

Original Research Article

Predictors of cholesterol gallstone formation among inhabitants of Port Harcourt in Nigeria

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ABSTRACT

Background: Various clinical and biochemical parameters have been hypothesized to predict cholesterol gallstone formation. Hence, this study was structured to evaluate the degree of some of these suggested predictors among inhabitants of Port Harcourt in Nigeria.

Methods: This was a retrospective study of the clinical and biochemical parameters of 42 cholesterol gallstones formers within a tertiary hospital in Nigeria. Records of age, gender, weight, height, calculated body mass index and plasma biochemical parameters (total cholesterol, total bilirubin, and total calcium) of cholesterol gallstone formers from 1st January 2008 to 31st December 2017 were abstracted from medical and laboratory records and analysed using SPSS version 20.

Results: There were more females (70%) than males (30%) with a ratio of 2.3:1. The age ranged from 31-64 with mean 46.78±9.33. Obesity was observed among 40.5% of study population. Female gender (OR = 2.823; 95% CI = 2.446-3.200; p<0.001), obesity BMI status (OR = 1.534; 95% CI = 1.436 - 1.632; p = 0.012) and abnormal plasma cholesterol status (OR = 3.011; 95% CI = 2.916 - 3.106; p<0.001) were significant predictors of cholesterol gallstone formation. Abnormal plasma cholesterol status was the strongest of the predictors with AUC of 0.920 (p<0.001), seconded by female gender (AUC = 0.889; p<0.001) and obesity BMI status (AUC = 0.834; p<0.001).

Conclusions: Abnormal plasma cholesterol status is the strongest independent predictor of cholesterol gallstone formation, seconded by female gender and high BMI status, among inhabitants of Port Harcourt in Nigeria.

Keywords: BMI, ETV, Cholesterol gallstone, Female gender, Plasma cholesterol

INTRODUCTION

Gallstone disease is one of the frequent surgical pathologies in the Western and Asian populations with a reported prevalence rate of 7.9% in men to 11.6% in women.¹ The disease is exceedingly more frequent among women and the aged individuals.^{2,3} For yet to be determined biologic reason, indigenous African descendants have been noted to exhibit a very low incidence and prevalence of the disease.³

The gallstones are traditionally classified into three basic types based on their biochemical constituents as cholesterol stones (CS), pigment stones (PS), and mixed stones (MS).⁴ Cholesterol stones basically contains greater than or equal to 50% lipid cholesterol, the pigment stones are composed of mainly bilirubin compounds while mixed stones are a combination of cholesterol and bilirubin calcium salts.⁵ Therefore, the three major constituents of gallstones are the cholesterol lipid, bilirubin compounds and various calcium salts.^{4,6}

The most predominant type of gallstone is reported to be the cholesterol variant.⁶ The initiating events in gallstone formation, in general, has eluded researchers for decades since its evolution, but seem to be multifactorial with genetic and environmental interactions.^{7,8} However, several authors had suggested that the supersaturation of one of the major biochemical constituents (cholesterol, bilirubin and calcium salts) in bile is one of the cardinal initiating effects in the formation of cholesterol gallstones in humans.⁹

In addition, some clinical and biochemical parameters have been reported to predict the incidence of cholesterol gallstone formation in the general population. These predictors include age, gender, body mass index (BMI) and the abnormal plasma concentrations of the major constituents of gallstones such as cholesterol, bilirubin and various calcium salts.^{9,10}

The degree of these predictors on the formation of cholesterol gallstones has mostly been reported within the Western and Asian populations. Hence, this present study was designed to assess the degree of these suggested clinical and biochemical predictors of cholesterol gallstone formation among inhabitants of Port Harcourt in Nigeria.

The objectives of the present study were to characterize the clinical and biochemical characteristics of cholesterol gallstones formers. And to determine the degrees of age, gender, BMI, plasma cholesterol, plasma bilirubin and plasma calcium to predict cholesterol gallstones. Also, to compare the findings of these studies with the literature.

