بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate studies

Assessment of Serum Gonadotropin (LH and FSH) Levels among Sudanese Women with Polycystic Ovary Syndrome

تقييم مستوى الهرمون المنشط للجسم الاصفر والهرمون المنبه للجريب في مصل الدم بين النساء السودانيات المصابات بمتلازمة تكيس المبايض

A Dissertation submitted in partial fulfillment for the requirement of M.Sc degree in medical laboratory science (Clinical chemistry)

Submitted by:

Rua Abdalmagid Alemam Abdalmageed

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Supervisor:

Dr.Abdelgadir Ali Elmugadam

Medical Laboratory Science - Clinical Chemistry

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الآية

بسم الله الرحمن الرحيم

قال تعالى:

(....وَقُلْ رَبِّ زِدْنِي عِلْمًا)

صدق الله العظيم

سورة طه الآية (١١٤)

DEDICATION

I dedicate This Modest for

My parent

who always pray for my success.....

Sister and brothers making every thing wonderful

my friends for thier love with great love and respect.

My Teacher

The reason of advancement and success

For my lovely friend .... To them I dedicate my accomplishment

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First, I thanks Allah for give me strength, patience and blessing to complete this work. peace upon the prophet, who helped and guided the mankind to the path of education and research.

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Abstract

Background:

Polycystic Ovarian Syndrome (PCOS) is one of the most common metabolic and reproductive disorders among women of reproductive age. The study was aimed to estimate Leutinizing hormone (LH) and Follicle Stimulating hormone (FSH) levels in women with PCOS.

Method: A case control analytical study was conducted at Alsir Abu Hassan fertility center in Khartoum State, included 100 blood samples, 50 samples from women with PCOS and 50 from apparently health individual as controls, serum LH and FSH levels were measured by ELISA (Enzyme linked immune sorbent assay) in case and control The data were analyzed using SPSS statistical analysis.

Results: This study showed that the levels of LH was insignificantly increased with (p-value 0.149) and means ±SD 12.19± 20.60 while there was no change in level of FSH in polycystic ovary syndrome patients compared to control group. The study showed that there was no effect in LH level across menstrual regularity, duration, infertility, family history, cosmetics and use of medication with (p-value 0.149). infertility with (p-value 0.350), family history with (p-value 0.350), cosmetics with (p-value 0.378) and use of medication with (p-value 0.286). Also there was no change is FSH level.

Conclusion: From the results and finding of this study, it is concluded that the Sudanese female with PCOs had normal levels of serum LH/ FSH hormone.

المستخلص

خلفية:

تعد متلازمة تكيس المبايض (**PCOS**) واحدة من أكثر الاضطرابات الأيضية والتناسلية شيوعًا بين النساء في سن الإنجاب. هدفت الدراسة إلى تقدير مستويات هرمون اللوتين (**LH**) وهرمون تحفيز الجريب (**FSH**) لدى النساء المصابات بمتلازمة تكيس المبايض.

الطريقة: أجريت دراسة تحليلية للحالة في مركز السير أبو حسن للخصوبة بولاية الخرطوم ، وشملت ١٠٠ عينة دم ، و ٥٠ عينة من النساء المصابات بمتلازمة تكيس المبايض ، و ٥٠ عينة من أفراد صحيين ظاهريًا كعناصر تحكم ، وتم قياس مستويات **LH** و **FSH** في الدم بواسطة **ELISA** (إنزيم فحص المواد الماصة المناعية) في الحالة والمراقبة تم تحليل البيانات باستخدام التحليل الاحصائي **SPSS** .

النتائج: أظهرت هذه الدراسة أن مستويات الهرمون اللوتيني ارتفعت بشكل طفيف مع (قيمة **p 0.149**) وتعني ± **20.60 SD 12.19** بينما لم يكن هناك تغير في مستوى **FSH** في مرضى متلازمة تكيس المبايض مقارنة بمجموعة التحكم. أظهرت الدراسة عدم وجود تأثير في مستوى الهرمون اللوتيني في انتظام الدورة الشهرية ، ومدتها ، والعقم ، والتاريخ العائلي ، ومستحضرات التجميل ، واستخدام الأدوية ذات القيمة الاحتمالية (**p-value 0.149**). العقم مع (القيمة الاحتمالية ٠.٣٥٠) ، التاريخ العائلي بـ (القيمة الاحتمالية ٠.٣٥٠) ، مستحضرات التجميل (القيمة الاحتمالية ٠.٣٧٨) واستخدام الأدوية مع (القيمة الاحتمالية ٠.٢٨٦). كما لم يكن هناك تغيير في مستوى **FSH**.

الخلاصة: من نتائج واستنتاجات هذه الدراسة ، استنتج أن الأنثى السودانية المصابة بـ **PCOs** لديها مستويات طبيعية من هرمون **LH / FSH** في الد

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List of Abbreviation

Abbreviations	Meaning
ACTH	Adrenocorticotropic Hormone
AYAs	Adolescent and Young Adult
BMI	Body Mass Index
CAH	Congenital Adrenal Hyperplasia
CVD	Cardio Vascular Disease
DHEAS	Dehydropiandrosteone Sulfate
DM	Diabetes mellitus
EAS	Embryology American Society
FNPO	Follicle Stimulating Hormone
FSH	Follicle Stimulating Hormone
FT4	Free Thyroxine
Gn RH	Gonadotropin releasing Hormone
HA	Hyper Androgenic
IR	Insulin resistance
LH	Luteinizing Hormone
Mc	Menstrual cycle
PCOM	Poly cystic ovary Morphology
PCOS	Poly cystic ovary syndrome

Chapter one

Introduction

Rationale

Objectives

1. Introduction, Objective and Rationale

1.1 Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous endocrine disorder which affects 5–15% of the general population, depending on the ethnicity and the applied classification system (Bozdag *etal*., 2016). (PCOS) is the most common endocrine disorder in women of reproductive age (Bozdag *etal*., 2016).

It is characterized by polycystic ovaries, oligo-amenorrhea, and hyperandrogenism (Bozdag *etal* ., 2016),(Lizeva *etal* ., 2016). PCOS is commonly related to features of metabolic syndrome, including obesity, impaired glucose metabolism, and insulin resistance (IR) (Couto Alves *etal.*, 2017) and is one of the main causes of anovulatory infertility (Ibanez *etal.*, 2017).The reported prevalence of PCOS in the community is between 6–10% depending on which criteria is used to define it (Bozdag *etal.*, 2016). Poly cystic ovary syndrome is characterized by ovulatory dysfunction, hyperandrogenism, and polycystic ovarian morphology (PCOM) based on ultrasounds (Teede *etal.*, 2018).

Polycystic ovary syndrome (PCOS) represents the most common endocrine dysfunction in fertile women and it is considered a heterogeneous and multifaceted disorder, with multiple reproductive and metabolic phenotypes which differently affect the early- and long-term syndrome's risks (Teede *etal*., 2018).

1.2 Rationale:

Systematic screening of women according to the National Institutes of Health (NIH) diagnostic criteria estimated that 4–10% of women of reproductive age suffer from PCOS. Although it was previously considered as a disorder of adult women, recent evidence suggests that PCOS is a lifelong syndrome, manifesting since prenatal age. In fact, according to the Rotterdam diagnostic criteria, the prevalence of PCOS in adolescents varies between a minimum of 3% and a maximum of 26% .However, the prevalence of the disease in children is still considered unknown .

The economic burden of PCOS is significantly huge. Around 4 billion dollars are spent annually in the United States to screen for the disease and treat its various morbidities, including hirsutism, infertility, and diabetes mellitus. The Australian Health System spends more than 800 million dollars every year to account for the disease.

Ability to measure LH and FSH level accurately is essential for establishing the diagnosis of true androgen excess . To add an information to help another researchers .

1.3 Objectives

1.3.1 General objective:

To assess LH and FSH level among women with Polycystic Ovarian Syndrome (PCOS).

