

**THE ASSOCIATION OF KISSPEPTIN WITH POLYCYSTIC OVARIAN
SYNDROME**

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DECLARATION

This thesis work is presented to University of Ghana, School of Graduate Studies through the College of Health Sciences, School of Biomedical and Allied Health Sciences, Department of Chemical Pathology. I hereby affirm that, not including references to former works by people whom I have properly recognized, this thesis is the work of my own research which took place at the Obstetrics and Gynaecology (Obs and gynae) department/clinic of the Korle-Bu Teaching Hospital and the department of chemical pathology, SBAHS, under the strict supervision of Professor Henry Asare-Anane and Dr. Ben D.R.T. Annan. This work shows evidence of original research undertaken by me and neither all nor parts of this thesis have been presented for another degree.

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DEDICATION

I dedicate this work to my parents, Mr. and Mrs. Ankumah, as well as Prof. Anthony Andrew Adjei for their profound contribution to my life.

ACKNOWLEDGEMENT

I give thanks to God almighty for life and for how far I have come. I owe so much appreciation to my supervisors, Professor Henry Asare-Anane and Dr. Ben Annan who have been a bundle of help to me. I want to sincerely thank my father Mr. Samuel K. Ankumah for his incessant support and my mother Mrs. Georgina Ankumah for her love and prayers. Also my special thanks go to Dr. Emmanuel Ofori, Comfort and Afua for their technical help in my assay analysis. My heartfelt thanks also go to lecturers and Staff members at the Chemical Pathology Department, College of Health Sciences- University of Ghana for their diverse input during this programme.

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ABSTRACT

Polycystic ovarian syndrome (PCOS) is a common endocrine abnormality in women of reproductive age which often varies from mild menstrual disorder to severe disturbance of reproductive and metabolic functions. It is characterized by a collection of signs and symptoms which include ovulatory dysfunction-related infertility, menstrual disorders or androgen-related symptoms.

Kisspeptin is a 54 amino acid neuropeptide that plays an essential role in human metabolism. It is encoded by the KISS1 gene and excites a reaction cascade in the hypothalamo-pituitary-gonadal (HPG)-axis, which is important in human reproduction. This study was aimed at determining the association of plasma kisspeptin with polycystic ovarian syndrome patients in Ghana. It was a descriptive cross-sectional study involving 81 participants. Of these subjects, 41 were diagnosed with PCOS while 40 were apparently healthy and without PCOS. Blood samples of subjects were collected and analysed for kisspeptin, luteinizing hormone (LH), follicular stimulating hormone (FSH), estradiol, prolactin and testosterone by ELISA method. Body mass indices (BMI) and fasting blood glucose were also assessed.

The difference between the means FBG (mmol/L) and kisspeptin (ng/ml) of PCOS patients and the controls (5.82 ± 0.06 vs 5.37 ± 0.14) [$p=0.001$], (20.21 ± 1.74 vs 12.15 ± 2.51) [$p=0.009$] were respectively significant. There were significances in the difference between the two groups for LH and FSH ($p= <0.0001$) respectively. The difference between means for testosterone between study groups (12.73 ± 0.37 vs 11.27 ± 0.52) was significant ($p=0.025$). Regression analyses showed that FSH (OR= 0.025, $p<0.0001$), FBG (OR= 3.33, $p=0.047$) and LH (OR= 0.162, $p=0.013$) were associated with kisspeptin levels in PCOS patients.

In conclusion, plasma kisspeptin levels were high in PCOS patients, relative to their nonPCOS counterparts. Fasting blood glucose and gonadotropins contributed significantly to the variances in kisspeptin levels seen among PCOS patients.

CHAPTER ONE

INTRODUCTION

1.1 Background

Kisspeptin is a neuropeptide hormone that is encoded by the KISS1 gene and plays an important role in human reproduction (Jayasena *et al.*, 2014). Its receptors have been found in organs such as testes, liver, pancreas, placenta, ovaries and the small intestine. It excites the neurons of the hypothalamus that lead to a cascade of reactions in the hypothalamopituitary-gonadal (HPG)- axis (Katagiri *et al.*, 2007).

Polycystic ovarian syndrome (PCOS) is a common endocrine abnormality in women of reproductive age (Teede *et al.*, 2010). These women sometimes develop multiple cysts in their ovaries that produce excess androgens, notably testosterone. It is a syndrome that is characterized by a group of signs and symptoms (Balen and Michelmore, 2002). These symptoms include ovulatory dysfunction-related infertility, menstrual disorders or androgen-related symptoms. The various symptoms of PCOS inform the management of the disorder (Badawy and Elnashar, 2011).

Androgen-excess contributes to the formation of more multiple cysts (fluid-filled sacs) in the ovaries (Balen and Michelmore, 2002). These cysts are about 2 to 5 mm in diameter upon ultrasound examination. The problems arising from PCOS may be due to some abnormalities in the hypothalamo-pituitary-gonadal (HPG) axis. These abnormalities vary from mild menstrual disorder to severe disturbances of the reproductive and metabolic functions as PCOS and insulin resistance (Umayal *et al.*, 2018). Gonadotropic releasing hormones (GnRHs) from the hypothalamus were known to be the primary regulators of the HPG-axis long before the discovery of kisspeptin (Yerlikaya *et al.*, 2013). In the HPGaxis,

the hypothalamus secretes the gonadotropic releasing hormones which stimulate the neurons of the pituitary to produce gonadotropins (the luteinizing and follicular stimulating hormones). The gonadotropins then cause the gonads (ovaries in the case of women) to produce the established normal amounts of reproductive hormones like testosterone, estradiol and progesterone for normal body and reproductive functions (Marshall *et al.*, 2012).

