Study of Bacterial Aetiogenicity of Pigment Gall Stones by Culture of Nidus of Calculus and correlation between Stone Culture and Bile Culture

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ABSTRACT

Background: Calculus disease of biliary tract is a major public health problem in north Kashmir and pigment gall stones form a significant portion of gall stones in our region. In this study we prospectively studied 84 cases of pigment gall stones to examine the role of bacteria in the formation of these stones.

Objective: To study the bacterial aetiology of pigment gall stones.

Methods: A hospital based prospective study of 84 consecutive cases of pigment gall stones was done from December 2012 to November 2019 and the results of stone culture and bile culture were statistically analysed. Informed consent was obtained from all the patients in the study group.

Results: A total of 84 cases studied included 32 (38.09%) male patients and 52 (61.91%) female patients. The median age was 48.5 years and mean age was 46.2 years. Of 84 patients, 21 (25.00%) had black pigment stones and 63 (75.00%) had brown pigment stones. 61 (72.62%) patients presented with biliary colic and 24 (28.57%) patients had chronic cholecystitis. Bile culture was positive in 46 (54.76%) patients and stone culture was positive in 70 (83.33%) patients.

Conclusion: A strong association was observed between stone culture and bile culture results (showing similar beta-glucuronidase producing bacteria) and pigment stone formation.

Key words: Gall stones, stone culture, bile culture, nidus

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INTRODUCTION

Gall stone disease is known to mankind since ancient times.¹ Pigment gall stones have been referred to as early as 500 AD by Alexander of Tralle². These constitute 10% to 27% of all types of gall stones found in general population and both intrahepatic gall stones and stones caused by bile stasis or cirrhosis are usually pigment stones³. Pigment stones are usually multiple, 2 to 5mm in diameter, irregular to smooth in shape, black to brown in colour and amorphous or crystalline on cross section. Pigment stones, especially the brown ones, can form within extra hepatic and intra hepatic ducts apart from their usual occurrence in the gall bladder

and are commonly associated with ascending cholangitis. Enteric bacteria have been found to play a significant role in pigment gall stone formation and these stones in general and brown ones in particular have a tendency to recur while black gall stone disease is usually non recurrent⁴. Unlike the causative factors like cirrhosis, ileal resection etc. observed in the West, infection has been found to be a predominant cause in South East Asia.¹⁰ In this study, we prospectively

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studied 84 patients with pigment gall stones disease to examine the role of beta glucuronidase producing bacteria in the formation of these type of stones. 'Pigment gall stones' included any black or dark brown stone containing less than 25% cholesterol, whether or not pigment was its major component and irrespective of the nature and proportion of other constituents¹³.

MATERIAL AND METHODS

A hospital based prospective study was carried out from December 2012 to November 2019 in the departments of General Surgery, Microbiology and Radio diagnosis of Govt. Medical College Baramulla, Kashmir. It included a total of 84 consecutive patients with pigment gall stones (confirmed by chemical analysis of extracted gall stones). A formal informed consent was taken from all of them. The patients were selected as they fulfilled the following inclusion criteria: confirmed to have pigment gall stones, that is, black to dark brown gall stones containing less than 25% cholesterol irrespective of the proportion of other constituents and whether are not pigment was its major constituent²⁰; not on any medication for gall stones for last three months and had not received antibiotics for two weeks prior to surgery.

A detailed account of history was taken and complete physical examination was performed. Routine investigations including complete blood count, liver function tests, serum amylase and calcium levels were requested as also an abdominal ultrasound performed to know about the location, number and size of gall stones and gall bladder wall thickness or oedema and any associated dilatation of hepato-pancreatic ducts was ruled out. In selected cases, an abdominal computerised axial tomogram or a magnetic resonance cholangiogram was also done to exclude or to elaborate on any added pathology.

