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Collage of Graduate studies

Assessment of Gonadotropin Levels and their Ratio in Gallstones Patients in Khartoum State

تقويم مستوى القونادوتروبين والنسبه بينها في مرضى حصاوي المراره بولاية الخرطوم

*A dissertation submitted in partial fulfillment for the requirement of
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قال تعالى:

"قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا إِنَّكَ أَنْتَ
الْعَلِيمُ الْحَكِيمُ"

صدق الله العظيم

سورة البقره :الآيه (32)

Dedication

To the source of proudness and appreciation

My great father

To the symbol of tenderness and patients

My amazing mother

To the pure supportive hearts

My sisters

To that who always standing next me

My fiance

I dedicate this research

Zainab

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Abstract

Background: Gallstone disease is the most common gastrointestinal disorder affecting the biliary system which occurs when hard fatty or mineral deposits of either cholesterol or bilirubin form in gallbladder. Many studies have shown the risk of estrogen hormone to be one of gallstone causes which can be reflected by the change in regulatory gonadotropin and their ratio. Therefore, present study aimed to evaluate gonadotropin (FSH and LH) level and their ratio and their correlation with study variables among gallstones Sudanese patients in Khartoum state.

Materials and methods: In cross sectional hospital base study n=60 subjects were enrolled, then classified into two groups, n=30 clinically diagnosed gallstone patients age ranged from (18 to 77) as case group, and n= 30 apparently healthy individuals as control matched. Serum level of FSH and LH were measured using sandwich ELISA technique and FSH/LH ratio was calculated.

Results: Analyses of frequency showed that, gallstone disease is more common in females (73%) than males (27%), abnormal high weight (70%) than normal weight (30%) and in Postmenopause (63.7%) than Premenopause (36.3%). Concerning contraceptive use, (60%) had used contraceptive in their life compared to (40%) never used. Moreover, independent t-test revealed that, the level of FSH is greatly higher in female than male (p-value 0.002), while FSH/LH ratio is significantly decrease in gallstone patients in comparison with control group (p-value 0.010). In addition person's correlation results noted positive correlation between FSH

and age (R-value 0.354 and *P*-value 0.045), moreover negative correlation was observed between FSH/LH ratio and BMI (R-value 0.424 and *P*-value 0.011).

Conclusion: The study concludes that, gallstones are common in obese postmenopausal Sudanese women also contraceptive used is a risk factor of gallstone formation in our population. Gallstone patients had lower FSH: LH ratio, thus monitoring of ratio is recommended.

المستخلص

خلفية الدراسة: مرض حصوة المرارة من أكثر أمراض القناة الهضمية التي تؤثر على الجهاز الصفراوي شيوعا والذي يحدث عندما تتكون ترسبات دهنية أو معدنة من الكوليسترول او الصفراوي داخل الحويصلة الصفراوية. أوضحت عديد من الدراسات خطورة هرمون الاستروجين في كونه واحدا من اسباب حصوة المرارة والذي يعكس بالتغير في مستوى القونادوتروبين والنسبه بينها. لذلك، تهدف هذه الدراسة لتقييم مستوى القونادوتروبين والنسب بينها وعلاقتها مع متغيرات الدراسة الاخرى في مرضى حصاوي المراره السودانيون في ولاية الخرطوم.

طرق ومواد الدراسة: في دراسة مقطعية تعتمد على المستشفى (n=60) شخصا شاركوا ثم تم تصنيفهم الى مجموعتين، (n=30) من مرضى حصوة المراره الذين تم تشخيصهم و تتراوح اعمارهم بين (18-77) كمجموعة الحالات و (n=30) افراد يظهر انهم اصحاء كمجموعة المقارنه . تم قياس مستوى هرمونات القونادوتروبين باستخدام تقنية الاليزا.

نتيجة الدراسة: أظهرت نتائج تحليل التردد ان مرض حصوة المرارة اكثر شيوعا في النساء (73%) من الرجال (27%)، في اصحاب الاوزان الثقيله من طبيعيي الوزن وفي اليائسات اكثر من النساء في ما قبل سن اليأس. فيما يخص استخدام موانع الحمل، (60%) قامو باستخدامها في حياتهم مقارنة ب (40%) الذين لم يستخدمو من قبل. اضافة لذلك اختبار T كشف عن ان مستوى الهرمون المحفز للاكياس تكوين البويضات (FSH) مرتفع وبشده عند النساء اكثر من الرجال (p-value 0.002). بينما النسبه بين القونادوتروبين (FSH/LH) منخفضه في المرضى اكثر من الاصحاء (p-value 0.010). اضافة الى ان انتائج اوجدت علاقة طردية بين الهرمون المحفز للاكياس تكوين البويضات (FSH) والعمر (R-value 0.354 and P-value 0.045) وعلاقة عكسية بين النسبه بين القونادوتروبين (FSH/LH) ومعامل كتلة الجسم (BMI) (R-value 0.424 and P-value 0.011).

خاتمة الدراسة: توصلت الدراسة الى ان، حصوة المرارة اكثر شيوعا عند النساء السوانيات اليائسات البدنيات وايضا استخدام موانع الحمل معامل خطورة لتكون حصاوي المراره في مجتمع هذه الدراسة. مرضى حصوة المراره لديهم انخفاض في النسبه بين القونادوتروبين (FSH/L) ، لذلك تمت التوصيه على متابعتها.

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CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Gallstone disease (Cholelithiasis) is the most common disorder affecting the biliary system and is one of the most prevalent gastrointestinal diseases which occur when hard fatty or mineral deposits (stones) form in gallbladder typically from either cholesterol or bilirubin. (Apstein and Carey, 1996)

Gallbladder and biliary related diseases occurred in about 104 million people (1.6%) in 2013 and they resulted in 106,000 deaths with incidence ranging from 10% to 20% of the world population and four times higher in women than in men. In Africa, the prevalence of GSD was reported from few countries and its prevalence in Sudan was 5.2% (Assefa, 2008).

The traditional risk factors for gallstone disease (GSD) are the four 'F's- 'female, fat, forty and fertile' - but additional risk factors are include birth control pills, pregnancy, family history of gallstones, obesity, diabetes, liver disease, or rapid weight loss (Saadeldin. *et al.*,2013).

Most people with gallstones (about 80%) never have symptoms. In 1–4% of those with gallstones, a crampy pain in the right upper part of the abdomen, known as biliary colic, occurs each year. Complications of gallstones include inflammation of the gallbladder, inflammation of the pancreas, biliary obstruction, gallbladder empyema or perforation and liver inflammation. Symptoms of these complications may include pain of more than five hours duration, fever, yellowish skin, vomiting, or tea-color urine. So gallstones may be suspected based on symptoms. Diagnosis is then typically confirmed by ultrasound and complications may be detected on blood tests [1w] (Ansaloni, 2016).

If there are no symptoms, treatment is usually not needed. In those who are having gallbladder attacks surgery to remove the gallbladder is typically recommended but in those who are unable to have surgery, medication to try

to dissolve the stones or shock wave lithotripsy may be tried (Apstein and Carey, 1996).

1.1.1 Definition of gallstone

Gallstone disease (GSD) is the most common disorder affecting the biliary system and is one of the most prevalent gastrointestinal diseases. Calculus disease of the biliary tract is the general term applied to diseases of the gallbladder and biliary tree that are a direct result of gallstones. Gallstone disease refers to the condition where hard fatty or mineral deposits (stones) form either in the gallbladder or common bile duct. The presence of stones in the gallbladder is referred to as cholelithiasis, from the Greek *chol-* (bile) *lith-* (stone) *iasis-* (process) (Apstein and Carey, 1996).

1.1.2 Epidemiology

Gallstone disease (GSD) is a worldwide disease with the incidence ranging from 10% to 20% of the world population and it remains to be one of the most common health problems leading to surgical intervention about 500,000 cholecystectomies are performed in the United States of America (USA) every year (Saadeldin. *et al.*, 2013).

In Western Europe the prevalence ranges from 5.9% to 21.9% with the highest prevalence seen in Norway, Sweden, Germany and the lowest in Simion, Italy. While in South America the highest prevalence is found in Chili with incidence of 1.2/100 women/year and 20% of those 40 years and older and 30% of those above 70 years old have biliary stone with female to male ratio of 4:1 in the USA. This discrepancy is narrowing in the older population (Schirmer. *et al.*, 2005).

The lowest prevalence is found in Asian and African countries. The reported prevalence in Asia ranges from 4.35-10.7%. While in Africa, the prevalence of GSD was reported from few countries. For instance, in a group of

antenatal women, the prevalence was 2.1% in Nigeria, 4% in Tunisia, 5.2% in Sudan and 10% in black women (above 50 years) of Soweto (Assefa, 2008).

1.1.3 Physiology

The gallbladder is a hollow, muscular organ that stores and releases bile into the duodenum of the small intestine. Under normal conditions the liver produces bile, which is a yellowish-green liquid composed mainly of cholesterol, bile acids, lecithin, and water that emulsifies dietary fat in the small intestine, and releases it into the duodenum when fat is present. When bile is produced but not yet needed in the intestine, it is stored and concentrated in the gallbladder. Normal functioning of the biliary system (liver, gallbladder, and associated ducts) ensures optimal release of bile into the GI tract (Apstein and Carey, 1996).

