SERUM IRON LEVELS IN GALLBLADDER STONE DISEASE : A COMPARATIVE STUDY

SUBMITTED BY

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P.G. IN GENERAL SURGERY

DISSERTATION SUBMITTED TO

B. L. D. E. (DEEMED TO BE UNIVERSITY)'s

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In partial fulfilment of the requirements for the degree of

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In

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UNDER THE GUIDENCE OF

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ABSTRACT

Background: Recent studies have pointed towards the role of trace elements like iron in the formation of gallstones. Serum Iron, serum ferritin and TIBC are the laboratory measurements that reflect the availability of iron for hemoglobin synthesis. Iron deficiency results in altered gallbladder and sphincter of Oddi (SO) motility and cholesterol crystal formation. In addition, gallbladder neuronal nitric oxide synthase (nNOS) has been shown to be markedly reduced after 8 weeks on an iron-deficient diet.

Objective: Our aim in this study is to correlate iron deficiency anemia with gallstone disease by estimating serum iron, serum ferritin, serum iron binding capacity.

Design: prospective case-control study

Method: Estimations of serum iron, serum ferritin and TIBC were done in the serum of 32 patients with chronic cholelithiasis and in 32controls admitted in B.L.D.E.(Deemed to be Univerity) Shri. B. M. Patil Medical College, Hospital and Research Centre, Vijayapur in Surgery Department from October 2016 to August 2018. The serum levels of the above parameters were compared in the two groups. Based on the hemoglobin of the patients and control group, all cases were divided into two groups: Non-Anaemic: (i.e., hemoglobin>11 g%), and Anaemic (i.e., hemoglobin ≤11). Serum cholesterol, iron and ferritin contents of both groups were analyzed and compared with each other

Results: Patients with gallstones were significantly Anaemic as compared to controls (p=0.0101). Patients with gallstones have statistically lower serum iron levels as well

as serum ferritin levels as compared to controls (p=0.001). The association between anemia and/or gender and having serum iron/ ferritin/ TIBC levels < normal, normal or > normal was not statistically significant in the cases group.

Conclusion: This study suggests that iron deficiency leading to anemia plays a significant role in super saturation of bile, leading to stone formation in the gall bladder. The serum cholesterol of the Anaemic group was found to be similar to the non-Anaemic group. Iron deficiency probably alters the hepatic enzyme metabolism, leading to super saturation of gall bladder bile with respect to cholesterol irrespective of serum cholesterol levels, hence promoting the cholesterol crystal formation.

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INTRODUCTION

Gallstone disease is a common clinical entity affecting the adult population of both sexes. The earliest known gallstones date back to the 21st Egyptian dynasty discovered in the mummy of a priestess of Amenen (1085-945 BC).Gallstones are classified into cholesterol stones, Black or brown pigmented stones or mixed stones. Conditions that favour the formation of cholesterol gallstones are super-saturation of bile with cholesterol, kinetically favourable nucleation and the presence of cholesterol crystals in the gall bladder long enough to agglomerate into stone. Recent studies have defined the role of trace elements (Fe, Ca, Zn and Cu) and defective pH in the formation of gallstones.[20]

As the old axiom says that a typical gall stone sufferer is a fatty, fertile, female of forty, is only partially true with a female: male ratio of 3:1 up to age 50 years, as the disease has been found in women soon after their first delivery and also in underweight and thin people. Gallstone disease has also been reported in infants, thus no age is immune[3]. Gallstones may produce symptoms or remain asymptomatic which are usually detected by abdominal ultrasound done for some other purpose. the presentation may range from flatulent dyspepsia and acute cholecystitis to its complications like empyema, chronic cholecystitis, gangrene, fistula and gallbladder carcinoma.[5]

Gallstone disease has troubled human lives since time immemorial. Cholecystitis and cholelithiasis are the most common disorders affecting the biliary system. Here are variations in incidence of gallstones according to geographical

distribution. The incidence varies from country to country and even in different parts of the same country.[3]

So while searching the literature for different factors, iron deficiency was found to be new and interesting etiological factor in the formation of gall stones. Gallstones hence produced may be symptomatic or asymptomatic. Over half the cases are asymptomatic, usually detected by abdominal ultrasound. today the incidence of gallstone disease has increased considerably with the invention of ultrasonography.[3]

Three conditions must be met to permit the formation of cholesterol gallstones.

- Bile must be supersaturated with cholesterol.
- Nucleation must be kinetically favorable.
- Cholesterol crystals must remain in the gall bladder long enough to agglomerate into stones.[11]

Iron deficiency 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. Iron acts as a coenzyme for nitric oxide synthetase (nos), and that is important for the maintenance of basal gallbladder tone and normal relaxation. it was found that iron deficiency resulted in altered motility of gall and sphincter of oddi (so), leading to biliary stasis and thus increased cholesterol crystal formation in the gall bladder bile.[11]

AIM AND OBJECTIVES OF THE STUDY

To correlate iron deficiency anemia with gallstone disease by estimating

- Serum Iron
- Serum Ferritin
- Serum Total Iron Binding Capacity.

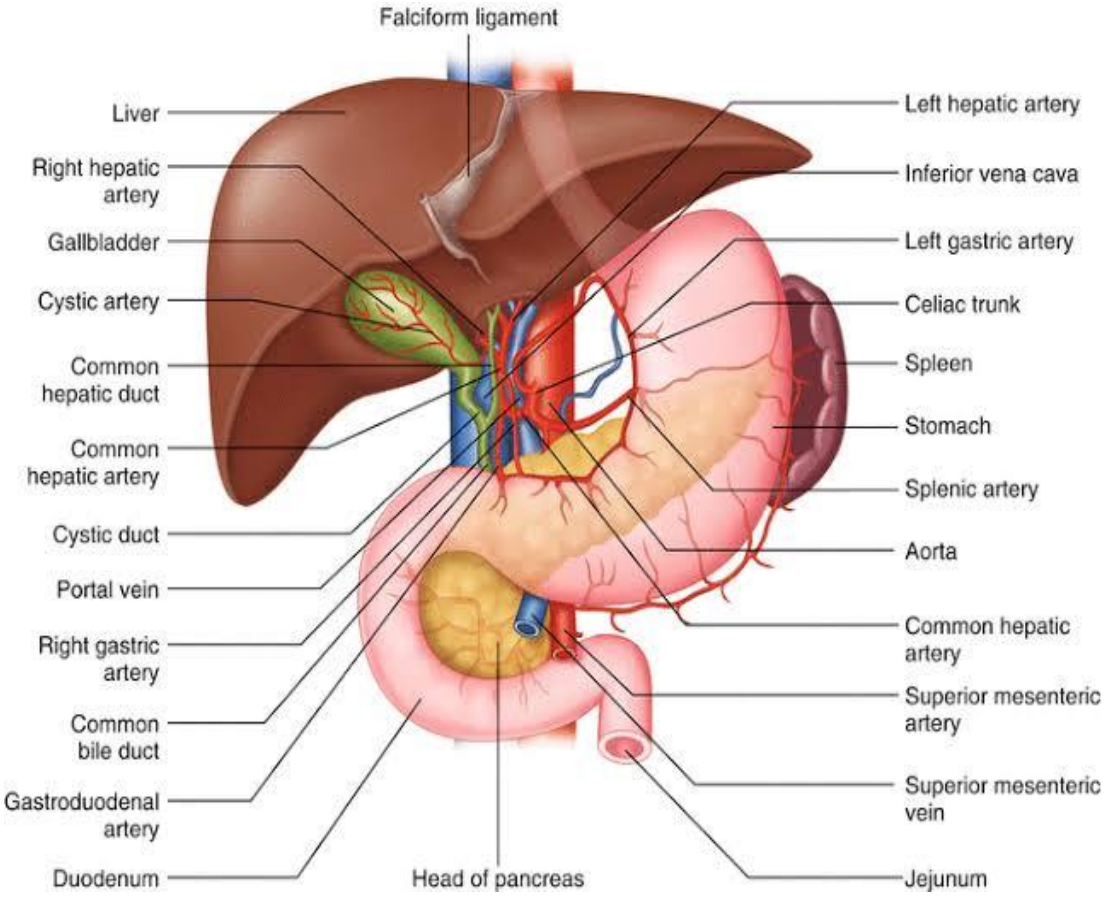
RESEARCH HYPOTHESIS

Iron deficiency is a factor in bile saturation and hence leading to Gallstone formation.

REVIEW OF LITERATURE

Gallbladder

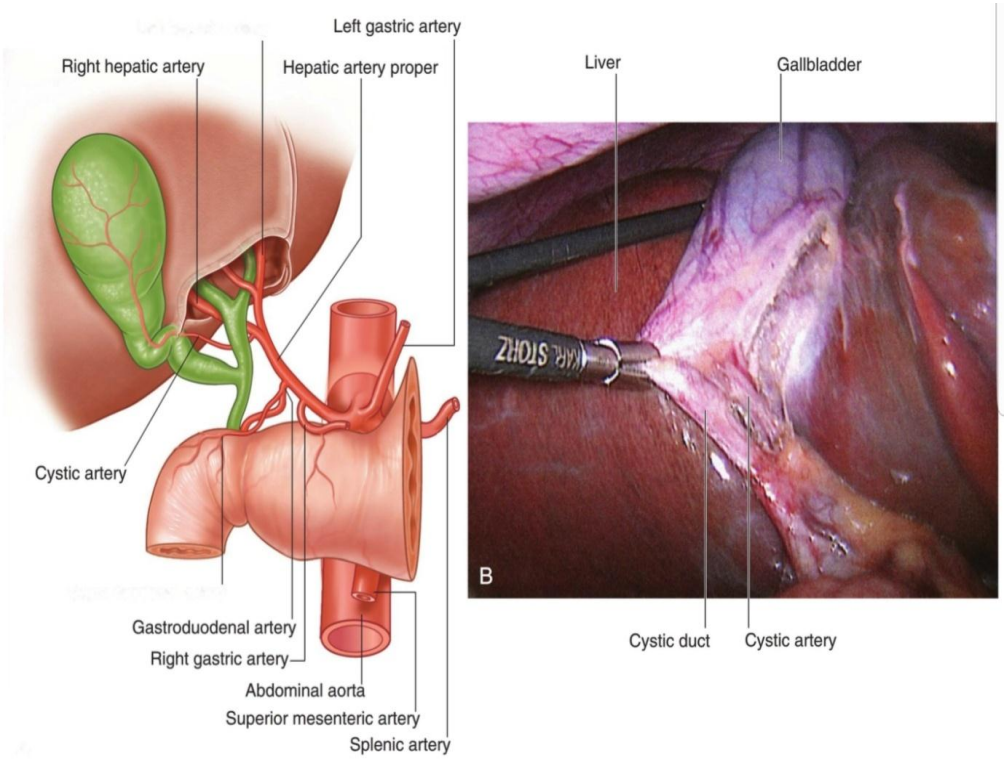
The gallbladder is a pear-shaped sac lying on the visceral surface of the right lobe of the liver in a fossa between the right and quadrate lobes (Abboud, Sleilaty, Tannoury, Daher, Abadjian, & Ghorra, 2011) .



It can be divided into three parts:

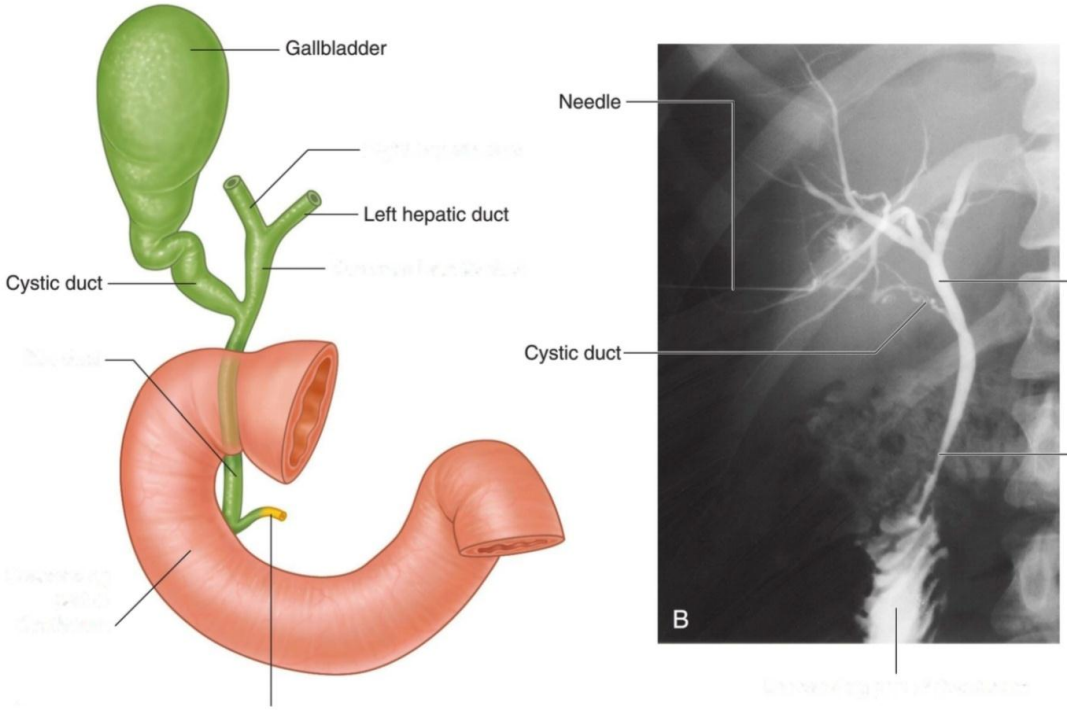
1. The fundus- the rounded end, which may project from the inferior border of the liver
2. The body of the gall bladder- a major part in the fossa, which may be against the transverse colon and the superior part of the duodenum
3. The neck of the gallbladder with mucosal folds forming the spiral fold.

