Original Research Article

COMPARATIVE SONOGRAPHIC EVALUATION OF THE GALLBLADDER IN SICKLE CELL DISEASE PATIENTS AND APPARENTLY HEALTHY NON-SICKLE CELL DISEASE INDIVIDUALS IN A NIGERIAN TOWN

ABSTRACT

BACKGROUND: Sickle cell disease (SCD) is an autosomal recessive blood disorder with multi-organ manifestations including the gall bladder. Studies have shown that individuals with sickle cell disease have strong tendency of developing pigment gallstones due to chronic red blood cell hemolysis and increased bilirubin levels.
AIM AND OBJECTIVES: The aim of this study was to evaluate and compare the gallbladder changes which includes volume, wall thickness, presence of biliary sludge and prevalence of calculi in sickle cell disease patients with age and sex-matched apparently healthy, normal non-SCD individuals.
MATERIALS AND METHODS: This was a cross-sectional comparative study of 50 known SCD patients attending the Haematology clinic of sub-urban tertiary health facility in Nigeria, and equal number of age and sex-matched apparently healthy, non-SCD volunteers attending the Well people clinic of the same hospital as controls. Each subject was evaluated for gallbladder volume, wall thickness, presence of biliary sludge and gallstones using a 3.5-5MHz curvilinear array transducer of a Mindray ultrasound machine, DC-6model, 2016.
DATA ANALYSIS: The data collected was analysed using the IBM Statistical Package for Social Sciences (SPSS) version 21.
Statistical test was considered significant at *p*-value ≤ 0.05 and a confidence interval of 95%.
RESULTS: The 100 subjects scanned were made up of 50SCD patients, comprising of29 (58.0%) males and 21 (42.0%) females with 50 non-SCD volunteers as controls, comprising of 24 (48.0) males and 26 (52.0) females, with an age range of 2–65 years. The mean age of the cases and controls was 22.1±14.7 years and 19.9±12.6 years respectively. Among the patients; 8 (16.0%) had cholecystitis and 10 (20.0%) had gallstones, with no detectable abnormality in the controls.
CONCLUSION: The ultrasonographic prevalence of gallbladder abnormality was recorded only in patients with sickle cell disease when compared to normal healthy controlsas shown with increased prevalence with age.

KEYWORDS: Sickle Cell Disease, cholelithiasis, gallbladder disease, ultrasound.

LIST OF ABBREVIATIONS

ISTH:	Irrua Specialist Teaching Hospital
USS:	Ultrasound Scan
HbAA:	Hemoglobin A Genotype
HbSS:	Hemoglobin SS genotype
SCA:	Sickle Cell Anemia

SCD: Sickle Cell Disease
HbSC: Hemoglobin SC genotype
HbCC: Hemoglobin CC genotype
BMI: Body Mass Index
GB: Gallbladder

INTRODUCTION

Sickle cell disease is an autosomal recessive disorder characterized by mutation in chromosome11, resulting in the replacement of glutamic acid which is hydrophilic, polar and negatively charged by valine which is a hydrophobic, less polar and neutral amino acid in position 6 of the globin chain.[1] This in turn, under deoxygenated conditions leads to gelation in which there is intra-erythrocytic hydrophobic interaction of sickle haemoglobin precipitation and then eventual formation of hemoglobin S (HbS).[1] The prevalence of SCD is about 2.39% and about 1.63% in the generalpopulation.

Individuals who are homozygous for HbS are said to have sickle cell anemia, thus, this repeated sickling and unsickling of red blood cells causes damage to the red blood cell membrane.[1] Other forms though rare, of sickle cell disease are HbSC, HbAC, HbCC, and beta-Thallasemia.[2] Malaria-endemic zones of West Africa have the highest prevalence of sickle cell disease.[3] In Nigeria, approximately 150,000 children are born yearly with SCD though it is more commonly diagnosed amongst adolescents and adults than children due to progression of the chronic haemolytic process.[4]

In Kano metropolis, the prevalence of sickle cell disease was about 11.87% for HbSS, 40.33% for HbAS, 0.22% for HbSC and 1.51% for HbAC as reported by Mustaphar *et al*.[5,6]

In a prevalence study in Benin by Odunvbun *et al*[7], SCD had a prevalence of 2.8% for HbSS and 0.2% for HbSC. This high prevalence has been attributed to survival advantage conferred by the sickle cell trait against Plasmodium falciparum.[7] This resistance to Plasmodium falciparum by individuals, creates a selective pressure that has maintained the sickle cell gene within human populations in malaria-endemic zones like sub-Saharan Africa and this is termed balanced polymorphism.[7] A recent evaluation from a retrospective study done by Nwogoh *et al*.[8] In Benin city, Nigeria revealed the prevalence of sickle cell disease to be about 2.39% and the carrier rate to be about 23%.

Sickle cell disease is characterized by chronic haemolytic anaemia and vaso-occlusive crises that are mainly precipitated by dehydration, temperature change, pH and deoxygenated haemoglobin as well as malaria.[9] The red blood cells become sickled in shape, abnormally long and slender because the haemoglobin present in them has reduce doxygen-carryingcapacity.[9]

The acutely painful episodes of SCD last for several days and are due to infarction attributed to vascular blockage by collection of erythrocytes which have undergone sickling as they meander through tiny capillaries due to capillary stasis resulting in hypoxia.[9] However, prophylactic anti-malaria medications given to these patients have reduced their crisis tendency.[10]

In general, patients with sickle cell disease can also have other systemic manifestations such as gallbladder changes, renal disease, hepatomegaly, splenomegaly, auto-

splenectomy, vaso-occlusive crisis.[10]
Other common manifestations include blindness due to plugging of tiny retinal vessels by sickle cells resulting in retinal damage; leg ulcers, deep vein thrombosis, pulmonary embolism, hand and foot syndrome as well as sgrowth retardation due to shortage of normal red blood cells which convey oxygen and nutrients needed for growth, thereby impeding growth and delaying puberty in teenagers.
Additionally, other complications from sickle cell disease includes; priapism, pregnancy-induced hypertension, miscarriages and increased gallstone formation due to excessive red blood cell breakdown; all these being more commonly reported among African patients with sickle cell disease.
Anatomically, the gall bladder is a hollow visceral elastic organ. Its fasting volume gives an objective measurement of its capacity. Dilatation of gall bladder due to stones at its neck, gallbladder wall thickening due to repeated inflammation with surrounding oedema as well as accumulation of bilirubin from excessive erythrocytic disintegration canal predispose to impairment in local blood circulation and biliary drainage.[11,12] This further worsens gall bladder dilatation and abnormally large volume when compared to other healthy normal individuals.[12]
Modern gallbladder imaging primarily involves real-time ultrasonography, computed tomography and magnetic resonance imaging.[13] Oral cholecystography which involves the use of contrast to study the biliary system was first used by Graham *et al*[14]
MRI is an effective modality for imaging the gallbladder, but it is cost-ineffective, images acquired may be affected by motion, it is not readily available especially in our environment, patient with severe allergy to contrast and those who have non-MRI compliant implants cannot benefit from it.[13]
Presently, ultrasonography is the most important imaging modality for gall bladder evaluation because it is relatively available, cost-effective, reproducible, reliable, non-ionizing, non-invasive and has high sensitivity for evaluating gall bladder dimension, functions and associated pathologies.[13,14] It is also a standard imaging modality to visualize other gall bladder changes in sickle cell disease such as biliary sludge, gallbladder polyps and other benign gallbladder tumors. The aim of this study was to evaluate the gallbladder in sickle cell disease patients and compare same with equal number of apparently normal, healthy, age and sex-matched non-SCD individuals in Irrua, Nigeria.
Nigeria has one of the largest population of children and adults where sickle cell disease is endemic in the world with almost 150,000 children born each year with this disease.[4] This creates enormous financial, emotional and health burden on the individuals, parents/caregivers and government at large. There are meagre resources to cater for them in developing and underdeveloped countries.[4]
There is still an enormous level of illiteracy and poor awareness on the socio-economic impact of sickle cell disease; hence an increasing number of cases of sickle cell disease with profound morbidity and mortality rates are still being recorded. Notably, despite the high disease burden in Africa and Sub-Saharan Africa, with more than 70% of sufferers living in these regions, only negligible expenditure and health care research have been achieved.[5] In Nigeria, carrier prevalence is about 20-30%.[34] Sickle cell disease affects about 2-5% of the Nigerian population of about 200 million.[15]
It is therefore important to evaluate the gallbladder as a component of the different

organ systems of the body typically affected by sickle cell disease.[15] This is because certain gallbladder pathologies could provoke emergency or increase the mortality rates in sickle cell disease patients.[4]

The aim of this study was to evaluate and compare the gallbladder changes which includes volume, wall thickness, presence of biliary sludge and prevalence of calculi in sickle cell disease patients with age and sex-matched apparently healthy, normal non-SCD individuals.

