Original Research Article

A study of etiology and role of bacteria in the pigment gall stone diseases at a tertiary care hospital

Rajendra Desai1, Earnest Daniel Prasad1*, Satyanarayana G.2, Lakshmi V.3, Nayana Joshi4

1Department of General Surgery, Shadan Institute of Medical Sciences, Peeramcheru, Hyderabad, Telangana, India
2Department of General Surgery Kamineni Academy of Medical Sciences and Research Centre, LB Nagar, Hyderabad, Telangana, India
3Department of Microbiology, Kamineni Academy of Medical Sciences and Research Centre, LB Nagar, Hyderabad, Telangana, India
4Department of Gastroenterology, Nizam’s Institute of Medical Sciences, Hyderabad, Telangana, India

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*Correspondence:
Dr. Earnest Daniel Prasad,
E-mail: prasad665@hotmail.com

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ABSTRACT

Background: Calculus disease of the biliary tract continues to be a significant health problem. Pigment stones are more common in Eastern studies. Predisposing factors such as cirrhosis, ileal resection are commonly seen in the West; while infection as a cause predominates in South East Asia. In this study we prospectively studied Pigment gall stones to examine the causes for and the role of bacteria in pigment Gall stone disease

Methods: A hospital based prospective study was carried out from January 1992 to December 1994 at Nizam’s Institute of Medical Sciences, Hyderabad. Institutional Ethics Committee permission was taken. Informed consent was obtained from all selected patients. During the study period a total of 25 consecutive patients with Pigment gall stones were analyzed. Details were recorded in a proforma. Stones were collected from the Gall Bladder with aseptic precautions.

Results: Total of 25 patients included, wherein 13 (52%) males and 12 (48%) females. The Median age was 55.5 years and the mean age was 50.2 years. Of 25 patients 11 (44%) had black pigment stones and 14 (56%) had brown stones. 22 (88%) patients presented with biliary colic, and 8 (32%) presented with chronic cholecystitis. At surgery 14 (56%) had a thickened wall suggesting inflammation, 7 (28%) had edema. Bile culture was positive in 11 (44%) patients and stone culture in 5 (20%) patient. Twenty two of 25 patients (88%) had evidence of bacteria on electron microscopy.

Conclusions: Whether black stones are associated with higher infection rates or bacteria cause black stones – this question probably would never be answered either on the bench or in the clinicians ward. We, however, can definitely say they are intimately related.

Keywords: Black stones, Gall stones, Infection rates, Pigment stones

INTRODUCTION

The digestive fluid i.e. bile may sometimes get hardened and it can lead to the formation of gall stones in the gall bladder. The size and shape can vary. It can vary from a size of small seed to that of cricket ball or golf ball. This is due to interaction of various factors.¹

The important cause of formation of gall stones is due to imbalance in the chemical constituents of bile. This leads
to precipitation of one or more components. In modern society, gallstone disease is becoming more common.2 Gallstone disease is known to mankind since ancient time.3,4 Though there are variations in the incidence of the disease, the disease is worldwide.5

Brown and black pigment gallstones are distinct from each other in terms of composition and morphology. Black stones are formed in sterile bile in the gallbladder. The chronic hemolysis, increasing age, hepatic cirrhosis, chronic alcohol use, total Parenteral nutrition and pancreatitis are the risk factors for black pigment gallstones.

Occurrence wise also, brown stones differ from black stones, in terms that brown stones can form within the extrahepatic and intrahepatic apart from usual occurrence in the gall bladder. They are associated with ascending cholangitis. Enteric bacteria are found to be uniformly infecting the brown stones. In terms of outcome the cure rate for black pigment stone disease is almost 100% compared to tendency to recur in brown pigment stone disease. Black pigment stone disease is usually non bacterial whereas the brown pigment stone disease is bacterial.6

Calculus disease of the biliary tract continues to be a significant health problem. Pigment stones are more common in Eastern studies. Predisposing factors such as cirrhosis, ileal resection are commonly seen in the West; while infection as a cause predominates in South East Asia.7 In this study authors prospectively, studied pigment gall stones to examine the causes for and the role of bacteria in pigment gall stone disease.

