ORIGINAL ARTICLE

A Comparative Study on Benign and Premalignant Histological Changes Induced by E. Coli and K. Pneumoniae in Gall Bladder Mucosa Having Pigment Stones

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ABSTRACT

Objective: To observe and compare benign and premalignant histological changes induced by Escherichia coli (E. coli) and Klebsiella pneumoniae (K. pneumoniae) in gall bladder mucosa having Pigment stones.

Study Design: Cross sectional comparative study.

Place and Duration of Study: This study was conducted at the department of Anatomy in collaboration with the department of Surgery, Al Nafees Medical College and Hospital (ANMC) from 14th November 2017 to 18th December 2019.

Materials and Methods: Out of total cholecystectomy specimens collected during study duration**,** only thirty gallbladder specimens having Pigment stones and bile culture positive for E. coli or K. pneumoniae were obtained. They were divided into two groups comprising fifteen specimens each (n=15). Group 1 had bile culture positive for E. coli, while group 2 was positive for K. pneumoniae. All specimens were processed in automated tissue processor after tissue processing. Paraffin embedded sections were stained with Haematoxylin and Eosin stain. Slides were examined by consultant pathologist under light microscope. Premalignant histological lesions included hyperplasia, metaplasia and dysplasia. Chi square was applied to compare the statistical association of histological lesions between group 1 and 2.

Results: Histopathological analysis revealed that hyperplasia, metaplasia and dysplasia in group 1 and group 2 were found to be statistically insignificant with p values of p= 0.715, p= 0.464 and p= 0.1 respectively.

Conclusion: The histological patterns of benign and premalignant findings in both group 1 and group 2 showed minimal variation. Although this study did not observe any progression of premalignant lesions to carcinoma, the eradication of bacteria remains crucial to prevent the development of histopathological lesions in the gallbladder.

Key Words: *Dysplasia, E. Coli, Hyperplasia, K. Pneumoniae, Metaplasia and Gallbladder.*

Introduction

Gall bladder is an organ connected to the hepatobiliary system. Simple columnar epithelium lines its perimeter.[1] The lamina propria and muscularis externa are located underneath the epithelium. Submucosa and muscularis mucosae are absent. The histopathological diseases found in gallbladder range from cholelithiasis on one side of spectrum and carcinoma on another end. Its malignancy is always fatal.[2] Gall bladder cancer is the most common cancer of biliary tract and fifth most common cancer among gastrointestinal tract related cancers.[3] It is ranked 21st most common cancer worldwide in terms of mortality. On gender basis, females are three time more affected by gallbladder cancer than male. Both benign and malignant diseases of gallbladder may have bacterial infections as an underlying cause.[4] Chronic bacterial infections, polyps, and other conditions are among well-established risk factors for gallbladder cancer[5]. The frequent bouts of gallbladder epithelial injury and healing encourage a backdrop of chronic inflammation that leads to increasing morphological abnormalities through a sequential cascade of metastasis-dysplasia till carcinogenesis.[6] Helicobacter and Salmonella species are established risk factors for gastric cancer and gallbladder

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dysplasia.[5] Certain virulent strains E. coli and Klebsiella are currently being investigated for their role in colorectal cancer. E. coli and Klebsiella species along with Enterobacter and Enterococcus are frequently found in bile cultured from gallbladder specimens having cholelithiasis and cancer but their association with histopathological lesions is yet not clear.[7] E. coli and klebsiella have a tendency to alter the normal histology of the gallbladder by producing beta glucuronidase and phospholipase. They encourage the formation of gallstones and their presence in bile may result in premalignant changes in the gallbladder.[8] The objective of this study was to compare benign and premalignant histological changes induced by E. coli and K. pneumoniae species in gall bladder mucosa, having Pigment stones. Gallbladder cancer is complex disease with poor prognosis and worst outcomes. If bacterial toxins produced by chronic bacterial infection are responsible for carcinogenesis, eradication of causative microbes by antibiotic therapy is essential in order to prevent chronic illnesses and patient health care.[9] So, the rationale was to identify the bacterial risk factors linked to gallbladder cancer before creating preventive and therapeutic strategies.