1.1.4 Pathophysiology

Cholelithiasis is the presence or formation of gallstones in the gallbladder. In general, gallstones are hard, calcified stones made of cholesterol, bilirubin, and calcium that form when the ratio of bile components is offset, leading to hardening of the bile into small stones (Apstein and Carey, 1996).

Many factors such as hypercholesterolemia, impaired metabolism of bilirubin, gall bladder dysfunction and bile stasis, genetic factors, age, oxidative stress, infections, inflammation and various metabolic disorders including hepatitis, pancreatic disease and obesity contributing to gallstone formation by increasing the amount of cholesterol in bile or a decreasing the total pool of bile acids which induces the formation of large unstable multiphospholipid vesicles, that can be aggregated and produce cholesterol monohydrate crystals. Like cholesterol, bilirubin is also insoluble in water and its transport in human bile is bile acid dependent. It was suggested that

biliary bilirubin acts as a 'nucleation core' for cholesterol in the bile, directly promoting stone formation and gallstones usually contain a pigment (unconjugated bilirubin) nucleus (Sanikidze and Chikvaidz, 2016).

1.1.5 Classification of gallstones:

Gallstones are classified by their location as intrahepatic, gallbladder (cholelithiasis) and bile duct (choledocholithiasis) stones and based on chemical composition and macroscopic appearance; gallstones are divided into two major types: cholesterol gallstone and pigment stones which subclassified to black and brown stone (Kim et al., 2003).

1.1.5.1. Cholesterol gallstones (CGS)

Cholesterol stones are predominate type and represent (> 85%) of gallstones and vary from light yellow to dark green or brown or chalk white and are oval, usually solitary, between 2 and 3 cm long, each often having a tiny, dark, central spot. To be classified as such, they must contain more than 50% cholesterol as cholesterol monohydrate crystals plus varying amounts of protein and calcium salts as precipitates of amorphous calcium bilirubinate, often with calcium carbonate or phosphate in one of the crystalline polymorphs. These stones are usually subclassified as either pure cholesterol or mixed stones that contain at least 50% cholesterol by weight (Kim *et al.*, 2003).

1.1.5.2 Black pigment stones

Black pigment stones (bilirubin stone) are small, hard gallstones composed of calcium bilirubinate as a polymer plus inorganic calcium salts (e.g., CaCO₃, CaPO₄) and The basis for their formation is excessive bilirubin excretion in bile which frequently associated with hemolysis or alcoholic cirrhosis and with old age. Curiously, this also occurs with bile salt malabsorption. When ileal disease or loss causes bile salts to escape into the

colon (especially the caecum) in large quantities which is a biological detergent can then solubilize bilirubin pigment and return it via the portal vein to the liver. This creates an enterohepatic circulation for pigment material whose excessive secretion into bile can then cause black pigment stones (Shaffer and Romagnuolo, 2006).

1.1.5.3 Brown pigment stones

Brown pigment brown (mixed) stones are soft and greasy gallstone composed of bilirubinate and fatty acids (calcium palmitate or stearate). The greasy texture comes from bacterial production of fatty acids from palmitic and stearic acid. These brown stones form in bile ducts in association with stagnation, inflammation, infection (often from a stricture or tumor) or parasitic infestation (e.g., liver flukes) of the biliary tract. Such conditions predispose to chronic cholangitis and eventually cholangiocarcinoma. Infection and inflammation increase β -glucuronidase, an enzyme that deconjugates bilirubin; the resultant free bilirubin then polymerizes and complexes with calcium, forming calcium bilirubinate in the bile duct system (Lee *at al.*, 2015).

1.1.6 Stages of formation of CGS

1.1.6.1 Chemical stage

Normally the bile that initially forms in the canaliculus contains unilamellar vesicles of lecithin and cholesterol. Bile salts meanwhile self-aggregate, forming simple micelles in the canaliculus. As bile flows along the biliary system and becomes more concentrated, these bile salts begin to solubilize the lecithin, from the vesicles forming mixed micelles. The lecithin, so incorporated, expands the solublizing capacity of the bile salt micelles (now termed mixed micelles) and incorporates cholesterol. The unilamellar vesicles take on more cholesterol, forming large multilamellar vesicles. This

stage, in which bile becomes supersaturated with cholesterol, may develop as early as puberty and is often associated with obesity. Supersaturated bile results from excessive cholesterol secretion (as in diabetes or obesity), a decrease in bile salt secretion (e.g., ileal disease or loss) or in export of lecithin (e.g., a mutation of the MDR3 gene responsible for lecithin transport) (Shaffer and Romagnuolo, 2006).

1.1.6.2 Physical stage

This physical stage (nucleation) involves the excess cholesterol precipitating out of solution as solid microcrystals. Precipitation of cholesterol occurs when cholesterol solubility is exceeded (cholesterol saturation index >1). That cause phospholipids vesicles become highly enriched with cholesterol and thermodynamically unstable, forming multilamellar vesicles. These results in the production of pronucleating proteins (including mucins) such as IgG and amino peptidase N secreted by the liver or gallbladder, which precipitate cholesterol micro crystals (shown as a notched rhomboid). Conversely, there may be a deficiency of antinucleating factors (such as apolipoproteins A-I or A-II) (Shaffer and Romagnuolo, 2006).

1.1.6.3 Gallstone growth

In this final stage, the cholesterol microcrystals precipitated from bile in the gallbladder are retained and aggregate and grow into macroscopic stones. Retention occurs in the gallbladder because of the long duration of time that bile is typically stored between meals and the epithelium in stone formers secretes excess mucus forms a colloidal mesh that entraps cholesterol microcrystals, preventing them from being ejected from the gallbladder. Mucin also creates a scaffold for the addition of more crystals. Furthermore, the excess cholesterol in bile accumulates in the sarcolemma and causes a defect in signal-transduction, impairing the contractile function of the

gallbladder smooth muscle and resulting in its failure to properly evacuate the solid material. Another motility defect is slowed intestinal transit. This allows the bacterial transformation of cholic acid to the secondary bile acid, deoxycholic acid, a hydrophobic bile acid that enhances cholesterol secretion and may help trigger crystal precipitation. The resultant stasis traps the microcrystals of cholesterol in a Mucin gel, allowing them to agglomerate, attract other insoluble components of bile, become biliary sludge and grow into overt gallstones (Shaffer and Romagnuolo, 2006).

1.1.7 Signs and symptoms

The natural history of gallstones is typically described in 2 separate groups of patients: those who have symptoms and those who are asymptomatic and the size and number of gallstones present does not appear to influence whether or not people are symptomatic or asymptomatic (Acalovschi *et al.*, 2003).

1.1.7.1 Asymptomatic Stones

Gallstones grow at about 1-2 mm per year over a five- to 20-year period before symptoms develop. They frequently are clinically “silent,” being incidentally detected on routine ultrasound performed for another purpose. Most people (80%) with gallstones never develop symptoms (Shaffer and Romagnuolo, 2006).

1.1.7.2 Symptomatic Stones

The cardinal symptom of gallstones is biliary pain (“colic”) which is described as pain in the right upper quadrant (RUQ) often radiating to the back, with or without nausea and vomiting. That ensues when an obstructing stone causes sudden distension of the gallbladder and/or the biliary tract. “Colic” is a poor term, as biliary pain typically does not increase and decrease spasmodically. Rather, the right upper quadrant or epigastric pain

begins rather suddenly, quickly becomes intense, remains steady for 15 minutes to some six hours and then gradually disappears over 30 to 90 minutes, leaving a vague ache. A person may also experience referred pain between the shoulder blades or below the right shoulder. These symptoms may resemble those of a "kidney stone attack". Often, attacks occur after a particularly fatty meal and almost always happen at night, and after drinking. If the stones block the duct and cause bilirubin to leak into the bloodstream and surrounding tissue, there may also be jaundice and itching. This can also lead to confusion. If this is the case, the liver enzymes are likely to be raised (Acalovschi *et al.*, 2003).

1.1.8 Complications

Complications are from stones in the gallbladder and includes: Obstruction of the cystic duct, leading to cholecystitis: this begins as a chemical inflammation and later may become complicated by bacterial invasion ,In addition to stone Passing out of the gallbladder into the common duct, causing biliary obstruction (cholestasis), sometimes accompanied by bacterial infection in the ductal system (cholangitis) which can cause purulent inflammation in the biliary tree and liver, and acute pancreatitis as blockage of the bile ducts can prevent active enzymes being secreted into the bowel, instead damaging the pancreas. Rarely, in cases of severe inflammation, gallstones may erode through the gallbladder into adherent bowel potentially causing an obstruction termed gallstone ileus. Symptoms of these complications may include pain of more than five hours duration, fever, yellowish skin, vomiting, or tea-color urine (Fitzgerald *et al.*, 2009).

1.1.9 Risk factors

Risk factors for gallstones include female gender, fecundity, and family history for gallstone disease which are strongly associated with the

formation of cholesterol gallstones. Estrogen-treatment enhances the risk, both in women when used for contraception or hormone-replacement and in men with prostatic cancer and significantly increased the biliary saturation of cholesterol and decreased the time required to form cholesterol crystals in bile in vitro (nucleation time). Also Fibrates, used for the treatment of dyslipidemia, interfere with cholesterol and bile acid synthesis and increase cholesterol secretion into bile (Meike *et al.*, 1998).

