“Assessing correlation between gallstone formation,iron deficiency and anemia in patients with gallstone disease”

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P.G in General Surgery

DISSERTATION SUBMITTED TO

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In partial fulfillment of the requirements for the degree of

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UNDER THE GUIDANCE OF

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I hereby declare that this dissertation **"Assessing correlation between gallstone formation, iron deficiency and anemia in patients with gallstone disease "** is a bonafide and genuine research work carried out by me under the guidance of **Dr. GIRISH KULLOLLI** , Professor, Department of general surgery at BLDE (Deemed to be university) Shri B. M. Patil Medical College Hospital and Research Centre, Vijayapura.

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LIST OF ABBREVIATIONS USED:

GSD	**GALLSTONE DISEASE**
NOS	**NITRIC OXIDE SYNTHASE**
NO	**NITRIC OXIDE**
nNOS	**NEURONAL NITRIC OXIDE SYNTHASE**
SO	**SPHINCTER OF ODDI**
IDA	**IRON DEFICIENCY ANAEMIA**
GB	**GALL BLADDER**
HMGCR	**3-HYDROXY-3-METHYLGLUTARYL-COA REDUCTASE**
NPC1L1	**NIEMANN-PICK C1-LIKE PROTEIN1**
ACAT	**ACYL-COENZYME-A-CHOLESTEROL ACYLTRANSFERASE**
CDCA	**CHENODEOXYCHOLIC ACID**
CA	**CHOLIC ACID**
BESP	**BILE SALT EXPORT PUMP**
MUC	**MUCIN GENES**
ABC	**ATP-BINDING CASSETTE**
SLE	**SYSTEMIC LUPUS ERYTHEMATOSUS**
FB	**FOREIGN BODY**
CCK	**CHOLECYSTOKININ**
CCK1-R	**CHOLECYSTOKININ 1 RECEPTOR**
NANC	**NONADRENERGIC NONCHOLINERGIC**
WBC	**WHITE BLOOD COUNNT**

USG	**ULTRASONOGRAPHY**
CT	**COMPUTED TOMOGRAPHY**
NADPH	**NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE**
GI	**GASTRO INTESTINAL**
TIBC	**TOTAL IRON BINDING CAPACITY**
BMI	**BODY MASS INDEX**
Fe2+	**FERROUS IRON**
DMT1	**DIVALENT METAL TRANSPORTER-1**
Fe3+	**FERRIC IRON**
ROS	**REACTIVE OXYGEN SPECIES**
NSAID	**NON-STEROIDAL ANTI-INFLAMMATORY DRUG**

ABSTRACT:

Title: Assessing Correlation Between Gallstone Formation, Iron Deficiency, and Anemia in Patients with Gallstone Disease

Background: Gallstone disease (GSD) is a prevalent hepato-biliary condition with multifactorial etiology. Iron deficiency and anemia have been hypothesized to contribute to gallstone formation through alterations in bile composition and gallbladder motility. This study investigates the correlation between iron deficiency, anemia, and the risk of developing gallstone disease.

Methods: A cross-sectional study was conducted at B.L.D.E. (Deemed to be University), Shri B.M. Patil Medical College, Hospital & Research Center, Vijayapura. A total of 122 patients diagnosed with gallstone disease via radiological evidence were included. Patients' hemoglobin, serum iron, and ferritin levels were assessed and correlated with gallstone prevalence. Data were statistically analyzed using Pearson’s correlation and Chi-square tests.

Results: The study found a significant correlation between gallstone disease and low serum iron and hemoglobin levels. Among gallstone patients, 72.1% had anemia, and 52.5% had low serum iron levels. Statistical analysis confirmed a significant association between iron deficiency and gallstone formation ($p < 0.05$).

Conclusion: The findings suggest that iron deficiency and anemia may contribute to the pathogenesis of gallstone disease. Screening for iron deficiency in at-risk populations could aid in early prevention and management strategies, potentially reducing gallstone-related complications.

Keywords: Gallstone disease, Iron deficiency, Anemia, Serum ferritin, Cholelithiasis, Hepato-biliary disorders.

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INTRODUCTION :

Gallstone disease (GSD) is a chronic relapsing hepato-biliary pathology, which involves formation of gall-stones anywhere in the hepatic part of bile duct, common bile duct, or within the gallbladder.

It affects our healthcare system substantially ,offering notable economic impact.

The prevalence of gallstones is related to age, sex and ethnic backgrounds and others.[1]

'The old axiom that a typical gall stone sufferer is a fat, fertile, female of fifty, is only partially true, as the disease is found in women soon after their first delivery and also in underweight and thin people.[2]

Conditions for the cholesterol gallstones occurence:

1. Supersaturation of the bile with cholesterol.

2. Nucleation must be kinetically favorable.

3. Cholesterol crystal stasis leading to agglomeration of stones in gall bladder.

4. Reduced entero-hepatic circulation of bile salts in the body.

'Iron deficiency i.e., low serum ferritin levels has been shown to alter the activity of several hepatic enzymes, leading to increased gall bladder bile cholesterol saturation and promotion of cholesterol crystal formation.[4,5]

Iron acts as a coenzyme for nitric oxide synthetase (NOS), which synthesizes nitric oxide (NO) and that is important for the maintenance of basal gall bladder tone and normal relaxation.[6,7]

The activity of neuronal gall bladder nitric oxide synthase (nNOS) is shown to be significantly decreased after a 2-month iron-deficient diet, leading to cholesterol crystal aggregation over time[5]

Iron deficiency (low blood ferritin levels) was discovered to cause sphincter of Oddi (SO) and gall bladder motility changes, which resulted in biliary stagnation and, consequently, even more cholesterol crystal formation in the gall bladder bile.[8]

Hence, testing of the hypothesis that iron deficiency and anemia (low hemoglobin, low serum iron) can be considered a risk factor for cholelithiasis is being tested in this study.

NEED FOR THE STUDY:

Gall stone disease is one of the most costly medical illnesses in the world among gastroenterological diseases.[9]

Gallstone disease (GSD) affects our healthcare system substantially,offering notable economic impact with the incidence of 4% of the Indian population.

This value is recorded highest among the western subcontinent , where cholelithiasis and its complications form a major chunk of medical cases .

In Indian subcontinent, owing to the recent epidemic of westernisation , incidences of GSD are bound to increase .

Female gender is more prone to develop cholelithiasis than males, owing to the hormonal and biochemical changes going in their bodies.

In India, about 53% of people have iron deficiency anaemia (IDA). The most frequent nutritional shortfall is iron deficiency, which is over 2.5 times more common whether anaemia is present or not. In developing nations, adolescents and women of adulthood are more frequently impacted.[57] Recurrence of gallstones is expected if gallstones are removed without cholecystectomy. Cholelithiasis can cause severe consequences like acute gallstone pancreatitis and gallbladder cancer if proper treatment is not received.[9].

Due to the increase in the incidence of cholelithiasis in India as well as due to its multifactorial etiogenesis, a study that can reveal the disease's prevalence and risk

factors is desperately needed in order to identify the population at risk early and start treatment, which will lower morbidity and mortality.

Therefore, gaining a better understanding of the factors that contribute to cholelithiasis could have drastic impact on public health, and can aid the Indian population with preventive measures thus preventing invasive interventions for the patient .

AIM OF THE STUDY :

- To establish a correlation between anemia, serum iron and ferritin levels in patients suffering from gallstone disease.

OBJECTIVE OF THE STUDY :

- To assess the efficacy of serum hemoglobin ,serum iron and ferritin levels as being a risk factor for development of gallstone disease .
- To assess the incidence of gallstone disease in iron-deficient vs non-iron deficient population .

REVIEW OF LITERATURE :

The word “Gall bladder” has its origins rooted in many ethnicities like latin(fel) , Greek , and german (chole-cyst) . The term "tartaric diseases" was used in the 16th century by Paracelsus to explain how chemicals from biological fluids precipitate to form stones in the human body, much like tartar accumulates in wine casks[58.]

Over the past century, gallstones have become far more common in the western world. Gallstones have been found in at least 20% of women and 8% of males over 40 in the United States, according to autopsy studies. It is thought to be about 4% in India[59.]

According to an epidemiological study limited to rail workers, cholelithiasis is seven times more common in North Indians as compared to South Indians.[10]

Gallstones are uncommon in Africa, where their prevalence falls lower than 1%, whereas they have increased from 2% to 7% in Japan[11,60.] An estimated 1 million new cases of cholelithiasis occur annually in the United States, and at least 20 million people are thought to have gallstones. According to autopsy study findings, the frequency in Europe is 18.5%, with Sweden having the highest prevalence and Ireland having the lowest. [12]

Modernisation, the accessibility of ultrasonography investigations in urban as well as rural setups, and increased affordability as a result of shifting socioeconomic structures and investigation costs are the primary causes of the changing incidence in India.[13]

From the intricate interplay of genetic variables, chronic carbohydrate overnutrition, dietary fibre depletion, and other inadequately characterised environmental influences, such as physical inactivity and infections, Gallstone disease phenotypes probably rise,all of which have been linked to 'westernised' nutrition.

Due to the growth in the incidence of cholelithiasis in both India and Western countries, there is an A study that can provide information on the disease's prevalence is desperately needed, the risk factors connected with the condition, so that the population at risk can be detected early and treatment could be initiated , thus reducing morbidity and mortality [14].

Anatomy :

The gall bladder is a 30-milliliter viscus that has a pear-like form.. It consists of three distinct sections: the fundus, the body and the neck.

On the visceral surface of the liver's right lobe, adjacent to the quadrate lobe, is the gall bladder fossa.

The fundus is the bulbous blind end that normally projects somewhat below the lower margin of the liver. It is located near the beginning of the transverse colon, adjacent to the hepatic flexure. The body ultimately touches the duodenum after moving upward and backward toward the right end of the porta hepatis. the right end of the porta hepatis, eventually coming into touch with the duodenum.

The neck is formed by the narrowing of the upper body and continues in the cystic duct (2-3 cm long; 2-3 mm broad).

Histologically, the wall of this fibromuscular sac exhibits smooth muscle. Its mucous membrane, which gives the body a honeycomb look, is a slack areolar tissue bordered with simple columnar epithelium (The neck is formed by the

narrowing of the upper body and continues in the cystic duct (2-3 cm long; 2-3 mm broad).

Histologically, the wall of this fibromuscular sac exhibits smooth muscle. Its mucous membrane, which gives the body a honeycomb look, is a slack areolar tissue bordered with simple columnar epithelium (secretes mucus). They are spirally organized in the neck and cystic cretes mucus). They are spirally organized in the neck and cystic duct (the "spiral valve of heister") .

Blood supply :

The arterial supply – 'the gastroduodenal and the right hepatic arteries, with major trunks running along the medial and lateral walls of the common duct (sometimes referred to as 3 o'clock and 9 o'clock).'[61]

The common bile duct receives the same nerve supply as the gallbladder, with the density of nerve fibres and ganglia increasing in the direction of Oddi sphincter.

The cystic artery (right hepatic artery branch)that travels through the calot's triangle to the gall bladder. Several variations have been reported.

Venous return occurs through several tiny veins in the GB bed into the liver and ultimately into the hepatic veins. Cystic veins travel independent of the cystic artery.

Lymphatic pathways from the gall bladder drain into the porta hepatis, the cystic node, and a node on the anterior edge of the epiploic foramen. Lymph travels from these nodes into the coeliac group of preaortic nodes.[85]

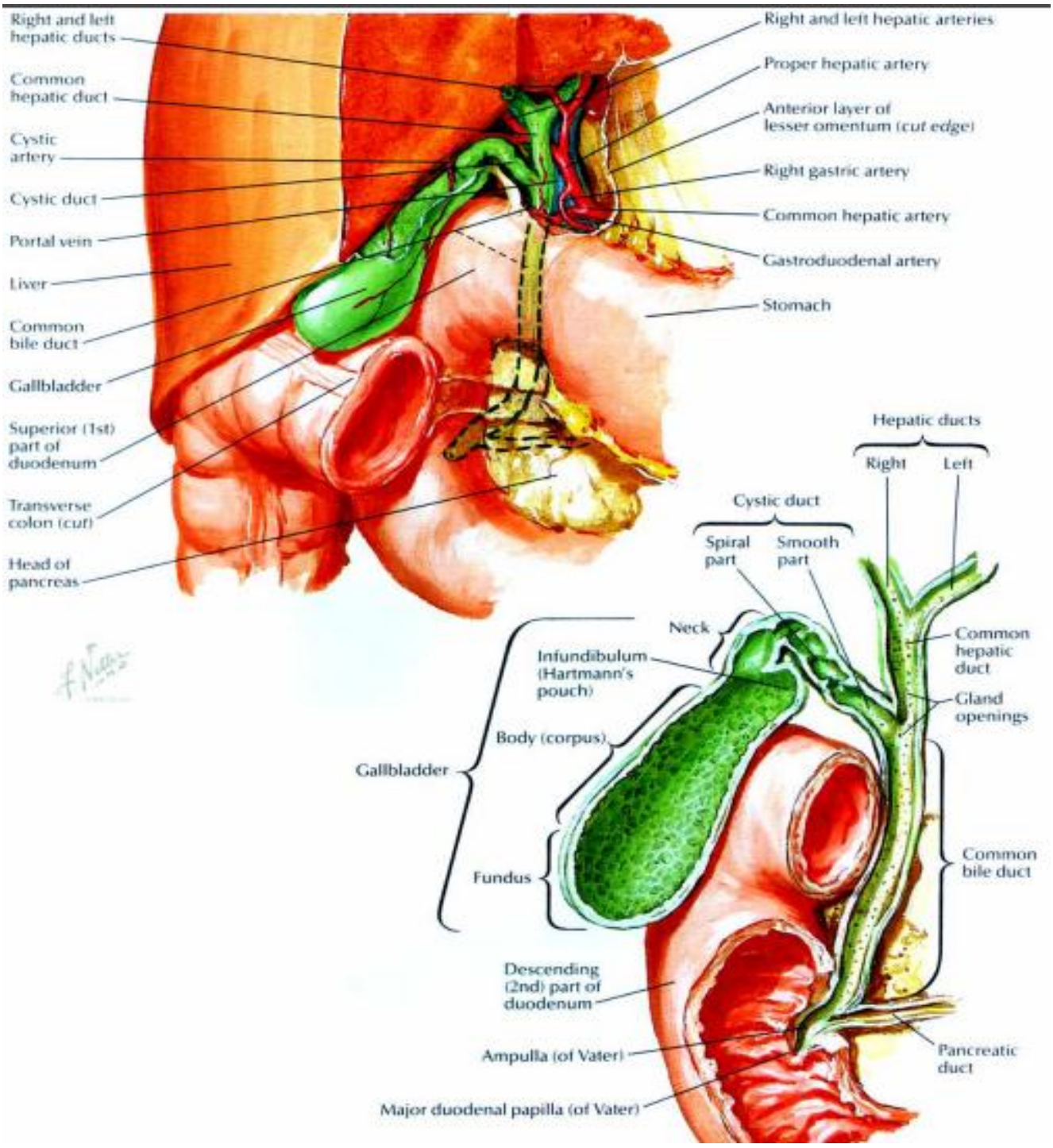


Figure.1 Anatomy of biliary system [15]

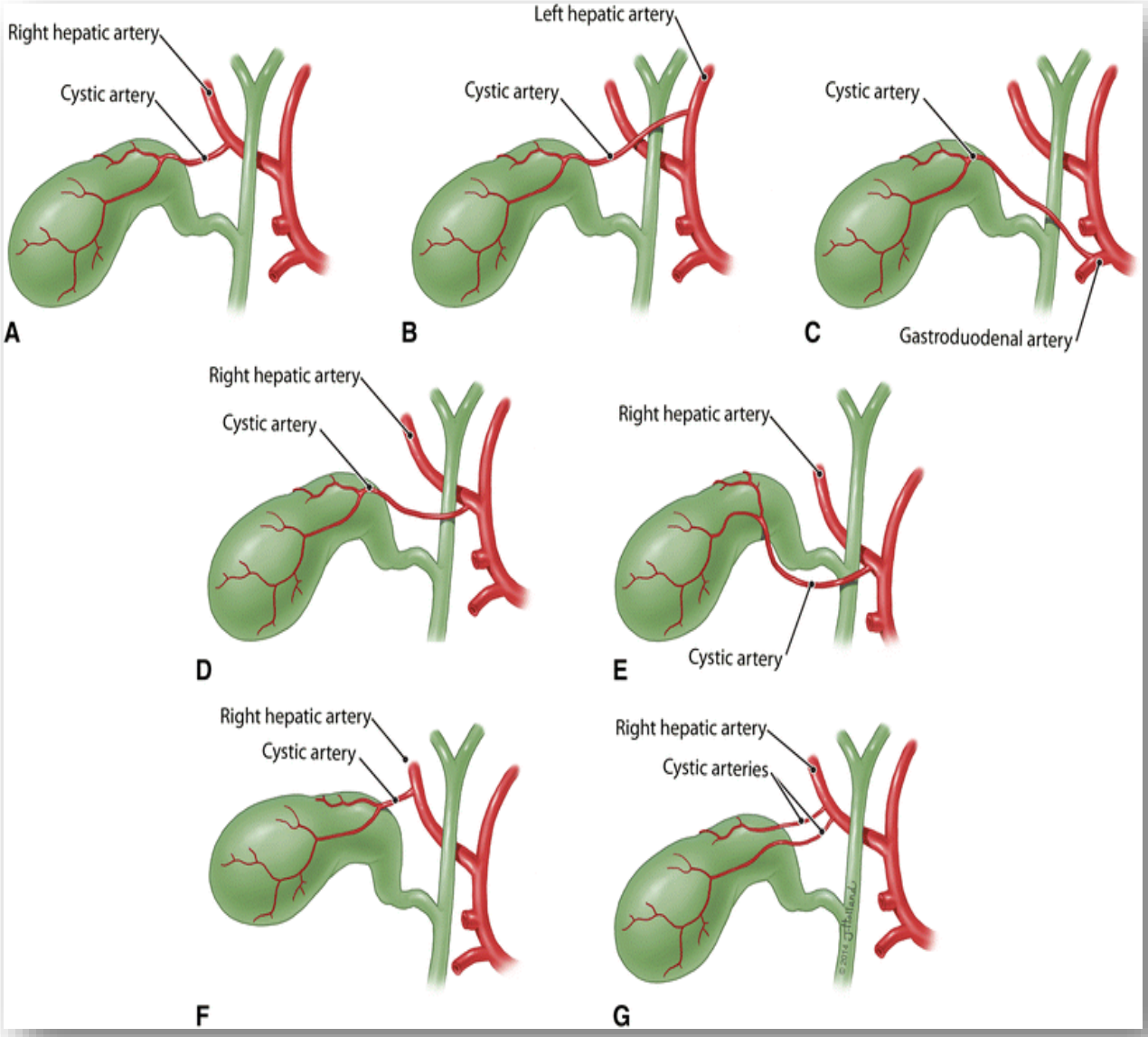


Figure 2 . Anatomical variations of the cystic artery[16]