The arterial supply to the gallbladder is the cystic artery from the right hepatic artery (a branch of the hepatic artery proper).



The gallbladder receives, concentrates , and stores bile from the liver.

The duct system for the passage of bile extends from the liver, connects with the gallbladder, and empties into the descending part of the duodenum. The coalescence of ducts begins in the liver parenchyma and continues until the right and left hepatic ducts are formed. These drain the respective lobes of the liver. The two hepatic ducts combine to form the common hepatic duct, which runs near the liver, with the hepatic artery proper and portal vein in the free margin of the lesser omentum. As the common hepatic duct continues to descend, it is joined by the cystic duct from the gallbladder. This completes the formation of the bile duct. The bile duct continues to descend, passing posteriorly to the superior part of the duodenum before joining with the pancreatic duct to enter the descending part of the duodenum at the major duodenal papilla



Gallstones

Gallstones are abnormal stone masses formed in the gallbladder or the intrahepatic bile ducts and infrequently also migrate to the common bile duct or the intestines (2). It is a common clinical entity present in approximately 10% of people over the age of 40 and is more common in women. The earliest known gallstones date back to the 21st Egyptian dynasty discovered in the mummy of a priestess of Amenen (1085–945 BC) (3). The first observation of gallstones in humans was reported by the Florentine physician Antonio Benivenius towards the end of the fifteenths century at an autopsy of a lady that had deceased with abdominal pain. Historical writings and autopsy findings indicate that Catherine the Great of Russia and the emperor Alexander the Great both suffered from gallstone disease with death of the latter ascribed to acute cholecystitis (2).

Gallstones are classified by their composition of major constituents into three types:

1. Cholesterol gallstones - composed principally of cholesterol monohydrate crystals
2. Pigment gallstones- the acid salt of calcium bilirubinate
3. mixed stones.

Cholesterol gallstones have been estimated to account for 75-90% of gallstones prevalence in Western countries (2). Cholesterol stones and the black variety of pigment gallstones form in sterile gallbladder bile whereas brown pigment gallstones form in infected bile.

There are three chemical species in bile relevant to the formation of cholesterol gall stones (4):

a. Cholesterol

Cholesterol is the major component of cholesterol gallstones. It is a sterol which is almost totally insoluble in water, the aqueous solubility being about 10-8mM[3]. Cholesterol accounts for about 95% of all sterols in bile and gallstones4. Cholesterol esters do not exist in bile.

b. Phospholipids

More than 95% of biliary phospholipid is diacylphosphatidylcholine (lecithin). Phospholipids also are very sparingly soluble in water as monomers, but swell in water to form a bilayer i.e. sheets two molecules thick, in which the fatty acid chains project toward the interior of the sheet and the polar choline groups point outward into the water phase.

c. Bile salts

Bile salts are detergent molecules. Their detergent properties are conferred due to the positioning of a large hydrophobic nucleus of the molecule on one side of the molecule and polar hydroxyl and carboxyl groups project on other side. Bile salts are soluble in water but above a very low concentration (about 5mM- the critical micellar concentration or CMC) associate into micelles. The common bile salts in man are cholate (trihydroxy) and chenodeoxycholate (dihydroxy) which are synthesized in the liver (primary bile salts), and deoxycholate (dihydroxy) and lithocholate (monohydroxy) produced from the primary bile salts in the intestine by bacterial dehydroxylation and referred to as secondary bile salts. Bile salts are also conjugated in the liver to taurine or glycine which, by lowering their pKa, allows them to remain ionized in the slightly acidic milieu of the upper small intestine, where otherwise they would be absorbed and not be available for fat digestion.

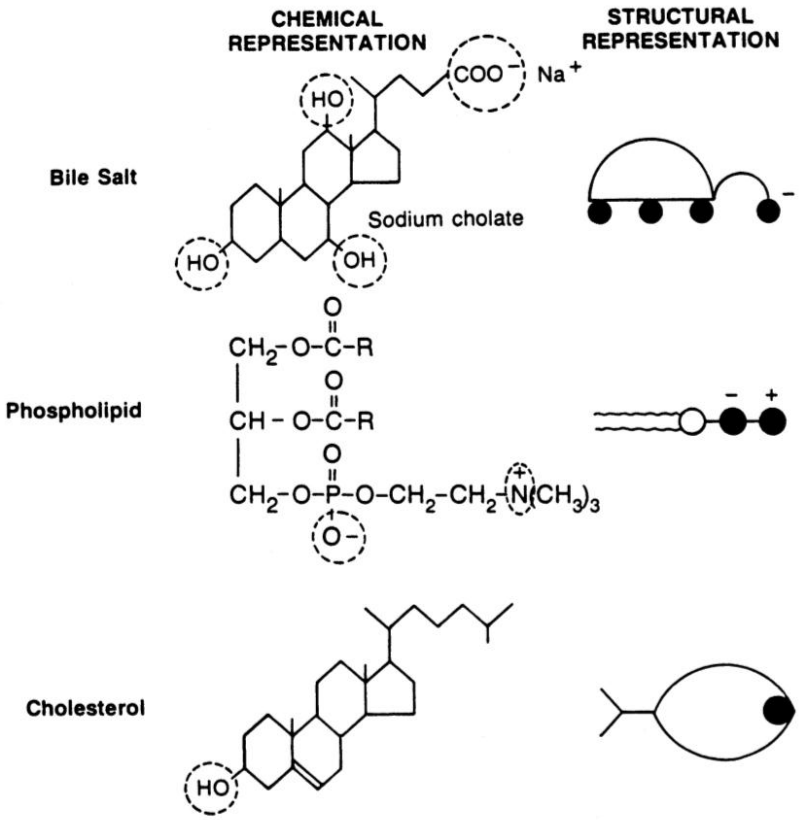


Figure : Chemical species in bile. The three major lipids of interest are shown. Polar groups represented in black in the structural representation have been circled by an interrupted line in the chemical representation.

For cholesterol gallstones to form, three conditions must be achieved;

1. the bile must become supersaturated with cholesterol,
2. the cholesterol must precipitate as solid cholesterol monohydrate crystals and
3. the crystals must aggregate with other elements of a stone to form the recognizable macroscopic concretion (Figure 1). [(4)]

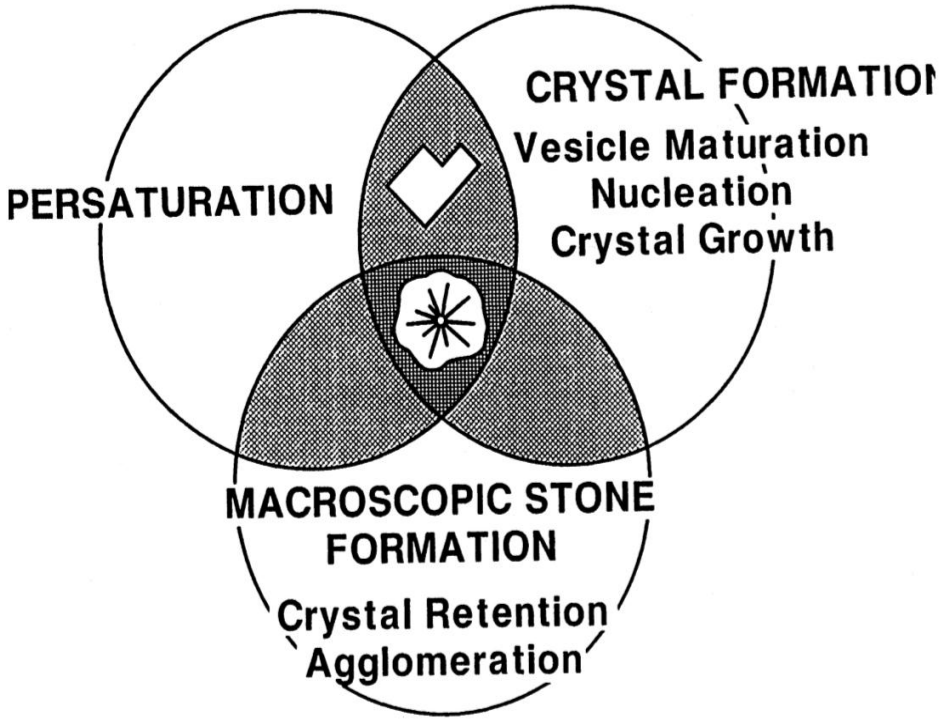


Figure : Venn diagram showing the conditions necessary for cholesterol gallstone formation.

The established risk factors for causing super-saturation of cholesterol in bile are elderly aged females, obesity with rapid weight loss, cirrhosis and diet related factors (5). For cholesterol stones, an imbalance in pro- and antinucleating biliary proteins, hypersecretion of gallbladder mucin and gallbladder dysmotility possibly from cholesterol "toxicity" to sarcolemma, all interact to promote nucleation. Crystallisation results in suspension of cholesterol crystals or bilirubinate salts in gallbladder mucin gel and is known as "biliary sludge". It is believed today that this stage is essential for evolution of both cholesterol and pigment stones.

Pigment stones:

Higher prevalence of black pigment than cholesterol gallstones are found in developing countries and in Asian populations. Although black pigment gallstones still are highly prevalent in Asia, the prevalence of cholesterol gallstones has been ever rising since the late 1960'ies – a trend ascribed to a westernized lifestyle. Brown pigment stones are uncommon in Western countries and reported with higher prevalence in Asia [(2)].

Brown pigment gallstones form principally in the bile ducts. These stones result from infection of the biliary tree, most commonly due to obstruction from migrating gallbladder stones. Chemical compositions of brown and black pigment stones are different: In black stones, calcium bilirubinate is polymerized and oxidatively degraded but in brown stones, calcium bilirubinate is present as the unpolymerised salt. Brown stones differ also from black stones in containing calcium fatty acid soaps, a result of bacterial phospholipase A1 hydrolysis of biliary lecithin. Both types of pigment gallstones may contain crystalline inorganic calcium salts especially carbonate (gallbladder stones) and phosphate (bile ducts stones) [(6)] .

The true presence or absence of gallstones can only be confirmed through surgery or autopsy. However, non-invasive radio-logical examinations have been developed in order to examine patients with suspected gallstone disease. Oral cholecystography was the examination of choice in the pre-ultrasound era, but somewhat unpractical since it required two days preparation, ingestion of tablets, exposure to radiation, and the cholecystogram was often inconclusive due to failed visualization of the gallbladder. The superiority of gallstone detection with ultrasound has been reproduced in the morbidly obese patients with sensitivity 91% and

specificity 100%. Inter-observer agreement for both detection and exclusion of gallstone disease is good (Kappa scores 0.78 and 0.73 respectively). Due to the many advantages, ultrasound examination has become the preferred non-invasive examination for gallstone disease [(2)].

Gallstones may produce symptoms or remain asymptomatic which are usually detected by abdominal ultrasound done for some other purpose. The presentation may range from flatulent dyspepsia and acute cholecystitis to its complications like empyema, chronic cholecystitis, gangrene, fistula and gallbladder carcinoma [(7)].

From time to time, gall stones impact in the region of Hartmann's pouch, which is a bulbous region of the neck of the gall bladder. When the gall stone lodges in this area, the gall bladder cannot empty normally and contractions of the gall bladder wall produce severe pain. If this persists, a cholecystectomy (removal of the gall bladder) may be necessary. Sometimes the gall bladder may become inflamed (cholecystitis). If the inflammation involves the related parietal peritoneum of the diaphragm, pain may not only occur in the right upper quadrant of the abdomen but may also be referred to the shoulder on the right side. This referred pain is due to the innervation of the visceral peritoneum of the diaphragm by spinal cord levels (C3 to CS) that also innervate skin over the shoulder. In this case, one somatic sensory region of low sensory output(diaphragm) is referred to another somatic sensory region of high sensory output (dermatomes) [(1)].

Trace elements:

Recent studies have found a role of calcium and trace elements such as iron, zinc and copper in the formation of gallstones [8]. It is important to understand Iron metabolism in the body to analyse the effects caused by its deficiency.

Iron metabolism [9]

Iron is important for the formation of hemoglobin as well as other essential elements in the body (e.g., *myoglobin, cytochromes, cytochrome oxidase, peroxidase, catalase).* The total quantity of iron in the body averages 4 to 5 grams, about 65 per cent of which is in the form of hemoglobin. About 4 per cent is in the form of myoglobin, 1 per cent is in the form of the various heme compounds that promote intracellular oxidation, 0.1 per cent is combined with the protein transferrin in the blood plasma, and 15 to 30 per cent is stored for later use, mainly in the reticuloendothelial system and liver parenchymal cells, principally in the form of ferritin.

