 Determination of chemical composition of gall bladder stones: Basis for treatment strategies in patients from Yaounde, Cameroon

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AIM: Gallstone disease is increasing in sub-saharan Africa (SSA). In the west, the majority of stones can be dissolved with bile salts, since the major component is cholesterol. This medical therapy is expensive and not readily accessible to poor populations of SSA. It was therefore necessary to analyze the chemical composition of biliary stones in a group of patients, so as to make the case for introducing bile salt therapy in SSA.

METHODS: All patients with symptomatic gallstones were recruited in the study. All stones removed during cholecystectomy were sent to Houston for x-ray diffraction analysis. Data on age, sex, serum cholesterol, and the percentage by weight of cholesterol, calcium carbonate, and amorphous material in each stone was entered into a pre-established proforma. Frequencies of the major components of the stones were determined.

RESULTS: Sixteen women and ten men aged between 27 and 73 (mean 44.9) years provided stones for the study. The majority of patients (65.38%) had stones with less than 25% of cholesterol. Amorphous material made up more than 50% and 100% of stones from 16 (61.53%) and 9 (34.61%) patients respectively.

CONCLUSION: Cholesterol is present in small amounts in a minority of gallstones in Yaounde. Dissolution of gallstones with bile salts is unlikely to be successful.

INTRODUCTION

Biliary lithiasis has been a common disease in Europe and the USA for decades. Over half of the cases are asymptomatic, usually detected by an abdominal ultrasound. For a very long time, biliary stone disease was said to be rare in Sub-Saharan Africa (SSA). Archampong reported a 0.4% prevalence of all admissions in the Korle Bu Teaching Hospital in 1969 in Ghana. Today the prevalence of gallstone disease has increased considerably with the widespread use of ultrasonography.

This increase has also been observed in African populations of Jamaica and SSA. Many studies to identify risk factors for biliary lithiasis in the West have focused on hypersaturation of cholesterol in bile in the nucleation process, a critical step in the genesis of bile stones. The high concentration of cholesterol in gallstones has been the basis for the widespread use of bile acids, a nonsurgical treatment for the dissolution of gall bladder stones. These stones account for as much as 80% of Western stones. Unfortunately, gall bladder stone composition is heterogeneous, and differs within and without populations around the world.

The increasing frequency of biliary stones in SSA, with its different epidemiological factors and diseases, prompted us to carry out a chemical analysis of gallstones. This study would demonstrate the role of cholesterol in our stones and therefore the necessity of using bile salts for gall stone dissolution in SSA.

MATERIALS AND METHODS

This was a cross-sectional study of a series of stones removed from patients at the University Hospital Center (UHC) of the University of Yaounde I from January 1, 1989 to December 31, 1998. All stones removed during surgery were placed on sterile gauze to air dry, transferred into a paper envelope bearing the name, age, and sex of the patient as well as the date. The first batch of stones from 19 patients was sent to the Urolithiasis laboratory in Houston, Texas in January 1996. A second collection of stones from 7 patients was sent to the same laboratory in May 1999.

All stone specimens were first examined for shape, size, and color. They were classified as cholesterol, black or brown pigmented stones, examined under a polarized microscope. The composition of the nidus, the internal and external shells was determined by X-ray diffraction as described previously. The percentage of cholesterol, calcium carbonate, and amorphous material such as black bilirubinate, black phosphate, glycopolymers and salts was determined. A descriptive analysis was done for stones from each patient. Patients who were able to pay for a serum cholesterol assay did so. Hemoglobin electrophoresis was not asked in this mainly adult population who did not give histories of sickle cell disease or crisis.

RESULTS

Patient population

There were 26 Cameroonian patients, all black Africans, aged between 27 and 73 years (mean 44.9 years). There were 16 women and 10 men, a 1.6 female to male sex ratio. The men were aged between 36 and 62 years whereas 6 women were less than 35 years (23.07%). All our patients resided in the city. They consumed mainly an African traditional diet made of local vegetarian menus mixed with imported processed Western items such as rice and wheat. The serum cholesterol level was normal in all 10 patients who did it.

Stone analyses

The percentage of cholesterol in the stones by weight is...
depicted in Table 1. Seventeen patients (65.38%) had stones with less than 25% cholesterol. Of these, 11 (42.30%) had cholesterol free stones. Seven patients (26.92%) and 9 others (34.61%) had stones with 50% and 80% cholesterol content, respectively.

Calcium carbonate was detected in stones from 4 (15.38%) patients, three of whom were female. Two of these females had mixed stones containing cholesterol, calcium carbonate and amorphous material.

Table 2 shows the distribution of amorphous material by weight in these stones. Stones from 16 (61.53%) patients contained more than 50% amorphous material. The entire stone was made of amorphous material in 9 (34.61%) cases.