Regarding weight, Obesity as well also other factors contributing to the metabolic syndrome such as dyslipidemia, hyperinsulinemia insulin resistance or overt type 2 diabetes are risk factors for the development of gallstones, itself supposed to be a complication of the metabolic syndrome. On the other hand, rapid active weight loss and weight cycling strongly increase the risk for the development of gallstones. Thus, weight reductions should not exceed 1.5 kg per week. Among specific dietary factors, short-time high cholesterol as well as high-carbohydrate diets were associated with increased risk for gallstones, and in highly prevalent areas, the intake of legume, while unsaturated fats, coffee, and moderate consumption of alcohol seem to reduce the risk (Marschall and Einarsson, 2007).

1.1.10 Diagnosis

Gallstones may be suspected based on symptoms a positive Murphy's sign is a common finding on physical examination during a gallbladder attack complications may be detected on blood tests.

Diagnosis is then typically confirmed radiologically (but not symptomatic disease) as Plain abdominal x-ray will only identify the 10-15% with high calcium content as radiopaque densities in the right upper quadrant, while ultrasonography is the most sensitive and specific method for detecting gallstones or a thickened gallbladder wall (indicating inflammation). In

suspected cases of acute cholecystitis, cholescintigraphy can assist the diagnosis by failing to fill the gallbladder with radionucleotide because of a stone obstructing the cystic duct (Shaffer and Romagnuolo, 2006).

1.1.11 Prevention

Cholesterol gallstone disease may be prevented by life-style changes, in particular by reducing total caloric intake, maintaining a healthy weight and some protective factors such as: Use of statins, synthetic FXR agonists, Ascorbic Acid as found increase in serum ascorbic acid levels was associated with a 13% lower prevalence of clinical gallbladder disease and oral UDCA during weight-loss which prevented cholesterol gallstone formation in man. In addition to Coffee after follow up subjects who consistently drank 2 to 3 cups of regular coffee per day were approximately 40% less likely to develop symptomatic gallstones (Marschall and Einarsson, 2007).

1.1.12 Treatment

If there are no symptoms, treatment is usually not needed. Once gallstones become symptomatic, some form of treatment is recommended such as:

1.1.12.1 Surgical (Cholecystectomy)

Cholecystectomy (gallbladder removal) has a 99% chance of eliminating the recurrence of cholelithiasis. The lack of a gallbladder may have no negative consequences in many people. However, there is a portion of the population between 10 and 15% who develop a condition called postcholecystectomy syndrome which may cause gastrointestinal distress and persistent pain in the upper-right abdomen, as well as a 10% risk of developing chronic diarrhea. Cholecystectomy may be either open cholecystectomy, that performed via an abdominal incision (laparotomy) to open the abdominal cavity below the lower right ribs for direct visualization and operation, or by

Laparoscopic cholecystectomy which is technique views the abdominal contents through a laparoscope performed via three to four small puncture holes for a camera and instruments and uses instruments inserted through trocars into the abdominal wall to perform surgical manipulation. The disadvantages include a somewhat higher complication rate, particularly from common duct injury and retained common duct stones, plus the potential for overuse.

1.1.12.2 Medical (Bile Salt Dissolution)

Administered orally, bile acids can dissolve cholesterol gallstones. Two bile acids reduce cholesterol saturation of bile are Chenodeoxycholic acid which is cheaper agent, but soon proved to have marked side effects, specifically dose-related diarrhea (20-40%) and more importantly, liver damage, all because of its more in addition to Ursodeoxycholic acid. Gallstone size largely determines the success rate as small stones with a relatively large surface area have the best result and ideal cases have tiny (< 0.5 cm) gallstones with a success rate greater than 80% while large stones in obese individuals have less favorable results. The reported success rate for complete dissolution varies from 13-80% with over one to two years of therapy. Medical therapy also reduces the frequency of episodes of biliary colic (Shaffer and Romagnuolo, 2006).

1.2 Gonadotropin

Gonadotropin are hormones produced by the gonadotroph cells and regulate the gonadal functions in males (testosterone biosynthesis and spermatogenesis in the testis) and females (estrogen and progesterone biosynthesis in the ovary, and the menstrual cycle). They include the two glycoprotein hormones follicle stimulating hormone (FSH) and luteinizing hormone (LH) (Richard and Neil, 2012).

1.2.1 Biochemistry

The gonadotropic cells of anterior pituitary gland secrete FSH (MW 30 KD) and LH (MW 32 KD) which are glycoprotein hormones composed of two peptide chains (usually referred to as α and β subunits) each with carbohydrate subunit groups attached and linked together by disulfide bond (Carl *et al.*, 2008).

The α - and β -subunits are encoded by different genes, located on chromosomes 6, 11, and 19 respectively. The α subunits of these hormones are similar to one another and are interchangeable while the β subunits display greater difference in amino acid sequence among them that confer the hormonal and immunological specificity. Isolated α subunits are devoid of biological activity and isolated β subunits may have slight intrinsic biological activity but The heterodimeric structure of the common α and unique β subunit is essential for attaining full activity .This suggests that the presence of both subunits is important for specific receptor recognition and that the β one responsible for eliciting the specific biological response (Carl *et al.*, 2008).

Disulfide linkages maintain no covalent subunit linkage, which determines the ultra structure of the mature folded molecule. After processing of hormonal protein precursors, glycosylation occurs by transfer of oligosaccharide complexes; that accounts for 15% to 13% of molecular weight and includes: fucose, mannose, galactose, glucosamine, galactosamine and sialic acid, to asparaginyl residues. Post-translational processing of carbohydrate side chains is critical for hormone signaling; it may be species specific and is not uniformly similar for human LH and FSH leads to substantial subtle variation (microheterogeneity) (Shlomo *et al.*, 2011).

1.2.2 Physiology

Gonadotroph cells constitute about 10% to 10 15% of the functional anterior pituitary cells and produce two gonadotropin (LH and FSH) those are grouped together under the generic term gonadotropins because they the gonadal cells in males and females to induce their functions (Larry, 2010).

1.2.3 Functions

The main function of gonadotropins is to regulate gonadal steroid hormone biosynthesis and to initiate and maintain germ cell development, in concert with peripheral hormones and paracrine soluble factors (Shlomo *et al.*, 2011).

In women, FSH induce growth and maturation of the ovarian follicles, regulates ovarian estrogen synthesis by inducing 17 β -hydroxysteroid dehydrogenase and aromatase and promotes the endometrial changes- the characteristics of the first phase (the proliferative phase) of the mammalian menstrual cycle .While LH concerned with ovulation and release of ovum from the ovarian follicles and cause luteinization of the ruptured follicles to form the corpus luteum which under influence of pulsatile LH release secretes both estradiol and progesterone, it also promotes the secretion and initiates the second (secretory) phase of mammalian menstrual cycle (Larry, 2010; Carl *et al.*, 2008).

In men, LH stimulates the development and functional activity of testicular Leydig cells and promotes androgen synthesis and secretion .While FSH function is not readily apparent but probably stimulates seminiferous tubule development, mediates the development of spermatozoa from spermatids in concert with testosterone, especially because failed spermatogenesis leads to elevated FSH levels and acts with LH to promote secretion of androgens (Shlomo *et al.*, 2011; Larry, 2010).

1.2.4 Action

The Gonadotropin hormones interact with their respective cell-surface G-protein-coupled receptors (GPCRs) linked to cAMP second messenger signaling expressed in the ovary and testis, evoking germ cell development and maturation and steroid hormone biosynthesis. (Larry Jameson .2010).

In female luteal cell LH receptors signal to enhance cAMP levels and induce cholesterol availability for ovarian steroidogenesis. The steroidogenic acute regulatory (StAR) protein is induced by LH and mediates cholesterol delivery to the inner membrane. LH enhances cytochromeP450-linked enzyme activity in the synthesis of pregnenolone and induces 3 β -hydroxysteroid dehydrogenase, 17 α -hydroxylase, and 17,20-ylase synthesis. The FSH receptor, a G protein-linked seven transmembrane molecule, shares 50% extracellular domains and 80% transmembrane homology with the LH receptor. FSH regulates ovarian estrogen synthesis by inducing 17 β -hydroxysteroid dehydrogenase and aromatase and also induces follicular growth. Estrogens are also permissive for FSH action and enhance FSH-induced cAMP levels. In Male Leydig cell LH receptor signaling induces intratesticular testosterone synthesis mediated by enhanced cAMP production. FSH function in male subjects is not readily apparent but probably mediates the development of spermatozoa from spermatids in concert with testosterone, especially because failed spermatogenesis leads to elevated FSH levels (Shlomo *et al.*, 2011).

1.2.5 Regulation

Deconvolution pulse analysis allows estimation of “real-time” hormone secretion rates, with an assumed disappearance rate constant. The characteristic secretary episodes characterized for LH and FSH indicate daily production rates of 1000 IU and 200 IU, respectively, and a

disappearance half-life of 90 and 500 minutes for each respective β -subunit (Shlomo *et al.*, 2011).