MATERIALS AND METHODS

STUDY DESIGN

This cross-sectional comparative study to evaluate the gallbladder changes in sickle cell disease patients and apparently healthy, non-SCD individuals in Irrua was carried out in the Radiology department of Irrua Specialist Teaching Hospital.

STUDY POPULATION

The study population comprised of already diagnosed SCD patients attending the Hematology clinic of the outpatient department of Irrua Specialist Teaching Hospital and control group of age- and sex-matched apparently healthy, non-SCD individuals attending routine clinic of the same hospital. Both groups were evaluated after a written consent was taken from them or their parents/guardians in the case of children.

STUDY LOCATION

The study was carried out in the ultrasound section of the Radiology Department of Irrua Specialist Teaching Hospital, Irrua, Edo State.

Irrua is the administrative seat of the Esan Central Local Government area of Edo State, Nigeria. It is located on the Esan plateau, some 87 km North of Benin city. Its vegetation is mixed rain fore stand transitional Guinea Savannah. This town has 3 major religions; Traditional (Ebor), Islam and Christianity. Like most other settlements in Edo state, it has 2 distinct weather conditions; dry and wet/rainy seasons. The temperature across the state is relatively high with narrow seasonal and diurnal variations, and ranges between 22-36° Celsius.

Irrua Specialist Teaching Hospital (ISTH) is a tertiary health care facility that caters for people in Irrua as well as referred patients from other parts of Edo and neighbouring states such as; Kogi, Delta, Ondo, Ekiti and other states.

INCLUSION CRITERION FOR SICKLE CELL DISEASE SUBJECTS

Subjects who were 6 months old and above who were confirmed heterozygous (HbSC) and homozygous (HbSS) for sickle cell disease via haemoglobin electrophoresis

EXCLUSION CRITERIA FOR SICKLE CELL DISEASE SUBJECTS

1. Subjects less than 6 months of age
2. Subjects with a previous history of hepato-biliarys surgery or subjects who have had elective or emergency cholecystectomy
3. Sickle cell disease patients with hypertension and/or diabetes mellitus
4. Sickle cell disease patients who were very ill

INCLUSION CRITERIA FOR HEALTHY VOLUNTEERS

Subjects 6 months and above who were confirmed to be either HbAA, HbAS or HbAC

EXCLUSION CRITERIA FOR HEALTHY VOLUNTEERS

1. Subjects less than 6 months of age
2. Subjects with a previous history of hepato-biliary surgery or subjects who have had elective or emergency cholecystectomy.
3. Healthy volunteers who did not consent to ultrasound scan
4. Individuals with diabetes mellitus, acute cholecystitis or chronic liver disease

SAMPLE SIZE DETERMINATION

The sample size was calculated as for a cross sectional study using the Cochran's formula with sickle cell disease prevalence of 2.39%.[8,16]

In Southern Nigeria

$N = Z^2pq/e^2$

Where N= total number of samples required

Z= abscissa of the normal curve or standard normal deviate = 1.96 critical value for 95% confidence level

p= estimated prevalence of an attribute that is present in the population i.e 2.39% = 0.0239q = 1-pis the confidence level at 95%

e= desired level of precision = 0.05 the estimated sample size is:

$N = \frac{(1.96)^2 \times 0.0239 \times 0.9761}{(0.05)^2}$

$N = \frac{3.842 \times 0.0233}{0.0025}$

N=35.8

With an attrition rate of 10%, minimum sample size for the study will be approximately 40.

However, 50 participants were recruited for each group to broaden the base of the study.

TECHNIQUE

All sonographic examinations were carried out by the researchers who are all consultant radiologists. The study was done using a 3.5-5MHz curve linear array transducer of a Mindray Ultrasound machine (DC-6 was, Shenzen Mindray Biomedical electronic company, Shenzen China, 2016).

The procedure was thoroughly explained to the subjects and their parents/guardians in the case of children, after which a written, informed consent as shown in the appendix was obtained. Subjects were required to fast overnight or for at least 4 hours for adults or just before the next meal for children to avoid post prandial contraction and allow proper visualization of the gall bladder. The details of current medications and biometric parameters such as age and sex, as well as the height were measured using stadiometer2006 model and weight using the standardized weighing scale 20011 model. To exclude hypertensive and diabetic patients, blood pressure estimation with a standardized sphygmomanometer, Caisson model, (2016) and fasting blood sugar using standardized glucometer (2018), were used.

During the examination, subjects were made to lie supine on the examination couch, exposing their abdomen from the xiphisternum to the pelvis with hands placed over the head to widen the intercostal spaces. When necessary, patients were placed in left lateral decubitus position to enhance adequate visualization of the gallbladder.

Acoustic coupling gel was applied over the abdomen. The transducer was gently placed over the right upper quadrant to visualize the gallbladder and was oriented such that the longest axis of the gallbladder was seen. The image was frozen on the screen.

Any mobile/non-mobile (adherent) or discrete intraluminal echogenic structure with distal acoustic shadowing was regarded as calculus. Its mobility was confirmed by varying patients' position (right/left lateral decubitus or upright), while keeping the probe in place in the right upper quadrant.
Also the gallbladder wall thickness was measured from inner to outer part of the posterior wall of the gallbladder body or vice-versa and any diameter greater than 3mm with or without hypoechoic halo around the gall bladder was considered abnormal and diagnostic of cholecystitis.
The probe was oriented perpendicular to the long axis of the gallbladder to obtain the transverse view of the gallbladder. The maximum diameter in this transverse section was viewed and the image was frozen. Measurements of the maximum width (W) and height (H) or anterior-posterior dimension was obtained and recorded in centimeters as shown in the image below. All measurements were taken from the inner wall of the gallbladder to the outer wall for gallbladder wall thickness but inner to inner wall for volumetric measurements. Images were magnified to minimize measurement error, where necessary. Three measurements were obtained for each parameter and the average was used to compute the mean gallbladder volume.
The inbuilt volumes software measurement on the ultrasound machine was used.

The body mass index (BMI) was calculated using the Lambert Adolphe's formula[17]. BMI = Height(m) / (weight)^2 (kg^2)

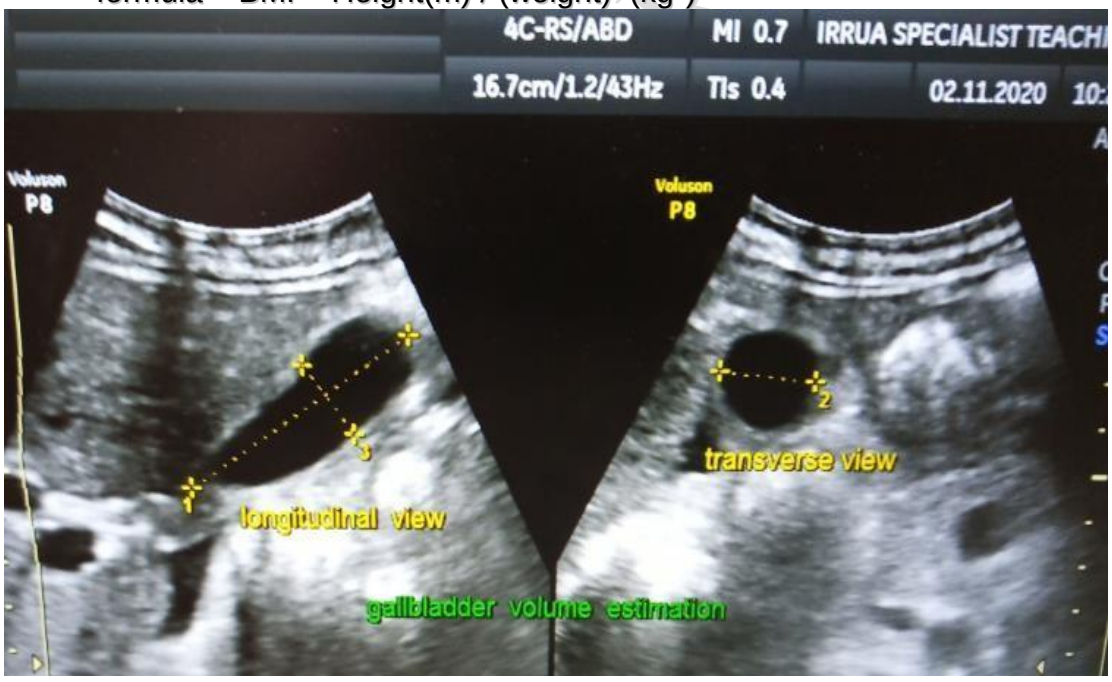


Figure 1: Longitudinal and transverse sonograms of the gallbladder for estimation of gallbladder volume. On the longitudinal view, the line running from fundus to the neck is the length (L). The linea cross the GB on transverse view is the width (W) and the line running from anterior to posterior is height (H)