Transport, storage, and metabolism of iron in the body are diagrammed in Figure can be explained as follows:

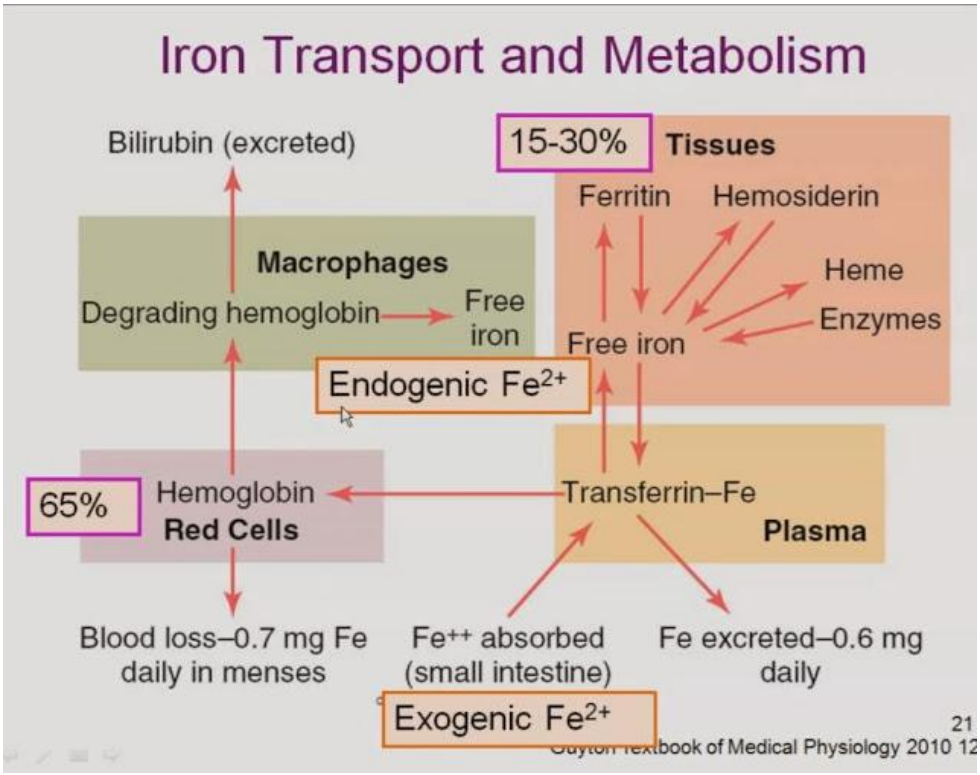


Figure : Transport, storage, and metabolism of iron in the body.

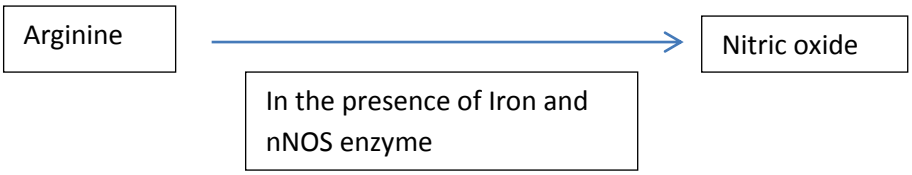
1. Iron is absorbed from the small intestine.
2. It immediately combines in the blood plasma with a beta globulin, *apotransferrin*, to form *transferrin*, which is then transported in the plasma.
3. The iron is loosely bound in the transferrin and, consequently, can be released to any tissue cell at any point in the body.
4. Excess iron in the blood is deposited *especially* in the liver hepatocytes and less in the reticuloendothelial cells of the bone marrow.
5. In the cell cytoplasm, iron combines mainly with a protein, *apoferritin*, to form *ferritin.* Apoferritin has a molecular weight of about 460,000, and varying quantities of iron can combine in clusters of iron radicals with this large molecule; therefore, ferritin may contain only a small amount of iron or a large amount. This iron stored as ferritin is called *storage iron.*

6. When the quantity of iron in the plasma falls low, some of the iron in the ferritin storage pool is removed easily and transported in the form of transferrin in the plasma to the areas of the body where it is needed.
7. A unique characteristic of the transferrin molecule is that it binds strongly with receptors in the cell membranes of erythroblasts in the bone marrow. Then, along with its bound iron, it is ingested into the erythroblasts by endocytosis. There the transferrin delivers the iron directly to the mitochondria, where heme is synthesized. [9]

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

Ferritin is the storage form of Iron. Iron deficiency has been shown to alter the activity of several hepatic enzymes leading to increased bile cholesterol saturation and promotion of crystal formation [10]. Iron acts as a cofactor for Nitric Oxide Synthase (NOS), which synthesizes Nitric Oxide (NO) [11] .

Nitric oxide (NO) is a major inhibitory nonadrenergic, noncholinergic (NANC) neurotransmitter in the gastrointestinal (GI) tract. It is a remarkably simple chemical. NO is synthesized from arginine by the activation of neuronal NO synthase (nNOS) enzyme in the myenteric plexus. NOS use NADPH as an electron donor and employ five enzyme cofactors to catalyze a five-electron oxidation of arginine to NO with stoichiometric formation of citrulline [12], one of the cofactor being Iron. NO released in response to nerve stimulation of the myenteric plexus causes relaxation of the smooth muscle. Released NO plays an important physiological role in various parts of the GI tract. NO regulates the muscle tone of the sphincter in the lower esophagus, pylorus, sphincter of Oddi, and anus [13].



The NOS acts as a calcium-calmodulin dependent enzyme. Hence, a deficiency in serum calcium causes deranged function of NOS resulting in altered gallbladder motility, leading to biliary stasis and subsequently increased crystal formation in bile [8]. Thus, deficiencies of serum iron and serum calcium can lead to increased risk of gallstone disease.

A study by Verma GR et al [8] suggested that higher biliary calcium and trace elements (Iron, copper and zinc) as well as a defective pH of gall bladder bile in patients with chronic cholelithiasis could be the underlying factor in the pathogenesis of gallstones.

Misra et al planned a study to analyse the exact role of serum iron and serum calcium in the pathogenesis of gallstone disease and to assess the relationship of biliary cholesterol super-saturation with levels of serum iron and calcium. Total 100 patients suffering from cholelithiasis were included in the study and were divided into four groups based on serum iron and serum calcium content; group A included patients with normal serum iron (the controls), group B included patients with iron deficiency (the cases), group A1 included patients with normal serum calcium and group B1 included patients with calcium deficiency (the cases).Five ml of blood sample was drawn intravenously before cholecystectomy, serum was analysed for parameters like serum iron, calcium, cholesterol and biliary cholesterol. The

prevalence of gallstone disease was much more in females as compared to males with male: female ratio being 1:4. 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.

Serum Iron, Serum Ferritin and TIBC

Serum Iron, TIBC and Transferrin Saturation are the laboratory measurements that reflect the availability of iron for hemoglobin synthesis. [14] In infection free situation, serum ferritin is a better indicator for diagnosis of iron deficiency than the aforementioned three [15].

Serum Iron

The serum iron level represents the amount of circulating iron bound to transferrin. The normal serum iron ranges from 9 to 27 μmol/L (50–150 μg/dL). [14]

A study conducted by Johnston et al [10] on Praire dogs suggested that an iron-deficient diet alters hepatic enzyme metabolism, which, in turn, increases gallbladder bile cholesterol and promotes cholesterol crystal formation.

Roslyn J et al [16] carried out a study to define the role of dietary iron in pigment gallstone formation. They found that consumption of diets rich in carbohydrates but deficient in iron alters hepatic metabolism of cholesterol and may

be an important etiologic factor in pigment gallstone formation. They suggested that Iron supplementation may prevent pigment gallstones in certain high-risk groups.

A similar result was obtained by Prasad et al [3] who concluded that the low serum iron level in one or the other way was leading to bile super saturation with respect to cholesterol, which lead to gallstone formation.

Earlier it was believed that a typical gall stone sufferer is a fat, fertile, female of fifty. But this is true only partially, as the disease is found in women soon after their first delivery and also in underweight and thin people. So while searching for other parameters, iron deficiency was found to be a new parameter of interest in the aetiology of gall stones. Kumar et al [17] conducted a study aimed at establishing the role of iron deficiency in the supersaturation of bile with cholesterol and thus formation of gallstones. 50 patients suffering from Cholelithiasis, confirmed by ultrasonography were divided into two groups. Group A consisted of patients with normal serum iron levels (non-anaemic) and group B, of patients with less than normal serum iron (anaemic). Serum cholesterol and gall bladder bile cholesterol of both the groups were studied and compared. The study revealed that Total serum cholesterol was not different in gall stone formers from that of the general population. However, the cholesterol level in the gall bladder bile was significantly higher in anaemic, than in non-anaemic individuals, leading the researchers to conclude that low serum iron levels lead to bile supersaturation with respect to cholesterol, which leads to gallstone formation.

On similar lines, Kshirsagar et al [18] studied one hundred and twenty patients prospectively over a period of two years in Department of General Surgery at Krishna Institute of Medical Sciences, Karad. Serum iron and serum cholesterol levels of

patients suffering from cholelithiasis were compared with healthy individuals. The researchers observed that most of the patients with gallstones had low serum iron levels. Serum cholesterol levels of patients suffering from cholelithiasis were not significantly different from that of normal healthy individuals. Hence they concluded that low serum iron levels lead to bile super-saturation with respect to cholesterol, which leads to gallstone formation.

Daddenavar and Daddenavar [19] carried out a study on 50 consecutive patients suffering from Cholelithiasis diagnosed by Ultrasonography to establish the role of iron deficiency in the super saturation of bile with cholesterol and thus formation of gallstones. The patients were divided into anemic and non-anemic groups, based on serum iron levels. Serum cholesterol and gall bladder bile cholesterol of both the groups were estimated.

Out of the total 50 patients, 42 (84%) were females and 08 (16%) were males. The majority of patients 31 (62%) presented with all the three symptoms of cholelithiasis i.e pain in the right upper quadrant, nausea/vomiting and flatulent dyspepsia. 32 (76.1%) out of the total female patients were multipara. 42 (84%) patients had body mass index (BMI) more than normal and 10 (20%) had normal or decreased BMI. Serum total cholesterol of gall stone formers was not different from that of the general population. There were no significant variations in the serum cholesterol contents of both the groups ($P=0.367$, $t=0.91$) whereas gall bladder bile cholesterol was significantly increased ($P<0.0001$) in the anemic, than in the non anemic group, thus suggesting that anemia may be contributing to the super saturation of gall

bladder bile with respect to cholesterol independent of serum cholesterol levels. Also, there was no significant variation of the above parameter in the male and female patients (P=0.082, t=1.77). The study concluded that probably anemia, obesity and sex hormones are independent risk factors operating for the causation of gallstones and if present together, they produce synergistic effects.

In a similar study by Sarhan, Hamed and Khalaf [(20)], out of the total 50 patients, 40 (80%) were females and 10 (20%) were males. The male to female ratio was 1:4. Anemia was more common in females than males, as 22 (55%) females were observed to be anemic, as compared to 1(10%) male. Thirty one (77.5%) out of the total female patients was multipara. Forty (80%) patients had body mass index (BMI) more than normal and 10 (20%) had normal or decreased BMI. All non anaemic gall stone sufferers (n=27) had a high average serum iron content of 91} 35 microg/dl, as compared to anaemic ones (n=23), where average serum iron was 26} 9.5 mg/dl. There were no significant variations in the serum cholesterol contents of both groups (P =0.367, t=0.91). The gall bladder bile cholesterol was significantly higher in the anemic individuals, as compared to that of the non-anemic ones (P <0.0001, t=4.53), suggesting that anemia may be contributing to the super saturation of gall bladder bile with respect to cholesterol independent of serum cholesterol levels.

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,

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:

Free iron is toxic to cells, and the body has established an elaborate set of protective mechanisms to bind iron in various tissue compartments. Within cells, iron is stored complexed to protein as ferritin. Apoferritin binds to free ferrous iron and stores it in the ferric state. Under steady-state conditions, the serum ferritin level correlates with total body iron stores; thus, the serum ferritin level is the most convenient laboratory test to estimate iron stores. The normal value for ferritin varies according to the age and gender of the individual. Adult males have serum ferritin values averaging 100 μg/L corresponding to iron stores of ~1 g, while adult females have levels averaging 30 μg/L , reflecting lower iron stores (~300 mg). As iron stores are depleted, the serum ferritin falls to <15 μg/L. Such levels are diagnostic of absent body iron stores. However, ferritin is also an acute-phase reactant and, in the presence of acute or chronic inflammation, may rise several-fold above baseline levels. As a rule, a serum ferritin >200 μg/L means there is at least some iron in tissue stores. [(14)] A lower-than-normal ferritin level can indicate an iron deficiency.

At the genetic level the expression of genes controlling ferritin levels viz Iron regulatory protein (IRP-1) might play a significant role in pathogenesis of cholelithiasis. Body ferritin levels, in contrast to haemoglobin, are not affected by residential elevation above sea level or smoking behavior.12 Therefore, ferritin can more closely reflect relationship with iron deficiency and can be its more specific

indicator, thus enabling to assess the relation between gall bladder stone and iron deficiency anaemia. [15]

Arora and Yadav [15] conducted a prospective study in the department of Surgery by dividing the study population into two groups; Case group with 200 patients with gallstone disease and control group with 50 patients without gallstone disease. Serum iron and ferritin contents of both groups were analyzed and compared with each other. They reported that the gallstones are more prevalent in female population than males in ratio of 5.4:1. They observed that low serum iron is not associated with Cholelithiasis in male. In males, serum ferritin was low in 64.5% of cases and 16.66% of controls. Serum ferritin levels were normal in 35.50% of cases and 66.66% of controls and above normal in 16.66% of controls suggesting that low serum ferritin is associated with gall stones in males. In this study, low serum iron was seen in 23.07% of females comparable to 23% low serum iron in control females and low ferritin was seen in 35.50% of female cases as compare to 15.38% of controls. They concluded that a low body store of serum iron is a risk factor for cholelithiasis in females and serum iron, serum ferritin may be used as marker of iron store so that low serum iron status could be diagnosed at early stage.

TIBC

The TIBC is an indirect measure of the circulating transferrin. The normal range for TIBC is 300–360 μg/dL (54–64 μmol/L). (14) It evaluates how well transferrin carries iron through blood.

A total iron binding capacity value above 450 mcg/dL usually means that there's a low level of iron in the blood. This may be caused by a lack of iron in the diet, increased blood loss during menstruation, pregnancy, or a chronic infection.

A total iron binding capacity value below 240 mcg/dL usually means that there's a high level of iron in the blood. This may be caused by liver damage, iron or lead poisoning, frequent blood transfusions, haemolytic anemia, which is a condition that causes red blood cells to die prematurely, sickle cell anemia, which is an inherited condition that causes red blood cells to change shape or hemochromatosis, which is a genetic condition that causes a build up of iron in the body.