Insulin resistance is another characteristic feature of PCOS (Madnani *et al.*, 2013). The abnormality in the HPG-axis has been reported to be the reason behind the unusual androgen excess and insulin resistance. There is an increased production of gonadotropin releasing hormones (GnRH) in the hypothalamus when kisspeptin receptors are oversensitized (d'Angelemont de Tassigny *et al.*, 2008; Liu *et al.*, 2008). The GnRHs excite the pituitary to release LH and FSH (gonadotropins). LH production becomes vigorous, activating the ovaries to produce elevated quantities of testosterone (Katulski *et al.*, 2018). The formation of follicular cysts then happens with a subsequently high insulin production (Bhuiyan *et al.*, 2015; Umayal *et al.*, 2019). Insulin resistance consequently occurs in PCOS.

There are numerous and varied symptoms that make it difficult to completely treat the disease. To make diagnosing the syndrome easier, the American Society for Reproductive medicine (ASRM) and the European Society for Human Reproduction and Embryology (ESHRE) consensus meeting came up with criteria (Gianaroli *et al.*, 2012). This was based on the association of at least two of the following: either the presence of polycystic ovaries on ultrasound, biochemical/clinical hyperandrogenism or oligo/amenorrhea, excluding other etiologies (Gianaroli *et al.*, 2012).

PCOS affects the quality of life of women through their self-esteem and general psychological health, which eventually have adverse effects on their socioeconomic lives (Himelein and Thatcher, 2006). Early detection and management of the syndrome is very crucial.

1.2 Problem statement

The problems with PCOS include reducing the quality of life of women in their reproductive years and also because the cause of this syndrome is unclear. This is because it shows varied clinical presentations that differ from one patient to another (Azziz *et al.*, 2006). This makes it difficult to completely treat the disease. Treatment is often symptombased (Madnani *et al.*, 2013) and so its route cause is not eliminated.

Another issue with PCOS is that it causes preeclampsia and even miscarriages (Leo *et al.*, 2016) in pregnant women. It is estimated to cause 70% of anovulatory infertility in Ghanaian women (Maya *et al.*, 2018). It is also capable of taking a psychological toll on patients at times because of unusual physical and hormonal features like alopecia, hirsutism and irregular menstruation, and sometimes their inability to meet societal expectations, especially in childbirth. They therefore do not have the sound mind to concentrate on productivity (Bhuiyan *et al.*, 2015).

1.3 Justification

PCOS affects 9% to 18% of women worldwide (Helm *et al.*, 2017) and 12.2% of women in Nigeria (Omokanye *et al.*, 2015). Polycystic ovarian syndrome has been recorded as a socioeconomic problem which causes pre-menopausal infertility (Madnani *et al.*, 2013; Omokanye *et al.*, 2015). There have been numerous studies conducted to identify a single cause of the disease so it can be effectively treated (Calley and Dhillon., 2014; Bhuiyan *et al.*, 2015; Umayal *et al.*, 2019). Studies done by Katulski *et al.*, (2018) and Umayal *et al.*,

(2019) reported high levels of kisspeptin in PCOS patients indicating a possible role of kisspeptin in the development of PCOS. Such conclusions cannot be drawn in Ghanaian PCOS patients until there are studies done in this regard. There may be possible variations in the kisspeptin levels in Ghanaian PCOS patients probably due to different genetic, geographic and environmental factors. There is, however, limited information on the role kisspeptin plays in the development of PCOS in Ghanaian patients. In view of the problems associated with PCOS, it is very important this study is done. This will contribute to the existing knowledge from other countries to help improve the management or treatment of the disease.

1.4 Hypothesis

The levels of kisspeptin will not be high in Ghanaian PCOS patients.

1.5 Aim

To determine the association of plasma kisspeptin with polycystic ovarian syndrome patients in Ghana.

1.6 Specific objectives

1. To assess serum levels of kisspeptin, estradiol, luteinizing hormone (LH), prolactin, follicle-stimulating hormone (FSH) and testosterone using the enzyme-linked immuneassay (ELISA) in study subjects, as well as their fasting blood glucose (FBG).
2. To assess the correlation between serum kisspeptin levels and luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol, prolactin, total testosterone and fasting blood glucose (FBG) in the PCOS subjects.
3. To determine the risk factors of PCOS.

