P: 1015 – 1025
   ed : Plorbent Tietz 2nd ed.
   W.B Saunders comp. Philadelphia, London , Toronto.

4. Hornof R, Pernegger C, Wenzl S, Bittermann G, Brinninger G, Tessadri R , Siegal H , Reckendorfer H, Feigl W, Losert U, Keiler A. Intraperitoneal cholelithiasis after laparoscopic cholecystectomy – behavior of 'lost' concrements and their role in abscess formation.Eur. Surg . Res .1996;28(3):179- 89

5. McDonald MP, Munson JL, Sanders L, Tsao J, Buyske J.Consequences of lost gallstone.Surg.Endosc. 1997Jul: 11(7):774- 7

6. McEntee G, Stuart RC, Byrne PJ,et al. Experimental study of starch induced intraperitoneal adhesions. Br. J. Surg. 1990;77:1113 – 1114

7. Memon Ma., Jenkins H.J. Jr, Fitzgibbons RJ Jr. Spontaneous erosion of a lost intra- abdominal gallstone through the back eight months following laparoscopic cholecystectomy .J. Soc. Laparoendosc. Surg. 1997 Apr-Jun; 1(2): 153-7

8. Najmaldin A.and Guillou P., Laparoscopic cholecystectomy. In:A Guide to Laparoscopic Surgery. Blackwell Science Ltd. 1998,P: 113 - 120

9. Nathanson LK,Shimi S, Cuschieri A. Laparoscopic cholecystectomy: the Dundee experience. Br. J. Surg. 1991:78:155-159.

10. Patterson EJ., Nagy AG. . Don't cry over spilled stones? Complications of gallstones spilled during laparoscopic cholecystectomy: case report and literature review.Can. J. Surg. 1997 Aug;(4): 300 – 4.

11. Sean Johnston, MB, FRCSI, Kieran O, Mally, MB, BCH, BAO, LRCP, SI, Gerry McEntee, MCH, FRCSI, Pierce Grace, MCH, FRCSI, Smyth,MRC.PATH.Professor David Bouchier-Hayes.MCH.FRCSI.Dublin, Ireland. The Need to Retrieve the Dropped Stone During Laparoscopic Cholecystectomy. The American Journal of Surgery Volume 167 June 1994

12. Warren CW, Wyatt JI. Gallstones spilled at laparoscopic cholecystectomy: a new cause of intraperitoneal granulomas.J. Clin. Pathol. 1996 Jan: 49(1): 84 – 5

13. Wilton PB,Andy OJ Jr,Peters JJ, Thomas CF, Patel VS , Scott – Conner CE . Laparsocopic cholecystectomy. Leave no (spilled) stone unturned.Surg. Endosc. 1993 Nov – Dec: 7 (6): 537- 8

14. Zamir G, Lyass S, Pertsemlidis D,Katz B . The fate of the dropped gallstones during laparoscopic cholecystectomy. Surg. Endosc. 1999 Jan;13 (1) :68 – 70