1. 3.2 Specific Objectives

1. To measure levels of LH and FSH in case and control .

2. To Calculate LH/FSH ratio.

3 To compare between BMI and PCOS

4. To compare between LH , FSH and the obesity and family history .

Chapter Two
Literature Review

2. Literature review

2:1. Polycystic Ovary Syndrome (PCOS)

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders in females. Polycystic ovary syndrome is a heterogeneous endocrine disorder which affects 5–15% of the general population, depending on the ethnicity and the applied classification system (Bozdag *etal* ., 2016). Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age (Bozdag *etal*., 2016). It is characterized by polycystic ovaries, oligo-amenorrhea, and hyperandrogenism (Bozdag *etal*., 2016),(Lizeva *etal*., 2016). Poly cystic ovary syndrome is commonly related to features of metabolic syndrome, including obesity, impaired glucose metabolism ,and insulin resistance (IR) (couto Alves *etal*., 2017) and is one of the main causes of an ovulatory infertility (Ibanez *etal*., 2017). The reported prevalence of PCOS in the community is between 6–10% depending on which criteria is used to define it (Bozdag *etal*., 2016). PCOS is characterized by ovulatory dysfunction, hyperandrogenism, and polycystic ovarian morphology (PCOM) based on ultrasounds (Teede *etal*., 2018). Despite the high prevalence of this condition, controversy surrounds the optimum diagnostic criteria and management for the adolescent population . Hyperandrogenism is the most consistent characteristic of PCOS in both adults and adolescents .(Couto Alves *etal*., 2017). Adult patients with PCOS commonly present with pathognomonic symptoms during adolescence. There is value in early identification of poly cystic ovary syndrome to optimally manage the associated long-term metabolic and reproductive health sequalae (Elhayek *etal*., 2016).

Treatment should be tailored to the individual and account for the nuances of this chronic condition when diagnosed in young women. Participants were evaluated either during the early follicular phase of the menstrual cycle (in women reporting regular menstrual cycles or the use of hormonal contraception) or at a random time when no dominant follicles or corpora lutea were present (in women reporting irregular menstrual cycles).

Poly cystic ovary was diagnosed according to the recommended thresholds of the 2018 International Evidence-based Guidelines for the Assessment and Management of PCOS (Bozdag *etal*., 2016).

2:1.1 Pathogenesis and Clinical Features:

Clinical features of PCOS include clinical hyperandrogenism in the form of hirsutism, acne, or alopecia. Menstrual irregularity encompasses primary or secondary amenorrhea, oligo menorrhea, irregular periods, and heavy menstrual bleeding. Clinical tests show poly cystic ovarian morphology (PCOM) on ultrasound, and / or metabolic derangement on blood testing , including insulin resistance, glucose intolerance, obesity and dyslipidaemia . There can be a marked heterogenicity in its clinical presentation (Morris *etal*., 2017).There is a complex relationship with genetic, metabolic, endocrine, environmental, and life style factors in poly cystic syndrome, and the a etiology that remains poorly understood(Rosenfield *etal*., 2016) (Bellver *etal*., 2018). Established the ories include disordered neuroendocrine gonadotropin secretion, hyper androgenism, insulin resistance , or a combination of these (Bellver *etal*., 2018).

2:1.2. Diagnostic Criteria:

In 2012 Embryology/American Society (EAS) for Reproductive Medicine Consensus work shop group reported that all three of the Rotterdam criteria

should be fulfilled for the diagnosis of poly cystic ovary syndrome (PCOS) in adolescents (Teed *etal*., 2018).In 2018, international evidence-based guidelines stated that for young patients<8 years post menarche, both hyper androgenism and ovulatory dysfunction must be present for the diagnosis of PCOS to be made (Teed *etal*., 2018). The appearance of polycystic ovarian morphology (PCOM) is of less value due to its lack of reliability as pathologic finding in the 8 years following menarche there was also a revision in the ultrasound measurement of the follicle number per ovary (FNPO), with a recommendation of a FNPO of at least 20 and/or ovarian volume of at least 10 mL to ensure no dominant follicles are present (Teed *etal*., 2018).

2:1.3. Laboratory test:

Measurement of total and free testosterone remains the mainstay of the biochemical analysis of hyperandrogenemia However, there is a lack of consensus in regard to the preferred androgen assay and reference values for adolescents. Laboratory workup should be individualized to exclude other causes of hyperandrogenism. Generally, this workup includes 17-hydroxyprogesterone (17-OHP) , androstenedione , free thyroxine (FT4), thyroid stimulating hormone (TSH), luteinizing hormone (LH), follicle stimulating hormone(FSH), and prolactin. Dehydroepiandrosteronesulphate (DHEAS) and androstenedione have a limited

role in assessment of PCOS, but are useful in the exclusion of other causes of hyperandrogenemia, including CAH, although this may be mildly elevated in AYAs with PCOS. If there is an abnormality in androstenedione or 17-OHP, a cosyntropin (ACTH) stimulation test should be ideally performed to screen for non-classical congenital adrenal hyperplasia (CAH) (Ates *etal*., 2018).

2:1.4. Prognosis of PCOS:

Women with PCOS are at risk for endometrial hyperplasia and endometrial cancer, insulin resistance and type II diabetes, hypertension, depression, psychological disorders, dyslipidemia, cardiovascular diseases, cerebrovascular stroke, weight gain, sleep apnea, non-alcoholic fatty liver disease, acanthosisnigricans (patches of darkened skin under the arms, in the groin, on the back of the neck) and autoimmune thyroiditis. Early diagnosis and treatment may reduce the risk of these complications, such as type 2 diabetes and heart disease (Orlowski *etal*., 2018).

2:1:5. Management of PCOS:

Treatment should be tailored to the needs of the individual. The aims of treatment are to improve quality of life and long term health outcomes, whilst balancing the side effects of treatment (Alkhalifa et al., 2016).

According to the consensus document, additional management considerations for adolescents with PCOS symptoms include:

A definitive diagnosis of PCOS is not necessary prior to initiating treatment. Treatment may decrease risk of future co-morbidity, even in the absence of a definitive diagnosis. Deferring the diagnosis of PCOS while symptomatic treatment and providing regular follow up is recommended. Obesity, hyperinsulinemia, and insulin resistance are recognized as common in adolescents with PCOS. Other causes of hyperandrogenemia and irregular menstrual periods must be ruled out before a diagnosis of PCOS can be established. Lifestyle modifications remain the first line treatment in Adolescent and Young adults (AYAs) who are overweight or obese.

2.1.6 Characteristic of PCOS

2.1.6.1 Hyperandrogenism

Puberty is characterized by physiological hyperandrogenism (Kahsar-Miller et al., 2001). Multiple. This can confound with pathological hyperandrogenism and therefore cloud the picture of PCOS (Moll and Rosenfield, 1983; Van Hooff et al., 2000; Mortensen et al., 2009; Rosenfield, 2013). Measurement of testosterone levels does not resolve this uncertainty because testosterone concentrations are highly influenced by the stage of puberty and the menstrual cycle along with other factors (Witchel et al., 2015). In addition, no cutoff values or reference ranges for androgen levels are well defined in female adolescents (Legro et al., 2013). Moreover, acne, which is largely seen during puberty, is not correlated with hyperandrogenism (Hickey et al., 2011). Furthermore, diagnosing hirsutism is challenging since the standardized scoring value does not take into consideration ethnic variations (Ferriman and Gallwey, 1961).

2.1.6.2 Menstrual irregularity

Adolescents frequently exhibit physiological menstrual irregularities such as oligomenorrhea (Powers et al., 2015), usually during the first 2 years after menarche, due to lack of maturation of hypothalamic-hypopituitary-ovarian axis (Tfayli and Arslanian, 2008). As such, menstrual irregularity can be sometimes an unreliable criterion for diagnosis of PCOS in adolescents (Kamangar et al., 2015). Through close observation of the menstrual cycle patterns, clinicians have to differentiate physiological anovulation associated with puberty from pathological anovulation as a dysfunction identified in PCOS (Franks, 2002; Wiksten-Almstromer et al., 2008). It has been suggested to postpone diagnosis at least 2 years after menarche to establish a persistent menstrual irregularity (Hardy and

Norman, 2013). However, this may delay the initiation of appropriate treatment (Powers et al., 2015).

2.1.6.3 Polycystic ovaries on ultrasonography

Normal physiological changes and variations in the volume and size of the ovaries during puberty make ultrasonography findings controversial for the diagnosis of PCOS (Dewailly et al., 2014). Also, performing a transrectal or transvaginal ultrasonography in adolescents may not be always applicable, which may delay diagnosis (Venturoli et al., 1995). For diagnostic purposes, normal ovarian volume in female adolescents is considered equal to or less than 10 ml (Carmina *etal* .,2010).

