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HISTOLOGICAL FACETS OF CHOLELITHIASIS IN IRAQI PATIENTS FACING CHOLECYSTECTOMY: POSSIBLE ROLE OF INFECTION

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ABSTRACT

Background: Cholelithiasis is a frequently encountered surgical problem that requires intervention. Infection may lead to deposition of bile and gallstones could enhance the bacterial growth. **Objectives**: Is to identify and characterize the infection in the gallbladder in cases with cholelithiasis, and to identify types of pathogens if present using both of the microbiological and histopathological assessments. **Materials and methods:** Cross-sectional observational study performed on 43 patients who underwent an elective cholecystectomy (laparoscopically) and age group ranging from 20-78 year for a period of 2 months from January1 to March 1, 2025, at Al-jumhori Teaching hospital in western side of Mosul Province, Iraq. All gallbladders were assessed microbiologically and histopathologically. Each gallbladder was incised and examined under light microscope and staining Microscopic examination was done to define the type of histological alterations. The cultures were obtained from several parts of gallbladder. **Results:** Females to males ratio equal to 5.1:1. Mean age was 56.5 ±1.2 year. Thirty eight out of 43 cases (88.3%) manifested at least one positive culture sample while the remaining 5(11.7%) were negative in all samples. The commonest microorganisms identified were *Escherichia coli* which was noticed in 13(33.4%) out of 39 cases followed by *Enterobacter aerogenes*. The cholecystitic changes were noticed in one (25%) out of 4 acalculus gall bladder using haematoxylin and eosin. Various histological abnormalities were shown in 42(95.5%) stones containing gallbladders which was significantly higher than that in acalculus gall bladder ($P<0.05$). **Conclusions:** *Escherichia coli* were the most frequent pathogen. There is a need for more than single antibacterial agent or a broad-spectrum therapy for these cases. Advanced techniques and modern diagnostic tools are crucial to characterized bacterial strain at our locality and to identify role of bacteria in the genesis of cholelithiasis. Rokitansky-Aschoff Sinuses were the most frequent lesions and this support the hypothesis that this lesion may have a role in the pathogenesis of gallstones formation.

KEYWORDS: Bacterial pathogens, Cholelithiasis, Infection, Pathology, Rokitansky-Aschoff Sinuses.

INTRODUCTION

Cholelithiasis is a widely distributed disease and represents one of the bread-and-butter surgical operations for most clinicians all over the world.[1,2] It is found in about 10-20% of adults with increase in its frequency with age and in female gender.[3]

The cholecystectomy had been conducted firstly by Carl Langenbuch in Germany in 1882, however, the management of gallstones still one of the crucial issues as the cholelithiasis is a frequent disease but there is a defect in the preventive strategies.[4] Moreover, the results of gallstones therapy were not satisfactory and associated with adverse sequel like iatrogenic injury of bile duct.[5] The pathogenesis of cholelithiasis is complicated and affected by many factors including genetic and environmental one beside the interaction between them. The Genetic factors represent 25% while the environmental one represents 62% of the liability.[6]

Wan *et al*., in 2025 suggested that infection may have a role in cholelithiasis, but this needs further clarification and that may permit finding an efficient treatment, prophylactic approaches and even lowering in the frequency of different possible complications that happened from these disorders.[7] Bacterial infection may lead to deposition of bile and gallstones could enhance the bacterial growth, however, the role of infection in gallbladder deposit is still not fully confirmed. Moreover, the organism that causes this frequent clinical enigma is not lucid yet.[8] The main objective of this work was to identify and characterize the infection in the gallbladder in cases with cholelithiasis presenting for cholecystectomy, and to identify types of pathogens if present using both of the microbiological and histopathological assessments.

MATERIALS AND METHODS

Ethical consideration: This study was approved by the I the scientific committee of college of medicine, University of Mosul. The requirement for informed consent was waived due to the use of anonymized clinical data and archived samples. The study adhered to the principles of the Declaration of Helsinki, and patient confidentiality was fully preserved.

This cross-section observational work was prospectively performed on 43 patients who underwent an elective cholecystectomy (laparoscopically) for cholilithiasis and enrolled age group subjects ranging from 20-78 years for a period of 2 months (January1 to March 1, 2025) at Al-Jumhori Teaching hospital in western side of Mosul Province, northern Iraq.

All cases were diagnosed with cholelithiasis with exclusion of patients who underwent an emergency cholecystectomy. The patients were admitted night prior surgery in the hospital and clinical examination and investigation were performed for operation for all patients with assurance of fitness for anesthesia. Agreement was obtained from all patients and preoperative dose of antibiotics (ceftriaxone 1gram) was given for all patients at the induction of anesthesia. Laparoscopic cholecystectomies were done according to standard surgical procedure. Then all gallbladders were assessed microbiologically and histopathologically.