Materials and Methods

It was cross sectional comparative study conducted at the department of Anatomy in collaboration with the department of Surgery at ANMC and hospital from 14th November 2017 to 18th Dec, 2019. A total of thirty(n=30) gallbladder specimens were selected out of cholecystectomy specimens collected during study duration based on selection criteria of pigment stones and bile culture positive for E. coli or K. pneumoniae. The sampling technique was convenient sampling. The study protocol was approved by Ethical Review Committee Board (ERCB) vide their letter dated F.2/IUIC-ANMC/EC-94/2015. The inclusion criteria were based upon cholecystectomy specimens having pigment stones and bile culture positive for E. coli or K. pneumoniae. Patients of male and female genders with mean age of 44$\pm$5 were selected for study purpose with informed consent. Patients diagnosed with biliary malignancy, prior biliary surgical procedures and gallbladder specimens with cholesterol stones were excluded from studies. 4-5 ml of bile was aspirated from each gallbladder sample by sterile syringes. Bile was subjected to gram staining procedure, bile culture and Analytical Profile Index (API 10s) to identify gram negative bacteria i.e. E. coli or K. pneumoniae. Thirty gallbladder specimens having Pigment stones were divided into two groups. Gallbladder specimens with Pigment stones were collected from patients after cholecystectomy having concomitant biliary infection with E. coli were included in Group 1 (n=15). Gallbladder specimens with Pigment stones were collected from patients after cholecystectomy having concomitant biliary infection with K. pneumoniae species. Were included in Group 2 (n=15). Longitudinal sections of gall bladder tissue were removed from body and fundus of gallbladder and fixed in 10% formalin for further tissue processing. Haematoxylin and Eosin stain (H & E) were used to study benign and premalignant histological lesions and dysplasia of gallbladder specimens in both group 1 and 2 under light microscope. Benign histological lesions observed were Hyperplasia (Increased number of cell production in normal tissue). Diffuse or local hyperplasia was noted in any gallbladder tissue section. Premalignant histological lesions observed were Metaplasia (conversion of one cell type into another) and Dysplasia (Presence of abnormal cells within a tissue). Pyloric (resembling pyloric glands) or intestinal metaplasia (showing goblet cells) was observed in each gallbladder tissue section. Low grade dysplasia showing stratification of epithelial cells and hyperchromatic nuclei was also noticed. High grade dysplasia showed marked cellular disorganization, pleomorphism, loss of polarity and increased mitosis of epithelium under light microscope. Both Benign and premalignant histological lesions of gallbladder compromise spectrum of chronic cholecystitis and gallbladder cancer.[10] The benign and premalignant histological lesions like hyperplasia, metaplasia and dysplasia in groups 1 and 2 were observed and compared with each other. The chi-square test or Fisher's exact test for categorical variables in group 1 and 2. A p value of <0.05 was considered statistically significant. All statistical analyses were performed by using SPSS version 22.

Results

Hyperplasia whether diffuse or focal was observed in

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gallbladder tissue under high power lens (10x40). Hyperplasia was present in 8 out of 15 (53%) percent of gallbladder specimens in Group 1. Hyperplasia was present in 7 out of 15(47%) percent of gallbladder specimens in group 2. Metaplasia was observed in gallbladder tissue under high power lens (10x40). Metaplasia was present in 9 out of 15(60%) percent of gallbladder specimens in group 1. Metaplasia was present in 47% (7 out of 15) percent of gallbladder specimens in group 2. Dysplasia was observed in gallbladder tissue under high power lens (10x40). Low grade focal dysplasia was present in 1 out of 15 (7%) of gallbladder specimens in group 1. Low grade focal dysplasia was present in 7% (1 out of 15(7%) of gallbladder specimens in group 2. There was no statistically significant association between benign and premalignant histological lesions of group 1 and 2.