CHAPTER TWO

LITERATURE REVIEW

2.1 An overview of Polycystic Ovarian Syndrome (PCOS)

PCOS defined as an endocrine-related syndrome that may be seen in women with irregular menses (Madnani *et al.*, 2013). The common features of PCOS, aside irregular or anovulatory cycles with signs of hyperandrogenism, include acne, hirsutism, alopecia, frank virilization, and sometimes with polycystic ovaries on pelvic sonography (Marshall *et al.*, 2012). Hyperandrogenaemia often results in hyperandrogenism with hirsutism as a characteristic feature. PCOS has been associated with obesity, insulin-resistance (IR) and a risk of developing Type 2 diabetes mellitus (Madnani *et al.*, 2013). The presence of multiple cysts in the ovaries contribute to the excessive production of androgens. It has been reported that hyperandrogenaemia leads to the formation of polycystic ovaries (Yan *et al.*, 2014). In PCOS, serum LH levels are often high. Testosterone is moderately increased with a decrease in SHBG, while estradiol and progesterone are usually in the normal mid-follicular range (Marshall *et al.*, 2012).

PCOS was estimated to have a prevalence ranging from 2.2% to 7% between the years, 1998 and 2004 (Knochenhauer *et al.*, 1998; Diamanti-Kandarakis *et al.*, 1999; Michelmore *et al.*, 1999; Asuncion *et al.*, 2000; Azziz *et al.*, 2004). As at the year 2014, the prevalence had increased from 7% to 10% (Barthelmess and Naz, 2014). This is because of constant changes in lifestyle, genetic and environmental factors (Barthelmess and Naz, 2014). The disease affects 9% to 18% of women worldwide (Helm *et al.*, 2017), 30% of women in the United States (Barthelmess and Naz, 2014) and 12.2% of women in Nigeria (Omokanye *et al.*, 2015). It is estimated to cause 70% of anovulatory infertility in Ghanaian women (Maya

et al., 2018). This shows the rate at which the burden of PCOS as a problem increases over the years.

2.3 Diagnosing and managing PCOS

Investigating PCOS is made through blood and ultrasound tests as diagnosis is usually based on the association of at least two of the following: Polycystic ovaries on ultrasound, biochemical or clinical hyperandrogenism and oligo or amenorrhea, with exclusion of other etiologies (Katulski *et al.*, 2018). Managing the disorder is often done by treating the symptoms (Madnani *et al.*, 2013; Marshall *et al.*, 2012; Katulski *et al.*, 2018). Treatment is done by targeting hormones as testosterone and progesterone, aside lifestyle modification (Sheehan, 2004).

2.4 Polycystic Ovarian Syndrome

2.4.1 Epidemiology

Polycystic ovarian syndrome has been shown to be a commonly occurring disease in premenopausal women (Azziz *et al.*, 2006) and is fast becoming a global problem as the prevalence is reported to be increasing over the years (Omokanye, 2015). The rate of occurrence of PCOS has approximately been doubling every two to five years (Barthelmess and Naz, 2014; Helm *et al.*, 2017). In the year 2013, there was a reported prevalence of 7.3% in Palestinian women (Musmar *et al.*, 2013). Engmann *et al.*, (2017) in their study in the United States of America reported that African and Hispanic women both have a high prevalence of the disease. Majority of women with PCOS are obese with a high body-mass index and a relatively higher LH: FSH ratio. The rate of incidence of PCOS has been attributed to an abnormal HPG-axis and certain lifestyle (Engmann *et al.*, 2017) hence lifestyle modifications contribute to reducing the incidence of the syndrome.

2.4.2 Factors that influence PCOS

2.4.2.1 Genetic component

Genetic factors play a major role in the development of PCOS. It has been established that this has a similar trait to type-2 diabetes mellitus and obesity (Unluturk *et al.*, 2016). PCOS has been viewed as an overall androgen excess disorder with gonadotropic and metabolic abnormalities of different degrees (Franks *et al.*, 1997; Unluturk *et al.*, 2016). Different families have recorded heterogeneous phenotypes of PCOS and even within the same family. The disease is complex in the traits it presents, making a single cause difficult to discover. The results from investigations into some families showed a common pattern within their ovarian morphology, menstrual irregularities, symptoms of hyperandrogenaemia and hyperandrogenism (Jahanfar *et al.*, 1996; Kahsar-Miller, 2001) in first-degree relatives of PCOS patients, compared with controls (Cooper *et al.*, 1968). Other studies reported a common pattern of hyperandrogenaemia, enlarged ovaries and hirsutism in a family-based studies (Givens *et al.*, 1988). In a study done by Hague *et al.*, (1988), 67% of the mothers and 87% of the sisters of probands were affected by hyperandrogenism, obesity and infertility.

There are increased insulin levels among first-degree relatives (Norman *et al.*, 1996) and a pancreatic beta-cell dysfunction in families of women with PCOS (Colilla *et al.*, 2001). PCOS women who had both hyperandrogenism and insulin resistance had hyperphosphorylated serine residues on their insulin receptors (Dunaif *et al.*, 2001).

2.4.2.2 Environmental factors

Environmental toxins play a role in disrupting reproductive health (Merkin *et al.*, 2016). Although there is limited research as to how these toxins may contribute to the development of PCOS, it has also been reported that PCOS symptoms are reduced with certain dietary

supplements and with weight loss among obese women (Merkin *et al.*, 2016). Environmental factors of socioeconomic, geographic and life-style nature have also been reported to play roles in the development of PCOS. Additional research is needed to determine how these various environmental determinants contribute to the onset of PCOS as well as ways these may be managed or controlled to prevent its development (Azziz, 2016).