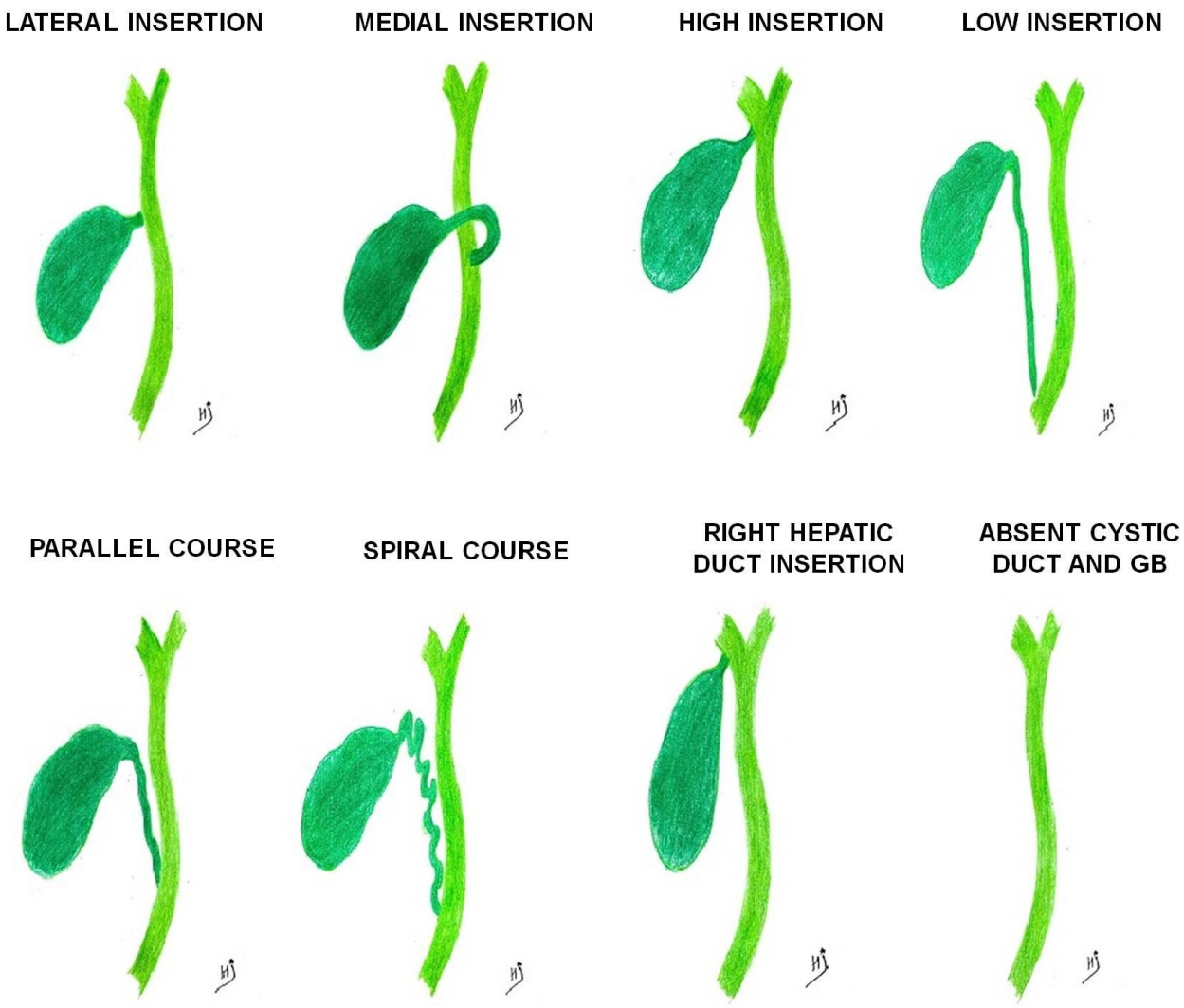


Figure 3. Variant anatomy of biliary tree [17]

There are three possible arrangements for the junction of the main pancreatic duct with the common bile duct. These ducts come together outside the duodenal wall and pass through it as a single duct in roughly 70% of individuals. About 20% of them open into the duodenum through the same aperture, but they unite inside the intestinal wall and have a short or nonexistent common duct. About 10% of them

leave through distinct apertures into the duodenum, known as the pancreas divisum. At the ampulla of Vater, the common bile duct is encircled by the sphincter of Oddi, a thick layer of circular smooth muscle. It regulates the passage of pancreatic juice and bile into the duodenum.[62]

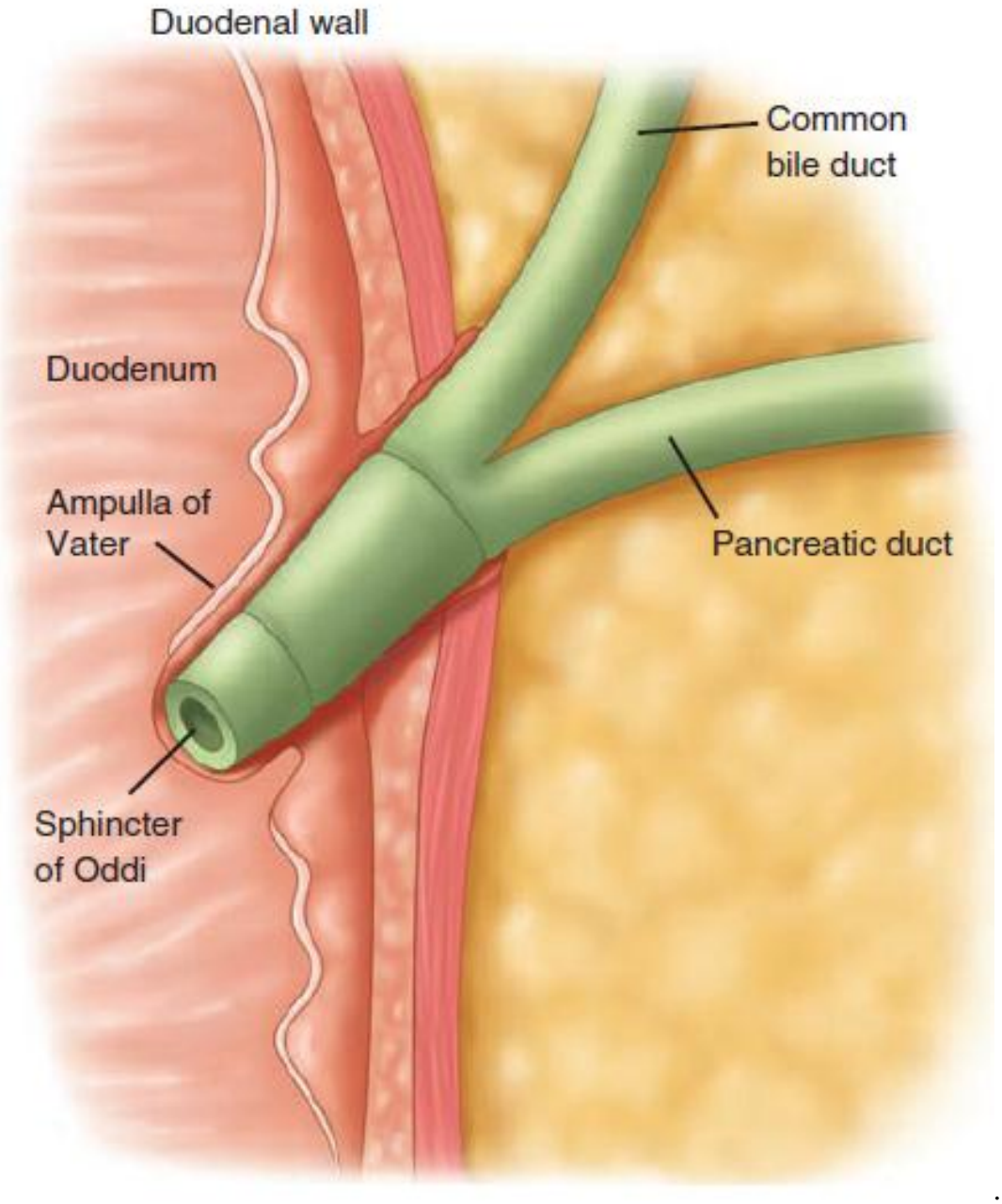


Figure 4 . Anatomy of common bile duct

Physiology :

One of the liver's numerous roles is bile secretion, which typically ranges upto 600 ml to 1000 ml each day.

Bile acids play a crucial role in lipid digestion and absorption. They cream large fat particles into smaller particles, which can then be acted upon by pancreatic juice lipase enzymes. Additionally, they aid in the absorption of digested fat end products through the intestinal mucosal membrane.

The gallbladder mucosa reabsorbed water and a major portion of the electrolytes (except calcium ions) during the concentrating process. Almost all other ingredients, particularly bile salts and lipid compounds (cholesterol,lecithin) aren't reabsorbed and hence become highly concentrated in bile.
Bile salts are synthesised by liver cells at a rate of around 6 grammes per day. The precursor to bile salts is cholesterol, which can be found in the diet or synthesised in liver cells during fat metabolism. Salts of these acids, primarily sodium salts, are then released into the bile.[63]

A metabolic deficit frequently results from the up to 40% of ingested lipids that are lost into the faeces when bile salts are absent from the intestinal system. About 94% of all bile salts are reabsorbed into the bile due to the enterohepatic circulation, which means that the salts make the entire circuit 17 times before they reach the feces. The rate of bile secretion increases with the amount of bile salts in the enterohepatic circulation, which is typically around 2.5 grammes. Indeed, consuming supplementary bile salts can enhance bile output by several hundred millilitres each day.

Every day, around 2 grammes cholesterol is taken from plasma and released into the secreted bile. The amount of cholesterol in the bile is somewhat determined by

the amount of fat consumed. Gallbladder epithelial inflammation, usually caused by a low-grade persistent infection. As a result, increased absorption of water and bile salts occurs while cholesterol accumulates in the gallbladder at increasing quantities. The cholesterol then starts to precipitate, creating many tiny crystals on the surface of the irritated mucosa before developing to massive gallstones.[18]

Circulation of Cholesterol and Bile Acid

Cholesterol Circulation

Cholesterol in the liver can pass through the biliary tract and into the small intestine, where it is reabsorbed and enters the bloodstream via the lymphatic system before returning to the liver. Cholesterol is produced in the body through two processes: biosynthesis and absorption. The liver is the primary site for cholesterol production. Enzymatic processes convert acetyl-CoA into cholesterol molecules. The endoplasmic reticulum transmembrane protein 3-Hydroxy-3-Methylglutaryl-CoA Reductase (HMGCR) and squalene monooxygenase are the rate-limiting enzymes in this pathway.[19] Hepatocytes convert some of their cholesterol into bile salts, while the remainder of their free cholesterol is pushed into the biliary system via ABCG5/8. Phospholipids produce microclumps, which are then secreted into the colon via bile.[20]

Niemann-Pick C1-Like Protein1 (NPC1L1) on intestinal epithelial membranes absorbs biosynthetic and dietary cholesterol. ACAT (also known as acyl-coenzyme A: cholesterol acyltransferase) further esterifies the cholesterol, This travels through the lymphatic system into the circulation before being taken up by the liver as chylomicrons.[21,22].

Efficient intestinal absorption of cholesterol and daily consumption of cholesterol are the primary determinants of the dietary proportion of total cholesterol. Depending on daily food consumption, cholesterol absorbed in the small intestine may use a negative regulatory mechanism to control the liver's production of cholesterol. [64]

Bile Acid Cycle

Bile acid and cholesterol are in balance in the bile. Bile acids are only synthesised by the liver (fig 5). The liver parenchymal cells use cholesterol to synthesise bile acids. This includes the unconventional path, which is facilitated by sterol 27-hydroxylase (CYP27A1) [23], and the conventional pathway, which is facilitated by cholesterol 7α-hydroxylase (CYP7A1). Chenodeoxycholic acid (CDCA) is the primary product of the non-classical pathway, whereas CDCA and cholic acid (CA) are produced by the classical system. The bile salt export pump (BESP (ABCB11)) secretes the freshly produced binding bile acids into the hepatocytes' capillary bile

channel, where they eventually enter the gut.[24.]

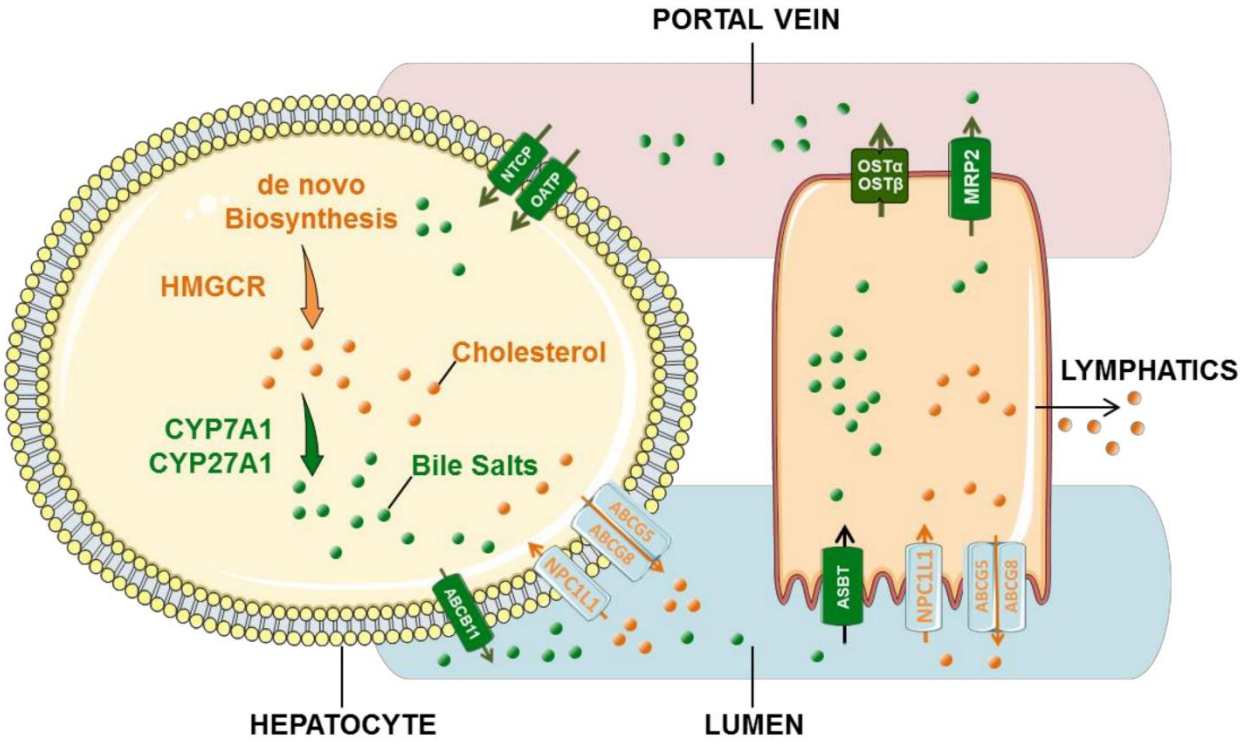


Figure 5 . Hepatoenteric circulation of bile acids and metabolism of cholesterol.

It is possible to synthesise cholesterol de novo. Hepatocytes convert some of their cholesterol into bile salts, and they also pump out the remaining free cholesterol into the biliary system via ABCG5/8. On intestinal epithelial membranes, NPC1L1 absorbs cholesterol, which subsequently travels via the lymphatic system to the bloodstream. ABCB11 secretes newly synthesised binding bile acids into the hepatocytes' capillary bile duct, which then allows them to enter the intestine. Bile acids are reabsorbed and sent to the liver through the portal vein when the terminal cavity of the basement membrane contains the heterodimer OSTα/β. Cholesterol is represented by orange. The bile acid cycle is represented by dark green. Furthermore, intestinal epithelial cells in the colon and small intestine passively reabsorb free bile acids. After that, OATP absorbs them via the hepatocytes' sinusoidal membrane, processes them, and secretes them into the capillary bile

ducts.[25]

The bile acid enterohepatic circulation is completed when bile acids pass through the biliary system and enter the intestinal lumen. Unreabsorbed bile acids circulate throughout the body and may ultimately be eliminated by the kidneys.[26]

Aetio-pathogenesis :

Gall stone Disease is a disease of multiple aetiologies. The risk factors for developing Gall stones are :

1. Gender: Women are more likely than men to have GSD.
2. Age: The incidence of Gall stone Disease is directly proportional to age.
3. Genes: Those who have relatives with Gall stone disease are 2-4 times more likely to develop GSD .Studies on mice have demonstrated the existence of a lithogenicity gene.[27] Through quantitative trait locus mapping in mice, a significant gallstone susceptibility locus (Lith6) was discovered in 2003.
4. Systematic fine mapping of the complete *Lith6* region is required to identify the causative genetic variants for gallstone in mice and humans [28] Several mucin genes (*MUC*) have been implicated in various diseases and gel-forming mucin genes (*MUC2, MUC5AC, MUC5B,* and *MUC6*) were recognized to be the important components of digestive mucus.

The Genetic Basis of Gallstone Development

The genetic components of gallstone formation have been better understood by using mice species that is vulnerable to cholelithiasis (C57L/J) and mice resistant to cholelithiasis (AKR/J). These mice have been used to explore the

potential involvement of lithogenic genes ONE and TWO (Lith1 and Lith2) in gallstone development. A key factor in determining hepatic cholesterol hypersecretion is Lith1, which is found on mouse chromosome The ATP-binding cassette (ABC) transporters ABCG5 and ABCG8 are highly expressed in intestine and hepatocyte cells.[30] These two proteins are taken from the endoplasmic reticulum, where they combine to form heterodimers.

In the endoplasmic reticulum, these two proteins combine to create heterodimers, which are then transferred to the apical membrane.

Because of the substantial decrease in bile cholesterol release caused by ABCG5/G8 inactivation, liver and plasma cholesterol levels are extremely sensitive to changes in dietary cholesterol intake. Consequently, this may raise the risk of early coronary heart disease, hypercholesterolaemia, and phytosterolemia.[31, 32] However, the gallbladder's cholesterol level rises due to the overexpression of the ABCG5/G8 protein, which raises the risk of cholesterol crystal precipitation.[33]

5. Race: Gallstones are widespread among American Indians (60–70%), less common among Hispanics of mixed Indian descent, and even less common among Black Americans.[65] Gallstones are present in 10–15% of white adults in developed nations, and they are even less common among Black Americans, East Asians, and sub-Saharan Africans. In Asians, the prevalence varies between roughly 3% and 15%, and it is almost nonexistent (less than 5%) among Africans.

   10 to 15 percent of white grown-ups in industrialized countries harbor gallstones. Incidence is further decreased in sub-Saharan Africa, black Americans, East Asia. It is virtually nonexistent (less than 5%) in Africans and varies between around 3% and 15% in Asians.[9,34].

6. Cholelithiasis prevalence varies greatly among Indian populations, with North Indians seeing a 2-4 fold greater frequency than South Indians.[35]

7. Helicobacter pylori infection has been associated with several non-gastric sequelae, including cholelithiasis [36]

8. Rapid weight loss: Increased mucus and calcium levels in the cystic bile accompany weight loss, which leads to biliary sludge and gallbladder stones.

9. Glucose intolerance

10. Insulin resistance

11. Obesity: Increased cholesterol production and excretion into bile are associated with obesity. However, being overweight is strongly correlated with the amount of cholesterol produced.[37]

12. High dietary glycemic load

13. Alcohol use

14. Drugs: The risk for GSD is increased by clofibrate,oestrogens, prednisolone, cyclosporin, nicotinic acid, azathioprine, and a few of other long-term medications[38,39]. Younger women who use oral contraceptives are more likely to develop GD, particularly when they are first starting to use them.[40].

Cholelithiasis was present in 68.8% of SLE patients receiving corticosteroid treatment.[41]

According to the information provided in these articles, oral contraceptives and corticosteroids,(including hormones related to steroid hormones), may be considered a model system for the development of gallstone disease in humans.

15. Pregnancy[42]

16. Diabetes mellitus.

17. Hypertriglyceridemia.

18. Iatrogenic: There are several accounts of foreign bodies (FBs) serving as nidus for biliary system stone development. Among the FBs recorded are parasites, metal and plastic fragments, surgical sutures, surgical clips, and swallowed debris. According to reports, the most frequent FBs that cause iatrogenic biliary stones are surgical clips. Since its initial usage in surgery, the occurrence of surgical clip migration followed by stone development has been widely acknowledged. Patients are usually initially diagnosed with biliary obstruction symptoms, which can be exacerbated by potentially fatal pancreatitis and cholangitis. Days to years may pass between the original surgical procedures and these. Unknown is the fundamental process by which FBs, including surgical clips, enter the biliary system. Entero-bilio reflux, penetrating wounds, direct introduction during surgery, or erosions that have been hypothesised to eventually migrate into the biliary system. Clip migration has also been attributed to infections, subclinical bile leaks, bile-duct damage, and improper clip placements.[43],[15]. Gallbladder stasis (from spinal cord injury or medications like somatostatin) and some illnesses (such cirrhosis and Crohn's disease).

