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EVALUATION OF INSULIN RESISTANCE IN PATIENTS WITH CHOLETHIASIS

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EVALUATION OF INSULIN RESISTANCE IN PATIENTS WITH CHOLETHIASIS

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November 2022

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ABSTRACT

EVALUATION OF INSULIN RESISTANCE IN PATIENTS WITH CHOLETHIASIS

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Master of Science in Chemistry

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Gallstone (GS) diseases highly prevalent with a complex and multifactorial pathogenesis that cholesterol gallstones is the most common disease associated with biliary lipid abnormalities. Fasting blood sample and gallstone were collected from 140 patients with gallstone submitted to cholcystoctomy in Azady Teaching Hospital in Kirkuk City from 1st April 2022 to the end of September 2022. They were divided into two study groups: GS patients with control (100 subjects with GS, 40 subjects control). Insulin, Lipid Profile(Cholesterol, Triglyceride, LDL-C, HDL- C, VLDL), Glucose, as well as measurement of HOMA-IR, BMI and stone analysis. The results of this study showed that the cholesterol gallstone was more common type than pigment or mixed stone, females have a greater risk of gallstone disease than males at all ages and the ratio of females to males was 3.1:1. The obesity is associated with gallstone formation showed that, BMI has high significant (p=0.001) difference between patients with gallstonecompared to control in both gender (males and females). Profound effects of lipid profile of serum lipids metabolism and lipoproteins on the gallbladder formation, showed : significant difference in TC, TG, LDL and VLDL for patients with gallstones compared with control. There was a high level of insulin in the serum of patients with gallstone compared with control, the mean level was,(10.2 ± 5.24μIU/mL).

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Keywords: Cholilithiasis, Insulin resistance, Obesity, Lipi profile

ÖZET

KOLETİYAZİ HASTALARINDA İNSÜLİN DİRENCİ DEĞERLENDİRİLMESİ

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Safra taşı (GS) hastalıkları, kolesterol safra taşlarının biliyer lipid anormallikleri ile ilişkili en yaygın hastalık olduğu karmaşık ve çok faktörlü bir patogenez ile oldukça yaygındır. Kerkük Şehri Azady Eğitim Hastanesinde 1 Nisan 2022'den Eylül 2022'nin sonuna kadar kolsistektomiye safra taşı ile başvuran 140 hastadan açlık kan örneği ve safra taşı alındı. İki çalışma grubuna ayrıldı: Kontrollü GS hastaları (GS'li 100 kişi, 40 denek kontrolü). İnsülin, Lipid Profili(Kolesterol, Trigliserit, LDL-C, HDL-C, VLDL), Glikoz, ayrıca HOMA-IR ölçümü, BMI ve taş analizi. Bu çalışmanın sonuçları, kolesterol safra taşının pigment veya karışık taştan daha yaygın bir tip olduğunu, kadınların her yaşta erkeklerden daha fazla safra taşı hastalığı riskine sahip olduğunu ve kadınların erkeklere oranının 3.1:1 olduğunu gösterdi. Obezitenin safra taşı oluşumu ile ilişkili olduğu, her iki cinsiyette (erkek ve kadın) kontrole göre safra taşı olan hastalar arasında yüksek anlamlı (p=0,001) fark olduğunu göstermiştir. Safra kesesi oluşumu üzerindeki serum lipid metabolizması ve lipoproteinlerin lipid profilinin derin etkileri, kontrol ile karşılaştırıldığında safra taşı olan hastalar için TC, TG, LDL ve VLDL'de anlamlı farklılık gösterdi. Safra taşı olan hastaların serumunda kontrol ile karşılaştırıldığında yüksek düzeyde insülin vardı, ortalama düzey (10,2 ± 5,24µIU/mL) idi.

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Anahtar Kelimeler: Kolilitiazis, İnsülin direnci, Obezite, Lipi profili

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CONTENTS

ABSTRACT ..... **i**
ÖZET ..... **ii**
PREFACE AND ACKNOWLEDGEMENTS ..... **iii**
CONTENTS ..... **iv**
LIST OF SYMBOLS ..... **vii**
LIST OF ABBREVIATIONS ..... **viii**
LIST OF FIGURES ..... **ix**
LIST OF TABLES ..... **x**
1. INTRODUCTION **1**
1.1 Aim of the Study **2**
1.2 Objectives Study **2**
2. LITERATURE REVIEW **3**
2.1 Gallbladder **3**
2.1.1 Historical review **3**
2.1.2 Anatomy and physiology of gallbladder **3**
2.1.3 Gall bladder Stones **5**
2.1.4 Classification of gallstones **5**
2.2 Cholesterol Stone **6**
2.2.1 Pigment stones **6**
2.2.2 Black pigment stones **6**
2.2.3 Brown pigment stones **7**
2.3 Pathogenesis of cholesterol gallstones **7**
2.3.1 Cholesterol saturation **7**
2.3.2 Nucleation **8**
2.3.3 Gallbladder Hypomotility **8**
2.4 Risk Factors of Gallstone Formation Disease **8**
2.4.1 Clinical Presentation **9**
2.4.2 Diagnosis and Treatment **9**
2.5 Lipids and Lipoproteins **10**
2.5.1 Chylomicrons **10**

2.5.2 Very low density lipoproteins 11
2.5.3 Low density lipoproteins–cholesterol 11
2.5.4 High density lipoproteins–cholesterol 11
2.5.5 Triglyceride 12
2.5.6 Cholesterol 12
2.5.7 Bile acid 13
2.6 Insulin 14
2.6.1 Insulin definition and structure 14
2.6.2 Biological function of insulin 15
2.6.3 Hyperinsulinaemia and type II diabetes mellitus 16
3. MATERIALS AND METHODS 18
3.1 Equipments 18
3.2 Subjects and Sample Collection 18
3.2.1 Serum Collection 19
3.2.2 Gallbladder Stones Collection 19
3.3 Methods 19
3.3.1 Estimation of insulin 19
3.3.2 Estimation of glucose 20
3.3.3 Estimation of total cholesterol 20
3.3.4 Estimation of triglycerides 21
3.3.5 Estimation of HDL- C 22
3.3.6 Estimation of LDL- C 22
3.3.7 Estimation of VLDL 22
3.3.8 Homeostasis model assessment index (HOMA-IR) 23
3.4 Stone Analysis 23
3.5 Physical Examination 24
3.6 Statistical Analysis 24
4. RESULTS 25
4.1 Basic Physical Characteristic of Study Population 25
4.1.1 Gender 25
4.1.2 Age 25
4.1.3 Body Mass Index 25

4.2 Physical Criteria of Gallbladder Stones.....26
4.3 Chemical Composition of Gallstones.....26
4.4 Gender Comparison of Variables in GS and Control.....28
5. DISCUSSION.....31
6. CONCLUSIONS AND RECOMMENDATION.....39
REFERENCES.....41
CURRICULUM VITAE.....48

LIST OF SYMBOLS

%	Percent
±	Plus-minus
°C	Degrees Celsius
dL	Deciliter
g	Gram
L	Liter
mg	Milligram
mL	Milliliters
mmol	Millimoles
ng	Nanogram
nm	Nanometer
nmol	Nanomoles
µL	Microliter

LIST OF ABBREVIATIONS

BA	Bile acid
BC	Before christ
BMI	Body mass index
CBD	Common bile duct
CCK	Cholecystokinin
CNS	Central nervous system
Con	Concentration
CYP7A1	Cholesterol 7α-hydroxylase
D.W	Distilled Water
ELISA	Enzyme-linked immunosorbent assay
F	Female
FBS	Fasting blood sugar
GGT	Gamma glutamyl transpeptidase
GLUT	Glucose transporter
GS	Gallstone
GSD	Gall stone disease
HD	Heart disease
HDL	High density lipoprotein
HMG- CoA	3-hydroxy-3-methylglutaryl-coenzyme a reductase
HOMA-IR	Homeostasis model assessment index
IgA	Immunoglobulin A
IR	Insulin resistance
JAK-STAT	Janus kinase-signal transducer and activator of transcription
Kg	Kilogram
LDL	Low-density lipoprotein
M	Male
Na+	Sodium ion
nm	Nanometer
P-value	Probability value
RBC	Red blood cell
SD	Standard deviation
TC	Total cholesterol
TG	Triglyceride
U.S.A	United State of America.
VLDL	Very-low density lipoprotein
WHO	World health organization
α	Alpha
β	Beta

LIST OF FIGURES

Figure 2.1 Anatomy of biliary tract..... 4
Figure 2.2 A- Cholesterol synthesis. B- Enterohepatic circulation of cholesterol and bile acids..... 14
Figure 2.3 Insulin structure 15
Figure 4.1 Chemcal reaction of gallstone analysis (Cholesterol) 27
Figure 4.2 Chemical reaction of gallstone analysis (Bilirubin) 27
Figure 4.3 Types of gallstones 28

LIST OF TABLES

Table 2.1 Gallstone formation risk factors..... 9
Table 3.1 Basic clinical characteristic of study population 24
Table 4.1 Basic clinical characteristic of study population 25
Table 4.2 Characteristic GS Patients (Females & Males)..... 26
Table 4.3 Level of FBS & HOMA- IR GS patients (Females &Males)..... 29
Table 4.4 Level of Lipid Profile, GS Patients (Females & Males)..... 30
Table 4.5 Level of Lipid Profile, GS Patients (Females & Males)..... 30

1. INTRODUCTION

The most common biliary system illness, gallstone disease, continues to be a major global health concern. Between 20 and 30 percent of Westerners and about 10 percent of non-Westerners worldwide are affected. (Attili *et al.* 1995).