All the 84 patients were subjected to cholecystectomy and gall bladder sample along with the stones within it was placed aseptically in a container. 5 to 10 ml of bile was aspirated from within the gall bladder and preserved for bile culture and sensitivity in all the 84 cases during the operative procedure. The gall stones were removed and immediately transferred to a McCartney bottle containing 5ml of normal saline for further processing. A portion of stone was sent for chemical analysis and the other portion preserved for microbiological evaluation. Only those

calculi were included in the present study whose chemical analysis results fulfilled the criteria already stated for the case of pigment stones.

A technique for culture of calculi as devised by Nemoy and Stamey²² in 1971 was done. The culture methods were designed for localization of bacteria to the substance of the calculus and to distinguish bacteria within a stone from surface colonization. Under all aseptic precautions, a portion of stone was washed consecutively in four test tubes containing 5 ml of sterile saline each and then crushed in another tube in a laminar air flow chamber. 0.1ml of fluid from washes 1 and 4 and of the fluid of crushed stones were quantitatively cultured in Blood Agar, McConkay Agar and Brain Heart Infusion Broth and Agar. The cultures were studied at 24 hours and 48 hours and organisms typed. An increased density of isolate in the fluid of crushed stone and increased colony count after culture compared with the fluid of wash 4 was considered evidence for bacteria within a stone. The spectrum of bacteria that colonized the pigment stone was detailed out to assess their relation with the genesis of pigment gall stones.

RESULTS

In the present study of 84 consecutive patients with pigment gall stones, confirmed by stone analysis, conducted in the department of General Surgery at Govt. Medical College Baramulla, 32(38.09%) patients were males and 52(61.91%) patients were females. The median age was 48.5 years and mean age was 46.2 years. The distribution of study subjects as per the type of stones found in 84 patients showed that 21(25.00%) patients had black pigment stones and 63(75.00%) patients had brown pigment gall stones. (Table 1).

Table 1: Distribution of 84 study subjects as per type of stone.

Type of pigment stone	Number	Percentage (%)
Black pigment stones	21	25.00
Brown pigment stones	63	75.00
Total	84	100

Of 84 study subjects, 61 (72.62%) patients presented with biliary colic, and 24(28.57%) patients presented with chronic cholecystitis. Acute infective conditions like acute cholecystitis were observed in 2(2.38%) patients. 16(19.05%) patients presented with ultrasonic evidence of mucocele or empyema of gall bladder prior to surgery. Only 1(1.19%) patient had a documented predisposing cause in

the form of glucose 6 phosphate deficiency haemolytic anaemia. (Table 2).

Table 2: Distribution of cases as per the presenting features .

Presenting feature	Number	Percentage (%)
Biliary colic	61	72.62
Chronic cholecystitis	24	28.57
Acute cholecystitis	02	2.38
USG evidence of infection	16	19.05
Predisposing causes in the form of G6PD deficiency haemolytic anaemia	01	1.19

Intra operative findings revealed thickened gallbladder wall suggesting chronic cholecystitis in 46 (54.76%) patients; 3(3.57%) patients had dense adhesions of gallbladder with surrounding viscera and 3 (3.57%) patients had gall bladder perforation with cholecystoenteric fistula formation. (Table 3).

Table 3: Presentation of subjects at the time of surgery.

Intraoperative presentation	Number	Percentage (%)
Thickened gallbladder wall	46	54.76
Gallbladder perforation	03	3.57
Adhesions	03	3.57

Of 84 study subjects, bile culture was positive in 46 (54.76%) cases while stone culture was positive in 70 (83.33%) cases. Both bile and stone cultures were positive in 32 (38.09%) cases; bile culture was negative but stone culture was positive in 38(45.24%) cases while in 14 (16.67%) cases both the cultures were negative.(Table 4). The stones which were positive showed uniform distribution of bacteria throughout the stone including its core as confirmed by the culture and sensitivity reports of the fragments of the stone including its core.

Table 4: Comparison of stone culture and bile culture results of 84 study subjects

Culture positivity	Number	Percentage (%)
Bile culture positive	46	54.76
Stone culture positive and bile culture positive	32	38.09
Stone culture positive and bile culture negative	38	45.24
Stone culture negative	14	16.67
Bile culture negative	38	45.24

Of 70(83.33%) culture positive stone culture results, 11(13.09%) black stones and 59(70.24%) brown stones were culture positive for bacteria (Table 5).

Table 5: Stone culture results of 84 study subjects.

Evidence of bacteria	Number	Percentage (%)
Stone culture positive	70	83.33
Stone culture negative	14	16.67
Presence of bacteria in black stones	11	13.09
Presence of bacteria in brown stones	59	70.24

Most of the bacteria were bacillary forms and *E. coli* was the commonest organism grown in 73.81% of stone cultures. Few cultures revealed other types of bacteria such as *Klebsiella* (3.57%), *Salmonella typhi* (3.57%) and *Staphylococcus aureus*. (Table 6). In some of the samples, there were groups of bacteria suggesting colony formation.

Table 6: Distribution of 70 patients with culture positive stone nuclei in relation to bile culture results.

Bacteria	Number of patients with culture positive stone cultures		Bile culture showing same bacteria	
	Number	percentage (%)	Number	Percentage (%)
<i>E.coli</i>	62	73.8	28	33.33
<i>Klebsiella</i>	3	3.57	1	1.19
<i>Salmonella Typhi</i>	3	3.57	2	2.38
<i>Staphylococcus aureus</i>	2	2.38	1	1.19
Total	70	83.33	32	38.09

DISCUSSION

In the present study, out of 84 patients studied, 32(38.09%) patients were males and 52 (61.91%) patients were females. The median age was 48.5 years and mean age was 46.2 years. Of 84 patients studied, 21(25.00%) patients had black pigment stones and 63(75.00%) patients had brown pigment stones. 72.62% patients presented with biliary colic and 28.57% patients presented with chronic cholecystitis. At surgery 54.76 % patients had thickened gallbladder wall suggesting chronic inflammation and 3.57 % cases presented with gallbladder perforation. These findings correlated with those of Akiyoshi T and Nakayama F²⁰. The central portions (nidus) of the stones collected from 84 patients were cultured as per the technique already described. It was observed that 83.33% of the stones from 70 patients were culture positive and 16.67% of the stones from 14 patients were cultured negative. Of 83.33 % culture positive patients, beta glucuronidase producing organisms were found in 73.81 % of patients. Of 70 culture positive stone cases, 32(38.09%) had bactobilia with same organisms. These findings suggest that bacterial infection with beta glucuronidase producing organisms plays a significant role in pigment gallstone formation and pigment gall stones are the tomb stones of bacteria; in addition it was observed that majority of black pigment stones were not associated with predisposing causes such as ileal resection, cirrhosis of liver or haemolytic disease unlike the observations in Western studies²¹ and were again found to be associated with infection of stone nuclei and bile. Authors

wanted to explore at this aspect and especially to explore the association of black pigment stones with bacterial infection of bile and stone material. It was found that a significant portion (25.00%) of our study subjects who had black pigment stones had no predisposing factors mentioned above in Western world and were again found to be associated with recurrent biliary infection.

Though it was observed that bacteria are possibly etiological agents due to the evidence at hand, we cannot exclude the possibility that they are innocent bystanders. The fact that bacteria were found in the nuclei of multi-layered stones favours the hypothesis that bacteria are associated with stones at a nascent stage of their development. These findings are supported by the work of Soloway RD et al^{24,25}. The possible mechanism in pigment stone formation could be a combination of cholecystitis and bile duct occlusion due to bacterial infection producing excessive mucous formation and release of deconjugated bilirubin, bile acids, hydrolyzing phospholipids and calcium, thereby leading to pigment stone formation^{5-9, 11, 12}. Similar observations have substantiated the close association of bacteria with brown pigment stone formation^{15-19, 14, 23}.

CONCLUSION

Considering all these findings, it could be suggested that hepatobiliary tract infection with beta glucuronidase producing bacteria has a significant role in the formation of pigment gall stones. Bacteriological study of a large number of stones especially in relation to their age, chemical composition and serial culture of patients might help in further establishing the role of bacterial infection in stone formation.

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