19. Bile levels rise with a high cholesterol intake. A diet deficient in fiber slows the passage of intestinal contents, which increases the production and utilization of secondary bile acids and improves the lithogenic qualities of bile. Bile's cholesterol saturation rises with refined carbs.

20.Bile levels rise when cholesterol consumption is high[44]. A diet deficient in fibre slows the passage of bowel contents, which increases the production and utilisation of secondary bile acids and Bile levels rise with a high cholesterol

intake44. A diet deficient in fiber slows the passage of intestinal contents, which increases the production and utilization of secondary bile acids and improves the lithogenic qualities of bile. 9. Bile's cholesterol saturation rises with refined carbs.

21. Cholecystokinin (CCK), a neuroenteropeptide hormone generated by the endocrine cells of the duodenum, goes to the gallbladder after a large meal that is heavy in fat and protein. It binds directly to the smooth muscle cells of the gallbladder wall's CCK1 receptor (CCK-1R). The gallbladder shrinks and as a result, releasing the concentrated bile into the intestine. Additionally, the pancreas, small intestine, gastric mucosa, pyloric sphincter, and sphincter of Oddi all have CCK-1R.

Intestinal absorption is enhanced, cholesterol calculi formation is markedly augmented, and gallbladder emptying and bile cholesterol metabolism are hindered in CCK or CCK-1R gene knockout mice[45].

Protective variables include diets high in alcohol, almonds, calcium, vitamin C, coffee, fiber, vegetable protein, and physical exercise.[46]

Gall Stone formation :

It is possible to identify four main categories of factors that, to varying degrees, contribute to the development of cholesterol gallstones: (1) those that cause cholesterol precipitation and crystallisation core formation; (2) those that impair the fundamental functions of the gallbladder (contraction, absorption, secretion, etc.); (3) those that impair the enterohepatic circulation of bile acids.[47,48]

Recurrence of gallstones is inevitable if gallstones are removed without removing the gallbladder because abnormal gallbladder emptying may facilitate the aggregation of nucleated cholesterol crystals[49].

The gallbladder's muscular contraction is the main mechanism regulating bile transport into the duodenum. A synchronized set of pressure relationships , dictate the direction of bile's movement following liver secretion. The sphincter of Oddi has a greater tone during fasting, which facilitates the passage of bile into the gallbladder.

Bile is concentrated in the gallbladder, which keeps the volume minimal. The gallbladder empties roughly every 120 minutes during the interdigestive phase, which is accompanied by forceful duodenal contractions as a component of the migrating myoelectric complex. This keeps bile salts in the enterohepatic circulation. These actions during fasting are mediated by motilin, which activates intrinsic cholinergic neurones. The primary modulator of gallbladder emptying after meals is cholecystokinin. Gallbladder contraction is triggered by pre-ganglionic cholinergic neurones.

By increasing intracellular calcium levels and causing the gallbladder smooth muscle to contract through signal transduction, agonists like cholecystokinin and acetylcholine engage the contractile machinery. Furthermore, the sphincter of Oddi is affected by cholecystokinin via pre-ganglionic cholinergic neurones, which

results in the release of nitric oxide and vasoactive intestinal polypeptide, which reduce the tone of the sphincter. Bile is supplied to the duodenum at the appropriate moment thanks to these activities, which are synchronised with motility and secretory functions in the upper gastrointestinal tract.[54]

Mammalian cells produce nitric oxide (NO), a mediator that is essential for blood pressure control, neurotransmission, and cellular defence systems. in the route from L-arginine to NO.

Through oxidation, nitric oxide synthases (NOSs) help transform L-arginine into nitric oxide (NO) and L-citrulline. The unique feature of these enzymes is that they combine an oxygenase and reductase domain, which share structural similarities with cytochrome P450 enzymes, within the same polypeptide chain. The production of NO and superoxide, as well as the stability of NOSs, is intricately regulated through interactions with Ca2+/calmodulin, the cofactor tetrahydrobiopterin, and the availability of substrates. Notably, significant competition exists between NOSs and arginases for L-arginine, underscoring strong interconnections among enzymes that metabolize L-arginine. Furthermore, an intermediary in the L-arginine to NO route, N(omega)-hydroxy-L-arginine, exhibits potent inhibitory effects on arginase activity, further highlighting the complex regulation of L-arginine utilization.[57,66]

The human body excretes cholesterol as either bile acids or as unmodified cholesterol after it has been converted to bile. The hepatic enzyme cholesterol 7ot-hydroxylase's ability limits conversion to bile acids. According to autopsy data, gallstone prevalence ranges from 11% to 36%.[55]

Nitric oxide (NO) has been shown to be a crucial inhibitory nonadrenergic, noncholinergic (NANC) neurotransmitter in the GI tract. Smooth muscle

relaxation results from NO produced in response to myenteric plexus nerve stimulation. Neuronal NO synthase (nNOS) in the myenteric plexus is activated to produce NO. Numerous areas of the GI tract depend on released NO for physiological functions. NO controls the tone of the sphincters in the pylorus, anus, sphincter of Oddi, and lower oesophagus. Additionally, NO controls the intestine's peristaltic reflex and the fundus' accommodation reflex. NOS inhibitors have been demonstrated in earlier research to postpone colonic transit and stomach emptying. GI tract motility issues may be caused by a decrease in nNOS expression, which is linked to a decreased local generation of NO. There is growing evidence that a number of GI disorders may be brought on by NO neurone malfunction in the myenteric plexus. [56]

Mammalian cells produce NO through a family of three NO synthases (NOS). The three mammalian enzyme isoforms—nNOS, iNOS, and eNOS—that correspond to the tissues of origin of the initial protein and cDNA isolates are identified in perspective articles.

Bilirubin, cholesterol, phospholipids, and bile salts are the primary organic constituents of bile. Gallstones are divided into two categories: pigment stones and cholesterol stones, depending on how much cholesterol they contain. There are two types of pigment stones: brown and black. ‘About 80% of gallstones in Western nations are cholesterol stones, with the remaining 15% to 20% being black pigment stones. Only a small percentage of stones are made of brown colour. In Asia, both varieties of coloured stones are more common.’[67]

Cholesterol gall stone :

Less than ten percent of gallstones are pure cholesterol stones. Usually, they come together as a single, sizable stone with a smooth surface. The majority of

cholesterol stones are mixed, comprising calcium, bile pigments, and at least 70% cholesterol by weight.

These stones can be uneven, multilobed, soft, or hard and faceted. They are usually several and vary in size. They can be green, black, or yellowish yellow in colour. While some cholesterol stones have a higher calcium carbonate content and are therefore radioopaque, more than 90% of cholesterol stones are radiolucent. The supersaturation of bile with cholesterol is the main cause of cholesterol stones.[68]

As a nonpolar material, cholesterol's solubility in bile and water is contingent upon the proportions of bile salts, lecithin (the primary phospholipid in bile), and cholesterol. Cholesterol is released into the bile where it forms a soluble vesicle complex with bile salts and phospholipids. When cholesterol secretion is excessive, either due to increased intake or impaired processing, supersaturation occurs, leading to stone formation.

When cholesterol levels surpass the capacity of bile salts and phospholipids to keep it in solution, the cholesterol precipitates out and forms a solid, resulting in a cholesterol stone. Cholesterol hypersecretion is typically the primary cause of supersaturation, rather than a decrease in the secretion of phospholipids or bile salts .[50]

Pigmented gall stones:

Calcium bilirubinate gives pigmented stones their dark colour and less than 20% cholesterol content. Stones with black and brown pigments are different from one another and belong to different categories.

Small and brittle, dark, and occasionally spiculated, black pigment stones are the norm.The stones form due to the supersaturation of unconjugated bilirubin in bile. Normally, bilirubin undergoes slow deconjugation in bile. However, conditions like hemolytic disorders (e.g., hereditary spherocytosis and sickle cell disease) lead to increased excretion of conjugated bilirubin, resulting in an elevated production of unconjugated bilirubin. Liver conditions such as cirrhosis can also increase the secretion of unconjugated bilirubin. This insoluble unconjugated bilirubin precipitates with calcium, forming insoluble calcium bilirubinate and creating a pigment stone. Because of their high calcium content, black pigment stones are often radiopaque. The gallbladder is where these stones mostly always originate. Black pigment stones are more prevalent in nations like Japan than the Western nations.[69]

Brown pigment stones are typically soft, mushy, brownish-yellow, and tiny (less than 1 cm). They can occur in the gallbladder or bile ducts, frequently due to bile stasis and bacterial infection. Bacteria such as *Escherichia coli* produce β-glucuronidase, which breaks down conjugated bilirubin into unconjugated bilirubin. Following its precipitation with calcium, this unconjugated bilirubin combines with dead bacterial cells to create soft brown stones in the biliary tree. More prevalent in Asian cultures, brown pigment stones are frequently associated with bile stasis brought on by parasitic illnesses such as Clonorchis sinensis (liver fluke) or Ascaris lumbricoides (roundworm). Patients with biliary strictures or other disorders that result in stasis and bacterial contamination are more likely to develop brown stones as primary bile duct stones in Western populations.[50]

Symptomatic Gallstones

Symptomatic Cholelithiasis. Patients with symptomatic gallstone disease often experience recurring pain bouts. When a stone blocks the cystic duct, the gallbladder wall's strain gradually increases as it contracts in response to food, causing pain. Biliary colic is the common term used to describe this postprandial right upper quadrant or epigastric pain.

A seemingly normal gallbladder with mild chronic mucosal inflammation to a shriveled, ineffective gallbladder with fibrosis and adhesions to surrounding structures are only a few examples of the pathological alterations, which frequently do not correspond well with the symptoms. Aschoff-Rokitansky sinuses develop when the mucosa becomes atrophic over time, with the epithelium pushing into the muscle layer. At first, the mucosa may appear normal or hypertrophied.

Clinical presentation :

The main symptom of symptomatic cholelithiasis is periodic discomfort, also known as biliary colic. This pain is constant and typically intensifies over the first 30 minutes after eating, lasting between 1-5 hours. It may radiate to the right upper back or between the shoulder blades, but it is typically felt in the epigastrium or right upper quadrant. The pain is intense and frequently starts abruptly, especially after a heavy meal or at night. Nausea and occasionally vomiting are common side effects. Usually, patients have distinct, recurring pain episodes interspersed with times when they are symptom-free. A physical examination during an episode may reveal minor pain in the upper right quadrant. The examination of the body is typically normal when the patient is not in pain. Patients with uncomplicated gallbladder stones typically have normal laboratory results, such as liver function and total leucocyte values.

In patients presenting with atypical symptoms, it is important to rule out other potential causes of upper abdominal pain, even if gallstones are present. Conditions that should be considered include, but are not limited to Herpes zoster, diverticular disease, inflammatory bowel disease, pancreatitis, peptic ulcer disease, gastro-oesophageal reflux disease, pleuritic discomfort, kidney stones, inflammatory bowel disease, and cardiac problems.

Investigations :

Blood tests :

Acute cholecystitis, or gallbladder infection, can be suspected or confirmed by an elevated white blood cell (WBC) count.

If bilirubin, alkaline phosphatase, and transaminases are elevated, cholangitis (biliary tree infection) should be suspected.

Alkaline phosphatase and conjugated bilirubin levels are typically elevated in cholestasis, which is an obstruction to bile flow, though transaminitis might not be present. Such a pattern may suggest choledocholithiasis (stones in the common bile duct) or an obstructing lesion such as a stricture or cholangiocarcinoma.

In patients with simple symptomatic cholelithiasis, biliary colic, or chronic cholecystitis (a chronic inflammatory state of the gallbladder without infection), blood tests will often be normal.

Plain abdominal radiograph:

Due to the fact that only 15–20% of gallstones are radio-opaque on X-ray, plain radiography has limited diagnostic capabilities. The "Mercedes Benz sign," which is an outside radio-opaque rim with a radiolucent core brought on by gallstone rim calcification and internal gas fissuring, is the traditional radiographic look. Increased calcium in bile causes gallstones to calcify.[51]

Transabdominal Ultrasonography

Little stones within the common bile channel regularly get held up at the distal conclusion of it, behind the duodenum, and are, in this manner, troublesome to identify.

A widened common bile channel on ultrasound, little calculi within the gallbladder, and a classic clinical presentation permits one to accept that a stone or stones are causing the obstacle.

Ultrasound is profoundly compelling in recognizing gallstones, with a affectability and specificity more prominent than 90%, and can moreover precisely distinguish other biliary tree anomalies. Gallstones are acoustically thick, reflecting ultrasound waves back to the transducer. Since they piece sound wave transmission to the zone behind them, they make an acoustic shadow (Fig.7). In contrast, polyps, which may be calcified, can deliver shadows but don't move with changes in pose. A few stones

frame a layered structure within the gallbladder, whereas others show up as silt or slime.

Highlights to hunt for on ultrasound examination of gallbladder are:

• Ultrasound Murphy's Sign (probe tenderness on compression of gallbladder).

•

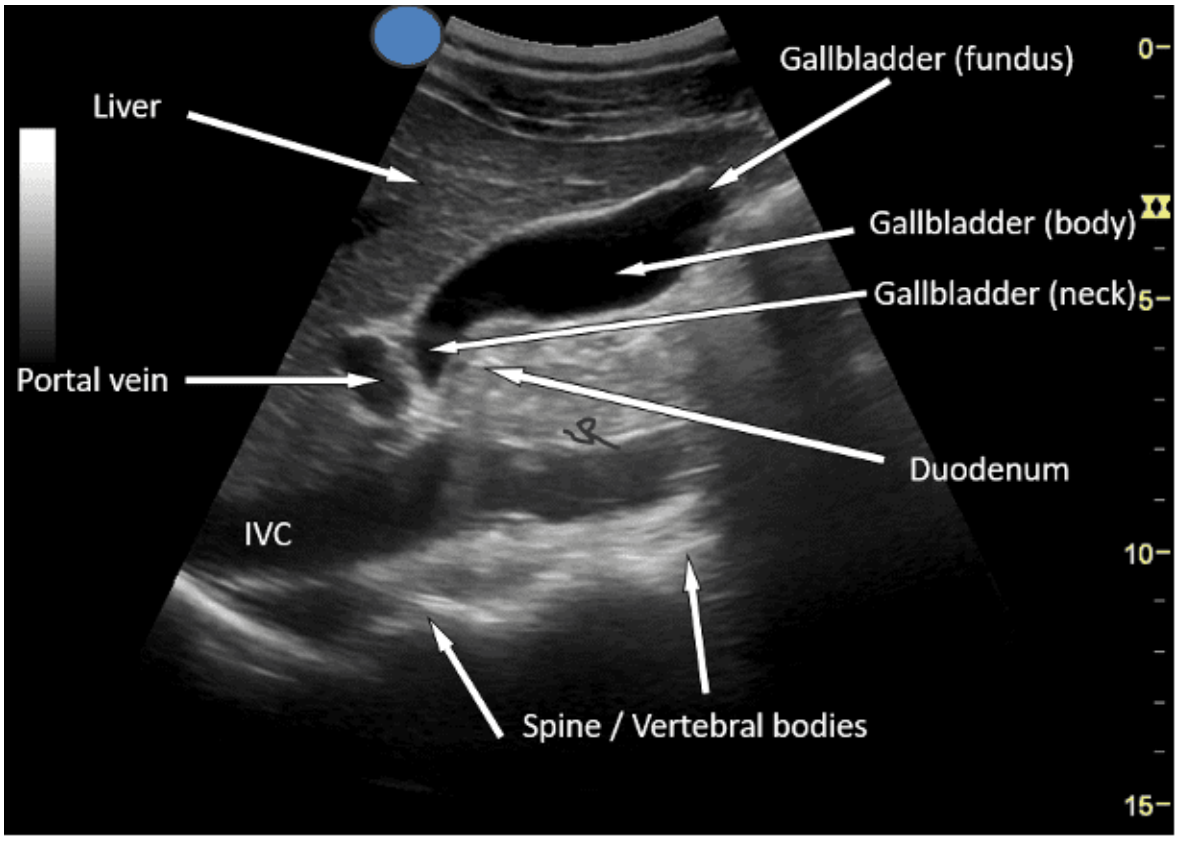


Figure 6. Normal gallbladder anatomy on USG[52]

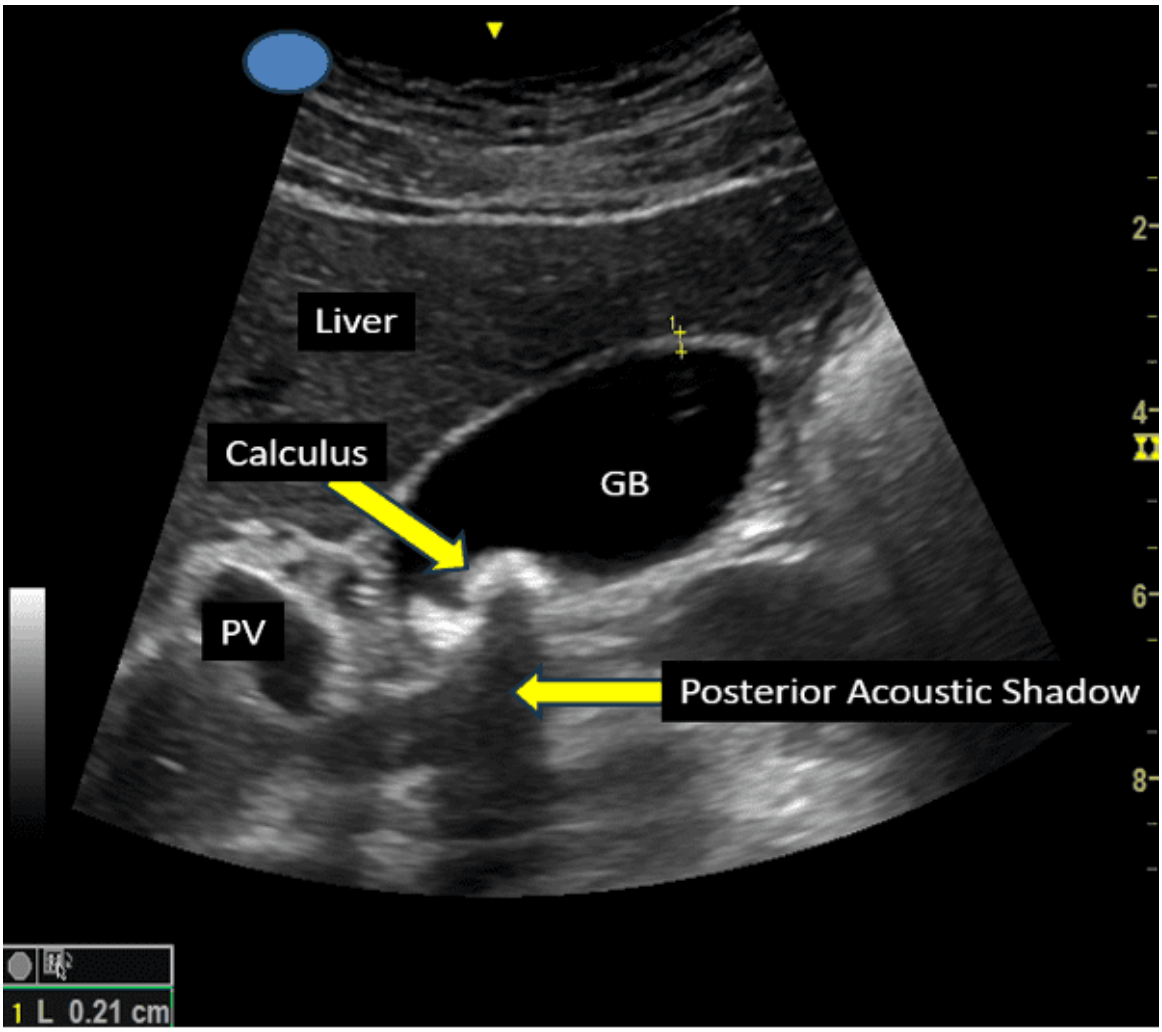


Figure 7. Cholelithiasis findings on USG abdomen and pelvis. [52.]

Computed Tomography

Abdominal CT scans are commonly used to diagnose gallbladder in cases of nonspecific abdominal pain. CT scanning is less sensitive than ultrasonography for identifying gallstones, however it is comparable for acute cholecystitis detection. The primary utility of a CT scan, however, is to determine the course and health of the extrahepatic biliary system and nearby organs, as well as to rule out other reasons of a patient's clinical presentation. CT is also the first test used to evaluate

individuals with suspected gallbladder cancer, extrahepatic biliary system cancer, or cancer of adjacent organs such as the pancreatic head.[70]

Management :

Surgical interventions :

For people who have symptomatic gallstones, surgical cholecystectomy provides the best long-term benefits. Following cholecystectomy, over 90% of patients with typical biliary symptoms and stones experience symptom relief. Patients experiencing unusual symptoms such dyspepsia, flatulence, belching, bloating, and dietary fat intolerance may experience less favourable outcomes. The laparoscopic technique has been shown safe and effective, and it has become the standard of therapy for symptomatic gallstone disease, replacing open cholecystectomy in routine patients. [71,72]

Laparoscopic cholecystectomy

A diseased gallbladder can be removed using a minimally invasive procedure called a laparoscopic cholecystectomy. This procedure has mainly replaced the open technique for cholecystectomies since the early 1990s. 'Acute cholecystitis, symptomatic cholelithiasis, chronic cholecystitis, biliary dyskinesia, acalculous cholecystitis, gallstone pancreatitis, and gallbladder tumours or polyps' are currently treated with laparoscopic cholecystectomy.[73]

Cholecystectomy is recommended for symptomatic individuals with gallbladder disease. The indications for laparoscopic cholecystectomy are similar to those for

open cholecystectomy. Cholecystectomy for moderate gallstone pancreatitis should be performed during the initial admission, but may be delayed for several weeks in patients with severe pancreatitis.

Small intestine obstruction brought on by gallstone ileus, coagulopathy are among the contraindications. As surgeons gain more minimally invasive experience, the number of relative contraindications decrease.[53]

Females whilst in pregnancy if diagnosed with cholelithiasis should be tried to be managed conservatively with dietary modifications. If it doesn't subside the symptoms, then laparoscopic cholecystectomy safely ,preferably in the second trimester. [54]

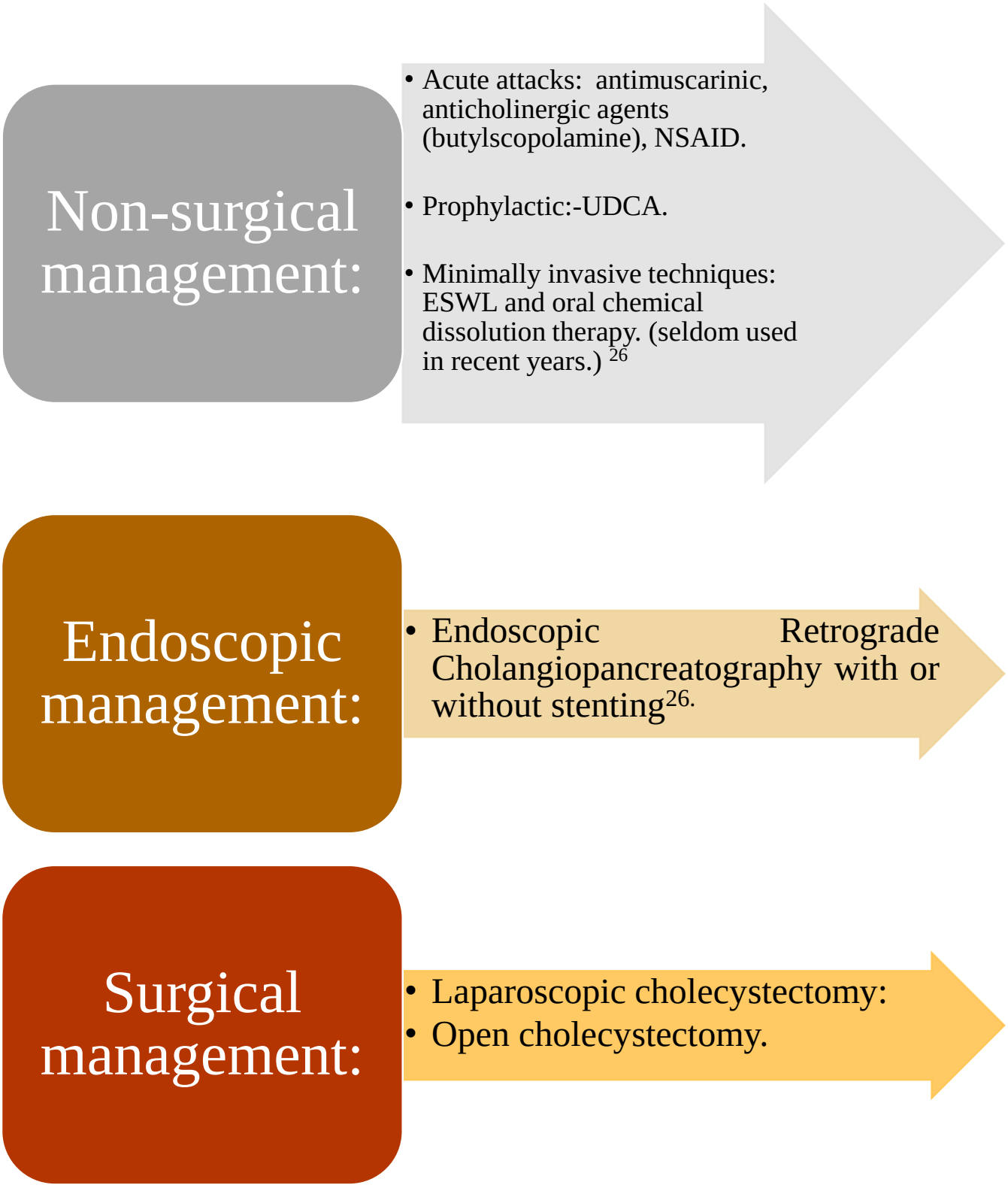


TABLE.1.Treatment modalities for cholelithiasis.[26]

Iron metabolism[18]:

Iron plays a crucial role in the synthesis of hemoglobin and other vital bodily components, including myoglobin, cytochromes, cytochrome oxidase, peroxidase, and catalase.

The body has an average of 4 to 5 grams of iron, with hemoglobin accounting for approximately 65%.

Diagrams depict the transport, storage, and metabolism of iron in the body.

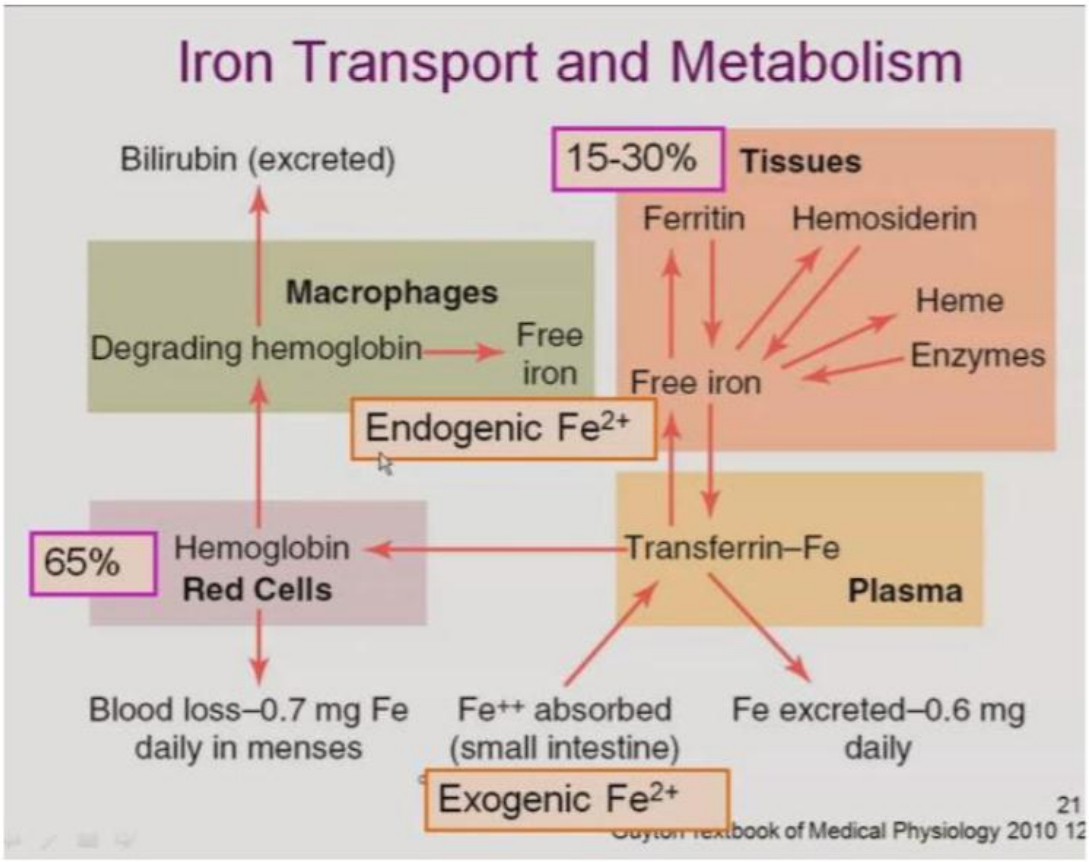


Figure 8.Iron metabolism in human body.

1.The small intestine absorbs iron.
2. It instantly forms transferrin in the blood plasma by combining with apotransferrin, a beta globulin, which is subsequently carried by the plasma.
3. The transferrin binds the iron loosely, allowing it to be released anywhere.
4. The liver hepatocytes receive the majority of the excess iron in the blood, whereas the bone marrow's reticuloendothelial cells receive less.
5. Iron mostly interacts with the protein apoferritin in the cytoplasm of cells to create ferritin. With a molecular weight of roughly 460,000, apoferritin can form clusters of iron radicals with different amounts of iron; Consequently, ferritin may have a high or low concentration of iron. We refer to this iron that is kept as ferritin as "storage iron."[18]

Action of Iron and ferritin on gall bladder and gallstone formation:

The storing form of iron is ferritin. 'It has been demonstrated that iron shortage changes the activity of a number of hepatic enzymes, increasing bile cholesterol saturation and encouraging the production of crystals'[7]. Nitric Oxide Synthase (NOS), which produces Nitric Oxide (NO), requires iron as a cofactor [5].

The neuronal NO synthase (nNOS) enzyme in the myenteric plexus is activated to produce NO from arginine. NOS catalyses a oxidation of arginine to NO with stoichiometric production of citrulline[74] using NADPH as an electron donor and five enzyme cofactors, one of which is iron.

Smooth muscle relaxation results from NO produced in response to myenteric plexus nerve stimulation. Numerous areas of the GI tract depend on released NO for physiological functions. NO controls the sphincter's muscular tone in the pylorus, anus, sphincter of Oddi, and lower esophagus[75].

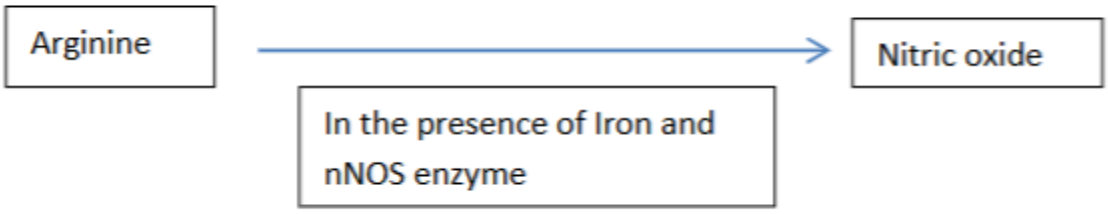


Figure 9.Nitric oxide synthesis.

According to a study by Verma GR et al.[76], patients with chronic cholelithiasis may have elevated levels of biliary calcium and trace elements (Iron, copper, and zinc) as well as a malfunctioning pH of gall bladder bile, which may be the root cause of gallstones.

A study was planned by Misra et al. to determine the precise function that blood iron and calcium play in the pathophysiology of gallstone disease and to evaluate the connection between biliary cholesterol super-saturation and serum iron and calcium levels. The study comprised 100 cholelithiasis patients, who were split into four groups according to their serum calcium and iron levels; Patients in groups A1 and B1 had normal serum calcium and calcium deficit, respectively; patients in group B1 had iron deficiency (the cases); while patients in group A1 had normal serum iron (the controls). Prior to cholecystectomy, five milliliters of blood were collected intravenously, and the serum was examined for factors such as biliary cholesterol, cholesterol, calcium, and serum iron.

The study concluded that 'the deficiency of serum iron and serum calcium along with increased cholesterol in bile results in supersaturation of bile resulting in increased crystal formation in gallbladder bile. Low serum iron,causing defective hepatic cholesterol metabolism and more stasis of bile because of decreased motility

of gallbladder leads to increased precipitation of cholesterol and hence gallstone formation.'

TIBC, serum ferritin, and serum iron

The laboratory measures that show the obtainability of iron for hemoglobin production are serum iron, TIBC, and transferrin saturation.[77] Serum ferritin is a more accurate marker for diagnosing iron shortage than the three previously discussed indicators when there is no ongoing inflammation[72].

Serum Iron

The amount of circulating iron bound to transferrin is indicated by the serum iron level. Serum iron levels typically fall between 9 and 27 μmol/L (50 and 150 μg/dL).[77]

According to a Johnston et al. study on Praire dogs, an iron-deficient diet changes the metabolism of hepatic enzymes, which raises gallbladder bile cholesterol and encourages the development of cholesterol crystals.

To determine how dietary iron contributes to the development of pigment gallstones, Roslyn J et al. conducted a study. They discovered that eating a diet high in carbs but low in iron changes how cholesterol is metabolized by the liver and could be a major contributing cause to the development of colored gallstones. They proposed that in some high-risk groups, iron supplementation may help avoid pigment gallstones.

Prasad et al.[77] additionally identified that low serum iron levels were either directly or indirectly contributing to biliary hypersaturation with respect to cholesterol, which in turn led to the formation of gallstones.
Previously, it was thought that a fifty-year-old woman who is overweight and fertile is the typical gallstone victim. This is only partially accurate, though, as the illness

is discovered in skinny and underweight individuals as well as in women shortly after their first delivery. Therefore, iron deficiency was discovered to be a novel parameter in the aetiology of gallstones while looking for other parameters. A study by Kumar et al.[4] sought to determine how iron deficiency contributes to the supersaturation of bile with cholesterol and the subsequent development of cholelithiasis.

Two groups of 50 patients with cholelithiasis, as determined by ultrasonography, were created. Patients in group A had normal serum iron levels (non-anemic), while patients in group B had lower-than-normal serum iron levels (anemic). Both groups' serum and gallbladder bile cholesterol levels were examined and contrasted. According to the study, gallstone formers' total serum cholesterol levels were the same as those of the general population.

The researchers came to the conclusion that low serum iron levels cause bile supersaturation with regard to cholesterol, which results in gallstone development, because the cholesterol content in the gall bladder bile was noticeably higher in anemic people than in non-anemic ones.

In a manner comparable, Kshirsagar et al.[78] prospectively examined 120 patients in the general surgery department at Krishna Institute of Medical Sciences, Karad, over a two-year period. Twenty cholelithiasis patients' serum iron and cholesterol levels were compared to those of healthy people. The majority of gallstone patients, the researchers found, had low serum iron levels. Patients with cholelithiasis had serum cholesterol levels that were not appreciably different from those of healthy, normal people. They came to the conclusion that low serum iron causes bile to become super-saturated with cholesterol, which in turn causes gallstones to develop.

In order to determine the function of iron deficiency in the hyper saturation of bile with cholesterol and the subsequent production of gallstones, Daddenavar and Daddenavar[79] conducted a study on 50 consecutive patients with cholelithiasis detected by ultrasonography. Based on their serum iron levels, the patients were split into anemic and non-anemic groups. Both groups' serum and gallbladder bile cholesterol levels were calculated.

Forty-two (84%) of the 50 patients were female, and eight (16%) were male.

The three symptoms of cholelithiasis—pain in the right upper quadrant, nausea/vomiting, and flatulent dyspepsia—were present in the majority of patients 31 (62%) at presentation. Of the total number of female patients, 32 (76.1%) were multipara. Ten patients (20%) had a normal or lower body mass index (BMI), while forty-two patients (84%) had a BMI that was higher than normal. Gallstone formers' serum total cholesterol levels were identical to those of the general population. While the anemic group's gall bladder bile cholesterol was significantly higher ($P<0.0001$) than that of the non-anemic group, there were no significant differences in the two groups' serum cholesterol contents ($P=0.367$, $t=0.91$). This suggests that anemia may be a factor in the super saturation of gall bladder bile with regard to cholesterol, regardless of serum cholesterol levels. Additionally, there was no discernible difference in the aforementioned parameter between the male and female patients ($P=0.082$, $t=1.77$).

Of the 50 patients in a related study by Sarhan, Hamed, and Khalaf (20), 40 (80%) were female and 10 (20%) were male. The ratio of men to women was 1:4. Females were more likely than males to have anemia; 22 (55%) of the females and 1 (10%) of the males were found to be anemic. Of the total number of female patients, thirty-

one (77.5%) were multipara. Ten patients (20%) had a normal or lower body mass index (BMI), while forty patients (80%) had a BMI that was higher than normal. Compared to anemic gallstone patients (n=23), whose average blood iron level was 26~9.5 mg/dl, all non-anemic gallstone patients (n=27) had a high average serum iron concentration of 91~35 microg/dl.

Of the 50 patients in a related study by Sarhan, Hamed, and Khalaf[80], 40 (80%) were female and 10 (20%) were male. The ratio of men to women was 1:4. Females were more likely than males to have anemia; 22 (55%) of the females and 1 (10%) of the males were found to be anemic. Of the total number of female patients, thirty-one (77.5%) were multipara. Ten patients (20%) had a normal or lower body mass index (BMI), while forty patients (80%) had a BMI that was higher than normal. Compared to anemic gallstone patients (n=23), whose average blood iron level was 26~9.5 mg/dl, all non-anemic gallstone patients (n=27) had a high average serum iron concentration of 91~35 microg/dl.

A prospective cohort study by Halgaonkar et al (21) in Mumbai included 100 consecutive patients over a period of 18-months (October 2013 to August 2015) with imaging studies suggestive of cholelithiasis. A detailed history was recorded from the patients as per the prescribed pro forma and thorough clinical examination was performed. The recorded data included demographics and details such as onset,22 duration, location, and progression of abdominal pain, associated symptoms of patient and relevant clinical findings. The study concluded that gallstones are more prevalent in females as compared to males with ratio of 5.6:1. Serum iron was found to be low in majority of the patients indicating iron deficiency as a cause of gallstone formation .

Serum ferritin:

Since free iron is poisonous to cells, the body has developed a complex system of defenses to bind iron in different tissue compartments. Iron is stored in cells as ferritin, a protein complex. Free ferrous iron is bound by apoferritin and kept in the ferric state. The serum ferritin level is the most practical laboratory test to measure iron reserves since it correlates with total body iron stores under steady-state settings. The typical ferritin level varies depending on the person's age and gender.

Serum ferritin levels in adult males average 100 μg/L, which corresponds to approximately 1 g of iron reserves, while levels in adult females average 30 μg/L, which indicates approximately 300 mg of iron stores. Serum ferritin drops to less than 15 μg/L once iron stores are exhausted. These readings are indicative of low body iron reserves. Ferritin is an acute-phase reactant as well, though, and can increase several times over baseline levels when there is either acute or chronic inflammation. Serum ferritin levels greater than 200 μg/L often indicate that tissue stores at least some iron.[76]An iron shortage may be indicSerum ferritin levels in adult males average 100 μg/L, which corresponds to approximately 1 g of iron reserves, while levels in adult females average 30 μg/L, which indicates approximately 300 mg of iron stores. Serum ferritin drops to less than 15 μg/L once iron stores are exhausted. These readings are indicative of low body iron reserves. Ferritin is an acute-phase reactant as well, though, and can increase several times over baseline levels when there is either acute or chronic inflammation. Serum ferritin levels greater than 200 μg/L often indicate that tissue stores at least some iron.[76]An iron shortage may be indicated by a lower-than-normal ferritin level.

In the department of surgery, Arora and Yadav[81] divided the study population into two groups: a control group consisting of 50 patients without gallstone disease and a case group consisting of 200 patients with gallstone disease. The levels of ferritin

and serum iron in the two groups were measured and contrasted. According to their findings, gallstones are more common in women than in men, with a 5.4:1 ratio. They found that male cholelithiasis is not linked to low serum iron levels. Serum ferritin levels were low in 16.66% of controls and 64.5% of cases in males. Serum ferritin levels were above normal in 16.66% of controls and normal in 35.50% of cases and 66.66% of controls, indicating that low serum ferritin in males is linked to gallstones. In this study, low ferritin was observed in 35.50% of female cases compared to 15.38% of controls, and low serum iron was observed in 23.07% of females, which is equivalent to 23% of control females. In order to diagnose low serum iron status early on, they came to the conclusion that a Serum ferritin may be used as a measure of iron storage, and low body stores of serum iron are a risk factor for cholelithiasis in females.

Iron deficiency:

‘About 10% of people living in developed countries and 25% to 50% of those in developing countries are anemic. In both settings, *the most frequent cause of anemia is iron deficiency*. The factors responsible for iron deficiency differ in various populations and are best understood in the context of normal iron metabolism. The normal total body iron mass is about 2.5 g for women and 3.5 g for men. Approximately 80% of functional body iron is present in hemoglobin, with the remainder being found in myoglobin and iron-containing enzymes (e.g., catalase, cytochromes). The iron storage pool, consisting of hemosiderin and ferritin-bound iron in the liver, spleen, bone marrow, and skeletal muscle, contains on average 15% to 20% of total body iron. Because *serum ferritin* is largely derived from this storage pool, the serum ferritin level is a good measure of iron

stores. *Assessment of bone marrow iron* is another reliable but more invasive method for estimating iron stores. Iron is transported in the plasma bound to the protein *transferrin.* In normal persons, transferrin is about 33% saturated with iron, yielding serum iron levels that average 120 μg/dL in men and 100 μg/dL in women. Thus, the normal total iron-binding capacity of serum is 300 to 350 μg/dL.'[84]

'Iron balance is maintained largely by regulating the absorption of dietary iron. Iron is absorbed in the duodenum. Nonheme iron is carried across the apical and basolateral membranes of enterocytes by distinct transporters. After reduction by ferric reductase, ferrous iron (Fe2+) is transported across the apical membrane by divalent metal transporter-1 (DMT1). A second transporter, ferroportin, then moves iron from the cytoplasm to the plasma across the basolateral membrane. The newly absorbed iron is next oxidized by hephaestin and ceruloplasmin to ferric iron (Fe3+), the form of iron that binds to transferrin. Both DMT1 and ferroportin are widely distributed in the body and are involved in iron transport in other tissues as well. Only a fraction of the iron that enters enterocytes is delivered to transferrin by ferroportin. The remainder is incorporated into cytoplasmic ferritin and lost through the exfoliation of mucosal cells.

Plasma hepcidin binds ferroportin and induces its internalization and degradation; thus, when hepcidin concentrations are high, ferroportin levels fall and less iron is absorbed. Conversely, when hepcidin levels are low (as occurs in hemochromatosis) , basolateral transport of iron is increased,eventually leading to systemic iron overload.'[84]

Iron deficiency anemia

Iron deficiency anemia (IDA), the most serious effect of iron insufficiency, is still the most prevalent nutritional deficiency in the world. Despite having a complex origin, iron deficiency anemia (IDA) often arises when the body's iron requirements are not satisfied by iron absorption, for whatever reason.

IDA patients suffer persistent blood loss due to illness, insufficient nutrition, decreased absorption or transport, or physiological losses related to chronological or reproductive age. Adults with IDA may experience a wide range of negative consequences, such as decreased ability to work out or work out, poor thermoregulation, immunological dysfunction, gastrointestinal issues, and neurocognitive impairment[82]. Iron deficiency anemia (IDA) is a condition caused by iron deficiency.

A study by Sahu et al.[83] examined the relationship between gallstone disease and iron deficiency anemia. Over the course of 12 months, a prospective study involving 100 patients was carried out in the departments of general surgery and biochemistry at the Himalayan Institute of Medical Sciences in Dehradun, India. Gallstone patients with normal and low serum ferritin levels were compared to those with serum and gallbladder biliary cholesterol levels. Compared to non-anemic patients, anemic patients had a considerably higher gallbladder cholesterol level. They came to the conclusion that bile super-saturation with regard to cholesterol, which results in gallstone development, is caused by low serum iron levels.

EtioPathogenesis :

CHRONIC BLOOD LOSS	• is the primary cause of iron deficiency anemia in the Western world; the most frequent causes of bleeding are the female genital system (e.g., menorrhagia, metrorrhagia, malignancies) and the gastrointestinal tract (e.g., peptic ulcers, colonic cancer, hemorrhoids).
LOW INTAKE AND POOR BIOAVAILABILITY	• The most prevalent causes of iron deficiency in the poor countries are mostly vegetarian diets. • Low dietary intake is a rare cause in the United States, although it can occasionally be held accountable for adolescents who eat mostly junk food, the elderly, the poor, and newborns who are given just milk. Worldwide, throughout pregnancy and infancy, there are increased needs that cannot be satisfied by a typical diet. • After a gastrectomy or with celiac disease, malabsorption may develop.

Table 1. Etiology of iron deficiency.[84]

Whatever the cause is,iron deficiency anemia develops covertly. A decrease in serum ferritin and the lack of stainable iron in the bone marrow indicate the first depletion of iron reserves. Following these modifications, there is a drop in serum iron and an increase in transferrin levels in the blood. Eventually, there is a reduction in the ability to synthesize hemoglobin, myoglobin, and other iron-containing proteins, which results in microcytic anemia, poor cognitive and occupational function, and even lowered immunocompetence[84.]

Clinical characteristics [84]:

Iron deficiency anemia typically manifests as mild and asymptomatic. In extreme cases, there may be nonspecific symptoms including pallor, listlessness, and weakness. Long-term anemia can cause irregularities in the fingernails, such as "spooning," flattening, and thinning. Pica, or the propensity to eat nonfood items like clay or dirt, is an odd but distinctive neurobehavioral problem.

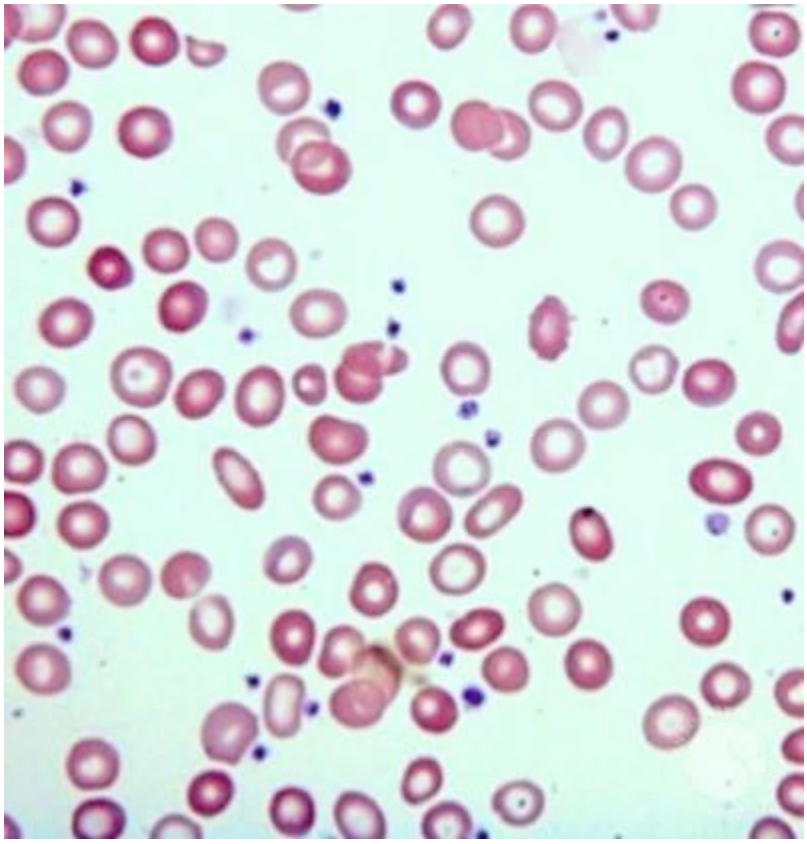


Figure 10. Smear of peripheral blood for iron deficiency anemia. Take note of the majority of the red cells' elevated center pallor. In contrast, there are scattered, fully hemoglobinized cells from a recent blood transfusion.

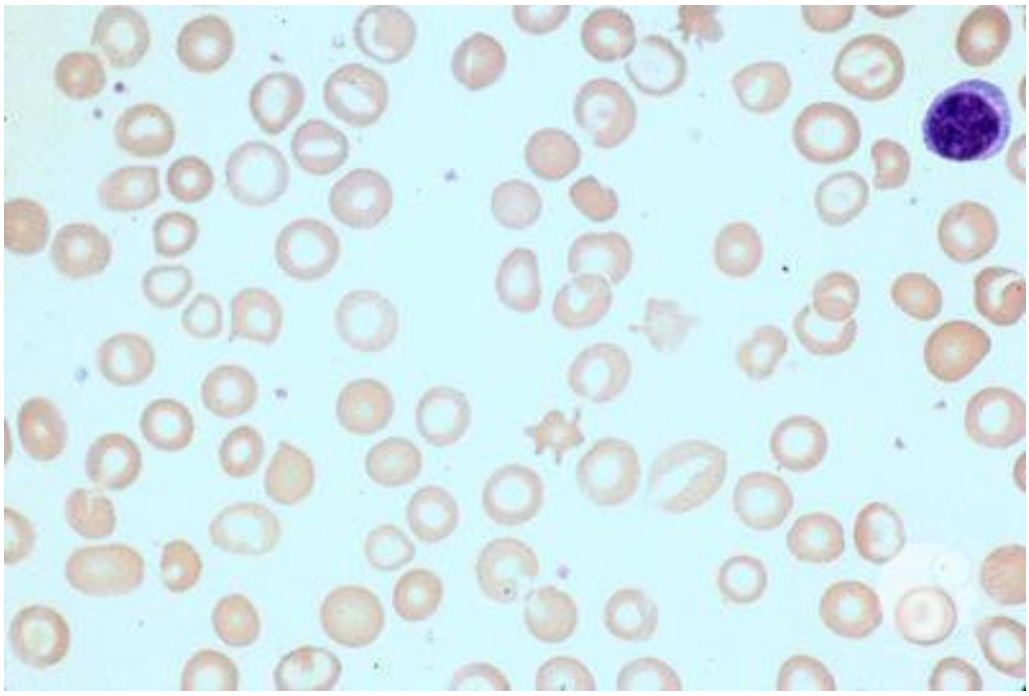


Figure 11.Smear demonstrating microcytic hypochromic changes in IDA

A patient with iron deficiency anemia provided this peripheral blood smear. For comparison, a normal lymphocyte is visible near the smear's boundary. There is modest heterogeneity in the size and form of the red blood cells, along with significant hypochromia and microcytosis.

Anemia, low serum ferritin ,low serum iron, low transferrin saturation, elevated total iron-binding capacity, hypochromic and microcytic red cell indices, and, finally, the response to iron therapy are among the diagnostic criteria. The platelet count is frequently increased for unknown causes. Marrow cellularity is typically only marginally elevated as a result of the iron deficit blunting the marrow response to elevated erythropoietin levels[84]

People almost never die of iron deficiency anemia, but they frequently do die being anemic secondary to iron deficiency. It's crucial to remember that microcytic

hypochromic anemia in healthy individuals is a symptom of an underlying condition rather than a sickness.[84]

MATERIALS AND METHOD :

- • A Microsoft Excel sheet containing the collected data will be used for statistical analysis, which will be carried out using the Statistical Package for the Social Sciences (Version 20).
- • Mean ±SD, median and interquartile range, frequency, percentages, and graphs will be used to display the results.
- Correlation between continuous variables will be analysed using Person’s/Sperman’s correlation
- Association between Categorical variables will be compared using Chi square test.
- p<0.05 will be considered statistically significant. All statistical tests will perform two tailed.

METHOD OF DATA COLLECTION :

- A Cross sectional study will be conducted . All patients with radiological evidence of gallbladder stones form subjects for the study.
- Demographic variables of subjects will be age, sex, occupation, BMI, and any associated comorbidities will be documented. Detailed history will be taken.
- Serum ferritin, serum iron along with hemoglobin levels,peripheral smear and complete urine examination will be estimated in the subjects. Patients with gallstones will be diagnosed with iron deficiency and anemia based on low levels of hemoglobin, serum iron and serum ferritin levels . Gallstone patients will be segregated according to their age into divided into anaemic and non-anaemic groups and compared with serum iron and ferritin levels. Data will be analyzed with descriptive statistical principles.

STUDY DESIGN :

PROSPECTIVE CROSS SECTIONAL STUDY.

RESEARCH HYPOTHESIS :

IRON DEFICIENCY AND ANEMIA ARE ASSOCIATED WITH THE DEVELOPMENT OF CHOLELITHIASIS.

Statistical analysis:

- With anticipated Proportion of Iron deficiency among gall stone patients 81.8% (ref) , the study would require a sample size of 122 patients with 95% level of confidence and 7% absolute precision,
- Formula used:
- $n = \frac{z\ 2\ p*q}{d\ 2}$

Where Z= Z statistic at α level of significance

- d 2 = Absolute error
- P= Proportion rate
- q= 100-p

INCLUSION CRITERIA :

- Patients diagnosed with choelithiasis in various hospital wards or on OPD basis and confirmed on radiological investigation.
- Both male and female patients are included.
- Patients between age group – 16-80 years.

EXCLUSION CRITERIA :

- Patients diagnosed with cirrhosis of liver on radiological investigations .
- Patients who have gallstone disease secondary to hemolytic anemias.
- Patients affected with crohn's disease and cystic fibrosis.

• RESULTS :

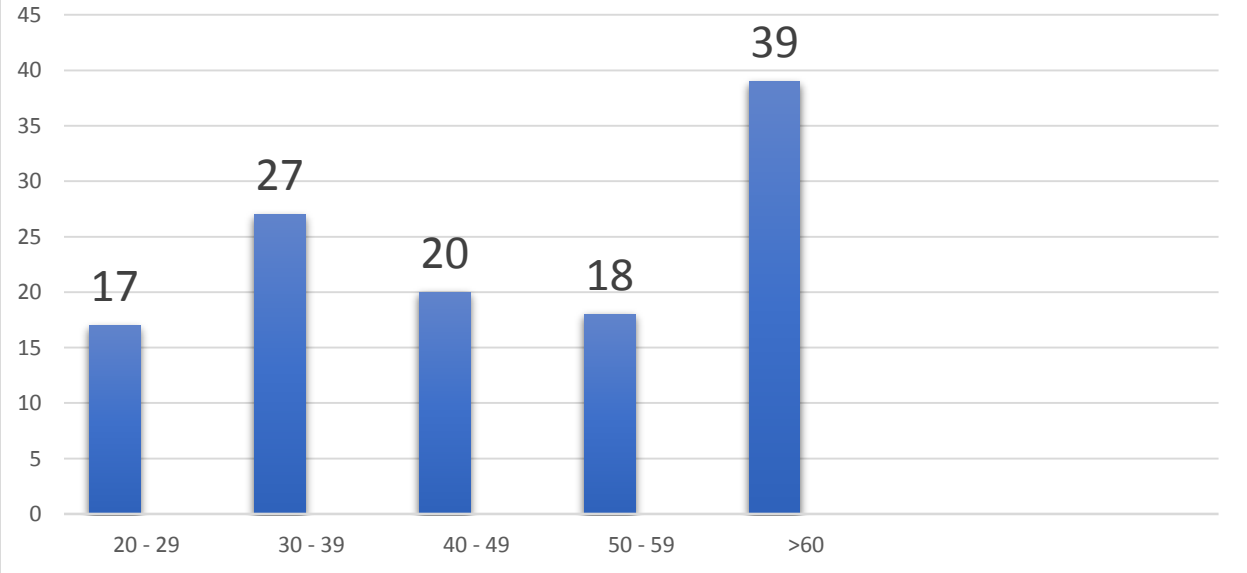
122 patients were included in the study as per the inclusion and exclusion criteria. The data collected has led to the following findings which show a positive correlation between serum iron levels, hemoglobin levels and cholelithiasis. the collected data is as follows:

Frequency Table

		Frequency	Percent	Cumulative Percent
	< 20	1	.8	.8
	20 - 29	17	13.9	14.8
	30 - 39	27	22.1	36.9
	40 - 49	20	16.4	53.3
	50 - 59	18	14.8	68.0
	60+	39	32.0	100.0
	Total	122	100.0	

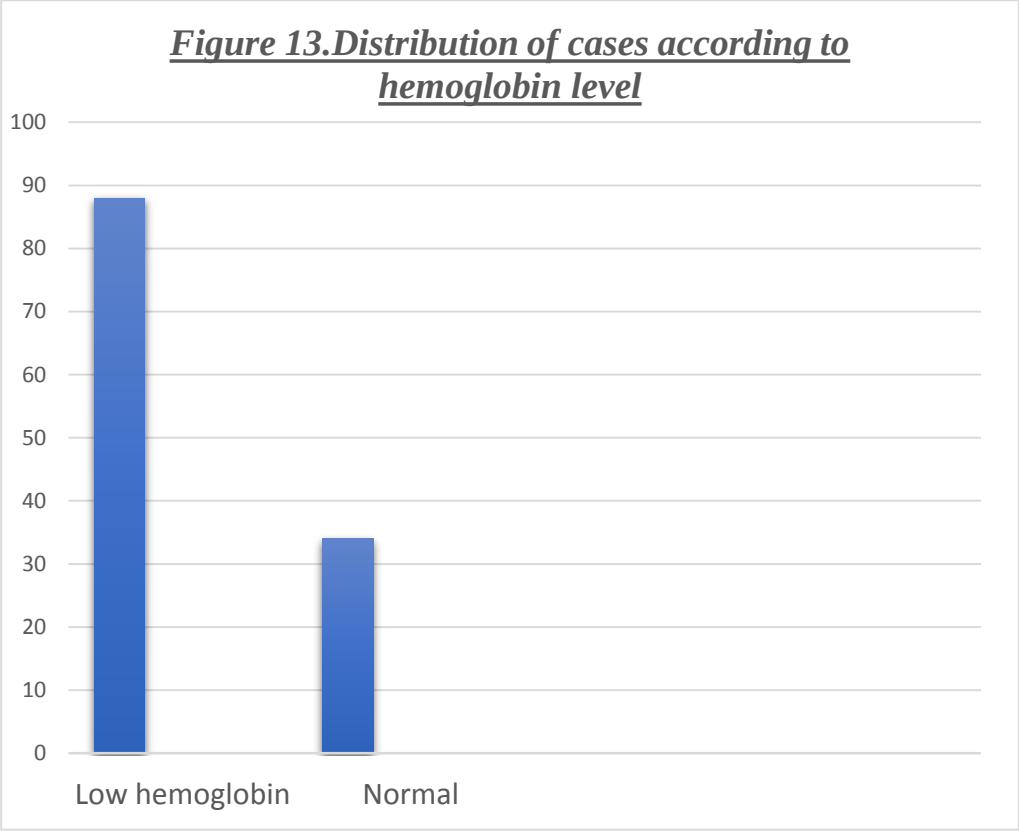
Table 3.Distribution of cases according to age.