Each gallbladder was incised and cultures were obtained from several parts as described and three tissue samples were obtained immediately after the operation (from fundus, body, and neck regions). In addition, other cultures were obtained from the stones and bile. Samples were collected using a sterile swab for 25 sec and swabs were immediately inoculated into the agar plates in microbiology department to be cultured in the appropriate media, which were as follows: Salmonella-Shigella agar, blood agar, chocolate agar, Mannitol salt agar and McConkey agar.[9] Incubation of plates was done under aerobic conditions at 37Cº for 24-48 hours, then swabs were cultured in another blood agar and incubation under anaerobic conditions for isolation of Streptococci at 37Cº for 24-48 hours was performed. The cultural growth was characterized and the cultural features were estimated by defining colonies, morphology and alterations in the media. Gram's staining of the fixed smears was achieved from the growth, and biochemical testing (for both gram stain positive and gram stain negative microorganisms) was performed to confirm the presence of bacteria. Data of the positive finding was reviewed by resident and senior in the department.

On the other hand, the obtained cholecystectomy specimens were put in 10% formalin and grossly examined, and the stones collected from the gall bladder were categorized principally depending upon the basis of their morphology: pale yellow or white stone as cholesterol, black and black to brown as pigmented stone, and brown yellow or green as mixed stone. Then the stones were analyzed by chemical examination.[10]

The gall bladders were categorized into two groups (calculus and acalculus) according to the presence of gall stones. Triple sections were taken from each gall bladder (body, neck, and fundus) for paraffin blocks and examination was done after staining with haematoxylin and eosin (H&E), Toluidine blue, and Gimsa stain. Microscopic examination was done to define the type of histological alterations including presence of inflammation, fibrosis, cholesterolosis, metaplasia, and tumor.

All sections were visualized in Ernst Leitz Wetzlar microscope (Germany). Photomicrographs were taken using digital camera (Acer, resolution 5.5 Mega pixels).

Statistical analysis

Fisher's Exact Test was applied to assess associations between gallstone presence and both histopathological and microbiological findings. The distribution of bacterial infection among different gallbladder sampling sites was also evaluated using Fisher's Exact Test. A two-tailed P-value < 0.05 was considered statistically significant. Statistical analysis was performed using SPSS software (version 28).[11]

RESULTS

During two months period total of 43 cholecystectomies were done in Mosul Province which included 36 females and 7 males, with females to males ratio equal to 5.1:1 (**Figure 1**). The Mean age was 56.5 ± 1.2 years. Thirty-nine patients (90.7%) presented with different types of gall stones while only 4 (9.7%) out of 43 patients, were free from gall stones. After operation, cultures were obtained from three different anatomical zones of the gallbladder mucosa from each patient who underwent cholecystectomy due to symptomatic cholelithiasis.

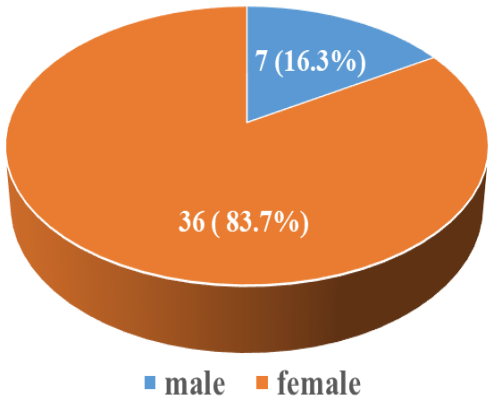

Figure 1: Sex distribution.

The commonest microorganisms identified were *Escherichia coli* which was noticed in 13 (33.4%) out of 39 cases followed by *Enterobacter aerogenes*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Enterococcus faecium* respectively as shown in Figure 2.

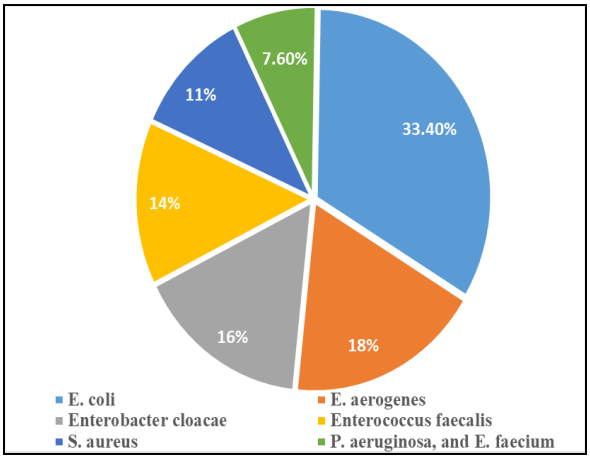

Figure 2: The percentage of each bacterial type identified in the gallstone.