Gonadotropin synthesis and release are dynamically regulated. This is particularly true in women, in whom the rapidly fluctuating gonadal steroid levels vary throughout the menstrual cycle that reflect the integration of sensitive and complex hypothalamic, pituitary, and peripheral signals in addition to leptin and prolactin are include:

1.2.5.1Gonadotropin releasing hormone

Hypothalamic GnRH is 10-amino acid hormone represent the pivotal integrator of peripheral signals in the regulation of the pituitary gonadal axis and the production of Gonadotropin is stimulated by it when binds to its G-protein–coupled receptor on the cell surface of the gonadotroph and is linked to cAMP second messenger signaling (Richard and Neil .2012).

GnRH is secreted in discrete pulses every 60–120 min, which in turn elicit LH and FSH, pulses. The magnitude of the LH response exceeds that of FSH and decreasing GnRH pulse frequency enhances LH pulse amplitudes, whereas increasing GnRH pulse frequency to more than every 2 hours down regulates the subsequent LH response. Based on this phenomenon, long-acting GnRH agonists are used to suppress Gonadotropin levels in children with precocious puberty and in men with prostate cancer and are used in some ovulation-induction protocols to reduce endogenous gonadotropins (Larry, 2010).

1.2.5.2 Gonadal hormones

Hormones secreted by the testis and ovary (steroid sex hormones and inhibin) exert negative feedback on the production of both GnRH and gonadotrophin (Richard and Neil, 2012).

Estrogens act at the hypothalamic and pituitary levels to control gonadotropin secretion that depending on the clinical situation, estrogen may either stimulate or inhibit pituitary gonadotropin synthesis and secretion, and it also can inhibit GnRH synthesis or action. As Chronic estrogen exposure is inhibitory, whereas rising estrogen levels, as occur during the preovulatory surge, exert positive feedback to increase gonadotropin pulse frequency and amplitude. This pattern is manifest in the cyclic control of gonadotropin secretion during the menstrual cycle and during puberty. Progesterone slows GnRH pulse frequency but enhances gonadotropin responses to GnRH (Shlomo *et al.*, 2011; Larry, 2010).

Testosterone feedback in men also occurs at the hypothalamic and pituitary levels and is mediated in part by its conversion to estrogens. After castration, elevated gonadotropin levels can be partially overcome by testosterone replacement. Mechanisms for these observations are complex, because testosterone also exerts a stimulatory effect on FSH β mRNA levels (Larry, 2010).

1.2.5.3 Gonadal Peptides

Pituitary Gonadotropin secretion is regulated by gonadal peptides which are members of the transforming growth factor β (TGF- β) family including inhibin: inhibin (Inhibin A, an α :Ba heterodimer; inhibin B, an α : β B heterodimer), activin (The activin A β A- and activin AB, β B homodimers) and follistatin peptides. Inhibin selectively suppresses FSH, whereas the

activin A β A- and activin AB (β B) homodimers stimulate in vitro FSH secretion (Shlomo *et al.*, 2011).

1.2.6 Pathophysiology

1.2.6.1 Excess Gonadotropin

Increased levels of gonadotrophin almost always reflect loss of negative feedback from the testis or ovary. Usually, primary testicular or ovarian failure yields serum LH and FSH levels several fold higher than the upper limit of normal. The commonest cause of this gonadotrophin over activity is physiological after the menopause when ovarian depletion of ova ends cyclical hormone production in women. Excess gonadotrophin secondary to increased GnRH stimulation is rare. In contrast, inappropriately timed rather than excessive production causes central precocious puberty. A pituitary adenoma secreting functional LH or FSH is incredibly rare. Commonly, however, non-functioning pituitary adenomas may stain by immunohistochemistry for the α -subunit, perhaps giving an indication of the developmental lineage, but little else (Richard and Neil, 2012).

1.2.6.2 Gonadotropin deficiency

During childhood, it is normal for the gonadotrophin to be low and relatively unresponsive to GnRH; however, continued gonadotroph inactivity will delay puberty and loss of gonadotropins after puberty causes secondary hypogonadism. In women, this is very common at some stage of the reproductive years as cyclical gonadotrophin secretion is very vulnerable to stress, excessive dieting or, most commonly, emotional anxiety of relatively minor proportions. A rise in prolactin levels is also sufficient to suppress LH and FSH production. Several syndromes from mutations in any one of a number of genes also result in loss of gonadotrophin because of absent GnRH. Clinically, it is important to realize that, in the face of significant

hypogonadal symptoms and signs, and low levels of sex hormones, gonadotrophin within the normal range are inappropriately low. In women, where significant fluctuation of gonadotrophin accompanies the normal menstrual cycle, this can be more difficult to identify. It tends to manifest as amenorrhea with low or undetectable serum estrogen. In both sexes the disorder is described as ‘hypogonadotropic hypogonadism (Richard and Neil, 2012).

1.3 Rationale:

Gallstone disease (GSD) is a worldwide disease and it remains to be one of the most common health problems leading to surgical intervention as every year about 500,000 cholecystectomies are performed in the United States of America (USA) with prevalence about 10% in its adult population.

Cholelithiasis incidence ranging from 10% to 20% of the world population and four times higher in women than in men , and this gender difference begins since puberty and continues through the childbearing years and many studies have shown that risk of developing cholesterol gallstones is increased by pregnancy, contraceptive steroids, and conjugated estrogens. These observations suggest that estrogen, a major female sex hormone, could be an important risk factor for the formation of cholesterol gallstones by increasing the cholesterol saturation index, altering bile salt and promoting nucleation of cholesterol into crystals, accordingly present research conducted to study the effect of estrogen in gallstone which can be reflected by the change in its regulatory gonadotropic hormones and this change may followed by its momentous effects on fertility and other biological functions.

1.4 Objectives:

1.4.1 General objective

- To assess the level of Gonadotropin and their ratio among gallstone patients in Khartoum state.

1.4.2 Specific objective

1. To estimate serum FSH and LH levels in Gallstone patients.
2. To calculate FSH/LH from the estimated levels
3. To compare mean of FSH and LH concentrations among cases and control individuals.
4. To correlate FSH and LH levels with variables (BMI, age and gender).

CHAPTER TWO

MATERIALS AND METHODS

2.1 Study design

This is descriptive cross-sectional study, conducted during the period of February to April 2017 in Ibn Sina hospital in Khartoum state.

2.2 Study population

Sixty individuals were enrolled in this study, classified into two groups, 30 gallstone patients as case group and 30 healthy individuals as control group.

2.3 Inclusion Criteria

Patients diagnosed with gallstone were included in this study.

2.4 Exclusion Criteria

Gallstone patients with diabetes mellitus or other endocrine disorders were excluded.

2.5 Collection of samples

Venipuncture blood specimen (6ml) were collected by using dry plastic syringes, tourniquet was used to make the veins more prominent from each volunteer under aseptic conditions and allowed to clot at room temperature for 2hours. Serum obtained by centrifugation at 4000 rpm then stored in -20 until the analysis.

2.6 Ethical Considerations

Study was approved from ethical committee of the Sudan University of Science and Technology, verbal informed consent was obtained and all patients were informed by the aims of the study.

2.7 Estimation of gonadotropin

The ELISA kits are designed for the in vitro quantitative determination of Follicle stimulating hormone and Luteinizing hormone concentration in human Serum by a microplate Immuno-enzymometric assay. The essential reagents required for the assay include high affinity and specificity

antibodies (enzyme and immobilized), with different and distinct epitope recognition, in excess, and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-FSH/LH antibody .Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex. Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody.

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

2.7.1 Procedure

Briefly according to manufacture all reagents, serum references and controls were brought to room temperature (20-27°C) and the microplate wells formatted for each serum reference, control and patient specimen to be assayed in duplicate. 50µl of the samples (or appropriate serum reference and control) were pipetted into the assigned well and (100µl) of FSH/LH-Enzyme Reagent solution was added to all wells and then plate was incubated for 60 minutes at room temperature. After incubation the contents of the microplate were discarded by aspiration and subsequently washed for three times using 300µl of wash buffer for each wash.100µl of Working

Substrate Solution was added to all washed well and incubated at room temperature for fifteen (15) minutes then (50µl) of stop solution was brought to each well and gently mixed for 15-20 seconds. Finally the absorbance in each well was measured at 450nm in a microplate reader within thirty (30) minutes of adding the stop solution.

2.7.2 Calculation of Results

Concentration of hormones obtained when dose response curve used to ascertain the concentration of FSH and LH in the specimens by recording the absorbance obtained from the printout of the microplate reader and plotting the absorbance for each duplicate serum reference versus the corresponding FSH/LH concentration in mIU/ml on linear graph paper then the best-fit curve drew through the plotted points. For determination the concentration of FSH/LH for each sample the average absorbance of the duplicates was located for each unknown on the vertical axis of the graph, the intersecting point on the curve was presented, and the concentration was read (in mIU/ml) from the horizontal axis of the graph .