2.4.2.3 PCOS and insulin resistance

PCOS is closely linked to insulin resistance. Insulin is a hormone that rises upon the consumption of food, enabling the cells to take up glucose from the blood for energy production. In insulin resistance, insulin is unable to bind to cells. There is no negative feedback in this regard and so the pancreas keeps secreting insulin. Excess insulin has been reported to cause inflammation and weight gain (Rojas *et al.*, 2013).

Seventy percent (70%) to 90% of obese PCOS patients have been reported to have insulin resistance whilst 30% to 75% of lean PCOS patients have insulin resistance (Nestler *et al.*, 1998; Corbould *et al.*, 2006). Insulin resistance causes impaired ovulation and is managed, aside surgery sometimes, by lifestyle modification, including weight-loss through healthy eating and exercising (Randeve *et al.*, 2012). Management with the metformin drug is another way of improving insulin sensitivity. Oral contraceptives are well-known management drugs for PCOS but it has been reported to interfere with blood glucose regulation and insulin resistance which underlie the disease (Cortes *et al.*, 2014).

2.4.2.4 PCOS and obesity

Obesity is defined by the world health organization as having a body mass index (BMI) greater than 30 kg/m² (W.H.O., 2019). Obesity is very noticeable in PCOS, amongst all the

presentations of the disease (Legro, 2012). The body is unable to assimilate the glucose due to insulin resistance. The accumulation of glucose in the blood eventually leads to weight gain.

Severe menstrual irregularities and anovulation in obese PCOS patients than non-obese PCOS patients have been recorded (Yerlikaya *et al.*, 2013) and at least a 5% weight loss has been proven to improve these conditions. A total weight loss of about 10% of the body weight has the potential to improve upon the menstrual cycle, especially of obese PCOS patients (Pasquali *et al.*, 2006).

2.4.3 Risk factors of PCOS

The factors that put most women at risk of developing PCOS include hormonal imbalances, obesity, hypercholesterolaemia, sedentary lifestyle, psychological stress, insulin resistance, menstrual irregularities, family history of infertility (Balen and Michelmore, 2002; Teede *et al.*, 2010; Badawy and Elnashar, 2011; Madnani *et al.*, 2013; Skorupskaite *et al.*, 2014). In another study the risk factors of the disease include menstrual cycle disorder, family history of diabetes and infertility as well as the lack of physical exercise (Ozay *et al.*, 2016). The enlargement and development of cysts in the ovaries are also considered an important risk factor (Qureshi *et al.*, 2016).

2.5 Kisspeptin

Kisspeptin is a 54-amino acid peptide, which comprises a diverse group of peptides with many different functions related to energy metabolism, reproduction and pubertal development (Yerlikaya *et al.*, 2013). It was initially isolated from placenta and called metastin due to its ability to slow down the spread of cancer cells (Roseweir *et al.*, 2009).

It is a hormone encoded by the *KISS1* gene, playing a critical role in human reproduction (Jayasena *et al.*, 2014). Kisspeptin is a principal regulator of the secretion of the gonadotropins, follicular stimulating hormone (FSH) and luteinizing hormone (LH), which play a key role in sexual development and the control of fertility (Roseweir *et al.*, 2009). Though kisspeptin stimulates the secretion of both FSH and LH, its effect on LH is more marked in women with fertility issues (Skorupskaite *et al.*, 2014).

Kisspeptin has an effect on metabolic function as it regulates the secretion of gonadotrophin releasing hormones (GnRH). Administering exogenous kisspeptin in various isoforms via different routes in the bid to manipulate its levels has the potential of being a new therapy in patients with high or low LH pulsatility (Skorupskaite *et al.*, 2014).

2.5.1 The structure and distribution of kisspeptin

Kisspeptin was discovered in Pennsylvania as a suppressing-gene called metastin (Lee *et al.*, 1996). It was later named ‘kisspeptin after the Hershey chocolate kiss. Kisspeptin is encoded by the *Kiss1* gene which is found in the 1q32 chromosome and has 145 major amino acid peptides which are cut to 54 amino acid peptides through antecedent proteolytic processing. The peptide of 54-amino acids is further cleaved to 14, 13 and 10 peptides for stability (West *et al.*, 1998; Muir *et al.*, 2001). This is because the full length of the kisspeptin-54 has pairs of basic residues surrounding it. These residues make the molecule bulky and unstable and so at the residues prohormone convertases cleave kisspeptin 54 to form the shorter and more stable forms (Kisspeptin -10, -13 and -14) (Kotani *et al.*, 2001). These short forms share a common motif with kisspeptin 54 and exhibit the same affinity and efficacy for *Kiss1r* (Amy *et al.*, 2009). Aside their stability, the true relevance of kisspeptins -10, -13 and -14 are unknown (Muir *et al.*, 2001).

Kisspeptin was identified as ligands for the G protein- coupled receptor, 54 (GPR54). The receptor was name kiss1r (Muir *et al.*, 2001; Gottsch *et al.*, 2009). Phospholipase C (PLC) is activated when kisspeptin binds the GPR54 system leading to the discharge of calcium which activates protein kinase C and brings about the kisspeptin signaling. There is an initial increase in intracellular calcium by the kiss1r then a gradual slower and steady production is achieved to prevent desensitization as mobilizing kiss1r is achieved (Constantin *et al.*, 2009).