Thirty eight out of 43 cases (88.4%) manifested at least one positive culture sample while the remaining 5 (11.6%) were negative in all samples. About 38 (97.4%) out of 39 neck samples were positive, while 36 (92.3%) out of 39 body samples were positive. On the other hand, samples of fundic region showed positivity in 34 (87.1%) out of 39 cases, bile swabs revealed positive results in 36 (92.3%) out of 39 cases, and finally those obtained from the stones presented with positivity in 30 (76.9%) out of 39 cases (P>0.05). The multiple microbial infections were not identified in any sample. The rates of the isolated bacteria with correlation with the sampling zone in all cases of cholelithiasis are presented in Figure 3.

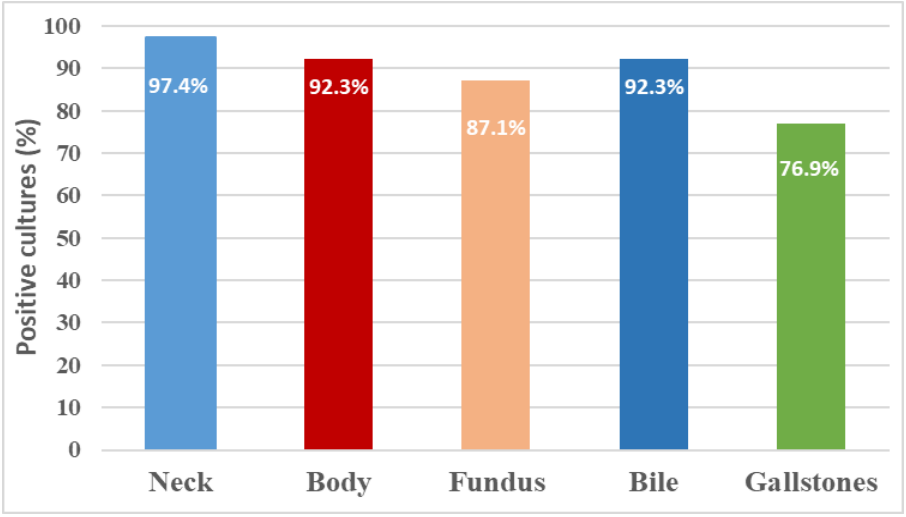

Figure 3: The percentage of isolated pathogens in relation to the sampling regions.

The cholecystitic changes were noticed only in one (25%) out of 4 acalculus gall bladder using hematoxylin and eosin (H&E), and the remaining 3(75%) samples were revealed normal gall bladder mucosa.

Examination under light microscope using haematoxylin and eosin (H&E) and Toluidine blue revealed that only one (2.6%) out of cases with gallstones showed normal gall bladder mucosa. On the other hand, various histological abnormalities were shown in the remaining 38(97.4%) out of stones containing gallbladders which was significantly higher acalculus gall bladder (P<0.05).

These findings include inflammation in 18 (46%) out of cases with gallstones followed by 15 (41%) out of calculus gall bladders showed presence of Rokitansky-Aschoff Sinuses**,** and finally features of cholesterlosis were noticed in 3 (7.6%) out of stones containing gallbladders**.** Figures (4, 5 and 6), Table 1.

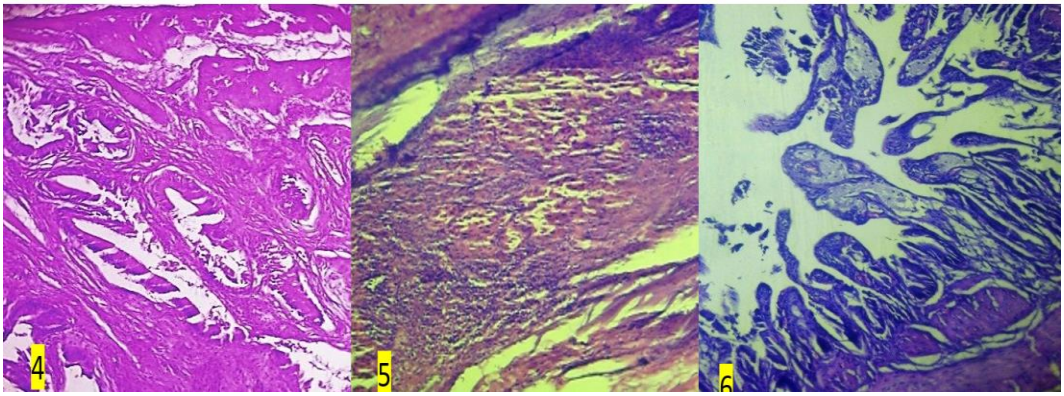

Figure 4: A Photomicrograph of gallbladder of 60 years –old male presented with features of cholelithiasis showed Rokitansky-Aschoff Sinus(arrow) (H&E X250).
Figure 5: A Photomicrograph of gallbladder of 40 years –old female. Accumulations of inflammatory cells are seen(arrow) (H&E X250).
Figure 6: A Photomicrograph of gallbladder of 47 years –old female with cholesterlosis (H&E X250).

Table 1: Association Between Gallstones and Histopathological Changes.

Variable	*Calculous (n=39)*	*Acalculous (n=4)*	*P-value**
Histological Changes Present	38 (97.4%)	1 (25%)	0.001
Normal Mucosa	1 (2.6%)	3 (75%)	

*Fisher's Exact Test (two-tailed)

DISCUSSION

Cholilithiasis is considered as a frequent surgical condition that need surgical interventions. The present work revealed that cholilithiasis was more frequent in females than in males (5.1:1). These findings are in accordance with those of others.[3,12] Gallstones are found commonly in females as they are genetically liable; physically inactive, beside the role of female hormones and diet habits.[12]

On the other hand, the mean age was 56.5 ±1.2 years. These findings are similar to those of others[7], who showed an increase in the frequency of gallstones with increasing age and that resulted from diminished in activity of cholesterol α reductase beside the increasing of HMG CoA reductase actions leading to cholesterol secretion and saturation of bile. In addition, as age increased there is a tendency to have diminished of physical actions so more liability of cholilithiasis.[7,13]

This work revealed that culture growth conducted on samples from different regions, bile, and stones obtained from patients who underwent surgery due to symptomatic cholelithiasis indicated presence of different types of bacteria. Thirty eight out of 43 cases (88.3%) manifested at least one positive culture sample while the remaining 5 (11.7%) were negative in all samples. About 38(97.4%) out of 39 neck samples were positive, while 36 (92.3%) out of 39 body samples were positive. So, the highest frequency of positivity was in samples obtained from neck region that lies near the bile duct. Similar observation was shown in those of Sebghatollahi *et al*., 2023[14] who reported that the percentage of bile duct infections is significantly high in cases of cholelithiasis.

On the other hand, bile swabs revealed positive results in 36 (92.3%) out of 39 cases, and finally those obtained from the stones presented with positivity in 30 (76.9%) out of 39 cases (P>0.05). In a previous work by Pagani *et al*., about 11 patients (27.5%) bile cultures were positive.[15] While Huang *et al*, (2022) reported that bile cultures were positive in 77.3% of patients with stones.[16] The differences in the values between the present study and those of others may be due to the difference in the number of the study sample beside the advance in the methods used for the diagnosis.

The present study showed that the most frequent pathogens include *Escherichia coli*, *Enterobacter aerogenes*, *Enterobacter cloacae.* These observations were comparable with those of other studies.[17-18]

A previous work tried to correlate the results of cultures with wound infection and reported that *Escherichia coli* was the frequent isolated bacteria.[16]

This study revealed that types of bacteria that identified in samples from stone are similar to that from the bile or from various parts of gallbladder so that support the idea that the gallstones were infected in the initial stage of gallstone formation. On the other hand, isolation of

Staphylococcus aureus, *Pseudomonas aeruginosa* in study samples reflected the potency of the body to defense against infection.[19]

This study revealed different histological abnormalities was shown in the 38(97.4%) stones containing gallbladders which was significantly higher than that in acalculus gall bladder (P<0.05). Rokitansky-Aschoff Sinuses were found in 15(41%) out of 39 cases of gallstones and represented the second frequent lesions after inflammation. These findings are similar to those of others.[20] Rokitansky-Aschoff sinuses are consequence of hyperplasia and herniation of epithelium into the fibromuscular layer of the gallbladder wall and they have a role in formation of brown pigmented gallstones.[21]

Study Limitations

This study was limited by a relatively small sample size and short study duration. Only aerobic and selected anaerobic cultures were performed, and advanced molecular techniques for bacterial identification were not utilized. Additionally, the observational design precludes establishing a causal relationship between bacterial infection and gallstone formation.

CONCLUSION

1. There is a growth of more than one pathogenic microorganism was grown in 88.3% of patients presented for elective cholecystectomy, however, the microscopic examination revealed that inflammations are shown in 46% out of 39 cases with gall stones.
2. The most frequently grown pathogens were *Escherichia coli*, *Enterobacter aerogenes*, *and then Enterobacter cloacae*, and the highest rate of isolates was in samples obtained from the neck region. Aseptic precaution should be considered during operation. Targeted treatment needs more than single antibacterial agent or a broad-spectrum therapy.
3. Advanced techniques and modern diagnostic tools are crucial to characterized bacterial strain and to identify role of bacteria in the genesis of cholelithasis.
4. Rokitansky-Aschoff Sinuses were the second most frequent lesions and this support the hypothesis that this lesion may have a role in the pathogenesis of gallstones formation.

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