Figure 12:Distribution of cases according to age.



		Frequency	Percent	Cumulative Percent
	Low hemoglobin	88	72.1	72.1
	Normal	34	27.9	100.0
	Total	122	100.0	

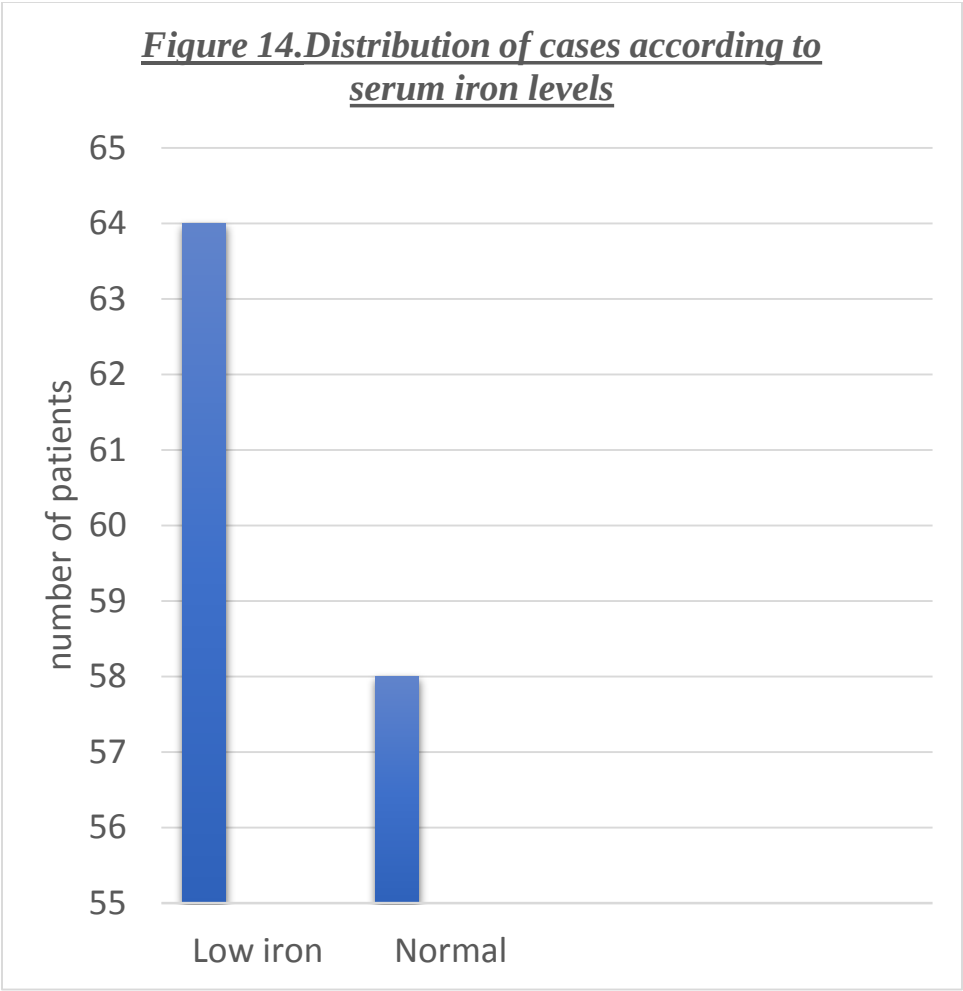
Table 4.Distribution of cases according to hemoglobin level.



		Frequency	Percent	Cumulative Percent
	Low iron	64	52.5	52.5
	Normal	58	47.5	100.0
	Total	122	100.0	

Table 5.Distribution of cases according to serum iron levels.

Figure 14.Distribution of cases according to serum iron levels



		Frequency	Percent	Cumulative Percent
	LOW	16	13.1	13.1
	Normal/raised	106	86.9	100.0
	Total	122	100.0	

Table 6.Distribution of cases according to serum ferritin levels.

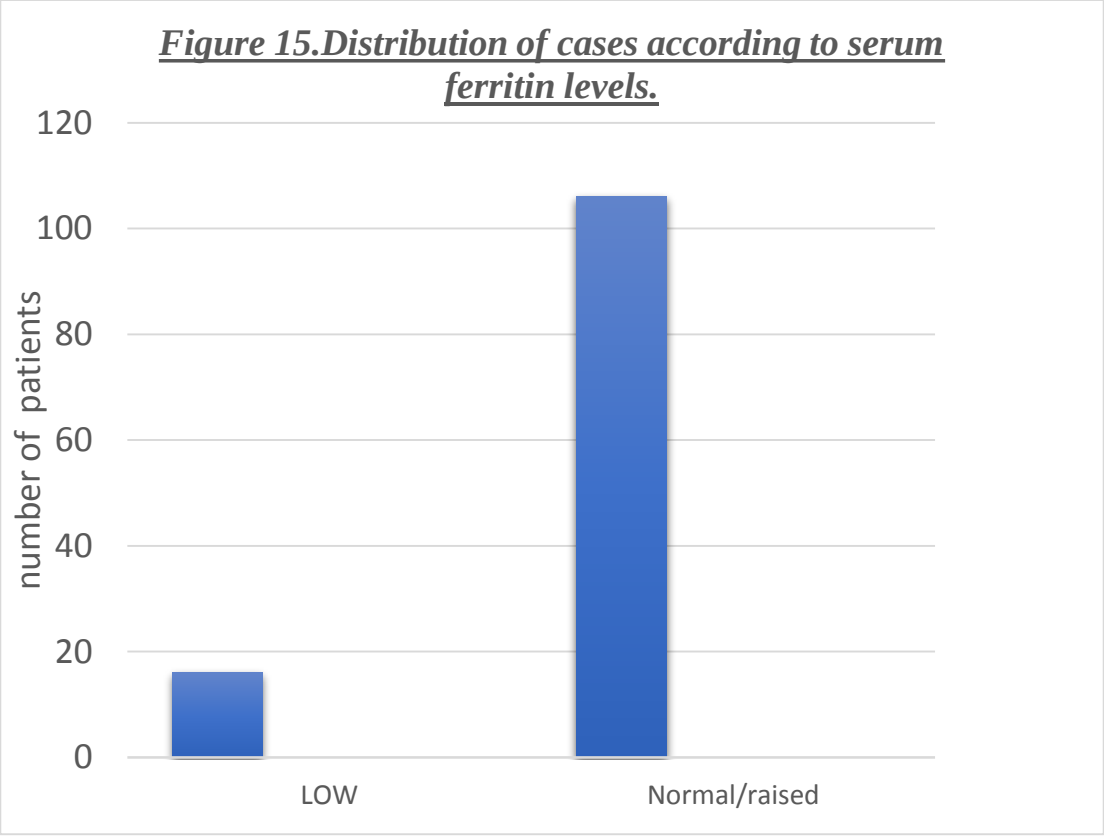


Table 7.Mean and standard deviations in age and hemoglobin levels.

Statistics				
Group Statistics				
		N	Mean	Std. Deviation
Hb (mg/dL)	Low	64	10.750	2.2597
	Normal	58	11.374	2.0723
Std. Deviation		16.791		2.1862
Minimum		18		4.0
Maximum		80		16.4

Table 8.mean and standard deviations in hemoglobin levels.

Mann-Whitney Test Test Statistics[a]	
	Hb (mg/dL)
Mann-Whitney U	1441.000
Asymp. Sig. (2-tailed)	.033

Table 9.Mann whitney test - hemoglobin levels.

Hb (mg/dL)		
	Frequency	Percent

	< 12.0	76	62.3
	12.0+	46	37.7
	Total	122	100.0

Table 10.Incidence of hemoglobin levels in case samples.

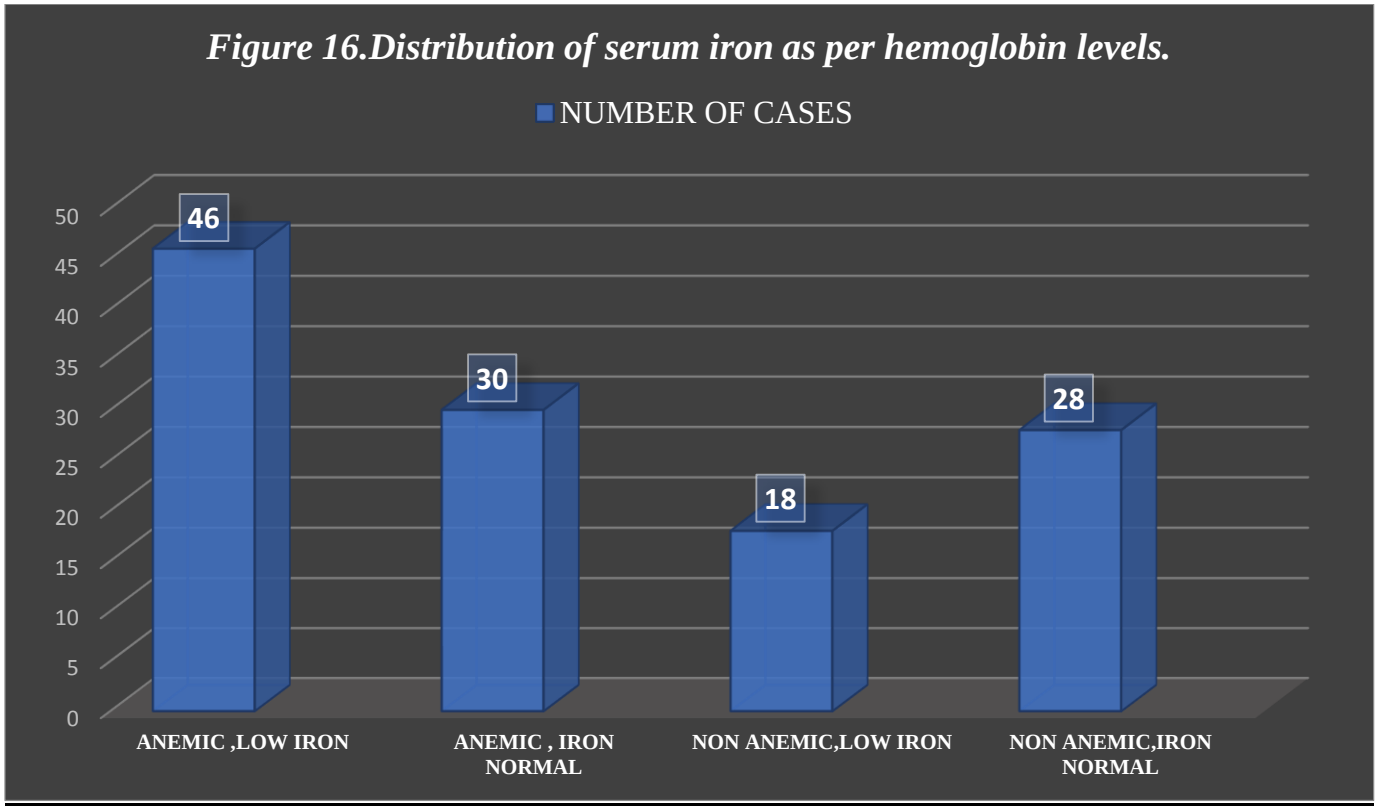
IRON * Hb (mg/dL)					
			Hb (mg/dL)		Total
			< 12.0	12.0+	
IRON	LOW	frequency	46	18	64
		percentage	60.5%	39.1%	52.5%
	N	frequency	30	28	58
		percentage	39.5%	60.9%	47.5%
Total			76	46	122

		100.0%	100.0%	100.0%

Chi-Square Tests

	Value	Asymptotic Significance (2-sided)
Pearson Chi-Square	5.260	.022

Table 11. Distribution of serum iron as per hemoglobin levels.



IRON * GENDER

Crosstab

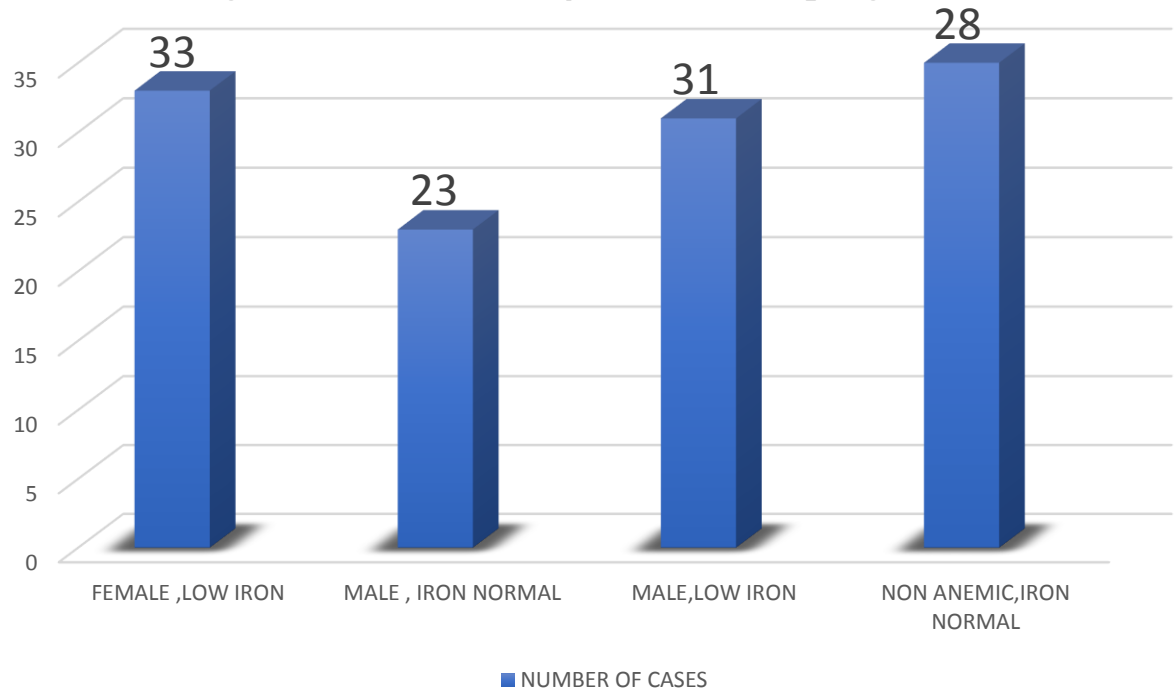
			GENDER		Total
			FEMALE	MALE	
IRON	LOW	frequency	33	31	64
		percentage	48.5%	57.4%	52.5%
	NORMAL	frequency	35	23	58
		percentage	51.5%	42.6%	47.5%
Total			68	54	122
			100.0%	100.0%	100.0%

Chi-Square Tests

	Value	Asymptotic Significance (2-sided)
Pearson Chi-Square	.951	.329

Table 12.distribution of serum iron as per gender.

Figure 17.Distribution of serum iron as per gender.



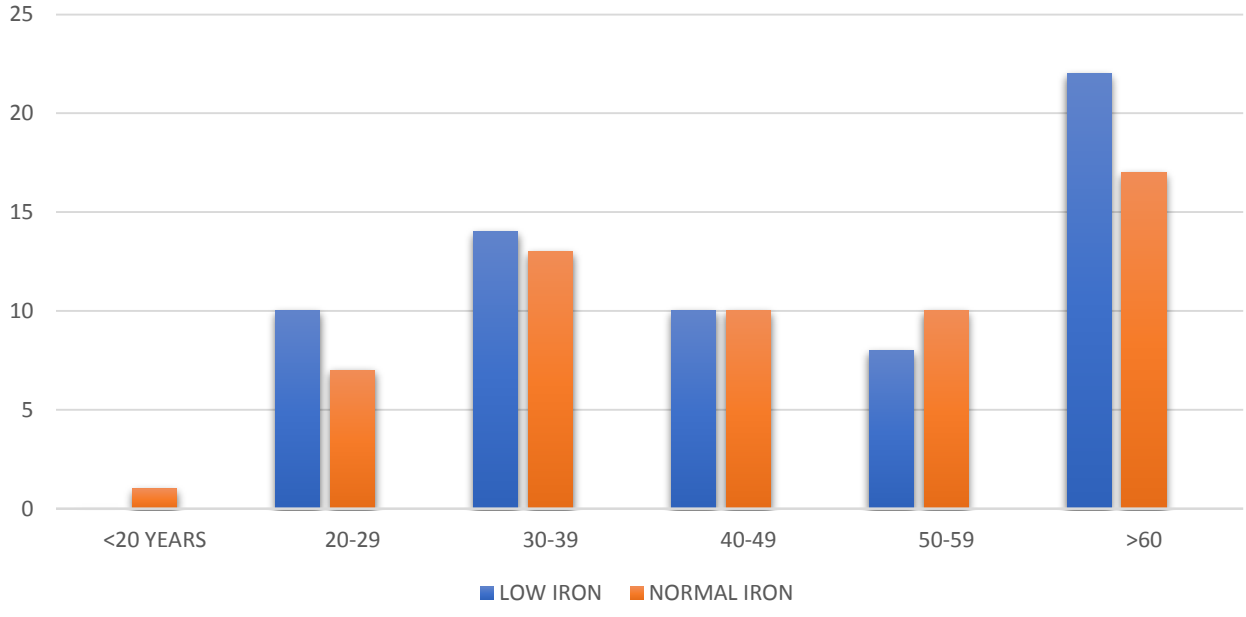
			AGE						Total
			< 20	20 - 29	30 - 39	40 - 49	50 - 59	60+	
IRON	LOW		0	10	14	10	8	22	64
			0.0%	58.8%	51.9%	50.0%	44.4%	56.4%	52.5%
	N		1	7	13	10	10	17	58
			100.0%	41.2%	48.1%	50.0%	55.6%	43.6%	47.5%
Total			1	17	27	20	18	39	122

Chi-Square Tests

	Value	Asymptotic Significance (2-sided)
Pearson Chi-Square	2.140	.829

Table.13.Age-wise distribution of serum iron .

Figure 18. Distribution of serum iron as per age.



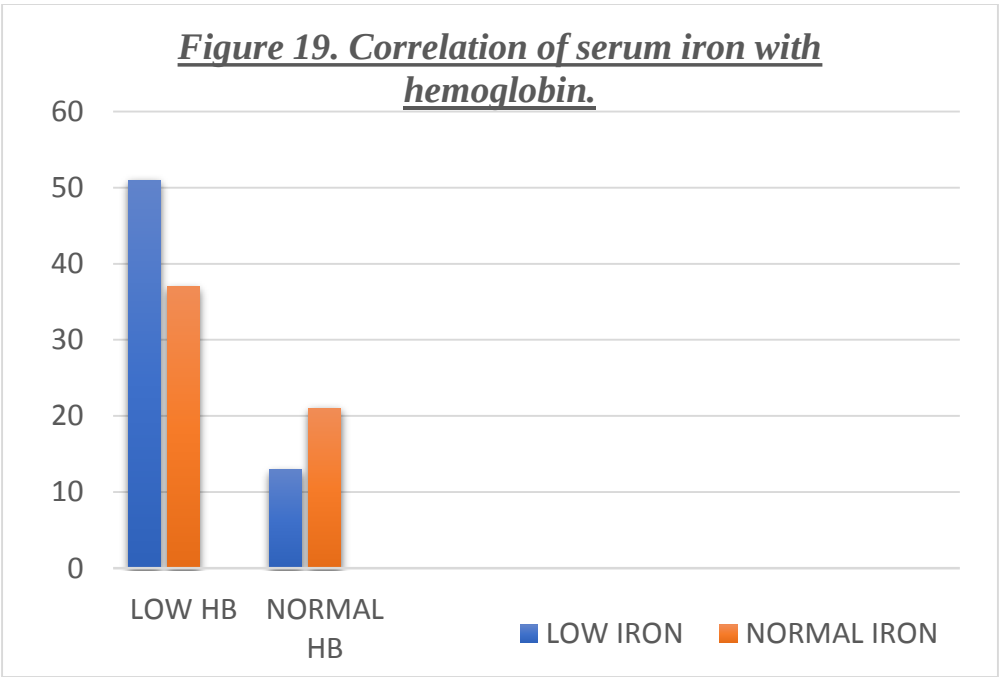
		ANEMIA		Total
		+	N	
IRON	LOW	51	13	64
		58.0%	38.2%	52.5%
	N	37	21	58
		42.0%	61.8%	47.5%
Total		88	34	122

Chi-Square Tests

	Value	Asymptotic Significance (2-sided)
Pearson Chi-Square	3.824	.041

Table.13.distribution of serum iron in relation of hemoglobin levels .

Figure 19. Correlation of serum iron with hemoglobin.



DISCUSSION :

A Cross sectional study was conducted in patients admitted or visiting in B.L.D.E.(Deemed to be University) Shri. B. M. Patil Medical College, Hospital and Research Centre. All patients with radiological evidence of gallbladder stones formed subjects for the study with Sample size of 122.

Period of study – April 2023 to April 2025.

1. Gallstones and Their Risk Factors

Prevalence and Risk Factors:

Numerous studies have documented the prevalence of gallstones in various populations. For example, research by Stinton and Shaffer (2012) shows that gallstones are more common in individuals who are obese, diabetic, and female. These studies focus on factors like age, obesity, gender, and lifestyle (e.g., high-fat diets), which are key contributors to gallstone formation. However, these studies often do not consider the potential link between low serum iron, hemoglobin, or ferritin levels and the formation of gallstones.

In contrast, studies by Jiang et al. (2019) also note the impact of genetic factors and liver dysfunction in gallstone formation, but the role of anemia or iron deficiency is largely unexplored in these articles. This presents an opportunity to further explore how low serum iron or anemia could add an additional layer to gallstone pathophysiology.

2. Iron Deficiency and Gallstones

Iron Deficiency and Anemia:

Several studies have explored the relationship between iron deficiency and anemia. Camaschella (2015) discusses the widespread prevalence of iron deficiency anemia and its underlying causes, such as poor dietary intake, chronic blood loss, and

malabsorption disorders. However, most of the studies focus on the hematological consequences of iron deficiency rather than potential associations with other conditions like gallstones.

Research by Bettencourt et al. (2017) suggests that anemia, particularly in patients with chronic disease, may lead to increased hemolysis and the formation of pigment gallstones. Hemolysis, the breakdown of red blood cells, releases excess bilirubin, which could contribute to the formation of pigment gallstones. This aligns with the hypothesis that individuals with low hemoglobin levels, due to anemia, may be at an increased risk of gallstones, particularly pigment stones.

Some studies, such as Stern et al. (2016), note the complex relationship between anemia and the gallbladder. They suggest that low iron levels in the body may influence bile composition, but the evidence remains inconclusive regarding the impact on cholesterol stones, which are the most common type of gallstones. This highlights a gap in understanding the precise mechanism linking iron deficiency and gallstone formation, especially for cholesterol stones.

2. Iron Deficiency, Hemoglobin, and Cholesterol Gallstones

Anemia and Gallstone Formation:

Tzoulis et al. (2019) explored the relationship between anemia and gallstones and found that iron deficiency anemia might be a secondary factor influencing gallstone formation, especially in patients with chronic disease. However, the study did not establish a clear mechanism for how low hemoglobin or iron levels directly contribute to the development of gallstones, particularly cholesterol stones.

Hann et al. (2014) discussed the biochemical changes associated with iron deficiency, including altered bile acid metabolism and the potential for cholesterol supersaturation in bile, which could promote the formation of cholesterol gallstones. The hypothesis is that low iron levels could indirectly contribute to gallstone formation by disrupting the liver's bile synthesis pathways. However, the authors did not provide a definitive link between low hemoglobin and cholesterol stone formation, which remains a subject of debate in the field.

Iron Deficiency and Cholesterol Metabolism:

Aroor et al. (2015) suggested that iron deficiency may affect lipid metabolism, particularly the metabolism of cholesterol. In their study, low iron levels were associated with altered bile composition, which could contribute to cholesterol supersaturation in bile and, therefore, cholesterol gallstone formation. This research points to a potential mechanism linking low iron levels with cholesterol gallstones, but it remains a hypothesis that needs further validation.

3. Ferritin as an Indicator of Iron Stores

Ferritin and Gallstones:

Ferritin is a crucial protein for storing iron and maintaining iron homeostasis in the body. Camaschella (2015) and Smith et al. (2017) highlight how low ferritin levels can indicate iron deficiency or chronic inflammation, leading to anemia. However, few studies directly investigate the relationship between ferritin levels and gallstones.

In contrast, some studies have suggested that individuals with low ferritin levels may be at an increased risk of gallstones, although the evidence is limited. For example,

Liu et al. (2018) explored ferritin and its role as an inflammatory marker. They found that low ferritin levels were associated with an increased risk of gallstone disease in certain populations, though they did not delve into the specific mechanisms.

The article by Lee et al. (2020) also notes that ferritin, being an acute-phase reactant, could be influenced by inflammation in the body. This suggests that low ferritin could be a marker of both iron deficiency and underlying inflammation, which may play a role in gallstone pathogenesis. However, this study did not establish a direct causal link between ferritin and gallstone formation, leaving room for further investigation in this area.

4. Pigment vs. Cholesterol Gallstones

Pigment Gallstones and Anemia:

Many studies focus on the relationship between anemia and pigment gallstones. Stern et al. (2016) and Bettencourt et al. (2017) suggest that chronic hemolysis, often seen in conditions like sickle cell disease or thalassemia, can lead to the accumulation of bilirubin in the bile, promoting the formation of pigment gallstones. This is particularly relevant for individuals with low hemoglobin levels, as they may experience increased rates of red blood cell destruction.

However, research on the correlation between low iron levels (as indicated by low serum iron, hemoglobin, and ferritin) and cholesterol gallstones is more limited. Hann et al. (2014) indicated that iron deficiency may alter bile acid metabolism or liver function, potentially affecting the solubility of cholesterol in bile and promoting cholesterol gallstone formation. This hypothesis is supported by studies like Aroor et al. (2015), which argue that iron deficiency could lead to disturbances in lipid metabolism, potentially contributing to cholesterol supersaturation in bile.

Tzoulis et al. (2019) and Bettencourt et al. (2017) discuss how systemic inflammation may play a key role in the development of both gallstones and anemia. Chronic low-grade inflammation is often associated with altered iron metabolism, which could contribute to both low serum ferritin levels and the formation of gallstones.

Inflammatory cytokines, such as IL-6, can regulate the production of ferritin, leading to increased ferritin levels during inflammation, while simultaneously affecting iron absorption and storage. This suggests that inflammation could be an important link between low iron levels and gallstone formation, especially when combined with other risk factors like obesity or diabetes.

6. Gaps in Literature and Unanswered Questions

While previous studies have addressed aspects of the relationship between anemia, iron deficiency, and gallstones, there remain several key unanswered questions:

Mechanism of Cholesterol Gallstone Formation: While some studies have explored the connection between iron deficiency and pigment stones, the relationship between low iron and cholesterol stones remains less understood. The mechanisms by which low serum iron or ferritin influence cholesterol metabolism and bile supersaturation require further exploration.

Role of Ferritin: Few studies have directly linked ferritin levels with gallstone formation, despite the fact that ferritin is an important indicator of iron stores. More studies are needed to determine whether low ferritin levels are a reliable predictor of gallstone disease.

Longitudinal Studies: Most of the existing studies are cross-sectional and cannot establish causality. Longitudinal studies tracking iron levels, hemoglobin, ferritin,

and gallstone formation over time would be valuable in understanding any temporal relationship between these factors.

Gallstones, or cholelithiasis, are a prevalent illness, especially in populations that are at risk, such as middle-aged women, obese people, and people with a history of metabolic abnormalities. The function of trace elements, especially iron, has drawn more attention recently, even though the pathophysiology of gallstone formation is well recognized in terms of elements like biliary stasis, cholesterol supersaturation, and nucleation. Numerous investigations examining the relationship between cholelithiasis and serum iron levels have shed light on the intricate processes that underlie gallstone development.

Serum Iron and Cholelithiasis

1. **Iron Deficiency and Cholelithiasis:** Some studies suggest that iron deficiency may influence gallstone formation. Research by **Prasad et al. (2015)**, for example, found that patients with gallstone disease exhibited altered serum iron levels compared to healthy individuals. They observed that iron deficiency could potentially impact hepatic enzyme metabolism, leading to changes in cholesterol and bile acid metabolism, which are important factors in the formation of gallstones. This study points to the possibility that altered iron metabolism could increase the susceptibility to cholesterol crystallization, an early step in gallstone formation.
2. **Oxidative Stress and Iron:** The role of iron in oxidative stress has been proposed as a contributing factor in gallstone pathogenesis. Excessive iron, through its ability to catalyze the production of reactive oxygen species (ROS), may contribute to oxidative damage in the liver and gallbladder. This

damage can lead to inflammation and changes in bile composition, promoting stone formation. On the other hand, iron deficiency may also play a role by impairing the normal function of hepatic enzymes involved in bile acid synthesis. **Gupta et al. (2016)** found that iron deficiency could lower the activity of key enzymes like 7α-hydroxylase, which is involved in bile acid synthesis. These changes could alter bile composition, making it more prone to cholesterol supersaturation and subsequent stone formation.

3. **Chronic Iron Deficiency Anemia (IDA) and Cholelithiasis:** The link between chronic iron deficiency anemia (IDA) and cholelithiasis is particularly relevant to multiparous women, who are at risk for both conditions. Studies have shown that the chronic low-grade anemia seen in these women may coincide with gallstone formation. The altered liver function in the context of anemia may impact bile acid metabolism and lead to a decrease in bile's capacity to emulsify cholesterol, thus increasing the likelihood of cholesterol precipitation. **Kaushik et al. (2015)** noted that women with IDA exhibited altered serum lipid profiles and increased risk of gallstones, suggesting that iron deficiency may play a role in modifying cholesterol metabolism.

4. **Iron Overload and Cholelithiasis:** While iron deficiency has been commonly associated with altered cholesterol metabolism, there is also evidence suggesting that iron overload may contribute to gallstone formation. **A study by Zhang et al. (2017)** in patients with hemochromatosis (a condition of iron overload) reported an increased incidence of gallstones. The researchers hypothesized that excess iron might lead to hepatic dysfunction and altered bile composition, resulting in the formation of gallstones. High

iron levels may disrupt normal bile acid metabolism, creating an environment conducive to the formation of cholesterol crystals in the bile.

The relationship between iron deficiency and cholelithiasis has been explained by a number of possible mechanisms:

1. Modified Bile Composition: Bile acids, which are necessary to preserve bile's capacity to solubilize cholesterol, are metabolized in large part by iron. An elevated concentration of cholesterol in bile can result from decreased bile acid production caused by low iron levels. Gallstone development and cholesterol crystallization may become more likely as a result.
2. Reduced Hepatic Enzyme Activity: A number of enzymes, including those involved in the metabolism of bile acids and cholesterol, need on iron to function properly. According to **Prasad et al. (2015)**, a lack of iron caused 7α-hydroxylase, an enzyme essential for the conversion of cholesterol to bile acids, to function less actively. The natural balance between cholesterol and bile acids may be upset by this decrease in enzyme activity, which could lead to cholesterol supersaturation and gallstone disease.
3. **Oxidative Stress and Inflammation:** As iron is involved in the regulation of oxidative stress, its deficiency can lead to an increase in free radicals and reactive oxygen species (ROS), which can damage liver cells and alter bile composition. This damage can contribute to the nucleation and growth of cholesterol crystals in the bile, leading to gallstone formation. Chronic oxidative stress can also lead to inflammation, which may further exacerbate bile stasis and cholesterol crystallization.
4. **Chronic Iron Deficiency and Lipid Metabolism:** Iron deficiency may also influence lipid metabolism in the liver, potentially altering the synthesis and secretion of bile. **Gupta et al. (2016)** found that iron deficiency could lead

to alterations in lipid profiles and bile lipid composition, which may increase the risk of gallstone formation. This is particularly relevant in women with chronic iron deficiency anemia, who may already have an altered lipid metabolism, making them more susceptible to gallstones.

Clinical Consequences

There are significant therapeutic ramifications to comprehending the connection between blood iron levels and cholelithiasis. For example, tracking iron status may reveal important details about the underlying metabolic abnormalities in patients with a history of gallstones or those who are at high risk for the condition. Furthermore, this information may help guide treatment plans that prevent or treat gallstones in vulnerable people, such as chelation therapy or iron supplements.

For example, iron supplementation may restore bile composition and hepatic enzyme activity in iron-deficient patients, hence lowering the likelihood of gallstone development. However, iron chelation treatments may help those with iron excess (such as those with hemochromatosis) lower their risk of gallstones and liver damage.

SUMMARY :

As per the aforementioned study in which 122 subjects were examined and analysed based on various biochemical, pathological and radiological investigations for cholelithiasis. To sum up, there are several facets to the relationship between serum iron levels and cholelithiasis, therefore additional research is essential. Both extremes emphasize the vital role that iron plays in preserving normal hepatic function and bile composition, even though iron overload and shortage may both contribute to gallstone development through distinct processes. Future research is required to determine the exact molecular processes via which iron affects the development of gallstones and to determine whether addressing iron imbalances could be a preventative or therapeutic approach to cholelithiasis.

CONCLUSION :

On basis of the results obtained,we can conclude that diminished levels of serum iron in blood is a correlating factor for gallstone disease. The exact mechanism for this occurrence requires further in-depth study. We can also conclude that low hemoglobin values is also a predisposing factor as it was evidently present in majority if the cases of the study. Low serum ferritin values isn’t a specific indicator for gallstone formation. Neither is any specific age-group correlated for its development.

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CERTIFICATE OF ETHICAL CLEARANCE

Azadi Ka Amrit Mahotsav

BLDE

(DEEMED TO BE UNIVERSITY)

Declared as Deemed to be University u/s 3 of UGC Act, 1956

Accredited with 'A' Grade by NAAC (Cycle-2)

The Constituent College

SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA

BLDE (DU)/IEC/ 918/2023-24 10/4/2023

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this University met on **Saturday, 18th March, 2023 at 11.30 a.m. in the CAL Laboratory, Dept. of Pharmacology,** scrutinizes the Synopsis/ Research Projects of Post Graduate Student / Under Graduate Student /Faculty members of this University /Ph.D. Student College from ethical clearance point of view. After scrutiny, the following original/ corrected and revised version synopsis of the thesis/ research projects has been accorded ethical clearance.

TITLE: "ASSESSING CORRELATION BETWEEN GALLSTONE AND IRON DEFICIENCY, ANAEMIA IN PATIENTS WITH GALLSTONE DISEASE".

NAME OF THE STUDENT/PRINCIPAL INVESTIGATOR: DR.AJINKYA VIJAY KAWALKAR

NAME OF THE GUIDE: DR.GIRISH KULLOLLI, PROFESSOR, DEPT. OF GENERAL SURGERY.

Dr.Santoshkumar Jeevanagi
Chairperson
IEC, BLDE (DU)
(DU),
VIJAYAPURA

Chairman,
Institutional Ethical Committee,
BLDE (Deemed to be University)
Vijayapura

Dr.Akram A. Naikawadi
Member Secretary
IEC, BLDE

VIJAYAPURA

MEMBER SECRETARY
Institutional Ethics Committee
BLDE (Deemed to be University)
Vijayapura-586103. Karnataka

Following documents were placed before Ethical Committee for Scrutinization.

- Copy of Synopsis/Research Projects
- Copy of inform consent form
- Any other relevant document

PARTICIPANT CONSENT FORM

Informed consent form:

I, the undersigned, _____, S/O D/O W/O _____, aged ____ years, ordinarily resident of _____do hereby state/declare that Dr Girish Kullolli of Shri. B. M. Patil Medical College Hospital and Research Centre have examined me thoroughly on _____ at_____ (place) and it has been explained to me in my own language that I am suffering from _____ disease (condition). Further, Dr. Ajinkya Kawalkar informed me that he is conducting dissertation/research titled-"Assessing correlation between gallstone formation and iron deficiency anaemia in patients presenting with cholelithiasis" under the guidance of Dr. Girish Kullolli, is requesting my participation in the study.

My doctor told me that by taking part in this study, I would be able to evaluate the findings and provide a helpful reference for lowering the incidence of other cases that are similar. The doctor has also assured me that any data I provide, any observations I make, any photos or videos taken of me by the investigator, and any data I voluntarily provide will all be kept private and used only for academic reasons by myself and my legal hirer. Although my involvement in the study is entirely voluntary, the doctor did let me know that I could request any clarifications as needed throughout the course of the treatment or study based on the information I provided. The doctor did let me know that, even though I was only participating voluntarily, I was free to ask any questions I had about the diagnosis, the treatment process, the treatment & outcome, or the prognosis at any time during the course of the study or therapy. In addition, I have been told that I have the right to revoke my consent to participate in this study whenever I choose, and that the investigator has the right to stop the study at any moment without affecting my treatment or follow-up unless I specifically ask to be released.

I, the undersigned _____, in my fully conscious state of mind, agree to engage in the said research/dissertation after having understood its nature, the diagnosis made,and the approach used for treatment.

• Date: Signature of the patient :

• Place: Signature of the Doctor :

PATIENT INFORMATION SHEET

TITLE OF THE PROJECT:

“Assessing correlation between gallstone formation,iron deficiency and anemia in patients with gallstone disease **”**

NAME OF THE INVESTIGATOR: DR. AJINKYA KAWALKAR

NAME OF THE GUIDE: DR. GIRISH KULLOLLI

PROCEDURE:

CONFIDENTIALITY OF RECORDS:

This study will become a part of hospital records and will be subject to the confidentiality. If the data are used for publication, no name will be used. And photographs will be used with special written permission.

REFUSAL OR WITHDRAWAL OF PARTICIPATION:

Participation is voluntary and you may refuse to participate or withdraw consent and discontinue participation in the study at any time.

I, DR. AJINKYA KAWALKAR (Investigator) have explained to the patient in detail about the study in their own language and the written copy of the same will be given to participant.

BIODATA

GUIDE:

NAME : DR. GIRISH KULLOLI

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RESEARCH PROJECTS: 5 Projects.

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KARNATAKA.