METHODS

This study was undertaken in the Department of Chemical Pathology and Metabolic Medicine of one of the tertiary hospitals in Nigeria (University of Port Harcourt Teaching Hospital, Port Harcourt). The hospital is situated in the south-south region of Nigeria. The Department of Chemical Pathology and Metabolic Medicine with an attached Metabolic Clinic, is one of the Departments of Pathology within the hospital where complex biochemical analysis are carried out. This retrospectively designed study was conducted between March and July 2018.

Records of age, gender, weight, height, calculated BMI and plasma levels of total cholesterol lipids, total bilirubin and albumin-corrected total calcium of all the cholesterol gallstone formers who presented to the Department of Chemical Pathology and Metabolic Medicine during a 10-year period (1st January 2008 to 31st December 2017) were recruited as study materials.

Inclusion criteria

All medical and laboratory records of cholesterol gallstone ($\geq 50\%$ of total cholesterol by weight) formers

who presented to the Department of Chemical Pathology and Metabolic Medicine during the study period (1st January 2008 to 31st December 2017).

Exclusion criteria

All records of those with gallstones $< 50\%$ by weight of total cholesterol content and records with incomplete data.

Specimen collection and biochemical analyses

All the cholecystectomy gallstones specimen presented to the department were all allowed to dry following collection and documentations. Prior to laboratory analysis, parts of the gallstones were powdered with pestle and mortar and dissolved in different solvents as previously described.⁶

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

To determine the total calcium and triglycerides, 30mg of powdered stone was dissolved in 3ml one normal HCl within 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 over an hour.

After allowing the solutions to come to room temperature, they were subsequently utilized for analysis. The analysis of total calcium and total bilirubin were done by non-enzymatic colorimetric methods while total cholesterol was analyzed using the enzymatic colorimetric methods. All reagents had been procured from Randox Laboratories, United Kingdom.

Fasting venous whole blood specimen were collected from each patient by phlebotomy prior to surgical removal of gallstones. Aliquots of the collected specimen were emptied into ethylenediaminetetracetic acid (EDTA) and lithium heparin specimen collection tubes and subsequently processed accordingly. Laboratory analysis for plasma total cholesterol was done using enzyme-catalyzed colorimetric methods with the plasma obtained from the EDTA specimen tubes. While total bilirubin, total calcium and plasma albumin were analyzed using non-enzymatic colorimetric methods with the plasma obtained from the specimen in the lithium heparin tubes. Same brands of laboratory reagents including three levels (levels 1, 2, and 3) of commercial quality control sera were procured from Randox Laboratories, United Kingdom through their agents in Nigeria.

Data acquisition, stratifications and definition

Data were abstracted from the medical and laboratory records of each patient and entered into Statistical Package for Social Sciences (SPSS) version 20. Records

of age in years, gender, weight in kilograms, height in meters, calculated BMI, clinical variables, gallstones macroscopic characteristic (color), and concentrations of plasma biochemical parameters (total cholesterol in mmol/l, total bilirubin in $\mu\text{mol/l}$, and albumin-corrected total calcium in mmol/l) were obtained.

The BMI was calculated as weight in kg divided by the square of height in meters.

The total plasma calcium was corrected using the following formula:

Albumin-corrected calcium = $0.02 (40 - \text{plasma albumin in g/l}) + \text{plasma calcium in mmol/l}$.

BMI was stratified as normal ($18.5 - 24.9 \text{ kg/m}^2$), overweight ($25 - 29.9 \text{ kg/m}^2$) and obesity ($>30 \text{ kg/m}^2$) based on World Health Organization definition.¹¹

Age was arbitrarily stratified as young (≤ 39 years) and old (≥ 40 years).

Total plasma cholesterol was stratified as normal ($< 5.17 \text{ mmol/l}$) or abnormal ($>5.17 \text{ mmol/l}$) based on the National Cholesterol Education Program-Adult Treatment Panel 111 (NCEP-ATP 111) classification.¹²

Total plasma bilirubin was stratified as normal ($<34 \mu\text{mol/l}$) or abnormal ($>34 \mu\text{mol/l}$).

Total plasma albumin-adjusted calcium as normal ($2.15 - 2.55 \text{ mmol/l}$) or abnormal ($>2.55 \text{ mmol/l}$).