FSH/LH ratio was calculated by the formula:

$$\text{FSH (mIU/ml)} \div \text{LH (mIU/ml)}$$

BMI obtained according to formula:

$$\text{Weight (kg)} \div \text{height}^2 \text{ (m)}$$

2.8 Quality control

Low, normal and high range controls treated as unknowns and values determined in every test procedure performed. Quality control charts maintained to follow the performance of the supplied reagents. Pertinent statistical methods employed to ascertain trends. Significant deviation from established performance used to indicate unnoticed change in experimental conditions or degradation of kit reagents.

2.9 Statistical analysis

The data was analyzed using statistical package of social science (SPSS) computer program using frequencies, independent T-test and person correlation results was expressed as percentage (%) and (mean \pm SD), and significance difference was consider as (*P* value <0.05)

CHAPTER THREE

RESULTS

3. Results

Sixty randomly samples were collected from study population to evaluate the level of FSH and LH, classified as 30 healthy apparently as control group for comparison and 30 gallstone patients as case.

Analyses of frequency showed that among gallstone patients (73%) was females and (27%) was males presented in **Figure3.1**.

Figure3.2 Shows frequencies of BMI among gallstone patients, classified as (30%) normal weight with (BMI of 18.5-26.5 kg/m²), (40%) overweight with (BMI of 26.5-30 kg/m²) and(40%) obese with (BMI >30 kg/m²) among gender (males and females).

Figure3.3 Presents the frequencies of female use contraceptives (60%) and that never use it (40%) among gallstone patient females.

Figure3.4 Shows frequencies of premenopausal (36.7%) and postmenopausal females (63.3%) among gallstone patients.

Table3.1 Presents the mean concentration of FSH in case (30.6±26.4) and control (39.6±28.1) with p-value=0.795, LH in case (10.5±7.56) and (13.5±7.94) in control with p-value=0.961 and FSH/LH among case (3.16±1.50) and control (3.68±2.88) with p-value=0.016.

Table3.2 Shows mean concentration of FSH (21.8±12.2) in males and (46.1±29.7) in females with p-value= 0.00^{**}, LH in males (7.22±6.36) and (15.7±7.30) in females with p-value= 0.731 and FS H/LH ratio (3.02±3.31) in males and (2.93±4.05) in females with p-value=0.576.

Table3.3 Indicates mean concentration of FSH, LH and FSH/LH (38.4±26.7) (14.5±6.88) (2.64±1.00) in Premenopause respectively and (53.7±31.7) (17.0±7.81) (2.92±1.02) respectively in Postmenopause with p-value= (0.339) (0.831) (0.933) respectively.

Table3.4 Teel the mean concentration of FSH, LH and FSH/LH ratio in female use contraceptive (43.6 ± 32.6) (15.0 ± 9.57) (2.82 ± 0.87) and in female not use contraceptive (47.8 ± 28.6) (16.2 ± 5.62) (2.76 ± 1.11) with p-value = (0.556) (0.190) (0.057) respectively.

Figure3.5 Personal correlation results show positive correlation between FSH and age with R-value 0.354 and *P*-value 0.045.

Figure3.6 Shows there is no correlation between LH and age with R-value 0.224 and *P*-value 0.234.

Personal correlation results show no correlation between FSH/LH ratio and age with R-value 0.163 and *P*-value 0.388 presented in **Figure3.7**.

Figure3.8 Presents no correlation between FSH and BMI with R-value 0.025 and *P*-value 0.895.

Figure3.9 personal correlation results show no correlation between LH and BMI with R-value 0.188 and *P*-value 0.319.

Negative correlation between FSH/LH ratio and BMI with R-value 0.424 and *P*-value 0.011 showed by personal correlation results in **Figure3.10**.

Frequencies of gender among gallstone patients

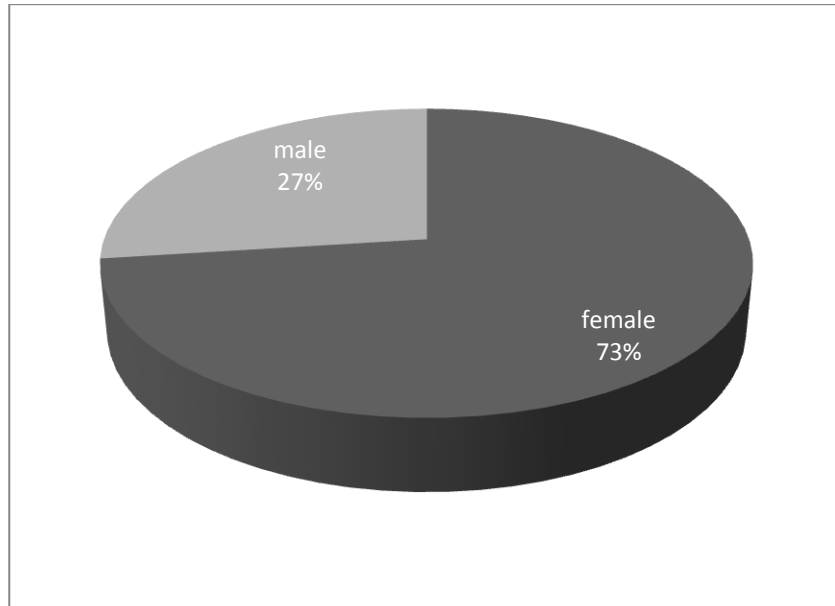
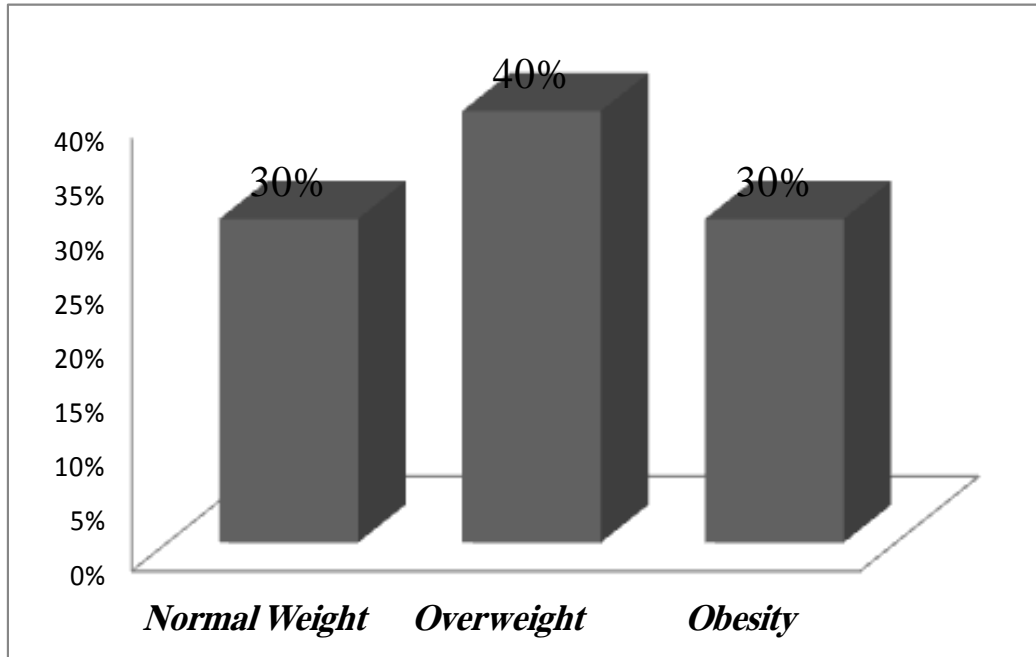


Fig.3.1 Shows frequencies of gender among gallstone patients, results expressed as percentage

Frequencies of BMI among gallstone patients



Frequencies of gender among gallstone patients, classified as normal weight (BMI of 18.5-26.5 kg/m²), overweight (BMI of 26.5-30 kg/m²) and obesity (BMI >30 kg/m²), results expressed as percentage presented in **Fig.3.2**.

Frequencies of female use contraceptives and female never use contraceptives among gallstone patients

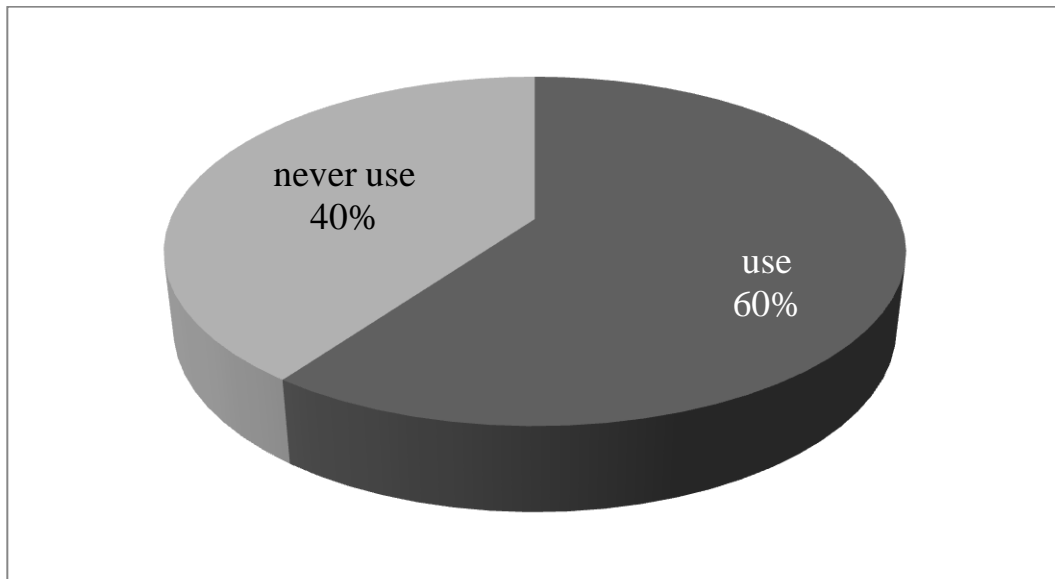
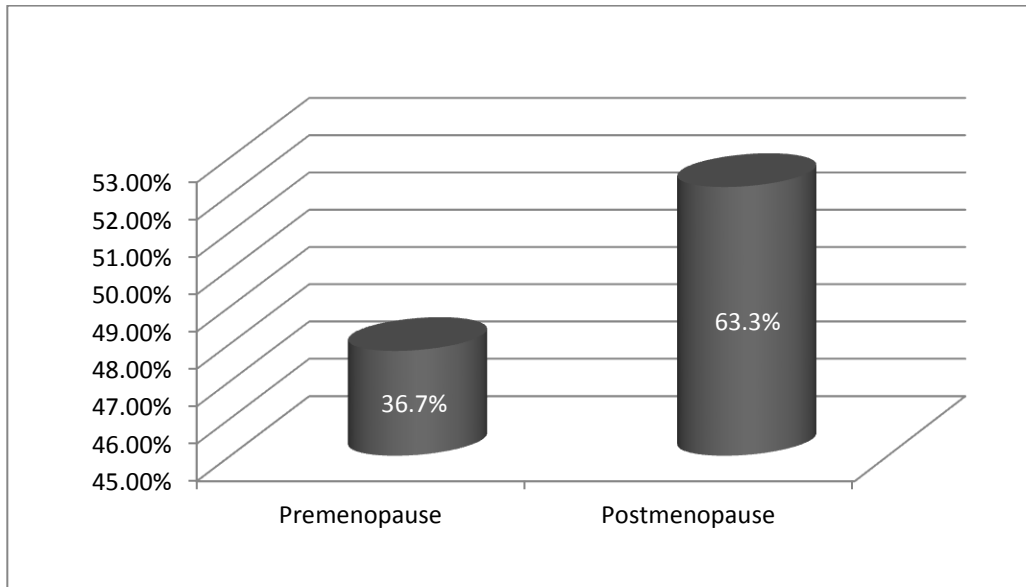


Fig.3.3 Presents the frequencies of female use contraceptives and that never use it among gallstone patient females, results expressed as percentage

Frequencies of premenopausal and postmenopausal females among gallstone patients



Analyses of frequency showed the frequencies of premenopausal and postmenopausal females among gallstone patients, results expressed as percentage in **Fig.3.4**.

Table3.1 Mean concentration of gonadotropins and their ratio in case and control groups

Parameters	Control (Mean \pmSD)	Case (Mean \pmSD)	<i>P-value</i>
FSH	39.6 \pm 28.1	30.6 \pm 26.4	0.795
LH	13.5 \pm 7.94	10.5 \pm 7.56	0.961
FSH/LH Ratio	3.68 \pm 2.88	3.16 \pm 1.51	0.016 ^{**}

Presenting the mean concentration of FSH, LH and FSH/LH ratio in case and control groups, significance difference consider as p-value ≤ 0.05 .

** indicate highly significance

Table3.2 Mean concentration of gonadotropins and their ratio among gender variation

Parameters	Male (Mean±SD)	Female (Mean±SD)	<i>P-value</i>
FSH	21.8±12.1	46.1±29.6	0.00**
LH	7.22±6.36	15.7±7.30	0.731
FSH/LH Ratio	3.02±3.31	2.93±4.05	0.576

Shows mean concentration of FSH, LH and FS H/LH ratio in male versus female, significance difference consider as $p\text{-value} \leq 0.05$.

Table3.3 Mean concentration of gonadotropins and their ratio in Premenopausal and Postmenopausal female

Parameters	Premenopause (Mean±SD)	Postmenopause (Mean±SD)	<i>P-value</i>
FSH	38.5±26.7	53.7±31.7	0.339
LH	14.5±6.88	17.0±7.81	0.831
FSH/LH Ratio	2.64±1.00	2.92±1.02	0.933

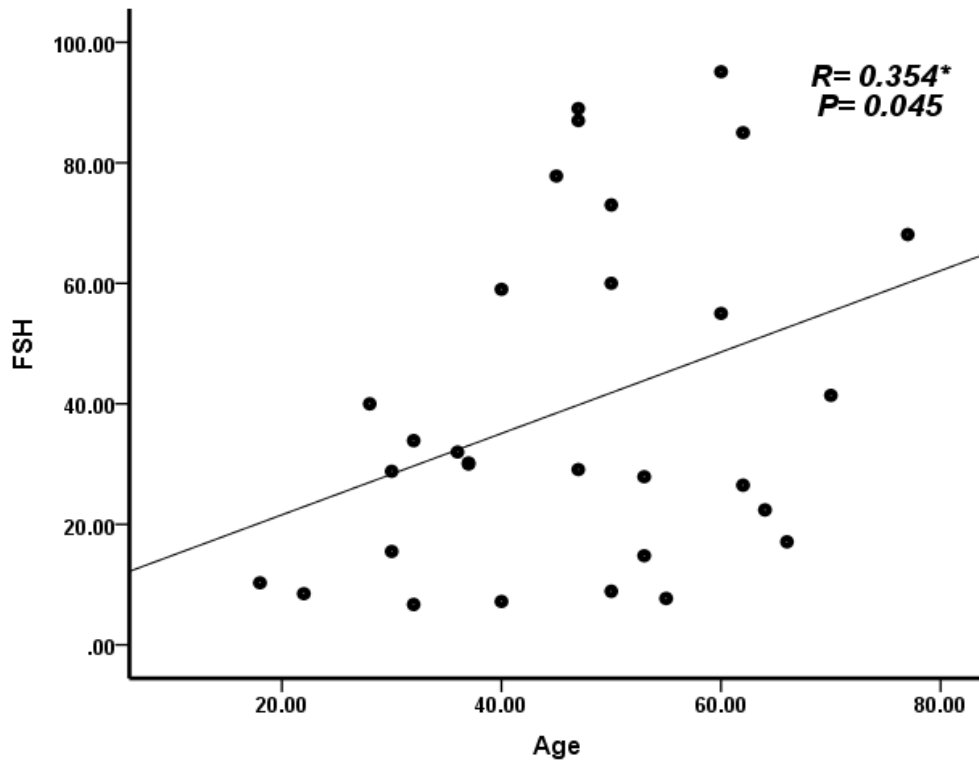
Shows mean concentration of FSH, LH and FSH/LH ratio in Premenopausal and Postmenopausal females, significance difference consider as p-value \leq 0.05.

Table3.4 Mean concentration of gonadotropins and their ratio in female use contraceptive and female not use contraceptive

Parameters	Use (Mean±SD)	Not Use (Mean±SD)	<i>P-value</i>
FSH	43.6±32.6	47.8±28.5	0.556
LH	15.0±9.57	16.2±5.6	0.190
FSH/LH Ratio	2.82±0.87	2.76±1.11	0.057

Shows mean concentration of FSH, LH and FSH/LH ratio in female use contraceptive and female not use contraceptive, significance difference consider as $p\text{-value} \leq 0.05$.

Relation between FSH and age



Personal correlation results showed positive correlation between FSH and age with R-value 0.354 and P -value 0.045 in **Fig.3.5**.

Correlation of LH ratio and age

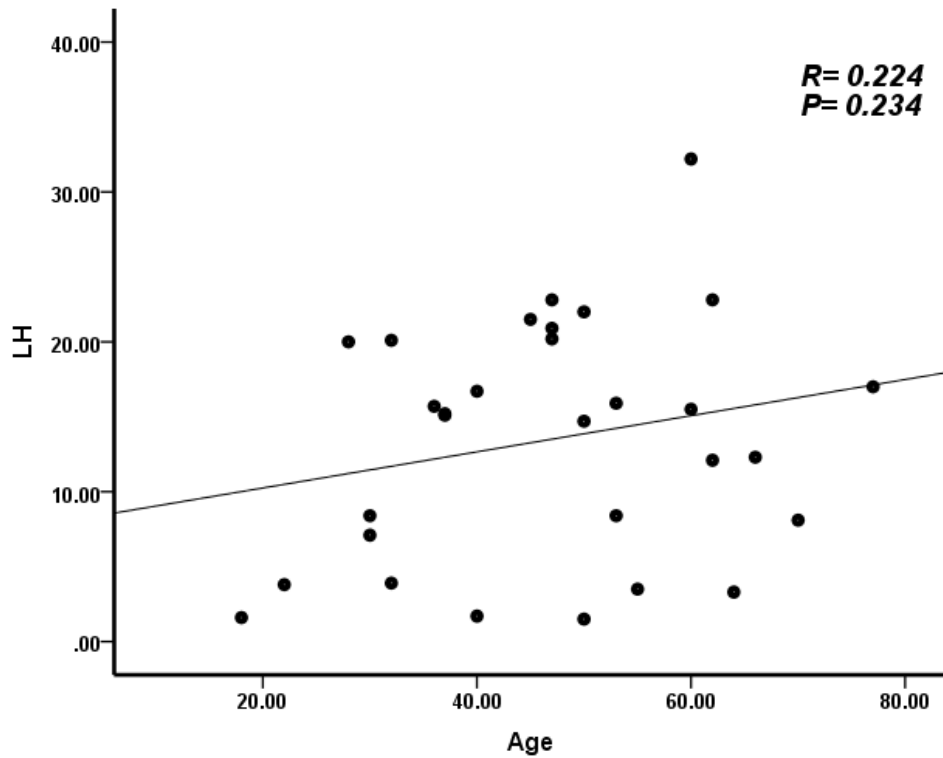


Fig.3.6 Shows no correlation between LH and age with R-value 0.224 and P-value 0.234

Relationship between FSH/LH ratio and age

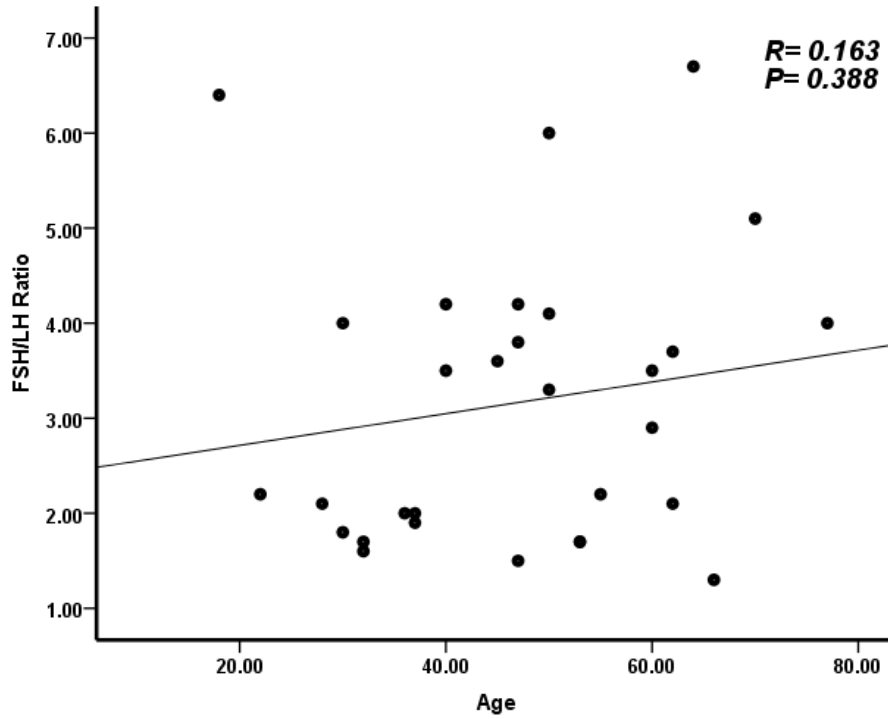


Fig.3.7 personal correlation results show no correlation between FSH/LH ratio and age with R-value 0.163 and P-value 0.388

Correlation of FSH and BMI

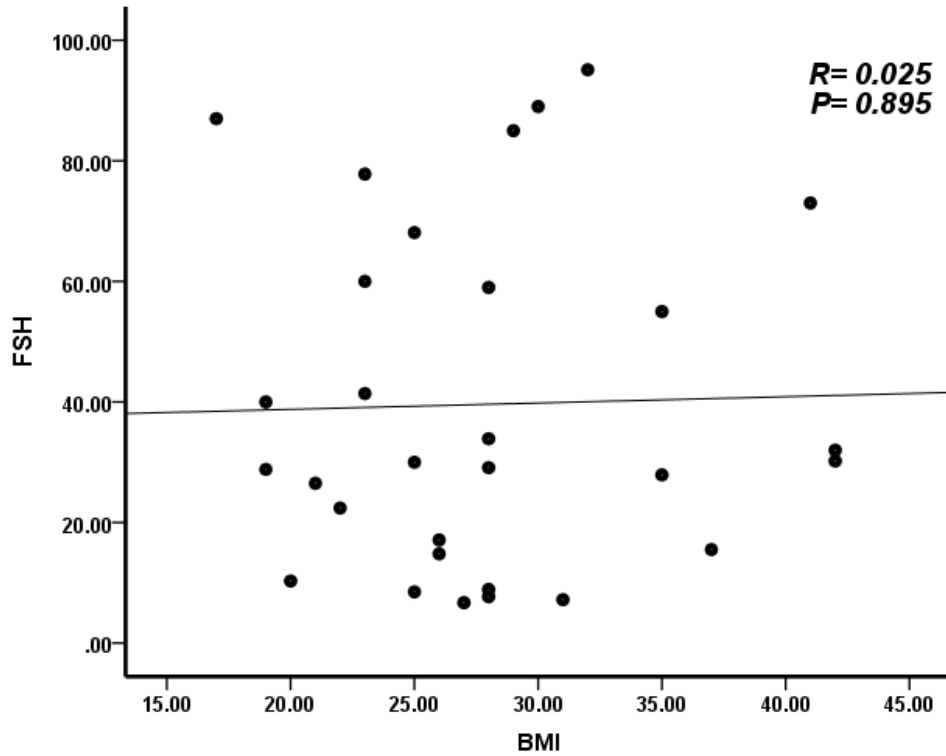
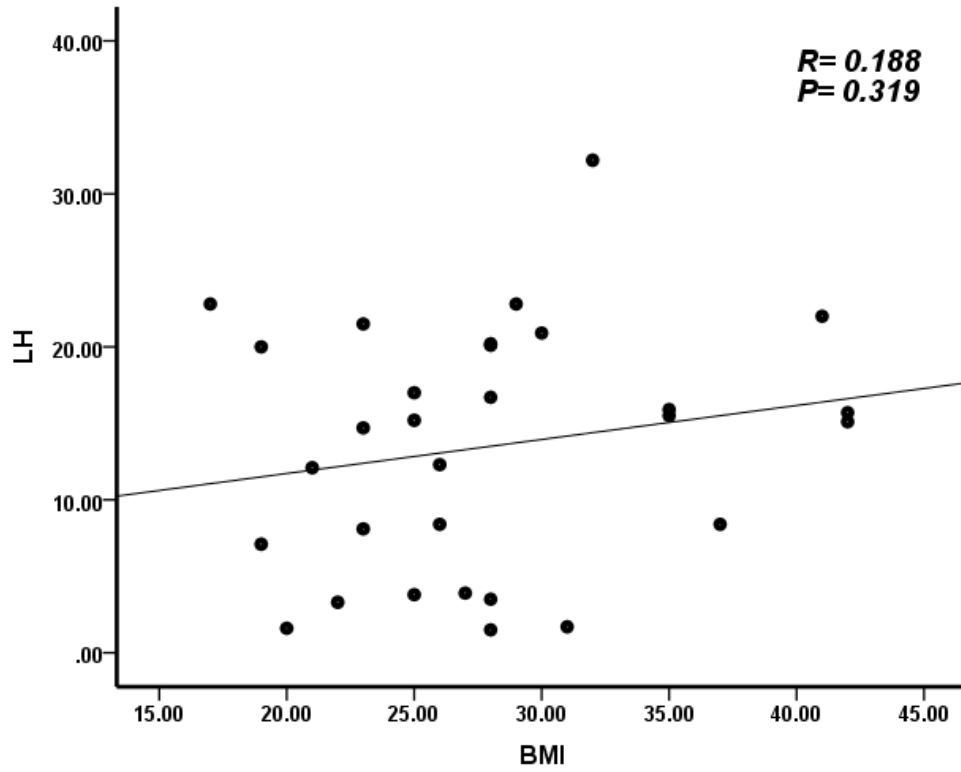


Fig.3.8 personal correlation results show no correlation between FSH and BMI with R-value 0.025 and P-value 0.895

Relation between LH and BMI



Personal correlation results show no correlation between LH and BMI with R-value 0.188 and *P*-value 0.319 presented in **Fig.3.9**.

