"HELICOBACTER SPECIES IN CHOLELITHIASIS AND CHOLECYSTITIS: HISTOPATHOLOGICAL AND SEROLOGICAL ASSOCIATION"

By

Dr. HARISH KUMAR G.

DISSERTATION SUBMITTED TO SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH, KOLAR, KARNATAKA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SURGERY

IN

GENERAL SURGERY

Under the guidance of

Dr. P.N. SREERAMULU
Professor

DEPARTMENT OF GENERAL SURGERY,
SRI DEVARAJ URS MEDICAL COLLEGE & RESEARCH,
TAMAKA, KOLAR-563101

MAY 2014

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation/thesis entitled **"HELICOBACTER SPECIES IN CHOLELITHIASIS AND CHOLECYSTITIS: HISTOPATHOLOGICAL AND SEROLOGICAL ASSOCIATION"** is a bonafide and genuine research work carried out by me under the guidance of **Dr.P.N.SREERAMULU** Professor, Department of General Surgery, Sri Devaraj Urs Medical College, Tamaka, Kolar.

Date:

Place: Kolar **Dr. HARISH KUMAR G.**

CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled **"HELICOBACTER SPECIES IN CHOLELITHIASIS AND CHOLECYSTITIS: HISTOPATHOLOGICAL AND SEROLOGICAL ASSOCIATION"** is a bonafide research work done by **Dr. HARISH KUMAR G.** in partial fulfillment of the requirement for the Degree of MASTER OF SURGERY in **GENERAL SURGERY.**

Date :

Place : Kolar

SIGNATURE OF THE GUIDE

Dr.P.N.SREERAMULU,

Professor,

Department Of General Surgery,

Sri Devaraj Urs Medical College

Tamaka, Kolar.

CERTIFICATE BY THE CO-GUIDE

This is to certify that the dissertation entitled **"HELICOBACTER SPECIES IN CHOLELITHIASIS AND CHOLECYSTITIS: HISTOPATHOLOGICAL AND SEROLOGICAL ASSOCIATION"** is a bonafide research work done by **Dr. HARISH KUMAR G.** in partial fulfillment of the requirement for the Degree of MASTER OF SURGERY in **GENERAL SURGERY.**

Date :

Place : Kolar

SIGNATURE OF THE CO-GUIDE

Dr.S.R.PRASAD,

Professor,

Department Of Microbiology,

Sri Devaraj Urs Medical College

Tamaka, Kolar.

CERTIFICATE BY THE CO-GUIDE

This is to certify that the dissertation entitled **"HELICOBACTER SPECIES IN CHOLELITHIASIS AND CHOLECYSTITIS: HISTOPATHOLOGICAL AND SEROLOGICAL ASSOCIATION"** is a bonafide research work done by **Dr. HARISH KUMAR G.** in partial fulfillment of the requirement for the Degree of MASTER OF SURGERY in **GENERAL SURGERY.**

Date :

Place : Kolar

SIGNATURE OF THE CO-GUIDE

Dr.T.N.SURESH,

Associate Professor,

Department Of Pathology,

Sri Devaraj Urs Medical College

Tamaka, Kolar.

ENDORSEMENT BY THE HOD,

PRINCIPAL / HEAD OF THE INSTITUTION

This is to certify that the dissertation entitled ***"HELICOBACTER SPECIES IN CHOLELITHIASIS AND CHOLECYSTITIS: HISTOPATHOLOGICAL AND SEROLOGICAL ASSOCIATION"*** *is a bonafide research work done by* ***Dr. HARISH KUMAR G.*** *under the guidance of* ***Dr. P.N.SREERAMULU****, Professor, Department Of General Surgery.*

Dr. M. MADAN MS
Professor & HOD,
Department of General Surgery,
Sri Devaraj Urs Medical College,
Tamaka, Kolar

Date:
Place: Kolar

Dr. M. B. SANIKOP MS
Principal,
Sri Devaraj Urs Medical College,
Tamaka, Kolar

Date:
Place: Kolar

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH CENTER, TAMAKA, KOLAR, KARNATAKA

ETHICS COMMITTEE CERTIFICATE

This is to certify that the Ethics committee of Sri Devaraj Urs Medical College & Research, Tamaka, Kolar has unanimously approved

Dr. HARISH KUMAR G.

Post-Graduate student in the subject of

GENERAL SURGERY *at Sri Devaraj Urs Medical College, Kolar*

to take up the Dissertation work entitled

"HELICOBACTER SPECIES IN CHOLELITHIASIS AND CHOLECYSTITIS: HISTOPATHOLOGICAL AND SEROLOGICAL ASSOCIATION"

to be submitted to the

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH, TAMAKA, KOLAR, KARNATAKA,

Date :
Place : Kolar

Member Secretary
Sri Devaraj Urs Medical College &
Tamaka, Kolar–563101

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH, TAMAKA, KOLAR, KARNATAKA

COPY RIGHT

DECLARATION BY THE CANDIDATE

I hereby declare that the Sri Devaraj Urs Academy of Higher Education and Research, Kolar, Karnataka shall have the rights to preserve, use and disseminate this dissertation/thesis in print or electronic format for academic/research purpose.

Date :

Place : Kolar **Dr. HARISH KUMAR G.**

ACKNOWLEDGEMENT

I am highly indebted to my guide ***Dr. P. N. SREERAMULU,*** *M.S., Professor, Department of General Surgery, Sri Devaraj Urs Medical College, Tamaka, Kolar, who guided me in bringing out this work with his thought provoking ideas and constant encouragement.*

It gives me immense pleasure to express my gratitude and sincere thanks to ***Dr. M. MADAN,*** *M.S., Professor and H.O.D., Department of General Surgery, Sri Devaraj Urs Medical College, Tamaka, Kolar, who took deep interest and gave constant support by encouraging in moulding this work.*

I also acknowledge my debt to ***Dr. K. MOHAN KUMAR.,*** *M.S.,* ***Dr. A. BHASKARAN****, M.S.,* ***Dr. K. N. NAGARAJ,*** *M.S. &* ***Dr. K. KRISHNA PRASAD,*** *D.N.B in General Surgery, Department of General Surgery, Sri Devaraj Urs Medical College, Tamaka, Kolar, who gave me moral support and guidance by correcting me at every step. I would like to extend this acknowledgement to my co-guides* ***Dr. S.R. PRASAD,*** *M.D, microbiology &* ***Dr. T.N. SURESH,*** *M.D, Pathology.*

I express my sincere thanks to all my teachers and Professors of Department of General Surgery, Sri Devaraj Urs Medical College, Tamaka, Kolar.

I remain thankful to all my assistant professors and lecturers for their support and encouragement. I acknowledge my sincere thanks to all my co-P.G.'s Dr.Lalith Sagar, Dr.Sharan Javali, Dr.Srikanth Talluri, Dr.Avinash Palreddy, Dr.Janhavi and all my juniors for their unconditional help and support at every step throughout my study.

I am much thankful to my parents ***Mr. Gopal Reddy*** *and* ***Mrs. Pushpamma*** *for their love, blessings and invaluable help. I thank my brother,* ***Dr. Monohar S,*** *for believing in me when I found it difficult to believe in myself and his support.*

I thank ***Dr. Naresh,*** *Department of Community Medicine for their invaluable help in helping me to deal with all the statistical work in this study.*

Last, but not the least, I thank the Almighty and ***my patients*** *for providing me the opportunity to carry out my study.*

Dr. HARISH KUMAR G.

ABSTRACT

BACKGROUND AND OBJECTIVES

Chronic cholecystitis is the most prevalent gall bladder disease in all population. Chronic cholecystitis and gall stones can cause epithelial hyperplasia of gall bladder mucosa or cancer.[1] Few studies have shown helicobacter can damage gall bladder mucosa and could be the key factor that leads to cholecystitis.[2]

Thus detection of helicobacter and association of helicobacter in gall bladder disease leads to new insight of treating cholecystitis and cholelithiasis. By treating helicobacter occurrence of post cholecystectomy syndrome can be minimized.

MATERIALS AND METHODS

An analytical prospective study on patients diagnosed with cholecystitis and cholelithiasis was done between December 2011 and august 2013. 65 patients were included in the study who met inclusion criteria. Gall bladder tissue collected by cholecystectomy and blood collected were investigated.

1. Serum IgM levels for helicobacter species.
2. Gall bladder tissue for hematoxylin and eosin, Giemsa staining for mucosal study.
3. Warthin-Starry silver staining for helicobacter detection

RESULTS :

3(4.6%) patients were tested positive for IgM class antibodies to the helicobacter and 3(4.6%) cases tested positive for helicobacter from cholecystectomy specimen by staining methods.

CONCLUSION :

According to our findings evidence of recent infection Helicobacter pylori as shown by demonstration of IgM class of antibodies to the organism was found in 4.6% of patients and histological evidence as demonstration in gall bladder mucosa could be optained in 4.6% of the patients with cholecystitis and cholelithiasis. Thus, the frequency of helicobacter infection seems to be low in the patient population studied.

KEYWORDS : helicobacter, cholecystitis, cholelithiasis.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF GRAPHS

INTRODUCTION

Biliary calculus disease is one of the most common disorder affecting the gastrointestinal tract and is important cause of morbidity. There has been marked rise in the incidence of gall stone disease in the west during the past century. In the UK, USA and Australia, the prevalence rates varies from 15- 25%.[1] In India, it is more common in the North India than in the South India.

Incidence of gallstones increases with the age. It is more common in female than male (M:F = 1:4) and about 50% of patients are asymptomatic.[1]

The pathogenesis of gallstones is multifactorial. It varies according to the type of gallstones. Primarily gallstones can be divided into two major groups.[2] First is pure gallstones contributing 10% of gallstones. Second is a mixed and combined gallstone which accounts for 90% of gallstones. Mixed gallstones frequently associated with cholecystitis.[2]

Helicobacter pylori is a gram negative and microaerophilic microorganism that can cause chronic gastritis, gastric and duodenal ulcers and gastric adenocarcinoma.[3] In the last few years, the scientists have been interested in studying the relationship between H. pylori infection and various extra-digestive diseases. Bile acids are generally known to have inhibitory effects on the adherence and growth of H. pylori in vitro. The in vitro bacteriostatic effect of bile has not been demonstrated to the same degree in vivo, suggesting the adaptive conditioning of H. pylori. It has been shown that a lower pH is more conducive to the survival of H.pylori.[4]

OBJECTIVES

To find histopathological association and sero positivity of helicobacter species in patients with cholecystitis and cholelithiasis.

REVIEW OF LITERATURE

The most well known member of the helicobacter genus, Helicobacter pylori is classified as a type 1 carcinogen and infects the human stomach and causes gastritis, peptic ulcer disease and gastric carcinoma.[5]

Besides helicobacter pylori, genus helicobacter contains more than 25 species, many of which cause extra gastric disease in humans and animals. These are named enterohepatic species and colonises in hepato biliary tract. Besides Several of these enterohepatic species are associated with the pathogenesis of chronic biliary disorders such as cholecystitis, cholelithiasis, gall bladder carcinoma and bile tract carcinoma.[6,10]

An experimental study has shown that helicobacter can damage human gall bladder epithelial cells in vitro and could be the key factor that leads to cholecystitis.[7,8]

Other study has shown 13.55% association of helicobacter in cholecystitis. Another study has shown negative association of helicobacter with biliary tract disease.[10]

ANATOMY, PHYSIOLOGY AND EMBRYOLOGY OF GALLBLADDER

EMBRYOLOGY[11]

At 3rd week when the embryo is 3mm in length and endodermal bud arises from the ventral aspect of the gut at the point between for foregut and midgut.

This endodermal bud enlarges and divides into pars hepatica and pars cystica. It passes through the septum transversum and grows into ventral mesogastrium.

Cranial portion is pars hepatica and caudal portion is pars cystica.

- Pars cystica develops into gall bladder and cystic duct.
- Pars hepatica cells grows into the transverse septum. The connection between hepatic diverticulum and foregut narrows and forms bile ducts.
- At 12th week of gestation liver function starts and cystic duct joins the hepatic duct and forms common bile duct (CBD).

ANATOMY[11,12,13]

Gallbladder is pear shaped organ (piriform shaped), sac like, hollow organ measuring 7.5 to 12 cm in length with capacity of about 50ml. It is capable of distension of about 50 times. It lies in the gallbladder bed of the inferior surface of liver. It extends slightly below the inferior margin of the liver.

Gallbladder is attached to the liver by loose areolar tissue called mesentery which is rich in small vessels and lymphatics. The extra hepatic portion of the bladder is covered by peritoneum.

Gall bladder has got four parts

- Fundus
- Corpus or body
- Infundibulum
- Neck

FUNDUS:

It is expanded portion of the gall bladder which projects beyond the inferior margin of liver. It is in contact with anterior abdominal wall. This portion is behind the right ninth costal cartilage where the lateral edge of the right rectus abdominis crosses the costal margin. Posteriorly it is related to transverse colon.

BODY:

It is directed upwards, backwards and to the left. It is continuous with the neck of the gallbladder. It is related to liver above, transverse colon and first and second part of duodenum below.

INFUNDIBULUM:

This small dilated bulbous part between neck and body of the gallbladder. It is also known as Hartman's pouch.

NECK:

Narrow part of gallbladder which curves up and forwards and then abruptly back and downward to become cystic duct.

HISTOLOGY

Gall bladder has three coats –

THE SEROUS COAT

Derived from peritoneum. It completely invests the fundus, under surface and sides of the body, neck of the gallbladder.

FIBROMUSCULR COAT

This layer of fibrous tissue mixed with unstriped muscular fibres which are longitudinal in direction though a few may be circular in arrangement.

MUCOUS COAT

It is loosely connected with the fibromuscular coat. Mucous membrane is elicited into minute rogue which give honeycomb appearance. It is yellowish brown in colour. Epithelium consists single layer of columnar cells of varying size. Apical surface contains microvilli which helps in absorption of H_2O and solutes from bile to make it more concentrated. Mucus granules are present in the apical half of some cells

which secretes mucus into the lumen.

CYSTIC DUCT

It starts from gallbladder and drains into common hepatic duct at an acute angle. Average length is 4 cm. The mucus membrane lining its inferior is thrown into series of crescentic folds from 5 to 12 numbers. These are called spiral valve of Heister.

Cystic duct joins common hepatic duct to become common bile duct which enters the 2nd part of duodenum in its medial aspect at the summit of ampulla of vater.

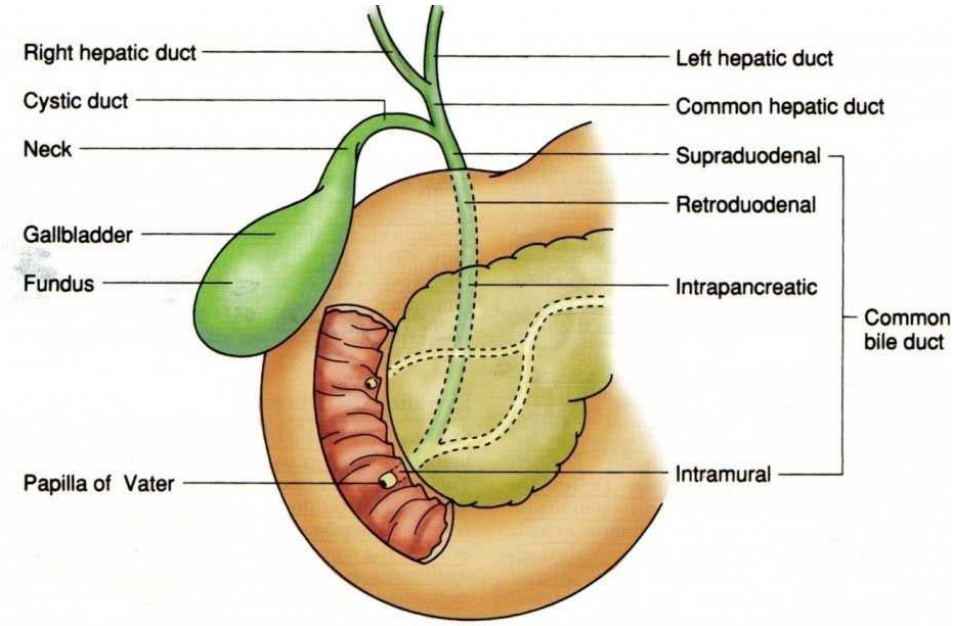


Fig.1: Course of Cystic Duct

SURGICAL IMPORTANCE OF GALLBLADDER :

- Fundus of the gallbladder is least vascular and it may undergo ischaemic changes and perforation is common.
- Gallstones may get impacted in the cystic duct and obstruct the flow of bile. Gallstones are commonly become impacted in Hartman's pouch.

ARTERIAL SUPPLY OF GALLBLADDER[14]

Major blood supply is from cystic artery which is branch of right hepatic artery. It runs in Callot's triangle closed to cystic duct. At the superior border of the neck of the gallbladder it divides into superficial and deep branches.

Accessory cystic artery may arise from common hepatic artery or from its branches. Occasionally cystic artery may arise from hepatic artery proper or rarely from gastroduodenal artery. Cystic artery also supplies branches to hepatic ducts and upper part of CBD.

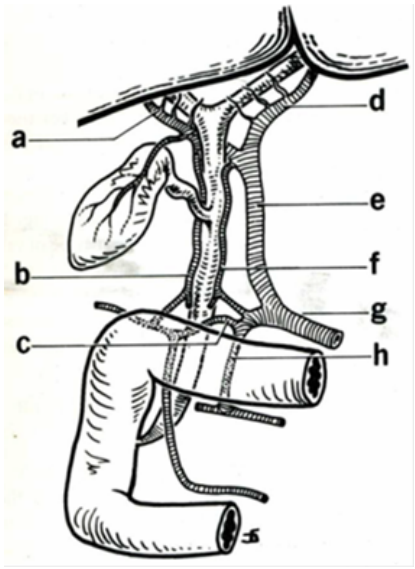


Fig 2 :Bile duct gall bladder blood supply

a) Right branch of hepatic artery
b) 9'O clock artery
c) Retroduodenal artery
d) Left branch of hepatic artery
e) Hepatic artery
f) 3'O clock artery
g) Common hepatic artery
h) Gastroduodenal artery

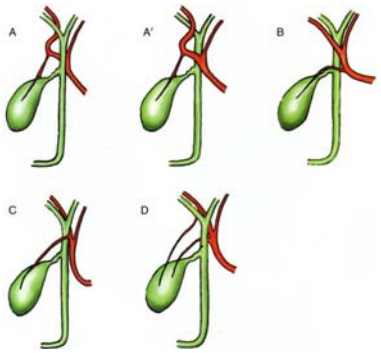


Fig.3: Cystic Artery Anomalies

A - Caterpillar hump right hepatic artery

B - Right hepatic artery anterior to common hepatic duct or CBD

C - Cystic artery anterior to common hepatic duct or CBD

D - Accessory cystic artery

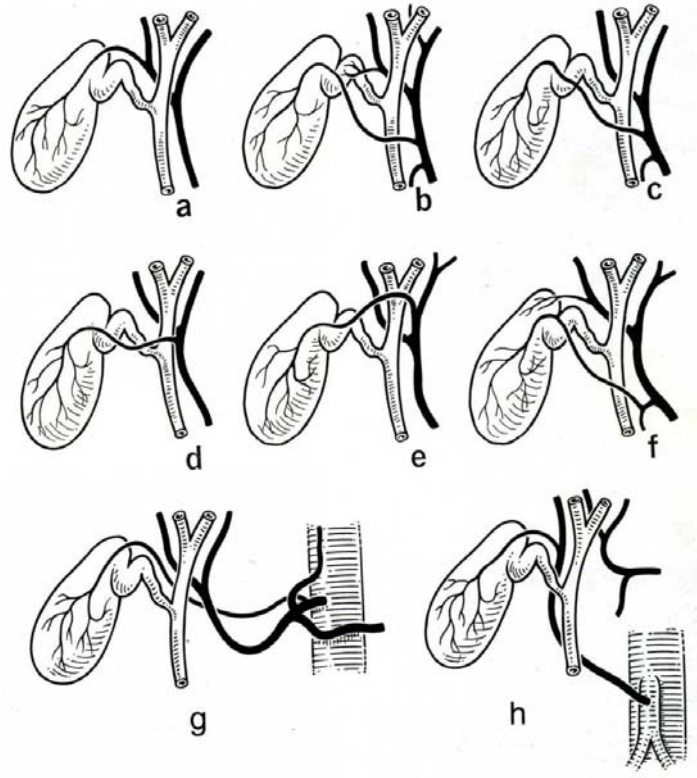


Fig.4 :Main Variations Of Cystic Artery

a) Typical course

b) Double cystic artery

c) Cystic artery crossing anterior to the main bile duct

d) Cystic artery originating from the right branch of hepatic artery and crossing the common hepatic duct anteriorly

e) Cystic artery originating from the left branch of hepatic artery

f) Cystic artery originating from the gastroduodenal artery

g) Cystic artery originating from the celiac trunk

h) Cystic artery originating from a replaced right hepatic artery

Venous drainage :

Venous drainage is carried out by small veins which enter directly from the gallbladder into the liver. Large cystic vein follows the cystic artery and joins the right portal vein.

Lymphatic drainage :

Lymphatics from subserosal and submucosal coats drain into the cystic lymphnode of lund which is the sentinel lymphnode which lies in the angle created by the junction of the cystic and common hepatic duct. Efferents from this pass through to the hilum of liver. Some lymphatics from the gallbladder drains to liver directly through subcapsular lymph channels of the liver.

Other lymphatics drains to porta hepatic lymph node which may enlarge in case of carcinoma gallbladder.

Nerve supply :

Supplied by both sympathetic and parasympathetic nervous system.

Parasympathetic -from vagus nerve through the coeliac ganglion.

Sympathetic - from T8-T9 levels and these fibres form plexus in the coeliac plexus from where the post ganglionic fibres supply the gallbladder along the hepatic artery.

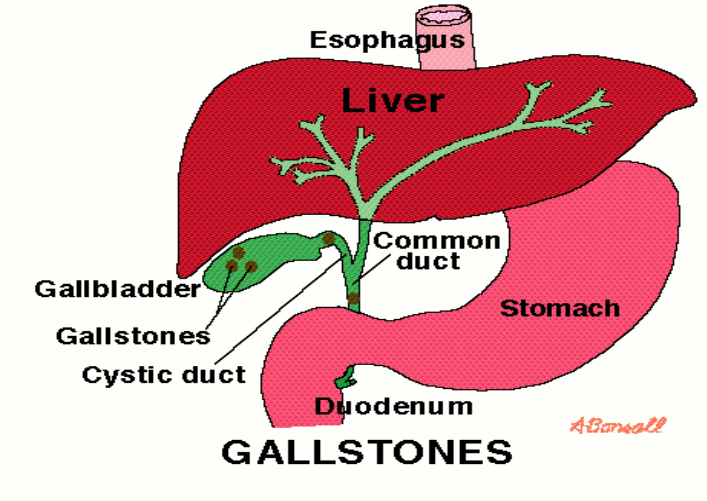


Fig.5: Gallstones locations

CONGENITAL ANOMALIES OF THE GALLBLADDER AND BILE DUCTS[15]:

1. Absence of GB: Occasionally GB is absent, failure to visualise the GB is not necessarily the pathological feature.
2. The Phrygian cap deformity: It is found in oral cholecystogram. Present in 2-6%. It refers to hats worn by the people of Phrygian.
3. Floating GB: Due to long mesentry, due to which GB may undergo torsion.
4. Double GB: Rare presentation, one of the GB part may be intrahepatic.
5. Absence of cystic duct: Rarely anatomical. This is usually pathological. It indicates the recent passage of stones or presence of stones at the lower end of cystic duct which

ulcerates into CBD. The danger lies during surgery because of injury to CBD.

6. Low insertion of the cystic duct: The cystic duct may open into CBD near the ampulla. Removal or ligation of cystic duct may lead to damage to the blood supply to CBD and lead to stricture.
7. An accessory cholecysto-hepatic duct: Rare anomaly. Ducts passing directly from GB into liver. Larger ducts should be closed but precise anatomy should be carefully ascertained.

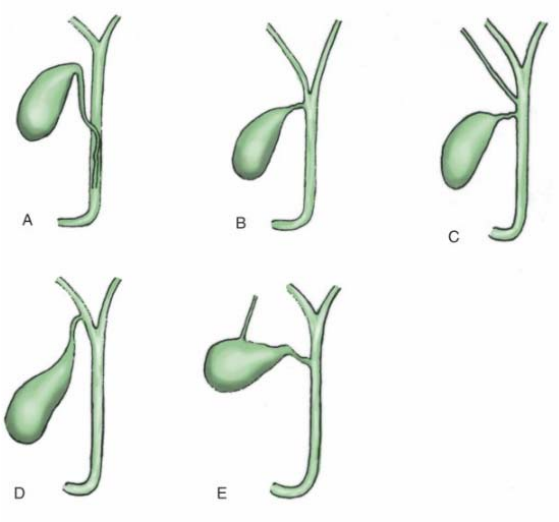


Fig 6 :Variations Of Cystic Duct

A) Long cystic duct with low fusion with common hepatic duct.

B) Abnormally high fusion of cystic duct with common hepatic duct (trifurcation).

C) Accessory hepatic duct.

D) Cystic duct entering right hepatic duct.

E) Cholecystohepatic duct

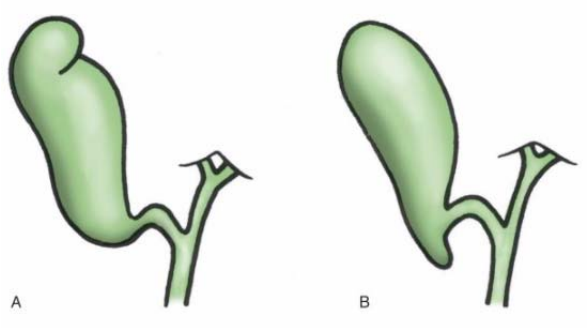


Fig 7 :

A) Phrygian cap deformity.

B) Hartmann's pouch of the infundibulum.

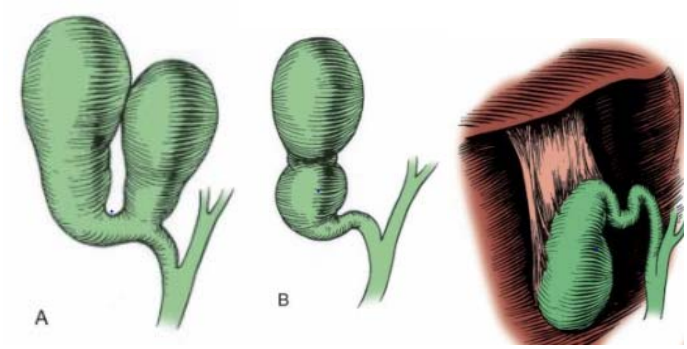


Fig 8 : Deformities Of Gall Bladder

a) Bilobed b)hour glass deformity c)floating gall bladder

SURGICAL PHYSIOLOGY OF GALLBLADDER[16]

Liver secretes bile at the rate of 40ml/hr.

Composition of bile are

- 1-2% bile salts
- 1% bile pigments, cholesterol and fatty acids
- 97% water

Secretory pressure of liver is approximately 375 mm of Hg

Contraction of Gallbladder:

1. Tonic contraction which lasts for 5- 30 minutes and elevates intravesical pressure to 300 mm H_2O
2. Rhythmic contractions – which lasts for 2-6 minutes, pressure does not exceed 30 mm H_2O

The maximal expulsive pressure of gallbladder is less than secretory pressure of liver and therefore filling and evacuation of the gallbladder is dependent upon reciprocal contraction and relaxation of the sphincter of oddi.

FUNCTIONS OF GALLBLADDER

1. **Reservoir of bile :**

During the intercibal period the sphincter of oddi is closed and bile is excreted by the liver and is directed to the gallbladder. After food, the resistance to flow through the sphincter of oddi is reduced, the gallbladder contracts and bile enters the duodenum. These motor responses are affected by the harmone cholecystokinin released by upper intestinal mucosa in response to food and fats.

Particular drugs:

1) Morphine, Pethidine increases the tone of the sphincter.
2) Anticholinergic drugs and glyceryltrinitrate decreases the tone of the sphincter

2. **Concentration of bile**:

By all absorption of H2O, NaCl and HCO3 by the mucous membrane of the gall bladder into blood stream and lesser extent into the lymphatics bile is concentrated in gall bladder upto 5-10 times. There will be corresponding increase

in the proportion of bile salts, bile pigments, cholesterol and calcium. The absorptive power of gall bladder is varies in disease. In disease, the gall bladder instead of absorbing fluid, pours out fluid rapidly. The absorption of bile salts is enhanced while more of calcium and cholesterol is seen in the lumen. This shows the relationship between the inflammation of gallbladder and formation of gall stones. Desquamated epithelium forms the nucleus of a stone, increase amounts of cholesterol provides raw material, increase absorption of bile salts result in the precipitation of cholesterol.

3. Secretion of mucus:

Gall bladder mucus at the rate of 20ml/24hr. This protects the mucosa from lytic action of bile and facilitates the bile through the cystic duct. This mucus secretion is increased in certain diseases like severe cholangitis or toxic hepatitis choledochal obstruction and hydrops of gall bladder (white bile). It does not contain bile salts or bile pigments but contains calcium carbonate. Gall bladder also secrets calcium in the presence of inflammation and cyst duct of obstruction resulting in calcification of gall bladder wall and calcium shells around the preformed stones.

4. Excretion of cholesterol:

Cholesterol ester is found in the connective tissue of gall bladder in cases of strawberry gall bladder or cholesterosis of gall bladder has led some pathologist to ascribe the function of cholesterol excretion. But it is not very definite through gall bladder adds cholesterol to the bile.

5. Change in the reaction of bile:

The bile excreted by liver is an alkaline in nature with pH 8.2. In gall bladder bile become acidic and its pH goes down to 7.4 to 7.0. This is due to selective absorption of various ions by the mucus membrane of gall bladder.

GALL STONES

CLASSIFICATION:

Gall stones are classified into two types:[17]

1. Pure gall stones:
    - **a.** Cholesterol gall stones 70%
    - **b.** Pigment gall stones 30%
    - **c.** Calcium carbonate gall stones
2. Mixed and Combined stones

Cholesterol stones: 10% gall stones are cholesterol stones. They are usually solitary with smooth surface, oval or round in shape, pale yellow in colour. They are thought to be formed in aseptic static bile and commonly found in Hartman's pouch. On section they shows radiating lines crossing the circular strata. In combined gall stone, the stone starts as pure cholesterol stones but ultimately receives mixed covering of pigment and cholesterol.

Fig 9: Cholesterol Stone

Pigment stones: May be pure or contain Calcium bilirubinate. They constitute of 80% of all gall stones. They are dark or black brown in colour, found exclusively in the gall bladder associated with excessive haemolysis like hereditary

spherocytosis, sickle cell disease, thalassemia etc. Excessive breakdown of Hb resulting in increase bilirubin which are excreted in bile and forms pigment stones in the gall bladder. Stones are usually appears as small soft fatty like masses.

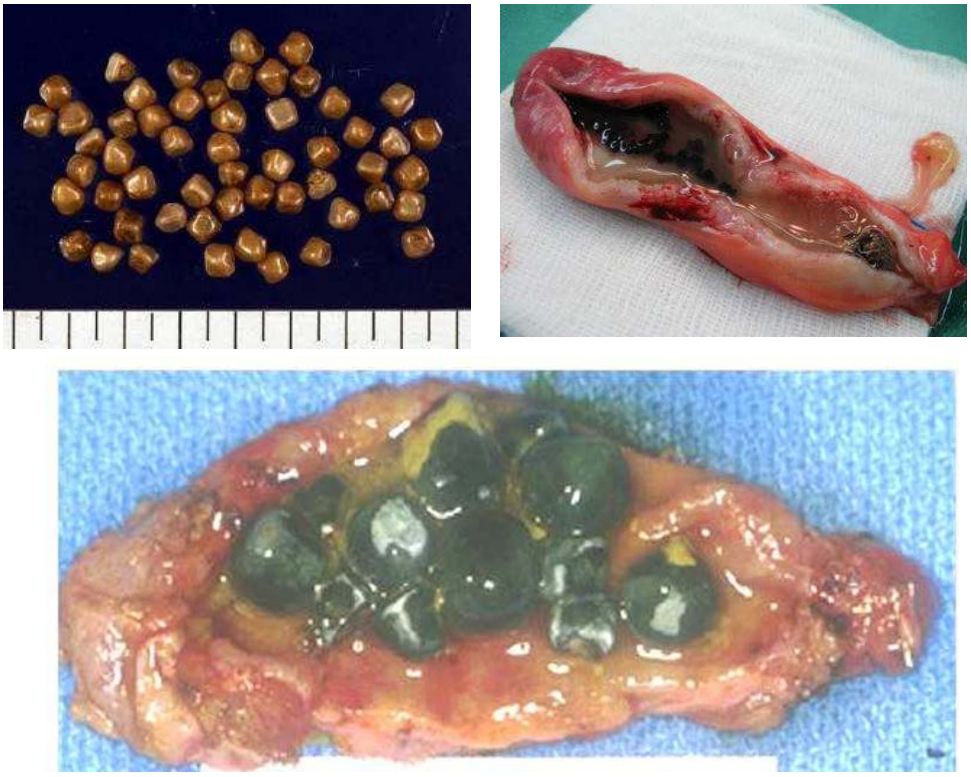


Fig 10: Pigmented Stones

Calcium bilirubinate stones are brown to orange in colour and soft in consistency. These stones are more often seen in bile ducts. These stones are often casued by infection (E.Coli and parasites)

Calcium carbonate stones - rarest type of stone they are grayish white in colour with smooth surface or articulated surface. Increase alkalinity of the bile favours this stone formation.

Mixed or Combined stones:

Mixed stones have varying proportion of all three of the stone forming constituents of the bile, eg- Cholesterol, bile pigment and calcium. They constitute about 10% of gall stones.

Combined stones are those in which central core of external layers are pure and the reminder of the stone is mixture of constituents. Combined stones may be solitary but mixed gall stones are invariably multiple with faceted surface. Stones may vary in size few cm in diameter. Colour of the stone depends on constituents of stones.

Pale yellow - Cholesterol

Black - Calcium bilirubinate

Grayish white - Calcium carbonate

On section of laminated central nucleus may contain epithelial debris and bacteria. This suggests inflammatory origin of stones. Mixed stones are frequently associated with cholecystitis. In about ½ the cases bacteria can be cultured from these gall bladder bile. Chemical inflammatory changes prepare the soil for bacterial invasion.

Fig 11: Mixed gall stones

EPIDIMENIOLOGY OF GALL STONES:[18]

It proves information about the prevalence and incidence of the disease

a. **True incidence**: 5 year incidence in women aged 30, 40 , 50, and 60 are (4%), (3.6%), (3%) and (3.7%) years and the same incidence rate in men were 0.3%, 2.9%, 2.5% and 3.3% at the same age. This shows that the incidence is more in women.

b. **Prevalence and incidence**: Gall stones are two times more common in women than in men. The incidence of gall stones in general population is 10%. Prevalence of gall stones in women between ages 20 to 55 varies 5%-20% and after 50 years 25%-30%. The prevalence in men is approximately half of that women.

c. **Ethnic predisposition**: Certain genetic factors play a key role in the pathogenesis of gall stone disease. Several genes that are associated with gall stone formation and resistance are identified in mice. The importance of these genes in human gall stone formation has not been established. Pima Indians in southern Arizona are an example of an extremely high risk population in which 70% of women less than 25 years are affected by the disease. Population at the lowest risk are sub Saharan Africans an Asians.

d. **Risk factors**: Gall stone disease is multifactorial in origin and occur sporadically. Specific risk factors predisposing gall stones have been identified.

Factors associated with gall stone formation:

Impaired gall bladder	Supersaturated bile
-Function	-Age
-Emptying	-Sex
-Absorption	-Genetics
-Excretion	-Obesity
	-Diet
Cholesterol nucleating factors	Enterohepatic circulation of bile acid
-Mucus	- Deoxycholate
-Glycoprotein	- Bowel transit time
-Infection	- Feacal flora
	- Ileal resection
	- Cholestyramine

1. **Age and gender:**

Gall stone disease increases with age as cholesterol secretion into bile incrases with age and bile acid formation decrease with age. Hence bile become more lithogenic with increasing age.

Most studied report that incidence and prevalence of gall stones is three to four fold higher in women than in men. But after age of 50 years the incidence may become equal in male and females. This may be due to increase oestrogen in young women lead to increased secretion of cholesterol into bile.

2. Pathophysiology of gall stone formation with aging:

Changes in bile composition with aging accounts for an increase risk of cholesterol gall stone formation. Biliary cholesterol saturation index (CSI) rises with age in both men and women.[19] This may be due to increase in hepatic cholesterol secretion, but bile salt and phospholipids secretion remains stable. An inverse relation was seen between the age and hepatic bile salt synthesis and activity of enzyme a-hydroxylase (rate limiting enzyme for bile salt synthesis).[20,21] Factors that change with the age like change in contraction of gall bladder, ability to concentrate bile are also incriminated in gall stone formation including pigment or mixed stones.

3. Obesity, weight loss and total parenteral nutrition:

Obesity is a well known risk factor for cholelithasis. Gall stone formation is directly related to body mass index (BMI=kg/m^2). Highest BMI (45kg/m^2) has got seven fold increased risk of gall stone formation as compare to non obese persons. This obesity related increase in gall stone formation is more in women than in men.[22]

Rapid weight loss is recognized risk factor for gall stone formation. Gall stone develop in approximately 25% of obese persons on restrict diet intake and in upto 50% of patients who undergone gastric by pass surgery. Gall bladder sludge or gall stones formation occur within 6 months of surgery. Around 40% of these patients experience the symptoms of gall stones.[22]

The physical alterations that lead to gall stone formation as a result of rapid weight loss are multiple

(i) Hepatic cholesterol secretion increase during caloric restriction

(ii) Increase secretion of mucin which is potent simulation of cholesterol crystal formation

(iii) Decrease gall bladder motality leading to biliary sludge formation

Gall stone formation can be prevented by administration of ursodeoxycholicacid in this patients. It is also found that there is decrease in gall stone formation in obese persons who are taking low caloric diet.[23]

TPN is associated with the development of acalculous cholecysttis, cholelithasis and cholecystitis. In 45% adults and 43% children gall stone develop after 3-4 months of TPN. Gall bladder sludge seen in TPN as early as after 3 weeks due to hypomatality with bile stasis and due to failure of sphincter of oddi to relax.[23]

4. Pathophysiology of gall stone formation in obese persons:

In obese persons hepatic cholesterol synthesis is increased and cholesterol saturation index (CSI) more. Gall bladder bile is supersaturated with cholesterol. Secretion of bile salts and phospholipids is either normal or increased. Gall bladder contractility may be decreased in the obese persons. So gall bladder stasis with supersaturated bile lead to gall stone formation.[24]

5. **Pregnancy and parity:**

Due to increase eostrogen level bile became more lithogenic due to increase in cholesterol secretion and supersaturation of bile. Gall bladder volume will be doubled and stasis develops with formation of biliary sludge. Higher progesterone levels also impairs gall bladder motility.

Both biliary sludge and stones are silent in nature but it may become symptomatic. After delivery in 60-70% pregnant woman biliary sludge disappears and gall stones disappears in 20-30%.[22]

6. **Drugs**:

Drugs with increases the gall stone formation are oestrogens, oral contraceptives, clofibrate octreotide, cefriaxone (third generation cephalosporin).

Oestrogen: The observations that gall stones are seen more in reproductive age group lead to initial hypothesis that oestrogen may promote gall stone formation.

Exogenous estrogen increase biliary cholesterol secretion by 40% causing cholesterol supersaturation of bile. Estrogen therapy also decrease plasma LDL and increase plasma HDL. There is increase LDL receptor expression by liver in estrogen therapy results in increased uptake of LDL by liver and increase secretion of cholesterol into bile.[24]

Clofibrate: Induces Cholesterol supersaturation in bile and decrease bile acid concentration by reducing the activity of enzyme a hydroxylase, the rate limiting enzyme in the pathway of bile acid synthesis. HMG Co-a reductase inhibitors reduce the biliary cholesterol saturation index but their role in prevention of or therapy of gall stone disease has not been clearly established. Octreotide, a

somatostatin analogue increase the gall stone formation. Decreased gall bladder motility and bile stasis are associated with octreotide treatment and leads to gall stone formation.

Ceftriaxone is generally excreted in the urine but utpo 40% secreted unmetabolised in the bile and reaches 100-200 times the concentration is serum. Once it exceeds the saturation level it combines with calcium and form insoluble salt. In 43% children who receive ceftriaxone in high doses (20-100 mg/kg/day) biliary symptoms are reported.[24]

7. **Diet**:

Hypertriglyceridemia is associated with an increase incidence of gall stone formation. High serum cholesterol does not seems to be a risk factor for gall stone formation. HDL levels are inversely correlated with development of gall stones.

Hence obese persons with hypertriglyceridemia with low HDL levels are at greatest risk for development of gall stones. Ingestion of refined sugars and physical activity are positively associated with the presence of gall stones in some studies. No association between alcohol, tobacco or caffeine ingestion and gall stone formation has been found.

8. **Systemic diseases**:

Gall stone formation is common in diabetic persons and its complications are also more. Insulin resistant diabetes mellitus is associated with hypertriglyceridemia, obesity, hypomotality of gall bladder leading to biliary sludge formation which intum may lead to gallstone formation.

The prevalence of gall stones in persons who had spinal cord injury is about

31% and biliary complications occur in 2.2%. The mechanism responsible for the association between spinal cord injuries and gall stone formation is not known. Gall bladder relaxation is impaired in these patients. Hence biliary stasis is likely the cause of gall stone formation.

9. Cirrhosis of liver:

Gall stone formation is 2-3 times greater in cirrhotic patients than a non cirrhotic population at all ages. In advanced cirrhosis there is marked reduction in bile salt secretion. It is stated that decrease in bile salt is matched by diminished biliary lecithin and cholesterol and bile is not lithogenic. Gall stone in cirrhosis and other chronic liver disease is usually due to chronic haemolysis and majority of the stones are pigment type. Jaundice in cirrhosis is more likely to be due to hepatic decompensaton than a stone in the CBD.

10. Ileal disease of resection:

In crohn’s disease with extensive involvement of ileum and major resection of ileum lead to malabsorption of bile salts. This inturn leads to increased cholesterol and supersaturated bile. Therefore gall stone formation is more. Gall stone are usually cholesterol type.

11. Gastric surgery:

Gastric bypass surgery for peptic ulcers and for gross obesity is complicated with increase prevalence in gall stone formation. Truncal vagotomy will adversely affect gall bladder emptying or bile lipid composition.

12. Haemolytic anaemia:

Patients with haemolytic anaemia and hereditary spherocytosis is associated with increased incidence of pigment gall stone formation due to haemolysis.

Prevalence rate:

- In hereditary spherocytosis is about 43.66%
- In sickle cell anaemia 37%
- Thalassaemia 10%

Saudiarabs with sickle cell anaemia have milder haemolysis due to increase alkali resistant Hb and has get low rate of gall stone formation.

13. Other conditions:

Children with cystic fibrosis have increased incidence of gall stones. Association with peptic ulcer and hyperparathyroidism-a firm evidence is not available.

PATHOGENESIS OF FORMATION OF GALL STONES

Pathogenesis of gall stone is multifactorial. There are significant difference in etiology of cholesterol and pigment gall stone. This understanding of this factor is important to prevent the disease and for treatment modalities. Gall stones are concretions and aggregations that are formed as a result of imbalance between bile acids and cholesterol in the ratio 1:10.[21,22]

CHOLESTEROL GALL STONE:

Cholesterol gall stone formation includes three stages-

1. Cholesterol saturation
2. Nucleation
3. Stone growth

Cholesterol is the major component of gall stones. It is held in solution in bile in the form of mixed micells comprising cholesterol, phospholipids and bile salts. Cholesterol calculi form as a result of dynamic interaction between the liver and gall bladder. Series of alteration in gall bladder function that promote nucleation and stone growth. Cholesterol gall stone formation involves. Super saturation of bile with cholesterol as a result of enhanced hepatic cholesterol synthesis, increase hepatic receptor uptake of apoliopoprotein, decrease hepatic catabolism of cholesterol, decrease diversion of cholesterol to cholesterol ester stores.[22]

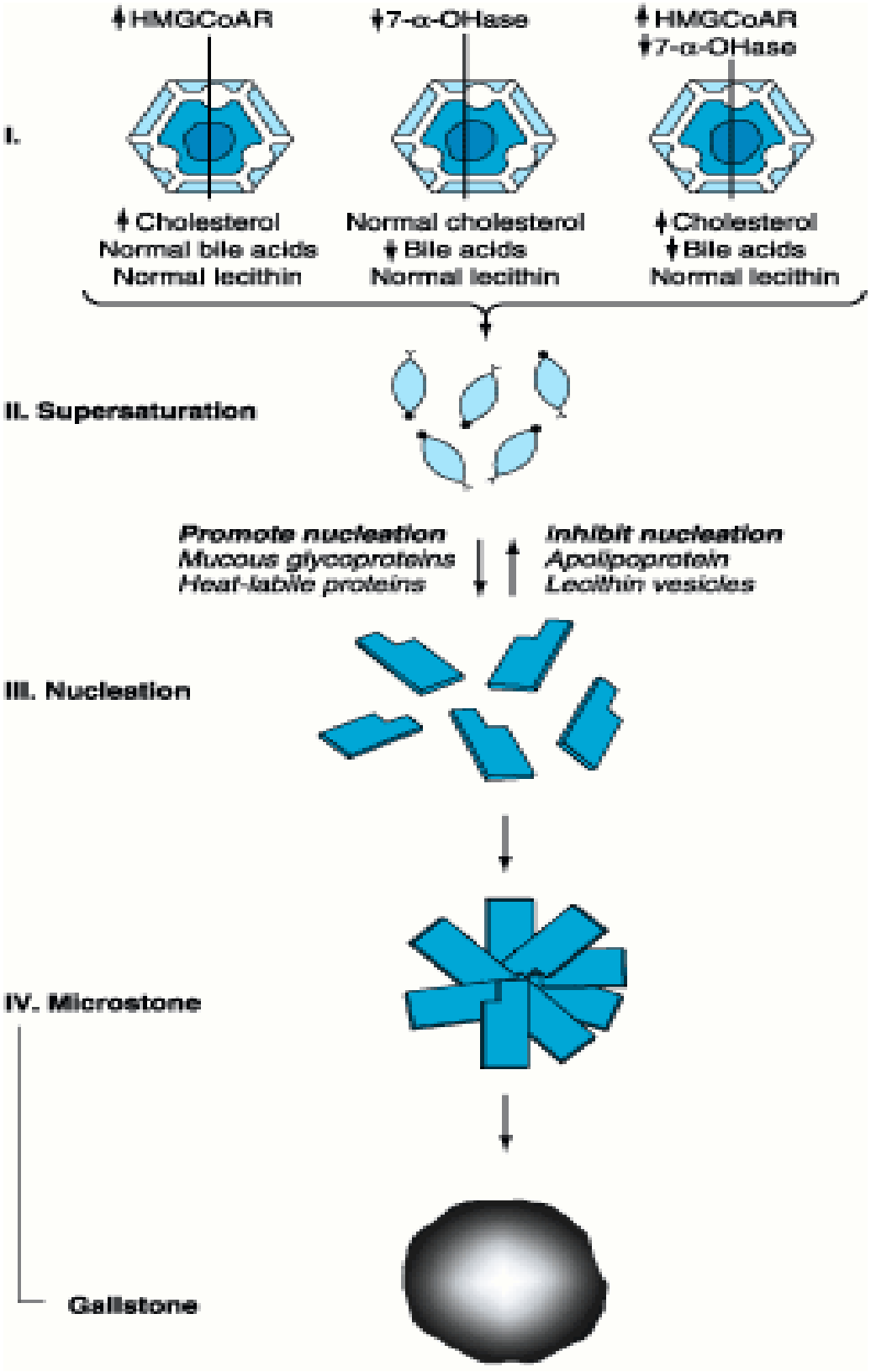


Figure 12-scheme of formation of gall stones

Other factors are:

- Incompetent transfer of cholesterol from the biliary vesicles to the bile salt-micells.
- Formation of high cholesterol containing abnormal vesicles
- Aggregation and fusion of unstable vesicles
- Cholesterol crystallization
- Biliary sludge formation
- Stone growth

GALL BLADDER FACTORS:

Gall bladder contributes in gall stone formation by a complex interaction of muscular and mucosal events.

a) Stasis:

Many patients with gall stones have gall bladder that empty more slowly, incompletely. This muscle abnormality precedes gall stone formation and persists after the gall stone have been removed by dissolution therapy. This stasis is a feature of both cholesterol and pigment stones. Other factors are like sequestration of bile acids within the gall bladder reducing the amount of bile salts available for cholesterol solubalisation, alteration in the secretory or absorptive function of gall bladder leading to biliary stasis.

b) Phospholipids in bile:

Studies indicate that gall stone formation is accompanied by an increase in arachidonic acid containing phospholipids. Increased hydrolysis of arachidonyl

lecithin provides the substrate for formation of prostanoids in the ball bladder wall. This activation of the prostanoid synthetic cascade is accompanied by reduced gall bladder motality and increase in mucin production by the gall bladder mucosa.

c) Bile mucus glycoproteins:

The excessive production of glycoproteins by gall bladder mucosa precedes stone formation. Mucin gel interferes with gall bladder contractility and emptying and acts as a nucleating matrix for cholesterol crystals to form cholesterol phospholipids vesicles.

d) Calcium:

Role of calcium is indicated by the presence of calcium salts in majority in gall stone, Preliminary results suggest the gall bladder bile from patients with cholesterol gall stones contain high levels of calcium. Exact mechanism by which biliary calcium increases the formation gall stones remains unknown but possible explanation includes enhanced absorption of H_2O and solutes by the gall bladder and increase gall bladder secretion of calcium, or decrease absorption of calcium. Crystalline structures of calcium carbonate and cholesterol monohydrate crystals provide frame work for gall stone formation. In addition to the structural role, data suggests the calcium promotes fusion of vesicles and evaluates cholesterol crystal growth.

STAGES OF GALL STONE FORMATION:

Cholesterol saturation:

Cholesterol is insoluble in bile. Cholesterol is held in solution by formation of mixed bile acid-lecithin-cholesterol micelles. Bile acids are emphipathic compounds with one end being hydrophilic and polar and other end being hydrophobic and nonpolar. These ionized molecules form micells in dilute solutions with hydrophobic end inwards and hydrophilic end outwards. Incorporation of lecithin into the micelle-allows H_2O to penetrate the structure causing swelling. This process increase the ability of the micelle to transport greater amount of cholesterol. Recent information indicates that no more than 30% of cholesterol is transported in micells. The relative amounts of cholesterol transported by vesicles and micells is related to the degree of bile saturation and crystal precipitation and stone formation.

Cholesterol supersaturation can occur secondary to secretion of hepatic bile with increased amounts of cholesterol or increased amounts of bile acids or lecithin.[21,22]

Sequence of events in cholesterol stone syntheses includes-

Nucleation- Aggregation or cholesterol crystals with in a supersaturated bile solution. Cholesterol monohydrate crystals form and agglomerate to become macroscopic stones. Mucin is pronuleating factor and act as a matrix on which crystals can conglomerate and clusterise.

Stone growth: It is natural consequence of cholesterol precipitation and conglomeration.

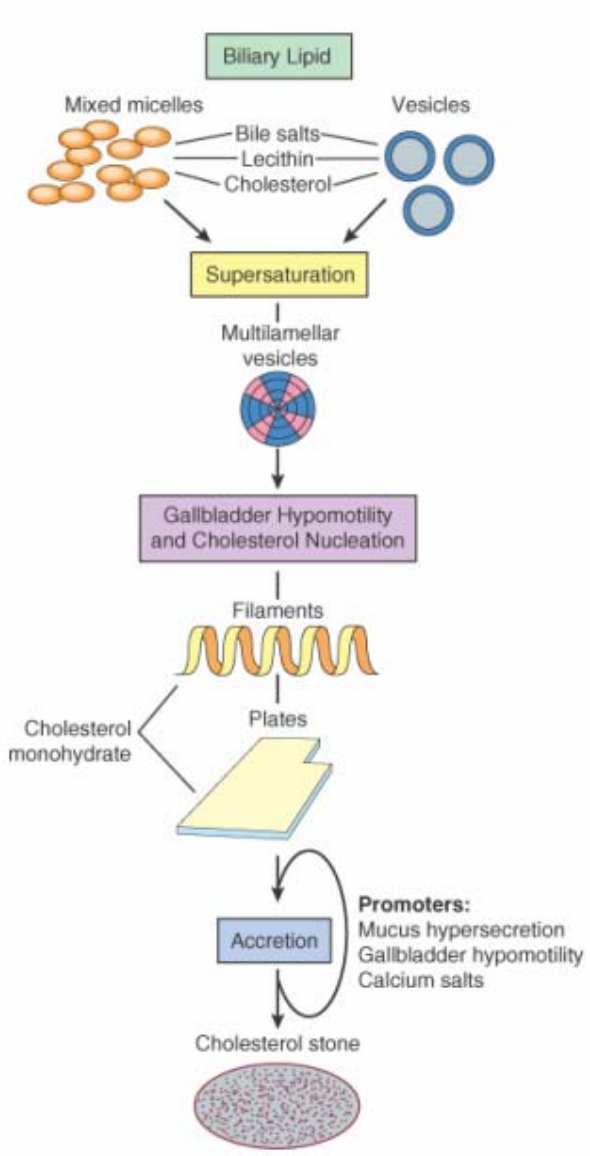


Fig 13 : Pathogenesis Of Gall Stones

PIGMENT STONES:

Pigment stones are black or brown in colour. They varies in shape and amorphous or crystalline on cross section.

80% of pigmented stones are black pigment stones that contain calcium hydrogen bilirubinate, calcium phosphate and calcium carbonate in a mucin glycoprotein matrix. Because of the polymerization of bilirubin these stone are

insoluble in all solvents. The conditions that causes black pigment stones are chronic liver disease, chronic haemolysis, TPN, Crohn's disease. Black pigment stones are formed in the gallbladder in sterile environment.[25,26]

BROWN PIGMENT STONES:

The pathogenesis of brown pigment stones is the enzymatic hydrolysis of biliary lipids by bacterial enzymes that produce bile supersaturated with the Ca, salts of unconjugated bilirubin, saturated long chain fatty acids and deconjugated bile acids.

Brown pigment stones constitute 20% pigment stones and develop from clinical conditions that produce an infected obstruction of the common bile duct including stones and structures.[27,28.29]

EPITAXY:

This is the phenomenon of the growth of one compound in one or more particular orientation on the substrate of another with near geometrical fit between respective networks which are in contact. Studies have shown that expitaxial role plays a significant part in almost all cases.

COMPOSITION OF DIFFERENT TYPE OF GALL STONES:

Chemical composition pathogenesis morphology:

Cholesterol stones (10%) - Super saturation of bile

- Bile stasis
- Infection (rare)

Large, smooth solitary, yellowish in colour, upto 4cm in diameter radiolucent[22]

Pigment stones (80%) - Haemolitic disorders (H.anaemia, infections, H.Sperocytosis, Sickle cell anaemia)

Multiple, jet black shiny, jack stones 0.5-1cm in diameter uniform in size and friable[24,27]

a) Calcium bilirubinate

b) Cholesterol with calcium bilirubinate

Mixed stones (5-10%) - Combination of bile constituents

- Bile stasis
- Infection

Multiple, hard faceted or irregular mulberry shape colour-yellow to green, black 10% radio-opaque.[28]

Cholesterol is the major constituents

Mixture of cholesterol, bile pigment, calcium salts interlaminated structure

Calcium carbonate (rare) - Excess calcium excretion in bile

Faceted, grayish in colour radio-opaque.[29,31]

CLINICAL MANIFESTATIONS

Majority of patients with gall stone are asymptomatic some will have atypical or non specific symptoms. Others will manifest with clinically significant symptoms of gall stones.[32]

Gall stones disease symptoms may be acute, chronic or totally absent. The differentiation between silent and symptomatic gall stones is important since this affects the management in individual case.

1) **Asymptomatic or silent stones**:

About 85-90% of patient with gall stones remains asymptomatic. The probability of a patient with silent gall stones developing biliary related pain is 1-2% per year and risk of developing complication like perforation and emphysema is even less (0.1% per year). The yearly risk of biliary pain decrease with time and gall stones in females are more likely to become symptomatic. In 90% of cases of carcinoma-gall bladder, gall stones are present.[32,33]

2) **Flatulant dyspepsia**:

This is the most common symptom and is described as feeling of fullness after food associated with belching and heart burn. This is more commonly qualitative dyspepsia-for fatty food. This symptom occurs irregularly and lacks the periodicity of peptic ulcer.

Other conditions like hiatus hernia, peptic ulcer and chronic pancreatitis should be ruled out before the diagnosis of cholelithasis is made.[32,33]

3) **Right hypochondria pain**:

In some it may be more discomfort and in some it may be excruciating pain. Pain radiates to interscapular region or right intra scapular area. Patient may complain of aching pain over the tip of the right shoulder. Due to distension of the gall bladder diffuse epigastric pain may be complained off. Localized pain may be due to inflammation of parietal peritoneum.

4) **Biliary colic:**

It is misnomer as the biliary symptoms are usually gradual in onset and

pain is localized to right upper quadrant (right hypochondrium) or epigastrium and not a colicky pain, colic occur when a stone is impacted in the cystic duct or at the Hartmann pouch. Episodes of biliary pain typically seen after meals and often associated with nausea and vomiting. Pain lasts for minute to hours an may radiate to the back or tip of the right scapula, pain resolves spontaneously or diminished with analgesics.[32,33]

5) Jaundice:

Cholestatic jaundice due to complete obstruction of common bile duct (CBD) and mild or incomplete obstruction.

6) Fever:

Occurs in 1/3 of patients may be raised during an attack of colic. Fever may seen without cholangitis, and may be associated with rigors.

PHYSICAL SIGNS:

1. Enlarged gall bladder may be palpable if there is mucocele or emphysema. Enlarged gall bladder is seen cholelithiasis when there is double impaction of stones i.e., one in cystic duct and other in CBD. Enlarged gall bladder felt as globular swelling projecting downwards just lateral to the right recuts muscle below the tip of ninth rib. It moves with respiration and side wards.[34]
2. Tenderness and rigidity in right hypochondria.
3. Murphy's sign (Moynihan's method)-Patient is asked to deep breath in and pressure is exerted with the fingers to palpate the fundus of the gall bladder. The gall bladder descends and hits the finger, the patient wince with pain and with a

catch in the breath. This examination can be done in sitting posture. This is present in acute cholecystitis.[34]

4. Bao's sign: Hyperasthesia between 9^{th} to 11^{th} rib posteriorly on the right side. It suggest acute cholecystitis.

COMPLICATION OF GALLSTONES[34]:

In the gall bladder

1. Cholecystitis with abscess, perforation, gangrene mucocele and emphysema
2. Chronic cholecystitis
3. Silent stones
4. Mirizzi syndrome
5. Porcelain gall bladder
6. Hydrops gall bladder
7. Carcinoma gallbladder

In the bile ducts:

1. Obstructive jaundice
2. Cholangitis
3. Acute pancreatitis

In the intestine:

1. Acute intestinal obstruction
2. Biliary enteric fistula
3. Gall stone ileus

INVESTIGATIONS[34]

To date there are no serum or other lab tests that are absolutely specific for the presence of gall stones. In acute cholecystitis due to gall stones patient will have leukocytosis. There may be mild elevation of transaminases and alkaline phosphatase. In CBD stones serum alkaline phosphatase will be elevated along with serum alkaline phosphatase will be elevated along with serum gamma glutamyl transpeptidase. Patient with fully developed biliary obstruction will show elevation of bilirubin, alkaline phosphatase and gamma glutamy transpeptidase.

Plan abdominal X-ray:

Only 10% gall stones are radio-opaque and can be visualized

Oral cholecystography:

For years this test was the mainstay and gold standard for the diagnosis of gall stone though now it has been replaced by USG except where function of the gall bladder has to be assessed. If cholecystography is more accurate than USG in terms of quantification of the number of stones and their sizes. The sensitivity for detection of radiolucent stones exceeds 90% but visualized of the ductal stone is obtained in only 20%.

Abdominal USG:

This is the preferred investigation for suspected cholelithiasis or cholecystitis

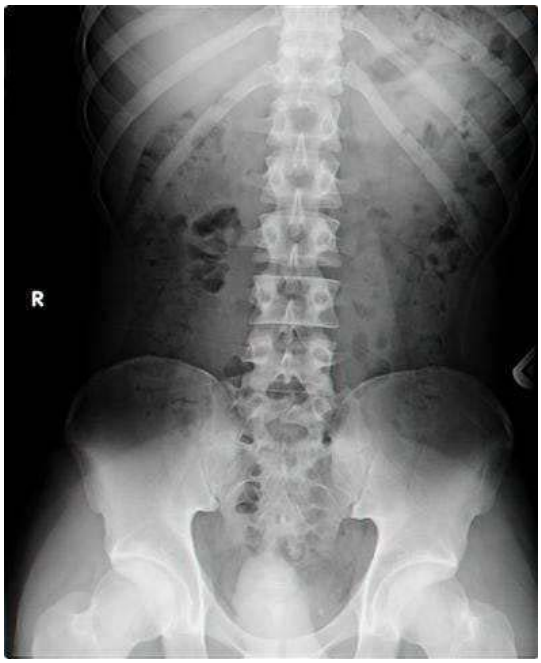


Fig 14: Plain X-ray of abdomen showing gall stones

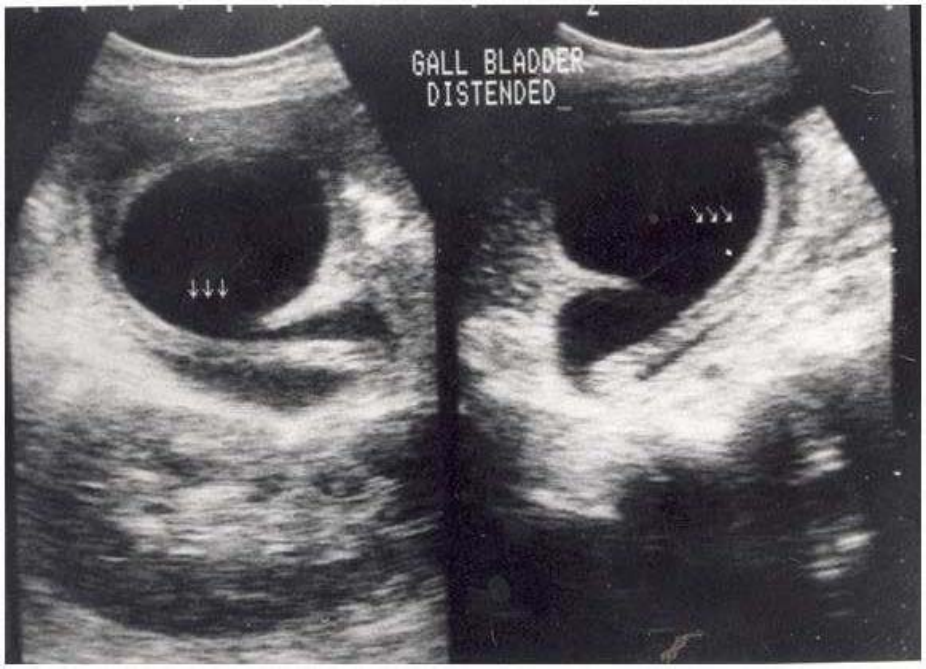


Fig 15 : Ultrasound abdomen showing gall stones in gall bladder

Sensitivity of USG to detect cholelithiasis is 95-99%. They are seen as echogenic foci with accosting shadowing and move with change in posture. This can detected the gall stones of about 1mm in size. The difficulty in USG is its limitation in measuring large gallstones and quantifying more than 3-5 gall stones in obese patients and in patients with ascites or gaseous distension.

CT scan:

This test provides more useful information than USG when there is extra hepatic obstruction avoiding to causes other than choledocholithiasis.

MRI:

Gall stone when sufficiently large may be visualized with MRI as low intensity filling defects with in the gall bladder bile. Majority of the stones however produce no signal and its role in the evaluation of patients with cholelithiasis remains nuclear.

ERCP:

ERCP is very accurate in the diagnosis of ductal calculi but is less accurate than USG and oral cholecystography in the diagnosis of gall bladder disease and gall stones.

Cholangiogram: For common bile duct stones

Operative biliary endoscopy:

A flexible endoscope can be passed down the cystic duct into the CBD enabling stone identification and removed under direct vision.

Oral cholecystography:

Visualization of gall bladder by giving radio-opaque dye. This test is useful in patients in whom USG is unsatisfactory.

Contrast media is given which is excreted by liver into the bile after its absorption in the intestine. (Contrast media-iodine containing preparation like telepaque or bioptin).

Uses:

Accuracy of gall stone detection 80-95%, number, size of stones, patency of the cystic duct, ability of the gall bladder wall to concentrate the bile and contraction of the gall bladder wall.

Contra indications:

1. Conjugated bilirubin level above 2mg/100ml
2. Failure of gall bladder filling with in 12 hour
3. Poor LFT
4. Previous cholecystecotmy
5. In the presence of renal disease

Technique:

1. Initial control X-ray is taken prior to cholecystography
2. A fatty meal is given to the patient in the previous day
3. 6 tablets of telepaque is are given orally at 9-00pm
4. The next day (after 12-16 hours) X-ray of abdomen is taken in erect and supine position. If gall bladder is visualized a fatty meal is given and one hour

later another X-ray of the abdomen is taken to seen the gall bladder contraction. If gall bladder is not visualized, double dose of the contrast is given.

5. Gall stones are seen as filling defects in the form of translucent areas in opaque shade of gall bladder.
6. If the gall bladder does not contract to1/3 of its size in response to fatty meal it indicates malfunction and often associated with stones.

Failure to visualize the gall bladder stones:

1. Failure of the patient to take telepaque tablets
2. Excessive diarrhea and vomiting due to contrast
3. Liver disease
4. Gastric surgeries and small intestinal anastomosis
5. Small intestine disease
6. Previous cholecystectomy
7. Blocked cystic duct
8. Poor preparation
9. Cholestasis

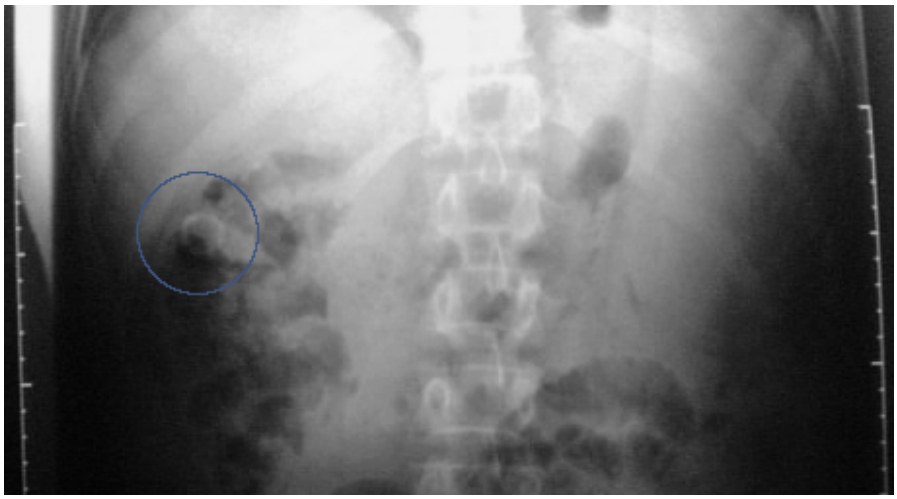


Fig 16: Oral cholecystogram showing gall stones in the gall bladder

CHOLANGIOGRAPHY:

When IV route is used the entire biliary tree can be visualized. Biligrafin is the contrast media used (20ml of 20% biligraffin). After doing a sensitivity test. It is used in whom oral cholecystography is unsuccessful. It is also used with oral cholecystography to visualize gall bladder and intra and extra hepatic biliary apparatus.

PTC:

It can be done in jaundiced patients. It is done by using chiba needle under fluoroscopy. Clotting time and platelet count should be done before PTC. Antibiotic cover is given before and after the procedure. Vitamin K infection is given if coagulation studies are abnormal.

In supine position patient is sedated and under lumbar aspiration (LA) needle inserted in 8^{th} intercostal space (ICS) in mid axillary line. Contrast media infected until it enters the biliary radicle to seen intra hepatic pathology and biliary calculus. Complications are haemorrhage and sepsis.

LFT:

Alterations is LFT may be due to long standing obstruction of CBD due to gall stone or due to repeated attacks of ascending cholangitis and hepatitis.

It includes:-

- Serum bilirubin
- Van Den Berg's reaction
- Serum alkaline phosphatase
- SGOT, SGPT
- Serum proteins
- Serum albumin
- PT (prothrombin time)

Investigations other associated pathological states:

- Urine routine
- RBS, RFT
- Serum cholesterol
- Upper GI endoscopy
- Serum amylase and urinary amylase for pancreatits

TREATMENT OF GALL STONE DISEASE

Treatment modalities

A) Non operative treatment (conservative)

B) Operative treatment

A) Non operative type[35]:

Medical: Oral dissolution-this is the old method of treatment since 1920. CDCA and UDCA are two agents used. UDCA is less toxic. These bile acids are ineffective in pigment stones.

Mechanism of action: It dissolves cholesterol gall stones by expansion of bile acid pools.

Chenodeoxycolic acid (CDCA): Specific inhibitor of HNG-COA reductase enzyme which is the rate limiting factor for cholesterol biosynthesis.

Ursodeoxycolic acid (UDCA): Facilitates conversion of hepatic cholesterol to bile acid and also reduces the cholesterol absorption in intestine.

Patient selection:

- Normal gall bladder function
- Stone size-<5mm, radiolucent
- Stone composition-cholesterol

Dose:

- UDCA dose is 8-10 mg/kg/day
- CDCA 12-15 mg/kg/day. Bed time dose gives maximum effect
- Most patients require life time maintenance therapy to prevent recurrence of disease

B) Indirect dissolution:

It involves percutaneous placement of pigtail catheter through liver and directly into gall bladder with rapid alternating infusion and aspiration of a specific agent that rapidly dissolves cholesterol. Agent used is MTBE (Methyl tert butyl ether)

which rapidly dissolves cholesterol in 4 to 5 hours. But this agent is potentially dangerous to bile ducts.

It is indicated in patients with high risk for surgery and symptomatic gall stones and in patients who refuses surgery. 10 ml is installed (MTBE) and exchanged every 45 minutes.

II) Shock wave lithostripsy[36]:

Criteria:

1. Three to four radiolucent gall stones with total diameter of<30mm in functional gall bladder in a symptomatic patients who is healthy.
2. The stones must be visible on USG and should be pin pointed by lithotripter. The shock wave path should avoid any lung or bone field.
3. Adjoining therapy with CDCA and UDCA may prevent recurrence of gall stone disease.

Procedure:

Anesthesia is not required. By various methods shock-waves are directed towards a focal point. Solid stones absorbs the energy and get fragmented.

Results: Only 20-25% patients will have satisfaction with this technique

Duration of treatment: 12 months

Complications:

- Biliary colic (30-60%)
- Skin pigmentation
- Haematuria
- Pancreatitis

Percutaneous cholecystolithotomy:

- It is developed from PCNL
- This technique removes gall bladder stones transperitonially
- Under GA, gall bladder is catheterized transperitoneally using fluoroscopy and USG screening
- Stones are removed after fragmentation with lithotripsy
- In non-functioning gall bladder also it can be used for remove gall stone
- 56-60% success rate
- Gall stone recurrence may occur
- Complications like bile leak, colon in bleeding, pancreatitis, common

III) Prostagladin synthatase inhibitors:

Parenteral indomethacin an diclofenac sodium is found to be effective in relieving biliary colic. It inhibits prostaglandin related inflammation of gall bladder wall and smooth muscle contraction also decrease the mucous production in gall bladder.

B) Surgical (Operative) treatment[37,38,39]:

a) Cholecystectomy

b) Open Cholecystectomy

c) Laparoscopic Cholecystectomy

In acute cholecystitis due to calculi conservative treatment is started. It includes analgesics, antibiotics, naso gastric tube aspiration, IV fluids, anti-emetics and low fat diet. This is followed by cholecystectomy.

a) Cholecystostomy[37]:

In some patients with a cholecystitis when the patient is aged and cannot tolerate major surgery due to renal and cardio-pulmonary disease cholecystectomy done.

Patient is preferred for surgery with antibiotics administration, high carbohydrate diet, adequate hydration to prevent renal failure and Vit K injection in jaundiced patient to prevent haemorrhage.

Procedure:

First the fundus is opened and inflammatory debris, gravel, stones are removed. Malecot's or Foley's catheter is passed into the gall bladder brought out through the small stab wound and connected to sterile bag. This tube is removed on 12^{th} or 15^{th} post operative day.

b) Cholecystectomy:

Indication:

- Calcium cholecystitis with or without symptoms
- Acute or chronic acalculus cholecystitis
- Trauma to the gall bladder
- Biliary peritonitis
- Carcinoma gall bladder

Position of the patient: Patient should lie in supine position with operating table slightly tilted to right

Incisions: Upper mid line, right paramedian or right sub costal (Kocher's) incision are used.

Adhesions between gall bladder and adjacent viscera are freed. If the gall bladder is tense is aspirated. The most important step is to pack the operative field with three gauze packs.

i) Placed over the hepatic flexure of colon

ii) Placed over the 1st part of duodenum and stomach

iii) Placed over the under surface of the right lobe of liver medial to gall bladder and held in place by means of retractor

Cholecystectomy done by two methods[38]:

1) Fundus first method
2) Duct first method (retrograde)

Fundus first method:

1. Gall bladder is emptied by trocar and cannula. Fundus held with sponge holding forceps all adhesions are cleared from gall bladder bed. Cystic artery is isolated, separated and ligated close to the gall bladder, cystic duct is dissected and traced to its junction with CHD and ligated and divided.
2. Gall bladder with its contents are removed without applying any clamp or haemostat. Bleeding from the raw surface liver may encountered.

Duct first method:

1. Fundus of the gall bladder held with sponge holding forceps and drawn downwards and outwards
2. Hartman's pouch is held with another tissue forceps and is drown downwards and to the right so that the calots triangle with cystic artery is exposed.
3. Cystic artery is ligated close to the gall bladder wall and divided.

4. By ligating cystic artery, subsequent dissection can be carried out without any danger of haemorrhage. The junction of cystic duct and CHD and bile duct is recognized and clearly dissected out. Cholangiography is carried out and then cystic duct is ligated and divided. Peritoneum is incised at the gall bladder margin and cleavage plane between live and gall bladder mad using blunt dissection. Vessels in the gall bladder bed is controlled by diathermy coagulation. Drainage tube is put in the region of divided cystic duct and brought outside through separate stab incision.

c) Partial cholecystectomy:

The cystic duct and CBD are exposed and gall bladder opened near its fundus. Contents evacuated. The gall bladder wall is excised from the fundus to cystic duct leaving behind the part of the gall bladder in relation to portahepatis and liver. The cystic duct is ligated after cholangiography.

Complication of cholecystectomy:

1. Subphrenic abscess
2. Bile peritonitis
3. Biliary fistula
4. Biliary stricture
5. Pancreatitis
6. Hepatorenal syndrome
7. Post-cholecystectomy syndrome
8. Biliary dyskinesia

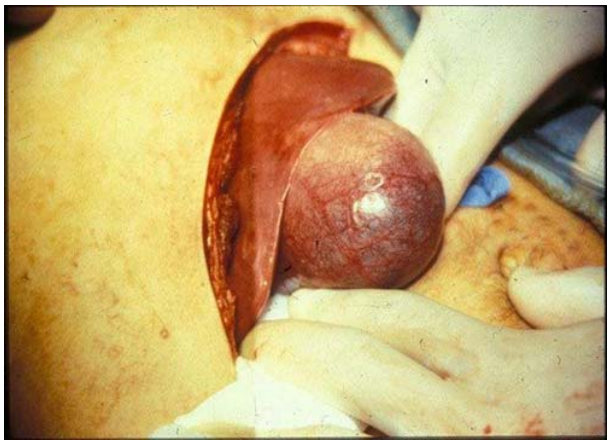


Fig 17 : Open cholecystectomy

Laparoscopic cholecystectomy[41]:

In 1987 first cholecystectomy was done in France and 1989 in US. This technique is gaining popularity in India as it is easy to perform, reduced hospital stay and economical one.

Indication:

- Cholelithiasis
- Chronic cholecystitis
- Non-functioning gall bladder

Contraindications:

- Acute cholecystitis
- Bleeding disorders
- Sepsis
- Portal hypertension
- Peritonitis

Equipments required:

1. High resolution camera
2. CO_2 insufflator
3. Light source
4. High resolution video monitor
5. Irrigation device
6. Electrocautery
7. Hand instruments-trocar, veress needle, scissors, dissectors, Maryland dissector, retractors, Grasper

Procedure:

The procedure is performed under general anesthesia or epidural anesthesia. Urinary bladder is emptied. Stomach is decompressed using nasogastric tube[42].

a) Operating Room Set-Up

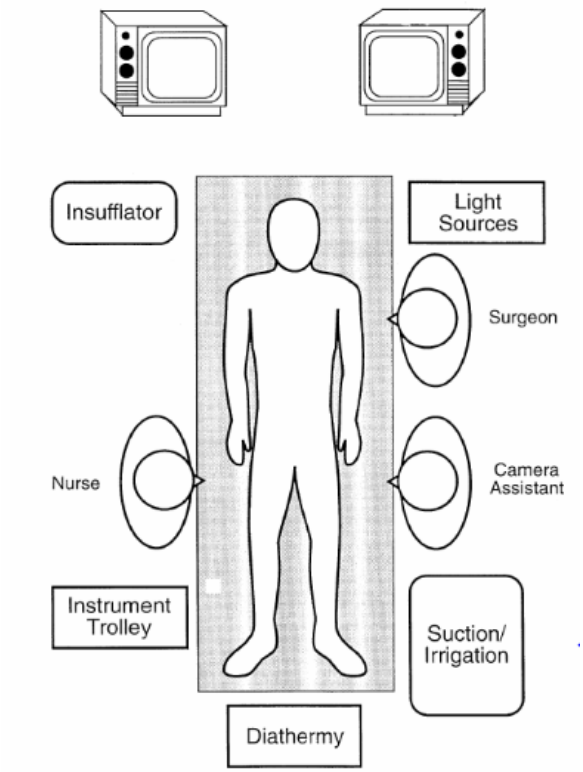


Fig 18 : Operating Room Set Up

b) Port Placement and Exposure

A 5- or 10-mm laparoscope is inserted into the abdomen through the umbilical port. The patient is then placed in a reverse Trendelenburg position of 30 degrees while rotating the table to the left by 15 degrees. This maneuver allows the colon and duodenum to fall away from the liver edge. The falciform ligament and both lobes of the liver are examined closely for abnormalities. The gallbladder can usually be seen protruding beyond the edge of the liver.

Two small accessory subcostal ports are then placed under direct vision. The first 5-mm trocar is placed along the right anterior axillary line between the twelfth rib and the iliac crest. A second 5-mm port is inserted in the right subcostal area in the midclavicular line. Grasping forceps are placed through these two ports to secure the gallbladder. The assistant manipulates the lateral grasping forceps, which are used to elevate the liver and to expose the fundus of the gallbladder.

The surgeon uses a dissecting forceps to raise a serosal "fold" of the most dependent portion of the fundus. The assistant's heavy grasping forceps are then locked onto this fold using either a spring or ratchet device. With these axillary grasping forceps, the fundus of the gallbladder is then pushed in a lateral and cephalad direction, rolling the entire right lobe of the liver cranially. This maneuver is complicated in patients with a fixed, cirrhotic liver or a heavy, friable liver because of fatty infiltration.

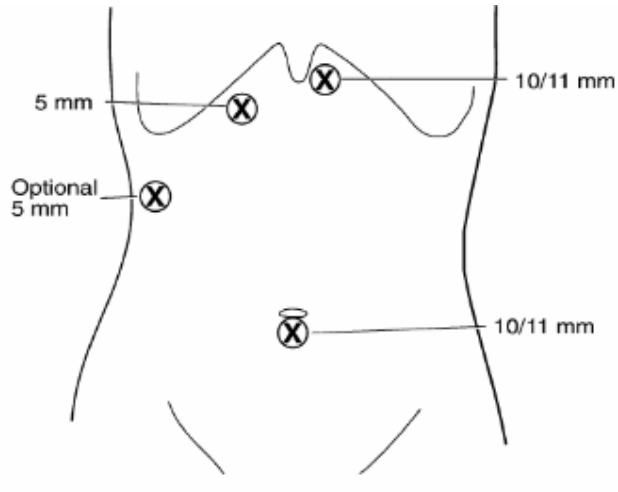


Figure 19- Port placement

c) Dissection

The infundibulum is grasped, placing traction on the gallbladder in a lateral direction to disalign the cystic duct and common bile duct. It is important to clearly identify the structures forming the sides of Calot's triangle, which include the cystic duct, cystic artery, and common hepatic duct.

After clearing the structures from the apex of the triangle, the junction between the infundibulum and the origin of the proximal cystic duct can be clearly identified. The strands of peritoneal, lymphatic and neurovascular tissue are stripped away from the cystic duct to clear a segment from the surrounding tissue. Curved dissecting forceps are helpful in creating a "window" around the posterior aspect of the cystic duct to skeletonize the duct itself

The cystic artery is separated from the surrounding tissue by similar blunt dissection at this time. If the cystic artery crosses anterior to the duct, the artery may require dissection and division prior to approaching the cystic duct. The neck of the gallbladder is thus dissected away from its liver bed, leaving only two structures entering the gallbladder—the cystic duct and artery. No structure should be divided

until the cystic duct and cystic artery are unequivocally identified. This is the "critical view" of safety essential to prevent bile duct injury during this procedure

Following clip ligation and division of the cystic duct, the cystic artery is dissected from the surrounding tissue for an adequate distance to permit placement of three clips

The ligated stumps of the cystic duct and the artery are then examined to ensure that there is no leakage of either bile or blood and that the clips are placed securely and compress the entire lumen of the structures without impinging on adjacent tissues

Separation of the gallbladder away from its hepatic bed is then initiated using an electrosurgical probe to coagulate small vessels and lymphatics.

The final attachments of the gallbladder are divided, and the liver edge is again examined for hemostasis the gallbladder is most easily removed at the umbilical port site where there are no muscle layers anterior to the fascial plane Occasionally, the fascial incision must be extended to extract larger stones or thick-walled gallbladders.

Advantages and disadvantages of laparoscopic surgery:

Advantage	**Disadvantages**
less pain	Lack of depth perception
Smaller incisions	View controlled by camera operator
Better cosmesis	More difficult to control haemorrhage
Shorter hospitalization	Decreased tactile discrimination
Earlier return to full activity	Potential co_2 insufflation complications
Decreased total costs	Adhesions/inflammation limit use and slight increase in bile duct injuries

Complications of laparoscopic surgery[44,45]

- Hemorrhage
- Bile duct injury
- Bile leak
- Retained stones
- Pancreatitis
- Wound infection
- Incisional hernia
- Trocar related - Abdominal wall bleeding, hematoma
  - Visceral injury
  - Vascular injury
- Pneumoperitoneum related - Co_2 embolism
  - Vaso –vagal reflex
  - Cardiac arrhythmias
  - Hypercarbic acidosis

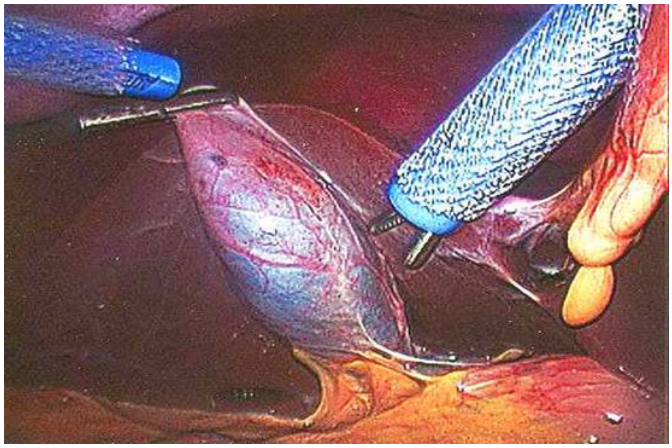


Fig 20: Laparoscopic cholecystectomy

HELICOBACTER PYLORI:

Helicobacter pylori (H. pylori) remains one of the most common worldwide human infections and is associated with a number of important upper gastrointestinal (GI) conditions including chronic gastritis, peptic ulcer disease, and gastric malignancy.[46]

The prevalence of H. pylori is closely tied to socioeconomic conditions and accordingly, this infection is more common in developing countries than in developed countries.[47]

MICROBIOLOGY:

H.pylori is a helix-shaped (classified as a curved rod, not spirochaete) Gram-negative bacterium about 3 micrometres long with a diameter of about 0.5 micrometres. It is microaerophilic; that is, it requires oxygen, but at lower concentration than is found in the atmosphere.[48]

H. pylori possesses five major outer membrane protein (OMP) families. The largest family includes known and putative adhesins. The other four families are porins, iron transporters, flagellum-associated proteins, and proteins of unknown function. Like other typical Gram-negative bacteria, the outer membrane of H. pylori consists of phospholipids and lipopolysaccharide.[49]

Fig 21 : Helicobacter Pylori

PATHOPHYSIOLOGY:

To avoid the acidic environment of the interior of the stomach (lumen), H. pylori uses its flagella to burrow into the mucus lining the stomach to reach the epithelial cells underneath, where there is a more neutral Ph. H. pylori is found in the mucus, on the inner surface of the epithelium, and occasionally inside the epithelial cells themselves. It adheres to the epithelial cells by producing adhesins, which bind to lipids and carbohydrates in the epithelial cell membrane. One such adhesion is BabA, which binds to the Lewis b antigen displayed on the surface of stomach epithelial cells.[50]

In addition to using chemotaxis to avoid areas of low pH, H. pylori also neutralizes the acid in its environment. It does this by producing large amounts of urease, which breaks down the urea present in the stomach to carbon dioxide and ammonia. The ammonia, which is basic, then neutralizes stomach acid.[51]

The inflammatory response caused by bacteria colonizing near the pyloric antrum induces G cells in the antrum to secrete the hormone gastrin, which travels through the bloodstream to parietal cells in the fundus. Gastrin stimulates the parietal cells to secrete more acid into the stomach lumen, and over time increases the number of parietal cells as well. The increased acid load damages the duodenum, which may eventually result in ulcers forming in the duodenum.[52,53]

When H. pylori colonizes other areas of the stomach, the inflammatory response can result in atrophy of the stomach lining and eventually ulcers in the stomach. This also may increase the risk of stomach cancer.[54]

INVESTIGATIONS[55-61]:

The methods used to diagnose the Helicobacter pylori infection are the microbiological, histopathological, immunological and methods based on demonstrating the activity of the bacterial enzyme urease. The methods can be grouped in two large categories:

1. Non invasive

a. Serology: Serological tests mostly based on ELISA or latex agglutination detects antibodies to H.Pylori or its products and are used to screen the patients with dyspepsia. IgM and IgG are commonly used. IgM is more specific and IgG is more sensitive, thus it can used as a good screening test. Serological tests are not confirmative.

b. Urea breath test: This test detects bacterial urease activity in the stomach by measuring the output of the carbon dioxide resulting from the splitting of urea into carbon dioxide and ammonia. A capsule urea labeled carbon -14 or -13 is fed to the patient and emission of the isotope in the carbon dioxide subsequently exhaled in the breath is measured. This test has excellent sensitivity and specificity, but carbon C-14 is radioactive, so not used in children. Carbon-13 is not radioactive but a mass spectrometer is needed for its assay.

c. Fecal antigen test: Stool antigen test that detects H.Pylori antigen in feces are available. This test is based on the use of monoclonal antibodies and is more accurate than polyclonal antibody test. This test has the potential to supplant serology for routine screening.

d. Polymerase chain reaction (PCR): DNA probes for the direct detection of H.pylori in gastric juice, feces, dental plaque and water supplies have been developed.

2. Invasive tests

a. **Biopsy urease test**: This is a simple and cheap test that can be performed at the bed side. The biopsy specimen is placed in to a small quantity of urea solution with an indicator that detects alkalinity resulting from the formation of the ammonia. Most infected patients (70%) give a positive result within 2 hours and 90% after 24 hours.

b. Histopathology and Microscopy: Histopathology provides permanent record of the nature and grading of the patients gastritis as well as detected H.Pylori. Organisms can be seen in sections stained with hematoxylin and eosin. But are more specific stains are available like, gram stain, geimsa stain.

c. **Culture**: Culture is no more sensitive than skilled microscopy of histological sections, but as several advantages as isolates can be tested for anti-microbial resistance and typed for epidemiological studies, Information about the virulence factors can inform clinical outcome. Selective agars and incubation condition similar to those used for campylobacters are used for primary isolation.Sensitive is increased if a non selective medium is used in parallel, high humidity is essential, plates are left un disturbed for 3 days and incubated for a week before being discarded as negative. H.Pylori forms discrete doomed colonies unlike the effuse colonies of C.jejuni and C.coli.

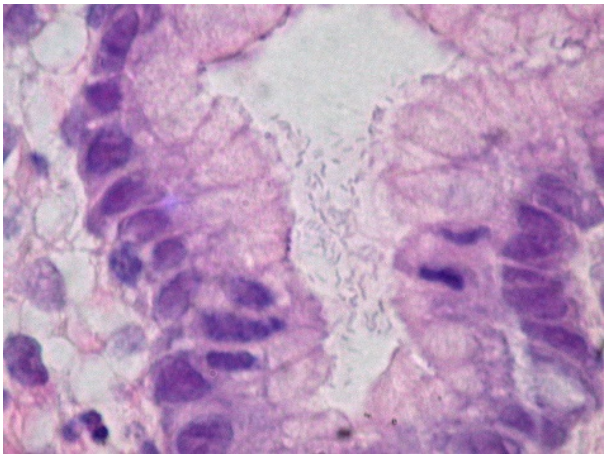


Fig 22 :Helicobacter in H and E method

TREATMENT REGIMENS:

THREE DRUG REGIMENS

Proton pump inhibitor (PPI) orally twice daily + Clarithromycin 500mg orally twice daily + Amoxicillin 1gm orally twice daily
Eradication 85-90%. Duration 10-14 days*

Bismuth subsalicylate 525mg four times daily +
Tetracycline 500mg four times daily
+ Metronidazole 500mg orally 3 to 4 times daily.
Duration=14 days.

Proton pump inhibitor orally twice daily +
Clarithromycin 500mg orally twice daily
+ Metronidazole 500mg orally twice daily.
Duration 10-14 days.

Ranitidine, bismuth citrate 400mg orally twice daily + Clarithromycin 500mg orally twice daily + Amoxicillin 1gm orally twice daily
Duration= 7-10 days

PPI orally twice daily + Amoxicillin 500mg orally
twice daily-orally three times daily
+ Metronidazole 500mg orally 2 or 3 times daily x 10-14days

Ranitidine, bismuth citrate 400mg orally twice daily + Clarithromycin 500mg orally twice daily + Metronidazole 500mg orally twice daily.
Duration= 7 days

TWO DRUG REGIMENS (FDA APPROVED)

PPI + either clarithromycin 500 mg orally three times daily OR Amox 1gm orally twice daily for 2 weeks then PPI for 2 more weeks (PPI = omeprazole 40mg QD or lansoprazole 30mg orally three times daily)

Ranitidine, bismuth citrate (RBC) 400 mg orally twice daily + clarithromycin 500 mg orally three times daily OR orally twice daily for 2 weeks then RBC for 2 more weeks.

FOUR DRUG REGIMEN.

Proton pump inhibitor orally twice daily + Bismuth subsalicylate 525mg four times daily + Tetracycline 500mg four times daily + Metronidazole 500mg orally 3 to 4 times daily.
Duration 2 weeks.

METHODOLOGY:

Sixty five patients presenting with clinical features of cholelithiasis and cholecystitis to department of surgery at R L JALAPPA HOSPITAL, TAMAKA, KOLAR during 21 months from November 2011 to august 2013 were included in the study.

INCLUSION CRITERIA

All cases who underwent surgery for Cholecystitis and cholelithiasis.

EXCLUSION CRITERIA

Patient who has been treated for helicobacter within 4 weeks

METHOD OF DATA COLLECTION:

Sixty five patients with cholelithiasis and cholecystitis were included in the study. A detailed history was elicited followed by general and systemic examination. Investigations as per the proforma made for the study were performed.

PATHOLOGICAL EXAMINATION TO DETECT H. PYLORI

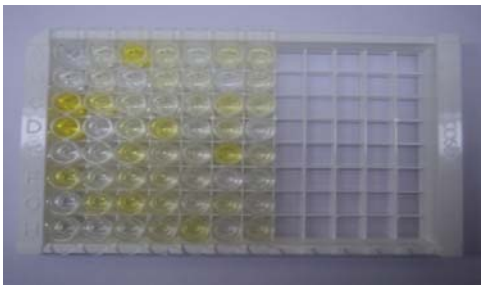
The Specimen of gall bladder after cholecystectomy was collected in sterile bottle with 10% formalin. The bottle was then sealed and sent for histopathological examination.

Gall bladder specimen was subjected for to haematoxylin and eosin, giemsa staining for mucosal study and Warthin-Starry silver staining to detect helicobacter.

SEROLOGICAL TESTING:

Serum IgM antibodies against helicobacter pylori were detected using the IgM ELISA commercial kit supplied by calbiotech inc, California. USA. For this purpose 3 ml of blood were collected prior to surgery before starting the patients on antibiotics.

Blood samples were allowed to clot at room temperature and centrifuged at 2500 rpm for 5 minutes in a REMI centrifuge after separating the clot from the wall of the test tube with a sterile loop. The sera were separated and stored frozen until they were tested as per the instructions of the manufacturer.



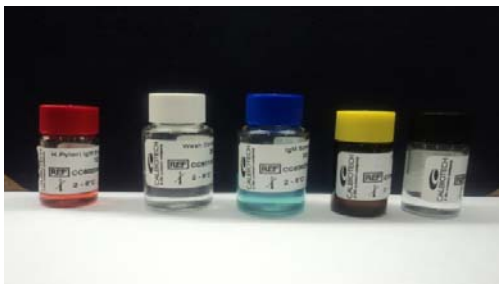


Fig 23 : Serological Methods

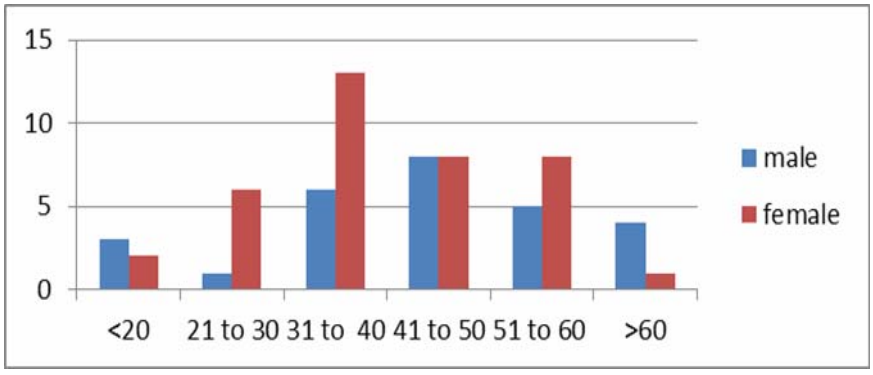
OBSERVATIONS AND RESULTS

Sixty five patients presenting with clinical features of cholelithiasis and cholecystitis to department of surgery at R L JALAPPA HOSPITAL, TAMAKA, KOLAR during 21 months from November 2011 to august 2013 were included in the study.

TABLE 1- AGE AND SEX DISTRIBUTION

AGE IN YEARS	MALE		FEMALE		TOTAL(N=65)
	NUMBER	%	NUMBER	%	TOTAL %
<20	3	11.1	2	5.2	7.6
21-30	1	3.73	6	15.7	10.7
31-40	6	22.2	13	34.2	29.2
41-50	8	29.6	8	21	24.6
51-60	5	18.5	8	21	20
>60	4	14.8	1	2.6	7.6
TOTAL	**27**	**100**	**38**	**100**	**100**
MEAN	**45.07**		**38.5**		**41.78**

Graph 1 – AGE DISTRIBUTION



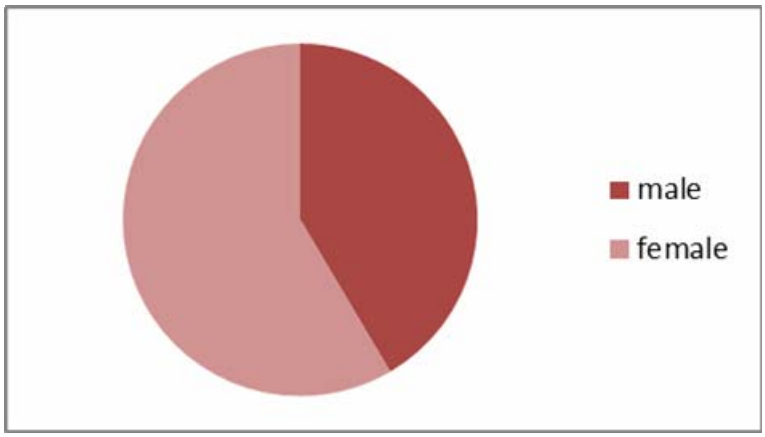
The youngest patient was 14 years old and the oldest patient was 77 years old. The mean age was 41.78. Bulk of the disease presented in the age group of 31-50 years.

TABLE 2- COMPARISON OF AGE DISTRIBUTION

AGE IN YEARS	OUR STUDY	Jafri Deeba et al. STUDY
< 20	5	1
21 – 30	7	26
31 – 40	19	22
41 – 50	16	19
>50	18	7
TOTAL	**65**	**75**
MEAN AGE	**45.07**	**42.32**

Our study was compared with the Jafri Deeba et al study, mean age group in our study was 45.07 years as opposed to 42.32 years in Jafri Deeba et al study. The majority of patients in our study was in the age group of 31-40 years where as in the Jafri Deeba et al study was 21-30 years old.

Graph 2 – SEX DISTRIBUTION



In this study 41.5% were males, and 58.5% were females, showing female preponderance in sex distribution of biliary calculi. Females outnumbered males in the ratio 1.4:1.

Table 3- COMPARISON OF SEX DISTRIBUTION

SEX	OUR STUDY	Arshad et al study
MALE	27	42
FEMALE	38	58
TOTAL	**65**	**100**

Sex distribution in our study showed female to male ratio 1.4 : 1, was comparable to Arshad et al study which had ratio of 1.38 : 1.

Graph 3- SYMPTOMS

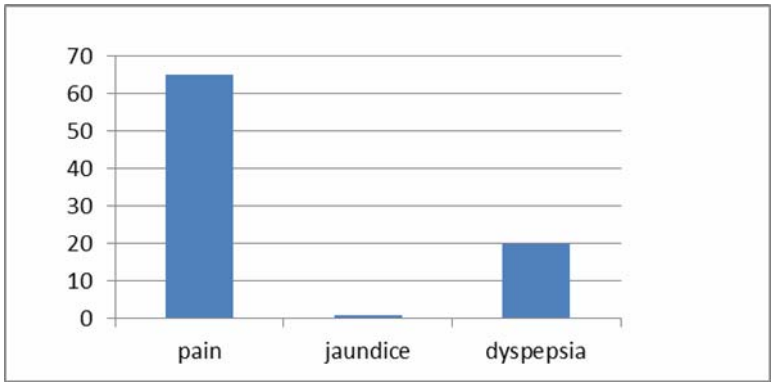
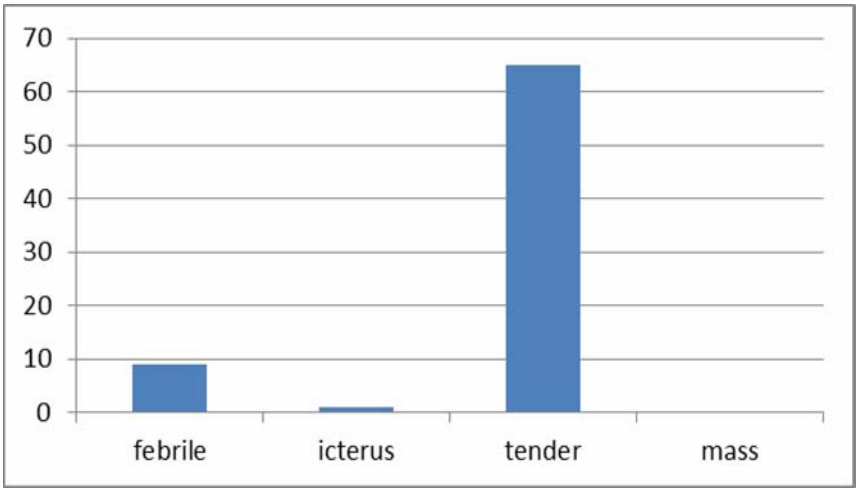


Table 4- CLINICAL PRESENTATION

SYMPTOMS			SIGNS			
PAIN	**JAUNDICE**	**DYSPEPSIA**	**FEBRILE**	**ICTERUS**	**TENDERNESS**	**MASS**
65	1	20	9	1	65	-

Graph 4- SIGNS



Pain was the most common presenting symptom whereas tenderness in the right hypochondrium was the most commonly elicited sign in these cases. All patients had pain in right hypochondrium, few were associated with pain in epigastrium as well.

Jaundice was present in one patient, however calculi in CBD was ruled out with imaging techniques.

20 patients presented with dyspepsia and 9 patients had fever, out of which 3 patients were associated with chills and rigors. These patients had septic focus in gall bladder or biliary tract.

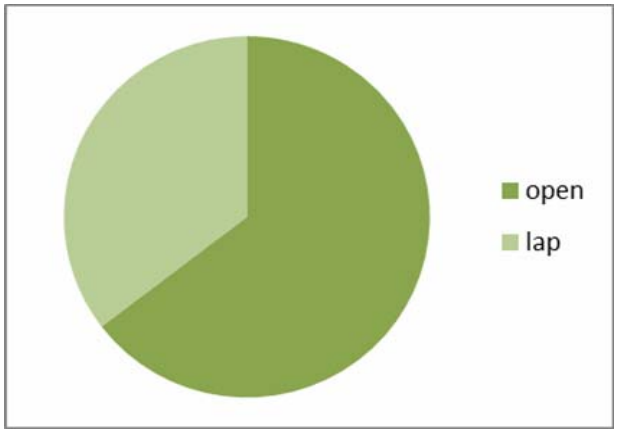
Table 5- COMPARISON OF SYMPTOM PRESENTATION

SYMPTOMS	OUR STUDY	Arshad et al STUDY
PAIN	100%	100%
JAUNDICE	1.5%	-
DYSPEPSIA	30.7%	68%

Pain was the commonest symptom of biliary calculi universally as evidenced by our study in comparison with study by Arshad et al present in 100% of cases. Pain was due to luminal obstruction from an impacted stone which is characteristically colicky or from inflammation which is burning type of pain.

Biliary dyspepsia was present in upto 30.7% of patients.

Graph 5- TYPE OF SURGERY

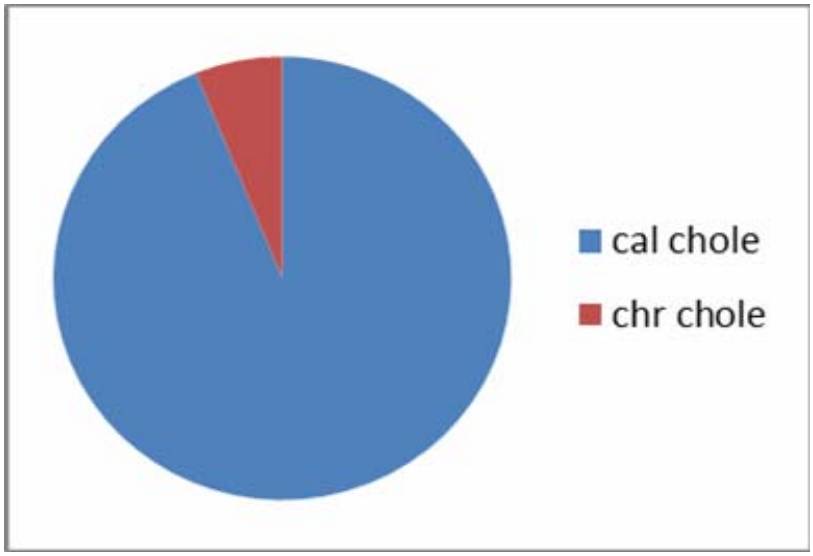


42 patients underwent open cholecystecyomy and 23 patients underwent laparoscopic cholecystectomy. Patients who underwent open cholecystectomy, kocher's right subcostal incision was used. patients taken for laparoscopic cholecystectomy were uncomplicated. In 2 cases laparoscopy was converted in to open cholecystectomy due to dense adhesions.

Table 6- HISTOPATHOLOGICAL FINDING

HISTOPATHOLOGY	NUMBER
CALCULUS CHOLECYSTITIS	61
CHRONIC CHOLECYSTITIS	4

Graph 6– HISTOPATHOLOGICAL FINDINGS

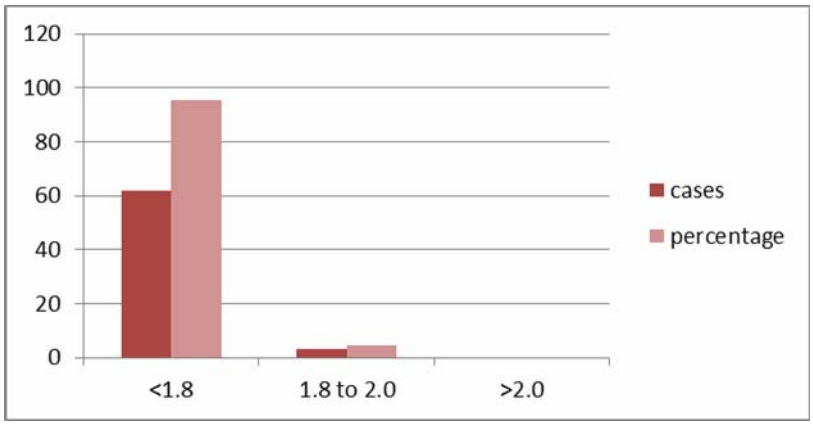


61 patients had calculus cholecystitis and 4 had chronic cholecystitis without calculi

ANTIBODY TITRES (SERUM IgM) (MEAN ABSORBENCE 450 NM)	GENDER		TOTAL
	MALE	FEMALE	
<1.8	25	37	62
1.8 - 2.0	2	1	3
>2.0	-	-	-
TOTAL	27	38	65

Table 7- SEROLOGICAL METHODS

Graph 7- SEROLOGICAL METHODS

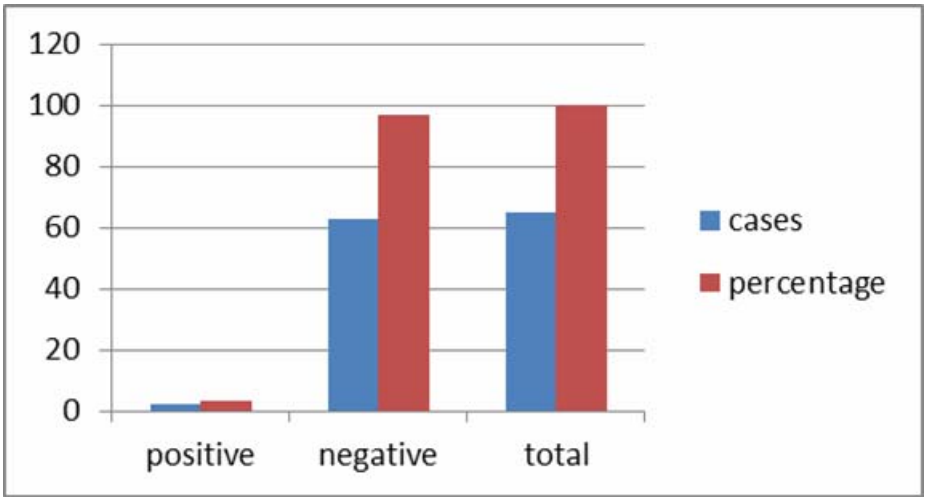


3(4.6%) patients were tested positive for helicobacter

Table 8 – STAINING METHODS

Cholelithiasis	Helicobacter pylori		Total
	Positive	Negative	
Male	2	25	27
Female	1	37	38
Total	3	62	65

Graph 8- STAINING METHODS

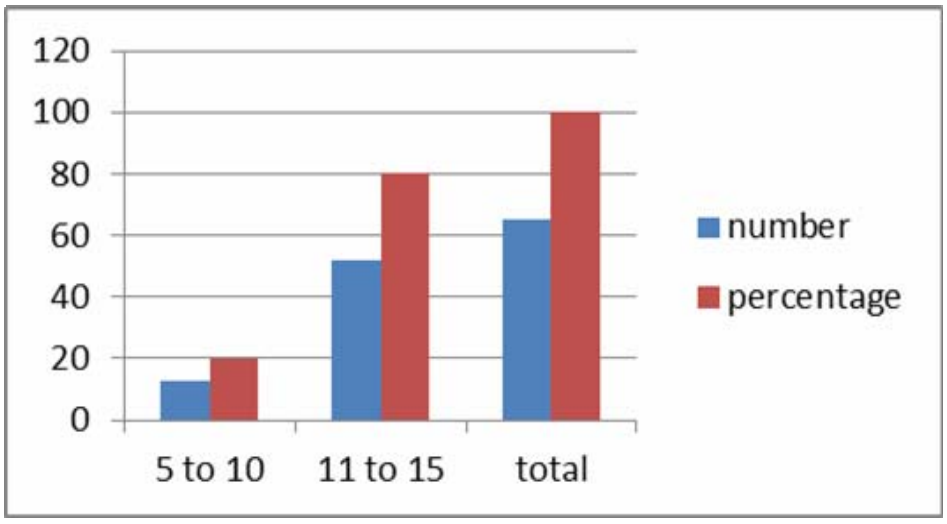


3(4.6%) cases tested positive for helicobacter from cholecystectomy specimen

Table 9 – DURATION OF HOSPITAL STAY

NUMBER OF DAYS IN HOSPITAL	NUMBER	PERCENTAGE(%)
5 – 10	13	20
11 – 15	52	80
TOTAL	**65**	**100**

Graph 9- DURATION OF HOSPITAL STAY



Most of the patients undergoing surgical intervention stayed in the wards for 1-2 weeks. Patients who underwent laparoscopic cholecystectomy had less than 10 days of hospital stay. Those who underwent open surgery had a little longer stay.

DISCUSSION

Detection of Helicobacter species in human bile has prompted a growing interest as to whether these organisms truly colonize the biliary tract of humans and cause hepatobiliary diseases. Bile-resistant Helicobacter species such as H. hepaticus, H. bilis and H. pullorum, have been discovered in man as well as animals. However, research in this area has been limited by the lack of gold standard in the diagnosis of these organisms in bile. A meta-analysis of some published work has shown strong association between Helicobacter and gall bladder disease . We, therefore, attempted to find a relation between Helicobacter pylori and gall bladder disease.[62]

This study was conducted in department of surgery, at Sri Devaraj Urs Medical College, Tamaka, Kolar. 65 cases with cholelithiasis and cholecystitis in the age group of 14-77 years were included in this study. Patients who had received treatment for H.pylori were excluded from the study. All these cases were stratified according to age, sex, presenting symptom, elicited signs, ultrasonogram, type of surgical intervention, histopathology, biochemical tests, staining and serology.

Most of the patients in our study were females 38(58.5%) and majority of the patients were in the age group of 31-40years (29.2%). Few patients were in younger age group as well, showing a decrease trend in the age for development for cholelithiasis. This could probably be due to change in lifestyle.[62]

Pain and tenderness in the right hypochondrium were the common symtom and elicited sign, 30.7 % cases had biliary dyspepsia. All this data was comparable with Arshad et al study, however biliary dyspepsia was present in more number of pateints (68%) compared to other study.

One patient had mild degree of jaundice, however obstructive causes were ruled out with imaging techniques. This may be due to transient calculi in the CBD which would have passed spontaneously.

42 (64.6%) cases underwent open cholecystectomy, rest underwent laparoscopic cholecystectomy. In 2 patients laparoscopic cholecystectomy was converted into open cholecystectomy due to dense adhesions.[63]

Majority of the patients (61) had calculus cholecystitis and 4 patients had features of chronic cholecystitis without calculi.

Curved bacteria were detected in three cases in direct microscopy with haematoxylin, eosin and gram staining, which was suggestive of helicobacter species. Those cases were confirmed by using warthin starry staining method.[64]

STAINING METHOD:

Table 10 : COMPARISON OF VARIOUS STUDIES

STUDY	NO. OF CASES	POSITIVE	PERCENTAGE
OUR STUDY	**65**	**3**	**4.6%**
Mendez sanchez N et al	95	1	1.05%
Griniatsos et al	89	4	4.5%
Lee J W et al	46	5	10.8%
Wafi attallah et al	94	21	22%
Arshad hussain et al	100	55	55%

In our study, 4.6% cases were positive species by staining methods. Data in our study, showing a low incidence of helicobacter species colonisation on the biliary mucosa, is in agreement with similar studies from different countries such as Turkey, Mexico, Germany, Canada and various other workers.

On the other hand it is in contrast with findings from other population and workers such as Wafi attallah et al (22%) and Arshad hussain et al (55%), where they found to have higher incidence of helicobacter species on gall bladder mucosa. [64]

This broad variation in the colonization rate cannot be explained only by the difference in seroprevalence of helicobacter species among different populations, also the methodology and sensitivity and specificity of the tests used to detect the organism have to be taken into consideration.

SEROLOGICAL METHODS:

In our study, 4.6% cases were positive for serum IgM antibodies against helicobacter, compared to other studies the prevalence is low. Few other workers have noted increase in antibody titres level. However they have used serum IgG antibodies against helicobacter. In our study controls were not used. Various workers have studied antibodies in healthy individuals as well as in patients of dyspepsia and have reported positivity ranging between 49 and 79%.[65,66]

The patients who were positive for helicobacter were treated with HP kit for 4 weeks postoperatively and were followed up at regular intervals.

We found that 3(4.6%) of the 65 patients screened for IgM class of antibodies in their serum gave positive results. The gall bladder tissue from these 3 patients also showed spiral organisms suggestive of helicobacter. Thus there seems to be good correlation between the presence of IgM antibodies in the serum to helicobacter pylori and histological demonstration of the organism in the gall bladder tissue.

Thus we think that in about 5% of the patients with calculus cholecystitis the disease maybe associated with helicobacter. Its quite possible that in a population there maybe individuals who have helicobacter species in the gall bladder mucosa which may not manifest as acute cholecystitis, but may go on to have chronic infection.

In this context, one needs to screen a population without gastritis for the presence of IgM class of antibodies to helicobacter pylori and those were positive should be followed up to understand the natural history of such colonization if found in the gall bladder.

SUMMARY

- The age incidence was found to be highest between 31 and 50 years.
- The incidence of cholelithiasis was more in females.
- All patients presented with pain abdomen.
- Majority of the patients had tenderness in right hypochondrium.
- Calculus cholecystitis was the most common mode of presentation.
- Open cholecystectomy was commonly done for cholelithiasis in our set up.
- Laparoscopic cholecystectomy has been used increasingly as a treatment modality.
- In our study, prevalence of helicobacter was 4.6% by staining methods and 4.6% by serological method.

CONCLUSION

According to our findings evidence of recent infection Helicobacter pylori as shown by demonstration of IgM class of antibodies to the organism was found in 4.6% of patients and histological evidence as demonstration in gall bladder mucosa could be optained in 4.6% of the patients with cholecystitis and cholelithiasis. Thus, the frequency of helicobacter infection seems to be low in the patient population studied.

BIBILOGRAPHY

1. Devarbhavi H, Nanivadekar S, Sawant P, Saraswathy K. Sensitivity of Helicobacter pylori isolates from Indian patients to different antibacterial agents. Indian J Gastroenterol. 1998;17(Suppl 1):S53.
2. Feldman RA, Eccersley AJ, Hardie JM.Epidemiology of Helicobacter pylori: acquisition,transmission, population prevalence and disease-to-infection ratio. Br Med Bull 1998; 54: 39-53.
3. Bulajic M, Stimec B, Milicevic M, et al.Modalities of testing Helicobacter pylori in patients with nonmalignant bile duct diseases. World J Gastroenterol 2002; 8:301-4.
4. Gasbarrini A, Franceschi F, Gasbarrini G, et al. Extraintestinal pathology associated with Helicobacter infection. Eur J Gastroentrole Hepatol 1997; 9: 231-3.
5. Silva CP, Pereia JC, Oliveria AG, Guerra JB, Marques DL, Sarmanho L. Association of presence of helicobacter in gall bladder tissue with cholelithiasis and cholecystitis.J,clin.Microbiol 2003;41:5615-5618.
6. Karagin PH, Stenramu, Wadstrom T, Ljungh A. Helicobacter species and common gutbacterial DNA in gall bladder with cholecystitis.World J Gastroenterol 2010;16:4817- 4822.
7. Chen DF, Hul, Yi P, Liu WW, Fang DC, Coa H.Helicobacter pylori damages human gall bladder epithelial cells in vitro.World J Gastroenterol 2008;14:6924-6928.

8. Soud WA, Stenram U, Ljungh A, Tranberg KG, Nillson HO, Wadstrom T.DNA of helicobacter species and common gut bacteria in primary liver carcinoma. Dig liver dis 2008;40:126-131.
9. Nillson HO, Mulchandani R, Tranberg KG, Stenram U, Wadstrom T.Helicobacter species identified in liver from patients with cholangiocarcinoma and hepatocellular carcinoma. Gastroenterology 2001;38:323-324.
10. Taneer J,Castedal M,Glatz E,Nillson HO,Olsson R,Wadstrom T. Identification of helicobacter pylori and other helicobacter species by PCR, hybridization, and partial DNA sequencing in human liver samples from patients with primary sclerosing cholangitis or primary biliary cirrhosis. J Clin Microbiol 200; 38:1071-1076.
11. Clemente CD: Gray's Anatomy. Philadelphia: Lea & Febiger, 1985 :132.
12. Klein AS, Lillemoe KD, Yeo CJ, et al: Liver, biliary tract, and pancreas, in O'Leary JP (ed): Physiologic Basis of Surgery. Baltimore: Williams & Wilkins, 1996: 441.
13. Scott-Conner CEH, Dawson DL: Operative Anatomy. Philadelphia: JB Lippincott, 1993:388.
14. Molmenti EP, Pinto PA, Klein J, et al: Normal and variant arterial supply of the liver and gallbladder. Pediatr Transplant 7:80, 2003.[PMID: 12581334]
15. Chen TH, Shyu JF, Chen CH, et al: Variations of the cystic artery in Chinese adults. Surg Laparosc Endosc Percutan Tech 10:154, 2000. [PMID: 10872977]
16. Boyer J: Bile secretion—models, mechanisms, and malfunctions. A perspective on the development of modern cellular and molecular concepts of bile secretion and cholestasis. J Gastroenterol 31:475, 1996.[PMID: 8726846]

17. Ko C, Lee S: Epidemiology and natural history of common bile duct stones and prediction of disease. Gastrointest Endosc 56:S165, 2002.
18. Brett M, Barker DJ: The world distribution of gallstones. Int J Epidemiol 5:335, 1976. [PMID: 1010661]
19. Brasca A, Berli D, Pezzotto SM, et al: Morphological and demographic associations of biliary symptoms in subjects with gallstones: Findings from a population-based survey in Rosario, Argentina. Dig Liver Dis 34:577, 2002. [PMID: 12502214]
20. Attili AF, De Santis A, Capri R, et al: The natural history of gallstones: The GREPCO experience. The GREPCO Group. Hepatology 21:655, 1995. [PMID: 7875663]
21. Small DM. Cholesterol nucleation and growth in gallstone formation.N Engl J Med 1980;302:1305-1311.
22. Tabata M, Nakayama F. Bacteria and gallstones: etiologic significance. Dig Dis Sci 1981;26:218-224.
23. Maki T. Pathogenesis of calcium bilirubinate gallstones: role of E. b-glucuronidase and coagulation by inorganic ions, polyelectrolyte and agitation. Ann Surg 1966;154:90-100.
24. Donovan JM, Carey MC. Pathogenesis and therapy of gallstone disease:physical – chemical basis of gallstone formation. Gastroenterol Clin North Am 1991;20:47-66.
25. Goodhart GL, Levison ME, Trotman BW, Soloway RD. Pigment vs Cholesterol cholelithiasis : bacteriology of gallbladder stone, bile, and tissue correlated with biliary lipid analysis in gallstone formation. Dig Dis Sci 1970;23:877-882.

26. Cetta F, DeNisi D, Petrini C, et al. Composition and possible pathogenesis of pigment gallstones. Gastroenterology 1984;86:A3.
27. Cettr M. The role of bacteria in pigment gallstone disease. Ann Surg 1991;213-:315-26.
28. Cetta F. The role of bacteria in pigment gallstones disease. Ann S 1991;213:315-26.
29. Stewart L, Smith AL, Pellegrini CA, et al. Pigment gallstones form as a composite of bacterial microcolonies and pigment solids. Ann Surg 1987;206:242-50.
30. Kaufman HS, Magnuson TH, Lillemoe KD, et al. The role of bacteria in gallbladder and common duct stone formation. Ann Surg 1989;209:584-91.
31. Maki T. Pathogenesis of calcium bilirubinate gallstone : role of E.coli, b-glucuronidase and coagulation by inorganic ions, polyelectrolytes and agitation. Ann Surg 1966;164:90-100.
32. Smith A, Stewart L, Pellegrini C, Way L. Gallstone disease : the clinical manifestations of infectious stones. Arch Surg 1988; 123.
33. Smith AL, Stewart L, Fine R, et al. Gallstone disease. The clinical manifestations of infectious stones. Arch Surg 1989;124:623-633.
34. Maingot's abdominal operations. 10th Edn., Section 12, Chapter 60-61,1717-1754.
35. Schoenfield I, Lachin J. Chenodiol (Chenodeoxycholic acid) for dissolution of gallstones: the National Cooperation Gallstone study. The Steering Committee TNCGSG. Ann Intern Med 1981;95:257-282.

36. Schoenfield I, et al. The effect of ursodiol on the efficacy and safety of extracorporeal shockwave lithotripsy of gallstones. N Engl J Med 1990;323: 1239-1245.
37. Scriven M, et al. Cholecystectomy : a study of patient satisfaction. JR Coll Surg Edinb 1993;38:151-170.
38. Cuschieri, Steele, Moosa. Essential surgical practice [Higher surgical training in general surgery] 4th Edn., Module 10, 375-452.
39. Bass EB, et al. Cost-effectiveness of laparoscopic cholecystectomy versus open cholecystectomy. Am J Surg 1993;165:466-471.
40. Schlump R, et al. A nation's experience in laparoscopic cholecystectomy. Surg Endosc 1994;8:35-41.
41. Scott TR, et al. Laparoscopic cholecystectomy : a review of 12,397 patients. Surg Laparosc Endosc 1992; 2:191-198.
42. Diez J, Arozamena CJ, Ferriaina P, et al. Relation between post operative infections and gall bladder bile leakage during laparoscopic cholecystectomies. Surg Endosc 1996;10:529-32.
43. Consensus Development Panel on Gallstones and Laparoscopic Cholecystectomy. Gallstones and laparoscopic cholecystectomy. JAMA 1993;269:1018-24.
44. Scott TR, et al. Laparoscopic cholecystectomy: a review of 12,397 patients. Surg Laparosc Endosc 1992; 2:108-125.
45. Diez J, Arozamena CJ, Ferriaina P, et al. Relation between post operative infections and gall bladder bile leakage during laparoscopic cholecystectomies. Surg Endosc 1996;10:568-600.

46. Schubert ML, Peura DA (June 2008). "Control of gastric acid secretion in health and disease". Gastroenterology 134 (7): 1842–60.
47. Suerbaum S, Michetti P (October 2002). "Helicobacter pylori infection". N. Engl. J. Med. 347 (15): 1175–86.
48. Blaser MJ, Atherton JC (February 2004). "Helicobacter pylori persistence: biology and disease". J. Clin. Invest. 113 (3): 321–33.
49. Stark RM, Gerwig GJ, Pitman RS et al. (February 1999). "Biofilm formation by Helicobacter pylori". Lett Appl Microbiol 28 (2): 121–6
50. Kusters JG, van Vliet AH, Kuipers EJ (July 2006). "Pathogenesis of Helicobacter pylori Infection". Clin Microbiol Rev 19 (3): 449–90
51. Olson JW, Maier RJ (November 2002). "Molecular hydrogen as an energy source for Helicobacter pylori". Science 298 (5599): 1788–90.
52. Amieva MR, El-Omar EM (January 2008). "Host-bacterial interactions in Helicobacter pylori infection". Gastroenterology 134 (1): 306–23.
53. Schreiber S, Konradt M, Groll C et al. (April 2004). "The spatial orientation of Helicobacter pylori in the gastric mucus". Proc. Natl. Acad. Sci. U.S.A. 101 (14): 5024–9.
54. Petersen AM, Krogfelt KA (May 2003). "Helicobacter pylori: an invading microorganism? A review". FEMS Immunol. Med. Microbiol. 36 (3): 117–26.
55. Bravos e.d, giman r.h, Accurate diagnosis of H pylori: other tests. Gastroenterol. Clin. En. Am., 2000; 29:925–30.
56. Midolo p, marsall b.j, Accurate diagnosis of H pylori: urease test. Gastroenterol. Clin. En. Am., 2000; 29:879–84.

57. Rasmussen LT, Labio RW, Gatti LLetal, Helicobacter pylori detection in gastric biopsies, saliva and dental plaque of Brazilian dyspeptic patients. Mem. Inst. Oswaldo Cruz., 2010.
58. Korwin j, Avantages et inconvenients des differentes methodes diagnositques de l'infection a H. pylori, Gastroenterol. Clin.Biol, 2003; 27:380–390.
59. Hala m, l-zimaity t, Accurate diagnosis of H. pylori with biopsy. Gastroenterol. Clin. En. AM., 2000; 29: 63–9.
60. Laine l, sugg j, neil g, Endoscopic biopsy requirement for post treatment diagnosis of H. pylori. Gastrointest. Endosc,2003; 51:664–6.
61. Roggero p, bonfigli a, luzzani s, balade a, corno g. et al., H. pylori stool antigen test: a method to confirm eradication in children. J. Pediatr., 2002; 140:775–7.
62. Jafri Deeba, Singhal Sanjay, Malik Abida, Khan Athar. Helicobacter pylori in gall bladder disease. Biomedical Research 2010; 21 (4): 437-440.
63. Arshad Hussain Abro, Irfan Zafar Haider, Sarfraz Ahmad. Helicobacter pylori infction in patients with calcular cholecystitis: A hospital based study. J Ayub Med Coll Abbottabad 2011;23(1)
64. Karagin PH, Stenram U, Wadström T, Ljungh A. Helicobacter species and common gut bacterial DNA in gallbladder with cholecystitis. World J Gastroenterol 2010;16:4817–22.
65. Myung SJ, Kim MH, Shim KN, Kim YS, Kim EO, Kim HJ, et al. Detection of Helicobacter pylori DNA in human biliary tree and its association with hepatolithiasis. Dig Dis Sci 2000;45:1405–12.
66. Maurer KJ, Ihrig MM, Rogers AB, Ng V, Bouchard G, Leonard MR, et al. Identification of cholelithogenic enterohepatic helicobacter species and their role in murine cholesterol gallstone formation. Gastroenterology 2005;128:1023–33.

ANNEXURES

PROFORMA

CASE NUMBER:	**IP NUMBER:**	**WARD:**
NAME:	**AGE:**	**SEX:**
ADDRESS:	**RELIGION:**	**OCCUPATION:**
DOA:	**DOS:**	**DOD:**

HISTORY

Chief Complaints

Abdominal pain

Nausea and vomiting

Jaundice

Pruritus

Colour of urine and stools

Fever

Dyspepsia

Mass abdomen

History of presenting illness:

1. Abdominal pain	2. Vomiting
Site	Onset
Character	Frequency
Time of onset	Projectile/Effortless
Duration	Quantity

Symptom free interval	Colour
Radiation	Contents
Aggravating factors	Odour
Relieving factors	Relation to pain
Relation to food	Nausea

3. Jaundice	**4. Fever**
Intermittent/Progressive	Time of onset
Duration	Duration
Obstructive/Non obstructive	Grade
Chills and rigors	Chills and rigors
Abdominal pain	Abdominal pain
Waxing and waning	Diurnal variation
Number of episodes	Sweating

Past history:

Recurrent abdominal pain	Recurrent fever	Intake of OCP's
Jaundice	Enteric fever	
Acid peptic disease	Hyperlipidemia	

Personal history:

Diet	Appetite(dislike for fatty foods)
Sleep	Bowel
Addictive habits	Bladder

Menstrual and Obstetric history:

Menarche	LMP
Amenorrhea	Obstetric formula

EXAMINATION:

General physical examination

Appearance - Ill / Well	Obesity
Pallor	Clubbing
Cyanosis	Generalised lymphadenopahty
Icterus	Oedema
Pulse rate	Blood Pressure
Respiratory rate	Temperature

ABDOMINAL EXAMINATION:

Inspection

Contour/Shape - Scaphoid / Distended	Umbilicus - Central / Displaced
Movement with respiration	Flanks – Free/Full
Scars - Operative / Branding	Dilated veins
VGP / VIP	Mass
Hernial orifices - Free / Full	Scrotum

Palpation

Local rise of temperature	Tenderness
Murphy's sign	Rebound tenderness
Guarding	Rigidity

Mass

Site	Size
Shape	Surface – Smooth/Nodular
Plane - Intra / Extra peritoneal	Movement – Mobile/Fixed
Consistency - Soft / Firm / Hard	Continuity with liver
Borders	
Pulsations	

Percussion

Liver span

Note over mass - Resonant / Impaired / Dull Free fluid

Movement - Mobile / Fixed

Auscultation

Bowel sounds - Normal / Increased / Decreased / Absent

Additional sounds - Present / Absent

Rectal examination - Normal / Abnormal

Cardiovascular system - Normal / Abnormal

Respiratory system - Normal / Abnormal

Central nervous system - Normal / Abnormal

PROVISIONAL DIAGNOSIS

INVESTIGATIONS

Hemoglobin: PCV:

Total Count Differential Count:

Bleeding time Clotting time:

Blood Urea Serum creatinine:

Blood Sugar (Fasting/Random)

Urine routine: Normal/ Abnormal

Bile salts and bile pigments

Urobilinogen

Liver function tests

Total bilirubin: Direct bilirubin:

SGOT: SGPT:

Total protein: Albumin:

Globulin: A: G ratio:

Alkaline phosphatase:

Ultrasound abdomen

Hepaticolithiasis Choledocholithiasis

Cholecystitis - Acute / Chronic Empyema

Cholelithiasis Others

Mucocele

Carcinoma

Endoscopic retrograde cholangiopancreatogram

CT abdomen

Oesophago gastro duodenoscopy

ECG

Chest X ray

FINAL DIAGNOSIS

TREATMENT

Observation Conservation

TPR, BP monitoring Intake / Output chart

Nil orally Gastric decompression

IV Fluids IV antibiotics

Operation

Elective / Emergency GA / Epidural

Incision Peritoneum - Normal / Inflamed

Viscera

Gall bladder Common bile duct

Acute / Chronic inflammation Normal / Dilated

Mass / Empyema / Gangrene Palpable duct stone

Cholelithiasis

Operation done

Histopathology;

1. H & E

2. Giemsa staining

3. Warthin starry silver staining

Serology;

IgM Antibodies for helicobacter

KEY TO MASTER CHART

JAUN	**JAUNDICE**
DYSP	**DYSPEPSIA**
FEB	**FEBRILE**
ICT	**ICTERUS**
TENDER	**TENDERNESS**
LFT	**LIVER FUNCTION TEST**
STB	**SERUM TOTAL BILIRUBIN**
SGPT	**SERUM GLUTAMATE PYRUVATE TRANSAMINASE**
ALP	**ALKALINE PHOSPHATASE**
USG	**ULTRASONOGRAM**
OPEN	**OPEN CHOLECYSTECTOMY**
LAP	**LAPAROSCOPIC CHOLECYSTECTOMY**
HPR	**HISTOPATHOLOGY REPORT**
SERO	**SEROLOGY**
GEIMSA	**GEIMSA STAINING**
WS	**WARTHIN STARRY SILVER STAINING**
DAYS IN H	**DAYS IN HOSPITAL**

MASTER CHART

SL. NO.	IP NO.	AGE	SEX	SYMPTOMS			SIGNS				LFT			IMAGING	TREATMENT				STAINING		DAYS IN H
				PAIN	JAUN	DYSP	FEB	ICT	TENDER	MASS	STB	SGPT	ALP	USG	Open	Lap	HPR	SERO	GEIMSA	WS	
1	711772	55	F	+	-	-	-	-	+	-	0.4	18	83	CL	+		CAL	-	-	-	11
2	718128	33	F	+	-	-	-	-	+	-	0.6	19	73	CL	+		CAL	-	-	-	12
3	720769	48	M	+	-	-	-	-	+	-	0.56	26	70	CC	+		CC	-	-	-	10
4	725201	45	F	+	-	+	-	-	+	-	0.6	21	76	CL		+	CAL	-	-	-	6
5	725202	45	M	+	-	+	-	-	+	-	0.5	19	97	CC		+	CC	-	-	-	5
6	724142	33	F	+	-	+	-	-	+	-	0.57	315	74	CL		+	CAL	-	-	-	7
7	732332	45	F	+	-	-	+	-	+	-	0.8	29	66	CL	+		CAL	-	-	-	10
8	731874	22	F	+	-	-	-	-	+	-	0.23	22	82	CL	+		CAL	-	-	-	11
9	733116	18	M	+	-	-	+	-	+	-	0.56	50	77	CL	+		CAL	-	-	-	12
10	735399	38	M	+	-	+	-	-	+	-	0.57	312	182	CL	+		CAL	-	-	-	11
11	735416	48	F	+	-	-	+	-	+	-	0.63	42	72	CL		+	CAL	-	-	-	6
12	735635	27	F	+	-	+	+	-	+	-	0.9	26	74	CC	+		CC	-	-	-	10
13	735216	55	F	+	-	+	-	-	+	-	0.5	19	97	CC	+		CC	-	-	-	11
14	735354	38	F	+	-	+	-	-	+	-	0.6	22	67	CL	+		CAL	-	-	-	12
15	727258	67	M	+	-	-	-	-	+	-	0.6	21	76	CL	+		CAL	+	+	+	11
16	747286	35	F	+	-	-	-	-	+	-	0.68	29	54	CL	+		CAL	-	-	-	10
17	780156	36	F	+	-	+	+	-	+	-	0.44	24	64	CL		+	CAL	-	-	-	6
18	751481	40	F	+	-	-	-	-	+	-	0.72	18	114	CL		+	CAL	-	-	-	7
19	752066	55	M	+	-	-	-	-	+	-	0.57	21	82	CL	+		CAL	-	-	-	10
20	753499	60	F	+	-	-	-	-	+	-	0.23	22	82	CL	+		CAL	-	-	-	11
21	761687	26	F	+	-	-	-	-	+	-	0.67	27	79	CL		+	CAL	-	-	-	6
22	762476	28	M	+	-	-	-	-	+	-	0.68	29	54	CL	+		CAL	-	-	-	11
23	767748	20	M	+	-	-	-	-	+	-	0.74	31	116	CL		+	CAL	-	-	-	7
24	764685	38	F	+	-	+	-	-	+	-	0.77	28	114	CL	+		CAL	-	-	-	10
25	750113	42	M	+	-	-	-	-	+	-	0.69	49	158	CL		+	CAL	-	-	-	5
26	776124	50	M	+	-	-	-	-	+	-	0.78	45	106	CL		+	CAL	-	-	-	7

MASTER CHART

27	778920	40	F	+	-	-	-	-	+	-	0.67	30	75	CL	+		CAL	+	+	+	12
28	780156	36	M	+	-	-	-	-	+	-	0.72	18	114	CL		+	CAL	-	-	-	5
29	785761	55	M	+	-	-	-	-	+	-	0.65	19	52	CL	+		CAL	+	+	+	11
30	778908	56	F	+	-	+	-	-	+	-	0.21	10	64	CL	+		CAL	-	-	-	12
31	760416	35	F	+	-	+	-	-	+	-	0.1	44	98	CL	+		CAL	-	-	-	10
32	785800	45	M	+	-	+	-	-	+	-	0.83	216	364	CL		+	CAL	-	-	-	6
33	738510	30	F	+	-	-	+	-	+	-	0.8	29	66	CL	+		CAL	-	-	-	10
34	796445	47	M	+	-	+	-	-	+	-	0.1	44	98	CL	+		CAL	-	-	-	11
35	797506	38	F	+	-	-	-	-	+	-	0.63	29	77	CL		+	CAL	-	-	-	5
36	800189	43	M	+	-	-	-	-	+	-	0.78	45	98	CL	+		CAL	-	-	-	12
37	797375	23	F	+	-	-	-	-	+	-	0.54	24	86	CL		+	CAL	-	-	-	6
38	804322	45	M	+	-	-	-	-	+	-	0.69	49	148	CL		+	CAL	-	-	-	5
39	804945	19	F	+	-	-	-	-	+	-	0.6	70	111	CL		+	CAL	-	-	-	6
40	804899	62	M	+	-	-	-	-	+	-	0.58	63	88	CL	+		CAL	-	-	-	10
41	805223	52	M	+	-	-	-	-	+	-	0.87	54	89	CL	+		CAL	-	-	-	12
42	805343	48	F	+	-	+	-	-	+	-	1.4	26	136	CL	+		CAL	-	-	-	11
43	823583	55	F	+	-	-	-	-	+	-	0.6	39	108	CL		+	CAL	-	-	-	6
44	864450	55	F	+	-	-	-	-	+	-	0.67	30	75	CL	+		CAL	-	-	-	10
45	848374	58	M	+	-	-	-	-	+	-	0.54	34	238	CL	+		CAL	-	-	-	11
46	789448	40	F	+	-	+	-	-	+	-	0.18	42	77	CL	+		CAL	-	-	-	12
47	803425	60	F	+	-	+	+	-	+	-	0.39	36	130	CL		+	CAL	-	-	-	7
48	780602	45	M	+	-	-	-	-	+	-	0.53	26	68	CL	+		CAL	-	-	-	12
49	801643	44	F	+	-	+	-	-	+	-	0.62	23	52	CL	+		CAL	-	-	-	10
50	840221	50	F	+	-	-	-	-	+	-	0.87	83	135	CL	+		CAL	-	-	-	11
51	846450	55	F	+	-	-	-	-	+	-	0.34	28	69	CL	+		CAL	-	-	-	10
52	840983	20	F	+	-	+	-	-	+	-	0.39	37	113	CL		+	CAL	-	-	-	5
53	814935	32	M	+	-	-	-	-	+	-	0.51	23	79	CL		+	CAL	-	-	-	7
54	819642	41	F	+	-	-	-	-	+	-	0.78	36	135	CL	+		CAL	-	-	-	12
55	807940	30	F	+	-	-	-	-	+	-	0.54	26	88	CL	+		CAL	-	-	-	10
56	887663	25	F	+	-	+	+	-	+	-	0.34	39	98	CL	+		CAL	-	-	-	11

MASTER CHART

57	887228	33	F	+	-	-	-	-	+	-	0.61	42	123	CL	+		CAL	-	-	-	10
58	813950	60	M	+	-	-	-	-	+	-	0.78	45	98	CL	+		CAL	-	-	-	12
59	900214	36	F	+	-	-	-	-	+	-	0.52	20	90	CL	+		CAL	-	-	-	11
60	893826	72	M	+	+	-	-	+	+	-	2.5	66	215	CL	+		CAL	-	-	-	12
61	907401	14	M	+	-	-	-	-	+	-	0.4	16	309	CL		+	CAL	-	-	-	6
62	962324	55	M	+	-	-	-	-	+	-	0.54	34	238	CL	+		CAL	-	-	-	12
63	878227	65	F	+	-	-	-	-	+	-	0.78	39	135	CL	+		CAL	-	-	-	10
64	946497	38	M	+	-	-	-	-	+	-	0.58	36	112	CL		+	CAL	-	-	-	7
65	947740	52	M	+	-	+	+	-	+	-	0.53	66	110	CL		+	CAL	-	-	-	5