Anywhere along the biliary tree, including the gallbladder and common bile duct, is susceptible to gallstones (GS) (Selvi *et al.* 2011). The solid mass known as GS is created when the composition of bile changes or becomes unbalanced. The bulk of GS is composed of cholesterol, which is by far the most prevalent type, with the remainder being mixed and colored. The clinical range of cholelithiasis includes asymptomatic conditions and deadly consequences (Sakorafas *et al.* 2007). Gallstones are often asymptomatic in two-thirds of cases, and there is a 14% yearly risk of developing biliary discomfort (Bornman and Beckingham 2001). Gallbladder hypomotility, abnormally high cholesterol production, and the buildup of mucin gel are all factors in the development of cholesterol gallstones (Dowling 2000).

Genetic, hormonal, and metabolic factors can directly affect the metabolic and dynamic process of cholesterol and bile acids (BA), which is associated to the etiology of gallstone disease (Abu Eshy *et al.* 2007). Gallstones risk rises significantly with age, however the difference in risk is more noticeable when a person is younger (Shaffer 2006). But at all ages, it is higher in women than in males (Attili *et al.* 1995). The higher risk in young women is likely related to pregnancy and childbearing, and it is believed that hormonal variables are mostly to blame for this association. While higher serum progesterone levels may cause gallbladder stasis, an additional risk factor for gallstone formation, increasing serum estrogen levels during pregnancy encourage the release of bile that is cholesterol-supersaturated (Cirillo *et al.* 2005).

But at all ages, it is higher in women than in males (Attili *et al.* 1995). The higher risk in young women is likely related to pregnancy and childbearing, and it is believed that hormonal variables are mostly to blame for this association. While higher serum

progesterone levels may cause gallbladder stasis, an additional risk factor for gallstone formation, increasing serum estrogen levels during pregnancy encourage the release of bile that is cholesterol-supersaturated (Cirillo *et al.* 2005). Dietary composition and energy content both have a role in how diet affects insulin sensitivity. 2008; C.-J. Tsai *et al.* An altered glucose metabolism may increase the chance of developing cholelithiasis in some patients, according to research on the relationship between diabetes mellitus and cholelithiasis (De Santis *et al.* 1997). A higher body mass index (and its complication, metabolic syndrome), as well as some newly discovered obesity-related hormones, were linked to an increased incidence of gallstones (Sakorafas *et al.* 2007).

The most effective method for treating symptomatic cholelithiasis is laparoscopic cholecystectomy. With decreased morbidity and mortality, the procedure has been demonstrated to be superior to open cholecystectomy(Verma *et al.* 2002).

1.1 Aim of the Study

In this study, patients and healthy controls were compared to see how hyprinsulimnia affected the formation of gallstones in patients.

1.2 Objectives Study

1. To clarify the relationship between level of serum insulin and incidence of gallstone formation.
2. To illustrate the prevalence of dyslipidiemia and other disorder in patient with gallstone disease.
3. To analyze and study gallstone types.

2. LITERATURE REVIEW

2.1 Gallbladder

2.1.1 Historical review

Egyptian mummies from 1000 BC have gallstones in their gallbladders, according to research. They have long been recognized as happening in people. Gallstones were reportedly discovered in the gallbladders of dissected human bodies in the 16th century, according to Vesalius and Fallopius. These observations showed that the cholelithiasis phenomena was well understood, but Langenbuch (1882), who performed the first cholecystectomy in the 19th century, advanced knowledge of gallstone illness. Muhe invented the method, and in 1985 he applied it for the first time in Germany.

Gallstone prevalence and risk factors have been determined by surveys employing non-invasive ultrasonography. Gallstones are more common in some ethnic groups and geographical regions than others. One ailment that is frequent in affluent countries is gallstones, while it is less common in underprivileged populations that still follow traditional diets (Caroli-Bosc *et al.* 1998).

2.1.2 Anatomy and physiology of gallbladder

The hepatobiliary system is made up of the liver parenchymal cells, bile canaliculi, bile ducts, and gall bladder (Figure 2.1). The interlobular bile ducts, which join to create the left and right main hepatic ducts, collect the bile that the hepatic cells secrete into the biliary canaliculi of the lobules. The common bile duct is made up of the hepatic ducts and the gallbladder's cystic duct. The gallbladder is where the majority of bile is stored and concentrated (Novikoff 1959).

The concentration, storage, and transportation of hepatic bile to the duodenum are the main duties of the gallbladder. It is a pear-shaped sac that is 7 to 10 cm long and

contains 30 to 50 ml of fluid. On the inferior surface of the liver, there is an anatomical fosse that marks the division between the right and left liver lobes. The hormone cholecystokinin (CCK), which is created in the duodenal lumen by the breakdown products of fat and protein, is primarily responsible for the gallbladder's post-meal contraction.The gallbladder shrinks throughout all stages of digestion as a result of vagal activity.

A healthy liver produces 500–1000 ml of bile each day on average, with the majority of that volume being made up of water, electrolytes, bile salt, proteins, lipids, and bile pigments. Cholesterol is used in the liver to make bile acids (Lefebvre *et al.* 2009).

Bile acids are physiological degreasers required for dietary lipid and fat-soluble vitamin absorption. They are also essential for biliary excretion and endogenous metabolite removal. substances that cannot be eliminated by the kidney, including chemicals, heavily protein-bound heavy metal cations, and other xenobiotic substrates (Kullak-Ublick *et al.* 2004).

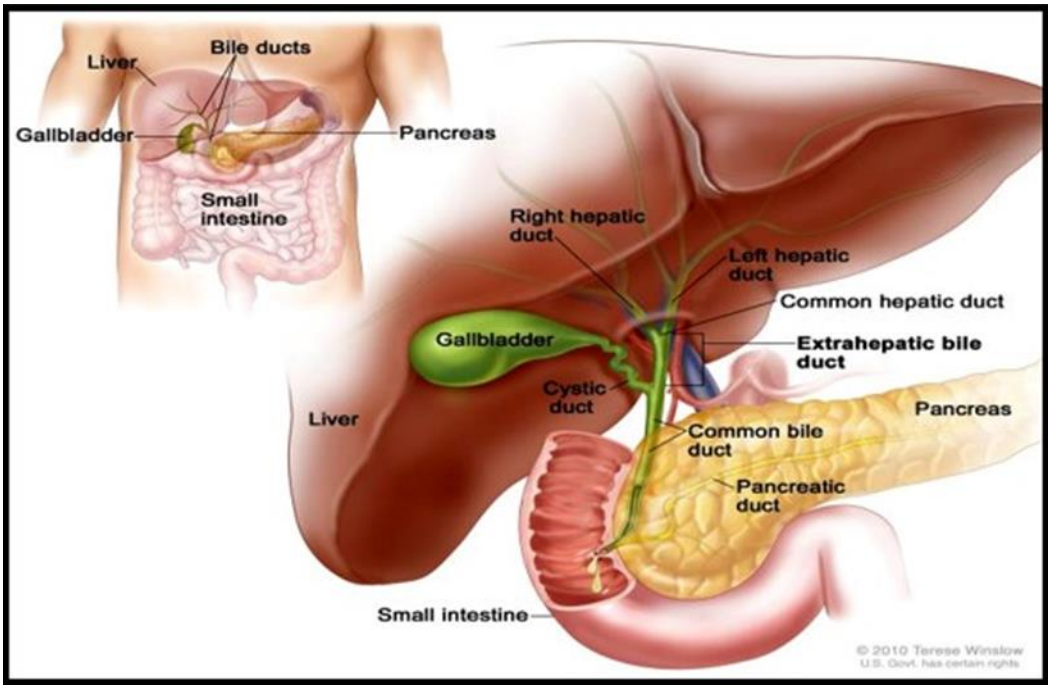

Figure 2.1 Anatomy of biliary tract (Kullak-Ublick *et al.* 2004)

2.1.3 Gall bladder Stones

Gallstone development is primarily caused by cholesterol, bile pigment, and calcium. Gallstones are made up of a complicated mixture of chemicals with a variety of chemical characteristics that is challenging to separate. A few trace metals like sodium, potassium, copper, magnesium, and iron as well as calcium salts of phosphates, carbonate, fatty acids, and phospholipids can also be found in gallstones. Along with the other elements described above, these chemicals are also present. These components are typically present in the sera and bile of kidney stone patients.

Gallstones can be as small as a grain of sand or as large as an inch, depending on how long they have been developing, and the pathological changes they cause can range from inflammation to malignancy (Verma *et al.* 2002).

2.1.4 Classification of gallstones

An optimal classification system is needed because the etiology, pathogenesis, clinical feature and treatment can be different according to the classes.In relation to the anatomical locations, stones may be found in gallbladder, intrahepatic duct (hepatolithiasis) or extrahepatic duct (choledocholithiasis).

In contrast to the more typical cholesterol gallstones observed in the West, hepatolithiasis is more frequently encountered in Asia, where the majority of these stones are composed of calcium bilirubinate. Due to dietary, socioeconomic, and demographic factors, the biliary calculi's chemical makeup and location vary around the globe and alter over time. Gallstones are categorized based on their chemical makeup (Kim *et al.* 2003).