Iron deficiency anemia

The most severe consequence of iron depletion is iron deficiency anemia (IDA), and it is still considered the most common nutrition deficiency worldwide. Although the etiology of IDA is multifaceted, it generally results when the iron demands by the body are not met by iron absorption, regardless of the reason. Individuals with IDA have inadequate intake, impaired absorption or transport, physiologic losses associated with chronological or reproductive age, or chronic blood loss secondary to disease. In adults, IDA can result in a wide variety of adverse outcomes including diminished work or exercise capacity, impaired thermoregulation, immune dysfunction, GI disturbances, and neurocognitive impairment [(22)].

Pamuk et al [(23)] determined the frequency of gallstones in iron deficiency anemia (IDA) patients and evaluated factors that could affect gall stones formation-like lipid levels and gallbladder motilities of the patients. One hundred and eleven IDA patients (88 females, 23 males; median age, 42) and 81 healthy controls (68

females, 13 males; median age, 42) were included into the study. The clinical findings of all IDA patients were recorded down; biochemical values and body mass index (BMI) were determined; and abdominal ultrasonography was performed. In addition, gall bladder emptying was monitored by ultrasound at 30-min intervals for 2 h after a mixed meal in randomly chosen, age-matched 25 IDA patients and 26 controls. Fasting volume (FV), residual volume (RV), and ejection fraction (EF) for all gall bladders were determined. The frequency of gall stones plus cholecystectomy was significantly higher in IDA patients (15 cases, 13.5%) than in the control group (five cases, 6.2%, $p = 0.048$). IDA patients with gall stones plus cholecystectomy were older than those without gall stones plus cholecystectomy ($p < 0.001$). FV and EF did not differ between IDA and control groups ($p > 0.05$). On the other hand, RV was significantly higher in IDA group than in controls ($p = 0.035$). The frequency of gall stones in IDA patients was significantly higher than in controls. The researchers suggested that the increased prevalence of gall stones in IDA might be explained with impaired gall bladder motility.

Prasad et al [(3)] conducted a study to correlate iron deficiency anemia with gallstone disease and to estimate the serum ferritin level as a diagnostic tool of iron deficiency anemia in patients with gallstone disease. The prospective study was conducted over a period of 24 months in the Department of General Surgery in a Delhi hospital. One hundred cases: 50 patients suffering from cholelithiasis admitted in the hospital confirmed by ultrasonography were included in this study. Fifty healthy volunteers were taken as the control group. Serum iron was estimated by the Ferrozine kit method. Serum cholesterol was estimated by the Enzopa kit based on the cholesterol oxidase/peroxidase method as devised by Allain et al. Based on the hemoglobin of the patients and control group, all cases were divided into two groups:

Non-anemic: (i.e., haemoglobin >11 g%), and anemic (i.e., hemoglobin ≤11). Serum cholesterol, iron and ferritin contents of both groups were analyzed and compared with each other and the following conclusions were drawn:

1. Serum total cholesterol of the patients of cholelithiasis was not different from that of general population. There were no significant variations in the serum cholesterol contents of both the groups. Also, there was no significant variation of the above parameter in the male and female patients.
2. The low serum iron level in one or the other way was leading to bile super saturation with respect to cholesterol, which leads to gallstone formation.
3. Serum ferritin cannot be taken as a sole diagnostic tool in the diagnosis of iron deficiency anemia as its value can vary due to other causes such as iron therapy, hepatocellular disease and inflammations (since cholecystitis is a inflammatory condition, this could be the reason for the high level of serum ferritin).

Sahu et al [(24)] conducted a study to correlate iron deficiency anemia with gallstone disease. The prospective study of 100 patients was conducted over a period of 12 months in the Department of Surgery and Department of Biochemistry, Himalayan Institute of Medical Sciences, Dehradun, India. Serum and gallbladder biliary cholesterol levels were compared with gallstone patients having normal and low serum ferritin level. Gallbladder cholesterol level was significantly higher in anemic, than in non-anemic patients. They concluded that low serum iron level is a factor in bile super-saturation with respect to cholesterol leading to gallstone formation.

MATERIALS AND METHODS

SOURCE OF DATA:

- The patients admitted in B.L.D.E.(Deemed to be University) Shri. B. M. Patil Medical College, Hospital and Research Centre, Vijayapur in Surgery Department.
- Period of study is from October 2016 to August 2018.

METHOD OF COLLECTION OF DATA:

The patients admitted in B.L.D.E. (Deemed to be University) Shri. B. M. Patil Medical College, Hospital and Research Centre, Vijayapur in Surgery Department during period of Oct 2016 – August 2018 with a sample size of 32 patients in each group comprising of a total of 64 patients.

Details of cases will be recorded including history, clinical examination, and investigations done. Following parameters of each patient will be recorded initially at the time of admission.

SAMPLING :

A Study Conducted by P.C.Prasad *et al* titled **To Study Serum Iron Levels in Patients of Gall Bladder Stone Disease and to Compare with Healthy Individuals** Published in the Indian J Surg 2012 was taken as the reference Study.[3]

On the basis of the study the serum iron in cases was 52% and among controls 6% were Anaemic, With 95% power and 1% significance level,

Related sample size is 32 using formula

Total Sample Size: 64
Case Study: 32
Control: 32

- Formula for estimating sample size : $n = \frac{(Z\alpha+Z\beta)^2*2*p*(100-p)}{d^2}$

where n = Sample size to be estimated.

Z α = Z value at α level

Zβ =Z value at β level

p = propotrtions of anemia in cases and controls

d= difference between 2 parameters.

Hence 32 cases and 32 controls will be included in the study.

Statistical Analysis:

Data will be analysed using-

- Mean±SD.
- Chi square test / Fisher's exact test

INCLUSION CRITERIA:

- All patients suffering from cholelithiasis confirmed by ultrasonography are included in this study.

EXCLUSION CRITERIA: following are the patients who will be excluded

- Cirrhosis of liver.
- Cystic fibrosis, Crohn's disease.
- Patients on long-term NSAIDS.
- On oral contraceptives or hormone replacement therapy.
- Pregnant females.

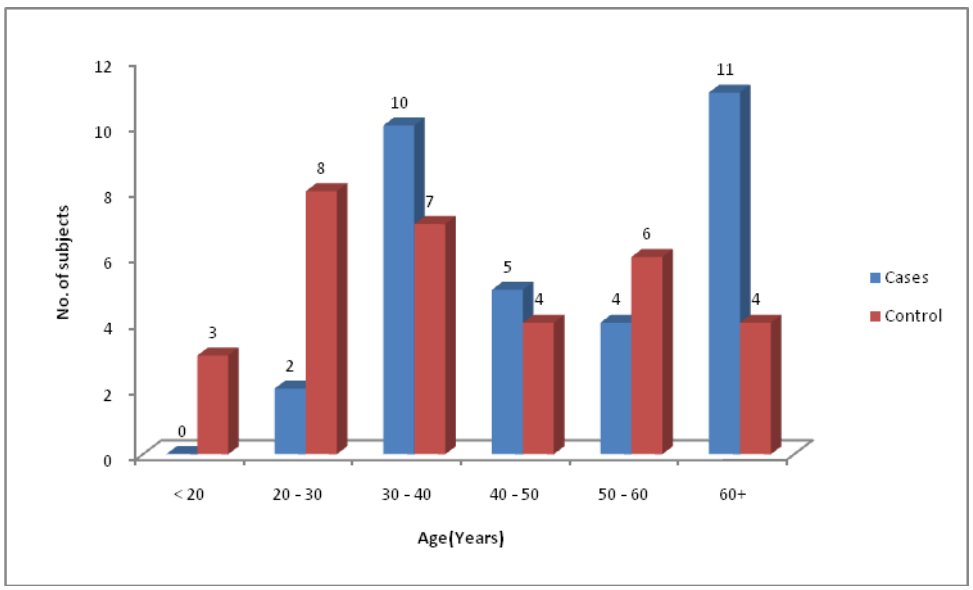
RESULTS

A total of 64 patients were included in the study and were divided into two groups, 32 patients having cholelithiasis (cases group) and 32 healthy individuals (Control Group).

Table 1: Distribution of subjects of cases and controls according to Age (Years)

Age(Years)	Cases No (%)	Control No (%).
< 20	0	3(9.3)
20 - 30	2(6.3)	8(25)
30 - 40	10(31.3)	7(22)
40 - 50	5(15.6)	4(12,4)
50 - 60	4(12.5)	6(18.8)
60+	11(34.4)	4(12.4)
Total	32(100)	100.0

Figure 1: Distribution of subjects of cases and controls according to Age (Years)

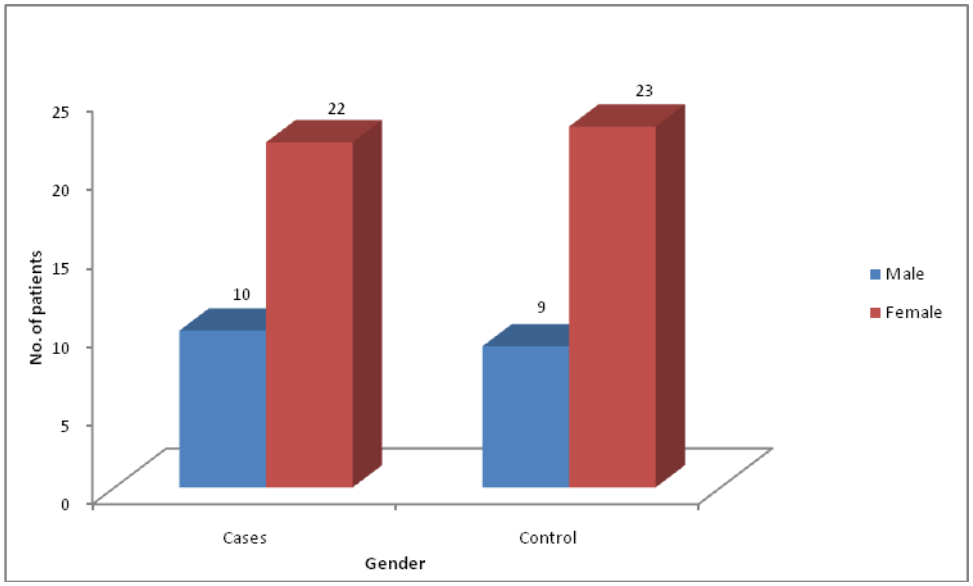


Maximum number of cases (34.4 %) were in the age group 60 years and above, followed by 31.3% in the age group 30 to 40 years. None of the cases were below 20 years of age. In the control group, maximum number of subjects (25 %) were in the 20 to 30 years age group, followed by 22 % subjects in the age group of 30-40 years. The distribution of cases and controls to be in a particular age group was not significant (p=0.0533).

Table 2: Distribution of subjects of cases and controls according to Gender

Gender	Cases No (%)	Control No (%).
Male	10(31)	9(28)
Female	22(69)	23(72)
Total	32(100)	32(100)

Figure 2: Distribution of subjects of cases and controls according to Gender



In our study, the male : female ratio among cases was 1: 2.2 whereas it was 1: 2.5 in the control group. The distribution of cases and controls to be of a particular gender was not significant (p=0.7844).

Table 3: Distribution of subjects of cases and controls according to Hb level

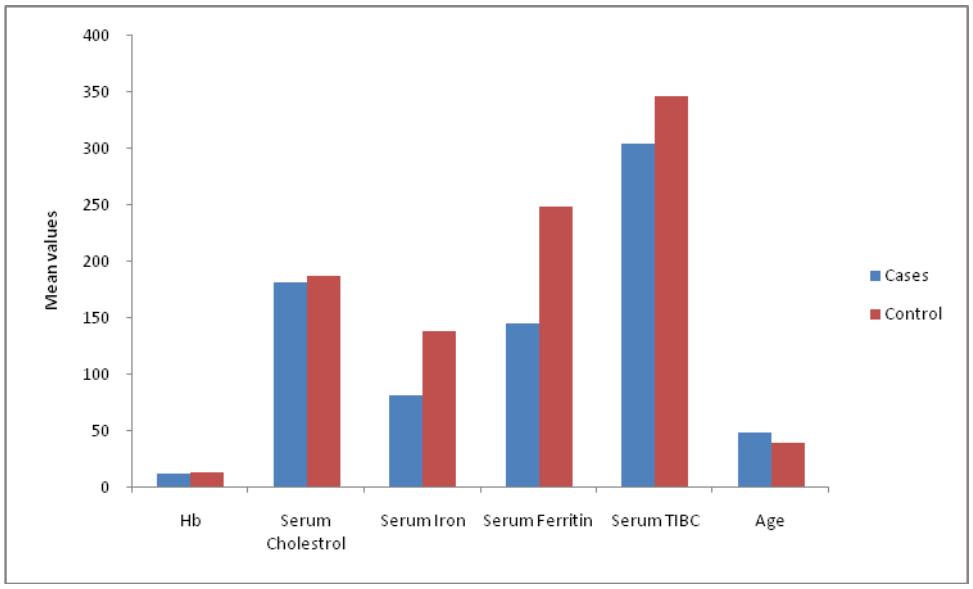
Hb level	Cases No(%)	Control No(%).
≤9.0	6(18.8)	0(0)
>9.0	26(81.3)	32(100)
Total	32(100.0)	32(100)

In the cases group, 81.3% cases were non-Anaemic (Hb > 9.0) while 18.8% were Anaemic (Hb ≤ 9.0). Among the controls, none were Anaemic (Hb ≤ 9.0). Having Hb levels ≤ 9.0 among cases as compared to controls was found to be statistically significant (p=0.0101).

