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Biochemical analysis of gallstones

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Abstract

Gallstone disease is the most common surgical problem encountered by general surgeons and constitutes the major portion of abdominal surgery in our practice. Gallstones are formed by super saturation of cholesterol in bile in the presence of other enucleating factors and calcium salts of bilirubin. In understanding the gallstone disease the knowledge of biochemical composition of gallstone is of huge importance. Broadly classified in 3 types (based on cholesterol content) that is cholesterol stone, mixed stone and pigment stone.

Keywords: Gallstones, laparoscopic surgery, mixed gallstones

Introduction

Gallstone disease is the most common surgical problem encountered by general surgeons and constitutes the major portion of abdominal surgery in our practice. Gallstones are crystalline deposits that are hard, pebble-like, usually made up of cholesterol or bilirubin, and are formed in the gallbladder [1]. Gallstones are formed by the super saturation of cholesterol in bile in the presence of other enucleating factors and calcium salts of bilirubin. In understanding the gallstone disease the knowledge of biochemical composition of gallstone is of huge importance [2]. Broadly classified in 3 types (based on cholesterol content): Cholesterol stone (cholesterol content >70%), Mixed stone (30% to 70% cholesterol) and Pigment stone (cholesterol content <30%) [3].

The prevalence of gallbladder stones varies widely across the world owing to the difference in diets preference. In the US at least 20 million have gall stones and approximately 1 million new cases of cholelithiasis develop each year. Cholelithiasis prevalence in Europe was 18.5% from the autopsy studies and the lowest prevalence from Ireland 5% and the highest from Sweden 38%. One epidemiological survey in rail and road workers showed that North Indians have a 7 times higher frequency of gall stone as compared with south Indians [4]. The majority of gallstones are cholesterol stones and remaining are pigmented. Cholesterol supersaturation of bile, decreased nucleation time and hypomotility of gallbladder leads to formation of cholesterol stones whereas pigment stones are brown and black. Black stones formed due to increased level of unconjugated bilirubin which binds with calcium carbonate to allow precipitation on the other hand brown stones are formed in bile ducts secondary to stasis and infection [5]. In understanding of gallstone disease or cholelithiasis the knowledge of chemical composition of gallstone is of paramount importance. Studies have been done so far but a study relating the clinical feature of cholelithiasis with type of gallstones has not been carried out in north India. Restriction of fat diet was used previously to restrict gallstone formation as well as the pain associated with gall bladder contraction [7]. In our study by defining the chemical composition of gallstone and establishing a correlation with its clinical feature we will open new windows for dietary modifications in gallstone disease as well implementation of non-surgical measures for it as gallstone is most common and costly of all digestive diseases.

Aims and Objectives

The present study was done in Department of General Surgery in collaboration with Department of Biochemistry in Maharishi Markandeshwar Medical College and Hospital, Kumarhatti-Solan (HP). 50 cases were admitted, examined, investigated and diagnosed as cholelithiasis during the period of November 2019 to September 2020.

The study was aimed at:

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1. To determine the biochemical composition of gallstone.
2. To advice dietary modifications for gallstone patients.

Materials and Methods

The present study has been done in Department of General Surgery in collaboration with Department of Biochemistry in Maharishi Markandeshwar Medical College and Hospital (MMMCH), Kumarhatti-Solan (HP). About 50 consecutive cases were admitted, examined, investigated and diagnosed as cholelithiasis during the period of November 2019 to September 2020.

These cases were selected after fulfilling the inclusion criteria. According to proforma all the basic history of 50 patients were collected. Information regarding the age, sex and nature of symptoms and duration of symptoms, dietary history and oral contraceptive pill intake, alcohol ingestion, smoking and associated morbidity like hypertension and diabetes were obtained. All patients' undergone detailed examinations underwent investigations such as complete blood count, liver function test, renal function test, blood sugar, lipid profile, urine analysis, chest X-ray, ultrasound of abdomen. Specialty consultations were taken for patients with associated comorbidities and their control was achieved before cholecystectomy.

