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Original Research Article

A micro bacteriological culture assessment of the histopathological alterations in liver and bile in gallstone disease

Pradeep Jaiswal, Rajeev Ranjan, Rinku Kumari, Kaushalendra Kumar, Ria Rajgaria, Pawan Kumar Jha*

Department of General Surgery, IGIMS, Patna, Bihar, India

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***Correspondence:**
Dr. Pawan Kumar Jha,
E-mail: jhapawankumar426@gmail.com

ABSTRACT

Background: The present investigation aims to evaluate both the intensity and occurrence of hepatic impairment in patients afflicted with gallstone disease, examining the involvement of bacteria in the progression of these alterations.
Methods: This prospective observational investigation was carried out on 189 patients scheduled for open or laparoscopic cholecystectomy at IGIMS in Patna, Bihar, India. In all the patients, laboratory and radiological investigations were performed. A healthy section of the liver border near the gallbladder fossa was chosen and grasped with non-traumatic forceps. Approximately 1 cm of the liver edge was excised using scissors and forwarded for histopathological analysis.
Results: An examination of 189 liver biopsy specimens revealed that 87 (46%) patients showed no abnormalities, while 102 patients (54%) exhibited one or more changes. Upon analysing the liver biopsy samples from the control cohort (41 patients) during autopsy, 37% of the cases were identified to display hepatic lipidosis, while 48% of the cases showed lymphocytic invasion. Importantly, no cases of acute inflammatory changes were detected in the control cohort. Microbiological analysis was conducted on 96 patients, of which 33 (34%) showed positive cultures, with one or more microorganisms isolated from either the biliary tract or liver. Among these, 74% (24 cases) originated from the bile or gallbladder, while 26% (9 cases) were isolated from the liver.
Conclusions: Gallstone disease induces significant liver histological changes, notably more prevalent in patients with prolonged symptoms. The present study clearly identifies this and underscores the importance of timely diagnosis and intervention for the effective management of this disease.

Keywords: Choledocholithiasis, Cholelithiasis, Gallstone disease, Liver biopsy, Liver pathology

INTRODUCTION

Gall stone disease is a pressing health concern affecting 3-6 % of the adult Indian population and approximately 10 to 15% of the general adult populace.[1] Dealing with this disease typically includes cholecystectomy, a frequently performed procedure by general surgeons. Given that 1 to 4% of people annually experience symptoms warranting therapeutic intervention, its prevalence remains significant.[2]

Gallstones form when the components in bile reach a saturation point and can no longer solubilize. As the bile concentration increases in gallbladder, these substances become oversaturated and eventually crystallize. The small crystals formed, then adhere to the gallbladder mucus, leading to the generation of gallbladder sludge. Gradually, these crystals enlarge and coalesce to create larger stones that obstruct the hepatic and biliary tree and eventually manifests as complications.[3]

Besides these, patients affect with gall stone disorders, generally depict additional changes in the liver, particularly, cholangitis. This adverse effect further results in comorbidities like fatty liver, secondary biliary cirrhosis, fibrosis, nonspecific inflammatory changes, reactive hepatitis, etc.[4] Furthermore, it has also been identified that patients affected with choledocholithiasis suffer significant liver damage in contrast to those with cholelithiasis and cholecystitis.[4]

The debate surrounding the incidence rates of hepatocellular damage in individuals with gall stone disease has persisted for many years.[5] In 1918, Graham was the first to recognize the potential impact of gallstone disease on the liver, emphasizing the need for thorough evaluation.[6] Building on this foundation, Orloff et al conducted pioneering and seminal research between 1942 and 1948, marking a significant milestone in the understanding of this connection.[7] However, subsequent studies by other groups, offered differing perspectives by challenging Orloff's assertion emphasizing on the connection between cholecystitis and cholelithiasis with substantial hepatic damage.[8,9] Contrarily, the findings of other researchers corroborated the results of Orloff's study.[10,11] This divergence of views underscores the ongoing exploration and refinement of our understanding of the relationship between gallstone disease and its potential impact on liver histology.