Correlation of FSH/LH ratio and BMI

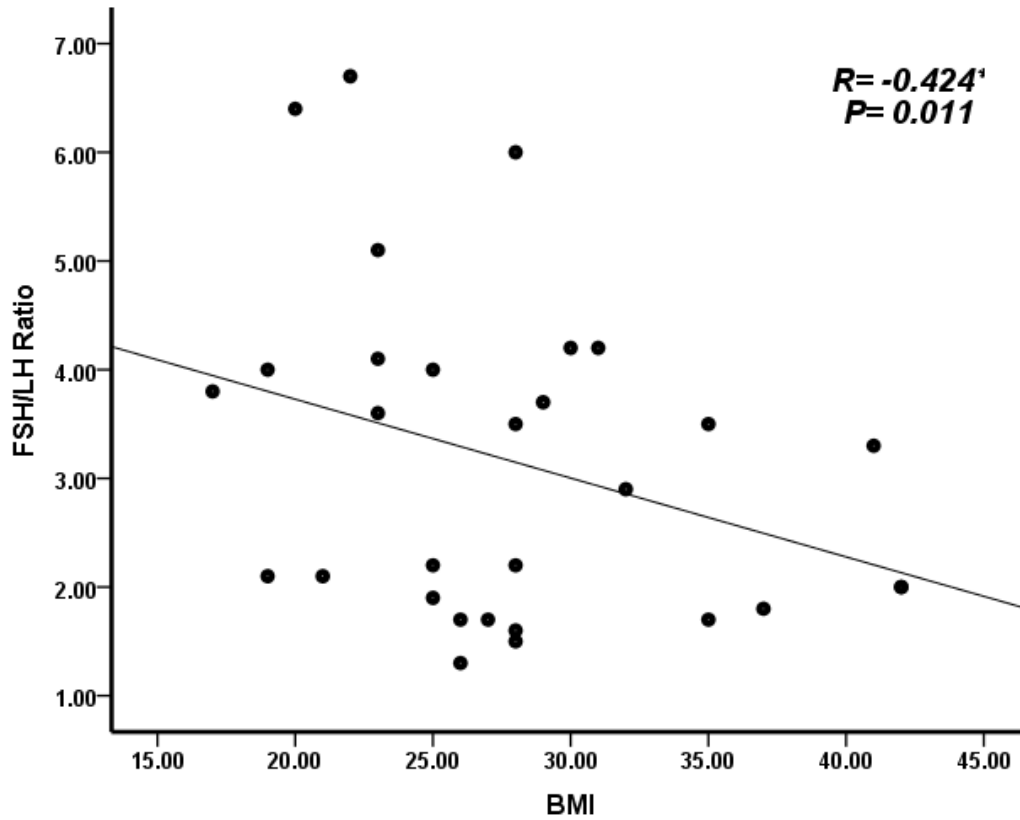


Fig3.10 Personal correlation results showed negative correlation between FSH/LH ratio and BMI with R-value 0.424 and P-value 0.011

CHAPTER FOUR

DISCUSSION, CONCLUSION AND

RECOMMENDATION

4.1 Discussion

Gallstone disease (GSD) is a worldwide disease and it remains to be one of the most common health problems leading to surgical intervention. Many studies have shown the risk of estrogen hormone to be one of gallstone causes which can be reflected by the change in its regulatory gonadotropic hormones and this change may followed by its momentous effects on fertility and other biological functions. Therefore, in descriptive cross-sectional study we aim to evaluate gonadotropin (FSH and LH) level and their ratio and their correlation with study variables among gallstones Sudanese patients in Khartoum state.

Analyses of frequency showed that gallstone disease is more common in females than males with percent (27% male and 73% female) witch approximately 1: 2.7 or 2.7 fold higher in females, this finding similar to previous finding that, out of 904 study population the predominantly are female which account of 798 (88%), and 106 (12%) were male (Nakeeb *et al.*, 2002). This difference between the sexes begins during puberty and continues through the fertile years, focusing attention on the effects of female sex hormones and mainly Estrogens have been hypothesized to be one important factor in the formation of gallstones (Peter *et al.*, 1989)

Moreover, the present study apprized that plurality of gallstone patients with abnormal high weight are 70% (40% overweight, 30% obese), while those with normal weight are (30%) which equal 2.31:. This finding attributed to the association of obesity and cholesterol level, which also act as precursor of bile salts biosynthesis, this disrupts the balance of bile composition and therefore increases the risk of gallstone formation (Mary *et al.*, 2011). In fact that, the risk of symptomatic cholelithiasis was proportional associated with increase BMI (Kharga *et al.*, 2016).

In addition 60% of gallstone females had use contraceptive, whereas the rest 40% never used, which approximately 1.5:1 fold. Therefore, our finding supported the association between contraceptive used and gallstone formation. Since estrogen administration increases the chances of women developing gallstones, through saturation index, alter bile acid composition, and decrease bile flow (Mary *et al.*, 2011). This proved by previous study findings that transcutaneous and oral estrogen administration of estrogen treatments significantly increased the biliary saturation of cholesterol and decreased the time required to form cholesterol crystals in bile in vitro (nucleation time) (Meike *et al.*, 1998).

Previously researchers explored that, postmenopausal female more susceptible to have gallstone due to the long-term exposure to other risk factors irrespective of locality or standard of living (Shlomo *et al.*, 2011). Since present study showed that the frequencies of premenopausal and postmenopausal females among gallstone patients are (36%) Premenopause and (64%) Postmenopause, this is similar to results of study in Taiwan which confirmed that increasing age had a direct relationship with the development of gallstones (ChenCY *et al.*, 1998).

The present study results revealed that, there were insignificant differences between mean concentration of both FSH and LH in gallstone patients in comparison with control group p-value (0.790 and 0.960) respectively, this finding disagreed with the results of previous study in patients with Cholelithiasis which suggested that the synthesis or metabolism of pituitary-gonad axis hormones was significantly abnormal (Rui Jing *et al.*, 1994). Changes in gonadotropins level connected mainly by administration of estrogen as contraceptives, hormonal replacement therapy and hormonal cancer therapy (Meike *et al.*, 1998), which cause of gallstone in previous

studies but not commonly used by Sudanese and their gallstone cause may return to other factors.

On the same manner, comparison analyses showed that, there was a highly significant decrease in FSH/LH ratio in gallstone patients in comparison with control group (p-value 0.010). This finding contradicts previous study that, men and postmenopausal women have lower LH in comparison with FSH (Russo *et al.*, 1993). Since our population does not used hormonal replacement therapy which known as causative factor of low LH level.

In addition independent t-test showed, there were insignificant differences of mean FSH, LH level and their ratio of postmenopausal when compared with premenopausal with gallstone (p-value 0.339, 0.831 and 0.933) respectively. These were no published study found compare between postmenopausal and premenopausal with gallstone.

The present study provided experimental evidence that, there was a very highly significant increase in mean concentration of FSH in female in comparison to males with gallstone (p-value 0.002). This finding attributed to, most of our population are postmenopausal, which have higher FSH level thus may lead to highly significant variation in result. Since aged-women with gallstones have higher FSH than healthy aged women (Zhoa, 1990). On the other hand there was an insignificant difference in mean concentration of LH and FSH/LH ratio of female with gallstone in comparison with males with (p-value 0.731) (p-value 0.576) respectively.

In contrast to fact that, feedback inhibition of estrogen or progesterone hormones components of oral birth control pills affect gonadotropin levels (Shlomo *et al.*, 2011) insignificant differences were noted in FSH, LH and FSH/LH ratio of females with gallstone used contraceptive and those never

use it (p-value 0.556, 0.190 and 0.057) respectively, which justified by the dose and durational variation in administrated estrogen.

In fact that as age increased, average FSH level exhibited a two phase linear increase (MacNaughton *et al.*, 1992), person's correlation results showed positive correlation between FSH and age (R-value 0.354 and *P*-value 0.045). Moreover no correlation between LH and age was observed (R-value 0.224 and *P*-value 0.234), which in concurrent with previous finding that LH levels did not differ significantly across age groups (MacNaughton *et al.*, 1992). In addition no correlation between FSH/LH ratio and age was noted (R-value 0.163 and *P*-value 0.388).

Personal correlation results showed no correlation between FSH and BMI (R-value 0.025 and *P*-value 0.895). This contradicted with previous study results which showed inverse relation between FSH and BMI in women in the early follicular phase (Zhoangua.1990), which justified by that most of our population are not in follicular phase.

Previous study results revealed that LH levels did not differ between lean and obese (Acien, 1999) consent with present study results which showed no correlation between LH and BMI (R-value 0.188 and *P*-value 0.319). Moreover, negative correlation was observed between FSH/LH ratio and BMI (R-value 0.424 and *P*-value 0.011), similar to previous observation that, BMI was inversely associated with FSH/LH ratio (Ecochard *et al.*, 2000).

4.2 Conclusion

The data suggests that, obese postmenopausal Sudanese women are more susceptible to gallstones also contraceptive used is a risk factor of gallstone formation in our population and FSH/LH ratio is lower in gallstone patients. Moreover, FSH correlates positively with age while the ratio is inversely associated with BMI. Thus patients with gallstone should be monitored for FSH, LH and their ratio for early diagnosis of related complications.

4.3 Recommendation

Estimation of estrogen hormone, lipid profile is recommended as further study and awareness by nutritional diet, lifestyle and all forms of estrogen administration are risk factors for gallstone is important.

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APPENDICES

Appendix (1)

بسم الله الرحمن الرحيم

Sudan University for Science and Technology

Collage of Graduated studies

Clinical Chemistry Department

Assessment of Gonadotropin levels and their Ratio in Gallstone patients in Khartoum state

Questionnaire NO: Date.....
Age: Sex:
Residence: urban (.....) Rural (.....)
Body weight (kg): Body height (cm):
Body mass index:
Duration of Disease:
Other disease:
Drugs use:
Have you ever been used contraceptive? Yes (.....) No (.....)
Type of contraceptive oral (.....) injection (.....)
Age at first menses: Age at last menses:
Premenopausal (.....) Postmenopausal (.....)

Investigations:

LH level:
FSH level:
Insulin level.....
Blood glucose level: