COMPARATIVE STUDY ON HELICOBACTER PYLORI INFECTION AS A CAUSATIVE AGENT IN GALL BLADDER TISSUE WITH SYMPTOMATIC CHOLECYSTITIS / CHOLELITHIASIS AND INCIDENTAL CHOLELITHIASIS

M.S. DEGREE EXAMINATION

BRANCH I - GENERAL SURGERY

Department of General Surgery

MADURAI MEDICAL COLLEGE AND GOVT RAJAJI HOSPITAL

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(Reg. No-221711107)

THE TAMILNADU DR.M.G.R. MEDICAL UNIVERSITY

CHENNAI, INDIA.

MAY 2020

CERTIFICATE BY THE GUIDE

This is to certify that this dissertation titled **"COMPARATIVE STUDY ON HELICOBACTER PYLORI INFECTION AS A CAUSATIVE AGENT IN GALL BLADDER TISSUE WITH SYMPTOMATIC CHOLECYSTITIS / CHOLELITHIASIS AND INCIDENTAL CHOLELITHIASIS"** submitted by **Dr C.JAGADISH** to the faculty of General Surgery, The Tamil Nadu Dr. M.G.R. Medical University, Chennai in partial fulfillment of the requirement for the award of MS Degree Branch I General Surgery, is a bonafide research work carried out by him under our direct supervision and guidance from November 2017 to September 2019.

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I **Dr.C.JAGADISH** hereby solemnly declare that this dissertation entitled **"COMPARATIVE STUDY ON HELICOBACTER PYLORI INFECTION AS A CAUSATIVE AGENT IN GALL BLADDER TISSUE WITH SYMPTOMATIC CHOLECYSTITIS/CHOLELITHIASIS AND INCIDENTAL CHOLELITHIASIS"** is a bonafide and genuine research work carried out by me. This is submitted to the TamilNadu Dr. M.G.R. Medical University, Chennai, in partial fulfillment of the regulations for the award of M.S. degree (Branch I) General Surgery.

PLACE: Madurai. **Dr.C.JAGADISH**

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CONTENTS

INTRODUCTION

Marshall and Warren were the first to prove conclusively that *H. pylori* was the etiological factor for gastritis and peptic ulcer disease. Since then, *H. pylori* has been implicated in the development of gastric adenocarcinoma and MALT lymphoma in the stomach. The prevalence of infection in the digestive tract by Helicobacter species varies in the population studied, suggesting epidemiological differences in the distribution of the bacillus in various countries. So far, *H. cinaedi, H. fennelliae, H. canis, H. rappini, H. pullorum,* and *H. canadensi* have been isolated from human intestinal tracts.

Helicobacter species isolated from the bile, gallbladder, or liver tissue of some animals, such as *Helicobacter pullorum* from poultry , *H. canis* from dogs , *H. cholecystus* from Syrian hamsters , "*Helicobacter rappini*" from sheep fetuses , and *H. hepaticus* and *H. bilis* from mice have been associated with hepatobiliary diseases. In the past few years, the presence of DNA of species of *Helicobacter*, including the well-known human pathogen *H. pylori,* has been identified in the bile, liver, and biliary epithelium obtained from patients with hepatobiliary diseases. More recently, the group isolated (for the first time) a *H. pylori* strain from the

liver of a patient with cirrhosis, demonstrating that bacteria of the genus *Helicobacter* may be viable in the human liver, as it is seen to be in animals.

In regard to the biliary diseases, few patients were evaluated in the first studies. In one of those studies, *ureB H. pylor*- specific DNA was detected in the gallbladder tissue of a Japanese patient with gallstone and cholecystitis. In another study evaluating the presence of *H. pylori ureA* genes in the bile by nested PCR, Lin et al. observed a positive result in three patients with primary or metastatic pancreatic tumor but not in four patients with biliary diseases.

In studies of the same subject that included a larger number of patients, discordant results have been observed. In some of them, the presence of DNA of enterohepatic *Helicobacter* or *H. pylori* has been detected. Fox et al. have found *H. bilis*, *H. pullorum*, or "*H. rappini*" DNA in bile or gallbladder tissue from Chilean patients with cholecystitis or cholelithiasis. More recently, the level of *H. bilis* DNA was seen to be higher in the bile of patients from Japan and Thailand with bile duct or gallbladder carcinoma than from those without malignant disease of the biliary tree. In another study from Yugoslavia, the presence of *H. pylori*-specific DNA in the bile was associated with biliary tract carcinoma but no association was seen between patients with gallstone and those without biliary disease.

Other studies from Germany and Mexico failed in detecting the presence of DNA of *Helicobacter* spp. in bile or gallbladder tissue from patients with biliary tree disease. In a Japanese study, furthermore, DNA of *Campylobacter* (rather than that of *Helicobacter*) was detected in the bile and biliary epithelium of patients with hepatolithiasis.

These discordant results may be explained by regional differences. However, it has to be emphasized that in most of the studies there was no control group or there were few patients included as controls. In other studies, patients that com- posed the control group had other disorders (such as pancreatic or gastric malignancies) that may have introduced bias (since the presence of *Helicobacter* DNA has been detected in the bile of patients with these diseases). Furthermore, in the studies aimed to investigate the presence of *Helicobacter* in the biliary tree as a risk factor for biliary disease, no adjustment for confounding factors was done.

So we did a comparative study on helicobacter pylori as a causative agent in gall bladder tissue with symptomatic cholecystitis / cholelithiasis and incidental cholelithiasis.

AIM AND OBJECTIVES

Aim of the Study

The study was undertaken to determine the presence of H.pylori as a causative agent of cholelithiasis.

Objectives:

A comparison regarding SYMPTOMATIC CHOLECYSTITIS / CHOLELITHIASIS AND INCIDENTAL CHOLELITHIASIS by PCR and Giemsa staining

1. Study about gall stones

2. To identify H.pylori association

Eligibility Criteria

A.Inclusion criteria:

- Patients more than 25 years and up to 60 years of age groups in both sexes presenting with cholelithiasis in GRH Madurai.
- Patient with BMI between 20 to 27
- Patients consented for inclusion in the study according to designated proforma

B.Exclusion criteria:

- Patients less than 25 years of age
- Patients more than 60 years of age.
- Macroscopic malignancy and perforation.
- Patient with severe co morbidities.
- Patients with BMI >27.
- Patient not consented for inclusion in the study.

DESIGN OF STUDY: Prospective Study

PERIOD OF STUDY: 2 Years

SELECTION OF STUDY SUBJECTS: Age between 25 and 60 years in both sexes, patients undergoing cholecystectomy surgery in GRH, Madurai.

DATA COLLECTION: Data regarding History, surgery done and outcome.

METHODS: Observation study

ETHICAL CLEARANCE: Approval obtained.

CONSENT: Informed and written consent from all patients.

ANALYSIS: using CHI SQUARE test – p value

CONFLICT OF INTEREST: none

FINANCIAL SUPPORT: NIL FROM THE INSTITUTION

PARTICIPANTS: Any case of cholelithiasis irrespective of sex and occupation were included in the study, (excluding the patients with age less than 25 and more than 60.).

Materials used:

1. Giemsa staining

2. Polymerase chain reaction.

REVIEW OF LITERATURE

HELICOBACTER

Helicobacter pylori is curved gram-negative rod that colonizes stomach and is associated with peptic ulcer disease and gastric carcinoma.

PATHOGENISIS OF THE BACTERIUM

H.pylori colonizes the stomach of 50% of the world's human population(30% in developed countries to nearly 80% in developing countries). Adhensins –Few(~2%) strains bind to mucosal epithelium by expressing Blood group antigen-binding adhesion & binds to Lewis blood group antigen.

Lipoprotein induces of pathological changes by producing toxins which includes Vacuolating cytotoxin (VacA): Induces the formation of vacuoles in the cytoplasm of epithelial cells, Cytotoxin : Associated Gene A (cagA).

Molecular mimicry

LPS of H.pylori cross reacts with Lewis blood group antigen , which contributes to pathogenesis of chronic active gastritis.

ENVIRONMENTAL RISK FACTORS:

Smoking increases the risks of ulcers and cancer in H.pylori colonized individuals.

Diets high in salt and preserved foods increase cancer risk, whereas antioxidants and vitamin C are protective.

CLINICAL MANIFESTATIONS :

- Acute gastritis Antrum is the most common site involved, cardiac end is not involved
- Antral gastritis : Predisposes to duodenal ulcers
- Pan gastritis : Predisposes to adenocarcinoma stomach
- Peptic ulcer disease : 80% of duodenal ulcers and 60% of gastric ulcers are due to H.pylori.
- Chronic atrophic gastritis
- Autoimmune gastritis
- Promotes pernicious anemia
- Adenocarcinoma of stomach
- Non-Hodgkin’s gastric lymphoma.

PROTECTIVE ROLE FOR H.PYLORI

Colonization of H.pylori (especially with cagA+ strains) has an inverse relation with the occurrence of Gastro esophageal reflux disease (GERD), Barrett's esophagus , Adenocarcinoma of esophagus and asthma

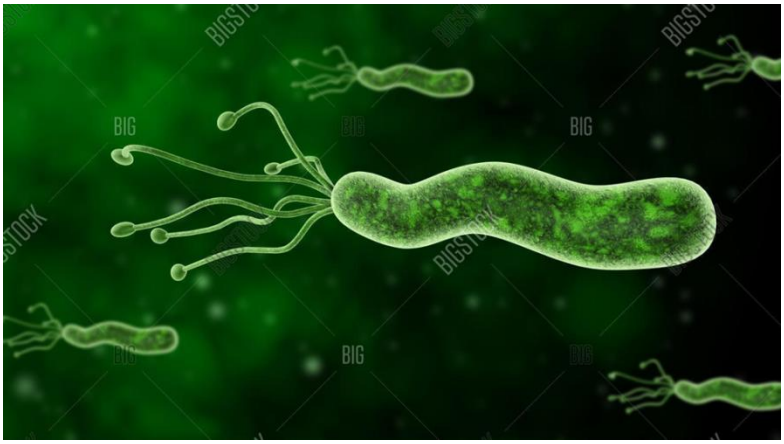

Figure 1 – 3 D image of Helicobacter pylori

LABORATORY DIAGNOSIS

Invasive test:

Endoscopy guided multiple biopsies can be taken from gastric mucosa (antrum and corpus) are subjected to

- Histopathology with Warthin Starry silver staining
- Microbiology - Gram staining - Curved gram-negative bacilli with seagull shaped morphology
- Culture media for H.pylori - Culture is the most specific test , however , it is not sensitive.

- Media for Campylobacter can be used, such as Skirrow's media
- Chocolate agar can be used
- Plates are incubated at 37 degree C under micoaerophilic condition.

- Biochemical tests : Oxidase, catalase and urease tests are positive.
- Biopsy urease test (rapid urease test) : Detects urease activity in gastric biopsies. It is sensitive and cheap.

Invasive Test

- Urea breath test : It is very popular now a days as it is noninvasive and is most consistent and accurate test. Most sensitive, quick and simple. Used for monitoring of treatment (becomes negative after treatment)

Stool antigen (co proantigen) assay : used for

Monitoring of treatment

Screening of children

Antibody (IgG) detection by ELISA : used for

Screening before endoscopy

Seroepidemiological study

TREATMENT OF H.PYLORI INFECTION:

Treatment in H.pylori infections is indicated for

Duodenal or gastric ulceration

Low grade gastric B cell lymphoma

However, treatment is not recommended for asymptomatic colonizers or primary prophylaxis for gastric cancer because of risk of adverse side effects and development of antibiotic resistance.

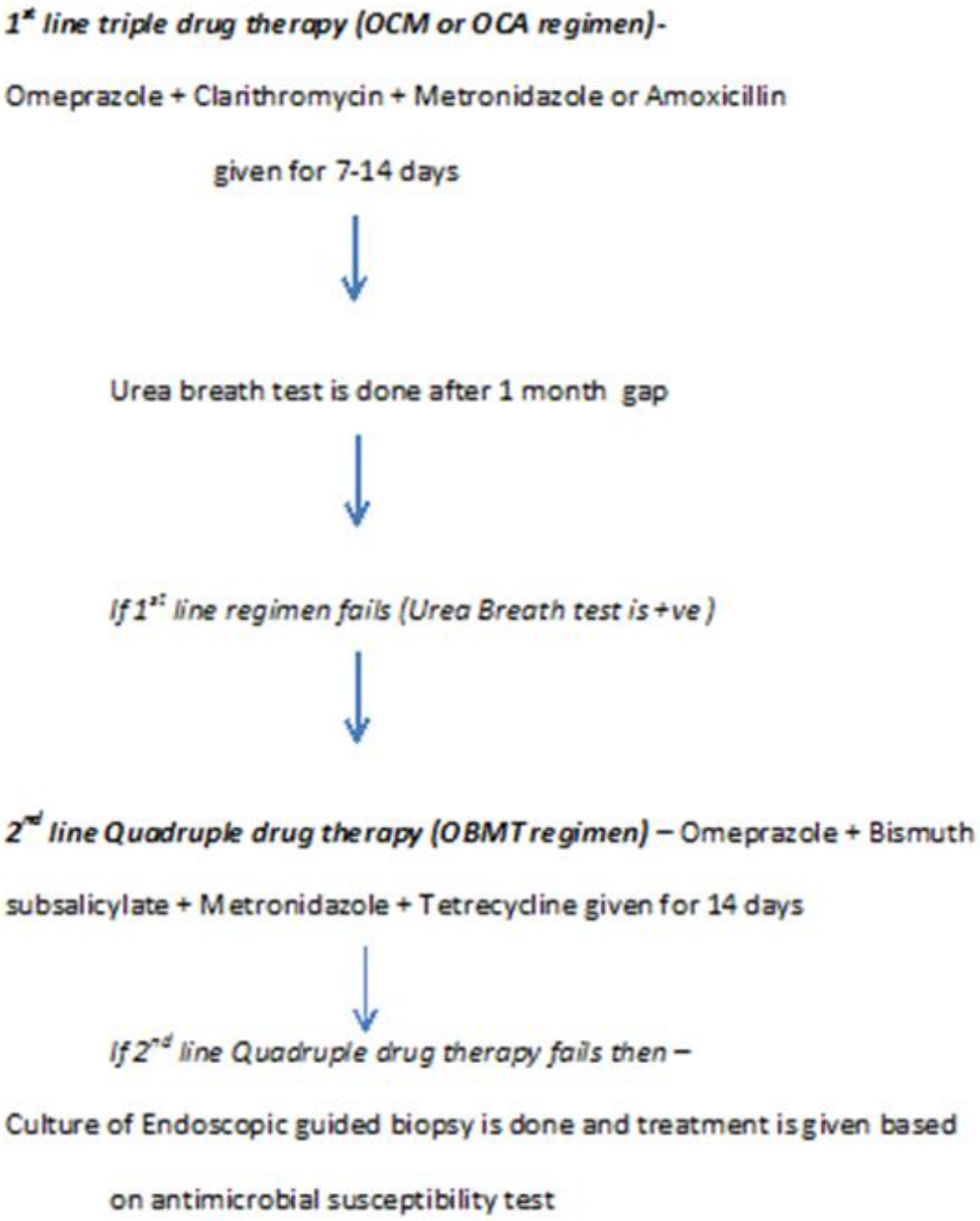

Figure 2 – Treatment Protocol for H. pylori

GALL BLADDER

Gall bladder is a pear shaped reservoir of bile situated in a fossa on the inferior surface of the right lobe of the liver. The fossa for the gall bladder extends from the right end of the porta hepatis to the inferior border of the liver.

The gall bladder is 7 to 10 cm long , 3cm broad at its widest part and about 30 to 50 ml in capacity. The fundus of the gall bladder is marked at the angle between the right costal margin and the outer border of the rectus abdominis called linea semilunaris.

The gall bladder is divided into (I) the fundus

(2) the body and

(3) the neck

The fundus projects beyond the inferior border of the liver , in the angle between the lateral border of the right rectus abdominis and the ninth costal cartilage. It is entirely surrounded by peritoneum, and is related anteriorly to the anterior abdominal wall, and posteriorly to the beginning of the transverse colon.

The body lies in the fossa for the gall bladder on the liver . The upper narrow end of the body is continuous with the neck at the right end of the portahepatis. The superior surface of the body is devoid of peritoneum ,

and is adherent to the liver. The inferior surface is covered with peritoneum and is related to the beginning of the transverse colon and to the first and second parts of duodenum.

The neck is the narrow upper end of the gallbladder. It is situated near the right end of the portahepatis. It first curves anterosuperiorly and then posteroinferiorly to become continuous

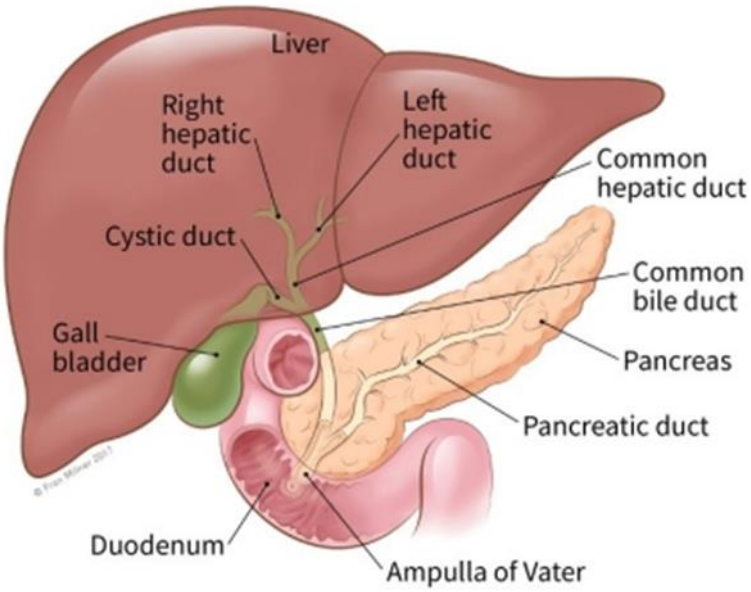

Figure 3 – Anatomical location of Gall Bladder

with the cystic duct. Its junction with the cystic duct is marked by a constriction. Superiorly, the neck is attached to the liver by areolar tissue in which the cystic vessels are embedded.

Inferiorly it is related to the first part of the duodenum. The mucous membrane of the neck is folded spirally to prevent any obstruction to the

inflow or outflow of bile.The posteromedial wall of the neck is dilated outwards to form a pouch called the Hartmann's pouch which is directed downwards and backwards.

Some regards this pouch as a normal feature of the gall bladder, but others consider it to be pathological. Gall stones may lodge in this pouch.

CYSTIC DUCT

Cystic duct is about 3 to 4 cm long. It begins at the neck of the gall bladder, runs downwards, backwards and to the left, and ends by joining the common hepatic duct at an angle to form the bile duct. The mucous membrane of the cystic duct forms a series of 5 to 13 crescentic folds, arranged spirally to form the so-called "spiral valve" of Heister. This is not a true valve.

BILE DUCT:

Bile duct is formed by the union of the cystic and common hepatic ducts near the porta hepatis. It is 8 cm long and has a diameter of about 6 mm.

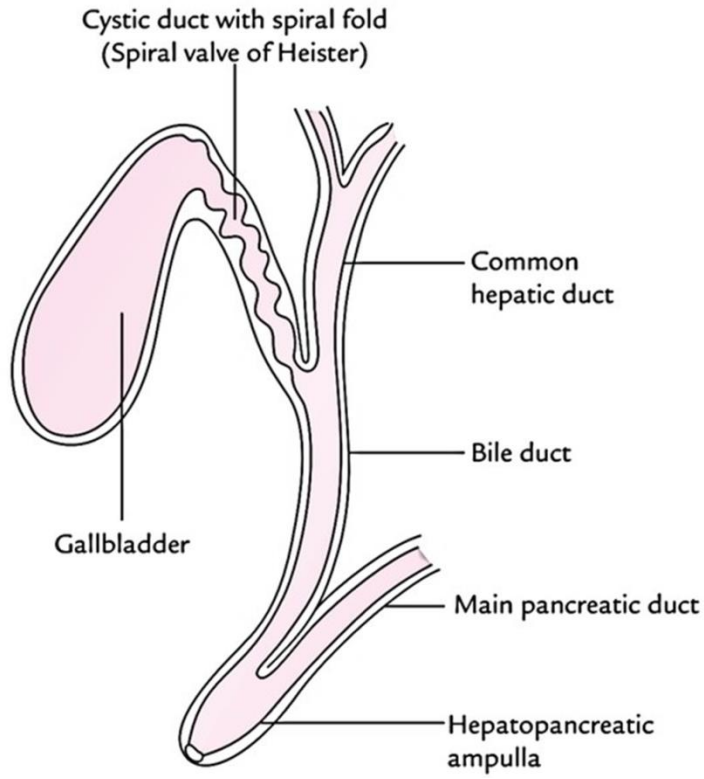

Figure 4 – Anatomy of Biliary Tree

The bile duct runs downwards and backwards, first in the free margin of the lesser omentum, supraduodenal part; then behind the first part of the duodenum the retroduodenal part; and lastly behind , or embedded in, the head of the pancreas infraduodenal part. Near the middle of the left side of the second part of the duodenum it comes in contact with the pancreatic duct and accompanies it through the wall of the duodenum, the intraduodenal part.

The course of the duct through the duodenal wall is very oblique. Within the wall the two ducts usually unite to form the hepatopancreatic ampulla, or ampulla of Vater. The distal constricted end of the ampulla

opens at the summit of the major duodenal papilla 8 to 10 cm distal to the pylorus. Quite often the bile and the pancreatic duct may open independently on the papilla.

Relations

Supraduodenal part in the free margin of lesser omentum.

- Anteriorly – Liver
- Posteriorly – Portal vein and epiploic foramen.
- To the left – Hepatic artery.

Retroduodenal part

- Anteriorly – First part of duodenum
- Posteriorly – Inferior vena cava.
- To the left – Gastroduodenal artery.

Infraduodenal part

- Anteriorly – A groove in the upper and lateral parts of the posterior surface of the head of the pancreas.
- Posteriorly – Inferior vena cava.

Intraduodenal part

SPHINCTERS RELATED TO THE BILE AN PANCREATIC DUCTS

The terminal part of the bile duct is surrounded just above its junction with the pancreatic duct by a ring of smooth muscle that forms the sphincter choledochus(choledochus = bile duct). This sphincter is always present. It normally keeps the lower end of the bile duct closed.

As a result , bile formed in the liver keeps accumulating in the gall bladder and also undergoes considerable concentration. When food enters the duodenum , specially a fatty meal, the sphincter opens and bile stored in the gall bladder is poured into the duodenum.

The sphincter choledochus is , therefore, essential for filling of the gall bladder. Another less developed sphincter , which is usually but not always present around the terminal part of the pancreatic duct is the sphincter pancreaticus. A third sphincter surrounds the hepatopancreatic ampulla and is called the sphincter ampullae. The sphincter ampullae may extend upwards to enclose the lower parts of the bile and pancreatic ducts.

The sphincters named above or often referred to collectively as the sphincter of oddi, although this term applies strictly only to the sphincter ampullae.

ARTERIES SUPPLYING THE BILIARY APPARATUS

- The cystic artery is the chief source of blood supply, and is distributed to the gall bladder, the cystic duct , the hepatics duct and the upper part of the bile duct.
- Several branches from the posterior superior pancreaticoduodenal artery supply the lower part of the bile duct.
- The right hepatic artery forms a minor source of supply to the middle part of the bile duct.
- An accessory cystic artery may arise from the common hepatic artery , or from one of its branches.

The cystic artery usually arises from the right hepatic artery , passes behind the common hepatic and cystic ducts, and reaches the upper surface of the neck of the gall bladder, where it divides into superficial and deep branches. Occasionally , the cystic artery arises from the hepatic artery proper, passes in front of, or behind, the bile duct or the common hepatic duct, to reach the upper surface of the neck of the gall bladder.

VENOUS DRAINAGE

- The superior surface of the gall bladder is drained by veins which enter the liver through the fossa for the gall bladder and join tributaries of hepatic veins.

- The rest of the gall bladder is drained by one or two cystic veins which commonly enter the liver, either directly or after joining with the veins draining the hepatic ducts and the upper part of the bile duct. Rarely the cystic vein opens into the right branch of the portal vein.
- The lower part of the bile duct drains into the portal vein.

LYMPHATIC DRAINAGE

- Lymphatics from the gall bladder, the cystic duct, the hepatic ducts and the upper part of the bile duct pass to the cystic node and to the node of the anterior border of the epiploic foramen. These are the most constant members of the upper hepatic nodes. The cystic node lies in the angle between the cystic and common hepatic ducts; it is constantly enlarged in cholecystitis.
- The lower part of the bile duct drains into the lower hepatic and upper pancreaticosplenic nodes.

NERVE SUPPLY

The cystic plexus of nerves, supplying the territory of the cystic artery , is derived from the hepatic plexus, which receives fibres from the coelic plexus, the left and right vagi and the right phrenic nerves. The lower part of the bile duct is supplied by the nerve plexus over the superior pancreaticoduodenal artery.

Parasympathetic nerves are motor to the musculature of the gall bladder and bile ducts, but inhibitory to the sphincters. Sympathetic nerves from thoracic seven to nine are vasomotor and motor to the sphincters.

Pain from the gall bladder may travel along the vagus, the sympathetic nerves, or along the phrenic nerves. It may be referred to different sites through these nerves as follows.

- Through the vagus to the stomach.
- Through the sympathetic nerves to the inferior angle of the right scapula.
- Through phrenic nerve to the right shoulder.

Functions of the Gall Bladder:

- Storage of bile, and its release into the duodenum when required.
- Absorption of water, and concentration of bile. Bile may be concentrated as much as ten times.
- The normal gall bladder also absorbs small amounts of a loose bile salt-cholesterol compound. When the gall bladder is inflamed, the concentration function becomes abnormal and the bile salts alone are absorbed leaving cholesterol behind. Bile salts have a powerful solvent action on cholesterol which tends to be precipitated. This can lead to the formation of gall stones.

- It regulates pressure in the biliary system by appropriate dilatation or contraction. Thus the normal, choledochoduodenal mechanism is maintained.

HISTOLOGY

Mucous membrane : It is projected to form folds. Epithelium consist of a single layer of tall columnar cells. Lamina propria contains loose connective tissue.

The fibromuscular coat: It consists of smooth muscle fibres and collagen fibres which rests on an outer fibroareolar coat.

DEVELOPMENT

Hepatic bud arises from the endoderm of caudal part of foregut. The bud elongates cranially. It gives rise to a small bud on its right side. This is called pars cystica forms the gall bladder and the cystic duct, which drains into the common hepatic duct (CHD).

Pars hepatica forms CHD and divides into right and left hepatic ducts. These ducts reach septum transversum and proliferate to form the hepatic parenchyma. The entire epithelium is endodermal and other layers are of splanchnic origin.

GALL STONE DISEASE – CHOLELITHIASIS

Gallstones:

Gallstone disease, or cholelithiasis, is one of the most common surgical problems worldwide. Gallstones are abnormal, inorganic masses formed in the gallbladder and, less commonly, in the common bile or hepatic ducts. They are a frequent cause of abdominal pain and dyspepsia. Although gallstones can form anywhere in the biliary tree, the most common point of origin is within the gallbladder.

Figure 5 – Gall Bladder with multiple Stones

Who is at risk for gallstones?

- Gender: Gallstones form more commonly in women than men.
- Age: Gallstone prevalence increases with age.

- Obesity: Obese individuals are more likely to form gallstones than thin individuals.
- Pregnancy: Women who have been pregnant are more likely to form gallstones than women who have not been pregnant. Pregnancy increases the risk for cholesterol gallstones because during pregnancy, bile contains more cholesterol, and the gallbladder does not contract normally.
- Birth control pills and hormone therapy: The increased levels of hormones caused by either treatment mimics pregnancy.
- Rapid weight loss: Rapid weight loss by whatever means-very low calorie diets or obesity surgery- causes cholesterol gallstones in up to 50% of individuals. Many of the gallstones will disappear after the weight is lost, but many do not. Moreover, until they are gone, they may cause problems.
- Increased blood triglycerides: Gallstones occur more frequently in individuals with elevated blood triglyceride levels.

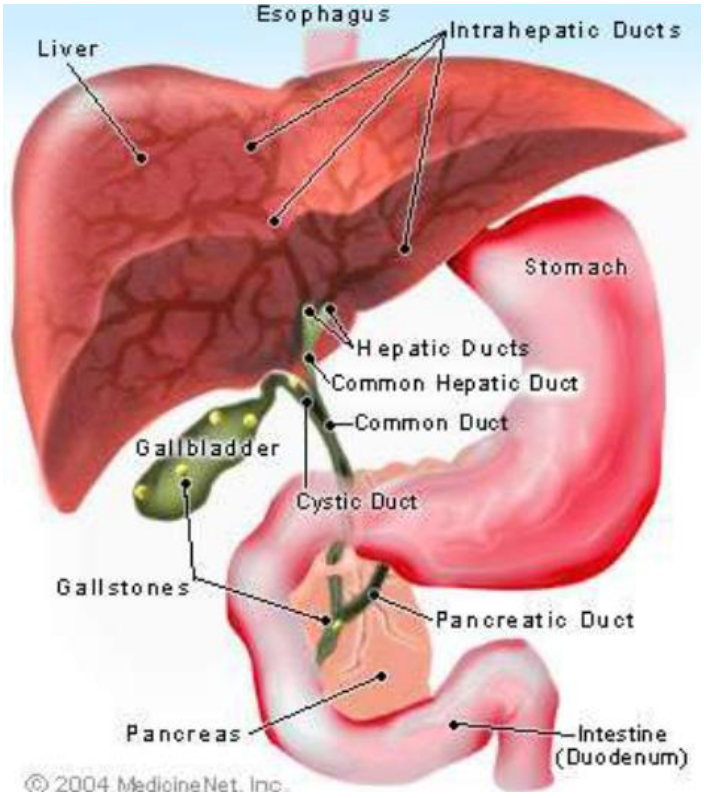

Figure 6 – Gall stone Disease

Types of Gallstones :

- Mixed (80%)
- Pure cholesterol (10%)
- Pigmented (10%)
- Black stones (contain Cabilirubinate, a/w cirrhosis andhemolysis)
- Brown stones (a/w biliary tractinfection)

Figure 7 – Types of Gall Stones

Pathogenesis:

Bile is composed of bile salts, phospholipids, cholesterol and also bilirubin which is conjugated before excretion. Gallstones are formed due to imbalance rendering cholesterol & calcium salts insoluble

Involves 3 stages:

1. cholesterol supersaturation in bile
2. crystal nucleation
3. stone growth

DEFINITIONS

Symptomatic cholelithiasis: Wax/waning postprandial epigastric /RUQ pain due to transient cystic duct obstruction by stone, no fever/WBC, normal LFT.

Acute cholecystitis : Acute GB inflammation due to cystic duct obstruction. Persistent RUQ pain +/- fever, ↑WBC, ↑LFT, +Murphy's = inspiratory arrest.

Chronic cholecystitis : Recurrent bouts of colic/acute cholecystitis leading to chronic GB wall inflammation/fibrosis. No fever/WBC.

Acalculous Cholecystitis : GB inflammation due to biliary stasis (5% of time) and not stones (95%). Seen in critically ill pts.

Choledocholithiasis: Gallstone in the common bile duct (primary means originated there, secondary = from GB).

Cholangitis: Infection within bile ducts due to obstruction of CBD. Charcot triad: RUQ pain, jaundice, fever (seen in 70% of pts), can lead to septic shock.

CAUSES OF GALLSTONE:

Prolonged fasting (5-10 days) can result in the formation of biliary sludge (microlithiasis) which resolves by itself when feeding is reestablished - but it can lead to biliary symptoms or gallstone formation

STAGES AND CLASSIFICATION

I. Initial stage or “prestone”

- Viscous, nonhomogeneous bile

- Bile sludge with formation of microstones

II. Formation of stones

- Localization:in gallbladder, in common bile duct, in hepatic ducts

- Amount: single, plural

- Composition: cholesterol, pigment, mixed

Clinical forms: asymptomatic (latent) and manifesting or symptomatic , Pain form with typical bile colic , Dyspeptic form , Masked form

III. Stage of chronic, recurrent cholecystitis with concremental

IV. Stage of complications

PREDISPOSING FACTORS FOR GALLSTONE FORMATION

- Cholesterol and mixed stones.
- Demographic and genetic factors – familial disposition; hereditary aspects; greater prevalence in Northern Europe and North America, lower – in Asia
- Obesity – increased biliary secretion of cholesterol .

- Weight loss – mobilization of tissue cholesterol leads to increased biliary cholesterol secretion while enterohepatic circulation of bile acids is decreased.
- Female sex hormones
- Estrogens stimulate hepatic lipoproteins receptors, increase uptake of dietary cholesterol, and increase biliary cholesterol secretion.
- Natural and synthetic estrogens lead to decrease bile salt secretion and decreased conversion of cholesterol to cholesterol esters.
- Ileal disease or resection – malabsorbtion of bile acids leads to decreased bile acids pool and decreased biliary secretion of bile salts.
- Increasing age – increased biliary secretion of cholesterol, decreased size of bile acid pool, biliary secretion of bile salts, and gallbladder motility.
- Gallbladder hypomotility leading to stasis and formation of sludge.
- Fasting
- Pregnancy
- Drugs: octreotide
- Prolonged parenteral nutrition.
- Clofibrate therapy - increased biliary secretion of cholesterol.
- Decreased bile acid secretion

- Primary bilary cirrhosis
- Chronic intrahepatic cholestasis.
- Miscellaneous
- High-calorie, high-fat diet.
- Pigment stones.
- Demographic/genetic factors: Asia, rural settings.
- Chronic hemolysis.
- Alcoholic cirrhosis.
- Chronic biliary tract infections, parasite infestation.
- Increasing age.

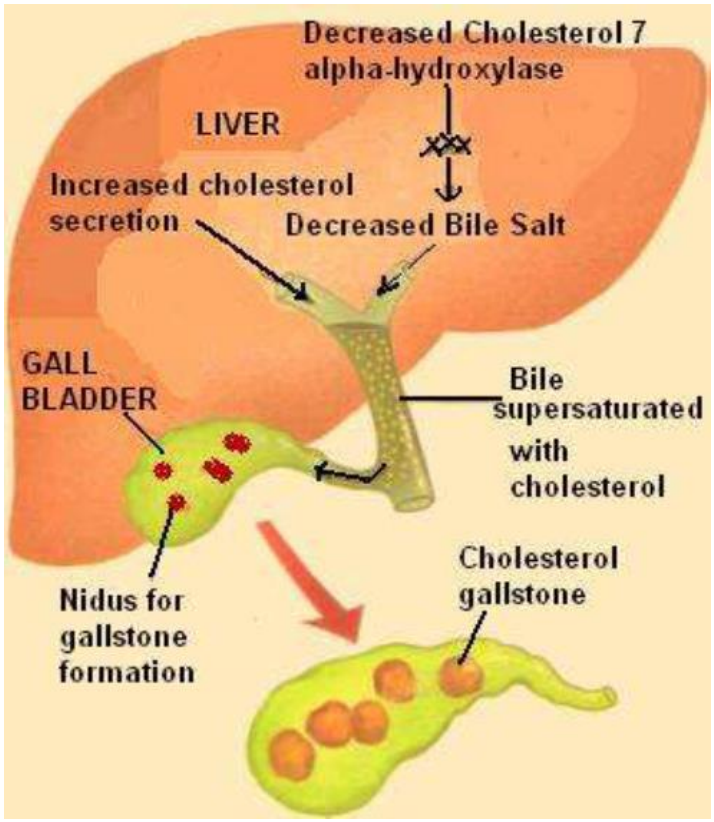

Figure 8 – Pathogenesis of Gall Stones

PATHOGENIC MECHANISMS

- Lithogenecity of bile in form of:

  ↑Cholesterol + normal bile acids and lecithin

  ↓Bile acids + normal cholesterol and lecithin

  ↑Cholesterol + ↓bile acids + normal lecithin

High concentration of bile acids may cause aseptic inflammation of gallbladder.

- Dysfuction of bile ducts and gallbladder – disturbances of gallbladder and sphincters synchronism

- Dysfunction of gallbladder
- Dysfunction of Oddi' sphincter
- Reflux of pancreatic juice into the gallbladder with the development of enzymatic cholecyctitis.
- Reasons for motor dystunction are:

Neurotic conditions which cause asynchronism.

Excessive intake of fatty and fried food lead to spasm of Oddi' and Lutkens' sphincters.

Long termed use of spasmolytics causes hypokinesia and atonia of Oddi' sphincter that in term lead to the reflux of duodenal contents into bile ducts.

Peptic ulcer disease with localization in the bulb.

- Genetic predisposition
- Occupational hazards (vibration, sedentary life)
- Infection: Escherichia coli, Staphylococcus, Enterococcus, Klebsiella, Clostridium, Proteus, Viruses of hepatitis, Parasite infestation in gallbladder and duodenum (amoebiasis, opisthorchiasis, fascioliasis, lonorchiasis, lambliasis) may activate infection in gallbladder.

- The route for bile contamination includes Hematogenous from portal vein or hepatic artery, Lymphogenous, Ascending from intestine.

SYNDROMES

- Pain syndrome
- Dyspeptic syndrome: Gastric & Intestinal
- Inflammatory syndrome (during exacerbation)
- Cholestatic syndrome (in obstruction of common bile duct)
- Dyslipidemia

COMPLICATIONS OF CHOLELITHIASIS

Cholangitis

Mechanical obstruction of bile ducts (choledocholithiasis)

Galbladder perforation and bile peritonitis

Empyema of gallbladder

Gallbladder hydrops

Pericholecystitis

- Passage of gallstones from the gallbladder into the common bile duct can result in a complete or partial obstruction of the common bile duct.
- Frequently, this manifests as jaundice.

- In all races, jaundice is detected most reliably by examination of the sclera in natural for yellow discoloration.
- Pancreatitis, another complication of gallstone disease, presents with more diffuse abdominal pain, including pain in the epigastrium and left upper quadrant of the abdomen.
- Cholecystitis means inflammation of the gallbladder. Like biliary colic, it too is caused by sudden obstruction of the ducts by a gallstone, usually the cystic duct.
- Cholangitis is a condition in which bile in the common, hepatic, and intrahepatic ducts becomes infected.
- Severe hemorrhagic pancreatitis occurs in 15% patients and carries a high mortality rate because of multisystem organ failure.
- In a few patients, the hemorrhagic pancreatic process and retroperitoneal bleeding induce discoloration around the umbilicus (cullen sign) or the flank (Grey-Turner sign).

CHARCOT TRIAD : (right upper quadrant pain, fever, and jaundice)

associated with common bile duct obstruction and cholangitis

- Alterations in mental status and hypotension, indicate Raynaud pentad, a harbinger of worsening, ascending cholangitis.
- Sharco symptoms: pain in the right upper abdomen, high fever, jaundice.

- Gangrene of the gallbladder is a condition in which the inflammation of cholecystitis cuts off the supply of blood to the gallbladder.
- Without blood, the tissues forming the wall of the gallbladder die, and this makes the wall very weak.
- The weakness combined with infection often leads to rupture of the gallbladder.
- The infection then may spread throughout the abdomen, though often the rupture is confined to a small area around the gallbladder (aconfined perforation).

CLINICAL MANIFESTATION

Pain syndrome depends on the stage of the disease:

In gallstones – biliary colic. Characterized by sudden onset of severe pain with duration from 30 min to 5 h, subsiding gradually or rapidly, localized in right hypochondria or epigastria, radiated in right scapula, right part of the chest, clavicula May be precipitated by fatty food, by consumption of a large meal following a period of prolonged fasting.

In **Dyskinetic Stage** – dull, mild pain in right hypochondria, epigastric fullness, related to emotional stresses.

Gastric dyspeptic syndrome: nausea and vomit with bile that don't improve condition, heartburn, belching, bitter taste, regurgitation with bile, loss of appetite.

Intestinal dyspeptic syndrome: steatorrhea, meteorism.

Inflammatory syndrome: fever, chills.

Cholestatic syndrome: skin etching

PHYSICAL FINDINGS

Pain syndrome: Superficial palpation demonstrates tenderness in right hypochondria, muscle rigidity. Deep palpation shows tenderness in the point of gallbladder, positive Ortner, Murphy, frenicus, Kehr symptoms.

Cholestasis: jaundice, skin pigmentation, xanthoma, xanthelasma.

Inflammation: fever, skin hyperestesia in right hypochondria and under the right scapula.

LABORATORY FINDINGS

For patients with uncomplicated cholelithiasis, blood work results usually are normal. However, labs can detect complications of gallstone disease; complications might alter the course of treatment.

- CBC

- Chemistry panel, including electrolytes, liver enzymes, and bilirubin.
    - Choledocholithiasis can manifest with only elevation of serum alkaline phosphatase or bilirubin.
    - Nearly 50% of patients with symptomatic gallstone disease will have abnormal transaminases.
- Serum lipase and amylase levels are helpful in cases of diagnostic uncertainty or suspected concurrent pancreatitis.

IMAGING STUDIES

X-Rays : Approximately 15% of gallstones are radiopaque and can be visualized on plain x-ray. A porcelain gallbladder (heavily calcified) should be removed surgically because of increased risk of gallbladder cancer.

Other causes of abdominal pain diagnosed with the assistance of x-rays include perforated viscus, bowel obstruction, calcific pancreatitis, and renal stones.

Ultrasound (US) is the most sensitive and specific test for the detection of gallstones. US provides information about the size of the common bile duct and hepatic duct and the status of liver parenchyma and the pancreas. Thickening of the gallbladder wall and the presence of pericholecystic fluid are radiographic signs of acute cholecystitis.

CT scanning often is used in workup of abdominal pain without specific localizing signs or symptoms. CT scanning is not a first-line study for detection of gallstones because of greater cost and the invasive nature of the test. When present, gallstones usually are observed on CT scan.

ULTRASONOGRAPHY EXAMINATION:

denotes gallstones by the acoustic shadow due to absence of reflected sound waves behind the gallstone

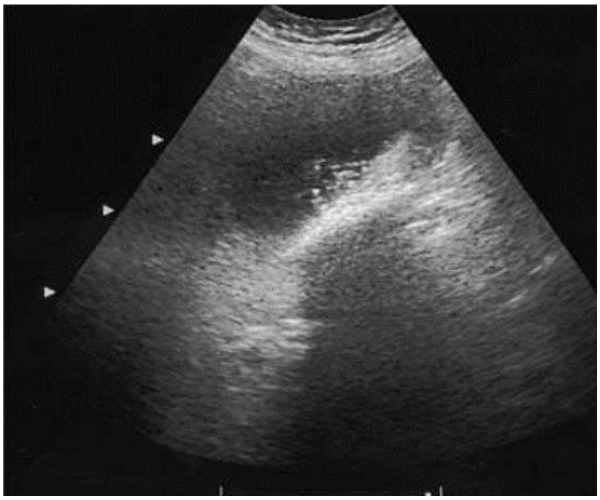

Figure 9 – USG finding in Gall stone disease

SPECTRUM OF GALLSTONE DISEASE:

Symptomatic cholelithiasis can be a herald to:

- An attack of acute cholecystitis
- Ongoing chronic cholecystitis
- May also resolve

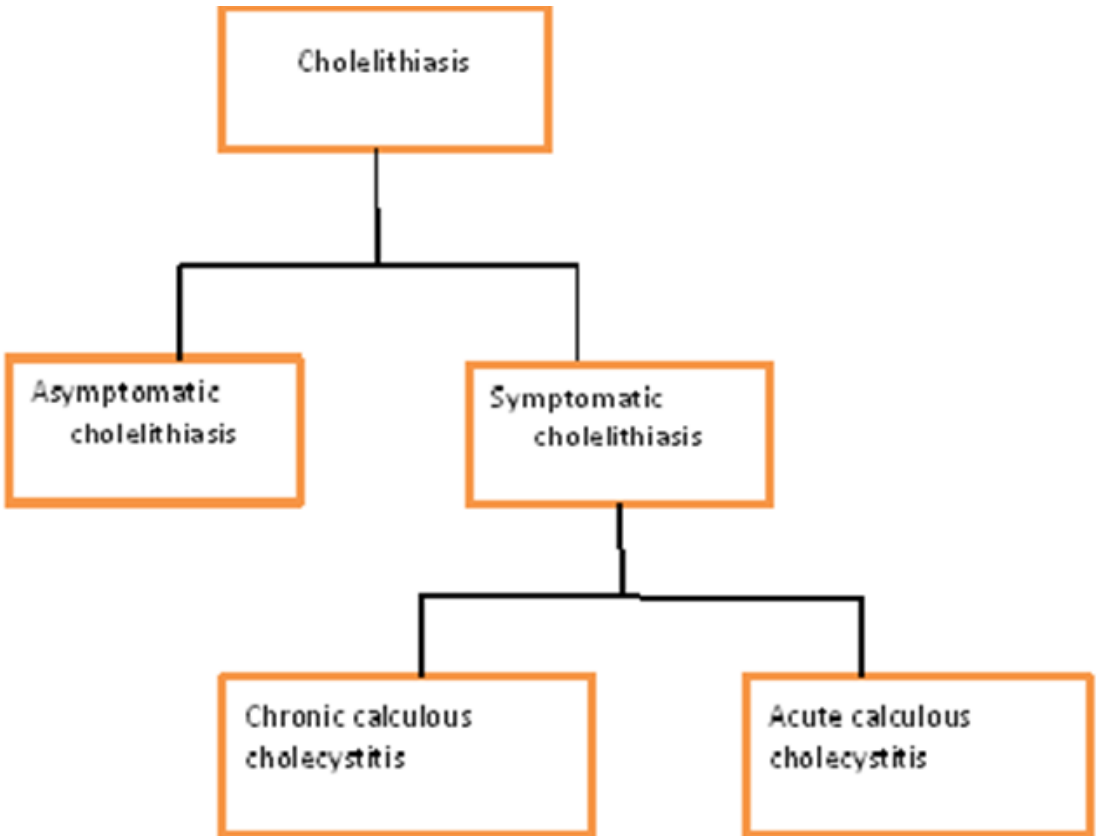

Figure 10 – Categorisation of Cholelithiasis

TREATMENT:

Removal of the gallbladder laparoscopic cholecystectomy is the treatment of choice for symptomatic gallbladder disease. Only gallstones that cause symptoms or complications require treatment.

There is generally no reason for prophylactic cholecystectomy in an asymptomatic person unless.

- the gallbladder is calcified
- gallstones are > 3cm in diameter

Laparoscopic Cholecystectomy:

MiniLap graspers used in laparoscopic cholecystectomy to grasp dome of gallbladder and dome of infundibulum. May include

- Single Incision Laparoscopic Cholecystectomy.
- Single Incision Laparoscopic Cholecystectomy with Two Umbilical Trocars.
- Two Port Laparoscopic Cholecystectomy.

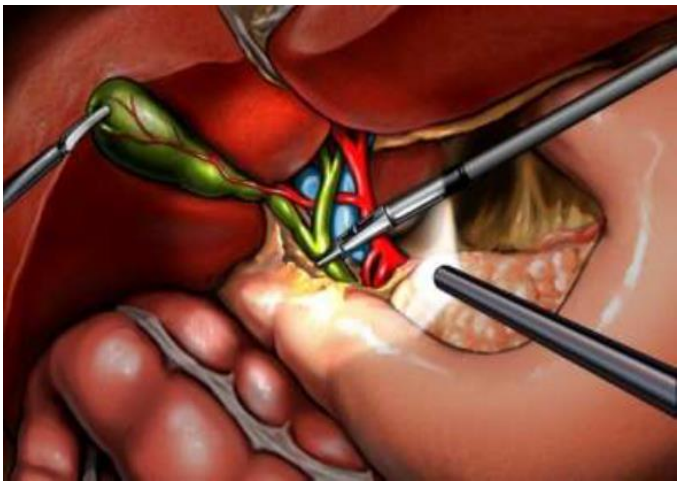

Figure 11 – Laparoscopic Cholecystectomy

CHOLECYSTECTOMY

In 1743, Jean Louis Petit did first cholecystostomy for gall stones. Until 1882, cholecystostomy was the procedure used to be practiced for gall stones. In 1882, Carl von Langenbeck did first cholecystectomy. In 1985, Prof Erich Muhe of Boblingen from Germany did first laparoscopic

cholecystectomy which he presented in congress of German surgical society. In 1987, Mouret of Lyon, France reported first laparoscopic cholecystectomy. Jacques Perissat (France) presented first paper on laparoscopic cholecystectomy.

Indications:

- Gallstones—symptomatic
- Cholecystitis—acute, chronic
- Acalculous cholecystitis
- Empyema gallbladder
- Mucocoele of gallbladder.

Prophylactic cholecystectomy is done in diabetic patients; congenital haemolytic anaemia; patients who has underwent bariatric surgery; while giving intraarterial chemotherapy through hepatic artery.

APPROACHES:

Open method:

Different incisions are—Right subcostal incision (Kocher's); Kehr hockey stick modification (medial end is extended upwards towards xiphisternum); upper midline incision; right paramedian; horizontal incision; Mayo-Robson incision. Right subcostal incision is commonly used.

Open approach is done when laparoscopic approach is not possible; or during laparoscopy difficulty arises and so conversion has been done to open method (failed laparoscopy); or if patient is not fi t for general anaesthesia or for pneumoperitoneum.

Laparoscopic Approach:

Laparoscopic cholecystectomy has become standard and ideal.

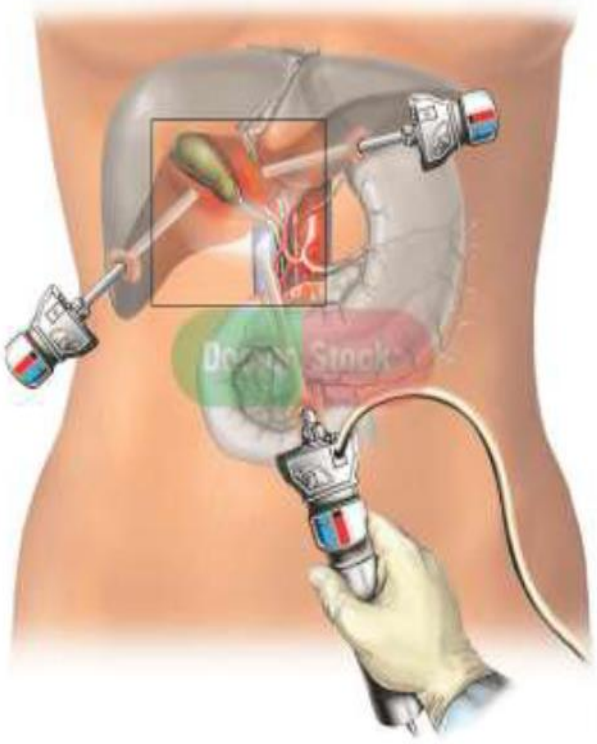

Figure 12 – Depiction Of Laparoscopic Cholecystectomy

PREOPERATIVE PREPARATION:

Evaluation by liver function tests, prothrombin time, diabetes, ultrasound (CBD should be assessed properly including its diameter); CT scan when needed to assess the biliary tree, pancreas; gastroduodenoscopy if hiatus hernia, oesophageal varices (portal hypertension) is suspected.

If CBD stone is present, ERCP and stenting should be done prior to laparoscopic cholecystectomy. If open cholecystectomy is planned then CBD exploration should be planned in these patients. Preoperative antibiotics are also necessary in cholecystectomy. Cardiac and respiratory assessment is required in these patients. Heparin as aprophylaxis for deep vein thrombosis may be a need.

TECHNIQUE OF OPEN CHOLECYSTECTOMY:

General anaesthesia or epidural anaesthesia is used. Nasogastric tube and urinary catheterisation is passed. Patient is in supine position. C-arm image intensifier/fluoroscopy should be kept in place or cassette changer top should be placed underneath the patient at the level of surgical fi eld. Preoperative antibiotics are given – tazobactum or amoxicillin as common organisms are Escherichia Coli and Streptococcus faecalis.

Heparin should be injected subcutaneously to prevent formation of deep venous thrombosis. Cleaning of entire abdomen is done; draping is

done in the incision area. Surgeon stands on the right side; two assistants on left side; during retraction of right costal margin, if needed, one assistant may have to come to right side towards left of the operating surgeon.

INCISION:

Right subcostal incision is used – 2 cm below the right costal margin. Skin, subcutaneous tissue, external oblique, internal oblique and transverse abdominis muscles are cut (transverse abdominis is often thin and can be split also). Medially rectus sheath is incised up to the midline; a haemostat is passed underneath the rectus muscle and is cut using monopolar cautery. 9th intercostal nerve is divided where it emerges lateral to the rectus muscle border. It causes only slight skin hypoesthesia but not muscle weakness (if possible it can be retained). Vessels underneath should be fulgurated. Rectus muscle after dividing will not retract in upper abdomen due to presence of tendinous intersections. Peritoneum is opened. Peritoneal cavity is explored. Oesophageal hiatus, gallbladder, pancreas, duodenum should also be palpated.

RETRACTION:

Right hand is placed between liver and diaphragm to retract liver downward and allow air to enter above the liver causing downward displacement of liver to make gallbladder more visible. Often colon and duodenum may be adherent to the gallbladder, they should be dissected by

sharp and finger dissection. Colon is pushed downwards; duodenum and stomach medially. Care should be taken not to injure the colon or duodenum.

Moist mops are kept on the colon and it is retracted downwards using a Deaver's retractor; another moist mop is kept on the stomach and duodenum and is retracted medially towards left using another Deaver's retractor.

Right costal margin isretracted upwards using an abdominal wall Morris retractor with a moist mop underneath. Liver is often retracted upwards from left of the falciform ligament or medial to the gallbladder using another Deaver's retractor.

Often gallbladder is very tense due to mucus or pus or bile which needs aspiration so that gallbladder is made easier to hold and handle.

Two Methods Used for Cholecystectomy:

Duct—first method (calot's first method):

Here Calot's triangle is dissected. Cystic artery is identified and ligated. Cystic duct is ligated close to the gallbladder. Gallbladder is separated from gallbladder fossa and removed. Haemostasis is maintained. Usually Calot's first method is used.

Fundus—first method:

It is done in difficult gallbladder due to dense adhesions. Fundus is separated from the liver bed. Dissection is carried proximally until cystic duct and cystic artery are identified, which are then ligated.

CALOT'S FIRST (CONVENTIONAL; BELOW UPWARD) METHOD:

Fundus of the gallbladder is held with Babcock's or sponge holding forceps or gallbladder holding forceps. Traction is given to fundus upwards and towards right (outwards). Hartmann's pouch is held with another Babcock's or sponge holding forceps and traction is given downwards and outwards.

In the Calot's triangle, peritoneum is incised using long curved scissor to expose the cystic duct and artery. With fi ne dissection using peanut (pledget in haemostat), haemostat and right angle clamp

Calot's triangle is dissected to identify the cystic artery, cystic duct, and junction where cystic duct joining the common bile duct. Cystic duct lymph node of Lund will be seen in Calot's triangle lying on the top of cystic artery.

Two silk or vicryl ligatures are passed around the cystic duct. Any stones in the cystic duct are felt and milked proximally into the gallbladder.

Entire length of the cystic duct is dissected using sharp and finger dissection. Ligature close to the Hartmann's pouch is ligated.

TECHNIQUE OF CYSTIC DUCT CHOLANGIOGRAPHY:

Distal ligature is not ligated instead is held apart with stretching. Cystic duct is incised near its beginning close to the gallbladder just distal to the tied ligature. A fine plastic tube (or infant feeding tube no 5-8 or ureteric catheter) or cholangiogram catheter (which has got a bead at the tip) of 2 meter length with 50 mL syringe filled with dilute sodium diatrizoate (1:1 with normal saline; 25 mL saline + 25 mL dye).

Entire syringe and plastic tube should be devoid of air bubble—very important. Plastic tube negotiated across the cystic duct for 5 mm close to bile duct. Narrow lumen or valve of Heister in the cystic duct may cause difficulty in negotiating the tube. Distal ligature is tied on the catheter with cystic duct. Bile should not be aspirated into the tube to prevent air bubble formation; if surgeon wants to confirm that tip is inside the lumen gently small quantity can be aspirated with care.

Left side of the theatre table is tilted upwards for 10 cm to avoid superimposition of the bile duct image with vertebrae. If C-arm is available, dye is injected and under fluoroscopy guidance; bile duct is visualized and also flow into the duodenum. A radiolucent area is identified as common bile duct stone.

Often dye will not enter the duodenum due to sphincter spasm; in such situations small dose of nitroglycerin or 1 mg of glucagon intravenously will relieve the spasm to allow the dye to pass into the duodenum. Even with this, dye has not entered the duodenum, then bile duct exploration is indicated. Findings to be observed in the cholangiogram are—diameter of the bile duct, any radiolucent area in the biliary tree, status of hepatic ducts, smooth tapering at ampulla of Vater and free flow of dye into the duodenum.

Cystic duct cholangiogram ensures and confirms ductal anatomy, may aid in limiting the ductal injury (even though it may not prevent) and decides need for common bile duct exploration.

If C-arm image intensifier is not available, initially 4 mL of dye is injected to take first X-ray film; 2nd X-ray film is taken after injecting remaining part of the dye.

INTRAOPERATIVE CHOLANGIOGRAM:

Cystic duct cholangiogram—commonly used

- Real time fluoroscopy—ideal
- Static film technique—two films should be taken
- Needle technique in laparoscopic cholecystectomy percutaneous needle is used

Gallbladder cholangiography

Infundibular approach (Kumar's)

Precautions while doing cystic duct cholangiography are—avoiding injecting more quantity and more concentrated contrast material as this will cause missing of small calculi and duodenum will be flooded with dye; air bubble should be avoided compulsorily; entire biliary system should be visualised including hepatic ducts; correction of sphincter spasm by drugs while doing the study if needed.

Cystic artery is doubly ligated using silk and divided. Usually cystic artery is above the level of cystic duct and in slight posterior plane than the cystic duct. Cystic artery is in close proximity to liver. Cystic artery should be adequately dissected for about 1 cm using right angled Mixter instrument; often its branches may need to be ligated independently.

Any variations, if possible, should be kept in mind. In adhesions, dissection of artery may take time; as far as possible artery should be dissected and ligated. Occasionally, in dense adhesions where dissection is not possible, then mass ligation may be done if it seems mandatory; but it carries higher chance of ligating or injuring right hepatic artery or hepatic duct.

While dissecting the cystic artery, artery may get torn inadvertently; then blind clamping the area with haemostat should be avoided; instead a

mop is kept in the place with pressure and after proper retraction and lighting, mop is gently removed to identify the bleeding proximal end of cystic artery which is held with right angle and carefully ligated.

Often in such situation, bipolar cautery fulguration helps in controlling the bleeding. If identification of cut end of cystic artery is still difficult, the incision should be extended medially or upward medially towards xiphisternum to get adequate exposure. Too large a cystic artery, if present, it is unlikely to be; rather it could be right hepatic artery (3 mm or more in diameter).

Cystic artery should be ligated first to allow subsequent Calot's dissection easier; to make cystic duct straight by eliminating the convolutions; to avoid its tearing due to traction on the gallbladder. Accessory cystic artery may be present in anomalous position. Bile duct should not be extensively dissected unnecessarily; but cystohepatic junction should be delineated properly. Monopolar cautery should be avoided close to bile duct; bipolar cautery is suitable.

Once bile duct exploration is not under strategy, then cystic duct is doubly ligated proximally after removing cholangiogram catheter. Simple ligature is placed using vicryl or silk close to cystohepatic junction; just distal to its transfixation suture is placed. Cystic duct is divided. It is ideal to place ligatures and then divide the cystic duct rather than clamping,

dividing and then ligating the duct. Many feel that cystic duct ligation is done only after complete mobilisation of the gallbladder from liver bed. However, if dissection is adequate in Calot's and assured then cystic duct can be ligated safely prior to dissection of gallbladder from liver bed.

DISSECTION OF THE GALLBLADDER FROM THE LIVER BED:

Usually it is easier to dissect the gallbladder from the liver by giving traction to fundus and Hartmann's pouch. Cystic duct stump is held with a haemostat to give outward traction to make peritoneal fold between gallbladder and liver stretched and fold is incised to proceed with the dissection. Dissection should be close to the gallbladder wall without injuring the liver tissue.

Dissection begins from Calot's area towards fundus. Dissection is done using scissor or monopolar cautery or using peanut. Plane of dissection is deep to mucosa of the gallbladder. All small bleeders from the liver surface should be fulgurated. Care is taken to look for direct entry of accessory duct from liver to gallbladder; if present, should be ligated securely to prevent bile leak. After removal of gallbladder, mop is kept in place for few minutes.

Often gelfoam or surgical may be kept to prevent oozing from the liver bed. Occasionally under running of the bleeding point using 4-0 vicryl may be useful. Catching with a haemostat and ligating should not be attempted in liver bed.

Complete haemostasis is achieved. Common bile duct is again palpated for confirmation that stones not missed. It is done by palpating supraduodenal portion of the common bile duct and also pancreatic portion by placing fingers behind the second part of the duodenum. Mops are removed; counts should be checked.

Tube or Jackson Pratt closed suction drain is placed when there is acute cholecystitis or in difficult gallbladder surgery. Abdominal wall is closed as single or two layers using vicryl. Skin can be approximated using stapler or nylon.

FUNDUS FIRST METHOD (ABOVE DOWNWARD):

Here after opening the abdomen and exposure with proper retraction instead of dissecting the Calot's, fundus of the gallbladder is held with sponge holding forceps. Using sharp scissor or monopolar cautery, fundus and body of gallbladder is dissected from its liver bed from fundus, to begin with, towards neck and Calot's triangle.

Blunt dissection using peanut or suction apparatus is very useful. One should avoid entering the parenchyma of liver. It is done in difficult cases wherein Calot's is difficult to dissect to begin with. Fundus first method ensures that common bile duct and hepatic ducts will not be injured. But oozing from the liver bed makes further dissection cumbersome.

Once Calot's is reached, cystic artery is ligated and divided. Cystic duct is ligated doubly after doing cystic duct cholangiography by similar method.

Postoperative Care:

Nasogastric tube is removed within 24 hours unless there is paralyticileus. In infected gallbladder, antibiotics are continued. Drain when kept is removed in 4 days.

PRECAUTIONS:

- Size of the common bile duct should not be overestimated; often it is small enough to mimic the cystic duct and inadvertent ligation by mistake is known to occur. Kinking at cystohepatic junction due to traction can cause clamping the junction with bile duct partially and completely.

- Anomalies of cystic duct (short or long; may enter right hepatic duct), of right hepatic duct; presence of accessory ducts should be remembered.
- Cystic artery while ligating care should be taken of variations of right hepatic artery and chances of ligating the right hepatic artery. Any artery if more than 3 mm in size, one should suspect it as right hepatic artery.
- Cystohepatic junction should be identified and dissected prior to ligation of the cystic duct. Caterpillar hump of right hepatic artery is known to exist and easily gets injured while dissecting.
- Dissection of gallbladder from liver bed often may be troublesome and is also should be meticulous. It is often observed that surgeon relaxes while dissecting the gallbladder from its bed once Calot's is addressed properly.
- Blind clamping and ligation should not be undertaken. If needed, incision should be extended accordingly. Cystic duct cholangiogram ideally should be done in all cases even though it is not practiced by many surgeons routinely.
- Cholecystitis in pregnancy: Ideally if possible cholecystectomy should be postponed and is done 3 months after delivery. If there is Specimen of gallbladder with

cholesterol stone. Secondary choledocholithiasis or if it becomes inevitable then ideal timing to do surgery is 2nd trimester. Cholecystectomy is safer. But if bile duct exploration is needed cholangiogram whether to be done or not is a debate. It is said that X-ray is safer after 1st trimester.

- Even then uterus shield should be used if cholangiogram is done. Bile duct stone after choledochotomy can be removed using choledochoscope. CT imaging should be avoided in pregnancy. Ultrasound is the main imaging modality in pregnancy. MRI is safer. Laparoscopic cholecystectomy is becoming an accepted method in 2nd trimester but needs expertise; open trocar placement; low pressure surgery and keeping PCO2 less than 40 mm Hg are the essential measures to be taken.
- Fetal monitoring during surgery, obstetrician help and follow up fetal monitor in immediate post-operative period is needed.

CHOLECYSTECTOMY IN CIRRHOTIC PATIENTS:

In cirrhotics, selection of the patient is important. Liver will be nodular. All adhesions are vascular and often bleed profusely. Dissection of the gallbladder from the liver bed may create technical problems. Preoperative preparation should be adequate like—injection vitamin K;

fresh frozen plasma transfusion, platelet transfusions, antibiotic therapy. Even then laparoscopic cholecystectomy is better than open approach in cirrhotics. Fundus first method should be used in cirrhotics; harmonic scalpel or other energy sources should be used. Doing partial cholecystectomy by ligating at the of Hartmann's pouch is much safer than dissecting Calot's.

Often conversion is needed; still often limiting the procedure towards cholecystostomy may be a compulsion due to technical hurdles. In cirrhotics, one should avoid placing T tube as localisation and track formation is poorer and chances of biliary peritonitis are higher. If at all. T-tube is placed then critical postoperative care is needed.

T tube should be removed only after 4 weeks. Profuse bleeding from the liver bed in cirrhotics is a real matter of concern.

Often it is difficult to achieve adequate required haemostasis and keeping a pack/mop in the bed to achieve compression for 24-48 hours is the only choice; such packs should be removed in 48 hours under anaesthesia gently with all precautions. Posterior part of the gallbladder can be left alone like that removing only remaining parts. Mucosa of such left out difficult gallbladder is ablated using ideally argon laser or bipolar cautery.

Opening of the cystic duct is invaginated using absorbable 3-0 suture material like vicryl.

In patients with coagulative disorders, cholecystectomy should be done by keeping ready adequate number of units of frozen plasma or platelets or factor VIII depends on the type of coagulopathy patient harbours.

COMPLICATIONS:

Injury to colon, duodenum and mesentery can occur whenever there are adhesions.

Bile leak:

It is the most distressing complication surgeon faces. Small bile leak through the drain is probably due to either cystic duct stump disruption or accessory duct leak. Usually it is 100-200 mL/day. It usually subsides in 7-10 days. If leak persists ERCP and stenting is needed; it also identifi es the bile duct and hepatic duct status. If bile leak is more than 400 mL/day it means probably it is bile duct or hepatic duct injury. It needs proper evaluation with CT scan/MRCP.It is addressed only at a later period once inflammation subsides after 3 months. Nutrition during this period should be maintained adequately. It needs expertise work and needed proper identification, dissection of biliary tree and hepaticojejunostomy.

Post-cholecystectomy jaundice may be due to cholangitis, missed bile duct stones, bile duct stricture due to trauma or ligation of bile duct or hepatic duct. ERCP, liver function tests proper imaging to identify the problem are needed to plan the eventual surgical correction.

Post-operative bleeding is not common. But can occur if cystic artery ligature is slipped or if there is sepsis at the gallbladder bed.

Occasionally it could be due to **anomalous right hepatic artery** which is injured due to inability to identify the vascular anomaly. Infection may cause hepatic artery pseudoaneurysm which may often be lifethreatening. It needs CT angiogram and intervention radiology to address the feeding artery of the pseudoaneurysm by glue or other embolisation devices. However, inadvertent embolisation or gluing of the right hepatic artery may cause acute fulminant hepatic necrosis of the liver and may kill the patient. Hogarth Pringle's manoeuvre is ideal to control bleeding on table by applying pressure using thumb and fingers over the Foramen Winslow.

Sepsis: Subphrenic, pelvic, hepatic abscess can occur occasionally when there is cholangitis, immunosuppression or if surgery done in a badly infected gallbladder. Blood culture, blood count, clinical assessment is a must. Higher generation antibiotics should be started to prevent them going for septicaemia. Abscess can be addressed usually by image guided catheter drainage or aspiration. Open drainage is needed only rarely.

Biliary stricture can occur as late sequelae after cholecystectomy due to bile duct injury. 80% of biliary strictures are post-operative. After cholecystectomy [open or laparoscopic, more common following laparoscopic (0.8%) than open method (0.35%)]. Clinical features are: Obstructive jaundice, pain abdomen, features of ascending cholangitis, profuse persistent bile leak.

Waltmann Walter syndrome – It is compression of IVC due to collection of fluid in gallbladder fossa and subphrenic space after cholecystectomy; it mimics coronary artery thrombosis.

Bismuth classification of biliary stricture

I. Low CBD stricture with stump (proximal to stricture) >2 cm

II. Middle CBD stricture with proximal stump <2 cm

III. Hilar. Confluence of right and left hepatic ducts is intact

IV. Separated right and left hepatic ducts

V. Stricture involving intrahepatic ducts

Causes of post-operative stricture:

- Too much traction to the GB during ' Fundus first cholecystectomy'
- Blind application of artery forceps in Calot's to control bleeding

- Anomalies of biliary tree
- Inflammatory adhesions at Calot's triangle
- Injury during other surgery like gastrectomy

Management: LFT, prothrombin time, ERCP, CT, MRCP; choledochoduodenostomy or choledochojejunostomy.

MINILAPAROTOMY CHOLECYSTECTOMY:

Here incision of 5 cm length is made lateral to the right rectus in subcostal region. It is done in olden days in thin individuals where easy retraction makes Calot's dissection possible. In present era of laparoscopic surgery, minilaparotomy cholecystectomy or any other surgeries should not be done.

When open surgery is done, adequate incision with adequate exposure of the dissecting surgical field should be the approach. Hence minilaparotomy has become a surgical legacy.

PARTIAL CHOLECYSTECTOMY:

It is done in severely adherent Calot's triangle difficult for dissections and biliary tree cannot be delineated. It is also useful in cirrhosis with portal hypertension. Here gallbladder is removed distal to the Calot's triangle at Hartmann's pouch level. When there is dense adhesion in the Calot's and dissection is impossible, it is better to do partial

cholecystectomy than injuring the bile duct or hepatic artery to lead into dreaded complications. Fundus first dissection is done carefully. When reaching towards the Calot's, care should be taken not to meddle and create complications. Hartmann's pouch is held with Babcock's forceps. Tissue just behind the Hartmann's is ligated and divided. Hartmann's pouch is divided. Gallbladder is removed. Any stones in the Hartmann's are removed; warm saline wash is given. Usually in such situations, cystic duct already sealed without communicating into the bile duct. Mucosa of the retained part of the Hartmann's pouch is fulgurated using bipolar cautery or argon laser. Hartmann's pouch on the cystic duct side is transfixed and ligated using vicryl.

SUBTOTAL CHOLECYSTECTOMY is a similar procedure but it is divided still further away from the Hartmann's pouch at the beginning of the body of the gallbladder. Technically there is hardly any difference.

LAPAROSCOPIC CHOLECYSTECTOMY

Laparoscopic cholecystectomy is the standard technique for removal of gallbladder.

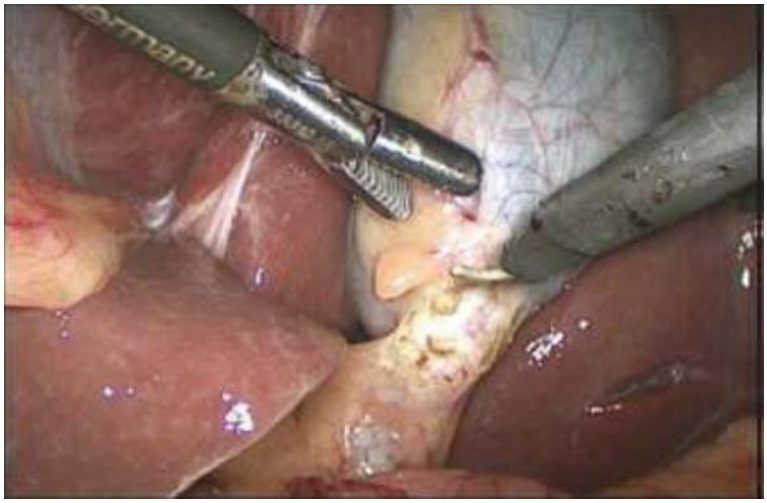

Figure 13 – Laparoscopic dissection of Calot's triangle

INDICATIONS

- Symptomatic gall stone diseases – pain, mucocoele, cholecystitis acute or chronic.
- Acalculous cholecystitis.
- Asymptomatic gall stones—when patient is undergoing bariatric surgery, diabetics, renal transplantation, haemolytic diseases, in children.
- Complications of the gall stones like gall stone ileus, empyema, and perforation also can undergo laparoscopic cholecystectomy; but conversion rate may be more. If during laparoscopic procedure, when dissection becomes difficult surgeon should not be hesitant to convert into open cholecystectomy to avoid serious complications.
- Gall stone dyspepsia where biliary pain is present without the presence of gall stones. Here patient may get relieved of the symptoms by

laparoscopic cholecystectomy. Biliary dyskinesia may be an indication.

- Gall bladder polyps—if polyps are multiple, if they are increasing in size, if size is more than 1 cm then laparoscopic cholecystectomy is indicated.

CONTRAINDICATIONS:

There is a debate about absolute contraindication for laparoscopic cholecystectomy. Patients who are unfit for general anaesthesia are contraindications. In patients with portal hypertension and coagulopathy laparoscopic cholecystectomy still be better than open but adequate precautions should be taken including correction of coagulopathy, need for plasma or platelet transfusions.

Proven gallbladder cancer is a sure contraindication for laparoscopic cholecystectomy. Multiple earlier laparotomies, severe dense adhesions in cholecystitis, acute pancreatitis, anomalies in the biliary system, cirrhosis, pregnancy, obesity are the real challenging situations while doing laparoscopic cholecystectomy.

Need for a conversion and non-hesitant to convert at right time prior to creation of critical problem are to be kept in mind by surgeons.

TECHNIQUE:

Patient is placed in 15º head up anti-Trendelenburg position with 15º right upward tilt. Probably this is done after insertion of the first camera port in supine position. Nasogastric tube should be passed to decompress the stomach. Urinary catheter is placed otherwise bladder should be emptied before patient is brought into the operation theatre table.

It is required in difficult cases or complications are suspected or when prolonged anaesthesia is needed. After clearing and draping, monitor is kept in the right up facing towards the table; surgeon stands on the left side; camera assistant on the left of the surgeon; scrub nurse on the right side near foot end of the patient. Ideally two monitors on right and left sides should be kept.

What is commonly practiced is North American approach with the position explained above. Radiolucent table is essential to do fluoroscopy whenever needed like on table cholangiogram.

INSTRUMENTS:

1. Veress needle
2. 10 mm port—2 in no.
3. 5 mm port—2 in no.
4. Maryland forceps.
5. Atraumatic (Universal) grasper.
6. Toothed grasper 5 mm.
7. Gallbladder extracting forceps 10 mm.
8. Clip applicator.
9. Suction irrigation.
10. Scissors.
11. Hook/spatula dissector.
12. Endo bag.

Alternatively patient may be kept in semilithotomy position with anti-Trendelenburg tilt; thighs should be kept parallel to ground. Surgeon stands in between legs. Port placements are almost similar but right hand working port is placed in the left hypochondrium to achieve triangulation.

This position is called as French or European position. Camera port (10 mm) is placed at upper umbilical crease line. But it can be placed at changed positions depending on a obesity, previous surgery. Open or

closed method can be used. A 10 mm working port is placed in the epigastrium just below the xiphoid process in the subcostal region.

After initially puncturing the rectus sheath, it is directed towards right to avoid entering through the falciform ligament. If it passes through the falciform ligament then bleeding may occur as falciform ligament is vascular; falciform ligament may act as a flap to interrupt proper functioning of the port. Third (5 mm) port is placed in the same level at midclavicular line; 4th lateral 5 mm port is placed at anterior axillary or still lateral depending on need.

Abdomen is inspected all round. Gallbladder is identified; presence of adhesions by omentum, colon, stomach and duodenum over the gallbladder is identified. Through lateral 5 mm port toothed instrument is passed and fundus of the gallbladder is held firmly and is pushed upwards towards diaphragm; this allows the opening and visualisation of the Calot's area.

If gallbladder is found tense then it is decompressed by aspirating the content using long needle passed directly through abdominal wall into the gallbladder under vision. Fine trocar and cannula also can be used but usually needle aspiration is sufficient. Release of adhesions is important. Through epigastrium port Maryland instrument is passed using a reducer. Through midclavicular port 5 mm non-toothed atraumatic grasper is

passed. Omentum is pulled down from the gallbladder by holding the edge using Maryland very close to the gallbladder. Cautery and blunt traction separates the omentum.

Similarly colon, stomach and duodenum are released from the field. Monopolar cautery may injure these structures due to heat generated and so should only be used judiciously. Once adhesions are released, infundibulum is held using the midclavicular port instrument. Infundibulum is held downwards and towards right.

Calot's triangle is dissected using Maryland forceps.

In infundibular technique, cystic duct is dissected carefully both in front and behind and traced towards infundibulum to visualise typical fl are when cystic duct joins the infundibulum and with a funnel shape. Cystic duct is further dissected from infundibulum towards the cystohepatic junction; i.e. from safety zone to danger zone.

Bipolar cautery should be used rather than monopolar cautery. Entire Calot's should be dissected including cystic artery. It should be made sure that only two structures enter the gland. Lymph node of Lund is a good marker to dissect cystic artery; node is anterior to the artery. Calot's dissection should not extend posterior to a constant landmark on the inferior surface of the liver—Rouvier sulcus. This oblique sulcus usually

is present in the liver; dissection should be in front of it; never extend posterior level of this landmark.

Another technique is ‘critical view of safety’ in which entire Calot’s triangle including Hartmann’s pouch is dissected off from the liver bed to identify the cystic duct and artery. Cystic artery is dissected using Maryland forceps; isolated. Any anomalies should be identified. Lymph node of Lund is a good marker to identify which is in front of the artery. Often anterior/superficial and posterior/deep branches are identified.

After dissection of entire Calot’s and to confirm that only two structures are entering the gallbladder cystic artery is clipped on both sides and divided with curved scissor. Harmonic scalpel can also be used to divide the cystic artery. Cystic artery is divided prior to cystic duct usually. Then cystic duct is clipped using 10 mm clip applicator through epigastric port. Two clips are applied proximally and one clip distally and divided in between.

Blind application of clip should be avoided; clip applied should be away from bile duct. Undue traction during clipping should be avoided. Before clipping gallbladder stone is milked into the gallbladder from the cystic duct if any stones are present.

Gallbladder is detached from the gallbladder using various instruments. Spatula with monopolar cautery is commonly used and safer.

Hook is also used; its bend is commonly used; to cut peritoneal edge sharp tip is hooked and fulgurated. Harmonic scalpel is an excellent tool for this. Hartmann's pouch is held using the 5 mm grasper (dolphin nosed) and alternatively changes its position according to its need.

Undue deeper fulguration may open up the liver tissue. It is safer to keep the instrument closer to the gallbladder than liver bed. When small peritoneal fold attachment is left near the fundus, bed is retracted to inspect the Calot's and gallbladder bed properly for the clip placement and oozing. Often gallbladder may get opened to spill the stones and bile. Bile should be sucked out properly and stones should be extracted or kept in an extracting bag kept in the peritoneal cavity.

Gallbladder is extracted through the epigastric port; 10 mm stout gallbladder holding instrument (claw-shaped) is used to hold at neck of the removed gallbladder; instrument with 10 mm port is removed with the gallbladder. Often port site rectus sheath and skin incision may need to be widened by few mm.

Gallbladder neck once comes out of the wound can be opened and suck out all bile. Stones in the gallbladder can be removed using an artery forceps to make its removal easier. Gallbladder can be held by passing curved Kocher's clamp through the epigastric port site wound after

removing the port temporarily. Retrieval bag can be used to remove the gallbladder and is probably ideal.

Once gallbladder is removed, gallbladder is checked for its opening and contents. Port is placed again. Gallbladder bed and Calot's triangle is checked again. Bed is irrigated and inspected for the final closure. Usually closure is not necessary. When there is difficulty in dissection or if sepsis is present, then drain is kept. A 14 French nasogastric tube is passed through the lateral 5 mm port is placed in gallbladder bed.

Key steps:

1. Place four standard ports.
2. Right up and head up position.
3. Maintain adequate traction on the gallbladder at all times.
4. Posterior dissection at Calot's triangle.
5. Cystic artery and duct are identified dissected properly from safe distal zone (infundibular) to danger proximal zone (CBD level)
6. Clip application after confirming anatomy.
7. Clip artery first then only duct.
8. Dissect gallbladder close to the wall.
9. Inspect gallbladder fossa.
10. Drain the gallbladder bed.

11. Fascial sheaths of all 10 mm port sites should be closed securely.

Trocars are removed, rectus sheaths are closed in 10 mm ports. Skin is sutured or sealed with cyancrylate glue.

The drain is removed after 24 hours and oral is started. The patient is mobilised as early as possible and discharged after 48 hours.

DIFFICULT GALLBLADDER:

The safest thing to do when the anatomy of the Calot's triangle is not defined is to convert to open surgery. Conversion should not be taken as a failure but as a wise decision in the interest of the patient. At times, the Calot's triangle is adherent and all planes are lost. In such cases, the suction irrigation device can be used as a blunt dissector and dissection can be done using jet of saline through the channel.

One should always be aware of the position of the duodenum and the CBD as they are likely to be damaged. Using a gauze piece to keep the field clean can be done provided utmost care is given and the same is retrieved at the end of the procedure. It is easy to lose track of the small piece and later struggle to find it.

Retrograde dissection can be done provided the surgeon is experienced but for the majority of us it may be safe to convert into an open

surgery. Subtotal cholecystectomy is an alternative which can be undertaken.

Additional instrument using additional port can be tried like fan liver retractor.

TROUBLE SHOOTING:

1. Difficulty in visualising the Calot's triangle
    - Reposition the patient.
    - Apply the traction cranially.
    - Clear the viscera away from the operative field.
    - Aspirate the Ryle's tube to deflate the stomach.
    - Apply lateral traction to the Hartmann's pouch.
2. Bleeding at Calot's triangle
    - Try to perform posterior dissection.
    - Apply compression using gauze piece and wait for 5 minutes.
    - Do not dissect the area blindly which you cannot visualise.
3. Short cystic duct
    - Confirm the anatomy.
    - Apply intra corporeal knots using silk or vicryl

4. Bleeding from liver bed

- Take the gallbladder on the spot and apply pressure
- keeping the gallbladder on it. Wait for 5 minutes.
- Stay close to the gallbladder while dissecting.

COMPLICATIONS OF LAPAROSCOPIC CHOLECYSTECTOMY:

Haemorrhage:

It can be from liver bed, slippage of cystic artery ligation or after formation of hepatic artery pseudoaneurysm.

Trocar site bleeding can also cause significant difficulty in controlling.

Spillage of stones:

They should be extracted carefully. Often it is left behind as stone gets migrated and it is difficult to pick up every stone. Such unidentified stone may cause real problem occasionally. It can cause abscess in subphrenic space or intra-abdominal region; can cause sinus formation, granuloma formation. It can erode various vital structures in the peritoneal cavity causing colocutaneous fistula, duodenal fistula, intestinal obstruction, major vessel thrombosis (middle colic artery), haematuria and septicaemia.

Biliary tree injuries are due to missed anatomy, over traction on the CBD, anomalies, adhesions, etc. If biliary tree (bile duct or hepatic duct) injury occurs conversion should be done. Type of injury is identified and classified. ERCP stenting may be tried on table. Otherwise choledochojejunostomy should be done. It is unwise to repair a completely transected common bile duct as stricture will develop eventually.

Injury to colon, stomach, and duodenum can occur. Complications specific to laparoscopy should be kept in mind.

Wound infection, incisional hernia can occur.

Pancreatitis, retained stones, biliary stricture are other complications.

METHODOLOGY

MATERIALS AND METHODS :

In this case-control study, patients who underwent cholecystectomy were divided into case and control groups. Case group consisted of patients who underwent cholecystectomy due to cholecystitis or cholelithiasis and the control group consisted of patients who underwent this procedure for incidental cholelithiasis . Participants included in this study were patients admitted to Govt Rajaji hospital at Madurai from November 2017 to September 2019.

Gallbladder tissue was taken from all patients immediately after cholecystectomy. The samples were immediately frozen at _80°C before processing for culture and DNA extraction was performed.

HISTOLOGICAL STUDY:

Gallbladder tissue specimens for histology were fixed in 10% buffered formalin immediately after cholecystectomy. The samples were then embedded in paraffin wax and 5-_m-thick histological sections were stained with hematoxylin and eosin for histological analysis.

The samples were examined by a pathologist who was unaware of their origin. The diagnosis of cholecystitis was based on the presence of mono- or mono- and olymorphonuclear inflammatory cells in the lamina propria, fusion of the mucosal folds giving rise to buried crypts of

epithelium, and the presence of Rokitansky-Aschoff sinuses. Gallbladder specimen stained with giemsa for Helicobacter species.

DNA ISOLATION:

Gallbladder tissue or bile DNA was extracted with a QIAamp DNA Mini kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's recommendations, with minor modifications. Briefly, approximately 25 mg of tissue and 500 _l of bile samples were suspended in 180 _l of lysis buffer (buffer ATL) and homogenized by vortexing.

A total of 20 ml of a proteinase K solution (20 mg/ml) was then added, followed by an overnight incubation at 56°C. A second lysis buffer (buffer AL) provided in the kit was added, and the sample was incubated at 70°C for 10 min. Next, 200 _l of ethanol was added; this mixture was then loaded on the QIAamp spin column and centrifuged at 6,000 _ g for 1 min.

The QIAamp spin column was placed in a 2-ml collection microtube, and the containing filtrate was discarded. The column material was washed twice (250 _l each time) with the first buffer (buffer AW1) and twice (250 _l each time) with the second washing buffer (buffer AW2) provided in the kit. Finally, the DNA was eluted with 100 _l of distilled water (2 _ 50 _l). The DNA concentration was determined by measuring the optical density at 260 nm.

PCR AMPLIFICATION WITH HELICOBACTER GENUS-SPECIFIC PRIMERS:

The 16S rRNA gene of the genus Helicobacter was amplified by a nested PCR assay. The outer primer pair (B37 and C70) (4) was used to generate 16S rRNA amplicons of approximately 1,500 bp. The nested inner primer pairs, which are specific for the Helicobacter genus, amplified fragments of 1,200 bp (primer pair C97 and C05) or 400 bp (primer pair C97 and C98) (3).

PCRs were performed in an Applied Biosystems thermal cycler in thin-wall tubes. A 10-_l amount of each DNA preparation was added to 100 _l of a reaction mixture containing 1% Taq polymerase buffer (50 mM KCl, 1.5 mM MgCl2, 10 mM Tris-HCl [pH 8.3]), a 0.5 _M concentration of each primer, a 200 _M concentration of each deoxynucleotide, and 2.5 U of Taq polymerase. The amplified product was identified by electrophoresis in a 1.0% agarose gel.

The DNA was stained with ethidium bromide and examined under UV light. In the second round, 1 _l of the PCR product was added to the reaction mixture. The sequences of the primers and PCR conditions are shown in Table 1. An Escherichia coli strain (clinical isolate) and a H. pylori strain (TX30A) served as negative and positive controls, respectively and distilled water was used as an internal reaction negative control.

16S rRNA GENE SEQUENCING:

The nested PCR products of 1,200 or 400 bp were purified using a Wizard PCR-Prep purification kit (Promega, Madison, Wis.) according to the manufacturer's directions. The purified amplicons were directly sequenced with an ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, Calif.) according to the manufacturer's instructions by using sequencing primers B35, B36, C01, C31, and X91 for the amplicons with 1,200 bp or C97 and C98 for those with 400 bp (3, 4).

The sequences were determined in an Applied Biosystems DNA automated sequencer (ABI PRISM 310; Applied Biosystems). The sequences were aligned using the CAP program at the INFOBIOGEN web server and compared (using the Blast Program at the National Center for Biotechnology Information computer server) with sequences listed in the GenBank database.

1] Kuruvammal 54 female who diagnosed as acute cholecystitis underwent open cholecystectomy. GB specimen sent for giemsa staining and PCR .

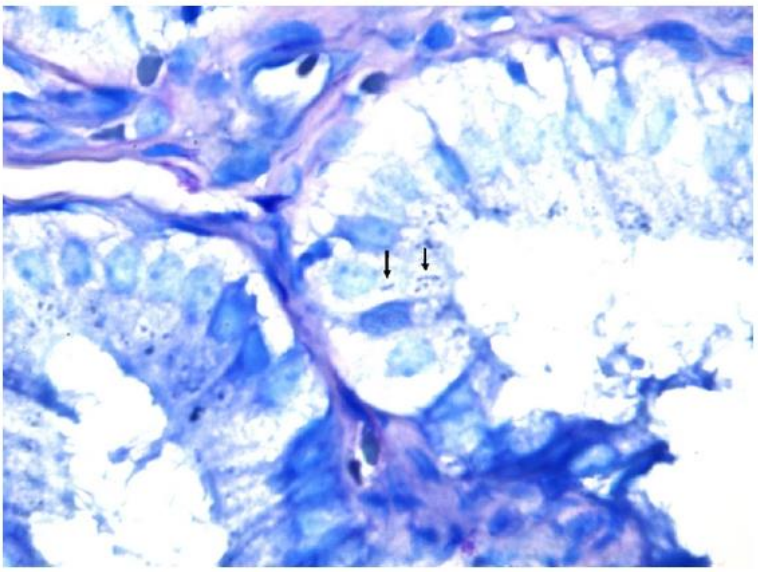

Figure 14 - Specimen positive for Helcibactor species with Giemsa stain.

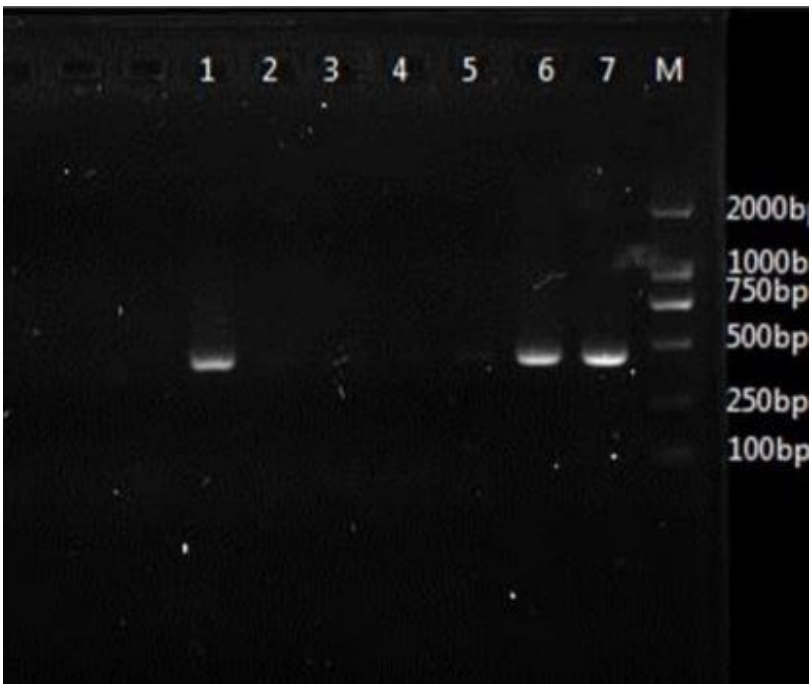

Figure 15 – PCR result of Kuruvammal 54 yrs / F

PCR product of helicobacter specific 16s rRNA gene from gall bladder and gastric mucosa sample. (lame M – step ladder marker ; 1- positive control of gastric biopsy derived h.pylori DNA ;2- negative control of gastric biopsy; 3-negative 16s rRNA gene: 4,5-negative for 16s rRNA gene in gall bladder in one individual patient: 6,7- positive from 16s rRNA gene in the study patient)

OBSERVATION AND RESULTS

Statistical analysis

All analysis were done using SPSS version 16(SPSS Inc., Chicago, IL). The clinical, demographic, diagnostic variables were compared for symptomatic and asymptomatic groups. Chi square test or Fisher's Exact Test was used to compare categorical variables.

Descriptive statistics was computed. Continuous data were tested for normality using Shapiro wilks normality test. Since the data levels were not normally distributed, a non-parametric test, [the Mann-Whitney U test] was used to compare age and BMI between groups. The confidence interval was set at 95%.

Table 1 - Comparison Of Variables Among Symptomatic And Asymptomatic Group

Fisher's exact test; shows (*p<0.05)

Variable		Symptomatic group N	(%)	Asymptomatic group N	(%)	p value
Diabetes	Absent	12	48.00%	10	40.00%	0.776
	Present	13	52.00%	15	60.00%	
USG Findings	Cholelithiasis	19	76.00%	0	0	
	Acute cholecystitis	6	24.00%	0	0	
Stone size	< 2.5	12	48.00%	16	66.70%	0.252
	> 2.5	13	52.00%	8	33.30%	
Wall thickness	Absent	17	68.00%	25	100.00%	0.004*
	Present	8	32.00%	0		
GB polyp	Absent	25	100.00%	21	84.00%	0.035*
	Present	0	0	4	16.00%	
LFT	normal	16	64.00%	19	76.00%	0.538
	Raised	9	36.00%	6	24.00%	
OGD sopy	No ulcer	19	76.00%	20	80.00%	0.5
	Ulcer present	6	24.00%	5	20.00%	
Type of surgery	Laproscopy	19	76.00%	23	92.00%	0.247
	Open surgery	6	24.00%	2	8.00%	

Demographic variables

Among the total of 50 patients included in the study, 25 symptomatic patients with cholelithiasis (76%) and acute cholecystitis (24%) and 25 asymptomatic patients were evaluated. There was no significant difference in the age of the subjects in the 2 study groups. The mean age of the Symptomatic patients was found to be 44.84± 6.5 (SD) yrs while asymptomatic patients averaged 46.4 ± 7.9 yrs (p = 0.386). In which Gender distributions were equivalent, with male/female distribution of 12/13 for the patients presenting with symptoms and 13/11 for the subjects in the asymptomatic group (p = 0.547).Similarly BMI also found to be similar in both the groups with mean BMI of 24.96±1.2 in symptomatic and 24.92±1.4 in asymptomatic patients (p=0.902)

Comparison of clinical and biochemical variables in study groups.

In symptomatic group, 52% patients were presented with stone size more than 2.5 cm and 33% in asymptomatic group but the difference was not statistically significant (p=0.252).But 32% patient in symptomatic group had wall thickness whereas none of them in asymptomatic group (p=0.04*). Furthermore LFT was found to be raised in 36% of symptomatic group and 24% in asymptomatic group (P=0.5). Although there was no significant difference in patients presented with ulcer between study groups (p=0.5). Among 50 patients (76 %) underwent

laparoscopic cholecystectomy for cholelithiasis/calculous cholecystitis in symptomatic group and only 8 patients underwent open surgery in which 6 of them were from symptomatic group. Among the asymptomatic group, 4(16 %) patients had gallbladder polyp but none of them in symptomatic group (p= 0.035*).

Table 2 - Association of the Presence of *Helicobacter* in Gallbladder Tissue with Asymptomatic and symptomatic group

	variables	Symptomatic group N	(%)	Asymptomatic group N	(%)	p value
Sex	Female	12	48.00%	13	52.00%	0.547
	Male	13	52.00%	11	44.00%	
GIEMSA staining	negative	17	68.00%	23	92.00%	0.074
	positive	8	32.00%	2	8.00%	
PCR	Absence of Helicobacter DNA in gall bladder tissue	15	60.00%	22	88.00%	0.06
	presence of Helicobacter DNA in gall bladder tissue	10	40.00%	3	12.00%	

Fisher's exact test; Not significant

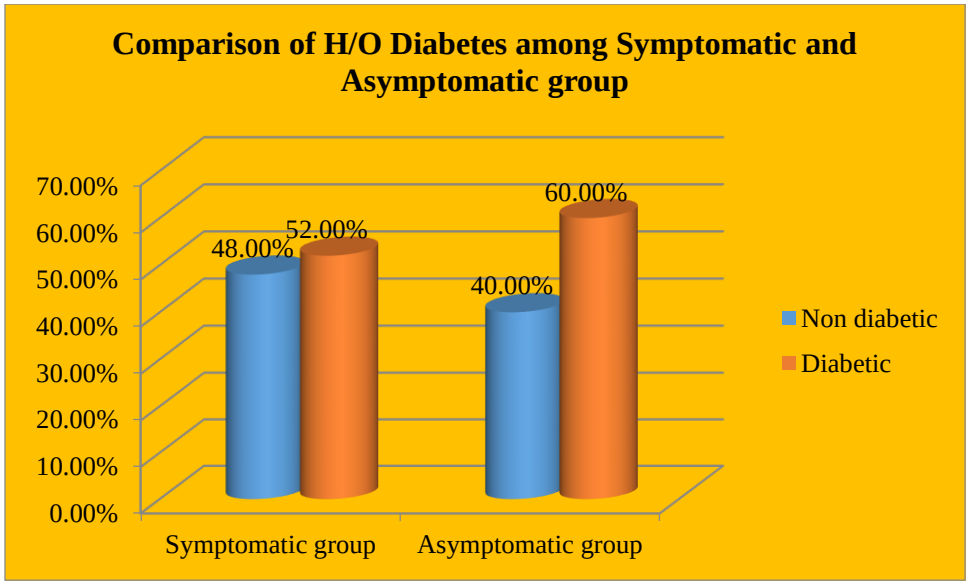

Chart 1 - Comparison of H/O Diabetes among Symptomatic and Asymptomatic group

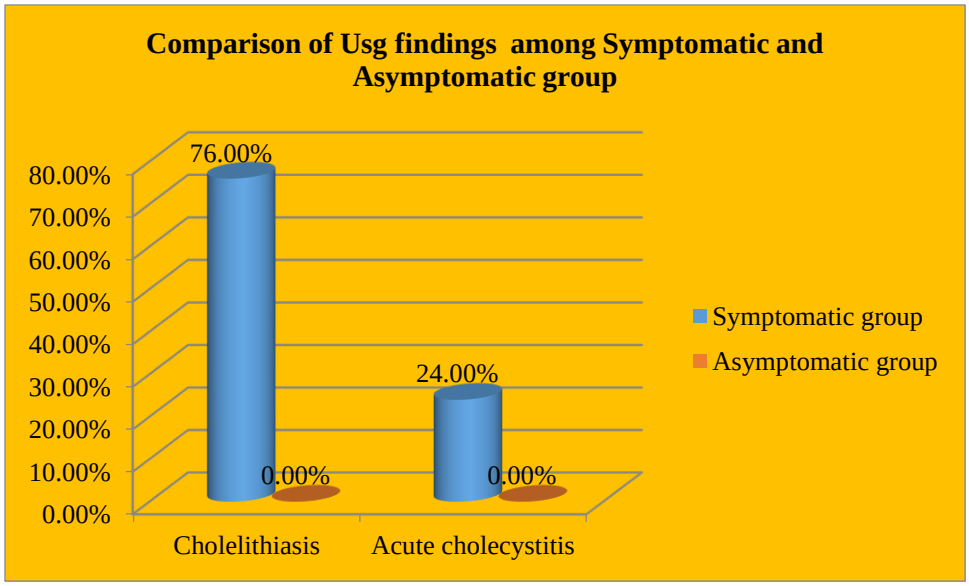

Chart 2 - Comparison of USG findings among Symptomatic and Asymptomatic group

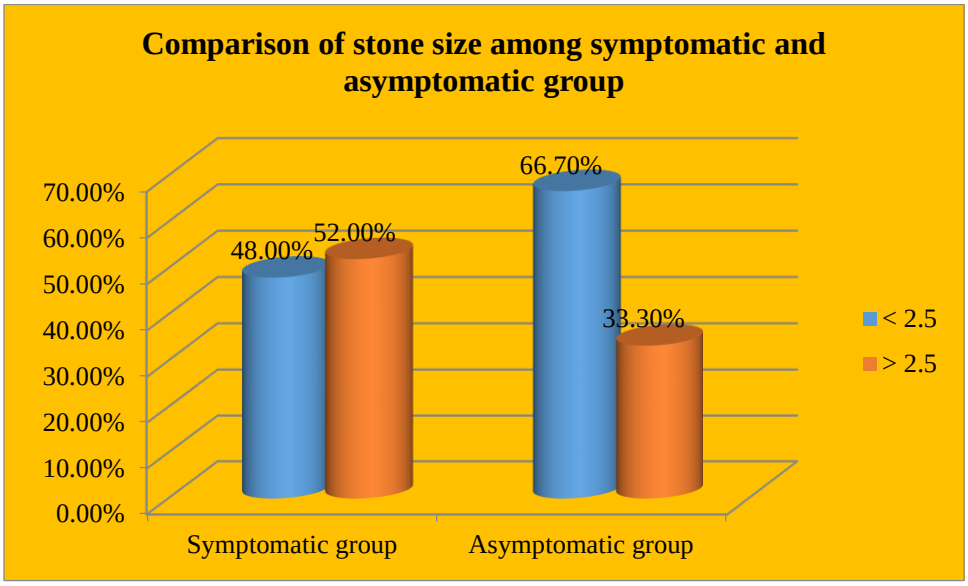

Chart 3 - Comparison of stone size among symptomatic and asymptomatic group

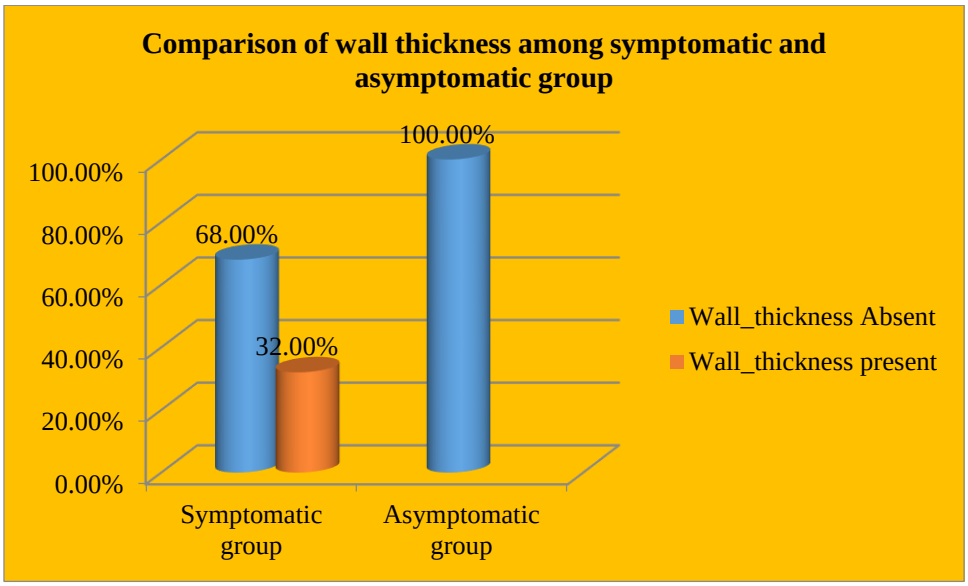

Chart 4 - Comparison of wall thickness among symptomatic and asymptomatic group

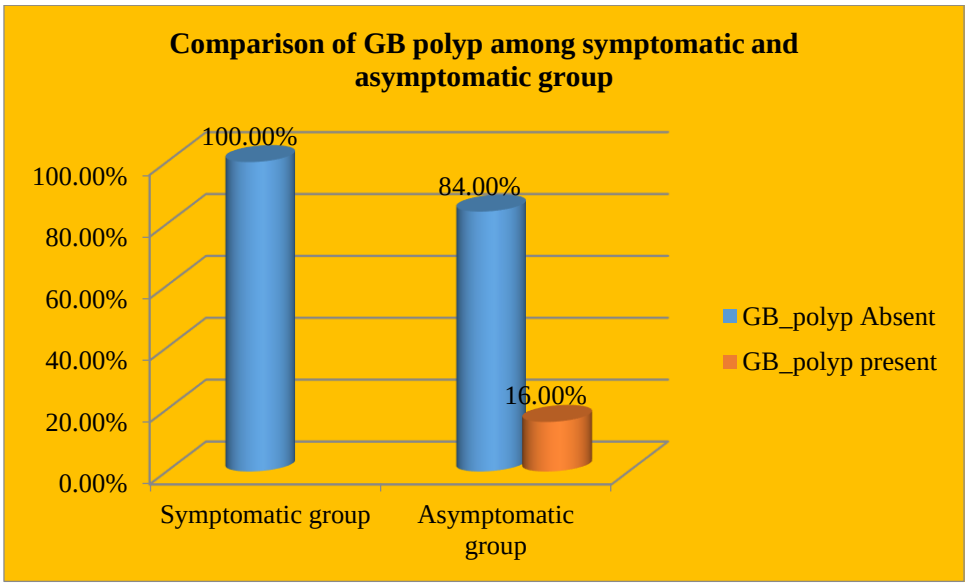

Chart 5 - Comparison of GB polyp among symptomatic and asymptomatic group

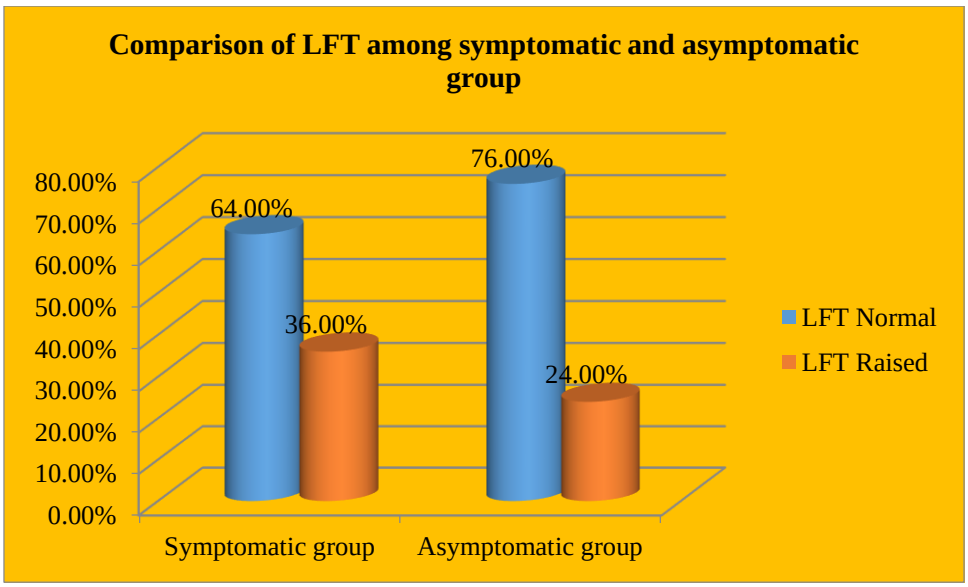

Chart 6 - Comparison of LFT among symptomatic and asymptomatic group

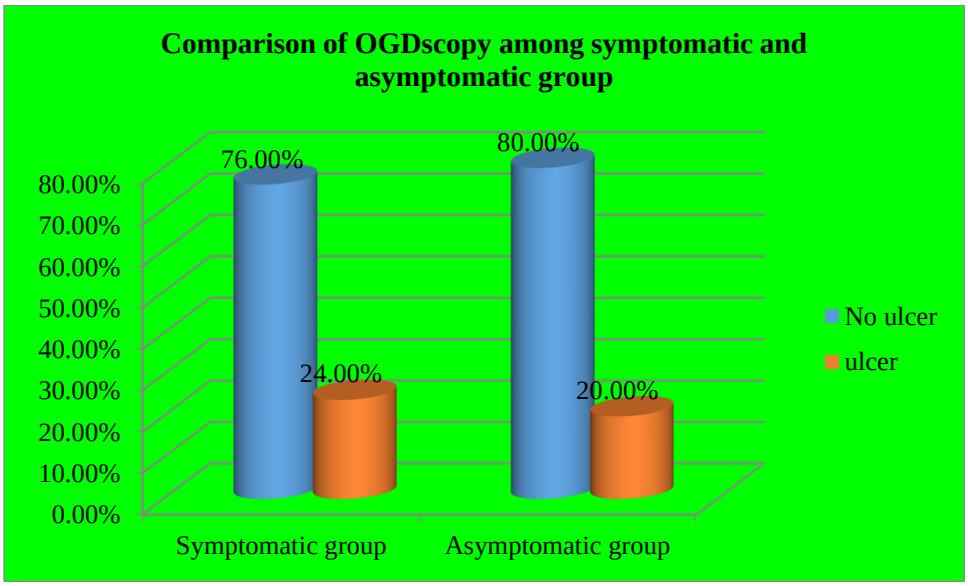

Chart 7 - Comparison of OGDscopy among symptomatic and asymptomatic group

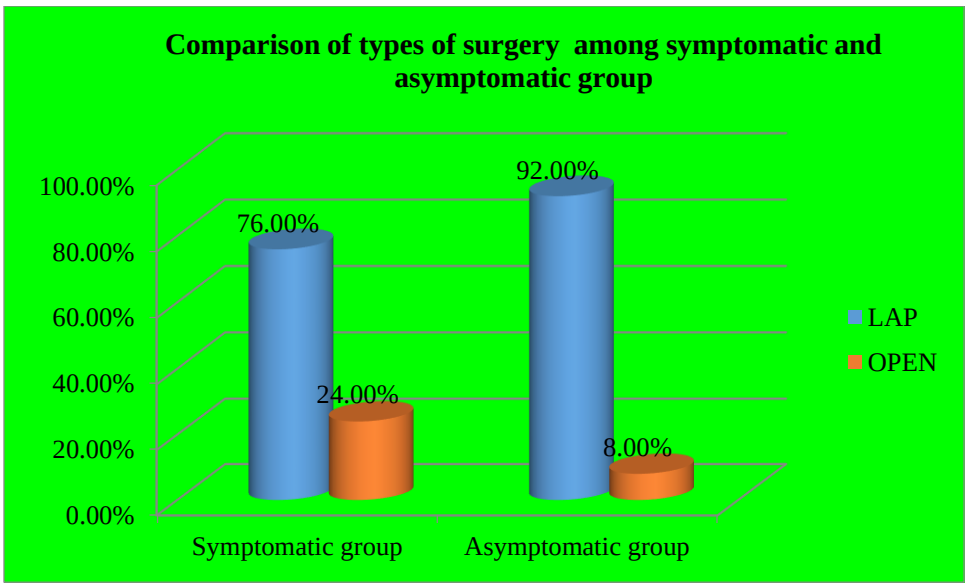

Chart 8 - Comparison of types of surgery among symptomatic and asymptomatic group

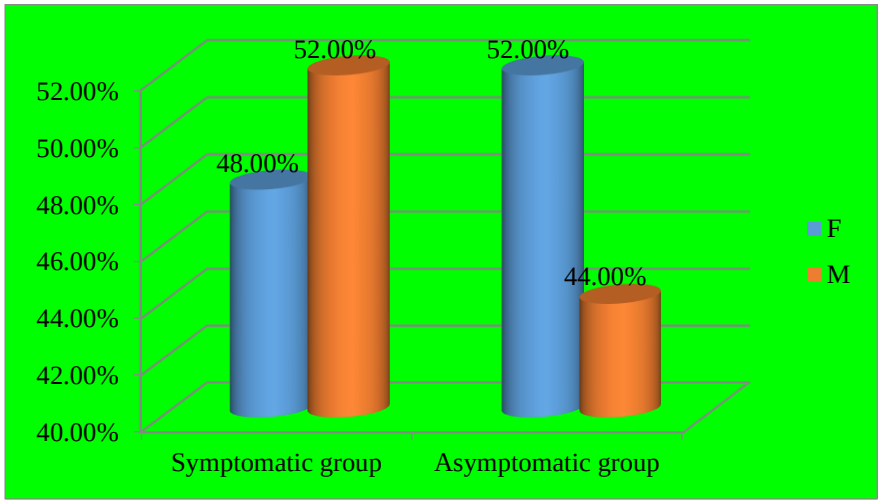

Chart 9 – Age distribution among both groups

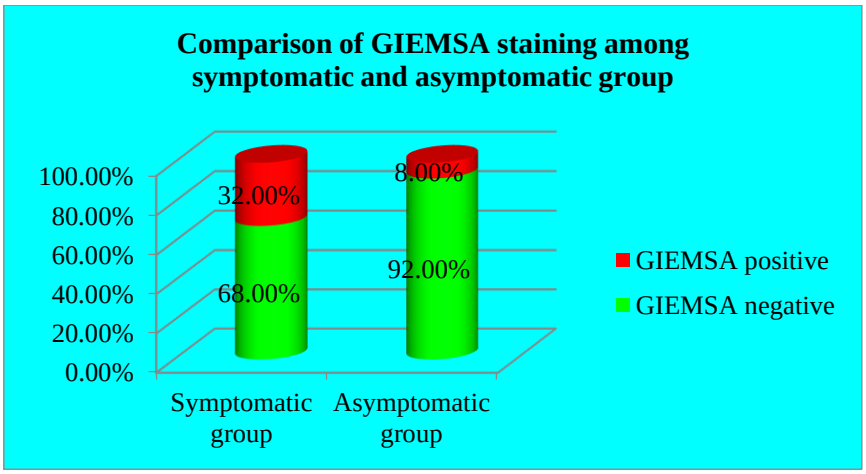

Chart 10 - Comparison of GIEMSA staining among symptomatic and asymptomatic group

Modified Giemsa staining detect *H. pylori* in 8 (32%) patients in symptomatic group and only 2 (8%) in asymptomatic group among the total 50 samples analyzed. Though the difference exist between the group which was not statistically significant (p=0.074).

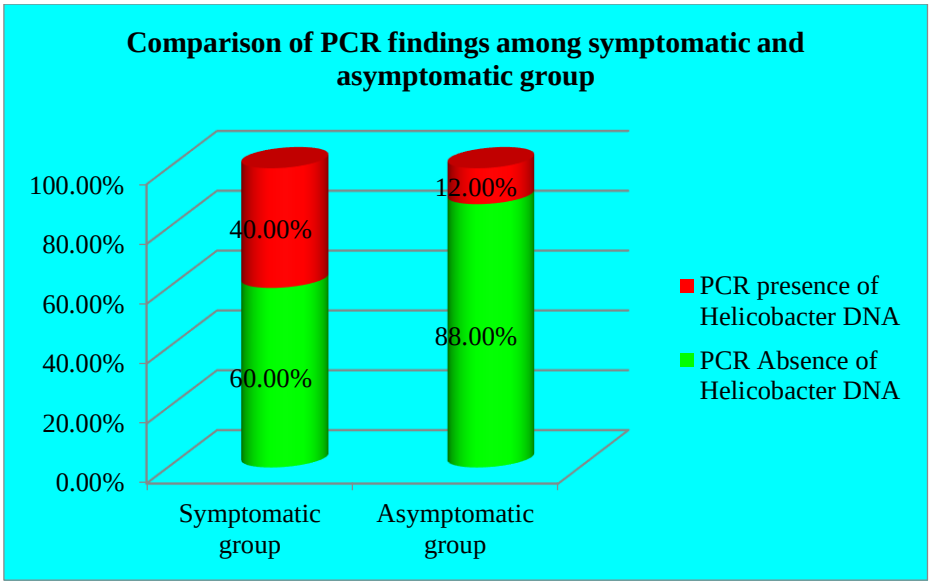

Chart 11 - Comparison of PCR staining among symptomatic and asymptomatic group

Helicobacter DNA was detected by nested PCR in the gallbladder tissue from 13 out of 50 patients in the study group. Among *Helicobacter* DNA-positive patients, 10 (33%) were from symptomatic group and 3 (12%) in asymptomatic group (p=0.06). Both the shorter (400-bp) and the longer (1,200-bp) amplicons were obtained in the samples of all positive patients.

Table 3 - Comparison of age and BMI among study groups

	Group	N	Mean	Std. Deviation	Std. Error Mean	p value
Age in yrs	Symptomatic group	25	44.84	6.562	1.312	0.386
	Asymptomatic group	25	46.64	7.926	1.585	
BMI	Symptomatic group	25	24.968	1.2628	0.2526	0.902
	Asymptomatic group	25	24.92	1.462	0.2924	

Mann whitney U test; Not significant

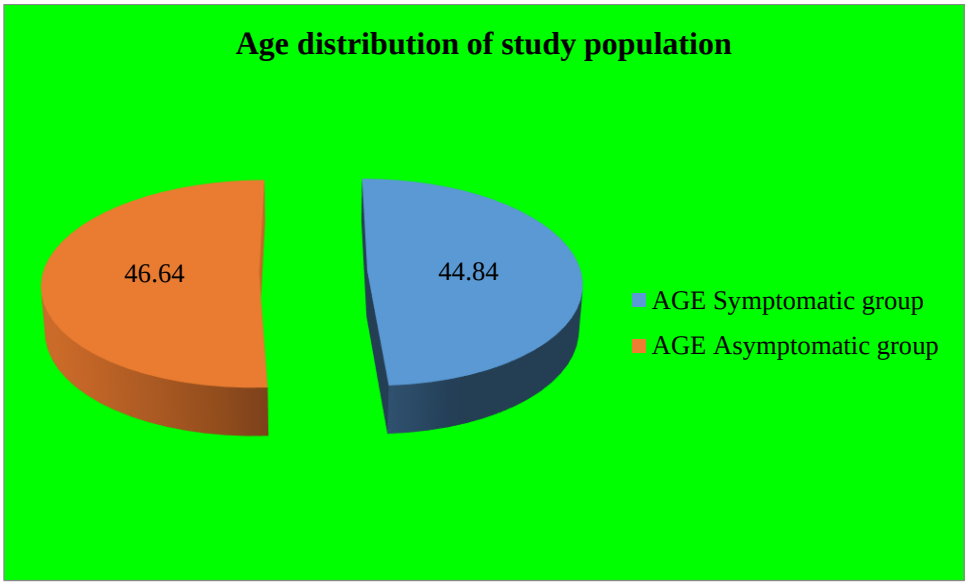

Chart 12 - Comparison of age and BMI among study groups

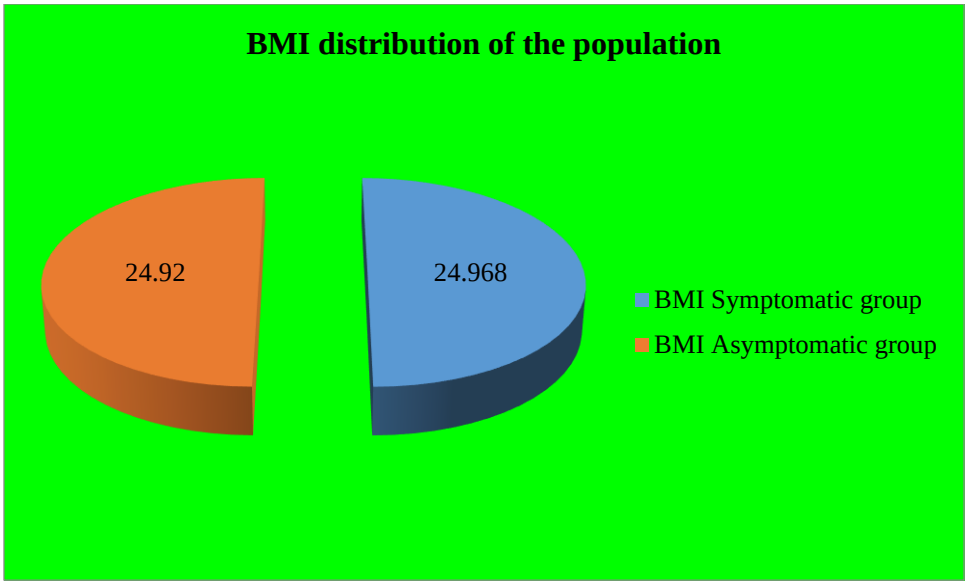

Chart 13 - BMI distribution of the population

DISCUSSION

1. H. pylori contributes to the formation of gallstones.

The relationship between H.pylori and gallbladder diseases, specifically gallstones, is still a controversial matter due to conflicting studies and inconclusive reports. However, there is enough evidence to show that bacterial population of H.pylori increases the risk of developing cholesterol-type gallstones. There are different mechanisms responsible for this condition but recent studies have highlighted the role of H.pylori.

According to a study published by the World Journal of Surgical Oncology, H.pylori releases a protein similar to that of an aminopeptidase enzyme which sets the stage for gallstone formation. This enzyme has cholesterol crystallization promoting abilities. Therefore, the presence of H.pylori can contribute to the formation of gallstones and serve as a starting point for infection around which a stone can develop. Aside from releasing proteins, it also produces soluble antigens that can lead to irregularities of the cycling of conjugated bile acids. This may result to abnormal transit time of bile acids.

Aside from the above-mentioned reasons, H.pylori's impact on overall immune system is said to contribute indirectly to lithiasis or stone formation.

2. H. Pylori aggravates gallbladder inflammation.

There are numerous known causes of chronic cholecystitis. One of them is the presence of bacterial infection in the biliary system. Various studies have shown that H. pylori is correlated with gallbladder inflammation, through the same mechanism that it contributes to the development of different gastrointestinal diseases.

Lab tests prove that in an H. pylori infected gallbladder, the cells lining the gallbladder are destroyed, with swollen mitochondria and dilated endoplasmic reticulum. These are crucial parts of the cell needed for energy, as well as the production and transport of proteins.

Rapid decrease in cell division, cell rupture, and cell death are all effects of H.pylori infection. The toxic factors in H pylori can activate factors inhibiting cell proliferation and ultimately lead to the death of cells.

Exposure of gallbladder cells to H. pylori also activates inflammatory cells in three different ways: via cellular immunity, humoral immunity, and autoimmunity.

3. H. pylori increases the risk of developing gallbladder tumors and gallbladder cancer.

H.pylori's role in inflammation leads to another gallbladder complication in which the bacteria contributes to the development of tumors and cancer of the gallbladder.

It is hypothesized that H.pylori plays a crucial role in the development of benign tumors and the higher prevalence of adenomyomatosis (GAM). GAM, also called adenomyomatous hyperplasia of the gallbladder, involves the wall thickening of the gallbladder wall, cholesterol accumulation, cholesterol crystallization, and/or enlargement of the gallbladder. Though GAM is usually asymptomatic, this condition can be an initial stage of a developing gallbladder cancer.

Gallbladder cancer, on the other hand, is characterized by chronic inflammation brought about by the presence of H.pylori. This leads to DNA damage, cell death and modulated enzyme activities. In 1994, the International Agency for Research on Cancer declared that Heliobacter pylori infection is associated with the development of stomach cancer. Aside from stomach and gallbladder cancer, H.pylori has been linked to non-Hodgkin's lymphoma.

Aside from the gastrointestinal tract and the gallbladder, other closely related organs within the biliary system like the liver and pancreas can also be severely affected by the proliferation of H. pylori.

Experiments in animal models have proven that the Heliobacter species can cause hepatitis, liver cancer, and severe damage to the immune system. H.pylori colonization is also a known culprit in pancreatic cancer.

Outside the biliary system, H.pylori has now been implicated in diverse conditions such as skin diseases, coronary artery disease, autoimmune diseases, and growth retardation in children. In this study, a total of 73 patients diagnosed with symptomatic gallstones have been admitted for laparoscopic cholecystectomy where a sample from stool and from bile were collected and tested for the presence of *H.pylori* antigens for all patients. There were 63 female (86.3%) and 10 (13.7%) males with age ranging from 28-63 years, mean age 41 (SD11.3) years.

Twenty three patients (31.5%) have positive *H. pylori* antigen in their stool samples, while 50 patients (68.5%) have negative test. Twenty one patients (28.8%) have positive *H. pylori* antigenin in their bile samples, while 52 patients (71.2%) have negative test. This shows the biliary colonization by *H. pylori* in patients with symptomatic gallstones.

Subgroup analysis revealed that sixteen patients (21.9%) have positive test for *H.pylori* antigen in their stool, but are bile-negative, and fourteen patients (19.2%) positive for *H.pylori* antigen in their bile, but are stool- negative. In contrast, only 7 patients (9.6%) revealed positive result in both specimens (stool and bile), with a P-value of 0.0002 which is highly significant

There was no correlation between the presence of *H.pylori* antigen in stool and bile with the sex of the patients with P- value =0.449.

This study showed the biliary colonization by *H. pylori* in patients with symptomatic gallstones was (28.8%), although it is an unusual anatomical site for H. pylori colonization. This is similar to Farshad *et al.,* (2004) who reported the presence of DNA but not antigen in 18.1% of gallstones and suggested that *H.pylori* infection may serve as initiating factor in development of gall stones (Farshad *et al*., 2004; Fox *et al*., 1998; Bulajic *et al.,* 1946; Sheta *et al*., Pandey, 2007; Figura *et al.,* 1998).

In our study,a total of 50 patients diagnosed with both symptomatic and asymptomatic gall bladder have been admitted for laparoscopic/open cholecystectomy and GB specimens were collected and tested for presence of H.pylori in GB wall with giemsa staining and PCR, with age

group ranging from 25-60,with mean age 44.84 in symptomatic group and 46.6 in asymptomatic group. there were 26 females and 24 males.out of 50 patients 25 were symptomatic and 25 were asymptomatic. Out of 25 symptomatic patient 8 were positive for giemsa staining (32%) and 10 were positive for PCR (40%). In asymptomatic group,out of 25, 2 were positive for giemsa staining (8%) amd 3 were positive for PCR (12%). This study also shows that out of 50 patients 26 were female. So female were slightly more common to develop cholelithiasis.

In this comparative study,giemsa staining for H.pylori in gall bladder specimen was positive in 8 patient in symptomatic group and 2 patients in asymptomatic group. The test of significance is 0.074. PCR test concludes 10 patient were positive in symptomatic group 3 patient were positive in asymptomatic group. The test of significance is 0.061. as p value is more than 0.05, this study is concluded insignificant.

LIMITATION: small number of cases.

CONCLUSION

Gall bladder colonization by H.pylori infection might be a insignificant factor in development of gall stones and cholecystitis. Whether eradication therapy for H.pylori infection may or may not be helpful in gall stone formation is yet not settled down.

- Further studies with larger samples of patients are needed to confirm a causal relationship between H.pylori infection and gallstone formation and other hepatobiliary diseases, especially if held in prospective way in asymptomatic patients who are harboring H. pylori, yet have normal gallbladder.

- Although it is not cost-effective, use of PCR to detect H.pylori DNA in bile as well as in gallstones themselves is worthy to try in further studies.

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PROFORMA

Name:- I. P. No

Age :- Unit

Sex :- D.O.A:

Occupation :- D.O.D:

Address :-

Phone no :

DIAGNOSIS:

PRESENTING COMPLAINTS

1) Duration of abdominal pain.

2) History regarding vomiting.

3) Co-existing comorbidities

4) Treatment history of APD

H/O Alcohol intake

H/O smoking

H/O NSAID's intake

H/O fever

H/O abdominal pain:

GENERAL PHYSICAL EXAMINATION

1. General survey

2. Body build and nourishment

3. Appearance

4. Attitude: Restless/ Quiet

5. Dehydration: Mild/ Moderate/ Severe/ Nil

6. Anaemia/ Jaundice/ Clubbing/ Cyanosis/ Lymphadenopathy/ Pedal oedema

7. Pulse

8. Temperature

9. Respiratory rate

10. Blood pressure

LOCAL EXAMINATION PER ABDOMEN

1. INSPECTION
2. PALPATION
3. PERCUSSION
4. AUSCULTATION

PROCEDURE:

INTRA OPERATIVE FINDINGS:

Thickness of GB:

HISTOPATHOLOGY Report:

Anatomical variant of GB (if any)

Nature of the GB stone

Multiple/single

Colour

Type

USG findings:

POLYMERASE CHAIN REACTION (PCR) Report:

GIEMSA staining:

CONCLUSION:

CONSENT

ஆராய்ச்சி தகவல் அறிக்கை

மதுரை அரசு இராசாசி மருத்துவமனையில் வரும் நோயாளிக்கு ஒரு ஆராய்ச்சி இங்கு நடை பெற்று வருகிறது. நீங்களும் இந்த ஆராய்ச்சியில் பங்கேற்க விரும்பிகிறோம் .

உங்களை சில சிறப்பு பரிசோதனைக்கு உட்படுத்தி அதன் தகவல்களை ஆராய்வோம். அதனால் தங்களது நோயின் ஆய்வரிகையோ அல்லது சிகிச்சையோ பாதிப்பு ஏற்படாது என்பதைதெரிவித்து கொள்கிறேன் .

முடிவுகளை வெளியிடும் போது அல்லது ஆராய்ச்சியின் போதோ தங்களது பெயரோ அல்லது அடையாளங்களோ வெளியிடமாட்டோம் என்பதை தெரிவித்துகொள்கிறோம்.

இந்த ஆராய்ச்சியில் பங்கேற்பது தங்களுடைய விருப்பத்தின் பேரில் தான் நடக்கும். மேலும் நீங்கள் எந்நேரமும் இந்த ஆராய்ச்சியில் இருந்து பின் வாங்கலாம் என்பதையும் தெரிவித்து கொள்கிறோம்.

இந்த சிறப்பு பரிசோதனை முடிவுகளை ஆராய்ச்சியின் போது அல்லது ஆராய்ச்சியின் முடிவின் போது தங்களுக்கு அறிவிப்போம் என்பதையும் தெரிவித்துகொள்கிறோம்.

பங்கேற்பாளர் கையொப்பம்

ABBREVIATIONS

LAP – laparoscopy

PCR – Polymerase Chain Reaction

H. pylori – Helicobacter pylori

DNA – Deoxyribo Nucleic Acid

CT – Computed Tomography

USG – Ultrasonography

RNA – Ribo Nucleic Acid

MASTER CHART

SNO	NAME	AGE	SEX	BMI	DIABETES	USG FINDINGS							OGD SCOPY GASTRIC ULCER /GASTRITIS	LAP/OPEN CHOLECYSTECTOMY	GIEMSA	PCR
						CHOLELITHIASIS	ACUTE CHOLECYSTITIS	STONE		WALL THICKING	PORCELIAN GB / GB POLYP	LFT				
								<2.5	>2.5							
1	KURUVAMMAL	54	F	26.5	1		✓			✓		↑	0	OPEN	✓	✓
2	MANOJ	43	M	24	0	✓		✓				N	0	LAP		✓
3	CHELLATHAI	52	F	24	0	✓			✓			N	0	LAP		
4	MADASAMY	44	M	23.8	0	✓		✓				N	0	LAP		
5	JANSIRANI	46	F	26.7	1		✓			✓		↑	0	OPEN		
6	PONNAMAL	49	F	23.8	0	✓		✓				N	1	LAP		
7	POORNARAJ	49	M	26.7	1		✓			✓		↑	0	OPEN		
8	PAPPATHI	49	F	26	0			✓				N	1	LAP		
9	JOHN	41	M	25.8	1	✓		✓				N	0	LAP		✓
10	SHANTHI	46	F	23.4	1	✓			✓			↑	0	LAP	✓	✓
11	SELVI	41	F	23.7	0	✓		✓				↑	0	LAP		
12	KUMARESAN	45	M	26.4	0	✓			✓			N	0	LAP		
13	SUMATHI	34	F	26.3	0	✓		✓				N	0	LAP	✓	✓
14	RAMASAMY	32	M	25.8	0	✓			✓			N	0	LAP		
15	RANJITH	35	M	25.6	1			✓				N	0	LAP		
16	HEMA	53	F	24.8	0		✓			✓		↑	1	OPEN		
17	PACHAKILI	45	M	25.4	1	✓			✓			N	1	LAP	✓	✓
18	DURAI	45	M	25.4	1	✓		✓				N	1	LAP		
19	NEELAKANDAN	42	M	26.9	0		✓			✓		N	0	OPEN	✓	✓
20	MARISELVI	47	F	24.3	1	✓		✓				↑	0	LAP	✓	✓
21	MAYILRANI	34	F	22.5	0	✓		✓				↑	1	LAP		
22	MANIKANDAN	53	M	24.3	1	✓			✓			↑	0	LAP	✓	✓
23	MOHAN	49	M	23.4	1	✓		✓				N	0	LAP		
24	SANTHOSAM	55	M	24.7	1	✓			✓			N	0	LAP		
25	GOMATHI	38	F	24	1	✓				✓		N	0	OPEN	✓	✓
26	CHANDRASEKAR	34	M	23	1			✓				N	0	LAP		
27	MANGLESWARI	49	F	24.5	0						✓	N	0	OPEN		
28	SETHUPATHI	30	M	21.9	1			✓				N	0	LAP		
29	KUMUTHA	46	F	21.3	1			✓			✓	N	0	LAP		
30	NANITHA	39	F	25.6	1			✓				N	0	LAP		
31	KANNAMMAL	43	F	24	1			✓				↑	0	LAP		
32	DINESH	34	M	25.1	0						✓	N	0	OPEN		
33	MUTHU VELLAM	49	F	25.9	1			✓				N	1	LAP		
34	PANDIAMMAL	36	F	23.5	1			✓				N	1	LAP		
35	RAJKUMAR	52	M	25.3	1			✓				N	0	LAP		
36	RANI	45	F	26.4	1			✓				N	1	LAP		
37	MEGALA	47	F	25.4	1			✓				N	0	LAP	✓	✓
38	VARALAKSHMI	55	F	22.6	1			✓			✓	↑	0	LAP		
39	MAYILAMMAL	57	F	25.7	1			✓				N	1	LAP		
40	INENNAMMAL	57	F	24.7	1			✓				N	0	LAP	✓	✓
41	KUMUTHA	53	F	25.7	1			✓				↑	0	LAP		✓
42	MAYAN	54	M	25.6	1			✓				N	0	LAP		
43	MAYILSAMY	56	M	26.1	0				✓			N	0	LAP		
44	MANNAN	58	M	26.3	0				✓			N	1	LAP		
45	SELVA KUMAR	42	M	26.5	0				✓			↑	0	LAP		
46	KARUPU	43	M	25.1	0				✓			N	0	LAP		
47	ROJA	51	R	24.7	0				✓			N	0	LAP		
48	VADIVEL	47	M	26.9	0				✓			↑	0	LAP		
49	MURUGAN	41	M	26.1	0				✓			N	0	LAP		
50	PALANIAMMAL	48	F	25.1	0				✓			↑	0	LAP		

ETHICAL COMMITTEE APPROVAL

MADURAI MEDICAL COLLEGE
MADURAI, TAMILNADU, INDIA -625 020
(Affiliated to The Tamilnadu Dr.MGR Medical University, Chennai, Tamil Nadu)

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8.Thiru.P.K.M.Chelliah, B.A., Businessman,21, Jawahar Street, Gandhi Nagar, Madurai.

ETHICS COMMITTEE CERTIFICATE

Name of the Candidate	:	Dr.C.Jagadish
Designation	:	PG in MS., General Surgery
Course of Study	:	2017- 2020
College	:	MADURAI MEDICAL COLLEGE
Research Topic	:	Comparative study on Helicobacter Pylori infection as a causative agent in Gall Bladder tissue with symptomatic cholecystitis/cholelithiasis and incidental cholelithiasis
Ethical Committee as on	:	08.04.2019

The Ethics Committee, Madurai Medical College has decided to inform that your Research proposal is accepted.

Member Secretary Chairman Dean / Convenor

Prof Dr.V.Nagaraajan
M.D., MNAMS, D.M., Dsc.,(Neuro), Dsc (Hon)
CHAIRMAN
IEC - Madurai Medical College
Madurai

DEAN
Madurai Medical College
Madurai-20

22 APR 2019

ANTI PLAGIARISM CHART

URKUND

Urkund Analysis Result

Analysed Document: Comparative Study on H.pylori on sypmtomatic & incidental Cholelithiasis.docx (D57496419)
Submitted: 10/23/2019 8:41:00 AM
Submitted By: c.jagadish19@gmail.com
Significance: 16 %

Sources included in the report:

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42

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