Table 4: Comparison of variables between study and control groups

Comparison between Study & Control	Cases Mean±SD	Control Mean±SD
Hb	11.18±1.55	12.17±1.68
Serum Cholestrol	180.91±41.73	187.25±38.63
Serum Iron	81.31±45.58	137.91±49.62
Serum Ferritin	144.57±142.25	247.90±97.55
Serum TIBC	304.72±129.81	346.03±77.12
Age	47.78±14.08	38.75±17.96

Figure 3: Comparison of variables between study and control groups



The mean Hb in studygroup was 11.18(SD=1.55), and that in control group was 12.17(SD=1.68). It was found statistically significant difference in cases and controls. (p=0.036)

Table 5 : Descriptive Statistics (Study Group)

Variables	N	Minimum	Maximum	Mean	Std. Deviation
AGE	32	23	70	47.78	14.075
Hb	32	8.9	14.3	11.175	1.5475
Serum Cholestrol (150-220mg/dl)	32	102	257	180.91	41.730
Serum Iron (60-160mcg/dl)	32	30	186	81.31	45.576
Serum Ferritin (7-282ng/dl)	32	1.6	600.0	144.572	142.2520
Serum TIBC (250-400mcg/dl)	32	230	800	344.72	129.810

Table 6 : Descriptive Statistics(Control group)

Variables	N	Minimum	Maximum	Mean	Std. Deviation
AGE	32	18	98	38.75	17.957
Hb	32	9.5	15.0	12.166	1.6802
Serum Cholestrol (150-220mg/dl)	32	126	280	187.25	38.631
Serum Iron (60-160mcg/dl)	32	52	230	137.91	49.620
Serum Ferritin (7-282ng/dl)	32	56.0	428.0	247.900	97.5468
Serum TIBC (250-400mcg/dl)	32	250	530	346.03	77.115

The **mean serum cholesterol** levels were lower in cases than controls (180.91 ± 41.73 vs. 187.25 ± 38.63). However, the difference was not statistically significant (p=0.586).

The **mean serum iron** level in cases was found to be significantly lower than in controls (81.31 ± 45.58 mcg/dl vs. 137.91 ± 49.62 mcg/dl, p=0.001).

The **mean serum ferritin** was significantly lower in cases than in controls (144.57 ± 142.25 ng/dl vs. 247.90 ± 97.55 ng/dl, p=0.001).

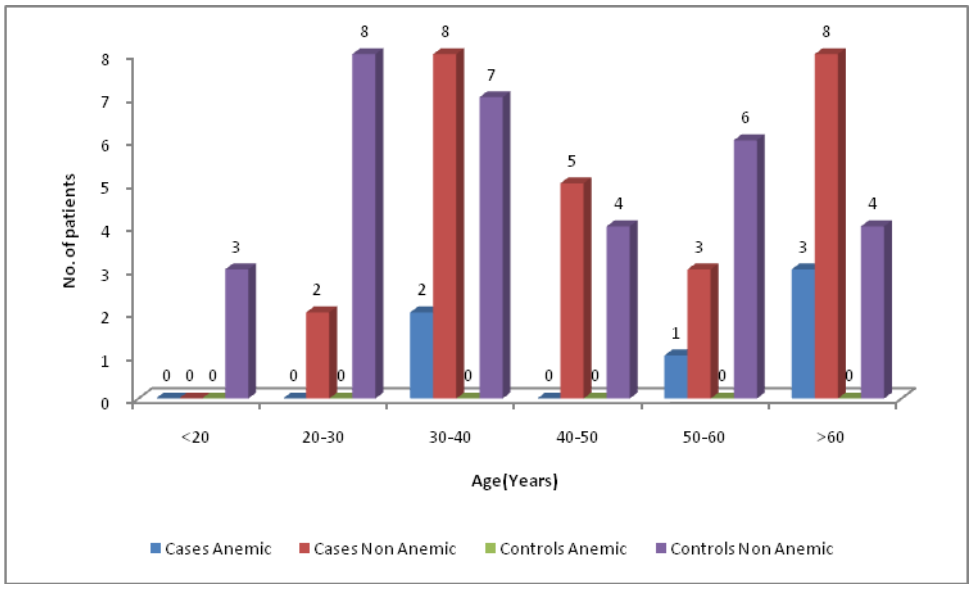
The **mean serum TIBC** levels were lower in cases than in controls, but the difference was not statistically significant (304.72 ± 129.81 mcg/dl vs. 346.03 ± 77.12 mcg/dl, p=0.273).

The **mean age** of cases was significantly higher than that of controls (47.78 ± 14.08 vs. 38.75 ± 17.96, p=0.005)

Table:7 Distribution of data according to Age and Anemia

Age(Years)	Cases			Controls		
	Anaemic	Non Anaemic	Total	Anaemic	Non Anaemic	Total
<20	0	0	0	0	3(9.4)	3(9.4)
20-30	0	2(6.5)	2(6.5)	0	8(25)	8(25)
30-40	2(6.5)	8(25)	10(31.3)	0	7(21.8)	7(21.8)
40-50	0	5(15.6)	5(15.6)	0	4(12.5)	4(12.5)
50-60	1(3.1)	3(9.4)	4(12.5)	0	6(18.75)	6(18.75)
>60	3(9.4)	8(25)	11(343.3)	0	4 (12.5)	4 (12.5)
Total	6(18.75)	26(81.3)	32	0	32	32

Figure 4: Distribution of data according to Age and Anaemia

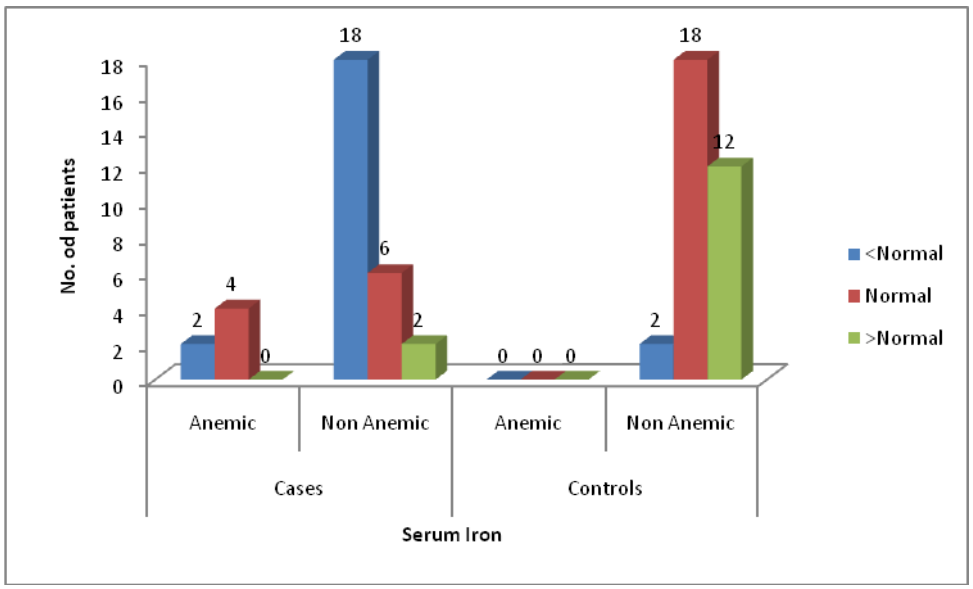


81.3% patients among the cases were non-Anaemic, of which maximum number, 25% each belonged to the age group 30-40 years and above 60 years. Among the Anaemic cases, Maximum number (9.4 %) were above 60 years of age. The cases being Anaemic or non- Anaemic was not statistically significant (p=0.6894). In the control group, none of the subjects were anaemic.

Table 8: Distribution of Serum Iron according to Anaemia

Serum Iron	Cases			Controls		
	Anaemic	Non Anaemic	Total	Anaemic	Non Anaemic	Total
<Normal	2(6.25)	18(56.25)	20(62.5)	0	2(6.25)	2(6.25)
Normal	4(12.5)	6(18.7)	10(31.25)	0	18(56.3)	18(56.3)
>Normal	0	2(6.25)	2(6.25)	0	12(37.5)	12(37.5)
Total	6(18.75)	26(81.3)	32	0	32	32

Figure 5: Distribution of Serum Iron according to Anaemia



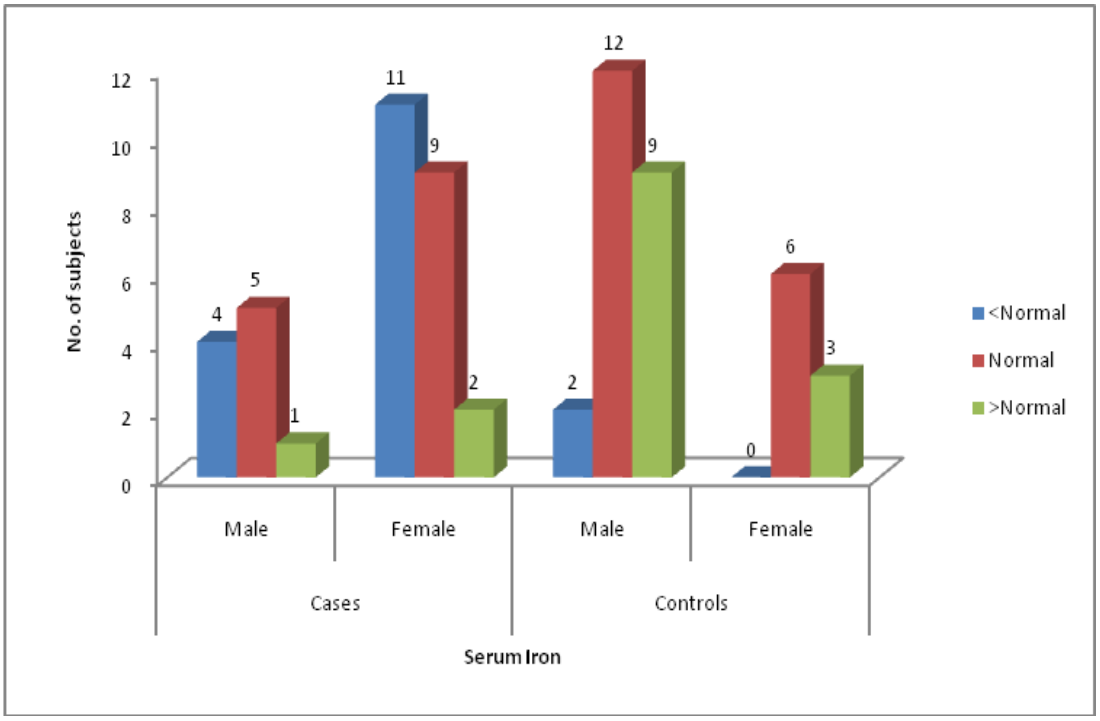
62.5% cases had serum iron levels < normal, of which 56.25 % were non Anaemic and 6.25 % were Anaemic. 31.25 % cases had normal serum iron levels, of which 18.7% were non Anaemic and 12.5 % were Anaemic. All of the 6.25% cases who had serum iron > normal were non Anaemic. The distribution of serum iron according to anemia among the cases was not statistically significant (p=0.3930).

In the control group, all of the 6.25% subjects who had serum iron < normal were non-Anaemic. Similarly, 56.3% subjects having normal serum iron levels were non Anaemic and 37.5% subjects having serum iron> normal were non-Anaemic.

Table 9: Distribution of Serum Iron according to Gender

Serum Iron	Cases			Controls		
	Male	Female	Total	Male	Female	Total
<Normal	4(12.5)	11(34.3)	15(46.8)	2(6.25)	0	2(6.25)
Normal	5(15.6)	9(28.1)	14(43.7)	12(37.5)	6(18.75)	18(56.3)
>Normal	1(3.1)	2(6.25)	3(9.4)	9(28.1)	3(9.4)	12(37.5)
Total	10(31.3)	22(68.7)	32	23(78)	9(28.1)	32

Figure 6: Distribution of Serum Iron according to Gender



46.8 % cases had serum iron levels < normal, of which 34.3 % were females and 12.5 % were males. 43.7 % cases had normal serum iron levels, of which 28.1 % were females and 15.6 % were males. 9.4% cases had serum iron levels > normal, of which 6.25 % were females and 3.1 % were males.

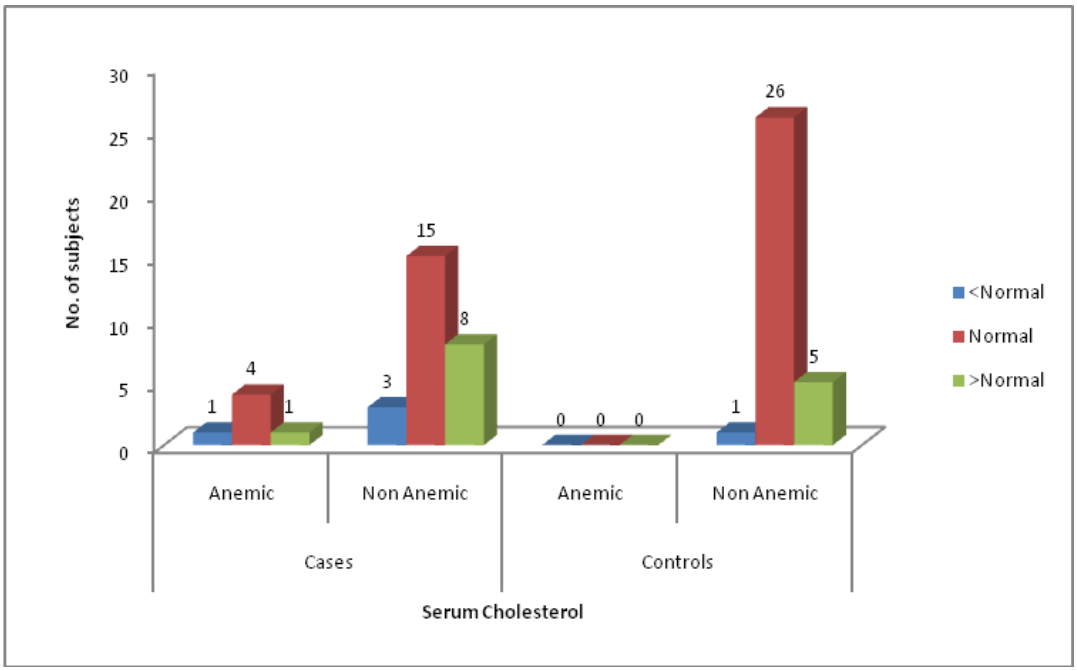
In the control group, all of the 6.25% subjects who had serum iron < normal were males. Of 56.3% subjects having normal serum iron levels37.5 % were males 18.5 % were females and of 37.5% subjects having serum iron> normal, 28.1 % were males.