2.5.2 Sexual dimorphism of kisspeptin neuron distribution

There is a report on the sexual difference in kisspeptin characteristics at the periventricular region and infundibulum pathway in the hypothalamus of humans (Pita *et al.*, 2011). It is well shown that the female hypothalamus has distinguished kisspeptin fibers in their infundibulum and ventral periventricular zone than in adult males with a significantly high collection of kisspeptin manifestation in the periventricular of female hypothalamus. There is also high kisspeptin in the hypothalamic infundibulum of females compared to males (Hrabovszky *et al.*, 2011).

2.5.3 Regulation of GnRH secretion by kisspeptin

There is a major release in GnRH when kisspeptin receptors are activated (d'Angelemont de Tassigny *et al.*, 2008, Liu *et al.*, 2008). This is so because the kisspeptin neuron in primates are in close proximity to the neurocytes of the GnRH. Nerves of the cells of kisspeptin extend to the GnRH neurocytes in the hypothalamic preoptic nerve (Clarkson *et al.*, 2006; Liu *et al.*, 2008). The effect of the release of GnRH is usually blocked by administering GnRH antagonists, though administering kisspeptin showed a massive rise in GnRH (Novaira *et al.*, 2009) leading to a subsequent increase in the secretion of the

follicle stimulating hormone (FSH) and luteinizing hormone (LH) both in human and animal studies (Dhillon *et al.*, 2005, Leonor *et al.*, 2012).

2.5.4 The role kisspeptin plays in Puberty

Sexual development in humans basically occurs as kisspeptin neurons exert an important function in starting a series of reactions involving the hypothalamic-pituitary-gonadal axis. (Hameed *et al.*, 2011). This is clear in the disadvantages of genetic change in kisspeptin signalling channel in individuals having congenital hypogonadotropic hypogonadism (CHH) (de Roux *et al.*, 2003). Individuals with heterozygous kisspeptin receptor mutation are also reported to have the CHH phenotype (Topaloglu *et al.*, 2012). It has been reported that an increase in the levels of LH, FSH and total testosterone (TT) occurs when kisspeptin is administered to healthy individuals with a relatively elevated surge in the levels of LH.

2.5.5 Maintaining hypothalamic gonadal steroid feedback with kisspeptin

A feedback signalling into the hypothalamus is generated by the reproductive organs which results in either a bad or good controlling effect on the secretion of LH and FSH. Before the discovery of kisspeptin, the gonadal steroid feedback mechanism was poorly understood. This was because the role of GnRH was not completely understood (Roseweir *et al.*, 2009). The discovery of kiss1r showed its relationship as the main mediator of gonadal steroid feedback in the hypothalamus. There is a feedback of estradiol on GnRH neurons that is caused by a type of estradiol receptor isoform ($ER\alpha$) which is expressed at the Anteroventral Periventricular (AVPV). This induces an increased production of LH. The LH produced early ovulation while in contrast, $ER\alpha$ on cell lines of kisspeptin at the Arcuate Nucleus (ARC) are part of the detrimental feedback pathway in preventing GnRH secretion and release in response to estradiol (Wintermantel *et al.*, 2006).

2.5.5 Kisspeptin association with PCOS

Kisspeptin as a hormone regulator of the GnRH is shown to have majority influence in reproductive conditions. PCOS being one of such endocrinological conditions, characterized by hyperandrogenaemia, the levels of kisspeptin may play a crucial role in the disease condition.

LH levels in a study done by Ozay *et al.*, (2016) showed that the levels of LH in PCOS patients were high and had a positive correlation with the high levels of serum kisspeptin. This result is consistent with recent studies done by Katulski *et al.*, (2018) and Umayal *et al.*, (2019) who went on to propose making serum kisspeptin concentration a marker for the early detection of PCOS. In their study, PCOS group's mean BMI was higher than for the control group (Umayal *et al.*, 2019).

2.5.6 Effects of kisspeptin on the gonads

All the stages of reproduction are controlled by the Hypothalamic-Pituitary-Gonadal (HPG) axis. The hypothalamus produces gonadotropin-Releasing Hormone (GnRH), which is transported to the anterior pituitary and stimulates the production of Luteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH). Slow GnRH pulsatility favours FSH secretion and fast pulse frequencies support LH secretion (Thompson and Kaiser, 2014).

The production of the steroid hormones (steroidogenesis) and gametes (gametogenesis) is controlled by LH and FSH. The steroids from the gonads then modify the neuronal function of the gonadotropic releasing hormones through negative and positive feedback action (d'Anglemont de Tassigny *et al.*, 2010). Reports show that hypothalamic kisspeptin acts

upstream of GnRH. This neuropeptide is required for reproductive development and maintenance as an impaired function of the kisspeptin receptor gene (*KISS1R*) are associated with pubertal failure (Lents *et al.*, 2008). Gonadal steroid feedback reduces GnRH, unless during pre-ovulatory LH surge. Besides activated progesterone receptors, elevated estradiol levels at the end of the follicular phase activate KISS1 neurons in the AVPV causing an increase in GnRH pulse frequency. This leads to the LH surge and ovulation (Thompson and Kaiser, 2014). After ovulation, as there is a rise in progesterone levels, GnRH pulse frequency slows, increasing FSH production.

2.5.7 Role of kisspeptin in metabolic diseases

The hypothalamus is the site of the brain for reproductive and metabolic functions. Kisspeptin neurons are localised in the arcuate nucleus of the hypothalamus and also expressed in peripheral tissues that are involved in metabolic functions. These tissues include pancreas, liver and adipose tissues. Metabolism involves the ability of the tissues to undergo biochemical reactions like the processing and transport of proteins, carbohydrates and lipids (Dudek *et al.*, 2018). Kisspeptin has been observed to play a role in metabolic imbalances such as under-nutrition, obesity and diabetes (Umayal *et al.*, 2019). These are said to be a result of low metabolism as against hyperphagia.

Hyperinsulinaemia, induced by glucose is reported to be caused by kisspeptin-54 which stimulates the beta cells of the pancreas to secrete insulin. The liver is the largest and one of the important organs in the metabolism of glucose. Aside storing glucose in the form of glycogen, it is involved in maintaining optimum blood sugar levels by providing glucose when needed by the body through glycogenolysis. This is achieved through the production of glucagon from the alpha cells of the pancreas (Hou *et al.*, 2017).

Adipose tissues store fat for periods of higher energy requirements and these are regulated by hormonal signals. KISS1 mRNA has been detected in human adipose tissue and shown to be involved in metabolic regulation but how exactly it plays a role in lipogenic and lipolytic processes is not clear. It has been reported, however, that there is a positive correlation between the body mass index and KISS1 mRNA levels in the adipose tissues in women (Cockwell *et al.*, 2013).

2.5.8 Kisspeptin and its functions with other neuropeptides in the HPG-axis regulation

The human infundibula nucleus shows that kisspeptin neurons co-concentrate neurokinin B (NKB) and Dynorphin (DYN) and this occurrence is much preserved in many mammals including humans (Hrabovszky *et al.*, 2011). Both NKB and DYN regulate HPG axis through its part in regulating feedback mechanism of GnRH expression (Clarke *et al.*, 2015). Neurokinin B (NKB) belongs to the peptide-related tachykinin (TAC3) family which is much observed on GnRH cells (Todman *et al.*, 2005) and directly stimulates GnRH neurons to greatly increase LH release (Hameed *et al.*, 2011). In humans, genetic changes in the receptor TAC3 have proven to cause hypogonadism (Topaloglu *et al.*, 2012). The endogenous opioid dynorphin (DYN) also regulates GnRH production through a negative feedback by progesterone (Goodman *et al.*, 2007). These infundibula nucleus neurons that together express all these neuropeptides are identified as KNDy neurons (Chen *et al.*, 2010) and these neurons are conserved across all species. The KNDy neurons make direct contact with GnRH cells to yield GnRH that is well regulated. Thus, neurokinin B exerts a stimulatory role and Dynorphin plays an inhibitory action all autosynaptically work together pulsate yield kisspeptin, and in turn, produce a pulsatile yield of GnRH and LH (Dahl *et al.*, 2009) as shown in figure 2.1.

Figure 2.1: Diagram displaying the Kisspeptin-GnRH trail and the association between KNDy neurons and GnRH neurons in human