Evidence suggests that patients experiencing cholangitis as a result of partial extra-hepatic large bile duct obstruction, regardless of the underlying cause, manifest both structural and functional liver changes.[12,13] It was noted that removing the bile obstruction, led to the restoration of bile flow and tackling of inflammation, and these were attributed to the observed alterations. This implies the existence of a direct link connecting the extent of hepatic inflammation and the obstruction within the biliary tree.[13] While earlier investigations demonstrated mild fibrosis in bile portal tracts, there were conflicting findings when examining the relationship between gallstone presence and liver histological changes.[12,13]

In a recent Indian study, cholelithiasis predominantly featured nonspecific reactive hepatitis (46.9%), whereas choledocholithiasis demonstrated notable modifications in both liver histopathology and liver function tests when compared to cholelithiasis.[12]

The current investigation intends to assess the impact of gallstone disease on liver parenchyma and the prevalence and extent of liver pathology in cholelithiasis in our population at the Department of General Surgery, IGIMS in Patna, Bihar, India.

METHODS

This prospective observational study was conducted at the Department of Surgery, Indira Gandhi Institute of Medical Science, Sheikhpura in Patna, Bihar, India following approval from the institutional ethical committee for a period of 2 years from August 2021 to September 2023. A total of 189 participants of either sex, scheduled for open or laparoscopic cholecystectomy were recruited.

Exclusion criteria were applied to ensure a homogeneous study population. Patients with a recent history of jaundice, use of hepatotoxic medication, hepatitis B or C, acute cholecystitis, altered coagulation profile, fatty liver, and portal hypertension, were excluded from the study.

Before enrolment, the patients and their relatives were comprehensively explained regarding the method of study and informed consent was obtained from them. Sociodemographic data of patients was obtained and were clinically and physically assessed. Subsequently, laboratory and radiological investigations were conducted for each participant. Both open and laparoscopic techniques were employed for gallbladder aspiration and cholecystectomy.

The hepatic pathology of 189 patients who were subjected to cholecystectomy for gallstone disease was studied. Among these, the liver and extrahepatic biliary tree cultures of the last 96 patients were also collected. Demographically, the patient cohort encompassed 147 (78%) women and 42 (22%) men; aged 16-86 years.

Further stratification regarding the participant’s health condition at the time of surgery revealed the presence of acute cholecystitis in 24 patients (12.7%), while common duct stones were seen in 18 patients (9.5%). Besides this, 44% of the patients (83 cases) were found to have chronic cholecystitis at the time of operation. In addition to these, 41 patients with no history of gallbladder or liver disease and calculi were taken as the control cohort. The cohort comprised of 26 men and 15 women between 15 to 84 years of age.

Following the removal of the gallbladder, hemostasis was carefully achieved around the gallbladder fossa (Figure 1 and 2). The process of achieving hemostasis around the gallbladder fossa involved the use of electrocautery to ensure a controlled and effective approach.

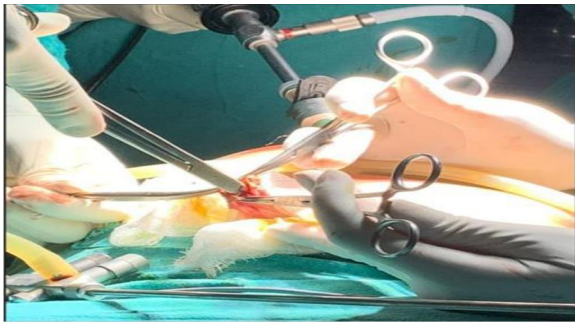

Figure 1: Removal of gall bladder through epigastric port.

Figure 2: Gallbladder specimen placed in sterile container.

Figure 3: Specimens send for histopathological examination with proper labelling, a) Gallbladder and b) Liver tissue.

To collect specimens for histopathological examination (Figure 3), an undamaged portion of the liver edge around the gallbladder fossa was selected. Atraumatic forceps were used to hold the selected portion, and approximately 1 cm of the liver edge was excised using sharp scissors (Figure 4). The excised liver tissue was then sent for histopathological examination (Figure 5).