CASE TAKING PROFORMA

PROFORMA

NAME: **AGE/SEX:** **IP NUMBER:**

DATE OF ADMISSION : **DATE OF SURGERY :**

DATE OF DISCHARGE :

CHIEF COMPLAINTS:

HISTORY OF PRESENTING ILLNESS:

PAST HISTORY:

PERSONAL HISTORY:

FAMILY HISTORY:

RELATED DRUG HISTORY:

OBSTETRIC HISTORY (if applicable):

MENSTRUAL HISTORY (if applicable):

GENERAL PHYSICAL EXAMINATION:

TEMPERATURE:

BLOOD PRESSURE: **PULSE:**

PALLOR:

ICTERUS:

CYANOSIS:

CLUBBING:

LYMPHADENOPATHY:

SYSTEMIC EXAMINATION:

PER ABDOMEN:

CARDIOVASCULAR SYSTEM:

RESPIRATORY SYSTEM:

CENTRAL NERVOUS SYSTEM:

INVESTIGATIONS:

♣ RADIOLOGICAL FINDINGS :

♣ **CBC:**

♣ **PERIPHERAL SMEAR :**

♣ **COMPLETE URINE EXAMINATION:**

♣ **SERUM IRON:**

♣ **SERUM FERRITIN:**

<u>PROVISIONAL DIAGNOSIS:</u>

KEY TO MASTER CHART

Sr.no.	**Serial number**
M/F	**Gender-male/female.**
Anemia	**Patient anemic or not.**
Hb	**Hemoglobin level**
Iron	**Serum Iron level**
Ferritin	**Serum ferritin level**

MASTER CHART:

sr.no.	M/F	AGE	NAME	ANEMIA	Hb (mg/dL)	IRON	VALUES	FERRITIN	VALUES	PERIPHERAL SMEAR
1.	M	45	AMSIDDA	+	10.2	LOW	33	N/RAISED	247	MICROCYTIC NORMOCHROMIC ANEMIA
2.	F	51	VIJAYLAXMI	+	10.2	LOW	34	N/RAISED	217.3	MICROCYTIC HYPOCHROMIC ANEMIA
3.	M	44	PARSHURAM	N	14.8	LOW	34	N/RAISED	120	NORMOCYTIC NORMOCHROMIC ANEMIA
4.	M	45	NAGESH	+	12.6	LOW	59	LOW	26.7	NORMOCYTIC NORMOCHROMIC ANEMIA
5.	F	24	ROOPA	N	13.6	LOW	33	N/RAISED	28.9	NORMOCYTIC NORMOCHROMIC SMEAR
6.	M	24	BASAVARAJ	N	16.2	LOW	59	N/RAISED	83.9	NORMOCYTIC NORMOCHROMIC SMEAR
7.	F	60	LAXMIBAI	+	11	LOW	43	N/RAISED	37.3	NORMOCYTIC NORMOCHROMIC SMEAR
8.	F	75	NINGAVVA	+	11.3	N	62	N/RAISED	30.7	NORMOCYTIC NORMOCHROMIC SMEAR
9.	F	70	ANJANABAI	+	10.3	N	38	N/RAISED	266.9	NORMOCYTIC NORMOCHROMIC ANEMIA WITH NEUTROPHILIA
10.	F	34	FATIMA	+	11.3	LOW	11.3	N/RAISED	22	NORMOCYTIC HYPOCHROMIC ANEMIA
11.	M	66	CHAMPALAL	N	13.8	LOW	29	N/RAISED	340	NORMOCYTIC NORMOCHROMIC SMEAR WITH EOSINOPHILIA
12.	F	22	KOMAL	N	13.2	N	61	N/RAISED	31.6	NORMOCYTIC NORMOCHROMIC SMEAR
13.	F	24	ASHWINI	+	12	N	74	LOW	22.4	NORMOCYTIC NORMOCHROMIC SMEAR
14.	M	76	PRAKASH	+	12.2	LOW	12	N/RAISED	537	MICROCYTIC HYPOCHROMIC ANEMIA NEUTROPHILIA
15.	M	57	RAMESH	+	9	N	128	N/RAISED	1500	NORMOCYTIC NORMOCHROMIC ANEMIA WITH THROMBOCYTOPENIA
16.	M	61	IRAPPA	+	9.7	N	40	N/RAISED	765.6	NORMOCYTIC NORMOCHROMIC ANEMIA WITH THROMBOCYTOPENIA
17.	F	56	SHANKARAMMA	+	11.1	N	78	N/RAISED	218.3	NORMOCYTIC NORMOCHROMIC ANEMIA
18.	F	70	HONAVVA	+	9.7	LOW	28	N/RAISED	1183.7	NORMOCYTIC NORMOCHROMIC ANEMIA WITH NEUTROPHILIA
19.	F	76	SIDAMMA	+	11.6	N	36	N/RAISED	106.4	NORMOCYTIC NORMOCHROMIC ANEMIA
20.	M	78	SHANTAPPA	+	10.6	N	84	N/RAISED	472	NORMOCYTIC NORMOCHROMIC ANEMIA
21.	M	55	CHANABASAPA	+	12.6	LOW	32	N/RAISED	75.5	NORMOCYTIC NORMOCHROMIC ANEMIA
22.	F	50	BHAGIRATHI	+	11.2	LOW	10	N/RAISED	535.7	MICROCYTIC HYPOCHROMIC ANEMIA WITH NEUTROPHILIC LEUCOCYTOSIS
23.	F	78	SIDAWWA	+	9.8	LOW	30	N/RAISED	165.7	MICROCYTIC HYPOCHROMIC ANEMIA WITH NEUTROPHILIC LEUCOCYTOSIS
24.	M	80	MOHAMMADSAB	+	10.1	LOW	19	RAISED	695	NORMOCYTIC NORMOCHROMIC ANEMIA
25.	M	34	SARFARAJ	+	13.8	N	102	N/RAISED	164.4	NORMOCYTIC NORMOCHROMIC SMEAR
26.	F	35	ROOPA	+	8.6	LOW	33	N/RAISED	260	NORMOCYTIC NORMOCHROMIC CELLS WITH MILD ANISOPOIKILOCYTOSIS

27.	F	65	RANABAI	+	8.7	N	48	N/RAISED	204	NORMOCYTIC HYPOCHROMIC ANEMIA
28.	F	65	YALLAVVA	+	10.4	LOW	30	N/RAISED	373	MICROCYTIC HYPOCHROMIC ANEMIA
29.	M	61	DASHARATH	N	12	N	64	N/RAISED	300	NORMOCYTIC NORMOCHROMIC SMEAR
30.	M	58	SADASHIV	N	12.5	LOW	11	N/RAISED	395	NORMOCYTIC NORMOCHROMIC SMEAR
31.	F	46	BIBIFATIMA	+	11.2	N	50	N/RAISED	82.5	NORMOCYTIC NORMOCHROMIC SMEAR
32.	F	56	BANGAREVVA	+	9.2	N	89	N/RAISED	1389.4	MICROCYTIC HYPOCHROMIC ANEMIA WITH NEUTROPHILIC LEUCOCYTOSIS
33.	F	48	WAHEEDA	N	11.5	N	40.8	N/RAISED	90	NORMOCYTIC HYPOCHROMIC ANEMIA
34.	F	37	LAXMI	+	6.6	LOW	19	N/RAISED	130.9	MICROCYTIC HYPOCHROMIC ANEMIA WITH NEUTROPHILIA
35.	F	60	SHARANBAI	N	13.2	N	34	N/RAISED	29.8	NORMOCYTIC NORMOCHROMIC SMEAR WITH NEUTROPHILIC LEUCOCYTOSIS
36.	F	29	SAVITA	N	11.5	LOW	25	N/RAISED	21.5	NORMOCYTIC NORMOCHROMIC SMEAR
37.	M	60	MANOJ	N	12	N	78	N/RAISED	131	NORMOCYTIC NORMOCHROMIC SMEAR
38.	M	38	BASAVARAJ	N	13.5	N	76	N/RAISED	204	NORMOCYTIC NORMOCHROMIC SMEAR WITH LEUCOCYTOSIS
39.	F	45	BIBIFATIMA	N	12.6	N	50	N/RAISED	82.5	NORMOCYTIC NORMOCHROMIC SMEAR
40.	F	25	NARAGEESH	N	11.1	LOW	12	N/RAISED	30	MICROCYTIC HYPOCHROMIC SMEAR
41.	F	24	RENUKA	+	10.1	LOW	30	N/RAISED	20.5	MICROCYTIC HYPOCHROMIC ANEMIA
42.	F	25	GAZAL	N	12	LOW	10	LOW	40.5	NORMOCYTIC NORMOCHROMIC SMEAR
43.	M	73	KALLAPPA	N	12	N	98	N/RAISED	209.8	NORMOCYTIC NORMOCHROMIC SMEAR WITH NEUTROPHILIC LEUCOCYTOSIS
44.	M	27	SHARANAYYA	N	14.5	N	72	N/RAISED	130	NORMOCYTIC NORMOCHROMIC SMEAR
45.	F	34	DANAMMA	N	12.1	N	46	N/RAISED	18.9	NORMOCYTIC NORMOCHROMIC SMEAR
46.	M	53	BASAVARAJ PUJARI	+	10.6	LOW	43	N/RAISED	86	NORMOCYTIC NORMOCHROMIC SMEAR
47.	F	42	RENUKA HOSAMANI	N	14.2	N	51	N/RAISED	44.8	NORMOCYTIC NORMOCHROMIC SMEAR
48.	M	31	YAMANAPPA	N	13.1	N	67	N/RAISED	168.5	NORMOCYTIC NORMOCHROMIC SMEAR
49.	M	38	MUTTU M	+	12.4	N	133	N/RAISED	378.1	NORMOCYTIC NORMOCHROMIC ANEMIA
50.	F	35	LAXMI KORE	+	9.6	LOW	<10	N/RAISED	14.1	MICROCYTIC HYPOCHROMIC ANEMIA
51.	M	62	VASUDEV NADAGOUDA	+	12.4	LOW	36.6	N/RAISED	390	NORMOCYTIC NORMOCHROMIC ANEMIA

52.	M	48	BASAVARAJ IRAPPA KAGAL	+	12.6	LOW	43	N/RAISED	86	NORMOCYTIC NORMOCHROMIC SMEAR
53.	M	61	MR.SHRISHAIL	+	9.1	LOW	30	N/RAISED	157	NORMOCYTIC NORMOCHROMIC ANEMIA WITH THROMBOCYTOPENIA
54.	M	41	VISWANATH IRAYYA MATHAPATI	+	6.4	LOW	19	N/RAISED	251.1	MICROCYTIC HYPOCHROMIC ANEMIA
55.	F	55	MRS.SALIMA	+	12.4	N	55	N/RAISED	100.5	NORMOCYTIC NORMOCHROMIC ANEMIA
56.	F	65	AMBAVVA SAGAYI	+	11.6	LOW	31.5	N/RAISED	250.8	NORMOCYTIC NORMOCHROMIC ANEMIA
57.	M	78	MOHAN KONDAGULI	+	12.4	LOW	22.5	N/RAISED	470.3	NORMOCYTIC NORMOCHROMIC ANEMIA
58.	F	40	RAMABAI PATEDA	N	12.1	LOW	10	LOW	98.4	NORMOCYTIC NORMOCHROMIC ANEMIA
59.	M	45	RAMESH RATHOD	+	6.1	LOW	10	LOW	19.8	HYPOCHROMIA,MICROCYTOSIS ,ANISOPOIKILOCYTOSIS.
60.	M	26	MANJUNATH DALAWAI	+	12.9	N	80	N/RAISED	100.6	NORMOCYTIC NORMOCHROMIC ANEMIA
61.	F	30	LAXMIBAI K	+	10.8	LOW	<10	LOW	14.1	MICROCYTIC HYPOCHROMIC ANEMIA WITH NEUTROPHILIA
62.	M	80	BASAVVA GARASANGI	+	11.7	LOW	46	N/RAISED	116.4	NORMOCYTIC NORMOCHROMIC ANEMIA
63.	F	32	MALA MANTYAL	N	12.1	N	65	N/RAISED	34.4	NORMOCYTIC NORMOCHROMIC ANEMIA
64.	F	30	BISMILLAH	+	11.2	LOW	21.2	N/RAISED	99.8	NORMOCYTIC NORMOCHROMIC SMEAR
65.	F	60	NURJAHAN .H.NAGARBOU DI	+	10.7	N	37	N/RAISED	26.5	NORMOCYTIC NORMOCHROMIC ANEMIA
66.	F	68	PUSHPA G.	+	10	LOW	30	N/RAISED	38	NORMOCYTIC NORMOCHROMIC ANEMIA
67.	F	52	SANGITA N.	+	12.2	N	108	N/RAISED	46.5	NORMOCYTIC NORMOCHROMIC ANEMIA
68.	M	67	MANU CHANDRAKAN T RATHOD	+	9.7	LOW	33.5	LOW	20	NORMOCYTIC NORMOCHROMIC ANEMIA
69.	M	35	BHIMU GANGANNA B	+	8.8	LOW	10	LOW	35	NORMOCYTIC HYPOCHROMIC ANEMIA,TEAR DROP CELLS+,ANIOSPOIKILOCYTOSIS
70.	M	35	PRAKASH DAVALAGI	+	10	LOW	37	N/RAISED	230.3	MICROCYTIC HYPOCHROMIC ANEMIA
71.	F	38	SHILPA GANDHI	+	11	N	113	N/RAISED	54.3	NORMOCYTIC NORMOCHROMIC SMEAR
72.	M	73	GURULINGAPP A KEMBAVI	+	5.8	LOW	10	LOW	22	PANCYTOPENIA WITH MODERATE ANISOPOIKILOCYTOSIS WITH TEAR DROP CELLS
73.	F	35	PARAMAVVA	+	10.7	LOW	10	N/RAISED	28.4	PANCYTOPENIA WITH MODERATE ANISOPOIKILOCYTOSIS
74.	F	18	AARTHI	N	13.5	N	46.5	N/RAISED	40	NORMOCYTIC NORMOCHROMIC SMEAR
75.	M	50	IRANNAGOUD A BIRADAR	N	13.5	N	139	N/RAISED	230	NORMOCYTIC NORMOCHROMIC SMEAR

76.	M	36	GYANAPPA	N	15.5	LOW	12.2	N/RAISED	400	NORMOCYTIC NORMOCHROMIC SMEAR
77.	M	30	BASANGOUDA BIRADAR	+	9.7	LOW	55	LOW	30	NORMOCYTIC NORMOCHROMIC ANEMIA WITH MILD ANISOPOIKILOCYTOSIS
78.	F	60	NOOR JAHAN BEGUM	+	9.8	LOW	26.8	N/RAISED	172.4	NORMOCYTIC HYPOCHROMIC SMEAR
79.	F	35	LAXMIBAI S.	+	11	LOW	43	N/RAISED	37.3	NORMOCYTIC HYPOCHROMIC SMEAR
80.	F	79	AMEENA SHAKH	+	11.9	N	99	N/RAISED	66.6	NORMOCYTIC NORMOCHROMIC SMEAR
81.	F	43	SHAMALA ABRAK	N	12.3	N	128	N/RAISED	92	NORMOCYTIC NORMOCHROMIC SMEAR
82.	F	38	KALPANA TAJNE	+	10	N	77	LOW	3.6	NORMOCYTIC HYPOCHROMIC SMEAR
83.	F	37	MAYURI NAVGAN	+	11	N	118	LOW	5.4	MICROCYTIC HYPOCHROMIC ANEMIA
84.	M	56	SIDAPPA	+	12.4	LOW	57	N/RAISED	554.2	NORMOCYTIC NORMOCHROMIC ANEMIA
85.	M	41	MUNAF MULLA	+	10.8	N	84	LOW	52	NORMOCYTIC NORMOCHROMIC ANEMIA
86.	F	24	AKSHATA TAVARAMATH	+	4	N	99	N/RAISED	174.9	NORMOCYTIC NORMOCHROMIC ANEMIA
87.	F	31	NETRAVATHI SALUNKHE	N	12.9	N	71	N/RAISED	29.4	NORMOCYTIC NORMOCHROMIC ANEMIA
88.	M	45	SOMASING	+	9.7	N	145	N/RAISED	294.1	MICROCYTIC HYPOCHROMIC ANEMIA
89.	M	73	SAHEBGOUDA	+	11.6	LOW	27	N/RAISED	193.8	NORMOCYTIC NORMOCHROMIC ANEMIA WITH THROMBOCYTOPENIA
90.	M	55	SIDAPPA TIGUNDI	+	11	LOW	57	N/RAISED	554.2	NORMOCYTIC NORMOCHROMIC WITH LYMPHOCYTOSIS.
91.	M	33	SHARANBASU	+	11.8	LOW	51	N/RAISED	75.6	NORMOCYTIC NORMOCHROMIC SMEAR
92.	F	44	GOURABAI WAGHAMARE	N	9.7	LOW	10	LOW	6.9	MICROCYTIC HYPOCHROMIC ANEMIA
93.	F	32	BOURAVVA NATIKAR	+	7.5	N	213	N/RAISED	83.4	MICROCYTIC NORMOCHROMIC ANEMIA
94.	M	80	ABDULRAZAK ALURKAR	+	10.4	LOW	35	N/RAISED	161.1	NORMOCYTIC NORMOCHROMIC ANEMIA
95.	F	60	MAHADEVI	+	9	LOW	20	N/RAISED	142.2	NORMOCYTIC NORMOCHROMIC ANEMIA
96.	F	23	JYOTI B.	+	6.8	LOW	30	N/RAISED	100.6	NORMOCYTIC NORMOCHROMIC ANEMIA
97.	F	46	KAJABI YALAWAR	+	11.2	N	99	N/RAISED	58.9	NORMOCYTIC NORMOCHROMIC ANEMIA
98.	F	65	SUSHILA KAMBLE	+	10.6	N	58	N/RAISED	30.3	NORMOCYTIC NORMOCHROMIC ANEMIA
99.	F	36	BOURAMMA	+	6.6	LOW	29	LOW	8.5	MICROCYTIC HYPOCHROMIC ANEMIA
100.	F	30	RENUKA PUJARI	N	12.4	N	56	N/RAISED	250	NORMOCYTIC NORMOCHROMIC ANEMIA
101.	F	24	RENUKA BIRADAR	+	10.1	N	112	N/RAISED	166.6	NORMOCYTIC NORMOCHROMIC ANEMIA

102.	F	65	SHANTAWWAW.	+	10.6	LOW	10	N/RAISED	63.2	NORMOCYTIC NORMOCHROMIC ANEMIA
103.	F	26	ASHWINI	+	11.6	LOW	10	N/RAISED	19.6	MICROCYTIC HYPOCHROMIC ANEMIA
104.	M	40	TULASHI S	+	10.9	N	70.2	N/RAISED	202.3	NORMOCYTIC NORMOCHROMIC ANEMIA
105.	M	65	SANGAPPA M	+	8.5	N	164	N/RAISED	136.5	MICROCYTIC HYPOCHROMIC ANEMIA
106.	M	40	PANDU KHARAT	N	15.9	LOW	18	N/RAISED	113.1	NORMOCYTIC NORMOCHROMIC SMEAR
107.	M	65	IRASANGAYYA B	+	12.8	N	78	N/RAISED	133.3	NORMOCYTIC NORMOCHROMIC SMEAR
108.	F	25	VIJAYALAXAMI	+	10.7	LOW	23	N/RAISED	21.8	NORMOCYTIC NORMOCHROMIC ANEMIA
109.	F	63	DUNDAMMA PALIDINI	N	13.4	LOW	18	N/RAISED	18	NORMOCYTIC NORMOCHROMIC SMEAR
110.	M	50	REVANSIDDAPPA	N	13.1	N	80	N/RAISED	180.6	NORMOCYTIC NORMOCHROMIC SMEAR
111.	M	52	NAUSHAD SHAIKH	+	11.4	N	62.5	N/RAISED	99	NORMOCYTIC NORMOCHROMIC SMEAR
112.	F	49	SANGEETA BIRADAR	+	10.2	LOW	11	N/RAISED	30.3	NORMOCYTIC NORMOCHROMIC ANEMIA
113.	F	45	NEELAMMA	+	11.3	N	48.3	N/RAISED	101.3	NORMOCYTIC NORMOCHROMIC ANEMIA
114.	M	68	HYDER HUSSAIN M	+	12	LOW	10	N/RAISED	231	NORMOCYTIC NORMOCHROMIC SMEAR
115.	M	53	AJAY AGRAWAL	N	16.4	N	77	N/RAISED	303	NORMOCYTIC NORMOCHROMIC SMEAR
116.	M	28	GURUPAD MUCHANDI	+	10.3	N	56.2	N/RAISED	109.2	NORMOCYTIC NORMOCHROMIC SMEAR
117.	F	52	AKKAMAHADEVI AADIN	+	8.7	LOW	20	N/RAISED	84	NORMOCYTIC NORMOCHROMIC SMEAR
118.	M	29	SANTOSH	+	5.9	LOW	15	LOW	2.1	MICROCYTIC HYPOCHROMIC ANEMIA WITH NEUTROPHILIA
119.	M	31	ROHITH GALGALI	+	12.1	N	195	N/RAISED	294.1	NORMOCYTIC NORMOCHROMIC SMEAR
120.	M	68	HYDER HUSSAIN M	+	12	LOW	10	N/RAISED	231	NORMOCYTIC NORMOCHROMIC SMEAR
121.	F	60	NILAVVA ASANGI	+	6.6	N	62	N/RAISED	554.6	NORMOCYTIC NORMOCHROMIC SMEAR WITH ANISOPOIKILOCYTOSIS
122.	F	70	RAJAMMA YALAGOD	+	6.8	N	197	N/RAISED	1448.9	NORMOCYTIC NORMOCHROMIC SMEAR WITH ANISOPOIKILOCYTOSIS