The distribution of serum iron according to gender among the cases or controls was not statistically significant (p=0.8682 and p = 0.5821 respectively).

Table 10: Distribution of Serum Cholesterol according to Anemia

Serum Cholesterol	Cases			Controls		
	Anaemic	Non Anaemic	Total	Anaemic	Non Anaemic	Total
<Normal	1(3.1)	3(9.4)	4(12.5)	0	1(3.1)	1(3.1)
Normal	4(12.5)	15(46.8)	19(59.4)	0	26(81.3)	26(81.3)
>Normal	1(3.1)	8(25)	9(28.1)	0	5(15.6)	5(15.6)
Total	6(18.75)	26(81.3)	32	0	32	32

Figure 7: Distribution of Serum Cholesterol according to Anaemia



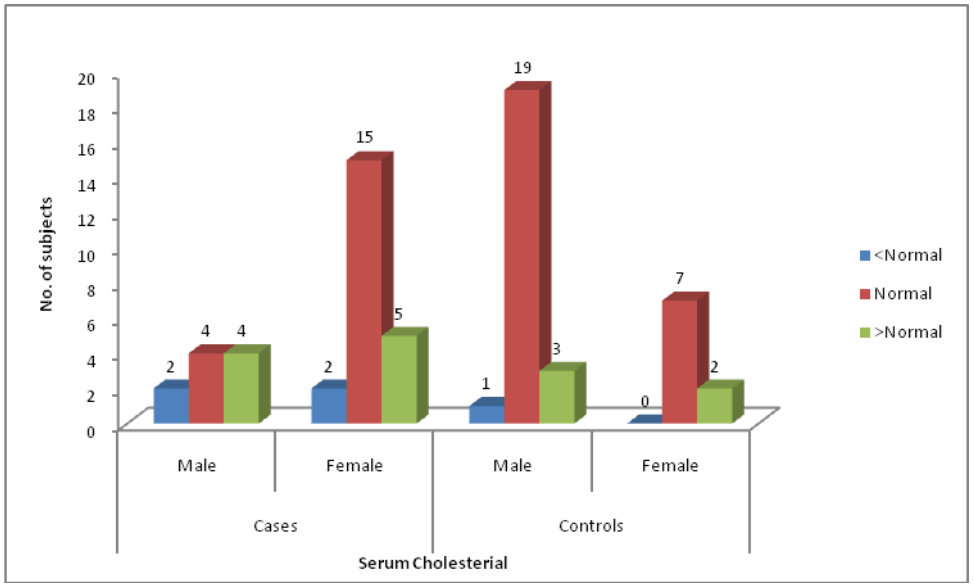
12.5% cases had serum cholesterol levels < normal, of which 9.4 % were non Anaemic and 3.1 % were Anaemic. 59.4 % cases had normal serum cholesterol levels, of which 46.8 % were non Anaemic and 12.5 % were Anaemic. Of the 28.1 % cases who had serum iron > normal, 25 % were non Anaemic. The distribution of serum cholesterol according to anemia among the cases was not statistically significant (p=0.7736).

In the control group, all of the 3.1% subjects who had serum cholesterol < normal were non-Anaemic. Similarly, 81.3 % subjects having normal serum cholesterol levels were non Anaemic and 15.6 % subjects having serum cholesterol> normal were non-Anaemic.

Table 11: Distribution of Serum Cholesterol according to Gender

Serum Cholesterol	Cases			Controls		
	Male	Female	Total	Male	Female	Total
<Normal	2(6.25)	2(6.25)	4(12.5)	1(3.1)	0(0)	1(3.1)
Normal	4(12.5)	15(46.8)	19(59.4)	19(59.4)	7(21.9)	26(81.3)
>Normal	4(12.5)	5(15.6)	9(28.1)	3(9.4)	2(6.25)	5(15.6)
	10(31.3)	22(68.7)	32	23(78)	9(28.1)	32

Figure 8: Distribution of Serum Cholesterol according to Gender



12.5 % cases had serum cholesterol levels < normal, of which 6.25 % were females and 6.25 % were males. 59.4 % cases had normal serum cholesterol levels, of which 46.8 % were females and 12.5 % were males. 28.1% cases had serum cholesterol levels > normal, of which 15.6 % were females and 12.5 % were males.

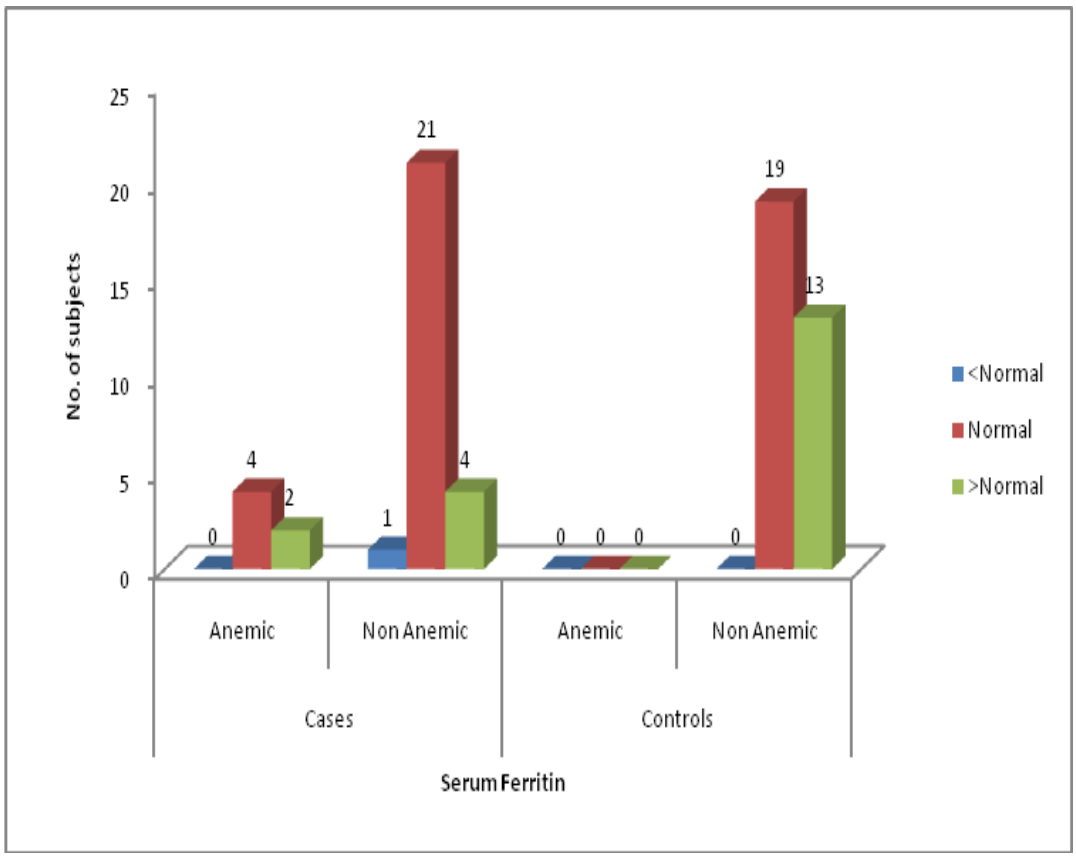
In the control group, all of the 3.1 % subjects who had serum cholesterol < normal were males. Of 81.3% subjects having normal serum cholesterol levels 59.4 % were males 21.9 % were females and of 15.6 % subjects having serum cholesterol> normal, 9.4 % were males.

The distribution of serum cholesterol according to gender among the cases or controls was not statistically significant (p=0.3161 and p = 0.6843 respectively).

Table 12: Distribution of Serum Ferritin according to Anemia

Serum Ferritin	Cases			Controls		
	Anaemic	Non Anaemic	Total	Anaemic	Non Anaemic	Total
<Normal	0	1(3.1)	1(3.1)	0	0	0
Normal	4(12.5)	21(65.,6)	25(78)	0	19(59.4)	19(59.4)
>Normal	2(6.25)	4(12.5)	6(18.75)	0	13(40.6)	13(40.6)
Total	6(18.75)	26(81.3)	32	0	32	32

Figure 9: Distribution of Serum Ferritin according to Anemia



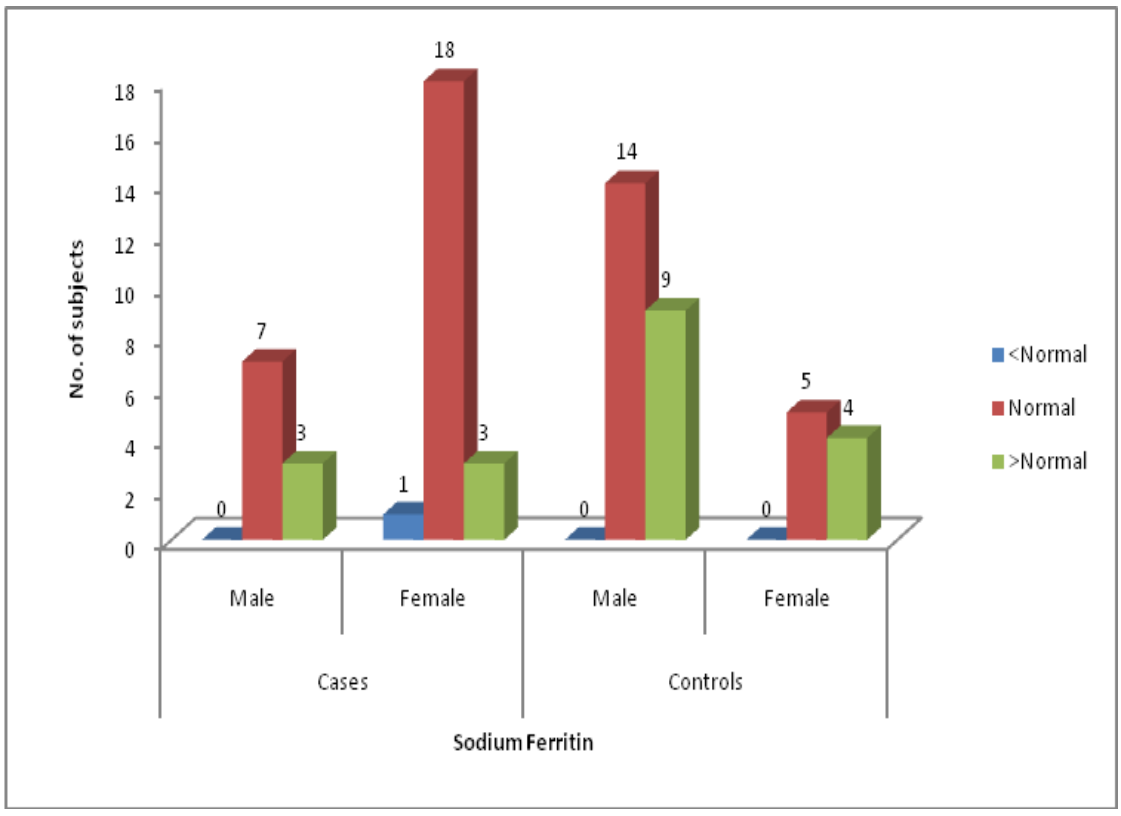
All of the 3.1 % cases who had serum ferritin levels < normal were non Anaemic. 78 % cases had normal serum ferritinl levels, of which 65.6 % were non Anaemic and 12.5 % were Anaemic. Of the 18.75 % cases who had serum ferritin > normal, 12.5 % were non Anaemic. The distribution of serum ferritin according to anemia among the cases was not statistically significant (p=0.5509).

In the control group, no subject had serum ferritin < normal. 59.4 % subjects having normal serum ferritin levels were non Anaemic and 40.6 % subjects having serum ferritin > normal were non-Anaemic.