Table 1 Percent by weight of cholesterol in the gallstones from 26 patients

| % by weight | Males | Females | Patient (%) |
|-------------|-------|---------|-------------|
| 0           | 6     | 5       | 11 (42.30) |
| <25         | 1     | 5       | 6 (23.07)  |
| >or =25 and <50 | 0   | 0       | 0           |
| >or =50 and <80 | 1   | 1       | 2 (7.69)   |
| >or =80 <100  | 0     | 4       | 4 (15.38)  |
| 100         | 2     | 1       | 3 (11.53)  |
|            | 10    | 16      | 26 (100)   |

Table 2 Percent by weight of amorphous material in the gallstones from 26 patients

| % by weight | Males | Females | Patient (%) |
|-------------|-------|---------|-------------|
| 0           | 2     | 1       | 3 (11.53)  |
| <25         | 0     | 5       | 5 (19.23)  |
| >or =25 and <50 | 1   | 1       | 2 (7.69)   |
| >or =50 and <80 | 1   | 0       | 1 (3.84)   |
| >or =80 <100  | 0     | 6       | 6 (23.07)  |
| 100         | 6     | 3       | 9 (34.61)  |
|            | 10    | 16      | 26 (100)   |

DISCUSSION

The results from this hospital-based nonrandomized study cannot be extrapolated to the community due to several limitations. In this group there were no children. Yet, it is known that children with hemolytic diseases develop cholesterol-poor, bilirubin-rich gallstones[2]. Even in children without hemolytic disease, the composition of gallstones was different from those of adults in Leeds, England[3]. Limitations to recruiting a potentially representative population of patients include poverty, in the absence of a financial scheme for health care coverage, ignorance and cultural factors that dissuade people from attending hospital services. Nonetheless, this pilot study permits us to raise the hypothesis that dissolution of gall bladder stones with bile salts is not a cost-effective alternative to surgical treatment.

A recent series of biliary lithiasis revealed a 4-fold increase of symptomatic gall bladder stone disease in Ghana from 1966-1999. This series also reported that the majority of Ghanaian stones were not cholesterol rich. Furthermore, cholesterol stones were more common in females and only 34% of their stones contained 75% or more of cholesterol by weight. They also showed that the external appearance of the stone was a poor predictor of its composition[21]. This means that even in the poorer regions of the world, such as Sub-Saharan Africa, all attempts should be made to chemically analyze stones.

The treatment of gallstone diseases runs the gamut from bile salt dissolution, to fragmentation with laser[18], pulverization with extracorporeal shock wave lithotripsy[21], endoscopic extraction, and classical surgery, whereas noninvasive medical therapy is appealing, bile acid therapy is only effective in some cholesterol gallstones. Bile acids are not effective in treating calcium bilirubinate or calcium carbonate/phosphate stones. It is therefore imperative that the composition of the stone be determined to tailor treatment for the individual patient[22].

To determine stone composition, there are many possibilities offered by different technologies. On simple X-ray, radiologically undetectable stone calcification reduces the probability of dissolution and calcified structures appearing in stones during treatment are composed of calcium carbonate. A radio-opaque stone would suggest that medical therapy is unlikely to succeed. Stone composition is also determined on computed tomography. Results from polarizing microscopy of gall bladder bile suggest that the presence of cholesterol crystals is a sensitive measure of cholesterol and vaterite microcrystalliths confirm presence of calcium carbonate in the gallstone[23].

In the less technically developed areas the chemical composition of stones was determined from its external appearance. This has been shown to be inaccurate. Frequently the stones are homogenized and chemically analyzed. Our stones were analyzed with X-ray diffraction where cholesterol, calcium carbonate, and amorphous material were detected.

The components of the amorphous material (bilirubine, glycerophosphates and bile salts) were not identifiable on X-ray diffraction. Infra-red spectroscopy and scanning electron microscopy were used to show that black and brown pigment gallstones differ in microstructure and micro-composition, suggesting that they form by different mechanisms. Black carbonate and brown stones layered structure suggests that stone growth is dependent on cyclical changes in biliary substances[24]. This may explain the permissive or causal role endogenous hormones have in gallstone formation[15]. Stone formation begins with nucleation where the interaction of pronucleators and antinucleators leads to formation of cholesterol crystals and these develop into gallstones[6,16]. Hepatic cholesterol hypersecretion is associated with the increased unsaturated fatty acid proportion in biliary phospholipids and gallbladder mucin secretion, thereby causing rapid crystal nucleation[17]. It is evident that gallstone disease has a multifactorial causation, including gall bladder infection[18], decreased gall bladder motility after surgery for obesity and/or weight loss[19], ileal disease (Crohn’s)[20], hemolytic diseases[2], familial hypercholesterolemia[21], and metabolic defects in hepatic bilirubin glucuronidation[22].

A shortcoming in our study is the absence of hemoglobin electrophoresis in a population where the prevalence of the sickle trait varies between 10% and 20% and that of the disease is about 1%[23]. We did not explore many of the risk factors mentioned above largely due to technical and financial limitations. The absence of calcium in the stones of 22 (84.61%) patients correlates with a small number of gallstones detected on plain X-ray of the abdomen in our region. The extremely infrequent occurrence of pure cholesterol gallstones is a strong argument against the introduction of oral dissolution agents in SSA.

CONCLUSION

There is a corresponding variation in prevalence of cholesterol rich stones as a variation in composition of gallstones. This variation seems to be related to genes and the environment. Stone composition determines the therapeutic approaches in each locality. This pilot study suggests that oral bile salt dissolution therapy would not be effective in 70% of our patients. As populations in SSA undergo epidemiologic transition from infectious diseases to noncommunicable diseases, there will be increasing prevalence of biliary lithiasis.
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Edited by MajY