2.2 Cholesterol Stone

consist of cholesterol, bile salts, bile pigments, calcium salts, and organic compounds containing glycoproteins. They can also be divided into two subtypes: pure (90–100% cholesterol) and mixed (50–90% cholesterol), which are produced when bile has an excessive amount of cholesterol but not enough bile salts. However, because they migrate from the gallbladder, cholesterol gallstones are the most prevalent and frequently accompanied by secondary common bile duct stones. A cholesterol stone discovered in the gallbladder is the initial gallstone (Kim *et al.* 2003).

2.2.1 Pigment stones

There are two sorts of stones that go by this name: black and brown; they both contain less than 30% cholesterol. Bilirubin, a calcium salt resembling calcium carbonate, and gallstones with between 10 and 20 percent pigmented gallstones make up the majority of the composition.

Although the pathophysiology of black and brown pigment stones is less understood than that of cholesterol gallstones, and each form of stone likely has a different pathogenesis, both types of pigment stones are colored as a result of anomalies in the metabolism of bilirubin. Similar to how individuals with cholesterol stones have their bile saturated with cholesterol, people with both types of pigment stones often have an excess of unconjugated bilirubin (Kim *et al.* 2003).

2.2.2 Black pigment stones

Black stones, which are primarily formed in the gall bladder, are more likely to develop in people with cirrhosis, biliary tract infections, genetic blood diseases like sickle cell anemia and cystic fibrosis, ileal Crohn's disease, or those who have had ileal resection (Vitek and Carey 2003).

2.2.3 Brown pigment stones

Other than calcium bilirubinate stones, brown stones have also been referred to as bilirubin stones, bile pigment calcium stones, earthy stones, and muddy stones. Brown stones are uncommon in the gallbladder and contain cholesterol, calcium bilirubinate, and calcium palmitate. They are caused by bile stasis and bile that has been contaminated with Escherichia coli. According to Williams and O'Connell (2008), the insoluble unconjugated bilirubinate precipitates after bacterial - glucuronidase deconjugates bilirubin diglucuronid (Williams and O'Connell 2008).

2.3 Pathogenesis of cholesterol gallstones

Cholesterol gallstones are formed by a number of processes, each of which is impacted by genetic and/or environmental factors. According to studies, neither the gallbladder nor the liver alone have a solely etiologic role in the development of calculi. Additionally, it seems that the liver and gallbladder's dynamic interplay results in the production of cholesterol calculi (Paumgartner and Sauerbruch 1991).

2.3.1 Cholesterol saturation

Under physiological circumstances, bile cholesterol is kept in solution by the incorporation of bile salts, phospholipids, and mixed micelles. Cholesterol leaves the solution and crystallizes when too many or not enough solubilizing bile salt and phospholipid molecules are released. This could lead to ailments that worsen the (Abu Eshy *et al.* 2007).

cholesterol in the bile is oversaturated. In addition to altered intestinal bile acid recycling, prolonged intestinal transit, and altered bile salt synthesis, disorders that increase hepatic cholesterol synthesis or absorption—which is likely obtained from lipoprotein rather than synthesis—could also be responsible for the supersaturation of bile (Bergheim *et al.* 2006).

2.3.2 Nucleation

The formation and agglomeration of crystals of cholesterol monohydrate is a process known as nucleation. Gallstone production still depends on the precipitation or nucleation of cholesterol microcrystals from supersaturated bile. The onset of nucleation and crystal formation is also influenced by the relative abundance of crystallization promoters and their relative insufficiency (Horton *et al.* 2003).

Human apolipoproteins A-I and IgA are antinucleating proteins, whereas immunoglobulins M and G, haptoglobin, 1-acid glycoprotein, aminopeptide, and mucin are pronucleating proteins. Although excessive gallbladder mucus secretion may encourage cholesterol crystallization, aspirin and nonsteroidal anti-inflammatory drugs prevent the formation of microcrystals and gallstones (Horton *et al.* 2003).

2.3.3 Gallbladder Hypomotility

Since expanded fasting gallbladder volume and decreased postprandial and interdigestive gallbladder emptying are frequent and unique symptoms in gallstone patients, the gallbladder is another important participant in the pathophysiology of gallstones in humans. Bile that is too saturated with cholesterol makes it easier for the gallbladder to absorb cholesterol and makes it easier for extra cholesterol to build up in the gallbladder wall. Gallbladder muscle function is inhibited by increased bile flow of cholesterol into these cells, which stiffens sarcolemmal membranes, decouples signal transduction, and stiffens sarcolemmal membranes (Bajwa *et al.* 2010).

2.4 Risk Factors of Gallstone Formation Disease

There are several known risk factors for developing gallbladder stones, as shown in the (Table 2.1). Environmental variables and genetic predisposition undoubtedly interact to cause gallstone formation, while some risk factors for gallstone formation may not be changeable (Abu Eshy *et al.* 2007).

Table 2.1 Gallstone formation risk factors

Age	**Increasing age**
Body routines	Rapid weight reduction, insufficient exercise, and obesity
Child bearing	Pregnancy
Drugs	Clofibrate, Ceftriaxone, Oral contraceptive, Octreotide
Ethnicity	Scandinavians and Pima Indians.
Genetics and ancestry	Gallstones run in the maternal family
Gender	Female
Ilea and other metabolic diseases	High triglycerides, diabetic mellitus, chronic hemolysis, alcoholic cirrhosis, biliary infection, Ilea disease (Crohn disease), resection or by pass
Hyperalimentation	Total parenteral nutrition, Prolonged fasting

2.4.1 Clinical Presentation

Asymptomatic gallstones, symptomatic gallstones, and consequences from gallstones are the three subtypes of gallstone disease. At the time of diagnosis, 60% to 70% of stones are asymptomatic; 1% to 4% of these start to exhibit symptoms every year. Within five and twenty years of diagnosis, respectively, ten and twenty percent of patients will eventually start displaying symptoms. Gallstone infections are characterized by right upper abdominal pain, fever, jaundice, feeling full after eating, clay-colored feces, nausea, and vomiting. Biliary colic, acute cholecystitis, chronic cholecystitis, choledocholithiasis, acute gallstone pancreatitis, gallstone ileus, biliary cirrhosis, and gallbladder malignancy are among the most prevalent clinical diseases brought on by gallstones (Kalloo and Kantsevoy 2001).

2.4.2 Diagnosis and Treatment

Biliary colic is the gallstone disease's most typical manifestation. The liver function tests should also be evaluated, but ultrasonography (US) is the ultimate inquiry for gallstone disease, particularly gallbladder stones. It is a precise and noninvasive approach with a sensitivity/specificity of better than 95%. Common bile duct (CBD)

stones might be detected if the liver's chemistry is abnormal or if the biliary tree is dilated in the United States.

Additionally, surgeons have access to a variety of operational methods for cholecystectomy. The laparoscopic surgery, the short incision mini-cholecystectomy, and the traditional open cholecystectomy are the three procedures that are still often used. The gold standard therapy of choice for symptomatic gallstones is laparoscopic cholecystectomy.

2.5 Lipids and Lipoproteins

Organic substances that are weakly soluble in water yet miscible in organic solvents are known as lipids. Plasma contains a variety of lipid types (triglyceride, phospholipid, cholesterol, cholesterol ester). Lipids are carried by lipoproteins because they are largely insoluble in bodily fluid (Attili *et al.* 1995).

Triglycerides and cholesterol esters, which are non-polar neutral lipids, make up the core of lipoproteins, which are complicated plasma lipid transporters. At the surface of lipoproteins are the more polar amphipathic lipids (free cholesterol and phospholipids). On their surface, they also have one or more specialized proteins called apolipoproteins. Size (by gel filteration), density (by ultra centerfugation), and net surface charge (by electrophoresis) are three factors that can be used to differentiate different classes (Kalloo and Kantsevoy 2001).

2.5.1 Chylomicrons

Chylomicrons, the largest lipoprotein particles, carry dietary cholesterol and triglycerides. They are produced by the enterocyte and secreted into the lymphatic system before entering the bloodstream. Lipoprotein lipase rapidly hydrolyzes chylomicron triglycerides in plasma. The LDL-receptor-related protein is used by the

liver to remove the chylomicron remnant, or residual triglyceride-depleted particle (LRP) (Kalloo and Kantsevoy 2001).

2.5.2 Very low density lipoproteins

VLDL particles, which are produced by the liver and contain endogenous triglycerides (55%–65%), cholesterol, phospholipids, and apolipoproteins, are secreted (B100 as well as C and E).

The triglycerides of VLDL particles are hydrolyzed in plasma by lipoprotein lipase. As VLDL eventually lose their triglyceride content, a portion of the surface, made up of phospholipids and apolipoproteins C and E, is transferred to HDL. As a result of this metabolic process, IDL particles (VLDL remnant) are produced, and they are either removed from circulation by liver receptors or go through additional metabolization to become LDL (Smith *et al.* 2002).

2.5.3 Low density lipoproteins–cholesterol

By eliminating the triglycerides from liver-derived (VLDL) particles through intravascular lipolytic processing, which is facilitated by several enzymes, LDL-C is produced (The LDL precursors). In actuality, LDL is the primary carrier of cholesterol in humans and is in charge of feeding the tissues with the highest sterol requirements. Additionally, LDLs are the lipoproteins that have been most directly linked to the development of atherogenic plaque. People who ingest a lot of saturated fat and/or cholesterol may have higher amounts of circulating LDL. One apo B molecule can be found inside of each LDL particle (Lei *et al.* 2008).