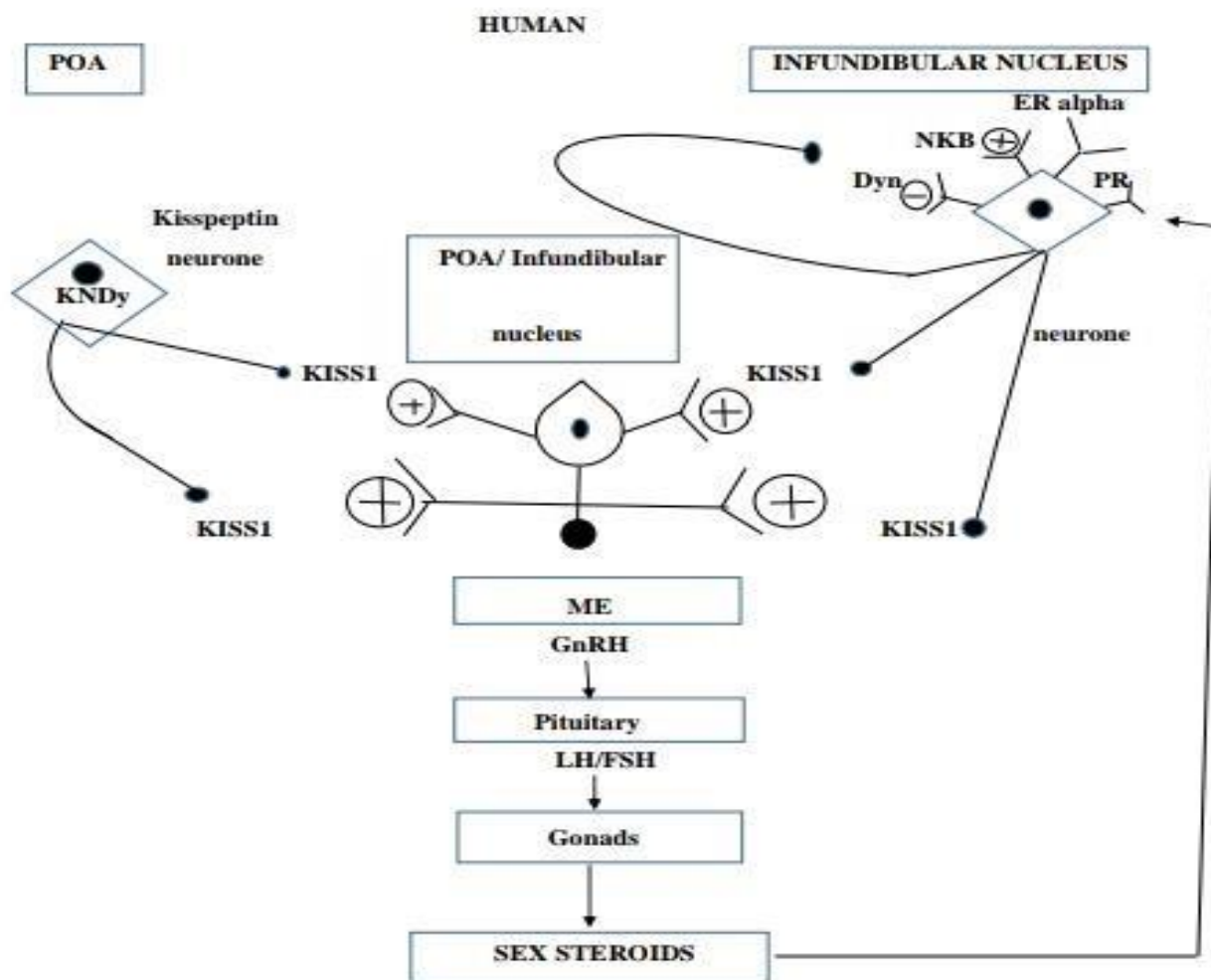


Figure 2.1: Diagram displaying the Kisspeptin-GnRH trail and the association between KNDy neurones and GnRH neurones in humans.

Kisspeptin neurones in the hypothalamus concentrate neurokinin B (NKB) and dynorphin (DYN) which regulate GnRH expression. GnRH then stimulates the pituitary to greatly increase the secretion of LH. LH and FSH, also produced by the pituitary then stimulate the gonads to produce sex steroids. These steroids, when they exceed their upper limits send a negative feedback to NKB and DYN (Skorupskaite *et al.*, 2014).

CHAPER THREE

METHODOLOGY

3.1 Study design

The study was a descriptive cross-sectional one involving 41 PCOS patients and 40 age-matched apparently healthy women.

3.2 Study site

The study was conducted at the Obstetrics and Gynaecology (ONG) clinic of the Korle-Bu Teaching Hospital, Accra Ghana. The Korle-Bu Teaching Hospital is a leading tertiary hospital in Ghana with about 2000 bed capacity. The hospital serves the city of Accra (with a population of about three million) and mostly the whole of the southern sector of Ghana. At Korle-Bu Teaching Hospital, there is an average daily out-patient attendance of 1,000 and 100 admissions daily (Personal Communication and Hospital Records). The ONG unit is located within the premises of the hospital to cater for the diagnosis and management of diseases peculiar to women and their reproductive health, in and outside the Greater Accra Region of Ghana. The gynaecology clinic is opened from Mondays to Fridays of every week, with five teams attending to patients on each of these days. There is an average daily attendance of 55 patients a day. Sample analyses were done at the Central Laboratory of the Korle-bu Teaching Hospital and the Chemical Pathology Department of the School of Biomedical and Allied Health Sciences.

3.3 Study participants

Diagnosing PCOS was based on the American Society for Reproductive medicine (ASRM) and the European Society for Human Reproduction and Embryology (ESHRE) consensus meeting criteria. This was based on the association of at least two of the following:

1. Polycystic ovaries on ultrasound
2. Biochemical/clinical hyperandrogenism
3. Oligo/amenorrhea, with exclusion of other etiologies

Apparently healthy women served as controls (were sampled from the St. Justin Anglican Basic schools at Ablekuma, Accra) were screened to make sure they did not have PCOS. Questionnaires were given to subjects to fill to obtain their demographic information (Appendix III).

3.4 Sample size and sampling techniques

The sample size formula used for comparison of groups, when testing the difference (d) between two sub-samples regarding a proportion with assumed equal number of cases ($n_1=n_2=n$) in two sub-samples was:

$$n = \frac{2z^2pq}{d^2} \quad n = \text{sample size} \quad z = \text{standard score for the confidence level} \quad p =$$

proportion in the target population estimated to have a particular characteristics.

Where $p = 0.122$ in Nigeria (Omokanye *et al.*, 2015) $q = 1 - p$ (proportion in the target population not having the particular characteristics)

With confidence level of 95%, $Z = 1.96$,

$d =$ degree of accuracy required (d is observed difference of 0.10 or more significant at the 0.05 level), $n = \frac{2(1.96)^2 \times (0.122 \times 0.878)}{(0.1)^2} \quad n = 82$

experimental subjects and another 82 control subjects

A total of 41 PCOS subjects and 40 non-PCOS subjects were recruited for this study through purposive sampling.