2.1.7 Associated morbidities

2.1.7.1 Obesity

Obesity is considered one of the most important features of PCOS. Its prevalence in diseased women varies between 61 and 76% (Glueck et al., 2005). The prevalence of obesity reaches 80% in the United States (Ehrmann et al., 1999) and 50% outside (Balen et al., 1995) which indicates that this figure depends on local environmental factors, ethnic backgrounds, and lifestyle, and not on the mere presence of PCOS itself. Childhood obesity is a well-documented risk factor for PCOS. Obese girls are at a higher risk of developing insulin resistance, metabolic syndrome, and PCOS later on in life (Pasquali et al., 2011). On the other hand, women with PCOS are at a higher risk of developing obesity (Randeva et al., 2012). Many studies explain that females with PCOS have increased visceral and subcutaneous body fat distribution due to increased androgen production rates (Kirschner et al., 1990); this central obesity follows a masculinized body fat distribution (Borruel et al., 2013) where the amount of visceral fat correlates with the degree of insulin resistance (Karabulut et al., 2012). Moreover, obesity plays a

significant role in expressing the metabolic features of PCOS. Women with PCOS have an atherogenic lipid profile, associated with elevated levels of low-density lipoprotein, triglycerides and cholesterol, along with decreased levels of high-density lipoprotein. They are also at a higher risk of developing atherosclerosis, arterial stiffness (Karabulut et al., 2012).

2.1.7.2 Insulin resistance

A great deal of attention has been given to the metabolic disturbances that accompany PCOS, as well as to the consequences of these disturbances later in life. Today, insulin resistance is considered the main pathogenic factor in the background of increased metabolic disturbances in women with PCOS (Siklar et al., 2015) which can explain hyperandrogenism, menstrual irregularity, and other metabolic manifestations seen in this disease (Baillargeon et al., 2003; Diamanti-Kandarakis and Dunaif, 201 2) In 1980, the association between hyperinsulinemia and PCOS was first noted by Burghen et al. who found a significant positive correlation between insulin, androstenedione and testosterone levels among PCOS women (Burghen et al., 1980). In fact, recent studies show that hyperinsulinemia is present in 85% of patients with PCOS, including 95% of obese and 65% of lean affected women (Teede et al., 2010, 2011; Stepto et al., 2013). Increased insulin levels in patients with PCOS may, along with the high levels of luteinizing hormone, trigger the arrest of follicular growth which contributes to anovulation (Dunaif, 1995). Hyperinsulinemia also alters the gonadotropin-releasing hormone (GnRH) pulse secretion pattern, suppresses the sex hormone-binding globulin (SHBG) and potentiates ovarian androgen production in women with PCOS (Bhattacharya and Jha, 2011; Hart et al., 2011; Huang et al., 2011; Lass et al., 2011; Rathsman et al., 2012; Wedin et al., 2012).

Multiple studies supported a correlation between diabetes and PCOS and showed that insulin-sensitizing drugs and dietary/lifestyle modifications improve hyperandrogenism in patients suffering from PCOS (Baillargeon et al., 2003; Diamanti-Kandarakis and Dunaif, 2012). When the hormone leptin is used as insulin-sensitizing agent, it decreases androgen levels and induces menstruation in affected lean women (Lungu et al., 2012). Other studies showed that 6 months of lifestyle modifications enhanced insulin sensitivity by 70% and significantly reduced anovulation in affected obese women (Baillargeon et al., 2003; Diamanti-Kandarakis and Dunaif, 2012). These studies provide support that insulin resistance aggravates hyperandrogenemia (Lungu et al., 2012). This is one of the critical junctures in the treatment of PCOS, which led to the consideration of insulin-mimetic or insulin-sensitizing agents as part of the management of the disease. These agents, as mentioned later in the review, include metformin, myo-inositol supplements, and thiazolidinedione. Finally, according to the Diabetes Prevention Program (DPP) Research Group (2002), PCOS patients should be tested for insulin resistance. A few biomarkers have been used to detect insulin resistance in PCOS women. For instance, insulin restrains the release of sex hormone binding globulin (SHBG) from the liver and the production of insulin-like growth factor binding protein 1 (IGFBP-1) (Kalme et al., 2003). It also affects the homeostatic model assessment (HOMA-IR) which is based on computations of fasting glucose and levels of insulin (Legro et al., 2004). Yet, different markers have variable sensitivities and specificities in insulin resistance testing (Nawrocka-Rutkowska et al., 2013). Thus, research should identify one universal marker for accurate diagnosis of decreased insulin sensitivity in patients with PCOS. If such a marker is identified, early detection of insulin resistance can lead to prompt treatment and prevention of any complication (Nawrocka-Rutkowska et al., 2013).

2.1.7.3 Type II diabetes mellitus

PCOS confers a substantially increased risk for type 2 diabetes mellitus and gestational diabetes from early ages (Randeva et al., 2012). About 1 in 5 women with PCOS will develop type II diabetes (Dunaif, 1999) making impaired glucose tolerance a common abnormality in this disease (Randeva et al., 2012). Cross-sectional and prospective longitudinal studies have consistently shown that women suffering from PCOS have a higher risk of developing type II diabetes mellitus or impaired glucose tolerance compared to control populations matched for age and ethnic background (Ehrmann et al., 1999; Legro et al., 1999, 2005; Boudreaux et al., 2006; Talbott et al., 2007; Lerchbaum et al., 2013). Furthermore, prospective longitudinal studies in young and middle-aged women with PCOS show a higher risk for developing diabetes later in life and is mainly due to an increased prevalence of obesity and insulin resistance among these patients (Dunaif, 1999).

Interestingly, family history of diabetes increases the prevalence of type II diabetes mellitus in PCOS patients. However, the prevalence of diabetes in PCOS patients with no family history of diabetes was still much higher than normal women (Ehrmann et al., 1995). Even though family history and obesity are major contributors in the development of diabetes in PCOS patients, diabetes can still occur in lean PCOS patients who have no family history, mainly secondary to insulin resistance (Dunaif, 1999).

2.1.7.4 Cardiovascular disease

In 1992, Dahlgren et al. identified a 7 times higher risk of myocardial infarction in patients with PCOS compared to healthy controls (Dahlgren et al., 1992). However, in 1998, an epidemiological study by Pierpoint et al. showed no difference in the risk between the two groups (Pierpoint et al., 1998).

More recent data showed that patients with PCOS have significantly elevated levels of circulating biomarkers of CVD, including C-reactive protein (Bahceci et al., 2004; Meyer et al., 2005) and lipoprotein A (Yilmaz et al., 2005; Bahceci et al., 2007; Berneis et al., 2009; Rizzo et al., 2009), in comparison to matched controls. Other studies demonstrated a higher burden of indicators of atherosclerosis with early onset cardiovascular dysfunction, i.e., arterial stiffness, endothelial dysfunction, and coronary artery calcification (Meyer et al., 2005; Moran et al., 2009).

In 2010, the Androgen Excess-PCOS society provided a consensus statement about increased risk of CVD in women with PCOS and developed a guideline to prevent such complication (Wild et al., 2010). Yet, despite the increased cardiovascular risk markers and the indubitable presence of CVD risk factors in this population, uncertainty remains regarding the increased cardiovascular morbidity and mortality in patients with PCOS (Legro, 2003; Wild et al., 2010; Schmidt et al., 2011; Sathyapalan and Atkin, 2012). Discrepancy between studies might be due to the heterogeneous nature of the populations studied. Therefore, supplementary methodologically rigorous trials are needed to determine the absolute risk of CVD in patients with PCOS throughout age ranges (Wild et al., 2010).

2.1.7.5 Infertility

Women with PCOS may have reduced fertility (Hart and Norman, 2006; Hart and Doherty, 2015) due to the associated endocrine and gynecologic abnormalities that impact ovarian quality and function (Hart and Norman, 2006). Accounting for up to 90% of ovulatory disorders (Hull, 1987), PCOS-associated persistent periods of anovulation are positively correlated with infertility (Imani et al., 1998). In 1995, a study reported up to 50 and 25% of women in a PCOS population suffering from primary and secondary infertility respectively (Balen et al., 1995). More recently in 2015, a study by Hart and Doherty showed that infertility is 10 times more

common among women with PCOS in comparison to healthy controls (Hart and Doherty, 2015).