Risk and complications of surgeries were explained to the patient in their own understandable language and a fully informed consent was taken in written prior to surgery. After cholecystectomy the shape, colour, surface and size of the gallstone were noted. The gallstones were then transferred into a urinary container and labeled with patients PMR number, name and sex.

The patient who underwent open cholecystectomy were discharged on 7th post-operative day and patient who underwent laparoscopic cholecystectomy were discharged on 2nd post operative day. Follow up was done in surgery outpatient department after 2 weeks.

Duration of Study: 11 months (November 2019 to September 2020)

Place of Study: Department of General Surgery and Department of Biochemistry Maharishi Markandeshwar Medical College and Hospital (MMMCH)

Study Design: Prospective

Sample size: 50

Source of Study: All patients fulfilling the inclusion criteria.

Inclusion criteria

- Patients diagnosed with cholelithiasis.
- Patients willing to be part of this study.
- Patients whose gallstones are sent for biochemical analysis.

Exclusion criteria

- Patients more than age 75 years.
- Patients not willing for follow up.

Biochemical analysis

Various physical parameters such as number of gallstone, shape of gallstone, color of gallstone, size of gallstone and texture were noted down. Then the stone was powdered in pestle and mortar. The powdered form of stone was then dissolved in respective solution depending upon the type of biochemical constituent to be analyzed. The tests were being performed in biochemical lab under the guidance of Dr Jai Prakash Bhartiya.

For cholesterol

- In a test tube, 10 mg of stone powder is dissolved in 1 ml of chloroform.
- After that, the test tube is placed in a boiling water bath for two minutes.
- Then 1 ml of this solution is taken in another dry test tube.
- 1 ml of acetic anhydride and 1 drop of concentrated H₂SO₄ added to it.
- Mix and observe for 2 minutes.
- Purple color is formed which soon changes to green.
- It may take 15-30 minutes for full development of green color.
- It is advisable to put the tube in dark during this procedure.

For triglycerides

- Diagnostic serum based on method of Wako and the modifications by McGowan *et al.* and Fossati *et al.* has been used.
- It determines triglycerides in human serum and plasma.
- Reagent is allowed to attain room temperature (15-30 degree Celsius).
- 1 mg of stone powder is mixed in the reagent and allowed to stand for 10 minutes at room temperature.
- It is advised not to shake prepared solution vigorously.

For phosphate

- In a test tube, 10 mg of stone was dissolved in 1 ml of HCL and graded up to 3.25 ml.
- With distilled water, it was reduced to a final amount of 10 mL.
- For 1 hour, the test tube was immersed in boiling water.
- 1 mg each ammonium molybdates and ascorbic acid added to the solution.
- Blue color signifies the presence of phosphate in the gallstone.

For bile pigment (bilirubin)

- In a test tube, 1 mg of stone powder was dissolved in 1 ml of chloroform.
- After that, the test tube is placed in a hot water bath for two minutes.
- In a test tube, 5 mL of sample is taken.
- Add a pinch of ammonium sulphate crystals.
- Add 2.5 ml of barium chloride.
- Take a filter paper and filter the solution.
- Spread the filter paper and air dry it.
- Add few drops of fouchet's reagent on the filter paper containing the residue.
- A green color will be obtained due to oxidation of bilirubin to biliverdin by FeCl₃.

For calcium

- 3 mg of stone powder was dissolved in 1 ml of HCL, graded into a 3.25 ml tube, and filled to a final volume of 10 ml with distilled water.
- The test tube was then kept in boiling water bath for 1 hour.
- Add 1 ml HNO₃ in the test tube.
- Now add ammonium oxalate in dissolved stone.
- White precipitates starts appearing which indicates presence of calcium in gallstone.

For fatty acids

- 1 mg of crushed stone was dissolve in chloroform-methanol mixture (2:1 ratio) and the ethyl alcohol-solvent ether in

(3:1 ratio) respectively.