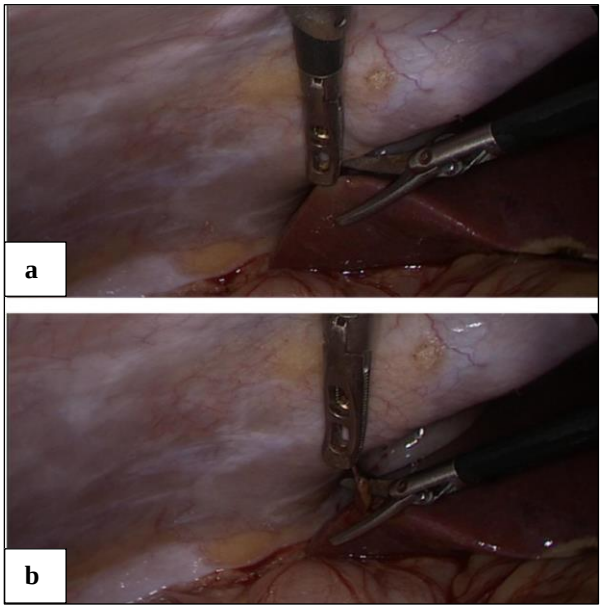

Figure 4 (a and b): Tissue taken from liver margin.

Figure 5: Liver tissue sample taken and put on a sterile gauge piece.

The study prioritized ethical standards, securing written consent and obtaining institutional ethics committee approval to uphold participant autonomy and informed consent principles.

Statistical analysis

The analysis of data utilized SPSS v21.0 (IBM, USA), representing quantitative data as mean and standard deviation, and categorical data as frequency and percentages. Statistical comparison between cohorts employed the Chi-square test, considering a p-value < 0.05 as indicative of statistical significance.

RESULTS

On thorough examination of the 189 liver biopsy specimens, it was revealed that 87 patients (46%) showed no abnormalities. The remaining 102 (54%) exhibited one or more changes. Among these, 53 cases (52%) displayed long-term inflammatory alterations in the portal triads. Hepatic lipidosis was detected in 27 patients (27%), while neutrophilic infiltration in the hepatic portal areas was observed in 20 patients (19.6%) (Table 1). Based on both clinical and pathological criteria acute cholecystitis was identified in 24 patients, with 75% (18 cases) of them displaying acute inflammatory changes in the portal triads. Furthermore, it was identified that out of these 18 patients with common duct stones, 10 cases (58%) exhibited acute hepatic inflammation. Among these 10, seven had both acute cholecystitis and choledocholithiasis.

Table 1: Histopathological examination of the liver in individuals with gallstone disease.

Pathological observations	Patient count (%)
No abnormalities	87 (46)
Hepatic abnormalities	102 (53.)
Abnormalities in the liver	
Chronic inflammatory alteration in liver	53 (52)
Hepatic lipidosis	27 (27)
Neutrophilic infiltration in portal triads	20 (19.6)

Analysis of liver biopsy specimens collected from the 41 patients of the control cohort during autopsy revealed that 37% (15 cases) exhibited hepatic lipidosis, while 48% (20 cases) showed lymphocytic infiltration. Notably, none of the participants of this cohort showed any acute inflammatory changes (Table 2).

Table 2: Findings of liver histopathology in control cohort.

Pathological observations	Patient count (%)
Chronic inflammatory alteration in liver	20 (48)
Hepatic lipidosis	15 (37)
Acute inflammatory alterations	0 (0)
No hepatic abnormality	6 (14)

Out of the 96 patients subjected to microbacteriologic examination, 33 patients (34%) showed positive culture results, indicating the presence of one or more microorganisms in the biliary tract or liver. Among these positive cultures, 74% (24 cultures) of the samples were collected from the gallbladder or bile, while 26% (9 cultures) were isolated from the liver. Notably, from the 9 hepatic cultures, 7 patients were identified to have acute cholecystitis or common duct stones.