On the other hand, some studies suggested that females with PCOS who conceive might suffer from pregnancy-related complications such as gestational diabetes (Bruyneel et al., 2014).

2.1.7.6 Cancer

Females suffering from PCOS present many risk factors associated with the development of endometrial cancer, such as obesity, insulin resistance, type II diabetes mellitus, and anovulation (Legro et al., 2013). Anovulation triggers an unopposed uterine estrogen exposure. This can subsequently trigger the development of endometrial hyperplasia and ultimately endometrial cancer (Hart and Norman, 2006). As a matter of fact, studies show that women with PCOS have a three-fold increased risk of developing endometrial cancer (Chittenden et al., 2009; Fauser et al., 2012; Haoula et al., 2012; Dumesic and Lobo, 2013) which is mostly well differentiated with a good prognosis (Fauser et al., 2012). Regardless, no data support ultrasound screening for endometrial thickness in women with PCOS, which comes in agreement with the American Cancer Society against screening for endometrial cancer in patients with average or increased risk. Yet women should be advised to notify their healthcare provider for any spotting or unexpected bleeding (Smith et al., 2001).

On the other hand, there are limited data to support any association between PCOS and breast and ovarian cancer (Chittenden et al., 2009; Fauser et al., 2012).

2.2.7 Psychological wellbeing

Psychological stress and PCOS have been shown to be intimately related. A vast number of studies showed that women with PCOS are more prone to suffer from

psychological disorders such depression (Veltman-Verhulst et al., 2012), anxiety (Jedel et al., 2010; Veltman-Verhulst et al., 2012), recreational drug-related incidents (Hart and Doherty, 2015), disordered eating, and psychosexual dysfunction (Deeks et al., 2010; Teede et al., 2011) in comparison to healthy female controls. In addition, females with PCOS have a lower self-esteem and body satisfaction (Weiner et al., 2004; Himelein and Thatcher, 2006) and subsequently tend to have more psychiatric hospital admissions than controls (Hart and Doherty, 2015). As a result, they display a low quality of life (Jones et al., 2008; Li et)

2.2 Luteinizing hormone (LH)

(LH, also known as lutropin and sometimes lutrophin) is a hormone produced by gonadotropic cells in the anterior pituitary gland. In females, an acute rise of LH ("LH surge") triggers ovulation and development of the corpus luteum. In males, where LH had also been called interstitial cell–stimulating hormone (ICSH), Häggström M (2014) it stimulates Leydig cell production of testosterone. It acts synergistically with FSH (Nosek, Thomas M. Essentials of Human Physiology. Section 5/5ch9/s5ch9_5).

2.2.1 Structure of LH:

LH is a heterodimeric glycoprotein. Each monomeric unit is a glycoprotein molecule; one alpha and one beta subunit make the full, functional protein.

Its structure is similar to that of the other glycoprotein hormones, follicle-stimulating hormone (FSH), thyroid-stimulating hormone (TSH), and human chorionic gonadotropin (hCG). The protein dimer contains 2 glycopeptidic subunits, labeled alpha and beta subunits, that are non-covalently associated (i.e., without any disulfide bridge linking them): *Häggström M (2014).*

- The *alpha subunits* of LH, FSH, TSH, and hCG are identical, and contain 92 amino acids in human but 96 amino acids in almost all other vertebrate species (glycoprotein hormones do not exist in invertebrates). *Häggström M (2014)* .
- The *beta subunits* vary. LH has a beta subunit of 120 amino acids (LHB) that confers its specific biologic action and is responsible for the specificity of the interaction with the LH receptor. This beta subunit contains an amino acid sequence that exhibits large homologies with that of the beta subunit of hCG and both stimulate the same receptor. However, the hCG beta subunit contains an additional 24 amino acids, and the two hormones differ in the composition of their sugar moieties. *Häggström M (2014.)*

The different composition of these oligosaccharides affects bioactivity and speed of degradation. The biologic half-life of LH is 20 minutes, shorter than that of FSH (3–4 hours) and hCG (24 hours). The biological half-life of LH is 23 hours subcutaneous or terminal half life of 10-12 hours (le Cotonnec JY, Porchet HC, Beltrami V, Munafo A (February 1998).

2.2.2 Genes

The gene for the *alpha subunit* is located on chromosome 6q12.21.

The luteinizing hormone *beta subunit* gene is localized in the LHB/CGB gene cluster on chromosome 19q13.32. In contrast to the alpha gene activity, beta LH subunit gene activity is restricted to the pituitary gonadotropic cells. It is regulated by the gonadotropin-releasing hormone from the hypothalamus. Inhibin, activin, and sex hormones do not affect genetic activity for the beta subunit production of LH. *Häggström M (2014)*

2.2.3 Function:

In females: ovulation, maintaining of corpus luteum and secretion of progesterone.

In males: testosterone secretion.

2.2.4 Effects in females

LH supports theca cells in the ovaries that provide androgens and hormonal precursors for estradiol production. At the time of menstruation, FSH initiates follicular growth, specifically affecting granulosa cells. With the rise in estrogens, LH receptors are also expressed on the maturing follicle, which causes it to produce more estradiol. Eventually, when the follicle has fully matured, a spike in 17α-hydroxyprogesterone production by the follicle inhibits the production of estrogens, leading to a decrease in estrogen-mediated negative feedback of Gn RH in the hypothalamus, which then stimulates the release of LH from the anterior pituitary. However another theory of the LH peak is a positive feedback mechanism from estradiol. The levels keep rising through the follicular phase and when they reach an unknown threshold, this results in the peak of the LH (Guyton and Hall Textbook of Medical Physiology 2006 page 1021).

This effect is opposite from the usual negative feedback mechanism presented at lower levels. In other words, the mechanism(s) are not yet clear. The increase in LH production only lasts for 24 to 48 hours. This "LH surge" triggers ovulation, thereby not only releasing the egg from the follicle, but also initiating the conversion of the residual follicle into a corpus luteum that, in turn, produces progesterone to prepare the endometrium for a possible implantation. LH is necessary to maintain luteal function for the second two weeks of the menstrual cycle. If pregnancy occurs, LH levels will decrease, and luteal function will instead

be maintained by the action of hCG (human chorionic gonadotropin), a hormone very similar to LH but secreted from the new placenta. *Häggström M (2014)*

Gonadal steroids (estrogens and androgens) generally have negative feedback effects on GnRH-1 release at the level of the hypothalamus and at the gonadotropes, reducing their sensitivity to GnRH. Positive feedback by estrogens also occurs in the gonadal axis of female mammals and is responsible for the midcycle surge of LH that stimulates ovulation. Although estrogens inhibit kisspeptin (Kp) release from kiss1 neurons in the ARC, estrogens stimulate Kp release from the Kp neurons in the AVPV. As estrogens' levels gradually increase the positive effect predominates, leading to the LH surge. GABA-secreting neurons that innervate GnRH-1 neurons also can stimulate GnRH-1 release. These GABA neurons also possess ERs and may be responsible for the GnRH-1 surge. Part of the inhibitory action of endorphins on GnRH-1 release is through inhibition of these GABA neurons. Rupture of the ovarian follicle at ovulation causes a drastic reduction in estrogen synthesis and a marked increase in secretion of progesterone by the corpus luteum in the ovary, reinstating a predominantly negative feedback on hypothalamic secretion of GnRH- (Norris DO *et al.,* 2013)

2.2.5 Effects in males:

LH acts upon the Leydig cells of the testis and is regulated by gonadotropin-releasing hormone (GnRH). The Leydig cells produce testosterone (T) under the control of LH, which regulates the expression of the enzyme 17β-hydroxysteroid dehydrogenase that is used to convert androstenedione, the hormone produced by the testes, to testosterone. The onset of puberty is controlled by two major hormones: FSH initiates spermatogenesis and LH signals the release of testosterone. an androgen that exerts both endocrine activity and intratesticular

activity on spermatogenesis. LH is released from the pituitary gland, and is controlled by pulses of gonadotropin-releasing hormone. When T levels are low, it stimulating the pituitary gland to release LH ("Male Medical Fertility Treatment: HCG + LH + Recombinant FSH To Increase Sperm Count Through Spermatogenisis". *Archived* from the original on February 19, 2015. Retrieved 6 April 2015). As the levels of T increase, it will act on the pituitary through a negative feedback loop and inhibit the release of GnRH and LH consequently. Androgens (T, DHT) inhibit monoamine oxidase (MAO) in pineal, leading to increased melatonin and reduced LH and FSH by melatonin-induced increase of Gonadotropin-Inhibitory Hormone (GnIH) synthesis and secretion. T can also be aromatized into estradiol (E2) to inhibit LH. E2 decreases pulse amplitude and responsiveness to GnRH from the hypothalamus onto the pituitary. Changes in LH and testosterone (T) blood levels and pulse secretions are induced by changes in sexual arousal in human males (Stoléru SG, Ennaji A, Cournot A, Spira A (1993). "LH pulsatile secretion and testosterone blood levels are influenced by sexual arousal in human males". *Psychoneuroendocrinology*. **18**(3): 205–18.).

2.2.6 Normal Levels

LH levels are normally low during childhood and, in women, high after menopause. As LH is secreted as pulses, it is necessary to follow its concentration over a sufficient period of time to get proper information about its blood level.

During the reproductive years, typical levels are between 1–20 IU/L. Physiologic high LH levels are seen during the LH surge (v.s.); typically they last 48 hours.

In males over 18 years of age, reference ranges have been estimated to be 1.8–8.6 IU/L (Mayo , 2012).

LH is measured in international units (IU). When quantifying the amount of LH in a sample in IUs, it is important to know which international standard your lot of LH was calibrated against, as they can vary broadly from year to year. For human urinary LH, one IU is most recently defined as 1/189th of an ampule denoted 96/602 and distributed by the NIBSC, corresponding to approximately 0.04656µg of LH protein for a single IU, but older standard versions are still widely in use.

The detection of a surge in release of luteinizing hormone indicates impending ovulation. LH can be detected by urinary ovulation predictor kits (OPK, also LH-kit) that are performed, typically daily, around the time ovulation may be expected. A conversion from a negative to a positive reading would suggest that ovulation is about to occur within 24–48 hours, giving women two days to engage in sexual intercourse or artificial insemination with the intention of conceiving.

The recommended testing frequency differs between manufacturers. For example, the Clearblue test is taken daily, and an increased frequency does not decrease the risk of missing an LH surge. On the other hand, the Chinese company *Nantong Egens Biotechnology* recommends using their test twice per day. If testing once per day, no significant difference has been found between testing LH in the morning versus in the evening, in relation to conception rates, and recommendations of what time in the day to take the test varies between manufacturers and healthcare workers. Tests may be read manually using a color-change paper strip, or digitally with the assistance of reading electronics (Martinez AR, Bernardus RE, Vermeiden JP, Schoemaker J (1994)).

Tests for luteinizing hormone may be combined with testing for estradiol in tests such as the Clearblue fertility monitor. The sensitivity of LH tests are measured in milli international unit, with tests commonly available in the range 10–40 m.i.u.

(the lower the number, the higher the sensitivity). As sperm can stay viable in the woman for several days, LH tests are not recommended for contraceptive practices, as the LH surge typically occurs after the beginning of the fertile window ("Ovulation Predictor Kit Frequently Asked Questions". Fertility Plus. Archived from the original on March 12, 2012. Retrieved 12 March 2012).

2.2.7 Excess of LH

In children with precocious puberty of pituitary or central origin, LH and FSH levels may be in the reproductive range instead of the low levels typical for their age.

During the reproductive years, relatively elevated LH is frequently seen in patients with polycystic ovary syndrome; however, it would be unusual for them to have LH levels outside of the normal reproductive range . Persistently high LH levels are indicative of situations where the normal restricting feedback from the gonad is absent, leading to a pituitary production of both LH and FSH. While this is typical in menopause, it is abnormal in the reproductive years. There it may be a sign of :

Premature menopause, Gonadal dysgenesis, Turner syndrome, Castration, Swyer syndrome, Polycystic ovary syndrome, Certain forms of congenital adrenal hyperplasia, Testicular failure or Pregnancy - BetaHCG can mimic LH so tests may show elevated LH .

2.2.8 Deficiency of LH

Diminished secretion of LH can result in failure of gonadal function (hypogonadism). This condition is typically manifest in males as failure in production of normal numbers of sperm. In females, amenorrhea is commonly observed. Conditions with very low LH secretions include:

Pasqualini syndrome , Kallmann syndrome, Hypothalamic suppression, Hypopituitarism, Eating disorder, Female athlete triad, Hyperprolactinemia, Hypogonadism and Gonadal suppression therapy (GnRH antagonist and GnRH agonist (inducing an initial stimulation (flare up) followed by permanent blockage of the GnRH pituitary receptor) (Valdes-Socin H *etal.*, 2017).

2.2.9 LH as a medication

LH is available mixed with FSH in the form of menotropin, and other forms of urinary gonadotropins. More purified forms of urinary gonadotropins may reduce the LH portion in relation to FSH. Recombinant LH is available as lutropin alfa (Luveris). All these medications have to be given parenterally. They are commonly used in infertility therapy to stimulate follicular development, the notable one being in IVF therapy.Often, HCG medication is used as an LH substitute because it activates the same receptor. Medically used hCG is derived from urine of pregnant women, is less costly, and has a longer half-life than LH (Luveris, 2006).

2.3 Follicle-stimulating hormone (FSH)

Follicle-stimulating hormone (FSH) secreted by the gonadotropic cells of the anterior pituitary gland ("Follicle-Stimulating Hormone". WebMD), and regulates the development, growth, pubertal maturation, and reproductive processes of the body. FSH and luteinizing hormone (LH) work together in the reproductive system ("Luteinizing and Follicle Stimulating Hormones". www.vivo.colostate.edu. Retrieved 2019).

2.3.1 Structure of FSH

FSH is a 35.5 kDa glycoprotein heterodimer, consisting of two polypeptide units, alpha and beta. Its structure is similar to those of luteinizing hormone (LH) thyroid-stimulating hormone (TSH), and human chorionic gonadotropin (hCG). The alpha subunits of the glycoproteins LH, FSH, TSH, and hCG are identical and consist of 96 amino acids, while the beta subunits vary. Both subunits are required for biological activity. FSH has a beta subunit of 111 amino acids (FSH β), which confers its specific biologic action, and is responsible for interaction with the follicle-stimulating hormone receptor. The sugar portion of the hormone is covalently bonded to asparagine, and is composed of mannose, N-acetylgalactosamine, N-acetylglucosamine, galactose, and sialic acid (Jiang X *etal*., 2012).

2.3.2 Genes

In humans, the gene for the alpha subunit is located at cytogenetic location 6q14.3. It is expressed in two cell types, most notably the basophils of the anterior pituitary. The gene for the FSH beta subunit is located on chromosome 11p13, and is expressed in gonadotropes of the pituitary cells, controlled by GnRH, inhibited by inhibin, and enhanced by activin (Online Mendelian Inheritance in Man (OMIM)).

2.3.3 Activity / functions

FSH regulates the development, growth, pubertal maturation and reproductive processes of the human body.

- In both *males* and *females*, FSH stimulates the maturation of primordial germ cells.

- In *males*, FSH induces Sertoli cells to secrete androgen-binding pr2.3.2oteins (ABPs), regulated by inhibin's negative feedback mechanism on the anterior pituitary. Specifically, activation of Sertoli cells by FSH sustains spermatogenesis and stimulates inhibin B secretion.
- In *females*, FSH initiates follicular growth, specifically affecting granulosa cells. With the concomitant rise in inhibin B, FSH levels then decline in the late follicular phase. This seems to be critical in selecting only the most advanced follicle to proceed to ovulation. At the end of the luteal phase, there is a slight rise in FSH that seems to be of importance to start the next ovulatory cycle (Dalkin *etal.,* 1999)).

Control of FSH release from the pituitary gland is unknown. Low frequency gonadotropin-releasing hormone (GnRH) pulses increase FSH mRNA levels in the rat, but is not directly correlated with an increase in circulating FSH. GnRH has been shown to play an important role in the secretion of FSH, with hypothalamic-pituitary disconnection leading to a cessation of FSH. GnRH administration leads to a return of FSH secretion. FSH is subject to oestrogen feed-back from the gonads via the hypothalamic pituitary gonadal axis (Sharma TP *etal.,* 2012).

2.3.4 Effects in females

FSH stimulates the growth and recruitment of immature ovarian follicles in the ovary. In early (small) antral follicles, FSH is the major survival factor that rescues the small antral follicles (2–5 mm in diameter for humans) from apoptosis (programmed death of the somatic cells of the follicle and oocyte). In the luteal-follicle phase transition period the serum levels of progesterone and estrogen (primarily estradiol) decrease and no longer suppress the release of FSH, consequently FSH peaks at about day three (day one is the first day of menstrual

flow). The cohort of small antral follicles is normally sufficient in number to produce enough Inhibin B to lower FSH serum levels. In addition, there is evidence that gonadotropin surge-attenuating factor produced by small follicles during the first half of the follicle phase also exerts a negative feedback on pulsatile luteinizing hormone (LH) secretion amplitude, thus allowing a more favorable environment for follicle growth and preventing premature luteinization (Fowler PA *etal.*, 2003)).

As a woman nears perimenopause, the number of small antral follicles recruited in each cycle diminishes and consequently insufficient Inhibin B is produced to fully lower FSH and the serum level of FSH begins to rise. Eventually, the FSH level becomes so high that downregulation of FSH receptors occurs and by postmenopause any remaining small secondary follicles no longer have FSH nor LH receptors. When the follicle matures and reaches 8–10 mm in diameter it starts to secrete significant amounts of estradiol. Normally in humans only one follicle becomes dominant and survives to grow to 18–30 mm in size and ovulate, the remaining follicles in the cohort undergo atresia. The sharp increase in estradiol production by the dominant follicle (possibly along with a decrease in gonadotrophin surge-attenuating factor) cause a positive effect on the hypothalamus and pituitary and rapid GnRH pulses occur and an LH surge results.

The increase in serum estradiol levels cause a decrease in FSH production by inhibiting GnRH production in the hypothalamus. The decrease in serum FSH level causes the smaller follicles in the current cohort to undergo atresia as they lack sufficient sensitivity to FSH to survive. Occasionally two follicles reach the 10 mm stage at the same time by chance and as both are equally sensitive to FSH both survive and grow in the low FSH environment and thus two ovulations can

occur in one cycle possibly leading to non-identical (dizygotic) twins (Dickerson LM *etal* ., 2008).

2.3.5 Effects in males

FSH stimulates primary spermatocytes to undergo the first division of meiosis, to form secondary spermatocytes .FSH enhances the production of androgen-binding protein by the Sertoli cells of the testes by binding to FSH receptors on their basolateral membranes, and is critical for the initiation of spermatogenesis (Boulpaep E *etal* ., 2005).

2.3.6 Measurement:

Follicle stimulating hormone is typically measured in the early follicular phase of the menstrual cycle, typically day three to five, counted from last menstruation. At this time, the levels of estradiol (E2) and progesterone are at the lowest point of the menstrual cycle. FSH levels in this time is often called *basal FSH* levels, to distinguish from the increased levels when approaching ovulation (*labtestsonline.org*. Retrieved 2019-05-06.).

FSH is measured in International Units (IU). For Human Urinary FSH, one IU is defined as the amount of FSH that has an activity corresponding to 0.11388 mg of pure Human Urinary FSH. For recombinant FSH, one IU corresponds to approximately 0.065 to 0.075 μg of a "fill-by-mass" product (Gonadotropin preparations: past, present, and future perspectives". *Fertility and Sterility*. **90** (5 Suppl): S13-20. November 2008).

2.3.7 High FSH levels

The most common reason for high serum FSH concentration is in a female who is undergoing or has recently undergone menopause. High levels of FSH indicate that the normal restricting feedback from the gonad is absent, leading to an unrestricted

pituitary FSH production. FSH may contribute to postmenopausal osteoporosis and cardiovascular disease (Zhu D *etal* , 2018)).

If high FSH levels occur during the reproductive years, it is abnormal. Conditions with high FSH levels include:

Premature menopause also known as Premature Ovarian Failure, Poor ovarian reserve also known as Premature Ovarian Aging, Gonadal dysgenesis, Turner syndrome, Castration, Swyer syndrome, Certain forms of CAH, Testicular failure, Klinefelter syndrome and Systemic Lupus Erythematosus also known as Lupus

Most of these conditions are associated with subfertility and/or infertility. Therefore, high FSH levels are an indication of subfertility and/or infertility(Li *etal* ., 2005)).

2.3.8 Low FSH levels

Diminished secretion of FSH can result in failure of gonadal function (hypogonadism). This condition is typically manifested in males as failure in production of normal numbers of sperm. In females, cessation of reproductive cycles is commonly observed. Conditions with very low FSH secretions are:

Polycystic Ovarian Syndrome ("Polycystic ovary syndrome: MedlinePlus Medical Encyclopedia". *medlineplus.gov*. Retrieved 2019), Polycystic Ovarian Syndrome + Obesity + Hirsutism + Infertility, Kallmann syndrome, Hypothalamic suppression, Hypopituitarism, Hyperprolactinemia, Gonadotropin deficiency and Gonadal suppression therapy.

Isolated FSH deficiency due to mutations in the gene for β-subunit of FSH is rare with 13 cases reported in the literature up to 2019 (Misgar RA *etal.*, 2019).

Chapter Three

Materials and Methods

3. Martials and Methods

3.1 Study design

case control study.

3.2 Study area:

Conducted at Khartoum state.

3.3 Study population:

The study population included women in reproductive age suffer from PCOS

3.4 Sample size:

One hundred sample have been collected 50 sample as test and 50 as control women selected by conventional random sampling because limited resource

3.5 Inclusion criteria:

women with PCOS.

3.6 Exclusion criteria:

Presence of other infertility disorders, pregnancy, and Diabetic women excluded.

3.7 Ethical consideration:

All samples and information are collected under agreement of patients; 100 sample have been collected from vinous blood collected in the plane container. this information is used for research only

3.8 Data collection method:

Data were collected by questionnaire (Appendix I) .

3.9 Blood sampling :

In this study 3ml of venous blood was collected by standard procedure plane container from each participant in this study.

3.10. Methodology:

LH

3.10.1 Principle (Appendix II)

Immunoenzymometric assay The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies 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.

3.10.2 Method:

Wash buffer;

Dilute content of wash concentrate to 1000 ml with distilled or deionized water in a suitable storage container. store at room temperature 20 -27 for up to 60 days.

Test procedure;

Before proceeding with the assay bring all reagent serum references and controis to room temperature

Format the micro plate wells for each serum reference, control and patients specimen to be assayed in duplicate.

Replace any unused micro well strips back into the aluminum bag seal and store at 2-8

Pipette 0.050 ml of the appropriate serum reference, control or specimen into assigned well,

Add 0.100 ml of LH-Enzyme reagent to all well

swirl the microplate gently for 20-30 second to mix and cover.

incubate 60 minute at room temperature.

Discard the contents of microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.

Add 0.3 ml of wash buffer, decant or aspirate. Repeat two additional times for a total of three washes.

Add 0.100 ml of substrate solution to all wells.

Incubate at room temperature for 15 minutes.

Add 0.05 ml of stop solution to each well and gently mix for 15-20 second.

Read absorbance in each well in 620-630nm.

3.10.3 Quality control:

Each Lab rotary should assay control at level s in the low, normal and range for monitoring assay performance. these control should be treated as unknowns and values determined in every test procedure performed. Quality Control charts should be maintained to follow the performance of the supplied reagent.

FSH

3.10.4 Method:

Wash buffer;

Dilute content of wash concentrate to 1000 ml with distilled or deionized water in a suitable storage container. store at room temperature 20 -27 for up to 60 days.

Test procedure:

Before proceeding with the assay bring all reagent serum references and controls to room temperature

Formate the micro plate wells for each serum reference, control and patient's specimen to be assayed in duplicate.

Replace any unused micro well strips back into the aluminum bag seal and store at 2-8

Pipette 0.050 ml of the appropriate serum reference, control or specimen into assigned well,
Add 0.100 ml of FSH-Enzyme reagent to all well

swirl the microplate gently for 20-30 second to mix and cover.

incubate 60 minute at room temperature.

Discard the contents of microplate by decantation or aspiration. If decanting , blot the plate dry with absorbent paper.

Add 0.3 ml of wash buffer, decant or aspirate. Repeat two additional times for a total of three washes.

Add 0.100 ml of single substrate solution to all wells.

Incubate at room temperature for 15 minutes.

Add 0.05 ml of stop solution to each well and gently mix for 15-20 second.

Read absorbance in each well in 620-630nm.

The result should be read within 30 minute of adding stop solution

3.10.5 Quality control:

Each Lab rotary should assay control at level s in the low, normal and range for monitoring assay performance .these control should be treated as unknowns and values determined in every test procedure performed .Quality Control charts should be maintained to follow the performance of the supplied reagent.

3.11. Data analysis:

Data was analyzed to obtain means and standard deviation of the result using statistical package for social science (SPSS) computer Programmed version 20.

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Chapter Four

Results

4 Result

4-1 Base line characteristics of pati

Table (4-1) the frequency analysis to Menstrual regulation showed that, 20(40%) are regular and 30 (60%) Irregular.

Frequency analysis to infertility showed that, 14(28%) are infertile and 36(72%) fertile.

Frequency analysis to medication showed that, 24(48%) take medication and 26(52%) do not take medication.

Frequency analysis to cream showed that, 30(60%) using cream and 20(40%) do not using cream.

Frequency analysis to Duration showed that, 41(82%) form one month to5year and 9(18%) from six to 10 year.

4.2 Distribution of patients according to BMI classification

Table (4-2) the frequency analysis to BMI showed that 14(28%) normal weight, 14(28%) over weight,modrate obse16(32%), 6(12%) obese.

4.3 Mean of study variable among study population

Table (4-3) shows mean of study variable, the range of age from 15to 45 years with mean 29.04±6.948 , the range of BMI from 19.5 to 41.4 kg/m2 with mean 29.02±5.168 , and range of duration from 1month to 5 years with mean 1.180±0.3880 .

4.4 Comparison of LH and FSH in case versus control group

Table (4-4) shows that LH was significantly increased in PCOS patients comparing with control group (p. value 0.149) while FSH in PCOS patients comparing with control group (p.value0.580) show no change , result expressed as (mean ± SD)

Comparison of LH and FSH across MC

Table (4-5) show there was significant increase in LH in patients with Irregular menstrual cycle compared to that regular (p-value 0.281).

Comparison of LH and FSH across Family history

Table (4.6) showed that there was significant difference between how have history & non have history in the level of LH in PCOS disease patients.

comparisons of LH and FSH across medication

Table (4.7) showed that there was insignificant difference between patients had use medication and not use medication in the level of LH in PCOS disease patients.

comparisons of LH and FSH across Cream

Table (4.8) showed that there was insignificant difference between patients had used cream and those are not use cream in the level of LH in PCOS disease patients.

Table (4-1) Baseline characteristics of patients

Variables	Frequency	Percentage (%)
Family History		
Yes	14	28%
No	36	72%
Infertility		
Yes	14	28%
No	36	72%
Medication		
Use	24	48%
Not use	26	52%
cosmetics		
Yes	**28**	**56%**
No	**22**	**44%**
Duration of Irregular Menstrual Cycle		
<5year	**28**	**56%**
5year- 10year	**22**	**44%**
Menstrual cycle		
Regular (R)	**20**	**40%**
Irregular (IR)	**30**	**60%**
Total	**50**	**100%**

Table (4-2) Distribution of patients according to BMI classification

Variable	Frequency	Percentage (%)
Under weight	0	0
Normal weight	14	28%
Over weight	14	28%
Moderate Obese	16	32%
Obese	6	12%
Total	**50**	**100.0**

*underweight<13kg/m2

*normal weight18-24 kg/m2

*over weight 25-29 kg/m2

*moderate obese 30-34 kg/m2

*obese>35 kg/m2

Table (4-3) Mean of age, BMI and duration in case group

Variables	Mean± SD
BMI kg/m2	29.02±5.17
Age (15-45) years	29.04±6.95
Duration (1 - 10) years	4.76±3.40

Table (4-4) Mean comparison of ,LH and FSH in case versus control group

Parameters	Case (Mean± SD)	Control (Mean± SD)	*P-value*
LH (ng/dI)	12.19 ±20.60	7.32 ±11.90	0.149
FSH (ng/dI)	6.83 ±6.08	6.35 ±2.36	0.580

Table (4-5) Mean comparison of LH and FSH across Menstrual cycle regularity

Parameters	**Regular Cycle N(20)(Mean± SD)**	**Irregular Cycle (30) (Mean± SD)**	***P-value***
LH (ng/dI)	9.17 ±11.69	16.74 ±29.16	0.281
FSH (ng/dI)	7.38 ±5.05	5.99 ±7.43	0.470

Table (4-6) Mean comparison of , LH and FSH family History

Parameters	**Yes N (14)(Mean±SD)**	**No (36) (Mean±SD)**	***P-value***
LH (ng/dI)	8.54 ±14.34	13.62 ±22.60	0.350
FSH (ng/dI)	7.92 ±5.79	6.40 ±6.22	0.425

Table (4-7) Mean○ comparison of LH and FSH across infertility medication

Parameters	**Yes N(24) (Mean±SD)**	**No N(26) (Mean±SD)**	***P-value***
LH (ng/dI)	9.00+11.73	15.14+26.21	0.286
FSH (ng/dI)	7.98+ 5.25	5.76+6.69	0.196

Table (4-8) Mean comparison of, LH and FSH across using cosmetics

Parameters	**Yes 28 (Mean±SD)**	**N0 22 (Mean±SD)**	***P-value***
LH (ng/dI)	7.67+11.95	15.40+28.07	0.378
FSH (ng/dI)	7.27 +5.21	6.26+7.14	0.580

. **Table (4-9) Mean comparison of LH and FSH across Infertility**

Parameters	**R N(20)(Mean± SD)**	**IR(30) (Mean± SD)**	***P-value***
LH (ng/dI)	8.54+14.34	13.62+22.60	0.149
FSH (ng/dI)	7.92+5.79	6.40+6.22	0.580

Table (4-10) The Ratio between LH and FSH in case and control

Parameters	**Case**	**Control**
LH / FSH Ratio	2:1	1:1

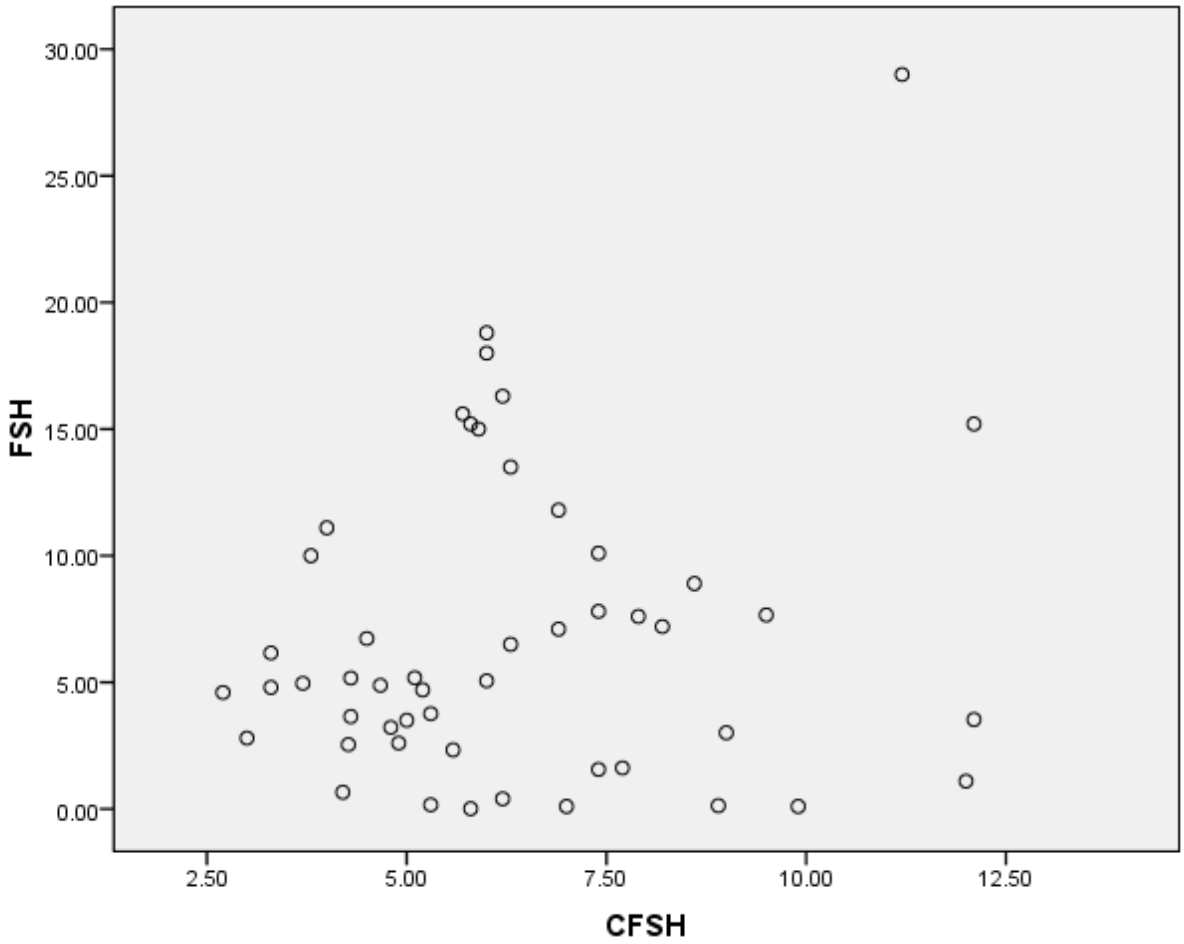

Figure 4.1(F) correlation between FSH and cFSH (R = 0.172**, P = 0.233)

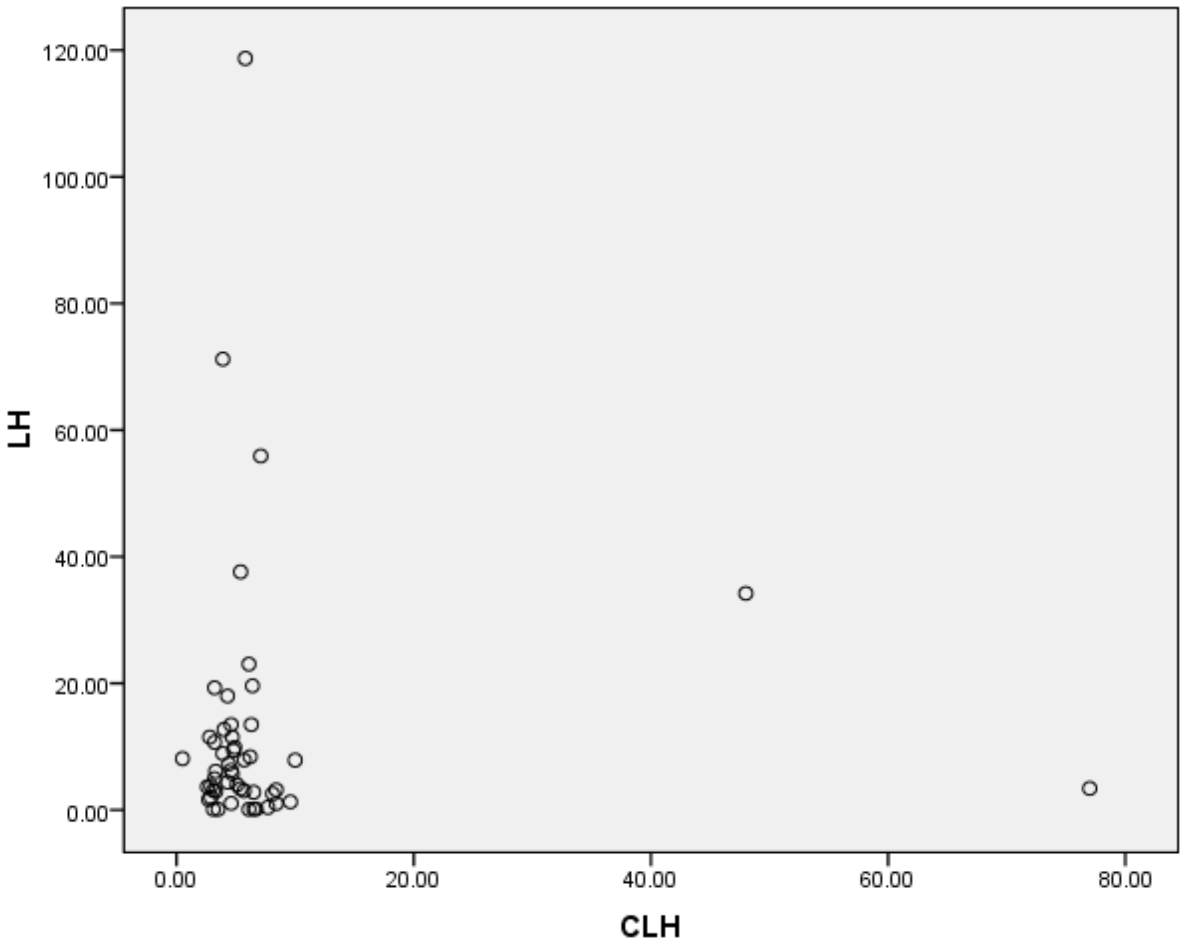

Figure 4.2 correlation between LH and CLH (R = 0.32**, P = 0.827)

Chapter Five
Discussion

5.1. Discussion:

Polycystic ovary syndrome (PCOS) is a common endocrine disease in women, characterized by heterogeneous presentation of hyperandrogenism, ovulatory dysfunction, and polycystic ovarian morphology (PCOM) (Palomba *etal.*, 2015).

Polycystic ovary syndrome (PCOS) disrupt the fertility hormones in females by increasing or decreasing them, this study conducted to get the disrupt of LH and assess of FSH among polycystic ovary syndrome (pcos) female patients.

In research, the ages ranged from 15 to 45 years. In the past they thought PCOs presents during the reproductive years only, but now can be diagnosed from f*etal* ., life. Although PCOS classically presents during the reproductive years with menstrual irregularities, hyperandrogenism and metabolic complications, we now understand that the origin of the disorder probably occurs very early starting from f*etal* life. In utero exposure to elevated testosterone levels coupled with gestational hyperglycemia may contribute to early differentiation of PCOS or may lead to amplification of the phenotype in genetically predisposed individuals. The spectrum of presentation of PCOS phenotype changes across the life span of a given individual. Improved understanding of the disease spectrum has allowed us to identify endocrine and metabolic changes in the very young subject with high risk of developing PCOS (Belinda George and M Ganapathi., 2016).

The present study revealed insignificant increase in mean of Luteinizing hormone levels among case when compared to control group with *p-value* 0.149, This finding agreed with studies done by the (oti-wilberforce *etal*., 2016) , (Ibrahim *etal*., 2015) also results of a study conducted by (Doldi *etal*., 1998). The ratio of LH to FSH in case equal 2:1 while the ration in control group 1:1 This finding agreed with studies done by the (E. SterlingNovember 8, 2011) and other study done by (Khushboo Brar *et al*., 2016)

5.2. Conclusion:

From the results and finding of this study, it is concluded that:

The serum levels of LH are insignificant higher in PCOs female patients, and there are no observed change in FSH concentration PCOs female patients.

5.3. Recommendations:

1- Therefore, we should not consider plasma LH as a very sensitive indicator of biochemical diagnosis hyperandrogenemia, LH can use with other parameter more than FSH as testosterone.

2- The measurement of LH and FSH ratio can be used as useful biochemical marker for diagnosis of polycystic ovary syndrome (PCOS).

3- AMH (Anti – mullerian Hormone) can be useful in diagnosis of PCOS.

4- More studies should be carried out on the LH and FSH in PCOS patient.

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Appendices

Sudan University of Science and Technology

College of Graduate Studies

QUESTIONNAIRE No ( )

Date: / /2019

Name: …….

Years.....Age:

Weight ……. BMI…… Height……..

Duration of disease …….

Menstrual regularity: Yes ….. No …….

Fertility : Yes ….. No …….

Cosmetics : Yes ….. No …….

History : Yes …… No ……

Medications: ……

Results

(ng/dl) ..... LH:

FSH …… (ng/dl)