- Take 0.5 ml chloroform in a test tube and add 0.5 ml sample drop by drop till the sample is fully dissolved.
- Add 1 drop of Sudan III reagent.
- Red color appears.
- The sample contains fatty acid.

Observations and Results

In this study, 50 patients with cholelithiasis were admitted in MMMCH, Kumarhatti, Solan (HP) between November 2019 to September 2020 were included. Well known literature on pathogenesis of gallstones and biochemical analysis of gallstone is reviewed. The results were then compared with different authors. After taking detailed history, clinical investigations an treatment, following observations were noted.

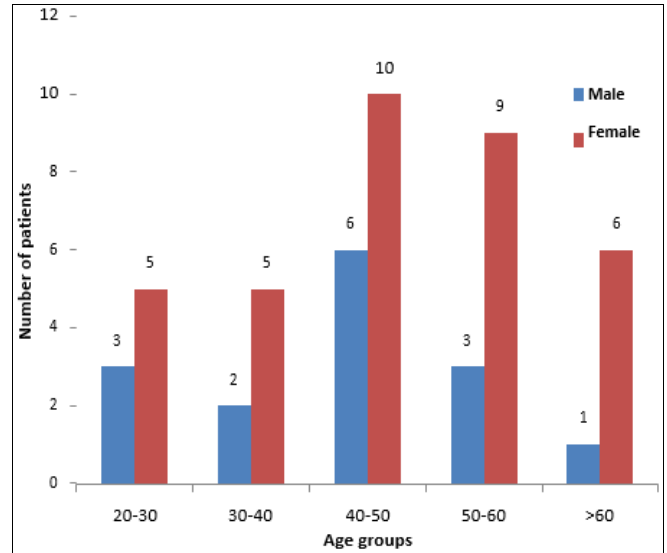
1. Age and gender distribution

Table 1: Age and Gender Distribution of Cholelithiasis

Age in years	Gender		Total	Percentage
	Male	Female		
21-30	3	5	8	16%
31-40	2	5	7	14%
41-50	6	10	16	32%
51-60	3	9	12	24%
>60	1	6	7	14%

In our study 15 patients were male and 35 patients were female. Maximum 32% of patients were in age group between 41 to 50 years. All cases in my study were between the ages of 22 and 75. The 5th and 6th decades have a higher occurrence, with the 5th

decade having the highest incidence.

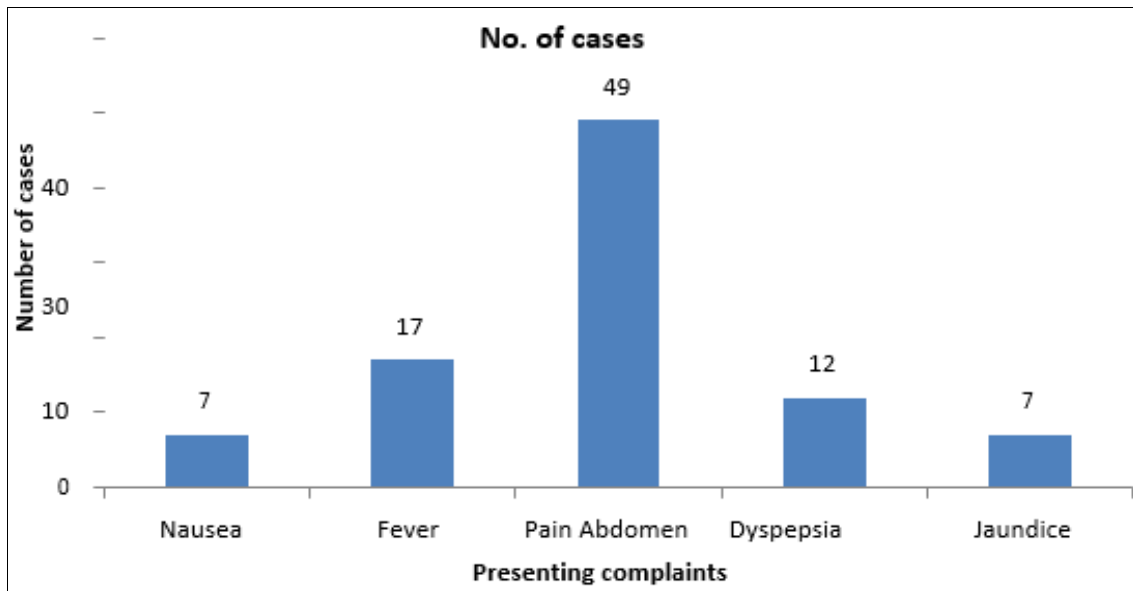


Graph 1: Age and gender distribution of cholelithiasis

2. Presenting symptoms

Table 2: Presenting symptoms of Cholelithiasis

	Pain Abdomen	Fever	Nausea/Vomiting	Dyspepsia	Jaundice
Number of cases	49	17	7	12	7
%	98	34	14	24	14



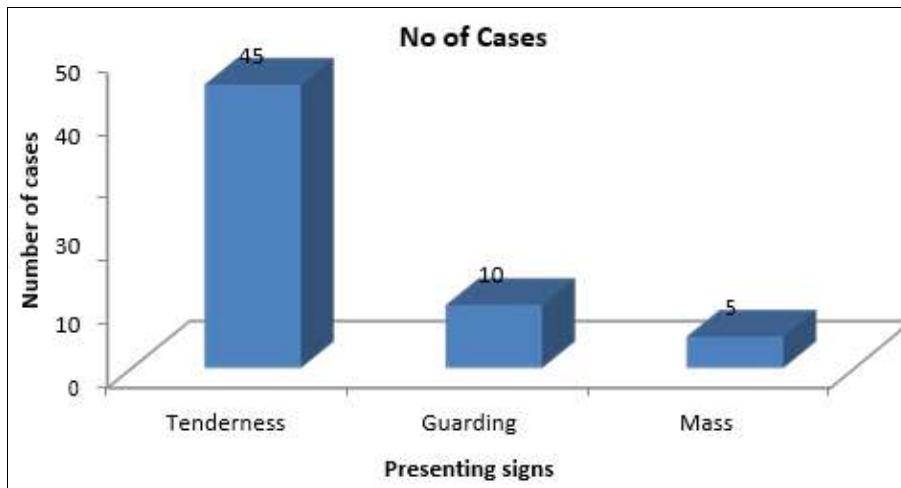
Graph 2: Presenting symptoms of cholelithiasis

Pain in abdomen was the most common presenting symptom in our study seen in 49 (98%) of the cases followed by fever in 17 (34%) cases. Dyspepsia was seen in 12 (24%) cases, 7 cases (14%) suffered from nausea/vomiting and 7 cases (14%) previously suffered from jaundice.

3. Signs of cholelithiasis

Table 3: Presenting signs of cholelithiasis

Signs	No. of cases	%
Tenderness	45	90
Guarding	10	20
Mass	5	10



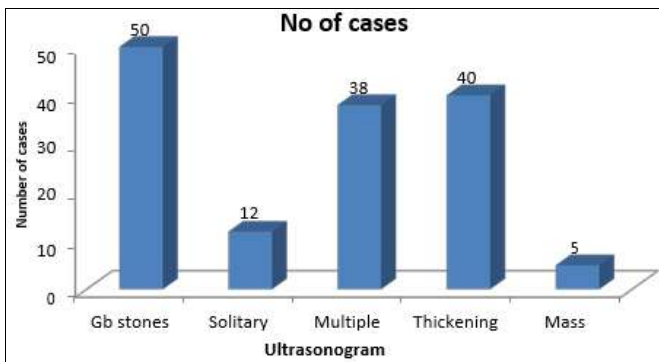
Graph 3: Presenting signs of cholelithiasis

Among the presenting signs seen in our study, tenderness was the most common sign seen in 45 cases (90%) followed by guarding in 10 cases (20%) and mass palpable in 5 cases (10%).