Moreover, it was noted that only 2 samples from the 96 cultures demonstrated bacterial growth despite lacking microscopic evidence. Within the subgroup of 83 patients with chronic cholecystitis, 29% (24 patients) tested positive for bile or gall bladder cultures. Among those showing signs of acute cholecystitis (24 patients), five patients were identified to have positive cultures, while all 18 patients with common duct stones showed positive cultures.

The bacterial isolates comprised 30 microorganisms of enteric origin, with 20 being aerobic and 10 anaerobic. The most prevalent organism identified in this set was *E. coli* which accounted for 40% of the aerobic microorganisms. The other major isolates identified were *Enterococci* and *Klebsiella pneumoniae*. Among anaerobic microorganisms, Propionibacterium was the most prevalent, representing 50% of isolates, alongside Clostridia, Peptococcus, and Bacteroides. Remarkably, 5 out of the 9 positive liver cultures identified Propionibacterium, a prevalence not previously reported within the biliary tract.

DISCUSSION

The observations presented in this study exhibits similarities to findings in several other studies.[14-16] The only notable microstructural alteration was acute inflammation, characterized by the infiltration of neutrophils into the portal triads. This inflammatory change was exclusively evident in patients with gallstone disease, whereas the occurrence of hepatic lipidosis, lymphocytic infiltration, and fibrosis, showed no significant difference between patients with gallstones and the control group. These findings underscore the crucial need for a well-matched control group in studies of this nature.

The study findings indicate a direct correlation between the development of hepatic alterations and gallstone disease aligning with the previous findings.[17,18] The evidence presented suggests that structural as well as acute inflammatory changes, along with abnormalities in hepatic functioning, are more prevalent and severe in cases of acute cholecystitis and choledocholithiasis compared to chronic cholecystitis. The severity of these alterations was evident both in histopathological and bacteriological analyses. Moreover, it has been observed that functional changes may diminish either through the removal of the acutely inflamed gallbladder or the spontaneous resolution of the inflammatory process within the organ. This further corroborates the existence of a direct connection of the hepatic degree of inflammation and that seen in the gallbladder.

The severity of hepatic damage was most pronounced in cases with common bile duct obstruction. Cholangitis is more commonly linked to partial rather than complete obstruction of the common duct.[19] The prevailing theory attributes cholangitis to enteric organisms ascending through the common duct as the primary source of infection in the biliary tract and liver.[20] The presence of biliary stasis is also considered a significant factor in fostering infection within the biliary tract.[21] The observations of the present investigation aligns with these fundamental concepts, showing a higher incidence of acute intrahepatic inflammation in more severe cases of gallstone disease, with 75% of patients having acute cholecystitis and 58% among these depicting signs of choledocholithiasis.

Furthermore, there was a significant increase in bacterial growth affecting 63% and 100% of patients in the disease and control cohorts respectively aligning with the study conducted by Kujiraoka et al.[22] The positive liver cultures were linked to these specific forms of gallstone disease, with only one exception. These findings imply a direct connection between the acute inflammatory changes seen in gallstone disease and the hindrance of bile flow within the biliary ductal system as established by prior investigations.[23,24] Acute inflammation in the gallbladder may lead to sufficient stasis within the biliary tract, fostering similar widespread intrahepatic inflammatory changes and promoting bacterial contamination in the liver. This analysis underscores the applicability of early cholecystectomy in cases of cholelithiasis and emphasizes the importance of such proactive measures in preventing potential hepatocellular damage linked to the primary complications of gallstone disease, including acute cholecystitis and choledocholithiasis.

The limitations of the study include a reliance on retrospective data, potentially introducing bias, and the absence of long-term follow-up data, limiting the assessment of sustained outcomes.

CONCLUSION

The present investigation highlights a clear correlation between the development of hepatic alterations in gallstone disease. Structural and acute inflammatory changes, coupled with functional abnormalities, are more prevalent and severe in cases of acute cholecystitis and choledocholithiasis compared to chronic cholecystitis. This underscores the importance of early cholecystectomy for cholelithiasis, emphasizing its pivotal role in preventing potential hepatocellular damage linked to the major complications of gallstone disease, specifically acute cholecystitis and choledocholithiasis.

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