The cholesterol gallstones were defined by their physical characteristics (pale yellow and whitish) and confirmed by the biochemical analyses of each stone (gallstones with $\geq 50\%$ by weight of total cholesterol content).⁵

Statistical analyses

The obtained data from medical and laboratory records were all transferred into SPSS version 20. The data were first tested for normality of distribution using Shapiro-Wilk statistical test. All the non-parametric distributed data were log-transformed prior to statistical analysis. The non-categorical data were presented as mean \pm standard deviations. While the categorical data were presented in numbers and percentages and compared with chi-square test or fisher’s exact test as appropriate. Forward stepwise logistics regression was used to determine the degree of relationship of each predictor with cholesterol gallstone formation (inclusion if alpha value is <0.05). Receiver operator characteristic (ROC) curve was employed to assess the predictive value of the different predictors of cholesterol gallstone formation. An alpha value of less than or equal to 0.05 was chosen as being statistically significant.

RESULTS

During the period (1st January 2008 to December 2017) under study, medical and laboratory records of 59 gallstones from gallstones formers were identified in the Department of Chemical Pathology and Metabolic Medicine of which 42 of these records met the inclusion criteria and were subsequently enrolled for the study.

Table 1: Stratifications of the study clinical and biochemical variables.

Variable	n	%	p value
Gender			
Male	13	30.0	<0.001*
Female	29	70.0	
Age (years)			
≤ 39	12	26.0	<0.001*
≥ 40	30	74.0	
BMI (kg/m²)			
Normal ($18.5 - 24.9 \text{ kg/m}^2$)	4	9.5	0.002* ^F
Overweight ($25.0 - 29.9 \text{ kg/m}^2$)	21	50.0	
Obesity ($>30 \text{ kg/m}^2$)	17	40.5	
Total plasma cholesterol			
Normal ($<5.17 \text{ mmol/l}$)	13	31.0	0.004*
Abnormal ($> 5.17 \text{ mmol/l}$)	29	69.0	
Total plasma bilirubin			
Normal ($<34 \mu\text{mol/l}$)	35	83.3	<0.001* ^Y
Abnormal ($>34 \mu\text{mol/l}$)	07	16.6	
Total plasma calcium			
Normal ($2.15 - 2.55 \text{ mmol/l}$)	29	69.0	<0.001*
Abnormal ($> 2.55 \text{ mmol/l}$)	13	31.0	

*Statistically significant; BMI=Body Mass Index; kg/m^2 = kilogram per meter squared; mmol/l = millimole per liter; $\mu\text{mol/l}$ = micromole per liter; ^FFishers exact test; ^YChi-square test with Yate’s continuity correction;

Following Shapiro-Wilks test, total plasma cholesterol (Z score = + 1.14; p = 0.417) and total plasma albumin-adjusted calcium (Z score = + 1.07; p-value = 0.221) concentrations were observed to be normally distributed while age (Z score = + 21.77; p = 0.002) and total plasma bilirubin (Z score = + 14.96; p = 0.010) were not normally distributed. Thereafter, the data of age and that of total plasma bilirubin were logarithmically transformed prior to further statistical analyses. The age of the study population ranged from 31 to 64 with mean SD of 46.78±9.33.

Table 2: Descriptive characteristics of the clinical and biochemical variables.

Variable	n	Mean±SD
Weight (kg)	42	84.07±8.60
Height (m)	42	1.71±0.19
BMI (kg/m ²)	42	28.39±3.32
Total plasma cholesterol (mmol/l)	42	5.39±0.66
Total plasma bilirubin (µmol/l)	42	4.25±1.7
Total plasma calcium ^a (mmol/l)	42	2.43±0.12

SD = Standard Deviation; BMI = Body Mass Index; kg/m² = kilogram per meter squared; mmol/l = millimole per liter; µmol/l = micromole per liter; kg = kilogram; m = meters
^aPlasma albumin-adjusted

In Table 1, the majority (70.0%) of the study population were females while the males constituted 30.0% with a

ratio of 2.3:1. The majority (74.0%) of the study population were more than forty years of age while 26% of the study cohorts were less than 39 years of age.

Based on the BMI stratification, 9.5% were of normal BMI while 50.0% and 40.5% were overweight and obese respectively. Abnormal levels of total plasma cholesterol, bilirubin and calcium were observed among 69.0%, 16.6% and 31.0% of study cohorts respectively.

In Table 2, the mean±SD of the weight, height, BMI, total plasma cholesterol, total plasma bilirubin and total plasma calcium of the study population were 84.07±8.60, 1.71±0.19, 28.39±3.32, 5.39±0.66mmol/l, 4.25±1.7 µmol/l and 2.43±0.12 mmol/l respectively.

In Table 3, age >40 years (OR = 5.115; 95% CI =1.796 - 8.434; p = 0.021), female gender (OR = 2.619; 95% CI = 1.641 - 3.597; p = 0.030), overweight BMI status (OR = 1.257; 95% CI = 0.954 - 1.560), obesity BMI status (OR = 1.686; 95% CI = 1.549 - 1.823; p = 0.004) and abnormal plasma cholesterol levels (OR = 3.21; 95% CI = 2.776 - 3.662; p = 0.001) of study population were statistically significant predictors of cholesterol gallstone formation on univariate logistic regression analyses. However, multivariate logistics regression analyses showed that female gender (OR = 2.823; 95% CI = 2.446 - 3.200; p <0.001), obesity BMI status (OR = 1.534; 95% CI = 1.436 - 1.632; p = 0.012), and abnormal plasma cholesterol level (OR = 3.011; 95% CI = 2.916 - 3.106; p <0.001) were significant independent predictors of cholesterol gallstone formation.

Table 3: Stepwise logistic regression analyses of predictors of cholesterol gallstones.

Variable	Univariate logistic regression		Multivariate logistic regression	
	OR (95% CI)	p value	OR (95% CI)	p value
Age (years)				
< 39	0.786 (0.586 - 0.986)	0.277	-	-
≥ 40	5.115 (1.796 - 8.434)	0.021*	0.944 (0.732 - 1.156)	0.412
Gender				
Male	0.455 (0.311 - 0.579)	0.176	-	-
Female	2.619 (1.641 - 3.597)	0.030*	2.823 (2.446 - 3.200)	<0.001*
BMI (kg/m²)				
Normal (18.5 - 24.9)	0.642 (0.513 - 0.771)	0.476	-	-
Overweight (25 - 29.9)	1.257 (0.954 - 1.560)	0.009*	0.915 (0.807 - 1.023)	0.079
Obesity (> 30)	1.686 (1.549 - 1.823)	0.004*	1.534 (1.436 - 1.632)	0.012*
Total plasma cholesterol				
Normal (<5.17mmol/l)	0.151 (0.106 - 0.196)	0.321	-	-
Abnormal (>5.17mmol/l)	3.219 (2.776 - 3.662)	0.001*	3.011 (2.916 - 3.106)	<0.001*
Total plasma bilirubin				
Normal (< 34µmol/l)	0.344 (0.288 - 0.400)	0.467	-	-
Abnormal (>34µmol/l)	0.815 (0.591 - 1.039)	0.027	0.667 (0.531 - 0.803)	0.143
Total plasma calcium^a				
Normal (2.15-2.55mmol/l)	0.509 (0.443 - 0.575)	0.273	-	-
Abnormal (> 2.55mmol/l)	0.717 (0.449 - 0.985)	0.032	0.562 (0.440 - 0.684)	0.319

*Statistically significant; BMI = Body Mass Index; OR = Odd ratio; CI = Confidence Interval; kg/m² = kilogram per meter squared; mmol/l = millimole per liter; µmol/l = micromole per liter; ^aPlasma albumin-adjusted

In Table 4, ROC analyses show that abnormal plasma cholesterol levels were the best predictor of cholesterol gallstone formation with AUC of 0.920 (95% CI = 0.901 - 0.939; $p < 0.001$). This is seconded by the female gender (AUC = 0.889; 95% CI = 0.865 - 0.913; $p < 0.001$) and obesity BMI status (AUC = 0.834; 95% CI = 0.813 - 0.855; $p = 0.011$).

Table 4: ROC analyses of predictors of cholesterol gallstone.

Variable	AUC	95% CI	p value
Abnormal plasma cholesterol (>5.17mmol/l)	0.920	0.901 - 0.939	<0.001*
Gender (Female)	0.889	0.865 - 0.913	<0.001*
BMI (>30 kg/m ²)	0.834	0.813 - 0.855	<0.001*

*Statistically significant; BMI = Body Mass Index; AUC = Area under the curve; CI=Confidence Interval; kg/m² = kilogram per meter squared; mmol/l = millimole per liter

DISCUSSION

In this index study, abnormal plasma cholesterol, female gender and high obesity BMI levels were independent predictors of the development of cholesterol gallstone and subsequent disease progression. During univariate logistic regression analysis, age >40years, female gender, overweight and obesity BMI levels and abnormal plasma cholesterol concentration were significant predictors of cholesterol gallstone formation among the study population. However, when these five predictors were all put into the multivariate regression test, female gender, obesity BMI and abnormal plasma cholesterol levels were identified as the three independent predictors, while the contribution of age and overweight BMI vanished. The predictive powers of abnormal plasma cholesterol, female gender and obesity BMI levels were confirmed during ROC analysis, however, total plasma cholesterol had the strongest predictive power with an AUC of 0.920 ($p < 0.001$).

Disorders of lipid homeostasis, specifically high plasma cholesterol concentrations, has frequently been noted as a potential predictor of cholesterol gallstones in several studies from the western and Asian populations.¹³⁻¹⁵ In a prospective study reported from Pakistan by Atamanalp et al, the authors had observed that high plasma cholesterol levels were associated with a high rate of cholesterol gallstone formation.¹³ A similar report had earlier been documented in another study reported from Saudi Arabia by Khairy et al.¹⁴ However, Thijs et al, found an inverse correlation between serum cholesterol and cholesterol gallstone risk.¹⁶ Several mechanisms had been documented as to the exact mechanism of high plasma cholesterol induction of cholesterol gallstone formation.^{13,14} However, the most widely accepted mechanism is the high plasma cholesterol induction of hepatic hypersecretion of the lipid cholesterol into bile

which culminates in the initiation of cholesterol gallstones formation.¹³

Gender, specifically the female gender, has long been recognized as a compelling risk factor for cholesterol gallstone formation in several reports.^{6,17-20} This is evident from this study where the females outnumbered the males by a 3:1 ratio. Although total plasma cholesterol was the strongest independent predictor of cholesterol gallstone in this study, this was followed by gender as the second predictor of cholesterol gallstone formation. Several factors had been adduced to the high prevalence of cholesterol gallstones among the female population.¹⁸ These factors include the effect of the high estrogen milieu in females which ultimately enhances hepatic cholesterol secretion into bile and the reduction of bile salts with resultant supersaturation of bile with the cholesterol lipid.¹⁷⁻¹⁹

The diminished motility of the gallbladder due to the effect of high progesterone levels, parity and the use of hormonal oral contraceptive pills are other factors contributing to the high cholesterol gallstones in the female gender.¹⁷

Obesity, as defined by BMI, is a frequent comorbid feature among gallstone formers in general and is one of the commonest metabolic predictive factors that occasion cholesterol gallstone formation.^{21,22} Its prevalence among gallstone formers is reported to be between 20-40% which is in accord with the finding of the present study.²¹ In this study, BMI greater than 30kg/m² was also an independent predictor of cholesterol gallstone which agrees with previously documented studies. Tirzu et al, had reported high BMI as the only predictor of cholesterol gallstone following adjusted logistic regression analysis in a case-control study.²³

In a recent prospective cohort study, Liu et al, observed an increased risk of cholesterol gallstone formation with high BMI.²⁴ Increased BMI enhances the activity of the rate-limiting enzyme (3-hydroxy-3-methyl-glutaryl co-enzyme A) of hepatic cholesterol synthesis which heightens the secretion of the lipid cholesterol into bile.²⁵

The limitation of the present study is that it is a retrospective study carried out in a single hospital setting, therefore its conclusion may not be representative of the entire population in the region. However, these limitations do not in any way undermine the credibility of this study.

CONCLUSION

Abnormal plasma cholesterol status, female gender and high BMI levels of obesity status are the major significant independent predictors of cholesterol gallstone formation among Port Harcourt inhabitants in Nigeria. However, abnormal plasma cholesterol status exhibited

the strongest independent predictive value than female gender and the high BMI levels.

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