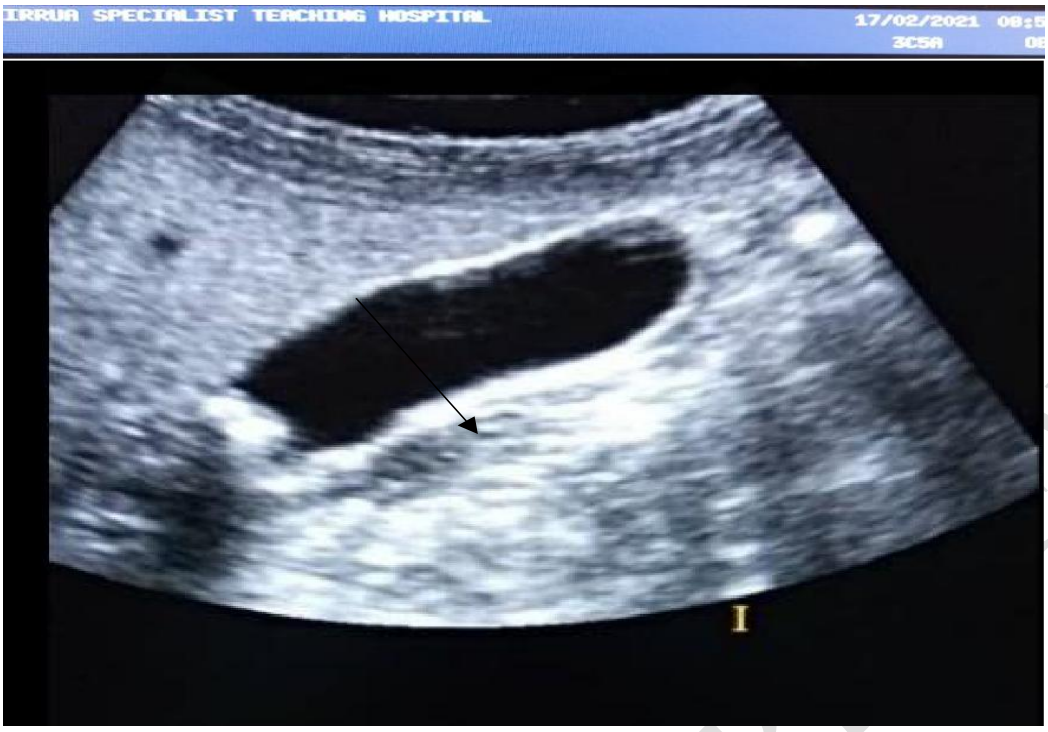
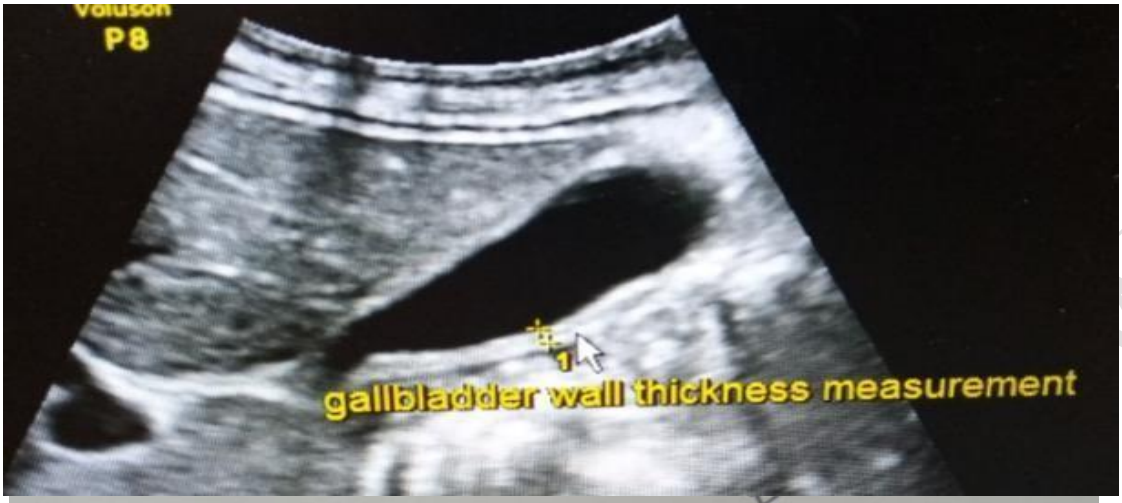


Figure2: **Longitudinal section of the gallbladder showing a fairly oval, smooth echogenic structure with distal acoustic shadow as well as thickened gallbladder wall consistent with calculous cholecystitis**



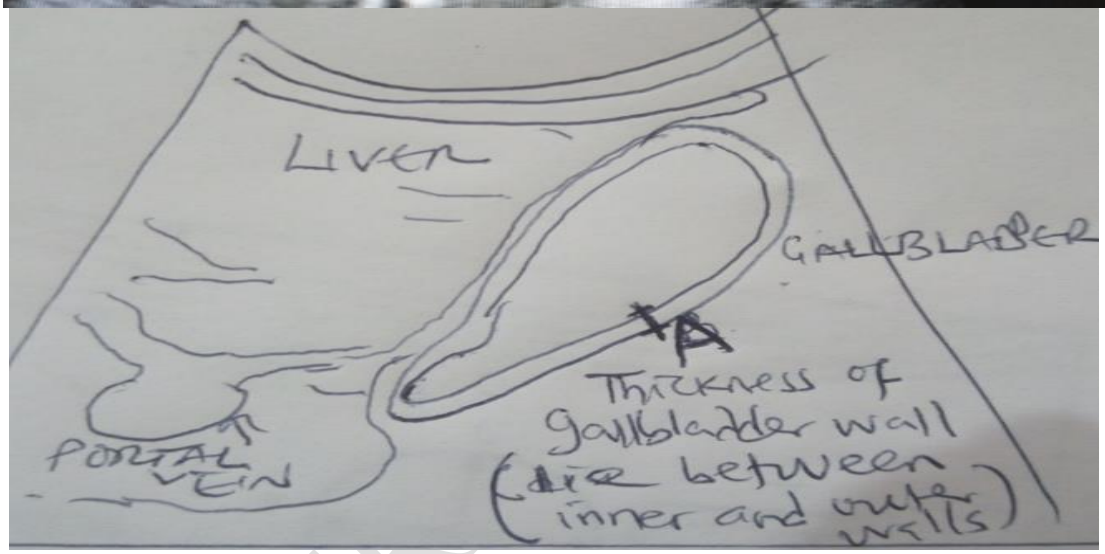


Fig3a: **Longitudinal sonogram of the gallbladder showing the thickness between the inner and outer walls of the gallbladder**

Fig 3b: **Corresponding line diagram showing the thickness between the inner and outer walls of the gallbladder, denoted A.**

DATA ANALYSIS

Data obtained from the subjects were entered into work sheet and subsequently registered into the computer spreadsheet, Microsoft Excel (Microsoft Corporation, USA) and IBM Statistical Package for Social Sciences (SPSS) Windows version 20.0 (ChicagoI L, USA). Entered data were double-checked for precision and data analysis were carried out using SPSS. Parametric data summarized using mean and standard deviation. Dichotomous variables were presented as frequency.

Data comparison (statistical test of significance) was done using chi-square test, student t-test, ANOVA (analysis of variance) and Spearman or Karl-Pearson's correlation coefficient where necessary. Confidence level of 95% was used and the statistical level of significance was set at $p<0.05$

STUDY LIMITATION

1. Getting the cooperation of patients and other healthy subjects to fast for about 4 hours was at times difficult, so they were counselled to come in the morning, having fasted overnight.
2. Intra and inter-observer variation were minimized by taking average of three measurements as in the case of the gallbladder measurement outlined.

RESULTS

One hundred (100) subjects consisting of 50 confirmed sickle cell disease patients (homozygous;HbSS, and heterozygous HbSC) aged 2 to 65 years and an equal number of age and sex-matched apparently healthy non-SCD subjects (homozygous [HbAA] and heterozygous [HbAS and HbAC]) aged 2 to 65 years were studied.

Socio-Demographic Characteristics of Participants

The age range for both study groups was 2 to 65 years as seen in table 1. The mean age for the sickle cell disease subjects was 22.1±14.7 years while the mean age for the control group was 19.9±12.6 years. The difference in the mean age between both groups was not statistically significant (p=0.441).

In the sickle cell disease group and control group, the largest number of subjects was in the age group 2 – 12 years, which was 16 (32.0%) and 17 (34.0%) respectively, followed by those aged 13–23 which was 13 (26.0) for sickle cell disease group and 15 (30.0) for the control group. The least represented with a frequency of 1 (2.0%) was found in those aged 35-45yrs and greater than 56yrs in both sickle cell disease and control groups (fig 4 and table 1).

When males and females were considered separately, the distribution was equally matched between the groups; 29(58.0%) males and 21(42.0%) females for sickle cell disease group and 24(48.0%) males and 26(52.0%) females in the control group. The differences in the sex distribution between the study groups was not statistically significant (*p*=0.316) (Figure 5 and Table 1).

Table 1: Socio-demographic Characteristics of Study Subjects

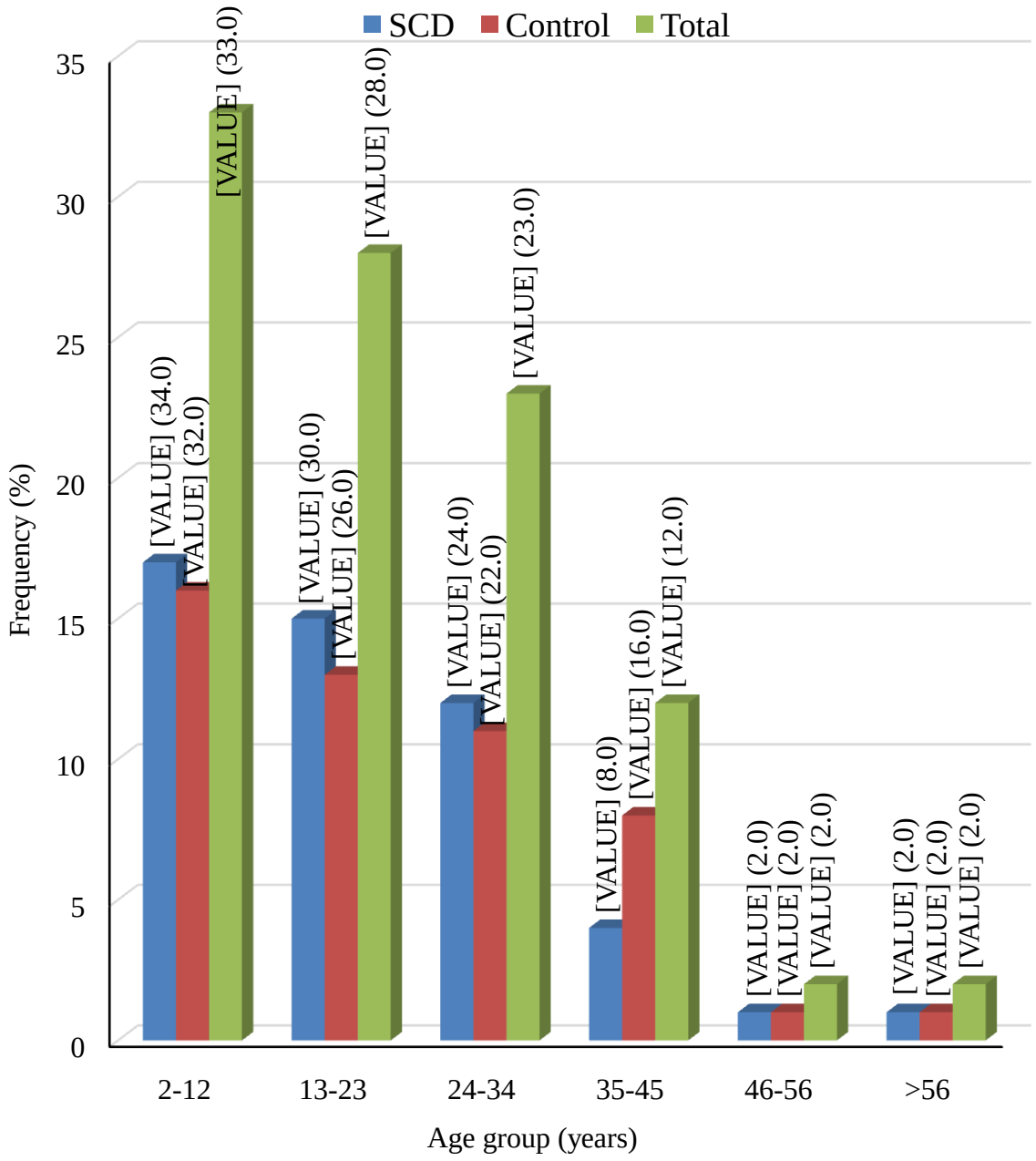
Parameters	Control (N = 50) Frequency (%)	SCD (N = 50) Frequency (%)	Chi-square (χ^2)	*p*-value

Age group				
2 – 12	17 (34.0)	16 (32.0)	2.032[§]	0.923
13 – 23	15 (30.0)	13 (26.0)		
24 – 34	12 (24.0)	11 (22.0)		
35 – 45	4 (8.0)	8 (16.0)		
46 – 56	1 (2.0)	1 (2.0)		
>56	1 (2.0)	1 (2.0)		
Mean age (years)	19.9±12.6	22.1±14.7	–0.774[†]	0.441
Gender				
Male	24 (48.0)	29 (58.0)	1.004	0.316
Female	26 (52.0)	21 (42.0)		
Religion				
Christianity	45 (90.0)	44 (88.0)	0.102	0.749
Islam	5 (10.0)	6 (12.0)		
Ethnicity				
Bini	0 (0.0)	1 (2.0)	9.343[§]	0.110
Esan	24 (48.0)	23 (46.0)		
Etsako	8 (16.0)	6 (12.0)		
Akoko Edo	0 (0.0)	1 (2.0)		
Owan	15 (30.0)	10 (20.0)		
Ibo	3 (6.0)	3 (6.0)		
⁞Others	0 (0.0)	6 (12.0)		

[§] Fisher's Exact Test [‡] Yates Continuity Correction [†] Independent t-test
⁞Others: Urhobo, Isoko, Itsekiri, Ijaw, Kwale, Yoruba

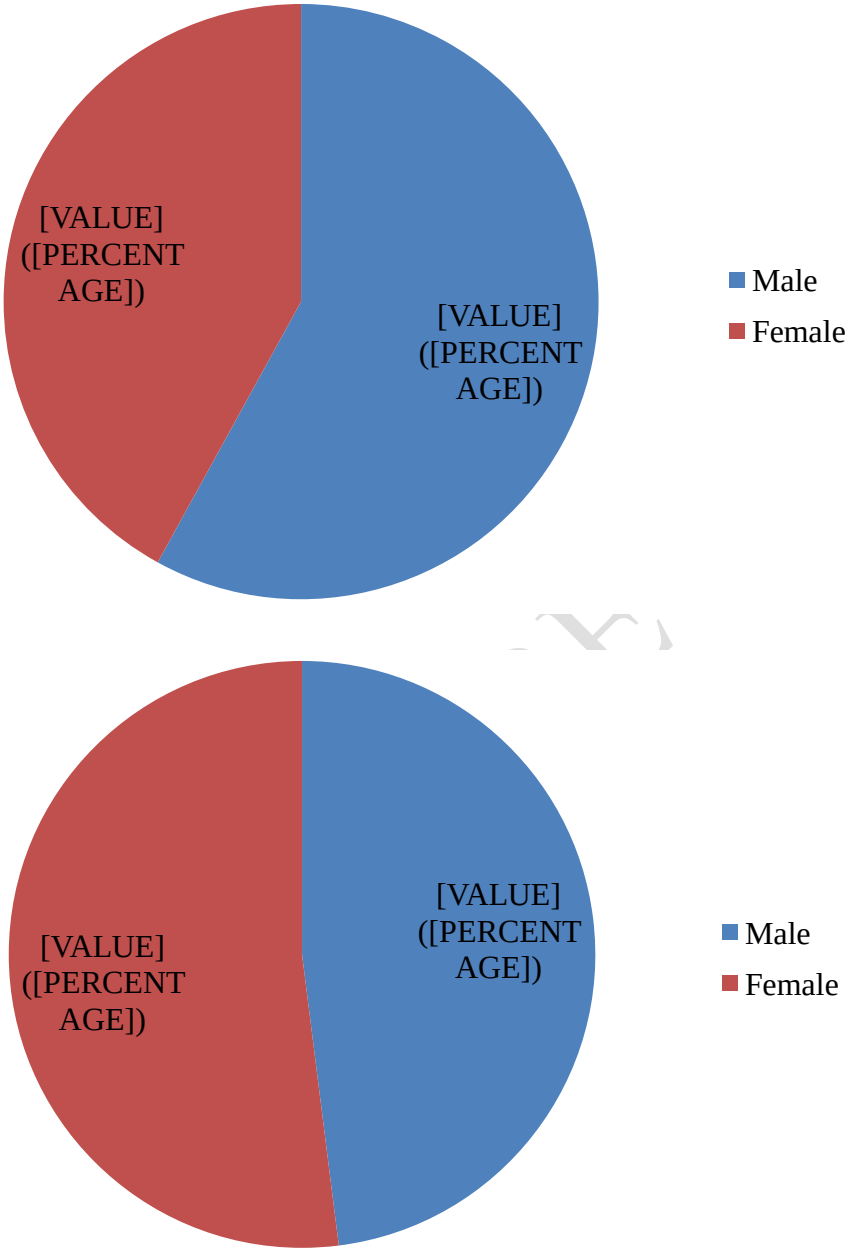
The majority of the participants were Christians 89 (89.0%); control group 45 (90.0%) and 44 (88.0) for sickle cell disease group while only 11 (11.0%) were Muslims, 5 (10.0) for control group and 6 (12.0%) for sickle cell disease group. This difference in the study groups was not statistically significant (p=0.749).

The ethnic group of most of the participants was Esan; 24 (48.0) in the control group and 23 (46.0%) for sickle cell disease group. This difference in ethnicity of the study groups was not statistically significant (p=0.110).



χ^2 Test = 1.468, p = 0.476

Figure 4: Bar chart showing age group distribution of the subjects



$\chi^2 = 1.004$, $p = 0.316$

Figure 5: Pie chart showing the distribution of the study subjects according to gender

Anthropometric Parameters of Study Subjects

The mean height, weight and BMI were higher among the control group compared with the SCD group but the differences were not statistically significant (1.52±0.23m against 1.48±0.28 m, p= 0.428, 52.4+/- 19.4kg against 49.2+/-21.3kg, 21.3+/-4.4kg/m^2 against 21.8 +/- 4.3kg/m^2, p= 0.584) (Table 2).

Analysis of the Haemoglobin Types

Table 3 shows that 29 (58.0%) were HbAA, 17 (34.0%) were HbAS. while HbAC were 4

(8.0%) each among the control group. Most patients were diagnosed with HbSS; 49(98.0%); and only one was diagnosed with HbSC; 1(2%). Of the SCD patients, 2 (4%) had been diagnosed at birth via routine neonatal Hb electrophoresis for offspring of sickle cell carrier parents, 3(6%) patients were diagnosed before reaching their first birthday, and the highest; 31 patients (62.0%) were diagnosed during their first to fifth year of life; 13 (26.0%) were diagnosed during their sixth to tenth year of life, while 1(2.0%) was diagnosed at age 40 years; the median age at diagnosis was 54 months.

Clinical Parameters of the Study Subjects

Almost all the patients with SCD had been admitted in the hospital as at the time of study; 46 (92.0%) with an average of 13.3 ± 2.9 days per year. More than half of patients; 28(56%) had a stay of 11 to 15 days.

Table 2: Comparison of Anthropometric Parameters of Study Subjects and type of haemoglobinopathy

Parameters	Control (N = 50) Mean ± SD	SCD (N = 50) Mean ± SD	t-test/ (χ^2)	df	*p*-value
Height (m)	1.52±0.23	1.48±0.28	0.796	98	0.428
Weight (kg)	52.4±19.4	49.2±21.6	0.786	98	0.434
BMI (kg/m^2)	21.8±4.3	21.3±4.4	0.550	98	0.584

***Statistically significant**

Table 3: HB genotypes of the study population

Characteristics	Number	Percentage
Genotype of Control group		
HbAA	29	58.0
HbAS	17	34.0
HbAC	4	8.0
Total	**50**	**100**
Genotype of SCD patient		
HbSS	49	98.0
HbSC	1	2.0
	50	**100**
Age diagnosed with sickle cell anaemia		
At birth	2	4.0
1month – <12 months	3	6.0
1 – 5 years	31	62.0
6 – 10 years	13	26.0
40 years	1	2.0
Median	54 months	
	50	**100**

More than half of patients with SCD had jaundice as at the time of study, 43 (86.0%), with18 (41.9%) of them having symptoms which lasted for 1 – 3 days while the remaining 25 (58.1) of them had symptoms lasting between4 and 8 days. The most common causes of the yellow eyes were malaria (46.0% of the subjects), sickle cell disease crisis (27.9% of the subjects). About 6 (14.0%) had malaria coexisting with sickle cell crisis, while another 5 (11.6%) had low back pain and anemia, coexisting with jaundice, as illustrated in table 4.

Majority of the SCD patients studied (86%) has had sickle cell crisis in the past.

In addition, only 9 (18.0%) subjects have had surgical procedures done before; 2(22.2%) have had caesarean section, herniorraphyin 3(33.3%), appendectomy in 3(33.3%) and cataract surgery in 1(11.1%) as illustrated in table 4 below.

Table 4: Clinical characteristics of patients with sickle cell disease

Characteristics	Number	Percentage
Admitted in hospital before		
Yes	46	92.0
No	4	8.0
	50	**100**
Duration of admission (days)		
5 – 10	8	17.4
11 – 15	28	60.9
16 – 20	10	21.7
Mean duration of admission (Mean ± SD) (days)	13.3 ± 2.9	
	46	**100**
Nature of illness		
Appendicitis	1	2.2
Malaria and Typhoid	1	2.2
a	1	2.2
Sickle cell crisis	29	63.0
Malaria with sickle cell diseases crisis	14	30.4
	46	**100**
Past history of jaundice		
Yes	43	86.0
No	7	14.0
	50	**100**
Ever had sickle cell crisis in the past		
Yes	43	86.0
No	7	14.0
Total	**50**	**100**
Length of time since last episode		
1 – 6 days	10	23.3
1 – 4 weeks	4	9.3
1 – 6 months	6	14.0
7 – 11 months	15	34.9
1 year	8	18.6
Total	**43**	**100**
Had any surgical operation done before		
Yes	9	18.0
No	41	82.0
Total	**50**	**100**
Nature of the operation done before		
Caesarean section	2	22.2
Herniorraphy	3	33.3
Appendectomy	3	33.3
Cataract surgery	1	11.1
Total	**9**	**100**

Comparison of the mean gallbladder volume, prevalence of gallstones and

prevalence of thickened gallbladder wall in SCD and control group.

The mean gallbladder volume of SCD subjects was significantly higher than the mean volume for the control group (35.7±23.3vs26.8±15.8cm^3; *p* = 0.029) with a range of 1.63-112.26cm^3 and 1.17-101.43cm^3 for SCD and control subjects respectively. (Table 5).

Of the 50 SCD subjects, gallstones were seen in 8 with a prevalence of 16.0%. This difference was statistically significant (p=0.012; Table 5).

Thickened gallbladder wall was seen in 10 out of the 50 sickle cell subjects with a prevalence of 20.0% while there was no case of gallbladder wall thickening in the control group. This was also statistically significant (p=0.001; Table 5).

Prevalence of cholelithiasis in sickle cell subjects according to age and gender

Table 6 showed that subjects within age range of 46 to 56years and those above 56 years had the highest prevalence of cholelithiasis of 100%, while those within 2 – 12 years of age had the least prevalence (0%). The prevalence of cholelithiasis increased with age from 0% at 2 – 12 years; to 7.7% at 13 – 23 years; 18.2% at 24 – 34 years; 37.5% at 35 – 45 years; and 100% at 46 – 56 and >56 years respectively. This difference in prevalence of Cholelithiasis by age classification of the subjects was statistically significant with p < 0.003 as shown on the table 6 below.

The prevalence of cholelithiasis was higher in the Christian religion; 15.9% than muslim religion; 1.7%. The difference was not statistically significant (*p* = 0.962).

The highest prevalence of cholelithiasis according to ethnicity was found among the Bini and Akoko Edo; 100%, followed by Owan; 90.0%, Etsako and others; 83.3% each, Esan 82.6% and the least Ibo; 66.7%. The difference was not statistically significant (*p* = 968).

Table 5: Mean gallbladder volume, presence of gallstone and presence of gallbladder wall thickening in SCD and controls.

Variables	Control (n = 50)	SCD (n = 50)	t-test/ (χ^2)	Df	*P.* value
Gallbladder volume (cm^3) (Mean ± SD)	26.8±15.8	35.7±23.3	–2.210†	98	**0.029***
Presence of gallstone (N(%))	0 (0.0)	8 (16.0)	6.658‡	1	**0.010***
Presence of thickened gallbladder wall N(%))	0 (0.0)	10 (20.0)	11.111	1	**0.001***

SD = Standard deviation
N = Number
% = Percentage
*** = Significant**
‡ Yates Continuity Correction † Independent t-test

Table 6: Prevalence of cholelithiasis in sickle cell disease patients by age

Age (years)	Number	Number (N) / % with stones	Male : Female
2 – 12	16	0 (0.0)	0:0
13 – 23	13	1 (7.7)	0:1
24 – 34	11	2 (18.2)	0:2
35 – 45	8	3 (37.5)	3:0
46 – 56	1	1 (100.0)	0:1
>56	1	1 (100.0)	1:0

Fisher's Exact Test = 13.950; $p < 0.003$

Prevalence of cholecystitis in sickle cell subjects according to age and gender

Those in the 46 – 56 and >56 years age groups had the highest prevalence of cholecystitis; 100% while those in the 2 – 12 years age group had the least prevalence of 0%. The prevalence of cholecystitis increased with age from 0% at 2 – 12 years; to 7.7% at 13 – 23 years; 27.3% at 24 – 34 years; 50.0% at 35 – 45 years; and 100% at both 46 – 56 and >56 years. This difference in prevalence of cholecystitis by age classification of the subjects was statistically significant with $p < 0.001$.

Comparison of age, height, weight, body mass index, gallbladder volume, the presence of gallstones and the presence of thickened gallbladder wall in male and female sickle cell disease and controls

Table 7 shows that there was no statistically significant difference in mean age (18.2±12.3 vs 24.6±15.2years, *p*=0.110), height (1.49±0.26 vs1.53±0.26m, *p*=0.532), weight (47.9±19.3 vs 53.9±22.7kg, *p*=0.310) and body mass index (20.7±4.1 vs 21.9±4.9kg/m², *p*=0.334). (Table10).

Also in Table 10, male sickle cell subjects had significantly higher mean fasting gallbladder volume in comparison with male control subjects (24.2±12.4 vs 37.0±21.6 cm^3, *p*=0.013). Although, female sickle cell subjects had higher mean fasting gallbladder volume in comparison with female control subjects, the difference was not statistically significant (29.3±18.4vs33.8±26.0 cm^3, *p*=0.481).

While male sickle cell subjects had 4 (13.8%) thickened gallbladder wall the male control subjects had no wall thickness 0%, but the difference was not statistically significant (*p* = 0.171). Similarly, the female sickle cell subjects had a total of 4 (19.0%) thickened gallbladder wall, but the female control subjects, had no wall thickening 0%. The difference was not statistically significant (*p* = 0.072).

Table 7 Comparison of age, height, weight, body mass index, gallbladder volume, the presence of gallstones and presence of thickened gallbladder wall in male and female sickle cell disease group and controls

Subject Gender	Age (years) (Mean ±SD)	Height (cm) (Mean ±SD)	Weight (kg) (Mean ±SD)	Body Mass Index (kg/m²) (Mean ±SD)	Gallbladder volume (cm³) (Mean ±SD)	Gallstones (N[%])	Wall thickness (N[%])
Males							
Control (n = 24)	18.2±12.3	1.49±0.26	47.9±19.3	20.7±4.1	24.2±12.4	0 (0.0)	0 (0.0)
SCD (n = 29)	24.6±15.2	1.53±0.26	53.9±22.7	21.9±4.9	37.0±21.6	4 (13.8)	6 (20.7)
p. value	0.110	0.532	0.310	0.334	**0.013***	0.171	0.054
Females							
Control (n = 26)	21.6±13.1	1.55±0.21	56.5±19.0	22.8±4.2	29.3±18.4	0 (0.0)	0 (0.0)
SCD (n = 21)	18.6±12.5	1.40±0.29	42.6±18.6	20.5±3.7	33.8±26.0	4 (19.0)	4 (19.0)
p. value	0.428	0.054	**0.015***	0.054	0.481	0.072	0.072

SD = Standard deviation, N = Number, % = Percentage
* = Significant

Comparison of gallbladder volume between male and female sickle cell disease with respective male and female controls across the different age groups

Mean gallbladder volume progressively increased with increasing age for male sickle cell subjects and female control subjects while the mean gallbladder volume of the male control was undulating at 13 – 23 years and 35 – 45 years categories and the female sickle cell disease had equally undulating volume at 35 – 45 years category (Table 8) With the exception of age group 2 – 12 in both male and female sickle cell subjects, similar gender and age groups; sickle cell disease patients had relatively higher mean gallbladder volume compared with control subjects, but only the difference in age in 13 – 23 (34.5±3.5 vs 46.4±5.9cm^3; p = 0.002) and 24 – 34 (26.8±3.9 vs 38.7±6.9 cm^3; p = 0.001) categories were statistically significant.

The highest gallbladder volume was however, found among the female control in the age >56 years category (101.4±0 cm^3). Moreover, the highest gallbladder volume was found among the male sickle cell disease group in age >56 years category (76.8±0 cm^3) (Table 11).

Comparison of gallbladder volume between male and female sickle cell disease patients among the different age groups

Male sickle cell subjects had progressively increased mean gallbladder volume with age as opposed to female counterparts which had fluctuations in the gallbladder volumes with respect to age. Overall, there was no statistically significant difference between the mean gallbladder volume across different age groups between the male and female categories of sickle cell disease patients (Table 12).

Table 8: Comparison of gallbladder volume between male and female SCD patients with male and female subjects among the different age groups

Gallbladder volume	Age Group (Years)					
	2 – 12	13 – 23	24 – 34	35 – 45	46 – 56	>56
Males	(C = 10, S = 8)	(C = 6, S = 7)	(C = 6, S = 6)	(C = 1, S = 7)	(C = 1, S = 0)	(C = 0, S = 1)
Control	14.0±9.8	5.2±2.1	34.5±3.5	33.9	50.9	
SCD	12.5±6.1	30.0±12.0	46.4±5.9	58.1±15.0		76.8
p. value	0.716	0.369	**0.002***	0.182		
Females	(C = 7, S = 8)	(C = 9, S = 6)	(C = 6, S = 5)	(C = 3, S = 1)	(C = 0, S = 1)	(C = 1, S = 0)
Control	12.9±7.2	26.8±3.9	33.5±0.8	41.9±12.3		101.4
SCD	11.0±4.2	38.7±6.9	55.9±33.8	48.8	62.2	
p. value	0.714	**0.001***	0.135	0.675		

SD = Standard deviation
* = Significant
C = Control
S = Sickle cell disease patients

Comparison of mean gallbladder volume, age and body mass index in sickle cell disease with calculi versus sickle cell disease patients without calculi

As shown in Table 9, mean gallbladder volume was statistically significantly higher in sickle cell disease who had calculi than in sickle cell disease patients without calculi (64.0±29.5cm^{3}vs30.2±17.8cm^3; *p*=0.000).Also, sickle cell patients with calculi had a higher statistically significant mean age compared to those without calculi (40.4±14.3 vs 18.6±12.0 years; *p* = 0.000) and statistically insignificant body mass index compared to those without calculi (22.4±6.2 vs 21.2±4.1kg/m^2; *p* = 0.485).

Comparison of mean gallbladder volume, age and body mass index in both male and female sickle cell patients with calculi

The mean gallbladder volume, age and body mass index were higher in male sickle cell disease subjects with calculi when compared to their female counterparts (gallbladder volume; 70.7±16.4 vs57.4±40.5 cm³, age; 48.0±11.7 vs 32.8±13.6 years and body mass index 27.6±1.3 vs 17.2±3.9 kg/m²). The differences was not statistically significant in gallbladder volume and age (p>0.05) but was statistically significant in body mass index (*p* = 0.002) (Table 10).

Table 9: Comparison of mean gallbladder volume, age and body mass index in sickle cell disease with calculi versus sickle cell disease without calculi

Variables	Sickle cell disease with calculi (N=8)	Sickle cell disease without calculi (N=42)	p. value
Gallbladder volume (cm^3) (Mean ± SD)	64.0±29.5	30.2±17.8	**0.000***
Age (years) (Mean ± SD)	40.4±14.3	18.6±12.0	**0.000***
Body mass index (kg/m^2) (Mean ± SD)	22.4±6.2	21.2±4.1	0.485

SD = Standard deviation
N = Number
* = Significant

Table 10: Comparison of mean gallbladder volume, age and body mass index in both male and female sickle cell disease with calculi

Variables	Male sickle cell disease with calculi (N=4)	Female sickle cell disease with calculi (N=4)	p. value
Gallbladder volume (cm^3) (Mean ± SD)	70.7±16.4	57.4±40.5	0.564
Age (years) (Mean ± SD)	48.0±11.7	32.8±13.6	0.141
Body mass index (kg/m^2) (Mean ± SD)	27.6±1.3	17.2±3.9	**0.002***

SD = Standard deviation
N = Number
*** = Significant**

Comparison of mean gallbladder volume, age and body mass index in sickle cell disease with thickened gallbladder wall versus sickle cell disease without thickened gallbladder wall

Table 11 shows that the mean gallbladder volume, age, and body mass index was statistically significantly higher in sickle cell disease who had cholecystitis than in sickle cell disease without cholecystitis (gallbladder volume; 64.4±22.8cm^{3}vs28.5±17.3cm^3; *p*=0.000, age; 38.0±14.5 vs 18.0±11.9; *p* = 0.000 and body mass index 24.4±4.4 vs 20.6±4.2; *p* = 0.014).

Table 11: Comparison of mean gallbladder volume, age and body mass index in sickle cell disease with thickened gallbladder wall versus sickle cell disease without thickened gallbladder wall

Variables	Sickle cell disease with thickened GB wall (N=10)	Sickle cell disease without thickened GB wall(N=40)	p. value
Gallbladder volume (cm^3) (Mean ± SD)	64.4±22.8	28.5±17.3	**0.000***
Age (years) (Mean ± SD)	38.0±14.5	18.0±11.9	**0.000***
Body mass index (kg/m^2) (Mean ± SD)	24.4±4.4	20.6±4.2	**0.014***

SD = Standard deviation
N = Number
*** = Significant**

Correlation of prevalence of cholelithiasis and cholecystitis with age and BMI

Table 12 shows correlation between age, BMI, cholelithiasis and cholecystitis. A positive moderate statistically significant correlation was noted between age and cholelithasis (r=0.550, n=50, p=0.0000, as well as between age and cholecystitis (r=0.548, n= 50, p=0.000)

BMI had a very weak positive correlation which is not statistically significant correlation with cholecystitis (r_{pb} = 0.101, n = 50, p = 0.485) while BMI had a, weak positive correlation which is statistically significant correlation with cholelithiasis (r_{pb} = 0.347, n = 50, p = 0.014) (Table 12).

Gender had a negligible strength of association with cholelithiasis (r = 0.071) and cholecystitis (r = 0.020). There was no statistically significant association between gender and cholelithiasis (p = 0.617) and also between gender and cholecystitis (p = 0.886), both males and females were equally likely to have cholelithiasis and cholecystitis (Table 12).

Table 12: Pearson's correlation coefficient scatter plot showing correlation of cholecystitis and cholelihiasis with age, sex and BMI

Sonographic gallbladder findings	Age			BMI			SEX		
	R	***P***	**N**	***R***	***P***	***N***	***R***	***P***	***N***
Cholecystitis	0.550	**0.000***	50	0.101	0.485	50	0.071	0.617	50
Cholelithiasis	0.548	**0.000***	50	0.347	**0.014***	50	0.020	0.886	50

r = Correlation coefficient, * statistically significant

Table 1 shows binary logistic regression to determine the factors associated with the occurrence of cholelithiasis. Age was 1.138 times more likely to affect the odds of cholelithiasis occurrence and was found to be statistically significantly (COR = 1.138; *p*= 0.004) related to the occurrence of cholelithiasis. As age increases, the occurrence of cholecystitis increases. After controlling for confounders, the multivariate analysis also shows that age was 1.340 times more likely to affect the odds of cholelithiasis occurrence and the only variable (AOR = 1.340; *p*= 0.030) found to be statistically significantly related to the occurrence of cholelithiasis. As age increases, the occurrence of cholecystitis increases.

BMI was 1.066 times more likely to affect the occurrence of cholecystitis. However, BMI was not statistically significantly (COR = 1.066; *p*= 0.477) related to the occurrence of cholelithiasis. After controlling for confounders, BMI was not statistically significantly less likely to affect the occurrence of cholelithiasis (AOR = 0.792; *p*= 0.225).

The females gender was not statistically significantly 1.471 times more likely to affect the occurrence of cholelithiasis while males were 0.680 times less likely(COR = 1.471; *p*= 0.618).After controlling for confounders, females were not statistically significantly 25.287 times more likely to affect the occurrence of cholelithiasis (AOR = 25.287; *p*= 0.073) while males were 0.040 times likely.

Age and BMI were statistically significantly more likely to affect the occurrence of cholelithiasis (Age: COR = 1.123; *p*= 0.002, BMI: COR = 1.259; *p*= 0.022). However, after controlling for confounders, age and BMI were more likely to affect the occurrence of cholelithiasis, but only age was statistically significantly (Age: AOR = 1.129; *p*= 0.006, BMI: AOR = 1.173; *p*= 0.191).

The female gender was not statistically significantly less likely to affect the occurrence of cholecystitis among the three variables (age, BMI and sex) (COR = 0.902; *p*= 0.886) while males were 1.109 more like to affect the occurrence of cholecystitis (COR = 1.109, CI = 0.270 – 4.550, *p* = 0.886). After controlling for confounders, the female gender was not statistically significantly more likely than the male gender (AOR = 3.981; *p*= 0.199).

Table 13: Logistic Regression analysis of predictor variables (age, BMI and sex) with response variable gallbladder stone and thickened GB findings in the study (sickle cell disease) group

Variable	Univariate models			Multivariate model (overall)		
	COR	**95% CI**	***p* value**	**AOR**	**95% CI**	***p* value**
Gallbladder stone						
Age	1.138	1.043 – 1.241	**0.004***	1.340	1.028 – 1.746	**0.030***
BMI	1.066	0.894 – 1.271	0.477	0.792	0.543 – 1.155	0.225
Sex						
Female	1.471	0.323 – 6.702	0.618	25.287	0.737 – 867.990	0.073
[R] Male	1			1		
Wall thickness						
Age	1.123	1.042 – 1.209	**0.002***	1.129	1.036 – 1.231	**0.006***
BMI	1.259	1.034 – 1.533	**0.022***	1.173	0.924 – 1.490	0.191
SEX						
Female	0.902	0.220 – 3.702	0.886	3.981	0.483 – 32.787	0.199
[R] Male	1			1		

[R]Reference category, COR: Crude odds ratio, AOR: Adjusted odds ratio, $p < 0.05$
***Statistically significant**

DISCUSSION

Sickle cell anemia is a genetically inherited autosomal recessive condition in which glutamic acid is replaced by valine in the position 6 of the beta chain of haemoglobin molecule.[1] SCD remains a disease of serious socio-economic importance in Nigeria as it imposes heavy burden on the patients, parents/carers and health institutions.[18]High incidence of pigment gallstones is caused by chronic haemolysis with accelerated bilirubin turnover.

Previous studies on cholelithiasis in SCD patients showed wide variation in the prevalence.[16,19,20,21,22,23]In many other parts of the world, varying figures given for the prevalence of gallstones, range from 4% to more than 20% (Tunisia 4.1%, Islamic Republic of Iran 4.7%, Bangladesh 5.4%, Peru 10.7%, Germany 7.8%), New Zealand 20.8% and United States of America 10%–15%)[24]. In this study, cholelithiasis was observed in 8 of 50 SCD subjects giving the overall prevalence of 16%. The prevalence of cholelithiasis has been observed to vary with age, gender, ethnicity and religion. These different figures could be due to differences in dietary cholesterol and/or fibre, though other factors such as genetic or environmental could also have some influence.[20]Prevalence of cholelithiasis in this study is in agreement with values observed by Agholor *et al*.,[16](16%), Ighodaro (14.3%)[21], Agunloye *et al*.,[22] (17.5%) in Ibadan and Adeniyi *et al*.,[23] (13.6%) in Lagos but much higher than the prevalence reported recently in North-East Nigeria (4.6%) by Ajani *et al*.[24]Akinyanju and Ladapo[25]in Ibadan and Akamaguna *et al*.[26]in Benin who employed the use of oral cholecystography documented a prevalence of 6% and 5% respectively, while Adekile and Makanjuola[27]in 1985 who employed a similar method in combination with ultrasonography in the diagnosis of cholelithiasisis also reported a lower prevalence (4.4%) in children below 17 years. The reason for the higher prevalence observed in the present study may be related to factors such as diets and lifestyle changes. Age and sample size can also contribute to variations in prevalence especially in studies where the participants included children and adults. Inah and Ekanem[28]equally documented a prevalence of 10% in an ultrasonographic study with subjects aged 1.5 to 55 years; while in Brazil, Gumiero *et al*[29]studied children and adults with SCD from age 7 months to 33 years and documented a much higher prevalence of 45%. The reported prevalence generally in the adult population is much higher than what has been reported in the paediatric age group and varies between 24% and 50%.[30]Generally-speaking, the documented prevalence of cholelithiasis in SCD may be related to the selection criteria, age group studied and possibly the diagnostic methods. Nevertheless, the ultrasonographic method has been proven to be a highly sensitive and specific method of diagnosing cholelithiasis.[27,30,31,32]

As reported in many of the previous studies.[21,24,28,30,32-35]prevalence of cholelithiasis was observed to increase with age. Similar finding was found in this index study as cholelithiasis was most prevalent in the older age group. Convincingly, it seems that the occurrence of cholelithiasis begins in late childhood and by the age of 18 years 30% of sickle cell patients would have developed cholelithiasis.[34] This trend can probably be explained by the repeated progressive sickling of the red cells with increasing age leading to deposition of pigment stones in the gall bladder.[33,36-39]The influence of puberty on the occurrence of the condition has also been postulated[24,28,30,32-35] and this may be a possible explanation for the findings in the present study. The prevalence of cholelithiasis has been reported to be more in females compared to their male

counterparts especially in the adult population[21,40,41]and this has also been observed in some paediatric studies.[28,30]This was also observed in this study.

In the present study, fasting gallbladder volume was significantly higher in sickle cell anaemia subjects than control group. Findings in our study was similar to finding in previous studies done in Benin, Lagos and in Tokyo.[12,21] These previous studies documented higher fasting gallbladder volumes in test subjects compared to control. It was noted that fasting gallbladder volume was higher in subjects with sickle cell disease compared to the controls probably due to the hepato-biliary system being overworked.

Furthermore, in this study, the mean gallbladder volume was significantly higher in sickle cell disease who had calculi (64.0±29.5cm^3) than in sickle cell disease without calculi (30.2±17.8cm^3), (p=0.000) thus presupposing that large gallbladder volume and poor biliary drainage is a risk factor for calculus formation in sickle cell disease, due to biliary stasis. This observation is corroborated by findings in the study by Ighodaro[21]who reported statistically significant difference in mean gallbladder volume of 73.73±20.75vs30.14±15.09 for sickle cell disease with calculi and sickle cell disease without calculi respectively and that of Olokoba *et al*[38]who examined 100 apparently healthy subjects and documented that the mean gallbladder volume in those with gallstones (26.5±14.7 ml) was higher when compared with the mean gallbladder volume in those without gallstones (24.1±12.7 ml).

In this study, male sickle cell disease with gallstones had higher mean age and BMI than male non-sickle cell disease without gallstones (24.6±15.2 vs 18.2±12.3, p=0.110 for age and 21.9±4.9 vs 20.7±4.1, p=0.334 respectively). Female sickle cell disease with gallstones on the contrary, had a lower mean age and BMI than female non-sickle cell disease without gallstones (18.6±12.5 vs 21.6±13.1, p=0.428 for age and 20.5±3.7 vs 22.8±4.2, p=0.054 for BMI respectively)[41]. This is similar to the findings by Ighadaro[21] and Billa RF *et al*[39] who reported that the mean age of sickle cell disease with gallstones was significantly higher than in those without gallstones. Overall, the mean age of case and control groups in this study were 22.1±14.7 years and 19.9±12.6 years respectively.

In this present study, there were 10 out of the 50 subjects who had thickened gall bladder wall, leading to a total prevalence of 20% and it was more than gallstone abnormality as outlined above. Out of these, six (6) were male subjects while four (4) were females. This is similar to a study by Saleh *et al*[40]in Kano where there were 19 subjects having thickened gall bladder wall, which was also more than gallstones prevalence; out of these, 11 were male subjects while 8 were female and a study by Nzeh et al[42]in Ilorin where 13 cases of thickened gallbladder wall were noted out of 161 subjects (i.e 8% prevalence) recruited for the study and that was more than those with gallstones (with 4.2% prevalence).The prevalence of cholecystitis is higher in this study compared to the studies by Ma'aji *et al*[43]in Sokoto where it was present in seven out of 72 patients making 9.7%, and by Bakhieta *et al*[41]in Sudan where only two patients out of 90(2.2%) presented with cholecystitis. This difference may be due to age range difference and smaller sample size in the latter two studies as well as differences in geographical location with regards to Sudan.

In a study carried out by Longo-Mbenza[44]in Congo among 190 patients, cholecystitis was found in 48 patients (25.3%) this was much higher than the findings in this study. This difference in number may be due to sample size and because the Congolese study

involved much older patients, with about 2/3 of their patients being 15 years and above while in the present study, more than half were children with only few adults.

The difference in the prevalence of cholecystitis (thickened gallbladder wall) between the subjects and controls in this study was statistically significant (p = 0.001). This is similar to the findings by Saleh *et al*[40] in Kano who found a statistically significant difference in the prevalence of cholecystitis between the subjects and controls (p = 0.000). In this study, none of the subjects had cholecystitis. This was in agreement with the report by Oguntoye *et al* [45]in their study in Ile- Ife.

The index study showed a positive correlation between age, gallstone and cholecystitis with Pearson's correlation coefficient (r = 0.550; p = 0.000 and0.548; p = 0.000, respectively. Similar results were also obtained from the studies in Kano and Egypt,[46]both of which showed statistically significant correlation between increasing age and gall stones. A study done by Walker et al[47]in Jamaica involving SCD patients also showed that gall stones are age- dependent: 15% in patients under 10 years of age, 22% in 10 and 14 years of age and 36% in 15 to 18 years of age, with a reported prevalence of 50% by age 22 years. The increasing prevalence of gallstones with age may be attributed to the chronic hemolysis and frequent use of antibiotics such as the quinolones for the treatment of infections in older SCDpatients.[19]There was no significant positive correlation between sex, gallstone and cholecystitis in this study with Pearson's correlation coefficients; (r = 0.071 and 0.020 and p= 0.617 and 0.886) for gallstones and cholecystitis, respectively.

The lack of correlation between gender and the gallbladder abnormalities may be because more than half of patients in this study were young patients where there was little effect of female sex hormones, no parity, and patients were not on oral contraceptives, which may increase the risk of gallbladder disease.[48]

In a case-control study in Australia among 100 SCD subjects, 50 of which were males and 50 females, there was no statistically significant difference in BMI and gallstones in men, while in women they found a positive association between BMI and gallstones. In another case-control study involving 80 adults done in Netherlands, a positive association was found in both men and women.[49,50] In the present study, SCD males with gallstones had higher BMI compared to their female counterparts and this was found to be statistically significant.

This present study showed that the presence of gallstones was statistically significantly related to increase in age as higher prevalence was recorded in adults than in children. Similar relationship was confirmed in a study carried out among 120 SCD patients in Taiwan, comprising of adults and children, with increasing prevalence of gallstones from the early adult age-group to the middle-aged group.[51]

The present study found a relationship with BMI and an increased risk of gallstone disease when considered alone, but when considered with other confounders (age and sex), BMI did not have any relationship with increased risk of gallstone disease. However, Meqdam *et al*[19] found a high statistically significant relationship between body mass index measurements of patients above 25 years of age in comparison with patients below 25 years of age with gallstones.

In another study, elevated body mass index (BMI) was found to be associated with an increased risk of cholelithiasis. Whether this association reflects a causal effect of obesity on gallstone development is unclear. It may be that another factor

simultaneously raises BMI and causes gallstone disease, and that elevated BMI is merely a marker of this other causal factor (in epidemiology, this common phenomenon is termed “confounding”).[52,53]

ETHICAL CONSIDERATION

Ethical clearance to proceed with this study was obtained from the Research Ethics Committee of the Irrua Specialist Teaching Hospital.

Subjects were examined after due explanation of the study objectives and methods of examination, following which a written informed consent was obtained from each participant.

Also volunteers and patient’s confidentiality were ensured as no personal information regarding them were divulged without their consent. Their names were also not disclosed at any point on the written materials in order to preserve anonymity.

The participants’ privacy was ensured and only chapter one agreeable to them was selected when necessary, in case of female participants.

Consent for children participants was obtained from their parents/guardians.

Also, the principal investigator bore the cost of the investigations where ultrasound was not indicated in the care and management of the SCD patients and volunteers.

CONCLUSION

This study has shown the usefulness of ultrasonography in evaluating the gallbladder in normal and sickle cell disease patients. It showed that there was an increased prevalence of gallbladder stones and cholecystitis in sickle cell disease patients when compared with normal non-SCD subjects; the prevalence of cholecystitis (20.0%) being more than that of gallstone (16.0%). When considered together, the prevalence of cholelithiasis and cholecystitis increases with age, BMI and gender, but gender does not affect the prevalence of choleystitis, when considered alone. After controlling for confounders, the prevalence of cholelithiasis and cholecystitis increases with age, BMI and gender while BMI does not affect the prevalence of gallbladder stone.

RECOMMENDATIONS

1. All sickle cell disease patients should be encouraged to have gallbladder ultrasonography done early, so that gallbladder changes associated with sickle cell disease can be diagnosed and treated on time.
2. Young children with SCD should have regular gallbladder assessment considering the increasing incidence of gallbladder changes with age.
3. Population-based study using large sample sizes should be done to validate the observations in this research.

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