Table I: Demographic Data

	E. coli (Group 1)	K. pneumoniae (Group 2)
Number of Cases (n)	15	15
Age (mean in years)	44±5	44±5
Gender Male	5	4
Gender Female	10	11

Table II: Premalignant Histological Lesions Observed in Gall Bladder Specimen of Group 1(E. Coli) and Group 2 (K. Pneumoniae)

	Group I		Group II		*p*-value
	Present	Absent	Present	Absent	
Hyperplasia	53% (8)	47% (7)	47% (7)	53% (8)	.715
Metaplasia	60% (9)	40% (6)	47% (7)	53% (8)	.464
Dysplasia	7% (1)	93% (14)	7% (1)	93% (14)	0.1

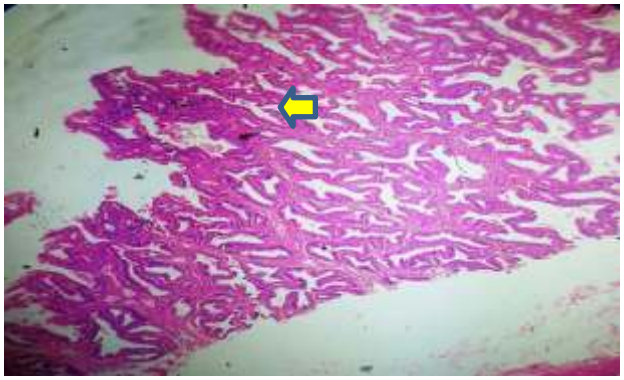

Figure 1: Photomicrograph Showing Gallbladder Specimen Having Hyperplasia (H&E stain) (Specimen No.6 of Group 2) (10x)

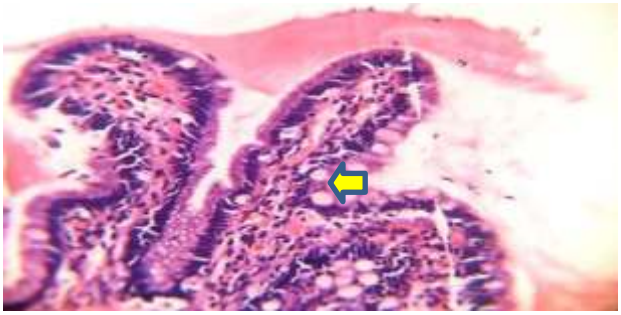

Figure 2: Photomicrographs Showing Gallbladder Specimen Having Metaplasia (H&E Stain). (Specimen No.5 of Group 1 (10x)

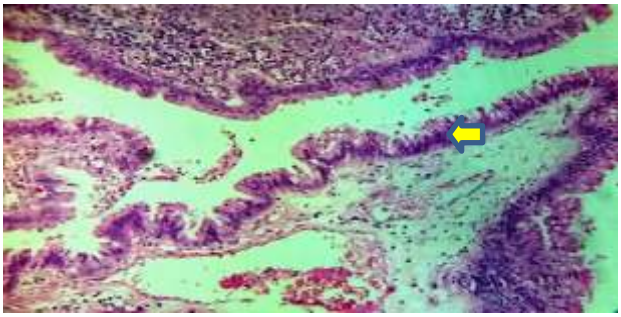

Figure 3: Photomicrographs Showing Low Grade Dysplasia in Gall Bladder (H&E stain ,10x)

Discussion

E. coli and K. pneumoniae were the most common bacteria in bile cultured from cholecystectomy specimens. Numerous regional and international studies support our findings that E. coli and K. pneumoniae are the most common bacteria isolated from bile.[11] Biliary bacteria in more predominantly present in gallbladder having pigment stones.[12,13] Hyperplasia was present in higher proportion i.e. 53% in group 1 gallbladders infected with E. coli but was statistically insignificant in both groups 1 and 2. Similarly, metaplasia was found in higher percentage i.e.60% in group 1 but was statistically insignificant in both groups. Low grade dysplasia was found only in single specimens of group 1 and 2.

Hyperplasia is a benign lesion frequently found in cholecystectomy specimens. It can be local, segmental, diffuse or annular. Adenomyomatous hyperplasia of the gallbladder is a benign condition characterized by hyperplasia of the gallbladder wall mucosa and muscularis propria with hallmark epithelial invaginations forming cystic pockets known as Rokitansky-Aschoff sinuses. This peculiar type of hyperplasia can mimic gallbladder cancer and present diagnostic challenge for health providers.

Hyperplasia is frequently in chronic inflammations of gallbladders.[14,15]

Metaplasia of both types i.e. pyloric and intestinal is found in histopathological lesions of chronic cholecystitis.[16] Pyloric gland metaplasia in found in association with bacterial infections, old age and gallstones. Intestinal metaplasia is less common of both types but with increased risk association for gallbladder cancer.[17] Bacterial infections with chronic cycle of inflammation and repair tend to increase metaplasia of gallbladder.[18,19]

Dysplasia is the second last step in development of gallbladder cancer following metaplasia.[20] Depending on other factors, whether gallbladder cancer eventually develops or not, dysplasia is incidental finding in bacterial cholecystitis. Low grade dysplasia is usually focal. High grade dysplasia is significantly related to adenocarcinoma of gallbladder.

Bacterial role has been implicated in many histopathological lesions of gastrointestinal tract leading to cancers.[21,22] H. pylori is a well-known and well-established risk factor for gastric cancers. It is responsible for the production of toxins, reactive oxygen species and inflammatory gastritis. Each component directly or indirectly contributes to altering normal host cellular pathways.[23] Salmonella species was recently declared as risk factor for gallbladder cancer by researchers. In chronic stages of cholecystitis, it releases toxins responsible for damaging DNA of host cells.[24] Similarly, Chlamydia infection is considered as risk factor for lung cancer.[24] Fusobacterium species is considered carcinogenic bacteria for colorectal cancers.[24] Certain pathogenic of E. coli modify host cell immune mechanism by virulence factors and inflammatory pathways in colorectal cancers. They can lead to altered cellular growth.[25] This role of bacterial infections in gastrointestinal diseases prompted our interest to explore the role of E. coli and K. pneumoniae infected bile and histopathological lesions of gallbladder in their presence.

In our study, the mean age of patients was 44 $\pm$ 5 years. Hyperplasia was more prevalent in Group 1 but not statistically significant. Supporting are findings are observation of hyperplasia in Pylori infected bile albeit to a lesser extent.[26] As comparison, one more study found hyperplasia to a lesser percentage in H. pylori bile positive samples.[27] One Indian study found hyperplasia to be prevalent in majority of cases.[28] One study demonstrated statistical association between degree of fibrosis and H. pylori.[29] This difference of more prevalence of hyperplasia in our study might be due to chronic stage of cholecystitis.

In our study metaplasia was more in group 1 as compared to group 2. Both of them were statistically not significant. In both groups, metaplasia was found in higher percentage overall as compared to one study conducted in Pakistan.[27] The study conducted in Pakistan only showed metaplasia only in 3% of H. Pylori positive gallbladder specimens. One study in India showed no metaplasia in H. Pylori infected gallbladder.[29] One study showed both pyloric and intestinal metaplasia to larger percentage in H. pylori infected gallbladder specimens supporting our findings.[30] Differences of metaplasia proportion in our study and other studies might be due age, sex and chronic phase of cholecystitis.

In our study, low grade focal dysplasia was present in single specimen both groups 1 and 2 but statistically insignificant. Dysplasia being incidental finding was not reported in one study conducted in Pakistan[27] and other in India.[29] One study conducted in Turkey found low grade dysplasia in 2% of gallbladder specimens.[30]

In our study, although statistically insignificant but benign and premalignant histopathological lesions are observed in gallbladder having Pigment stones and bile infected with E. coli or K. pneumoniae. Exact pathological mechanisms and virulence factors of E. coli and K. pneumoniae contributing to gallbladder lesions is poorly understood due to paucity in research. They have carcinogenic potential for premalignant lesions in gallbladder like salmonella is not known till date. Our limitations of research study are primarily due to fact that it is observational study. A definite link from premalignant lesion to dysplasia cannot be established only with bile having E. coli or Klebsiella species. Molecular, host cell genetics and virulence factors of pathogenic strains of E. coli and Klebsiella need to be studied and explored on research basis to assess their risk.

Conclusion

The histological patterns of benign and premalignant findings in both group 1 and group 2 showed minimal variation. Although this study did not observe any

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progression of premalignant lesions to carcinoma, the eradication of bacteria remains crucial to prevent the development of histopathological lesions in the gallbladder.

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CONFLICT OF INTEREST

Authors declared no conflicts of Interest.

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DATA SHARING STATMENT

The data that support the findings of this study are available from the corresponding author upon request.