2.5.4 High density lipoproteins–cholesterol

By using a variety of enzymes to remove the triglycerides from liver-derived (VLDL) particles, a process known as intravascular lipolytic processing produces LDL-C. (The

LDL precursors). Actually, LDL is the principal sterol carrier in humans, providing sterol to tissues with the highest sterol requirements. LDLs are also the lipoproteins that have been shown to be most directly responsible for the development of atherogenic plaque. People who consume a lot of saturated fat and/or cholesterol may see an increase in their circulating LDL levels. One apo B molecule is present in each LDL particle (Laakso *et al.* 1990).

2.5.5 Triglyceride

Triglycerides are a kind of fatty acids that are used for storage and transportation (TG). They are mostly present in adipocytes and are composed of three fatty acid molecules linked to a glycerol backbone. Lipolysis is the process by which triglycerides are converted into free fatty acids and mono-acylglycerols (Nagle *et al.* 2009).

2.5.6 Cholesterol

As a component of cell membranes and as a necessary precursor to numerous other compounds, including vitamins, steroid hormones, and bile acids, cholesterol is an integral constituent of all tissues. Cells produce cholesterol, and when we eat, we also absorb it.

The homeostasis of cholesterol, which is meticulously regulated by a multitude of feedback systems, is crucially maintained by the liver. Molecules that reach the liver through lipoprotein receptors include low density lipoproteins and chylomicrons (LDL). The most major enzyme in the liver's involvement in the de novo production of cholesterol from acetyl coenzyme A is 3-hydroxy-3 methyl glutaryl CoA reductase (HMG- CoA), which is necessary for the entire process (Figure 2.2).

When an organism is functioning normally, the intake and output of cholesterol are balanced and coordinated with the goal of ensuring that enough cholesterol is available to meet the requirements of the various tissues. Pathological processes including

hyperlipidemia, atherosclerosis, and bile stones are caused by an imbalance between these mechanisms, which occurs under pathological conditions and raises the amounts of cholesterol in the blood (Caterina *et al.* 2001).

2.5.7 Bile acid

The two most effective ways to remove body cholesterol, according to general agreement, are the liver's biliary release of the substance and conversion of the substance to bile acids. However, the liver alone is responsible for eliminating cholesterol since it converts cholesterol to bile acids. This biotransformation converts a water-insoluble cholesterol molecule into a water-soluble bile acid molecule (Figure 2.2).

Several different enzymatic processes are used to produce bile acids. The cholesterol 7 - hydroxylase enzyme, which is encoded by the CYP7A1 gene, catalyzes the early stage of both the catabolism of cholesterol and the production of bile acids.

Either the neutral (traditional) method or the acidic (alternative) pathway can be used to produce BAs. The two main bile salts in humans, cholate and chenodeoxycholate, are "primary" species produced in the liver from cholesterol. Deoxycholate and lithocholate, referred to as the "secondary" bile salts, are modifications of the primary bile salts produced by specialist anaerobic gut bacteria. Both of these substances circulate continuously in humans through an exceptionally effective enterohepatic pathway. The basic BA synthesis can be broken down into several steps using the following diagram: 1)initiation, which typically involves a precursor sterol's 7-alpha hydroxylation. 2) further ring structural alterations. 3) Side chain oxidation and shortening. 4) Typically, the amino acid to which the BA is conjugated is turin or glycine (Lefebvre *et al.* 2009).

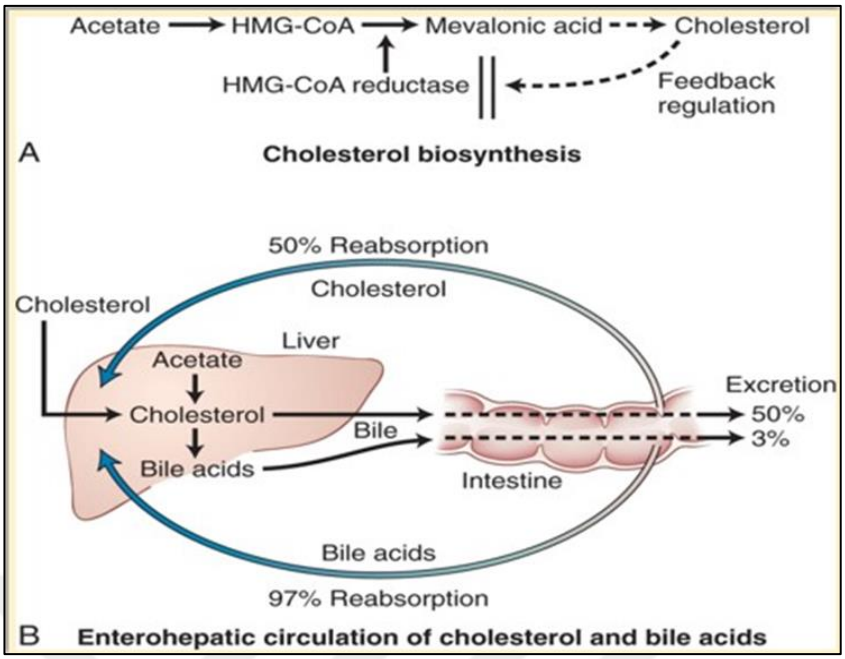

Figure 2.2 A- Cholesterol synthesis. B- Enterohepatic circulation of cholesterol and bile acids (Lefebvre *et al.* 2009)

2.6 Insulin

2.6.1 Insulin definition and structure

The insulin gene, which is found on human chromosome 11, is used to make the hormone insulin, which is synthesized as a prohormone by the islets of Langerhans of the pancreas. Chain A and Chain B, two polypeptide chains that are connected by the C-peptide chain, make up the structure of proinsulin. When C-peptide is broken down by enzymes, the chains A (which have 21 amino acid residues) and B chain are left behind (with 30 aminoacide residues). C-peptide levels in urine and serum are helpful markers of beta-cell health (Figure 2.3). Insulin is normally released into the bloodstream continuously in modest pulsatile increments (a basal rate), with an enhanced release (a bolus) after ingesting meals.

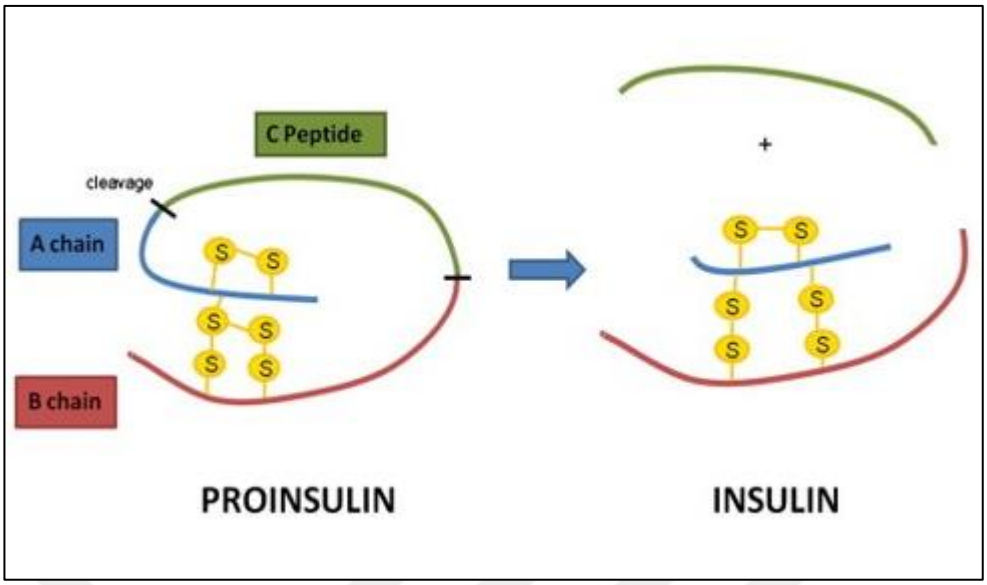

Figure 2.3 Insulin structure (Friedman 2002)

2.6.2 Biological function of insulin

The anabolic hormone insulin has a profound impact on the metabolism of protein, fat, and carbohydrates. Actually, when blood glucose levels rise, pancreatic cells generate and release insulin into the portal circulation, where it rises quickly. By inhibiting hepatic synthesis and promoting glucose absorption in skeletal muscle and fat, insulin lowers blood sugar via the glucose transporter. Blood glucose levels are lowered by the action of released insulin, helping to maintain a stable, normal blood sugar range of 70 to 120 mg/dL (3.9 to 6.66 mmol/L). An average adult secretes between 40 and 50 U per day, or 0.6 U per kilogram of body weight.

Sterol response element-binding proteins (SREBPs) regulate the expression of over 30 genes, which in turn regulates the synthesis and absorption of fatty acids, triglycerides, cholesterol, and phospholipids. A rise in plasma insulin levels after eating stimulates protein synthesis, slows the gluconeogenic process, encourages the creation of fat in adipose tissue, and makes it easier to store glucose as glycogen in the muscles and liver. Additionally, it stops the gluconeogenic process from taking place. Additionally, during a typical overnight fast, the decline in insulin levels promotes the release of fat, protein,

and glucose from muscle, the liver, and adipose tissue. As a result, insulin has an effect on numerous metabolic processes and organ systems.

However, insulin also affects the gastrointestinal tract, kidneys, heart, pancreas, vasculature, central nervous system, and kidneys. The traditional "big three" tissues of the liver, adipose tissue, and skeletal muscle are known to be affected by signaling through the insulin receptor with regard to metabolism. Insulin signaling, which also influences appetite, hydration and electrolyte balance, lipoprotein metabolism, and vascular tone, affects the metabolism of all three dietary macronutrients.

Regulation of insulin activity affects the integration of processes other than just glucose metabolism. This observation has the consequence that certain chronic diseases have a causal relationship with dysregulation in insulin production or insulin activity (Friedman 2002).

2.6.3 Hyperinsulinaemia and type II diabetes mellitus

Insulin resistance is thought to be indicated by hyperinsulinemia. Reduced sensitivity or response to the metabolic effects of insulin is known as insulin resistance (IR). It is crucial in the pathophysiology of conditions like diabetes mellitus (DM), dyslipidemia, obesity, hypertension, and coronary artery disease. Additionally, IR is a characteristic of numerous syndromes connected to abnormal reproductive endocrinology, including polycystic ovarian syndrome and premature adrenarche.

Type II diabetes mellitus is described as a complex metabolic condition with variable etiology with susceptibility effects being masked by social, behavioral, and environmental risk factors. It is a widespread illness that, as a result of its complications, is linked to high morbidity and early mortality. It is a significant burden on both individuals and society.

Insulin resistance can be detected by the insulin suppression test, hyperinsulinemic euglycemic clamp test, Homeostasis Model Assessment for Insulin Resistance (HOMA-IR), and Quantitative Insulin-Sensitivity Check Index (QUICKI).

A disturbance in the metabolism of fat is intimately related to insulin resistance. There are many factors that can contribute to an imbalance in lipid metabolism, including high dietary cholesterol/fat, obesity, diabetes, and genetics. Dyslipidaemia, characterized by increased triacylglycerol and decreased HDL cholesterol, is one of its symptoms. Furthermore, it is well known that the establishment of insulin resistance is influenced by a rise in the availability of free fatty acids. Fat may accumulate in insulin target tissues like the liver and skeletal muscle as a result of an increased fatty acid supply. This "ectopic" fat storage and the onset of lipotoxicity, commonly known as insulin resistance, are closely associated (Bickerton *et al.* 2008).

3. MATERIALS AND METHODS

3.1 Equipments

- Spectrophotometer-CECIL –England.
- Incubator-Memmert- Germany.
- Centrifuge-Kokusan – Japan.
- Automatic pipette-Slamed.
- ELISA Microplate washer-Biotek- U.S.A.
- ELISA Microplate reader-Biotek-U.S.A.
- Auto-analyzer-Architect/plus-USA.

3.2 Subjects and Sample Collection

Blood samples were taken by Biotek-U.S.A. from 140 cholelithiasis patients who were being treated for cholecystectomy at the surgical ward of the Azady Teaching Hospital in Kirkuk City between April 2022 and the end of September 2022. The age range of the participants in this study for both gender factors—male (M) and female (F)—is 20 to 65 years, and they are chosen based on the study questionnaire (appendix A).

The serum lipid profiles (total cholesterol, TG, HDL-C, LDL-C, VLDL) of the patients and controls as well as glucose were assessed. These samples were divided into many diagnostic categories, such as:-

- Group One: GS patients group, included 76 (F) and 24 (M).

- Group Tow:- Healthy included 30 (F) and 10 (M).

Note: Asymptomatic participants without gallstones or prior cholecystectomy were included in all control groups. Age, gender, and BMI (Body Mass Index) were used to match asymptomatic people with cases.

3.2.1 Serum Collection

A sterile disposable syringe was used to draw 10 ml of venous blood from each participant after they had fasted for eight to twelve hours. The blood was then placed in a simple tube and allowed to coagulate at room temperature. After 15 minutes of centrifugation at 3000 rpm, the serum was aspirated, split into aliquots, and kept at -20 C until the time of estimation.

3.2.2 Gallbladder Stones Collection

Stones were collected from patients after surgical removal of gallbladder. Each one was given a serial number, they were washed with D.W. 2-3 times until washing became clear, stones were light protected and air dried. The different physical characteristics of the stones, including their quantity, variety, size, texture, and cross-section, were observed.

3.3 Methods

3.3.1 Estimation of insulin

A solid phase (ELISA) based on the sandwich concept is the DRG Insulin ELISA Kit. A monoclonal antibody that is specific to a particular antigenic location on the Insulin molecule coats the microtiter wells. An enzyme conjugate, which is an anti-Insulin

antibody conjugated with biotin, is incubated in the coated well with a patient sample aliquot that contains endogenous Insulin. The unbound conjugate is removed after incubation. The streptavidin peroxidase enzyme complex binds the biotin-anti-Insulin antibody during the second incubation stage. When the substrate solution is applied, the amount of color that results is related to how much insulin is present in the patient sample (details are found in Appendix B).

3.3.2 Estimation of glucose

According to the advice of the business Randox GOD/PAP-U.S.A., fasting blood sugar (FBS) was calculated using the enzymatic method. The following reaction serves as the basis for the method Equation 3.1 and Equation 3.2:

Glucose Oxidase

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{Peroxidase}} \text{gluconic acid} + H_2O_2 \quad (3.1)$$

$$2H_2O_2 + \text{4-Aminophenazone} + \text{Phenol} \rightarrow \text{Quinoneimine} + 4H_2O \quad (3.2)$$

The intensity of the pink to red color that appears from measuring glucose concentration colorimetrically at 500 nm is proportionate to that amount(details are found in Appendix C).

3.3.3 Estimation of total cholesterol

Although measurements of the latter are more frequently employed, cholesterol estimation is based either on their free fatty acid content or their cholesterol level. These are determined enzymematically by Biolabo kit CHOD/PAP - France. The following reaction serves as the method's foundation Equation 3.3, Equation 3.4 and Equation 3.5:

Cholesterol esterase:

$$\text{Esters of cholesterol} \rightarrow \text{Cholesterol} + \text{Free fatty acids} \quad (3.3)$$

Cholesterol oxydase

$$\text{Cholesterol} + O_2 \rightarrow \text{Cholesten 4 -1- 3} + H_2O_2 \quad (3.4)$$

Peroxidase:

$$2H_2O_2 + \text{Phenol} + \text{4- Amino} \rightarrow \text{Antipyrine Quinoneimine (pink)} + 4\ H_2O \quad (3.5)$$

The intensity of the pink to red hue that is produced when cholesterol content is determined colorimetrically at 500 nm is proportional to that value(details are found in appendix D).

3.3.4 Estimation of triglycerides

Although measurements of the latter are more commonly employed, estimating triglycerides is based on on their fatty acid content or their glycerol level. These measurements are made enzymematically by (Biolabo kit GPO-France). The following reaction serves as the basis for the method Equation 3.6, Equation 3.7, Equation 3.8 and Equation 3.9:

Lipase

$$\text{Triglycerides} \rightarrow \text{Glycerol} + \text{Fatty acid} \quad (3.6)$$

Glycerol kinase:

Glycerol + ATP → Glycerol-3-Phosphate + ADP (3.7)

Oxidase:

$$\text{Glycerol-3-phosphate} + O_2 \rightarrow \text{DihydroxyacetonePhosphate} + H_2O_2 \quad (3.8)$$

Peroxidase:

$$2H_2O_2 + \text{4-Chlorophenol} + \text{4-amino anti pyrine} \rightarrow \text{Quinoneimine} + 4H_2O \quad (3.9)$$

At 500 nm, the colored complex's absorbance is measured. This absorbance is proportional to the specimen's triglyceride content (details are found in Appendix E).

3.3.5 Estimation of HDL- C

Phosphatugsticacids and magnesium chloride are added to the sample to precipitate the chylomicron and lipoproteins (VLDL-C) and (LDL-C) that are present. After centrifugation, total cholesterol reagent is used to test the HDL-C detected in the supernatant using the Biolabo kit HDL-Cholesterol -PTA (details are found in Appendix F).

3.3.6 Estimation of LDL- C

Utilizing the following formula (85) to determine LDL-C: Total cholesterol - HDL cholesterol - Triglyceride/2.2 = LDL-C (mmol/L).

3.3.7 Estimation of VLDL

The following formula (8) was used to determine VLDL-C: Triglyceride/2.2 = VLDL-C (mmol/L)

3.3.8 Homeostasis model assessment index (HOMA-IR)

HOMA-IR was calculated using the following equation (181): [fasting insulin (U/mL) X fasting glucose (mmol/L)] X 22 .523].

3.4 Stone Analysis

Depending on the kind of chemical ingredient that needed to be studied, the stones were ground into a powder in a pestle and mortar and then dissolved in various solvents. Gallstones' organic components, such as cholesterol and bilirubin, are quantitatively analyzed using an enzymatic colorimetric approach (commercial kit from Biolabo). (Wentrup-Byrne *et al.* 1997) .

Reagents:

[Chlorophorm (CHCL3) M=119.38 g/mol, Cholesterol kit, Bilirubin kit (total & direct bilirubin)].

Procedure:

To determine TC & bilirubin:

- In test tube, 30 mg of stone powder was dissolved in 3 cc of chloroform.
- The tube was heated for two minutes in a pot of boiling water.
- The subsequently obtained stone solution was used to calculate the total cholesterol and total bilirubin. Appendices D and G contain instructions for measuring bilirubin and cholesterol, respectively.

- The dissolved stone solutions were maintained at 2 to 8 degrees Celsius when not in use.

3.5 Physical Examination

The work contained data on the patient's height, weight, and measured BMI (weight in kilograms divided by height in meters squared). Obesity is measured using BMI, the WHO, and the global obesity task force classifications Table 3.1 (Field *et al.* 2001). (Appendix H).

Table 3.1 Basic clinical characteristic of study population

BMI(kg/m_2)	Classification *	Risk of co-morbid obesity
18.6-24.8	Conventional range	Negligible
25.1 – 29.7	Overweight	Mildly increased
>30.0	Obese	Moderate
30.0 – 34.8	Class I	Severe
35.0 – 39.8	Class II	Very Severe
>40.0	Class III	

*Classification of the World Health Organization (WHO) and International Obesity Task Force.

3.6 Statistical Analysis

The statistical application for social research SPSS for Windows, version 20.0, was used to determine the mean and standard deviation. The independent t test was used to compare the independent variables. The threshold for statistical significance was a P value of 0.05 or lower.

4. RESULTS

4.1 Basic Physical Characteristic of Study Population

4.1.1 Gender

76 of the 100 patients with gallstones were female, and 24 were male. There were 3.1 more females than males, Table 4.1.

4.1.2 Age

Four age groups of gallstone patients and controls were created. The age group under 50 years old has the highest gallstone prevalence, Table 4.1.

4.1.3 Body Mass Index

Gallstones were common in subjects with BMI ≥ 30 kg/m^2, Table 4.1.

Table 4.1 Basic clinical characteristic of study population

Variable		**Patients with GS**		**Control**	
		No=100	%	No=40	%
Gender	Males	24	24	10	25
	Females	76	76	30	75
Age	20-29	10	10	4	10
	30-39	13	13	6	15
	40-49	26	26	11	27.5
	≥ 50	51	51	19	47.5
BMI kg/m^2	18.5-24.9	19	19	24	60
	25-29.9	30	30	16	40
	≥ 30	51	51	/	/
Cholesterol stones (79)	Females	42	42	/	/
	Males	13	13	/	/
Pigment stones (5)	Females	3	3	/	/
	Males	1	1	/	/
Mixed stones (56)	Females	31	31	/	/
	Males	10	10	/	/

4.2 Physical Criteria of Gallbladder Stones

The gallstones varied in their size (Plate 1.1). The weight of gallstones ranged from 0.3 gm to 6 gm. The cholesterol gallstones were varied in color from yellowish white to light brown and were either single or multiple. Solitary stones are usually round or mulberry, and may be larger than 2.5 cm in diameter (Plate 1.2), when multiple stones were faceted and smooth plate (Plate 1.3).On cross section they are crystalline or laminated with a distinct dark nucleus (Plate1.5).

In (plate 1.1), pigment stones are depicted; nearly all of these stones are of the black pigment kind, which is numerous, tiny, and black. The mixed stones are typically numerous (Plate 1.4), faceted, and come in shades of yellow and dark brown. Occasionally, they also have sections that are cut with black shell and lamina (Plate 1.6)

4.3 Chemical Composition of Gallstones

The chemical make-up of the gallstones that were extracted from the study's participants is displayed in Table 4.2, Figure 4.1, Figure 4.2 and Figure 4.3.

Table 4.2 Concentrations of metabolites in the different types of gallstones.

Stone type	**Cholesterol (mg/gm)**	**Bilirubin (mg/gm)**
Cholesterol stone	485.10 ± 33.11	0.8 ± 0.64
Mixed stone	329.8 ± 25.0	1.1 ± 0.56
Pigment stone	240.29. ± 13.17	1.82 ± 0.83

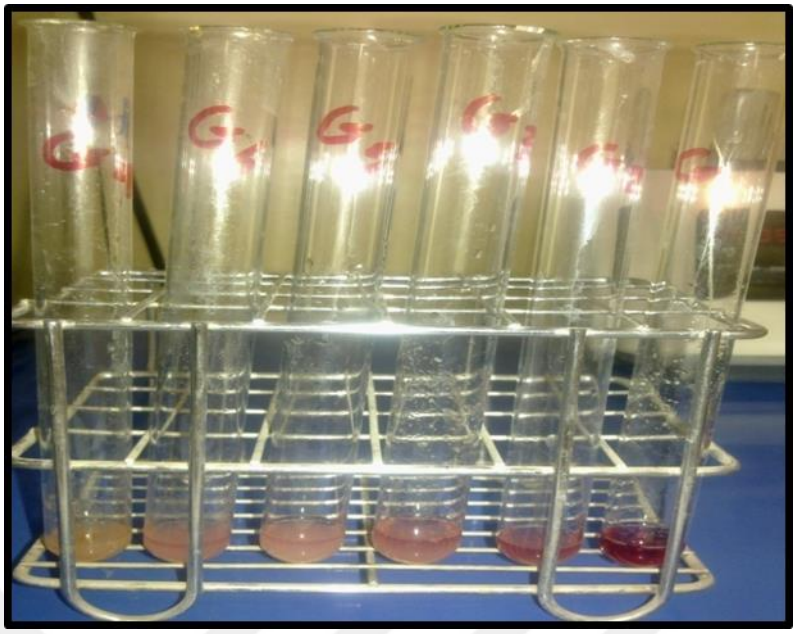

Figure 4.1 Chemcal reaction of gallstone analysis (Cholesterol)

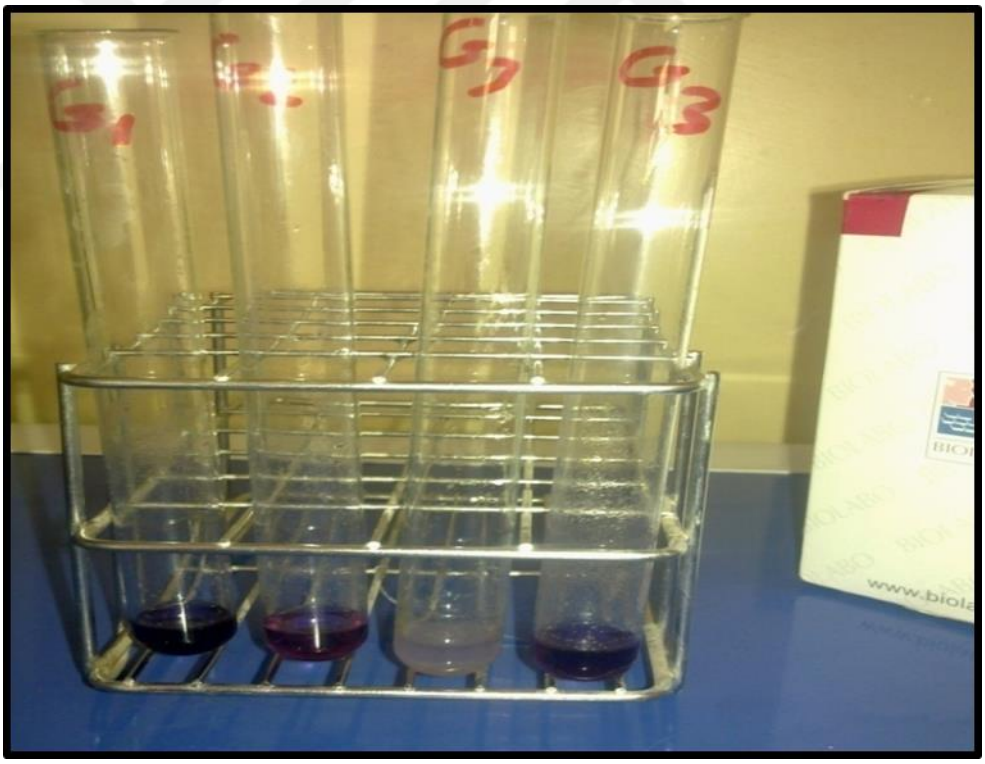

Figure 4.2 Chemical reaction of gallstone analysis (Bilirubin)

Figure 4.3 Types of gallstones

4.4 Gender Comparison of Variables in GS and Control

Age, mean, and SD were significantly different (p= 0.001) between female GS patients (42.23 10.66 years) and female controls (34.60 8.20 years), but not significantly different (p= 0.025) between male GS patients (43.91 13.56 years) and male controls (35.80 10.90 years).

In addition there was a significant (p= 0.000) difference in BMI ,mean± SD in females GS patients (28.42 ± 3.10 kg/m2) and females control (24.92 ± 0.75 kg/m2), and there was a significant (p= 0.001) difference, mean ±SD in males GS patients (26.71 ± 3.33 kg/m2) and males control(24.26 ± 1.14 kg/m2), Table 4.3.

In FBS, there was a significant difference in mean and SD between female GS patients (4.34 0.65 mmol/L) and female controls (3.88 0.35 mmol/L) and between male GS patients (4.12 0.59 mmol/L) and male controls (3.52 0.25 mmol/L), which was significant (p=0.001). In HOMA-IR, there was a significant (p= 0.001) difference in mean and SD between female patients with GS (2.11 1.47) and the control group (1.07 0.35), and between male patients with GS (1.80 0.807) and the control group (0.91 0.18), Table 4.4.

TC level in females GS patients was (4.72 ± 0.88 mmol/L versus 3.5 ± 0.66 mmol/L in females control ; p= 0.001),while in males GS patients (4.50 ± 0.74 mmol/L versus 3.59 ± 0.07 mmol/L in control; p= 0.001). In TG mean level in females GS patients was (1.19 ± 0.53 mmol/L versus 0.82 ± 0.52 mmol/L in control ; p= 0.002),while in males GS patients mean level was (1.34 ± 0.39 mmol/L versus o.78 ± 0.38 mmol/L in control ; p= 0.000).

HDL-C level in females GS patients was (1.30 ± 0.48 mmol/L versus 1.32 ± 0.17 mmol/L in control ; p= 0.863),while in males GS patients was (1.10 ± 0.20 mmol/L versus 1.09 ± 0.26 mmol/L in control ; p= 0.858), in LDL-C mean level in females GS patients (2.89 ± 0.93 mmol/L versus 1.86 ± 0.57 mmol/L in control ; p= 0.000),while in males GS patients mean level(2.75 ± 0.79 mmol/L versus 2.33 ± 0.68 mmol/L in control ; p= 0.049), in VLDL mean level in females GS patients (0.49 ± 0.23 mmol/L versus 0.32 ± 0.24 mmol/L in control;p= 0.002),while in males GS patients mean level(0.60 ± 0.18 mmol/L versus 0.16 ± 0.50 mmol/L in control ; p= 0.001), Table 4.5.

Table 4.3 Characteristic GS Patients (Females & Males)

Variable	Females (Mean± SD)		P value	Males (Mean ± SD)		P value
	GS patients	Control		GS patients	Control	
Age	42.23 ±10.66	34.60±8.20	**0.001**	43.91±13.56	35.80 ±10.9	**0.025**
BMI kg/m^2	28.42 ± 3.10	24.92±0.75	**0.001**	26.71 ± 3.33	24.26 ±1.14	**0.00**

Table 4.4 Level of FBS & HOMA- IR GS patients (Females &Males)

Variable	Females (Mean ± SD)		P value	Males (Mean ± SD)		P value
	GS patients	Control		GS patients	Control	
FBS mmol/L	4.34 ± 0.65	3.88 ± 0.35	0.001	4.12 ± 0.59	3.52 ± 0.25	0.001
HOMA-IR	2.11 ± 1.47	1.07 ± 0.35	0.001	1.80 ± 0.80	0.91 ± 0.18	0.001
Insulin μIU/mL	10.16±6.18	6.40 ± 1.65	0.003	9.66 ± 4.17	5.98 ±1.2	0.001

Table 4.5 Level of Lipid Profile, GS Patients (Females & Males)

Variable	Females (Mean ±SD)		P value	Males (Mean± SD)		P value
	GS patients	Control		GS patients	Control	
TC mmol/L	4.72 ± 0.88	3.5 ± 0.66	0.001	4.50 ± 0.745	3.59 ± 0.07	0.001
TG mmol/L	1.19 ± 0.53	0.82 ± 0.52	0.002	1.34 ± 0.394	0.78 ± 0.38	0.000
HDL-C mmol/L	1.30 ± 0.48	1.32 ± 0.17	0.863	1.10 ± 0.206	1.09 ± 0.26	0.858
LDL-C mmol/L	2.89 ± 0.93	1.86 ± 0.57	0.000	2.75 ± 0.795	2.33 ± 0.68	0.049
VLDL mmol/L	0.49 ± 0.23	0.32 ± 0.24	0.002	0.60 ± 0.188	0.16 ± 0.50	0.001

5. DISCUSSION

There were both single and many stones that met the physical criteria for gallstones in this investigation. Compared to pigment and mixed stones, pure cholesterol stones were solitary and larger in size. The nidus for the growth of the cholesterol stone is the nucleated cholesterol monohydrate crystal, and over the course of several years, recurrent cholesterol deposition on the nidus causes the stone to increase. (Wentrup-Byrne *et al.* 1997) A sudden crystallization event is necessary for the production of numerous stones, and nucleation flaws have been hypothesized to grow from free-floating crystal plates in a subgroup of individuals with multiple gallstones. Despite the fact that nucleation did occur more quickly in patients with several gallstones than in those with a single gallstone, Jungst *et al.* discovered that this was not because of increased cholesterol saturation (Jüngst *et al.* 1992).

Understanding the chemical composition of a gallstone helps identify risk factors that make some persons more vulnerable to the development of calculi and gives vital evidence for the cause, etiology, and metabolic foundation of a gallstone's production. (Gustafsson *et al.* 2000).

The three forms of gallstones that can form in the gallbladder are cholesterol, pigmented, and mixed gallstones based on their chemical composition. In the current study, the main component of cholesterol gallstones was studied. The most prevalent component was cholesterol, which was shown to contain (485.10 33.11 mg/gm) for cholesterol stone, (329.8 55.0 mg/gm) for mixed stone, and (285.36 36.01 mg/gm) for pigment (Shareef *et al.* 2009).

Gallstones are primarily composed of cholesterol, according to literature from Western nations like Germany and several Scandinavian countries, however research from Africa and China has documented a diversity of gallstone compositions. The variations in dietary habits of the people living in these two countries as well as metabolic anomalies of cholesterol metabolism may be to blame for the differences.The cholesterol saturation index is higher than 1 between cholesterol and bile salts. The

discovery that cholesterol stones had the highest cholesterol content supports the theory that the primary cause of these types of stones is cholesterol supersaturation in the bile, which crystallizes as a stone (Schafmayer *et al.* 2006).

In contrast, this study found that mixed stones contained more cholesterol than pigment stones but less cholesterol than cholesterol stones. This may be because these stones contain both cholesterol and bile pigment. This study found that while bilirubin level was highest in pigment stone, it had the lowest cholesterol content among the three types of stones. Numerous past investigations have proven this (Ostrow 1984).

Because the conjugated form of bilirubin is water soluble and eliminated through the bile, the bulk of the bilirubin in these stones is unconjugated. The key contributing causes are infection of the biliary system, an increase in unconjugated bilirubin secretion into bile, and a decrease in biliary acid secretion. Age is a common factor in the development of GSD in all ethnic groups. This is consistent with the findings of the study. From 21 to 80 years of age, there are age-related increases in incidence, and female prevalence is higher than male prevalence(Gokulakrishnan *et al.* 2001) Gallstone disease rarely affects newborns and children before the age of 20; the most prevalent stones are pigment stones, which are linked to hemolysis or long-term conditions including cystic fibrosis, thalassemia major, and sickle cell anemia (Bajwa *et al.* 2010). In this study, 44.28 % of the patients have a life expectancy of less than 50 years.

These findings are in accordance with those reported by Selvi RT et al that, the mean age of cholelithiasis presentation was 45.90 years, while Maskey CP et al discovered that the most common age group for cholelithiasis was below 30 years containing 37.5%. In a study from Brazil, The majority of patients (51%), who were between the ages of 41 and 60, were over 60 when they first presented. A study conducted in Taiwan by Chen CY *et al.* demonstrated that gallstone development was directly related to aging simply because of prolonged exposure to other risk factors, regardless of location or way of living. In elderly participants compared to younger subjects, gallstones occur 4–10 times more frequently. Because cholesterol 7-hydroxylase, the rate-limiting enzyme for bile acid production, becomes less active with age, biliary cholesterol

saturation rises. It is true that bile acid synthesis declines, biliary cholesterol output rises, bile cholesterol saturation rises, and gallbladder hypomotility occurs in older people, both men and women (Paumgartner *et al.* 1989). From a biological standpoint, it is more likely that HMG Co-A reductase, the enzyme that limits the creation of cholesterol, will exist as people age. As a result, the liver will secrete more cholesterol, which will increase the amount of cholesterol in the bile (Einarsson *et al.* 1985).

In general, gender is a major risk factor for gallstone development. In comparison to men, women are more than twice as likely to have gallstone disease. Gallstone prevalence was found to be 22.9% in males and 77.1% in women in the current study. Women are more likely than men to have gallstone disease at all ages, with a ratio of 3.4:1 between the sexes. These matched earlier studies with an extremely low female-to-male ratio of 1.2:1, 10:1 for Pima Indians, and 2.3:1 in European women. Particularly between the ages of 20 and 30, female hormones seem to be involved. Another study that looked at the relationship between estrogen and cholesterol biosynthesis discovered that estrogen, in particular, activated the HMG Co-A reductase enzyme, increasing the production of cholesterol and increasing the risk of hyper saturation in women. Gallstone incidence was shown to be higher in postmenopausal women receiving estrogen replacement treatment, supporting the hypothesis that there is a relationship between estrogen and gallstones. By preventing gallbladder contraction and encouraging hypomotility and gallbladder stasis (Hulley *et al.* 2002), progesterone may also be a factor in gallstone disease.

Gallstone production also appears to be influenced by parity. Gallstones appear to be more common in people who have more pregnancies and longer reproductive cycles than women who stay nulliparous. Women under the age of 25 who had less than four pregnancies had a 4 to 12 times higher risk of developing cholesterol stones than nulliparous women of the same age and weight, according to a research (Valdivieso *et al.* 1993).

While the bulk of research carried out in the West have determined that females are more likely than males to acquire cholelithiasis, there is still some debate regarding

gender as a risk factor for the condition (Méndez *et al.* 1990). While research on Asian patients has not been able to find a gender-related difference. Gallstone incidence discrepancies between the sexes were observed to diminish with age, according to Attili AF *et al.* Cholelithiasis is more common in men than in women under the age of 50, but in women than in men in age groups after 50, according to Liu CM et al (Liu *et al.* 2006). For pigment lithiasis, which affects both men and women equally, there is no substantial association between femininity and parity and cholesterol gallstones because the factors impacting pigment lithiasis are unrelated to the levels of female hormones and their potential impact on gallbladder emptying (Berci 2004).

Grave co-morbidities associated with obesity include osteoarthritis, heart disease, diabetes mellitus, hypertension, stroke, and gallstone disease. According to studies by Field AE *et al.*, the incidence of diabetes, gallstones, hypertension, heart disease, colon cancer, and stroke (in men only) increased during the course of a 10-year follow-up period. Adults who were overweight but not obese had a markedly increased risk of contracting a number of illnesses (Field *et al.* 2001).

Furthermore, even among those who were in the upper half of the healthy weight range, a link between BMI and the risk of acquiring chronic diseases was evident. Although it is yet unclear how exactly obesity raises the risk of gallstone diseases, it is one of the major risk factors for the development of cholesterol gallstones (Tsai *et al.* 2005).

Additionally, people with central obesity and those who acquired their obesity earlier in life than later show a stronger association between gallstone disease and obesity. The results of the current investigation showed that obesity is linked to a linear rise in gallstone production in both male and female cholelithiasis patients (males and females). This result was consistent with what other people had reported. It has been hypothesized that obesity may be linked to gallstone disease due to increased cholesterol production and hepatic secretion, which causes bile to become too saturated with cholesterol (Mabee *et al.* 1976). Additionally, several investigations have found that obese people have impaired gallbladder motility. Hypomotility results in

incomplete and irregular emptying of the gallbladder's contents, which promotes bile stasis and crystal formation (Mokdad *et al.* 2003).

By eating the right foods, the pathogenesis of gallstones can be halted. According to study, a high dietary fiber consumption may lower the risk of gallstone formation, whereas a high calorie, protein, carbohydrate, refined sugar, and calcium intake may raise it. Moreover, Gustafsson U et al found that vitamin C administration changed the composition of bile and prolonged the nucleation time in patients with gallstones, suggesting that vitamin C intake may possibly affect how people manufacture cholesterol (Simon 1993).

Consumption of insoluble fiber is inversely associated with gallbladder disease, according to epidemiological studies. Research on nutrition and gallstone disease in people is severely constrained by two fundamental problems with gallstone etiology. First off, gallstones usually don't create any symptoms and develop gradually. Gallstones were observed to grow at a rate of about 2 mm each year (Paumgartner and Sauerbruch 1991).

The chronic hyperglycemia of diabetes can cause long-term damage, dysfunction, and failure to a variety of organs, including the heart, kidneys, nerves, eyes, gallbladder, and blood arteries. Dysfunction of the digestive system has a substantial impact on the morbidity of this condition (Saxena *et al.* 2005). Insulin encourages the synthesis of lipids and inhibits their breakdown, which is consistent with its role as an anabolic hormone. Sterol response element-binding proteins (SREBPs), which activate the expression of more than 30 genes involved in the synthesis and absorption of fatty acids, triglycerides, cholesterol, and phospholipids, control the lipid homeostasis that is controlled by insulin (Horton *et al.* 2003).

Additionally, as insulin controls the Na +-K+ pump, which may negatively impact the ionic and osmotic equilibrium of smooth muscle, hyperinsulinemia may possibly be a significant contributor to these observations. Numerous investigations have revealed

inadequate gallbladder emptying and increased fasting gallbladder capacity (Gitelson *et al.* 1963).

This connection may not exist in people without diabetes. A recent hypothesis advanced by Shebl FM *et al.* suggests that the primary cause of gallstone development may not be diabetes but rather insulin resistance (Shebl *et al.* 2011). This result is consistent with the current investigation, which discovered a highly significant difference in insulin levels (p=0.000) in both males and females with NDM gallstones compared to controls. This study backs up other studies that show how insulin resistance and hyperinsulinemia contribute to the formation of cholesterol gallstones (Lei *et al.* 2008).

This link with non-diabetes is uncertain. A recent hypothesis advanced by Shebl FM *et al.* suggests that insulin resistance, rather than diabetes, may be the primary factor in the development of gallstones (Shebl *et al.* 2011). This result is consistent with the current investigation, which discovered a highly significant difference in insulin levels (p=0.000) between NDM gallstone patients and controls in both sexes. This result is consistent with a number of studies that looked at the involvement of insulin resistance and hyperinsulinemia in the formation of cholesterol gallstones (Lei *et al.* 2008). Although it has been shown that cholecystokinin (CCK)-stimulated gallbladder movement is decreased by both hyperglycemia and euglycemic hyperinsulinemia, insulin may potentially enhance the incidence of gallstones by decreasing both baseline and CCK-stimulated motility (Gielkens *et al.* 1998).

According to Matthews DR et al, the classic euglycemic hyperinsulinemic clamp's evaluation of insulin sensitivity, which can be easily measured from a single fasting blood sample, has a substantial correlation with HOMA-IR. The HOMA-IR score, which is calculated as follows, indicates the level of insulin resistance: With a result of >2.5, HOMA-IR is a strong sign of insulin resistance. Patients with GS and the control group in this study showed a substantial difference in HOMA-IR (Matthews *et al.* 1985). Hepatic insulin resistance thus provides a crucial link between the metabolic syndrome and an increased incidence of cholesterol gallstones (Biddinger *et al.* 2008). that cholelithiasis may affect how the metabolic syndrome develops.

The fundamental cause of cholesterol supersaturation, according to the epidemiology of gallstone disease, is hypersecretion of cholesterol, which increases with aging. Hypersecretion may result from abnormalities in the metabolism of hepatic cholesterol, such as increased hepatic absorption, increased de novo synthesis, and/or imperfect conversion to bile acids or cholesterol esters (Schwartz *et al.* 1982).

Méndez-Sánchez and Uribe (2001) claim that the instability of cholesterol carriers in bile and the formation of cholesterol crystals are caused by metabolic changes in hepatic cholesterol output, modifications in gallbladder motility, and intestine bacterial degradation of bile salts. It is now generally acknowledged that a change in lipid metabolism, which results in a relative rise in cholesterol levels compared to other lipids produced by the liver into the bile, is the key factor in the development of cholesterol gallstones. Information provided by Kern FJ supports the claim that people with gallstones have a different hepatic metabolism of cholesterol than people without stones (Kern 1994).

The results of the current investigation are consistent with those of earlier studies in that they show that changes in plasma lipid and lipoproteins have been seen in relation to gallstone development, and that triglyceride concentration, total cholesterol, LDL, and gall bladder illness are related. This study found an association between high LDL levels and the risk of gallstones, which was not supported by other studies (Völzke *et al.* 2005). However, Laakso M et al showed that individuals with gallstone disease had low levels of TC and LDL-C, which would be at least in part attributable to the alteration of their lifestyles, particularly those connected to nutrition (Laakso *et al.* 1990). The outcomes of earlier investigations have not been reliable when assessing potential relationships between total cholesterol levels and GSD. There have been several reports of relationships that lack statistical significance. In a recent study, Sabanathan S *et al.* (Duque-L *et al.* 1999) discovered a positive correlation between serum triglyceride levels and gallstones, although other studies found no such correlation (Rao *et al.* 2012).

Although the majority of the gallstone patients in this study had elevated serum TG levels, their average levels were practically within the normal range, and Méndez-

Sanchez N et al found that none of the lipid variables (TC, LDL-C, HDL-C, and TG) were statistically associated with the risk of gallstone disease.(236) A recent study by Rao PJ *et al.* found that serum HDL-C significantly increased in comparison to the control group (Rao *et al.* 2012). that contradicts both our study, which revealed no association between GSD and HDL-C concentration, and earlier studies that identified a connection between the development of gallstones and low blood HDL concentrations (Duque-L *et al.* 1999). VLDL is stimulated by hyperinsulinemia, which is present in the insulin resistant condition. Gallstone disease is a danger in patients with hypertriglyceridemia who are frequently overweight and insulin resistant (Rao *et al.* 2012).

The alteration of serum lipids in cholesterol gallstones is suggestive of metabolic syndrome and underscores the critical role that serum lipids play in the pathophysiology of cholelithiasis. Obesity (particularly belly fat), low HDL cholesterol, high triglycerides, high blood pressure, and high blood sugar are all part of the metabolic syndrome, a group of diseases. Gallstones may be at risk due to metabolic syndrome, according to research (Saxena, Sharma, and Dubey 2005). That patients with cholesterol stones did not exhibit excessively high serum cholesterol was of particular note. One reasonable explanation for these data could be because the serum-derived lithogenic bile contains too much cholesterol, which lowers the levels of these chemicals in the serum (Klass *et al.* 2006).

6. CONCLUSIONS AND RECOMMENDATION

Conclusions:

From the outcome of the present study the following conclusions be found:

- Cholesterol stones are the most common gallstones.

- Obesity and female gender increase incidence of cholesterol gallstone formation.

- Gallstone production in patients without a clinical diagnosis of diabetes mellitus is linked to hyperinsulinemia, insulin resistance, and normal serum glucose levels.

Recommendations:

From the outcome of the present study the following recommendations may be suggested:

- To other research students in this field we suggested to study of other markers that affect on gallbladder motility and markers that affect on the level of serum lipid in gallstones patients like enzymes which is responsible for the transport and metabolism of lipid .

- As a routine component of their clinical evaluation, we advise that all patients with cholelithiasis now receive a comprehensive lipid profile, including HDL and LDL.

- People who are at risk factor (such as multiparity, female sex, advancing age, and obesity), may modify their diet by encouragement of vegetable and fruit intake and simultaneously the restriction of animal protein, fat intake, as these, may have an important effect in preventing or minimizing stones formations.

- While certain activities, such brisk walking or intense exercise, lessen the risk of gallstone disease, having less physical activity raises the chance of gallstones.

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