4. Ultrasonogram findings

Table 4: Ultrasonogram findings of cholelithiasis

Findings on Ultrasonogram	No. of cases	%
Stones in gallbladder	50	100
Solitary Stone	12	24
Multiple stones	38	76
Thickening of Gallbladder	40	80
Mass	5	10



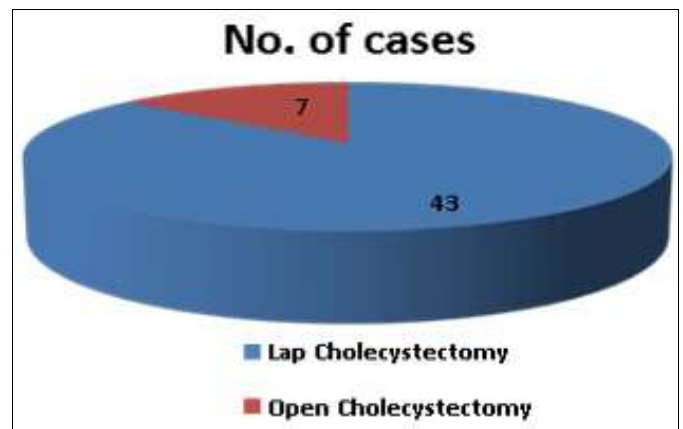
Graph 4: Ultrasonogram findings of cholelithiasis

Ultrasonographic findings revealed most common finding was thickened gallbladder seen in 40 cases (80%). 76% cases (38) had multiple stones and 24% cases (12) had solitary stone. Mass was seen in 5 cases (10%).

5. Type of cholecystectomy

Table 5: Type of cholecystectomy

Type of operation	No. of cases	Percentage
Laparoscopic Cholecystectomy	43	86
Open Cholecystectomy	7	14



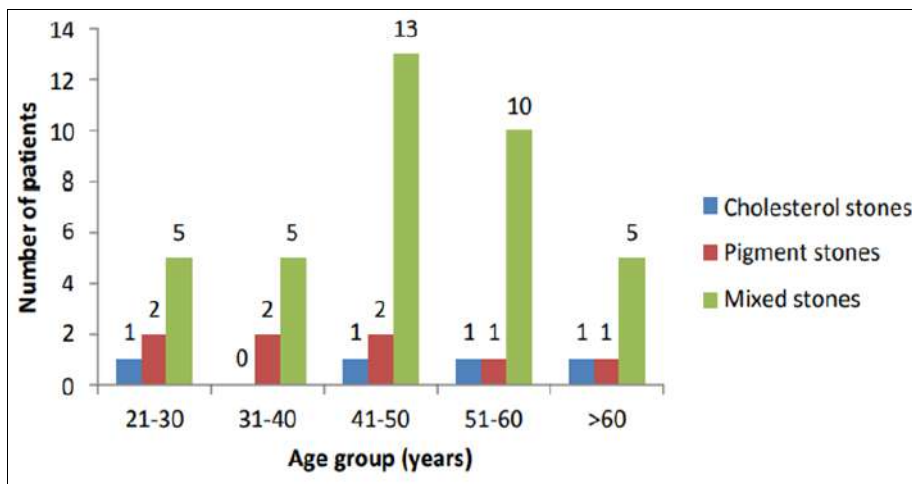
Graph 5: Type of cholecystectomy

Out of the total 50 cases who underwent cholecystectomy 43 (86%) underwent laparoscopic cholecystectomy and rest 7 (14%) underwent open cholecystectomy. Laparoscopic cholecystectomy because of its less postoperative pain, less hospital stay and complications was popular surgical operation among the patients.

6. Gallstones in cholelithiasis

Table 6: Type of gallstones in cholelithiasis

Age	Cases	Cholesterol Stones	Pigment Stones	Mixed Stones
21-30	8	1	2	5
31-40	7	0	2	5
41-50	16	1	2	13
51-60	12	1	1	10
>60	7	1	1	5
Total	50	4	8	38



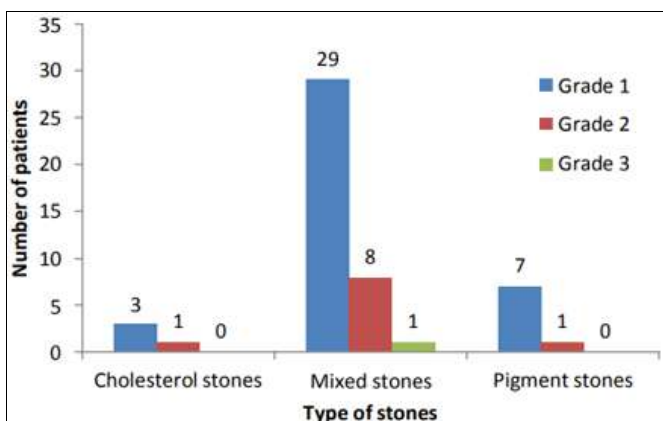
Graph 6: Type of gallstones in cholelithiasis

Out of 50 cases with cholelithiasis 38 patients (76%) had mixed stones, followed by pigment stones in 8 patients (16%) whereas cholesterol stone was only present in 4 cases (8%). Majority of the patients were in age group 41-50 (32%) followed by 51-60 age group (24%).

7. Gallstone and severity of cholecystitis

Table 7: Correlation between type of gallstone and Severity of cholecystitis

	Grade1	Grade2	Grade3
Cholesterol Stones	3	1	0
Mixed stones	29	8	1
Pigment stones	7	1	0



Graph 7: Correlation between type of gallstone and severity of cholecystitis

In our study grade 1 cholecystitis was associated maximum with mixed stones whereas least with cholesterol stone 7 cases only and 1 mixed patient was having grade 3 cholecystitis. 7 cases of pigment shown grade 1 cholecystitis, only 1 had grade 2 cholecystitis and none had grade 3 cholecystitis. 3 patients with cholesterol stone had grade 1 cholecystitis, 1 with grade 2 cholecystitis and none with grade 3 cholecystitis.

Discussion

In this study, 50 patients with cholelithiasis admitted in MMMCH, Kumarhatti, Solan (HP) between November 2019 to September 2020 were included. The available literature on biochemical analysis is reviewed and results of our study are compared with those authors. After detailed history, clinical

investigations and treatment, following observation were noted. The aim of the study was to compare the available literature present on gallstone disease and its biochemical composition with our present study.

Age distribution of Gallstone disease

Patients fall between 22 and 75 years. There is an increased incidence in the fifth and sixth decade with maximum incidence in the fifth decade. Similar incidence was seen in Abdalla M Jarari *et al.* in which the peak incidence was in 41 to 50 years of age [18]. Bashir M Jarrar *et al.* shown similar prevalence of age from 19 to 75 years [19]. Similar incidence of prevalence was seen in El Shahat A Toson and Marihan A Helal *et al.* [20]

Sex distribution of Gallstone disease

In this present study 35 out of 50 cases were female while the rest were male. Similar prevalence of stones in female was seen by Priyesh Halgaonkar *et al.* in their study. In their study 85 cases out of 100 cases were females which is in relevance with our present study [22]. Kalghatgi S *et al.* in their study concluded that females are more in comparison with male [24]. G P Naveenthan and Praveen Mallikarjunan found similar sex preponderance in their study [26].

Presentation of Gallstone disease

Pain was predominant symptom in the present study with 98% incidence. The commonest site was in the right hypochondrium. Similar presentation was seen in G P Naveenthan and Praveen Mallikarjunan series [26] and DrSidduraj C Sajjan series [38]. Pain with vomiting in our study was similar to Ganey *et al.* series [39]. The dyspepsia was relieved by in our patients after cholecystectomy. The incidence of dyspepsia in present study was similar to Ganey *et al.* series [39] and Alok Sharma *et al.* series [40].

Number of gallstones

Similar to our studies all the other studied reviewed by me it was found that ultrasound was main modality for investigation of gallstones. In our study out of 50 patients 12 had solitary stones and 38 were multiple. In similar study done by S.K Bansal *et al.*, it was found that out of 24 gallstones only 6 stones were solitary whereas 18 were found to be multiple [15]. This finding was in accordance to a study done by Alok Sharma *et al.* series [40].

Operation done for Gallstone disease

In the present study 7 patients underwent open cholecystectomy

and 43 patients underwent laparoscopic cholecystectomy. The most common incision used in open cholecystectomy was right subcostal incision which was used in 5 patients of open cholecystectomy. The result of my study was similar to the study done by J A Lujan *et al.* in their study ^[41].

Type of Gallstones found and it's Prevalence

In our present study 76% had mixed stones, 16% had pigment stones and only 8% cases had cholesterol stones. The same result was inferred by P Chandran *et al.* in their study. They found that out of 200 patients 76 were mixed calculi, 72 were pigment calculi and 52 were cholesterol stones. They also found higher incidence of mixed stone in their study ^[16]. Kalghatgi S *et al.* in their case series found that incidence of mixed stones is more than other two types of stones that is pigment stone and cholesterol stone ^[24]. G P Naveenathan and Praveen Mallikarjunan in their study found that out of 51 patients, mixed stones were present in 92% of the patients while rest 8% had cholesterol stone ^[26].

Dietary Association of Gallstone disease

Gallstone disease has appeared to increasing in incidence over the years in India as well as western world and the reason is clearly defined that is the dietary intake of patients that is in increase of intake of fatty and high calorie diet and increased consumption of alcohol. Mairlyn Tseng *et al.* series ^[35], Ada Cuevas, Angela M madden *et al.* ^[36] and Hemlata Sharma *et al.* series ^[42] support to limit the use of dietary fat, high caloric diet, simple sugars and alcohol to restrict the formations of gallstones.

Summary

The study consists of only 50 cases gallstones therefore there may be some variations in the statistics as number of my cases is small for full statistical evaluation. In this study, 50 patients with cholelithiasis admitted in MMMCH, Kumarhatti, Solan (HP) between November 2019 to September 2020 were included. The available literature on biochemical analysis is reviewed and results of our study are compared with those authors. After detailed history, clinical investigations and treatment, following observation were noted. The aim of the current study was to compare the available literature present on gallstone disease and its biochemical composition with our present study.

1. The highest age incidence of cholelithiasis was seen in the patients of 5th and 6th decade with maximum incidence in the 5th decade.
2. There was an increased incidence in female as compared to males.
3. Pain in right hypochondrium was found to be the predominant symptom seen in present study.
4. Ultrasound was main modality for investigation of gallstones disease.
5. Maximum number of patients had multiple gallstones while only few had solitary gallstone.
6. Laparoscopy cholecystectomy was done in majority of patients and it was treatment of choice in my study.
7. In our present study 76% patients had mixed stones, 16% had pigment stones and only 8% cases had cholesterol stones.
8. Severity of sign and symptoms of cholelithiasis is independent of type of gallstones found in the patients.
9. Increase in dietary fat, high calories and consumption of alcohol are positively associated with gallstone disease.

Conclusion

The incidence of gallstones is higher in 5th and 6th decades of life. The incidence of gallstones is more common in females as compared to males. The commonest symptom of gallstones is pain in right hypochondrium and ultrasonography is the modality of choice for detection of gallstones. Multiple gallstones are present in majority of the patients in our present study as compared to a solitary stones.

Laparoscopic cholecystectomy is popular and most done procedure nowadays with reduced stay in hospital, less pain and disability as in comparison with open cholecystectomy.

Out of three different types of gallstones, biochemical analysis in my current study revealed that mixed type of gallstones are commonest type of stones present in this region. Reduction in dietary fat, high caloric diet, simple sugar and alcohol intake can be beneficial in reduction of gallstones formation.

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