Table 13: Distribution of Serum Ferritin according to Gender

Serum Ferritin	Cases			Controls		
	Male	Female	Total	Male	Female	Total
<Normal	0	1(3.1)	1(3.1)	0	0	0
Normal	7(21.9)	18(56.3)	25(78)	14(43.8)	5(15.6)	19(59.4)
>Normal	3(9.4)	3(9.4)	6(18.75)	9(28.1)	4(12.5)	13(40.6)
Total	10(31.3)	22(68.7)	32	23(78)	9(28.1)	32

Figure 10: Distribution of Serum Ferritin according to Gender



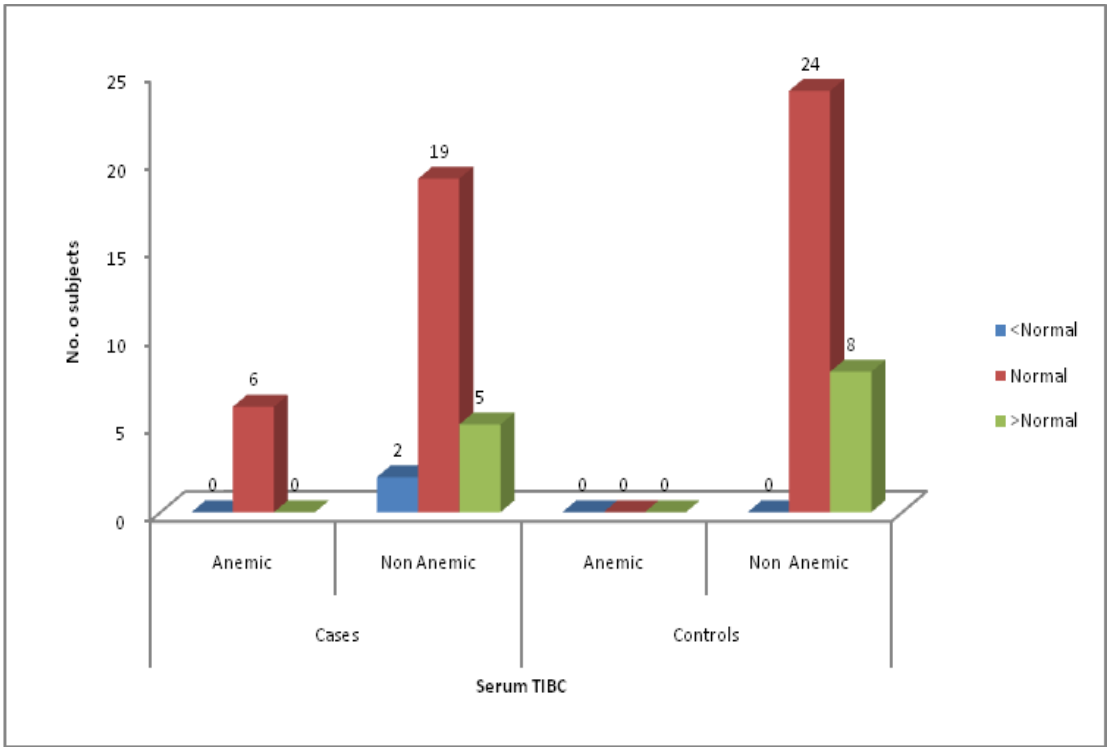
All the 3.1 % cases who had serum ferritin levels < normal were females. 78 % cases had normal serum ferritin levels, of which 56.3 % were females and 21.9 % were males. 18.75% cases had serum ferritin levels > normal, of which 9.4 % each were females and males.

In the control group, none of the subjects had serum ferritin < normal. Of 59.4 % subjects having normal serum ferritin levels 43.8 % were males 15.6 % were females and of 40.6 % subjects having serum ferritin> normal, 28.1 % were males. The distribution of serum ferritin according to gender among the cases or controls was not statistically significant ($p=0.4586$ and $p = 0.7832$ respectively).

Table 14: Distribution of Serum TIBC according to Anemia

Serum TIBC	Cases			Controls		
	Anaemic	Non Anaemic	Total	Anaemic	Non Anaemic	Total
<Normal	0	2(6.25)	2(6.25)	0	0	0
Normal	6(18.75)	19(59.4)	25(78)	0	24(75)	24(75)
>Normal	0	5(46.8)	5(46.8)	0	8(25)	8(25)
Total	6(18.75)	26(81.3)	32	0	32	32

Figure 11: Distribution of Serum TIBC according to Anemia



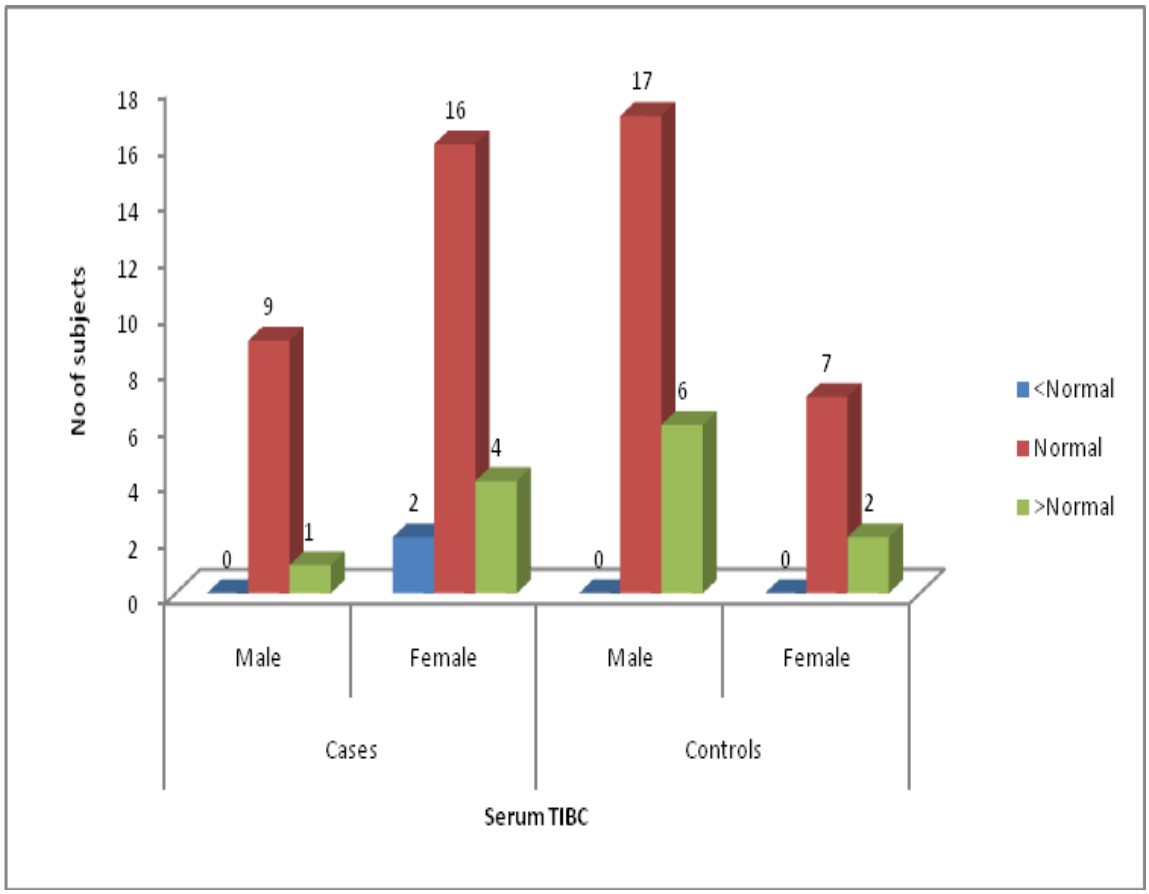
All of the 6.25 % cases who had TIBC levels < normal were non Anaemic. 78 % cases had normal serum TIBC levels, of which 59.4 % were non Anaemic and 18.75 % were Anaemic. All the 46.8 % cases who had serum TIBC > normal were non Anaemic. The distribution of serum TIBC according to anemia among the cases was not statistically significant (p=0.3556).

In the control group, no subject had serum TIBC < normal. All of the 75 % subjects having normal serum TIBC levels as well as 25 % subjects having serum TIBC> normal were non-Anaemic.

Table 15: Distribution of Serum TIBC according to Gender

Serum TIBC	Cases			Controls		
	Male	Female	Total	Male	Female	Total
<Normal	0	2(6,25)	2(6.25)	0	0	0
Normal	9(28.1)	16(50	25(78)	17(53)	7(21.8)	24(75)
>Normal	1(3.1)	4(12.5)	5(4.8)	6(18.75)	2(6.25)	8(25)
Total	10(31.3)	22(68.7)	32	25(78)	9(28.1)	32

Figure 12: Distribution of Serum TIBC according to Gender



All the 6.25 % cases who had serum TIBC levels < normal were females. 78 % cases had normal serum TIBC levels, of which 50 % were females and 28 % were males. 4.8 % cases had serum TIBC levels > normal, of which 12.5 % were females and 3.1 % were males.

In the control group, none of the subjects had serum TIBC < normal. Of 75 % subjects having normal serum TIBC levels 53 % were males 21.8 % were females and of 25 % subjects having serum TIBC> normal, 18.75 % were males.

The distribution of serum TIBC according to gender among the cases or controls was not statistically significant (p=0.4804 and p = 0.8204 respectively).

DISCUSSION

Our study was conducted in patients admitted in B.L.D.E.(Deemed to be University) Shri. B. M. Patil Medical College, Hospital and Research Centre, Vijayapur in Surgery Department during period of Oct 2016 – August 2018 with a sample size of 32 patients in cases group and 32 patients in control group comprising of a total of 64 patients.

Distribution according to age group

In our study, there was uniform distribution of cases and controls among various age groups. The distribution of cases and controls to be in a particular age group was not significant (p=0.0533).

Distribution according to gender

In our study, 69 % cases and 72% controls were females. The distribution of cases and controls to be of a particular gender was not significant (p=0.7844).

As in our study, several studies have reported that gallstones are more prevalent in female population than males [15,19,20,21,5].

Distribution according to diagnosis

In our study, acute gastritis was the most common diagnosis in controls (37.5 %), followed by acid peptic disease (28.1%). In the cases group, all the subjects presented with some form of cholelithiasis.

Distribution according to Hb level

In our study, all the controls (100%) had Hb more than 9, while in the cases group, only 81.3% cases had Hb more than 9. 18.8% cases had Hb levels less than 9

as compared to nil among the control group. Having Hb levels ≤ 9 among cases as compared to controls was found to be statistically significant (p=0.0101).

Comparision of Hb levels amongst groups

In our study, the mean Hb level in cases was found to be significantly lower than in controls (11.18 ± 1.55 vs. 12.17 ± 1.68, p=0.036). Pamuk et al [23] also reported that the frequency of gall stones plus cholecystectomy was significantly higher in IDA patients (15 cases, 13.5%) than in the control group (five cases, 6.2%, p = 0.048).

Comparision of serum cholesterol

In our study, though the mean serum cholesterol levels were lower in cases than controls, the difference was not statistically significant (180.91 ± 41.73 vs. 187.25 ± 38.63, p=0.586).

Similar to our finding, Kumar et al [17] reported that Total serum cholesterol was not different in gall stone formers from that of the general population. However, the cholesterol level in the gall bladder bile was significantly higher in anaemic, than in non-anaemic individuals, leading the researchers to conclude that low serum iron levels lead to bile supersaturation with respect to cholesterol, which leads to gallstone formation.

Comparision of serum Iron

In our study, the mean serum iron level in cases was found to be significantly lower than in controls (81.31 ± 45.58 mcg/dl vs. 137.91 ± 49.62 mcg/dl, p=0.001).

Several studies have reported that deficiency of serum iron leads to gall stone formation [5,16,3,18]. Halgaonkar et al [21] also reported that serum iron was found to be low in majority of the patients indicating iron deficiency as a cause of gallstone

formation. Sahu et al [24] concluded that low serum iron level is a factor in bile super-saturation with respect to cholesterol leading to gallstone formation.

Comparision of serum ferritin

In our study, the mean serum ferritin was significantly lower in cases than in controls (144.57 ± 142.25 ng/dl vs. 247.90 ± 97.55 ng/dl, p=0.001)

Comparision of serum TIBC

In our study, though the mean serum TIBC levels were lower in cases than in controls, the difference was not statistically significant (304.72 ± 129.81 mcg/dl vs. 346.03 ± 77.12 mcg/dl, p=0.273).

Comparision of age

In our study, the mean age of cases was significantly higher than that of controls (47.78 ± 14.08 vs. 38.75 ± 17.96, p=0.005)

D**istribution of data according to age and anemia**

The distribution of Anaemic patients and non Anaemic patients among the cases in a particular age group was not statistically significant (p=0.6894).

Distribution of serum Iron according to anemia

Of the 62.5% cases who had serum iron< normal levels, 6.25% were Anaemic. Of the 31.25% cases who had normal serum iron levels, 12.5 % were Anaemic. Of the 6,25 % cases who had > normal serum iron levels, none were Anaemic. 6.25 % of the healthy volunteers have value of serum iron less than normal (59–158 μg/dl). None of the healthy volunteers who have value of serum iron less than normal (59–158 μg/dl)

are Anaemic. This distribution of serum iron according to anemia was not statistically significant (p=0.6894).

Several researchers have reported results which are not in line with our findings. Sarhan, Hamed and Khalaf (20) reported that all non anaemic cases (n=27) had a high average serum iron content of 91± 35 µg/dl, as compared to anaemic ones (n=23), where average serum iron was 26 ± 9.5 µg/dl.

Distribution of Serum Iron according to Gender

There are 34.3 % female patients with gallstone disease who have serum iron levels below the normal value (59–158 µg/dl). There are no females in the healthy volunteer group whose serum iron levels are below normal. There are only 28.1 % female patients with gallstones whose serum iron levels are normal, which is 18.75 % females in the healthy control group. Most of the patients with gallstone disease whose serum iron levels are subnormal are females.

However, the association between gender and having serum iron levels < normal, normal or > normal was not statistically significant in both the cases and control groups (p=0.8682 vs. p=0.5821)

Arora and Yadav (15) have reported that low serum iron is not associated with cholelithiasis in male. In their study, low serum iron was seen in 23.07% of female cases comparable to 23% low serum iron in control females.

Distribution of serum cholesterol according to anemia

In our study, the association between anemia and having serum cholesterol levels < normal, normal or > normal was not statistically significant in the cases (p=0.7736).

Kshirsagar et al [18] and Daddenavar and Daddenavar [19] also reported that there were no significant variations in the serum cholesterol contents of Anaemic and non-Anaemic groups (P=0.367, t=0.91). Sarhan, Hamed and Khalaf [20] reported that there were no significant variations in the serum cholesterol contents of Anaemic and non-Anaemic groups (p=0.367, t=0.91). Prasad et al [3] reported that Serum total cholesterol of the patients of cholelithiasis was not different from that of general population. Also, there were no significant variations in the serum cholesterol contents of Anaemic and non Anaemic groups.

