INTRODUCTION

Gallstones also referred to as cholelithiasis, are stone-like crystalline deposits in the gallbladder, supersaturated with varying concentrations of the primary biochemical constituents of hepatic bile. Gallstones occur due to an imbalance in the biochemical constituents of bile with resultant precipitation of one or more component of bile.\(^1,2\) It is one of the commonest causes of surgical emergencies in the western world and more prevalent among women and advancing age.\(^3,4\) The condition is rare among indigenous Africans including Nigerians.\(^5,7\) However, a recent epidemiologic review of gallstone incidence in Nigeria suggests an increasing trend of the condition owing to the westernization of dietary habits among Nigerians.\(^6,8\)

The mechanism of gallstone formation is vague in the literature.\(^9,10\) The proposed theories of its etiopathogenesis include supersaturation of bile and resultant precipitation of one or more of the primary biochemical constituent of bile (cholesterol, triglycerides, bilirubin, calcium salts, proteins, and other lipids and minerals), biliary infection, drugs, and impaired motility.
of the gallbladder secondary to spinal cord injury.1,2 However, the theory of supersaturation of bile and resultant precipitation of one or more of the primary biochemical constituent especially cholesterol, bilirubin, and calcium salts is the most accepted among other suggested theories.1,9,10

Based on its primary biochemical constituents, gallstones are classified into three types namely cholesterol stone (CS) with cholesterol lipid making up more than >90% of the stone which occurs in alteration of lipid metabolism especially in obesity, pigment stone (PS) with conjugated bilirubin making up about 90% of the stone which occurs in hemolytic states, and mixed stone (MS) with varying concentrations of cholesterol, bilirubin, and calcium salts.1,11

The evaluation and quantification of these constituents in gallstones enables the understanding of its origin, etiology, metabolic basis, and pathogenesis.12,13 However, there is a paucity of clinical data on the biochemical constituents of gallstones among Nigeria patients. Hence, this present study is conducted in essence to investigate these biochemical constituents among indigenous blacks of Nigerian origin.

**Objectives**

- To determine the demographic characteristics of records of study cohorts with gallstone disease.
- To determine the types of gallstones in records of patients with gallstones.
- To determine the biochemical constituents of each gallstones type.
- To compare the biochemical constituents of each type of gallstone.
- To compare the findings with similar studies in the literature.

**METHODS**

**Study area and site**

This study was carried out in the Department of Chemical Pathology and Metabolic Medicine of the University of Port Harcourt Teaching Hospital (UPTH), Port Harcourt, Nigeria. The hospital is one of the tertiary hospitals located in the south-south region of the country providing advanced specialist medical care to the populace in the region. The Department of Chemical Pathology and Metabolic Medicine has a well-equipped clinical chemistry laboratory were complex biochemical analysis is undertaken with an attached Metabolic Clinic.

**Study design**

This was a retrospective, descriptive, and cross-sectional study, designed to meet the specific objectives of the study. The study was conducted between June and September 2017. Informed consent and ethical approval are not required in UPTH due to the retrospective design of the study.

**Study material**

The laboratory records of all the gallstone biochemical analysis in the Department of Chemical Pathology and Metabolic Medicine of UPTH during a 10-year period (1st January 2007 to 31st December 2016) were recruited as study materials. The gallstones samples had come from the Department of surgery of UPTH and the peripheral tertiary hospitals in the region, including some private hospitals.

**Study eligibility criteria**

Inclusion criteria include all records of gallstone biochemical analysis irrespective of age, sex and comorbid condition during the study period (1st January 2007 to 31st December 2016)

Exclusion criteria include all records with incomplete data.

**Collection of specimen and biochemical analysis**

Postsurgical gallstone specimen presented to the department was all allowed to dry for two weeks prior to analysis. Before the analysis, parts of the gallstones were powdered with pestle and mortar and dissolved in different solvents as described by Chandra et al and Shalayel et al.12,13

To determine the total cholesterol and total bilirubin, 30 mg of powdered stone was dissolved in 3ml chloroform in a test tube. The tube was placed in a boiling water bath for 2 minutes.

To determine the total calcium and triglycerides, 30 mg of powdered stone was dissolved in 3 ml I N HCl (one normal HCl) in a graduated 10ml test tube and its volume was made up to 10ml with distilled water. The tube was placed in a boiling water bath for one hour.

The different solutions were allowed to come to room temperature and subsequently used for analysis of each biochemical constituent. The analysis of total calcium and total bilirubin were done by non-enzymatic colorimetric methods while total cholesterol and triglycerides were analyzed using the enzymatic colorimetric methods.14,17

All reagents had been procured from Randox Laboratories, United Kingdom.

**Collection and classification of data**

Data were abstracted from the laboratory records of each patient and entered into Statistical Package for Social Sciences (SPSS) version 15. Data on age, sex, clinical
variables, gallstones macroscopic characteristic (color), and concentrations of gallstone biochemical constituents (total cholesterol, triglyceride, total bilirubin, and calcium) were obtained from laboratory records. Gallstones were classified based on color as follows:

1) Pale yellow and whitish as cholesterol stone (CS)
2) Black and blackish brown as pigment stone (PS)
3) Brownish yellow or greenish as mixed stone (MS)

Statistical analysis

The obtained data from laboratory records were imported into SPSS version 15. The data were first tested for normality using Shapiro-Wilk statistical test. All non-parametric data were log-transformed before analysis. Continuous data were presented as mean±standard deviations and compared with independent t-test or one-way analysis of variance where appropriate, while categorical data were presented in numbers and percentages and compared with Chi-square test or Fisher’s exact test as required. A cutoff p value of <0.05 was chosen as being statistically significant.

RESULTS

During the period (1st January 2007 to December 2016) under study, 51 samples of gallstones were submitted to the Department of Chemical Pathology and Metabolic Medicine for biochemical analysis. Records of 48 of these samples met the inclusion criteria and were recruited as study materials in this study.

The gallstone samples came from 14 (29.2%) males and 34 (70.8%) female patients with a statistical significance of 0.004 and a male to female ratio of 1:2.4. The overall mean age of the study cohorts was 46.88±7.96 and ranged from 31 through 64 years. However, the age range for men was 40-64 years, while female age ranged from 31–63 years. There was no difference in the mean age between males and females (males 50.39±8.31 versus females 45.47±7.52; p=0.056), however, the mean age of the men was higher than the females.

Table 1: Distribution of classes of gallstones.

| Gallstone type               | n  | %   |
|------------------------------|----|-----|
| Cholesterol stone (CS)       | 31 | 64.6|
| Pigment stone (PS)           | 6  | 12.5|
| Mixed stone (MS)             | 11 | 22.9|

Chi-square test with Yate’s continuity correction=21.875; p≤0.001*; *Statistically significant.

In Table 1, based on the physical color characteristics of the gallstones, the most common of the gallstones were cholesterol stone which comprised 64.6% of all the study gallstones, followed by the mixed stone with 22.9% and pigment stone being the least with 12.5%.

In Table 2, The females predominated across all the three types of stones with a higher proportion of both sexes presenting with cholesterol stones.

Table 2: Sex distribution of gallstones.

| Sex     | Types of gallstone | Total n (%) |
|---------|--------------------|-------------|
|         | Cholesterol stone (CS) n (%) | Pigment stone (PS) n (%) | Mixed stone (MS) n (%) |
| Male    | 8 (25.8)           | 2 (33.3)     | 4 (36.4)     | 14 (29.2) |
| Female  | 23 (74.2)          | 4 (66.7)     | 7 (63.6)     | 34 (70.8) |

Fisher’s exact test=0.753; p value=0.897

Table 3: Distribution of biochemical constituents in each type of gallstone.

| Gallstone constituents | Types of Gallstone | Cholesterol stone (CS) Mean±SD | Pigment stone (PS) Mean±SD | Mixed stone (MS) Mean±SD | P value  |
|------------------------|--------------------|-------------------------------|-----------------------------|--------------------------|---------|
| Cholesterol (mg/gm)    | 649.18±86.94       | 403.30±40.70                 | 488.93±64.91                | <0.001*                  |
| Triglycerides (mg/gm)  | 57.37±9.44         | 42.30±0.80                   | 78.12±0.89                  | <0.001*                  |
| Bilirubin (mg/gm)      | 3.06±1.51          | 5.64±0.64                    | 1.54±0.06                   | <0.001*                  |
| Calcium (mg/gm)        | 15.59±6.89         | 20.05±5.70                   | 38.86±7.10                  | <0.001*                  |

*Statistically significant; mg/gm=milligram per gram

Table 4: Sex distribution of the biochemical constituents of gallstones.

| Gallstone Constituents | Male            | Female          | P value |
|------------------------|-----------------|-----------------|---------|
| Total Cholesterol (mg/gm) | 550.97±45.56   | 593.37±10.43   | 0.277   |
| Triglyceride (mg/gm)    | 59.92±14.10     | 60.37±13.42    | 0.917   |
| Total Bilirubin (mg/gm) | 2.45±1.38       | 3.28±1.77      | 0.125   |
| Total Calcium (mg/gm)   | 21.42±12.87     | 21.50±11.43    | 0.989   |

mg/gm=milligram per gram
In Table 3, the concentration of cholesterol lipid was more in the cholesterol stone, moderate in mixed stone while the pigment stone had the least cholesterol. Triglyceride concentration was more in the mixed stone, moderate in the cholesterol stone, but least in the pigment stone. The bilirubin concentration was more in the pigment stone, moderate in the cholesterol stone but least in the mixed stone. Calcium was more in the in the mixed stone, moderate in the pigment stone and least in the cholesterol stone.

In Table 4, the concentrations of all the four biochemical constituents of the examined gallstones were all higher in females than in the males. However, there was no statistical difference observed.

**DISCUSSION**

Gallstone disease is one of the many reasons for intra-abdominal surgical emergencies. It is associated with the western world, advancing age, and more prevalent among the female gender. In this study, there were female preponderance among the study cohorts which is in accordance with the numerous global findings of gallstone disease epidemiology. The calculated male sex to the female sex ratio obtained was 1:2.5 which agrees with some local report but at variance with others. The reason for the preponderance for gallstone disease among females is unclear. Some authors have suggested the influence of higher and extended period of exposure to estrogen hormone. This is supported by the fact that younger females in their reproductive years, when estrogen is at its peak, are more likely to present with gallstone disease. This fact is also augmented by the findings in this study, where we observed that the females were younger with lower age range than their male counterparts.

Traditionally, gallstones are classified based on their biochemical components as cholesterol stones (CS), pigment stones (PS), and mixed stones (MS). Though more advanced methods of classification have been described in the literature to typify gallstones. Cholesterol stones is the most common of all three gallstones reported in the literature especially in the western population, followed by MS and the PS. In this study, 64.7% of gallstones were CS, followed by MS with 22.9% and PS with 12.5% of the gallstones. This finding is in contrast with a report from Sudan where PS was more predominant followed by the MS and CS but is in accord with a recent study documented among Iraqi patients with gallstones. Most (64.7%) of the stones in this study in both sexes were CS and the females predominated with 74.2% of those with CS which reinforces the female preponderance of the disease.

Several mechanisms of gallstone formation have been described and put forward in the literature, however, the sequence of events leading to gallstone formation is very unclear. Among the several suggested mechanisms, the imbalance in the biochemical constituents of bile with resultant precipitation of one or more component of bile seem the most plausible. Cholesterol stones result from un-physiologic supersaturation of bile with cholesterol due to hypersecretion or impaired cholesterol metabolism. Pigment stones result from pigment supersaturation resulting from hypersecretion of bilirubin conjugates in hemolytic states or enterohepatic cycling of unconjugated bilirubin in non-hemolytic states. While MS results from a combination of the factors involved in CS and PS evolutions, in addition with various calcium salts involvement.

In accordance with other studies, cholesterol lipid is the most prevalent biochemical constituent of the three types of gallstones and was significantly higher in CS than in MS and PS in descending order. This finding is in accord with other local and foreign reports and underscores the role of cholesterol lipid in CS and PS evolution. The triglyceride lipid was significantly higher MS, followed by the CS and least in PS which is accord with the report by Chandra et al who suggested that triglycerides get accumulated along with cholesterol salts in MS due to more deposition of calcium salts of cholesterol and esters of fatty acids. Bilirubin, which gives the PS its color, was unsurprisingly higher in PS than in the other two stones which are attributed to the higher bilirubin salts in the PS. Calcium level was significantly more in MS, moderate in PS and least in CS which agrees with similar studies. The precipitation of calcium salts is the most critical event in gallstone formation.

**CONCLUSION**

Cholesterol gallstones (CS) are the most common of the three types of gallstones in this study and the cholesterol lipid is the most prevalent constituent of the gallstones. The females presented with more of the gallstones disease than the males. Therefore, the risk of gallstone disease suggested to be mostly cholesterol lipid-dependent and more prevalent in female gender is reinforced in this study. However, further studies with large sample size are advised to confirm these findings.

**Limitations**

It is a retrospective hospital-based study conducted in a single center, therefore its conclusion may not reflect the pattern of gallstone parameters of the entire population in the region. However, the hospital is the only center in the region where gallstones are presented for analysis from other peripheral health centers and could, therefore, be easily assumed to be representative of the population in the region since gallstone disease is extremely rare in the region.

**ACKNOWLEDGEMENTS**

We, the authors extend our gratitude to Dr. Owamagbe M. Emmanuel for his critical assessment, review, and corrections of this manuscript before publication.
**Funding:** No funding sources  
**Conflict of interest:** None declared  
**Ethical approval:** Not required

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Cite this article as: Amadi C, Wichendu PN. Biochemical constituents of gallstones from indigenous blacks of Nigerian origin. Int J Sci Rep 2018;4(5):104-8.