**METHODS**

A hospital based prospective study was carried out from January 1992 to December 1994 at Nizam’s Institute of Medical Sciences, Hyderabad. Institutional Ethics Committee permission was taken.

Informed consent was obtained from all selected patients. During the study period a total of 25 consecutive patients with Pigment gall stones were analysed. Details were recorded in a proforma. Stones were collected from the Gall Bladder with aseptic precautions. The stones were collected in 4 sterile bottles:-

- Washed in saline and stored in a dry bottle
- Washed in saline and stored in Povidone iodine 10%
- Physical analysis with electron microscopy and
- Chemical analysis.

The chemical composition of the stone was used to categorize the stones into cholesterol stones, brown pigment stones or black pigment stones.

Microbiological analysis was done by using the stones stored in betadine after washing them with 3 aliquots of sterile saline. Surface culture was done by plating to rule out contamination, then the stones were crushed and cultured in blood agar, Macconkey agar, brain Heart infusion broth and agar and Mueller Hinton agar. The cultures were studied at 24 hrs and 48 hrs and organisms typed.

Electron microscopy was done after each sample was numbered, the sample cut with a sterile blade, and mounted on a brass stub with conducting glue. The whole specimen was made conducting by sputter coating it with a very thin layer of Gold. The samples were then scanned in the Electron microscope at 15 KeV and an amperage of 6 x 10^-16 A and photomicrographs were obtained of areas of interest. Bacteria were accepted as positive based on the morphology conforming to coccoid or bacillary form and dimensions corresponding to bacterial dimensions.

Data was entered in Microsoft Excel worksheet and analysed using proportions.

**RESULTS**

| Table 1: Sex wise distribution of study subjects. |
|------------------------------------------------|
| Sex     | Number | Percentage (%) |
|---------|--------|----------------|
| Male    | 13     | 52             |
| Female  | 12     | 48             |
| Total   | 25     | 100            |

Twenty five patients included 13 (52%) males and 12 (48%) females. The median age was 55.5 years and the mean age was 50.2 years (Table 1).

| Table 2: Distribution of study subjects as per type of stone. |
|---------------------------------------------------------------|
| Type of pigment stone | Number | Percentage (%) |
|-----------------------|--------|----------------|
| Black pigment stone   | 11     | 44             |
| Brown pigment stone   | 14     | 56             |
| Total                 | 25     | 100            |

Of 25 patients 11 (44%) had black pigment stones and 14 (56%) had brown stones (Table 2).

Twenty two (88%) patients presented with biliary colic, and 8 (32%) presented with chronic cholecystitis. Acute infective conditions like acute cholecystitis were present in 8 (32%) patients. Only one patient (4%) had a predisposing cause in the form of Glucose 6- phosphate deficiency Hemolytic anemia. 3 (12%) patients had fever prior to surgery of which two had leukocytosis. Of the 25 patients only five (20%) showed ultrasound evidence suggestive of infection (Table 3).

At surgery 14 (56%) had a thickened wall suggesting inflammation, 7 (28%) had edema. 15 (60%) had
significant adhesions. 4 patients (16%) had gall bladder perforations (Table 4).

**Table 3: Distribution of study subjects as per the presenting features.**

| Presenting features               | Number | Percentage (%) |
|-----------------------------------|--------|----------------|
| Biliary colic                     | 22     | 88             |
| Chronic cholecystitis             | 08     | 32             |
| Fever prior to surgery            | 03     | 12             |
| Ultrasound evidence of infection  | 05     | 20             |
| Predisposing cause in the form of Glucose 6-phosphate deficiency | 01 | 04 |
| Hemolytic anemia                  |        |                |

**Table 4: Presentation of subjects at the time of surgery.**

| Presentation of subjects at the time of surgery | Number | Percentage (%) |
|-------------------------------------------------|--------|----------------|
| Thickened gall bladder wall                      | 14     | 56             |
| Edema                                            | 07     | 28             |
| Gall bladder perforation                         | 04     | 16             |

**Table 5: Culture positivity of the study subjects.**

| Culture positivity          | Number | Percentage (%) |
|-----------------------------|--------|----------------|
| Bile culture positive       | 11     | 44             |
| Stone culture positive      | 05     | 20             |
| Stone culture negative      | 05     | 20             |
| Bile culture negative       | 11     | 44             |

Bile culture was positive in 11 (44%) patients and stone culture in 5 (20%) patients (also positive for bile culture). Fourteen (56%) had stone cultures negative and 11 (44%) bile cultures negative (Table 5). The stones which were positive showed uniform distribution of bacteria throughout the stone including the core (Table 6).