Distribution of serum cholesterol according to gender

6.25 % each of male and female patients with gallstones have below normal serum cholesterol levels, which is 3.1% for males and 0 % for females in normal healthy individuals. 12.5 % of male and 46.8 % of female patients with gallstones have normal serum cholesterol levels, which is 59.4 % and 21.9 % in normal healthy individuals, respectively. The association between gender and having serum cholesterol levels < normal, normal or > normal was not statistically significant in the formation of gallstones (p=0.3161 vs. p=0.6843).

Similar to our findings, Daddenavar and Daddenavar [19] also reported that there was no significant variation of serum cholesterol in the male and female patients (P=0.082, t=1.77).Prasad et al. [3] also reported that there was no significant variation of serum total cholesterol in the male and female patients.

Distribution of serum ferritin according to anemia

78 % of patients with gallstones have normal value of serum ferritin; of which 12.5 % are Anaemic whereas 59.4% in the control group have normal serum ferritin values

and none are Anaemic . In patients with gallstones who have serum ferritin levels > normal, 6.25 % are Anaemic whereas in the control group of the 40.6% patients who have serum ferritin levels more than normal, none are Anaemic. The association between anemia and having serum ferritin levels < normal, normal or > normal was not statistically significant in the cases (p=0.5509).

Distribution of serum ferritin according to gender

In the case group, 0 % of male and 3.1 % of female patients have less than normal serum ferritin levels. In the control group neither males nor females had serum ferritin below normal values. In the case group, 21.9 % of male and 56.3 % of female patients have normal serum ferritin levels. This is 43.8 % and 15.6 %, respectively, in the control group. In the case group, 9.4 % each of males and females have greater than normal serum ferritin levels. This is 28.1 % and 12.5% respectively in the control group. The association between gender and having serum ferritin levels < normal, normal or > normal was not statistically significant in the cases or controls (p=0.4586 vs. p=0.7832).

Arora and Yadav [15] reported that low ferritin was seen in 35.50% of female cases as compared to 15.38% of female controls. In males, serum ferritin was low in 64.5% of cases and 16.66% of controls. Serum ferritin levels were normal in 35.50% of cases and 66.66% of controls and above normal in 16.66% of controls suggesting that low serum ferritin is associated with gall stones in males.

Distribution of serum TIBC according to anemia

78 % of patients with gallstones have normal value of serum TIBC; of which 18.75 % are Anaemic whereas 75 % in the control group have normal serum ferritin values and none are Anaemic . All of the 46.8% patients having gallstones who have TIBC values > normal are non Anaemic. Similarly, in the control group, all the 25 % controls who have TIBC values > normal are non- Anaemic. The association between anemia and having serum TIBC levels < normal, normal or > normal was not statistically significant in the cases (p=0.3556).

Distribution of serum TIBC according to gender

In the case group, 0 % of male and 6.25 % of female patients have less than normal serum TIBC levels. In the control group neither males nor females had serum TIBC below normal values. In the case group, 28.1 % of male and 50 % of female patients have normal serum ferritin levels. This is 53 % and 21.8 %, respectively, in the control group. In the case group, 3.1 % of males and 12.5 % females have greater than normal serum TIBC levels. This is 18.75 % and 6.25 % respectively in the control group. The association between gender and having serum TIBC levels < normal, normal or > normal was not statistically significant in either cases or controls (p=0.4804 vs. p= 0.8204).

CONCLUSION

- Subjects with gallstones have statistically lower serum Iron levels as compared to controls (p=0.001)
- Subjects with gallstones have statistically lower ferritin levels as compared to controls (p=0.001)This study suggests that iron deficiency leading to anemia plays a significant role in super saturation of bile, leading to stone formation in the gall bladder. The serum cholesterol of the Anaemic group was found to be similar to the non-Anaemic group. Iron deficiency probably alters the hepatic enzyme metabolism, leading to super saturation of gall bladder bile with respect to cholesterol irrespective of serum cholesterol levels, hence promoting the cholesterol crystal formation.
- Serum total cholesterol of the patients of cholelithiasis was not different from that of general population. There were no significant variations in the serum cholesterol contents of both the groups. Also, there was no significant variation of the above parameter in the male and female patients.
- Serum ferritin cannot be taken as a sole diagnostic tool in the diagnosis of iron deficiency anemia as its value can vary due to other causes such as iron therapy, hepatocellular disease and inflammations (since cholecystitis is a inflammatory condition, this could be the reason for the high level of serum ferritin).

SUMMARY

Gallstone disease is a common clinical entity affecting the adult population of both sexes. Gallstones are classified into cholesterol stones, black or brown pigmented stones or mixed stones. Conditions that favour the formation of cholesterol gallstones are super-saturation of bile with cholesterol, kinetically favourable nucleation and the presence of cholesterol crystals in the gall bladder long enough to agglomerate into stone. Recent studies have pointed towards the role of trace elements (Fe, Ca, Zn and Cu) and defective pH in the formation of gallstones. Serum Iron, TIBC and Transferrin Saturation are the laboratory measurements that reflect the availability of iron for hemoglobin synthesis. The serum iron level represents the amount of circulating iron bound to transferrin. The normal serum iron ranges from 9 to 27 μmol/L (50–150 μg/dL). Free iron is toxic to cells, and the body has established an elaborate set of protective mechanisms to bind iron in various tissue compartments. Within cells, iron is stored complexed to protein as ferritin. Apoferritin binds to free ferrous iron and stores it in the ferric state. Under steady-state conditions, the serum ferritin level correlates with total body iron stores; thus, the serum ferritin level is the most convenient laboratory test to estimate iron stores. The normal value for ferritin varies according to the age and gender of the individual. Adult males have serum ferritin values averaging 100 μg/L corresponding to iron stores of ~1 g, while adult females have levels averaging 30 μg/L , reflecting lower iron stores (~300 mg). The TIBC is an indirect measure of the circulating transferrin. The normal range for TIBC is 300–360 μg/dL (54–64 μmol/L). (14) It evaluates how well transferrin carries iron through blood.

Our study attempted to correlate iron deficiency anemia of patients admitted in B.L.D.E. (Deemed to be University) Shri. B. M. Patil Medical College, Hospital and Research Centre, Vijayapur in Surgery Department from October 2016 to August 2018 with gallstone disease by estimating serum iron, serum ferritin and serum Total Iron Binding Capacity in 32 cases and 32 matched controls.

In our study, subjects with gallstones were found to be significantly Anaemic as compared to controls (p=0.0101). They also had statistically lower serum iron levels as well as lower serum ferritin levels compared to controls (p=0.001).

This study suggests that iron deficiency leading to anemia plays a significant role in super saturation of bile, leading to stone formation in the gall bladder. The serum cholesterol of the Anaemic group was found to be similar to the non-Anaemic group. Iron deficiency probably alters the hepatic enzyme metabolism, leading to super saturation of gall bladder bile with respect to cholesterol irrespective of serum cholesterol levels, hence promoting the cholesterol crystal formation.

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ANNEXURES

ETHICAL CLEARANCE CERTIFICATE

Institutional Ethical Committee
BLDE.U's Shri B.M. Patil Medical College
No. 61/2016
Date 06/10/16

B.L.D.E. UNIVERSITY'S
SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR-586 103
INSTITUTIONAL ETHICAL COMMITTEE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 04-10-2016 *at* 03pm *to scrutinize the Synopsis of Postgraduate Students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected & revised version synopsis of the Thesis has been accorded Ethical Clearance.*

Title "Serum iron levels in gallbladder stone disease : A comparative Study"

Name of P.G. student Dr. Gopashetty Sheeraj
Dept of Surgery

Name of Guide/Co-investigator Dr Basavaraj Navarangi
Asso. Prof of Surgery

DR.TEJASWINI. VALLABHA
CHAIRMAN
INSTITUTIONAL ETHICAL COMMITTEE
BLDEU'S, SHRI.B.M.PATIL
MEDICAL COLLEGE, BIJAPUR.

Following documents were placed before E.C. for Scrutinization
1) Copy of Synopsis/Research project.
2) Copy of informed consent form
3) Any other relevant documents.

SAMPLE INFORMED CONSENT FORM

B.L.D.E(DEEMED TO BE UNIVERSITY)

SHRI B.M. PATIL MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTRE, VIJAYAPUR – 586103, KARNATAKA

TITLE OF THE PROJECT : **SERUM IRON LEVELS IN GALLBLADDER STONE DISEASE: A COMPARATIVE STUDY**

PRINCIPAL INVESTIGATOR : **Dr.GOPASHETTY DHEERAJ**

PG GUIDE : **Dr.BASAVARAJ NARASANAGI**
M.S. (General surgery)
Professor
Department of Surgery

PURPOSE OF RESEARCH:

I have been informed that this study will analyse SERUM IRON LEVELS IN GALLBLADDER STONE DISEASE : A COMPARATIVE STUDY. I have been explained about the reason for doing this study and selecting me/my ward as a subject

for this study. I have also been given free choice for either being included or not in the study.

PROCEDURE:

Patient will be explained about the need of the study and patient will also be explained about the required investigations as per standard protocol. I am aware that in addition to routine care received I will be asked series of questions by the investigator. I have been asked to undergo the necessary investigations and treatment, which will help the investigator in this study.Patients who met the inclusion criteria were randomly assigned a study group (Group A) or control group (Group B).

RISKS AND DISCOMFORTS:

I understand that I may experience some pain and discomforts during the examination or during my treatment. This is mainly the result of my condition and the procedures of this study are not expected to exaggerate these feelings which are associated with the usual course of treatment.

BENEFITS:

I understand that my participation in the study will help to correlate the iron deficiency aneamia with gallstone disease by estimating serum iron, serum ferritin, serum total iron binding capacity.

CONFIDENTIALITY:

I understand that medical information produced by this study will become a part of this hospital records and will be subjected to the confidentiality and privacy regulation of this hospital. Information of a sensitive, personal nature will not be a part of the medical records, but will be stored in the investigator's research file and identified only by a code number. The code key connecting name to numbers will be kept in a separate secure location.

If the data are used for publication in the medical literature or for teaching purpose, no names will be used and other identifiers such as photographs and audio or video tapes will be used only with my special written permission. I understand that I may see the photograph and videotapes and hear audiotapes before giving this permission.

REQUEST FOR MORE INFORMATION:

I understand that I may ask more questions about the study at any time. Dr.GOPASHETTY DHEERAJ is available to answer my questions or concerns. I understand that I will be informed of any significant new findings discovered during the course of this study, which might influence my continued participation. A copy of this consent form will be given to me to keep it and for careful reading.

REFUSAL OR WITHDRAWL OF PARTICIPATION:

I understand that my participation is voluntary and I may refuse to participate or may withdraw consent and discontinue participation in the study at any time without prejudice to my present or future care at this hospital.

I also understand that Dr.Gopashetty Dheeraj will terminate my participation in this study at any time after he has explained the reasons for doing so and has helped arrange for my continued care by my own physician or therapist, if this is appropriate.

INJURY STATEMENT:

I understand that in the unlikely event of injury to me/my ward, resulting directly to my participation in this study, if such injury were reported promptly, then medical treatment would be available to me, but no further compensation will be provided.I understand that by my agreement to participate in this study, I am not waiving any of my legal rights.

I have explained to _____ the purpose of this research, the procedures required and the possible risks and benefits, to the best of my ability in patient's own language.

Date: Dr. Basavaraj Narasanagi (Guide) Dr. Dheeraj G (Investigator)

STUDY SUBJECT CONSENT STATEMENT:

I confirm that Dr.Gopashetty Dheeraj has explained to me the purpose of this research, the study procedure that I will undergo and the possible discomforts and benefits that I may experience, in my own language.

I have been explained all the above in detail in my own language and I understand the same. Therefore I agree to give my consent to participate as a subject in this research project.

_____ _____

(Participant) Date

_____ _____

(Witness to above signature) Date

PROFORMA FOR CASE TAKING

SL NO

NAME

AGE IP NO

SEX UNIT

RELIGION DOA

OCCUPATION DOO

ADDRESS DOD

SOCIO-ECONOMIC STATUS

Complaints:

HISTORY OF PRESENT ILLNESS

1)Pain abdomen

Duration

Location

Nature

Aggravating factors

Relieving factors

2)Nausea Present/Absent

3)Vomiting Present/Absent

Duration

Episodes

Content

4)Fever Present/Absent

Duration

Type

Chills/Rigors

5)Other symptoms

PAST HISTORY: H/O Previous surgery- yes/no

H/0 Drug intake -yes/no

H/O Chronic illness -yes/no

PERSONAL HISTORY:

Appetite: increased/decreased/normal Sleep: Disturbed/normal

Diet : Bowel/Bladder:

Habits :

GENERAL PHYSICAL EXAMINATION

BUILT: WELL/MODERATE/POOR

NOURISHMENT: WELL/MODERATE/POOR

PALLOR

ICTERUS

FEBRILE

PEDAL EDEMA

GENERAL LYMPHADENOPATHY

VITAL DATA:

TEMPERATURE:

PULSE

RESPIRATORY RATE

BLOOD PRESSURE:

SYSTEMIC EXAMINATION:

PER ABDOMEN:

Inspection:

Skin

Umbilicus

Contour

Visible veins

Visible peristalsis

Hernial orifices

Genitals

Palpation:

Temperature

Tenderness

Mass

Organomegaly

Percussion:

Free fluid

Liver dullness

Auscultation:

Bowel sounds

RESPIRATORY SYSTEM;

CARDIOVASCULAR SYSTEM:

CENTRAL NERVOUS SYSTEM;

CLINICAL DIAGNOSIS:

LABORATORY TESTS

HB%

TOTAL COUNT

DIFFERENTIAL COUNT

N/L/E/B/M:

URINE ROUTINE:

RBS

B.UREA

S.CREATININE

HIV

HBsAg

CHEST X RAY:

ULTRASONOGRAPHY OF ABDOMEN AND PELVIS:

SERUM CHOLESTEROL:

SERUM IRON:

SERUM FERRITIN:

SERUM TOTAL IRON BINDING CAPACITY: