

QUANTITATIVE ANALYSIS OF SERUM LIPID PROFILE IN PATIENTS WITH GALLSTONE DISEASE

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ABSTRACT: Gallstone disease is one of the most prevalent gastrointestinal diseases; with a substantial burden to health care system. Biliary diseases are a common place occurrence in the present day surgical practice. In recent years, effort has been made to knowing the pathophysiological basis of gallstone formation. The role of serum lipids in the aetiology of cholelithiasis is very important and in cholesterol gallstones serum lipids are altered, hence the aim of this study is to determine and analyze the changes in the lipid profile parameters which are responsible for the causation of biliary calculi, with risk factor correlation. This study was conducted in MKCG Medical College & Hospital Brahmapur, Odisha from December 2012 to November 2014. Out of 12073 surgical admissions December 2012 to November 2014 in our hospital, The 50 cases of gallstone disease of different age group were selected randomly. Fifty patients of different disease other than gallstone disease of different age group were taken as controls from the indoor of the Department of General Surgery were included in this study. The patients were in the age group of 20 to 70 years, of both sexes, who presented with acute abdomen, with a high degree of suspicion of biliary calculi. A detailed history was taken from the patients to analyse the risk factors. The gallstones received after cholecystectomy examined grossly and categorized according to their morphology; the serum of the patients was collected for analysis of fasting lipid profile. The statistical operations were done through Graph pad InStat (© 2013 Graph pad Software, Inc.) and SPSS (Statistical Presentation System Software) for Windows, version 20.00 (SPSS, 2011. SPSS Inc: New York) to find out the descriptive parameters. The highest age group of the presentation of cholelithiasis was 41 to 50 years, the incidence in females being more common than males. All the patients presented with pain in abdomen and majority of them had tenderness in the right hypochondria. Chronic cholecystitis was the most common mode of presentation and ultra-sonogram is the most common imaging modality of choice. Mixed stones were predominant.

KEYWORDS: Cholelithiasis, Serum lipid profile.

INTRODUCTION: Gall stones disease (GD) or cholelithiasis (chole=bile, lithier=stone & sis=process) is a very common gastro-intestinal disorder which is present commonly in worldwide.^{1,2} Gallstones may form because of many different disorders.³ Gallstone disease is a chronic recurrent hepatobiliary disease, the basis for which is the impaired metabolism of cholesterol, bilirubin and bile acid, which is characterized by formation of the gallstone in hepatic bile duct, common bile duct or gallbladder.⁴ Among gastro-enterological diseases, gallstone disease is one of the world's most expensive medical conditions.⁵ Most of the gallstones patients

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present with severe abdominal pain requiring investigations and treatment. Many of them need surgical intervention by the time they are symptomatic.⁶ The chemical composition of gallstones is essential to study the aetiopathology of gallstone disease.⁷ Hence, Gallstones are classified into: cholesterol stones, pigment stones and mixed stones. However, all stones even pure cholesterol gall stones also usually contain small amounts of bilirubin. The prevalence of gallstones continues to rise with age, and it is higher in women than in men. This may be due to the increase of cholesterol content in the bile by the effect of estrogen.⁸ There are three stages of gallstones formation, i.e. super saturation, nucleation & aggregation. The relative concentration of cholesterol, bile salt & phospholipids determine cholesterol solubility in bile. Cholesterol precipitation results from an imbalance of these three components in bile i.e. cholesterol, bile salt & phospholipids. The changes in bile composition are closely related to the disorder of lipid metabolism in liver. However during formation of cholesterol gallstone, different links in the disturbance of lipoprotein cholesterol metabolism and their effects in lithogenesis, still have many controversies. Some investigators reported that gallstones patients have hyperlipidemia. Mixed and pigment stones are common in northern India.⁹ Epidemiological studies have shown that demographic characteristics and social customs did not contribute to the pigment gall stone formation.¹⁰ Studies from northern India have looked into the dietary factors which predispose to cholesterol gall stones,¹¹ while such information is not available from southern India. Although this disease has a low mortality rate, its economic and health impact is significant due to its high morbidity.¹² The major elements which are involved in the formation of gall stones are cholesterol, bile pigments, calcium, hepatic bile composition, biliary glycoprotein, infection, age, sex, genetics, oestrogen, dietary factors, geographical prevalence, and cirrhosis of the liver.¹³ Now -a -days, the prevalence of gall stones is highly seen in southern Odisha, especially in the rural population. Most of the gall stone patients are asymptomatic and thus the present study describes an extensive outlook into the biochemical analysis of the stones, their incidence, age and sex distribution, the risk factors, the type of stones, the lipid profile. The dietary habits of people in our country, especially in this locality have quite changed a lot towards the western style in the last few decades the aim of my study is to establish the relationship between hyperlipidemia or dyslipidemia and gallstone diseases.

MATERIALS AND METHODS: The present piece of work "Quantitative Analysis of Serum Lipid Profile in Patients with Gallstone Disease" was undertaken in the Department of General Surgery, M.K.C.G. Medical College & Hospital, Brahmapur, prospectively from December 2012 to November 2014. The selection of cases and procedure adopted in this study are detailed below. This is a prospective study which was done on biliary calculi that were removed either laproscopically or by open surgery. Out of 12073 surgical admissions December 2012 to November 2014 in our hospital. The 50 cases of gallstone disease of different age group were selected randomly. Fifty patients of different disease other than gallstone disease of different age group were taken as controls from the indoor of the Department of General Surgery were included in this study. The patients were in the age group of 20 to 70 years, of both sexes, who presented with acute abdomen, with a high degree of suspicion of biliary calculi. The confirmation of cholelithiasis and its complications was done by ultrasonography. Patients with intra-hepatic

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calculi and patients, who were not undergoing either open or laparoscopic cholecystectomy with or without choledocolithotomy, were excluded from this study.

A detailed history was elicited, with particular attention to the hepatobiliary system, to assess the various risk factors. Each patient was examined physically to assess the general condition and the vital data was recorded. Per abdominal examination was done according to the standard protocol and the findings were documented. Based on the severity of the signs and symptoms and the USG reports, the treatment modality was decided. Patients who presented with acute pain abdomen, guarding, rigidity and obstructive symptoms with cholelithiasis were managed with intravenous fluids; nil by mouth and Ryle's tube aspiration. After taking the patient's consent, they were taken up for surgery.

The study was approved by Institutional ethics committee. Informed consent was taken from all the patients. The gall stones were sent for biochemical analysis. The controls were not suffering from gallstone disease

INCLUSION CRITERIA:

- 1) Cases: Fifty (50) cases of clinically and radiologically diagnosed gallstone patients included in the study.
- 2) Controls: Fifty (50) no's, selected randomly between 20 to 70 years of age, they were admitted to this hospital for treatment for other disease were included in the study.

EXCLUSION CRITERIA:

- 1) Patients not willing for study.
- 2) Patients on anti- lipidemic drugs.

After collection of 5ml of fasting venous sample from a large peripheral vein under aseptic precautions the sample was subjected to centrifugation and serum was obtained. Serum lipid parameters such as Total serum cholesterol (T-C), High density lipoprotein cholesterol (HDL-C), Very low density lipoprotein (VLDL), Low density lipoprotein cholesterol (LDL-C), Serum triglyceride (TG) were estimated in the department of Biochemistry of MKCG Medical College & Hospital, by using TBA-120 FR Toshiba autoanalyser.

The fasting serum lipid profile estimation was done. Total cholesterol was estimated by using CHOD-PAP, triglycerides were estimated by GPO-TOPS, HDL- C was estimated by HDL-C plus second generation, HDL Cholesterol by using kit from Agappe diagnostics. LDL-C was calculated by using Friedwald's formula after taking into consideration its limitation, $LDL-C = TC - \{HDL-C + (TG/5)\}$. VLDL was calculated by $TG/5$.

ANALYSIS OF GALLSTONE: Gallstones were collected after cholecystectomy. The stones were divided into 3 groups depending upon their colour: pale yellow and whitish stones as cholesterol calculi, black and blackish brown as pigment calculi and brownish yellow or greenish with laminated features as mixed calculi. The various physical parameters of stones such as number, shape, size, texture and cross-section were noted.

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BIOCHEMICAL ANALYSIS OF GALLSTONE: The stones were washed by distilled water and dried. A selected number of stones were picked randomly, and then they were crushed on a dry mortar and pestle. The power was then transferred into a test tube and warmed with successive small portion of ether, 5 ml at a time, by heating the test tube in a beaker of warm water. The extract was filtered and the filtrate was evaporated to dryness. The residue was then dissolved in 3 ml of chloroform and Liebermann bruchard reaction was carried out by adding 2 ml of mixture to 10ml of acetic acid anhydrite and 1 ml of concentrated sulphuric acid. Development of green colour indicated presence of cholesterol in the calculi.

The residue that remained after the ether extraction was treated with 10 ml of 25% hydrochloric acid to dissolve inorganic salts which might be present. The solution was filtered and divided into two 5 ml proportions. To one portion 2 -3 ml of 5% ammonium molybdate and few drops of concentrated nitric acid was added. The presence of phosphate was demonstrated by formation of yellow precipitate.

Second portion was neutralised with concentrated ammonia solution and acidified by adding acetic acid. 4 -5 drops of saturated solution of ammonium oxalate was added to the solution. A white precipitate indicated presence of calcium. The residue remaining after treatment with hydrochloric acid was tested for bile pigments. The residue on the filter paper was washed with water, dried and extracted with 5 ml chloroform. To this extract 5 ml Diazo reagent (made by mixing 10 ml of Diazo A and 0.3ml of Diazo B) was added. Volume increased by adding 10 ml of 80% alcohol. The content were thoroughly mixed and allowed to stand for 30 minutes. The presence of bilirubin was indicated by red colour due to formation of azo bilirubin. Each case was studied as per the Performa.

STATISTICS AND RESULTS:

Cases (50)		Controls(50)	
Female	38(76%)	Female	35(70%)
Male	12(24%)	Male	15(30%)

Table 1: sex distribution

The male to female ratio for various pathologies varied with maximum being female. The overall sex ratio in this study group 3.16:1 as female being (76%) (n=38) and males a mere 24% (n=12) of the study group. As can be seen, there is remarkably increased number of females patients (76%) in the cases. Therefore, the controls were taken with a bias towards the fairer sex.

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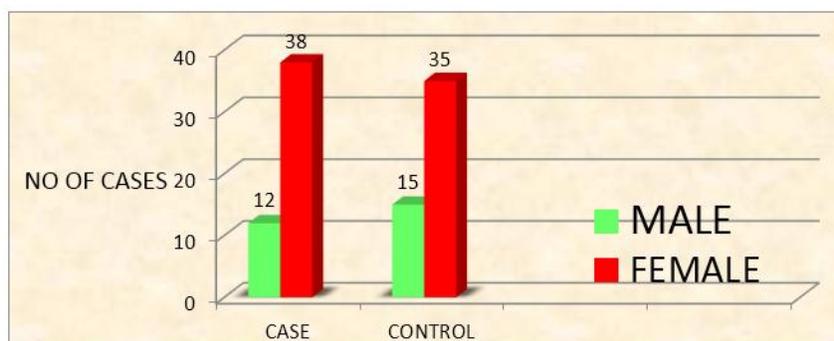


Fig. 1: Sex distribution of cases and control group

Age	Female	%	Male	%	Total	%
21-30	4	8	2	4	06	12
31-40	9	18	1	2	10	20
41-50	16	32	4	8	20	40
51-60	6	12	3	6	09	18
61-70	3	6	2	4	05	10

Table 2: Gallstone patients' distribution according to age

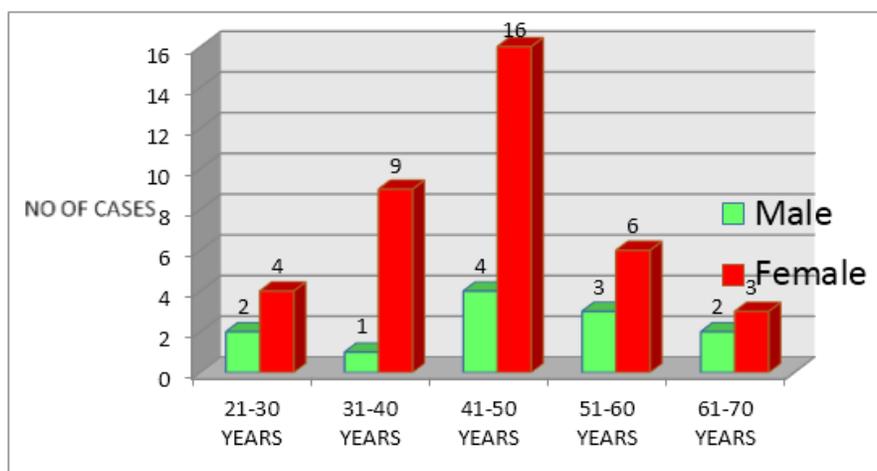


Fig. 2: Distribution of gallstones cases according to age & sex

It was found that incidence of gallstone disease increasing after adulthood in this study. The mean age was 45.28 ± 11.12 . The age group in controls varies from 20-70 years. The mean age of all control cases was 44.84 ± 9.721 . In this study male constitute 24% and female 76% in contrast to the literature females' outnumbered males in the ratio of 3.16:1. The bulk of the disease (40%) presented in the age group of 41-50 years. Female are distributed around 25 to 50 years. There is a female dominance which gradually goes down with advancing age. In this study youngest patient was 22 years and the oldest was 70 years old.

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Types of cases	No.	Percentage	Male: Female
Acute cholecystitis	11	22	1:10
Chronic cholecystitis with cholelithiasis	24	48	4:20
Chronic cholecystitis with choledocholithiasis	12	24	6:6
Empyema gallbladder	02	04	1:1
Malignancy of gallbladder	01	02	0:1

Table 3: Pathological distribution of Cases

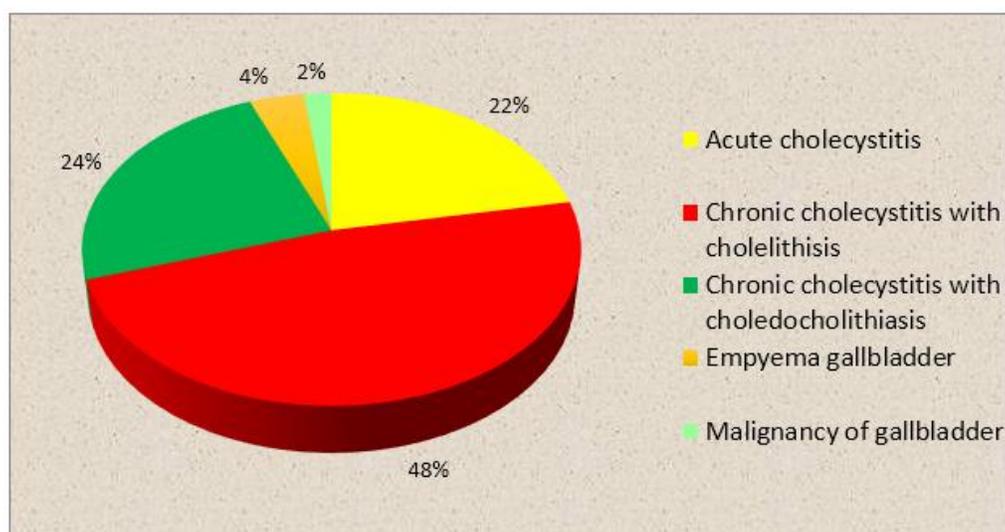


Fig. 3: Pathological distribution of cases

Most of the patients belong to the category of chronic cholecystitis. In this group chronic cholecystitis with gallstones was most common presentation (48%). As expected, the female cases are more in all types of cases especially in chronic calculus cholecystitis. The disparity in the ratio closes down in cases with chronic cholecystitis with CBD stones, empyema gallbladder.

Symptoms	No. of cases	Percentage
Pain abdomen	42	84
Dyspepsia	32	64
Pain abdomen with vomiting	18	36
Pain abdomen, vomiting, fever	09	18
Pain abdomen, vomiting, jaundice	06	12
Pain abdomen, jaundice, fever	04	08
Vomiting, jaundice, no pain abdomen	04	08
Pain, jaundice, fever with feature of shock	00	00

Table 4: Different Symptoms at Presentation

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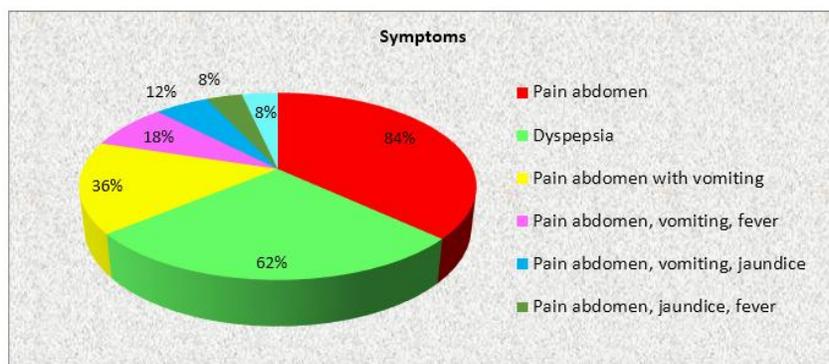


Fig. 4: Symptoms of presentation

In this study around 84% of patients presented with abdominal pain as chief complaint. Abdominal pain ranges from mild pain to severe pain lasting 12-24 hours. 62% presented with dyspepsia as their chief complaint. It was present in other cases too. Jaundice was a predominant feature in cases with CBD stones. But classical Charcot's triad was present in 08% cases and Reynolds's pentad was absent. Fever was also associated other symptoms, it was never the chief complain.

Signs	No. of cases	Percentage
Right upper quadrant tenderness	37	74
Clinical jaundice	12	24
Fever	14	28
Murphy's sign	16	32
Palpable gallbladder	11	22
Shifting dullness	01	02
Hepatomegaly	01	02

Table 5: Physical Signs Elicited

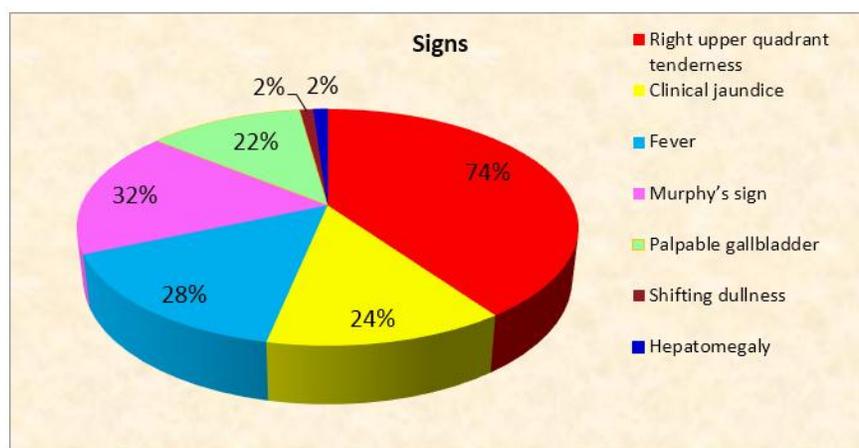


Fig. 5: Physical signs elicited

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The most common clinical sign elicited was right upper quadrant tenderness (37/50) i.e.74%. It was present in all 11 cases of acute cholecystitis, 14 cases of cholelithiasis, 10 cases of chronic cholecystitis with CBD stone and all 2 cases of empyema gallbladder. Jaundice was present in 12 cases (24%) out of 50, out of which one was carcinoma gallbladder. Murphy's sign was positive in 10 out 11 i.e. 90.90% cases of acute cholecystitis. Palpable gallbladder was present in 11(22%) cases. Shifting dullness and hepatomegaly was present in the same case, having carcinoma gallbladder.

Sl. No.	Serum parameters	Range	Mean	SD
1.	T—C(mg/dl)	120—200	146.8	18.19
2.	TG- (mg/dl)	80 - 180	128.9	37.69
3.	HDL - C (mg/dl)	35 - 50	40.28	5.253
4.	LDL - C (mg/dl)	Less than 120	76.12	14.08
5.	VLDL - C (mg/dl)	15 -40	30.56	8.728

Table 6: Lipid profile of control (N = 50)

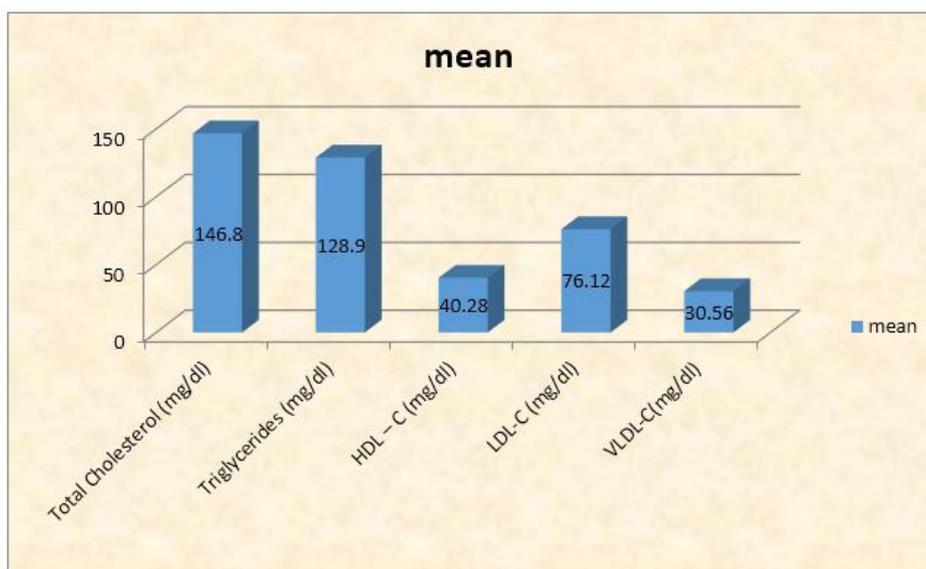


Fig. 6: Lipid profile of controls

It is evident from the above table that means value of serum lipid profiles fall within the normal range. Here values are compared after using two times of standard deviation (2SD). This includes 95% of controls.

Group	No	Cholesterol Mean±2SD	Triglyceride Mean±2SD	HDL Mean±2SD	LDL Mean±2SD	VLDL Mean±2SD
Patient	50	165.6± 59.66	184.2±146.20	35.50±13.292	95.86±47.86	37.10±22.02
Control	50	146.8±36.38	128.9±75.20	40.28±10.506	76.12±28.16	30.56±17.456

Table 7: The comparison of serum lipid profile between gallstone patients and control subject

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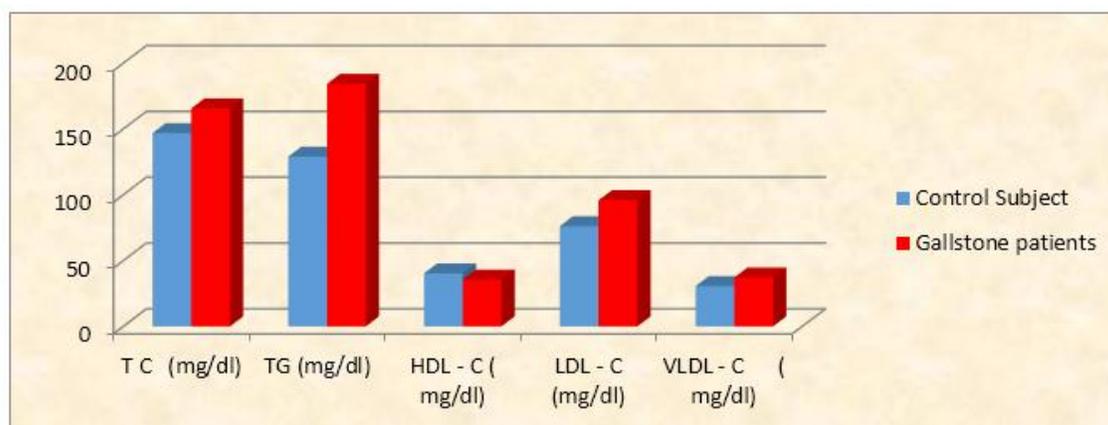


Fig. 7: Comparison of serum lipid profile between control subject and gallstone patients

The values of T-C, TG, LDL-C and VLDL were more than two standard deviations of control and were found to be highly significant (p value = p <0.01). The values of HDL-C when compared with controls were decreased, and more than two standard deviations found to be highly significant (p value = p <0.01).

Group	No	Cholesterol Mean± 2SD	Triglyceride Mean± 2SD	HDL Mean± 2SD	LDL Mean± 2SD	VLDL Mean± 2SD
Male	12	168.0± 50.60	201.8±143.90	35.50±12.664	92.33±30.76	41.25±19.93
Female	38	164.9±62.78	178.70±147.48	37.50±14.564	96.97±52.26	35.29±22.24

Table 8: Serum lipid profile male VS female cases of gallstone disease

The values of T-C, TG, LDL-C, HDL- C and VLDL were more than two standard deviations of male and female patients were found to be non-significant.

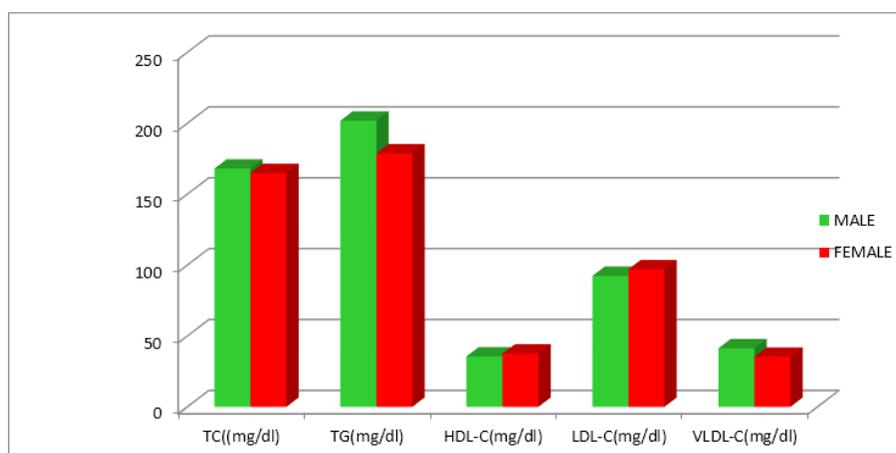


Fig. 8: Serum lipid profile Male vs. Female cases gallstone disease

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Age Group	No.	Cholesterol Mean± 2SD	Triglyceride Mean± 2SD	HDL- C Mean± 2SD	LDL- C Mean± 2SD	VLDL- C Mean± 2SD
20-45 yrs	27	163.3±56.06	175.9±151.02	34.81±13.12	94.70±40.9	35.96±23.86
46-70 yrs	23	168.4±76.46	194±141.86	36.30±12.64	97.22±55.76	38.43±19.834
Control	50	146.8±36.38	128.9±75.20	40.28±10.506	76.12±28.16	30.56±17.456

Table 9: The concentration of lipid profile in two age groups in patients with gallstone

The mean values of serum T-C, TG, LDL-C, HDL- C and VLDL - C are higher in age group 46-70 yrs than 20-45yrs age group, and more than two standard deviations between these two group found to be non-significant (p value = $p < 0.05$). The values of T-C, TG, HDL -C, LDL- C and VLDL-C is significantly higher (p value = $p < 0.01$) than the controls.

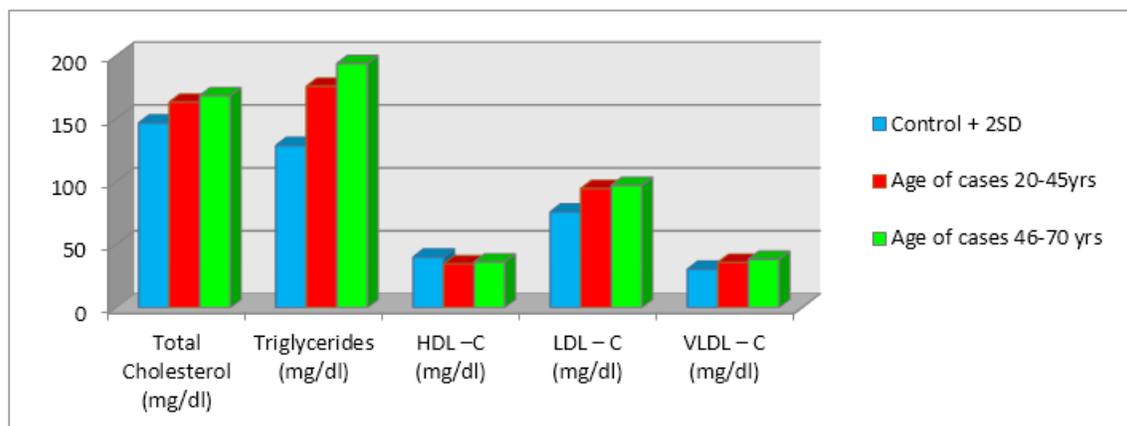


Fig. 9: The concentration of lipid profile in two age groups in patient with gallstone

TYPES OF STONE:

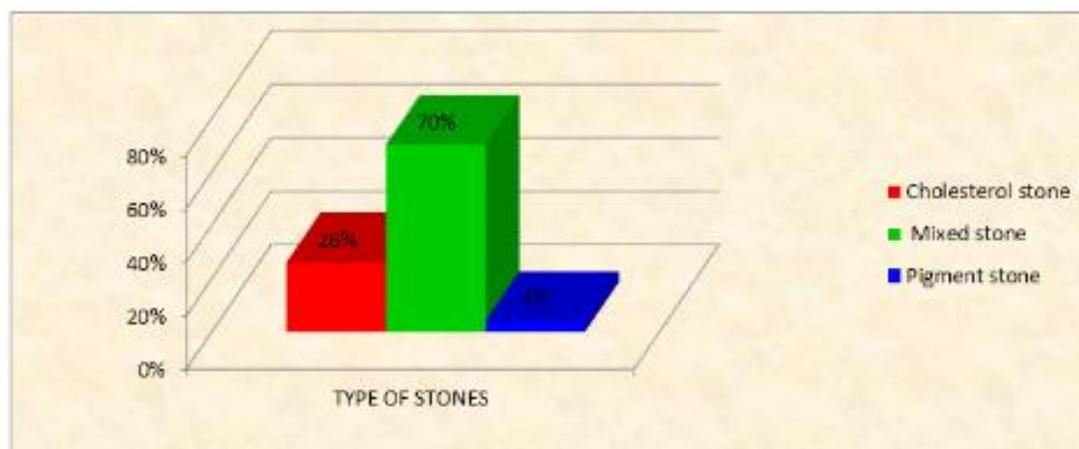


Fig. 10: Percentage of types of stone

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Types of stone	No. of cases	Percentage
1. Cholesterol	13	26
2. Mixed	35	70
3. Pigment	02	04

Table 10: Types of stones



Fig. 11: Percentage of types of stone

Parameter	Mixed stones (n=38)	Cholesterol stone (n=10)	P value
Total Cholesterol (mg/dl)	155.5±23.17	201.2±27.08	NS
Triglycerides (mg/dl)	185.0±80.12	183.6±50.91	NS
HDL – C (mg/dl)	34.39±7.008	39.70±3.653	NS
LDL – C (mg/dl)	88.45±16.09	121.8±31.91	NS
VLDL – C (mg/dl)	36.29±10.97	40.00±12.21	NS

Table 11: Comparison of serum lipid profile among Mixed stone patients and Cholesterol stone patients

Although the triglyceride, total cholesterol, LDL cholesterol in cholesterol patients are higher than that of mixed stone patients, The values of T-C, TG, LDL-C, HDL- C and VLDL were more than two standard deviations of mixed stone patients and cholesterol stone patients were found to be non-significant (p value = $p < 0.05$).

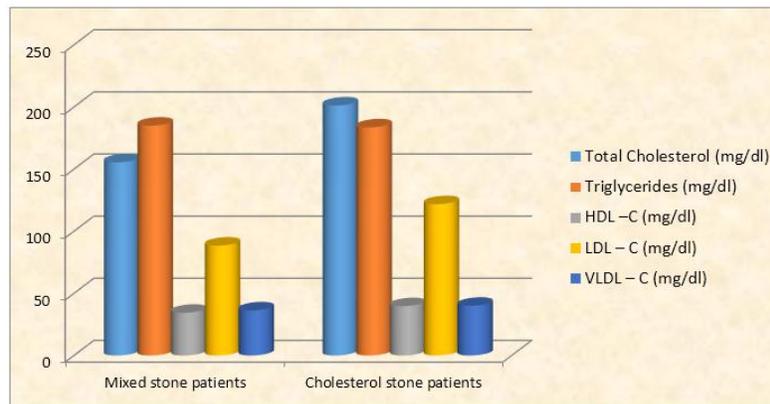


Fig. 12: Comparison of serum lipid profile among mixed stone patients and Cholesterol stone patients



Fig. 13: Variuos morphological features of gall stones post cholecystectomy specimens

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DISCUSSION: Several studies have been done on the gall stone status worldwide, till date. Indian studies showed that the incidence of gall stones was more in northern India as compared to that in southern India. This study was done in south Odisha. Many significant findings were observed in our study.

The youngest patient in the study was 22 years old and the oldest patient was 70 years old. The mean age of presentation was 48.00 ± 13.48 in males and 44.68 ± 10.64 in females. The bulk of the disease presented in the age group of 41-50 years i.e. 40% of the total number of cases. In this study showed a female preponderance with 76% females and 24% males, with a ratio of 3.16:1. Our study correlated with the studies conducted by Bockus HL et al and Nagaraj SK et al.^{14,15} Cholelithiasis can present from the young age to the centenarian. In my study, cholelithiasis had a peak incidence in the age group of 41-50 years, but no age group was found to be exempted from the disease process. We observed a lower age limit of 22 years as compared to the findings of the Nagaraj SK et al and Brazilian series.^{15,16} Studies have shown that pain was the most common presenting symptom, as was universally evidenced; I also observed the same symptoms in 84% i.e. 37 out of 50 cases. This pain was either due to the luminal obstruction from an impacted stone, which was characteristically colicky or from an inflammation, which was a burning type of pain. Dyspepsia was the second most common presentation (64%); it was mainly a feature of chronic cholecystitis cases. This symptom frequently in patients with and without gallstones¹⁷. Many of them had received earlier treatment for APD without facing proper investigation. This study also correlated with the studies conducted Nagaraj SK et al.¹⁵ Jaundice was a predominant feature in cases with CBD stones. But classical Charcot's triad was present in 08% cases and Reynolds's pentad was absent. Fever was also associated other symptoms, it was never the chief complaint. The most common clinical sign elicited was right upper quadrant tenderness (37/50). It was present in all cases of acute cholecystitis. Cholecystitis 14 cases, 10 cases of chronic cholecystitis with CBD stone and all 2 cases of empyema gallbladder. Studies conducted by Gosh SK et al,¹⁸ reported tenderness in the right hypochondriac region as the most common sign. Even in my study, this finding was consistent, 4 out of 5 patients had tenderness in the right hypochondriac region. Jaundice was present in 12(24%) cases out of 50, out of which one was carcinoma gallbladder. Murphy's sign was positive in 10 out 11(90.90%) cases of acute cholecystitis. Palpable gallbladder was present in 11(22%) cases. Shifting dullness and hepatomegaly was present in the same case, having carcinoma gallbladder. These results are similar to those seen in a study by Nagaraj SK et al.¹⁵ All the patients were subjected to the determination of the serum lipid profile like total cholesterol, HDL cholesterol, LDL cholesterol, VLDL cholesterol, and Triglyceride. The results so obtained were compared with the lipid profile of healthy persons taken as control. The result of serum total cholesterol, triglycerides, and LDL cholesterol, VLDL cholesterol show significant increase whereas serum HDL cholesterol shows significant decrease as compare with control subject.

The serum lipid parameters were compared to that of different studies conducted by different authors and it was quite similar to their findings.

In this study the mean values of serum T-C, TG, LDL-C, HDL- C and VLDL - C are higher in age group 46-70 yrs than 20-45yrs age group, and more than two standard deviations between these two group found to be non-significant.

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Male to female ratio in gallstone patients was 1:3. Mean plasma cholesterol and triglyceride values were higher in male gallstones patients as compared to controls. No significant difference was observed in the mean serum triglyceride values between male and female patients. In our study. The values of T-C, TG, LDL-C, HDL- C and VLDL were more than two standard deviations of male and female patients found to be non-significant.

Comparison was made among the lipid levels of patients on the basis of morphology of gallstones, the changes were not significant (pigment gallstones were excluded in comparison due to very less samples). Although the triglyceride, total cholesterol, LDL cholesterol and in cholesterol stone patients are higher than that of mixed stone patients, but it is not statistically significant.

CONCLUSION: The present prospective study of quantitative analysis of lipid profile shows a higher level of serum total cholesterol, triglycerides, and LDL cholesterol and VLDL cholesterol, whereas serum HDL cholesterol shows significant decrease as compare with control subject. This is similar to those published in foreign and other Indian literatures.

This study will definitely throw some light in this regard and help our patients to have a clear knowledge of relationship between dietary fat and gallstone diseases. High lipid profile may be risk factor and also a predictor of prognosis of gallstone disease. The risk of disease may be lowered by life style modification by avoiding sedentary habits and dietary modification by reducing consumption of saturated fat and control of dyslipidemia and control of obesity, hence a step ahead in the possible prevention of these diseases. Evaluation of lipid profile should be done routinely in gallstone diseases since literature suggest improvement of symptoms with a low fat diet and use of anti-hyperlipidemic drugs.

So, finally it is concluded that gallstone disease is associated with altered serum lipid profile namely hyperlipidemia or dyslipidemia which requires correction by dietary management, use of anti-hyperlipidemic drugs to achieve long lasting cure and to prevent possible future recurrences.

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