**Table 6: Evidence of bacteria in patients.**

| Evidence of bacteria          | Number | Percentage (%) |
|-------------------------------|--------|----------------|
| Positive                      | 22     | 88             |
| Negative                      | 03     | 12             |
| Presence of bacteria in black stones | 08     | 72.7           |
| Presence of bacteria in brown stones | 14     | 100            |

Most of the bacteria were bacillary forms and in some authors had groups of bacteria suggesting colony formation. 3 patients had clinical or laboratory evidence of infection at the time of sample collection. Only 3 patients did not have either past or present evidence of infection in any form. 72.7% of black and 100% of brown stones had bacteria on electron microscopy (Table 6).

**DISCUSSION**

Total of 25 patients included 13 (52%) males and 12 (48%) females. The Median age was 55.5 years and the mean age was 50.2 years. Of 25 patients 11 (44%) had black pigment stones and 14 (56%) had brown stones. 22 (88%) patients presented with biliary colic, and 8 (32%) presented with chronic cholecystitis. At surgery 14 (56%) had a thickened wall suggesting inflammation, 7 (28%) had edema. Bile culture was positive in 11 (44%) patients and stone culture in 5 (20%) patient. 22 of 25 patients (88%) had evidence of bacteria on electron microscopy.

Gallstones have been described as the tombstones of bacteria. It is now clear that brown pigment stones are indeed so and numerous studies have demonstrated the close association of bacteria with brown pigment stones. Authors wanted to look at this aspect and especially look at black pigment stones since we found that majority of stones collected from our patients were black stones and in almost all of them no predisposing factor for their formation, as reported in literature, was found. Authors confirmed this general impression in our study and discovered that the majority of patients with black pigment stones did not have an associated predisposing cause such as ileal resection, cirrhosis of the liver or hemolytic disease. Most Western studies in contrast show evidence of these diseases in patients with black pigment stones. Authors also found that brown pigment stones in many patients had a central core occupied by a black pigment stones. Similarly, from studies in South East Asia conditions such as recurrent pyogenic cholangitis, Fasciola hepatica infection etc are shown. In contrast authors have found a very high incidence of bactobilia in individuals with black pigment stones in addition to bacteria on electron microscopy. The possible mechanisms that could explain this include a higher incidence of infectious complications of biliary disease once initiated due to poor immunity. This is due most often to a poor nutritional status. However, there is lack of any objective evidence to suggest impaired immunity through community based studies have shown cell mediated immune deficiency in individuals. In Asian countries a higher concentration of bacteria in the upper gastrointestinal tract has been observed. This may be related to unhygienic conditions due to poor sanitary conditions and due to contamination of water.

Though authors feel that the bacteria are possibly etiological due to the evidence at hand we cannot exclude the possibility that they are innocent bystanders. The fact that bacteria are present in stones throughout the matrix of the stone and the fact that they are associated with the nucleus of multi-layered stones goes in favor of the hypothesis that bacteria are associated with stones at a nascent stage of their development if not the cause of...
their birth. These findings find support in the work of Stewart et al, unlike Soloway.\textsuperscript{12}

Experimental models developed by Maki T, where bacteria were injected into the gall bladder leading to the formation of pigment stones lend support to present study hypothesis.\textsuperscript{8} Beta-glucuronidase activity either of bacterial origin or endogenous deconjugation is the probable etiological mechanism. Soloway has not reported a high incidence of infection subsequent to the development of stone disease. This means that stones per se do not lead to a higher incidence of Infection.

CONCLUSION

Whether black stones are associated with higher infection rates or bacteria cause black stones - this question probably would never be answered either on the bench or in the clinicians ward. We, however, can definitely say they are intimately related.

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