3.4.1 Inclusion criteria

1. Women 18 years old and above, who have been diagnosed with PCOS (for cases).
2. Women 18 years old and above who have not been diagnosed with PCOS.

3.4.2 Exclusion criteria

1. Pregnant women. Pregnant women have a different hormonal pattern and usually with high kisspeptin levels due to the presence of the placenta which produces kisspeptin (Reynolds, 2009).
2. Diabetic patients. This was to rule out obvious insulin resistance (Reynolds *et al.*, 2017).

3.5 Sample collection and storage

Five (5) ml of venous blood samples were collected from participants into gel separator tubes, processed by centrifugation at 2500 revolutions per minute with resulting sera stored at -20°C until required for use.

3.6 Method of sampling, biochemical and statistical analyses and information publication

- The reason behind the research was explained to the participants in a language to their understanding.

- Consent was sought from participants before proceeding.
- Demographic information and blood samples of patients were taken during clinical consultation upon acquisition of their signed consents.
- Biochemical analyses of the samples were run at the central laboratory of the Korle-bu teaching hospital.
- Information of participants was only known to the principal investigator and clinicians and protected under lock and key.
- Results of the laboratory tests were printed and made available to the participants.

3.7 Demographic measurements

Height and weight of participants were measured using a standardized stadiometer and standardized weighing scale respectively. Body mass index (BMI) was then calculated as body weight (in kilograms) divided by square of the height (in meters). This information was attained by the principal investigator or from the medical records of patients.

3.8 Biochemical analyses

Serum levels of kisspeptin, luteinizing hormone (LH), follicle-stimulating hormone (FSH), total testosterone, estradiol and prolactin were determined using the enzyme-linked immunosorbent assay (ELISA) kit. Fasting blood glucose was also analysed with the glucose oxidase method.

The assays utilised monoclonal antibodies (MAbs) directed against distinct epitopes of estradiol, luteinizing hormone, follicle-stimulating hormone and total testosterone.

3.8.1 Principle and method of measuring blood glucose

The glucose concentrations were quantitatively analyzed based on the glucose oxidase method using the VITROS GLUCOSE (GLU) slide technique. The VITROS GLU is a multi-layered, diagnostic component that has been layered on a polyester support. Five microliters (5 μ l) of the sample was placed on the slide and was distributed uniformly by the distribution layer to the fundamental layers. The oxidation of glucose in the sample is sped up by glucose oxidase to form hydrogen peroxide and gluconate. The reaction was then preceded by an oxidative link catalysed by peroxidase in the presence of dye precursors to produce a colour. The intensity of the colour was measured by reflected light (Curme *et al.*, 1978).

Reaction sequence:

B-D-glucose + O₂ + H₂O

glucoseoxidase → D-gluconic acid + H₂O₂

2H₂O₂ + 4-aminoantipyrine + 1,7-dihydroxynaphthalene

peroxidase → Red dye

3.8.2 Principle and method of measuring kisspeptin

The test was based on the double-antibody sandwich enzyme-linked immunosorbent onestep process assay (ELISA) in the analyses of Kisspeptin-54 in samples. The reaction of kisspeptin present in sample simultaneously reacting with antigen conjugate and antikisspeptin coated wells. This is what the principle of the test was based on Standards, test samples and horseradish peroxidase (HRP)-labelled kisspeptin-54 antibodies were added to enzyme wells which were pre-layered with kisspeptin-54 and incubated at 37oC for one hour. It was then washed to remove uncombined enzyme. Chromogen solution A and B, blue in colour were used for colouring after thorough washing of the reactant to

eliminate any unbound enzyme. The reaction with the acid changed the colour blue of Chromogen Solution A and B to yellow. The intensity of the color developed was determined spectrophotometrically at $450\text{nm} \pm 10\text{nm}$ and the optical density (O.D) of standard curve was used to determine kisspeptin concentration.

3.8.3 Principle and method of measuring testosterone

The test was based on the solid phase enzyme-linked immunosorbent assay which followed the typical competitive binding schematic to assay levels of testosterone in samples. There was competition between unlabelled antigen (existing in standards, controls and subject samples) and enzyme-linked antigen (conjugate) for a restricted quantity of antibody binding sites on the micro-well plate. The well plates were then washed with buffer and decanted to eliminate any unbound materials. After washing, the enzyme substrate 3, 3', 5, 5'-tetramethylbenzidine (TMB) was added to each well at timed intervals, incubated on a plate for 10-15 minutes at room temperature until desired dark blue color appears. A stop solution was then added to the enzymatic reaction to terminate the reaction. The absorbance was then estimated on a microliter plate reader. The intensity of color developed was inversely proportional to the concentration of testosterone in the samples. A set of standards was used to design a standard curvature to read testosterone concentration from it.

3.8.4 Principles and methods of measuring luteinizing hormone (LH)

The test utilised the 'sandwich' type enzyme immunoassay that engaged a double extremely specific monoclonal antibody. A monoclonal antibody specific for LH (horseradish peroxidase) was restrained onto the micro-well plate and another monoclonal antibody specific for a dissimilar zone of LH was bonded to horse radish peroxidase (HRP).

Luteinizing hormone (LH) in standards and samples were permitted to attach to the well plate, cleaned with distilled water and further incubated with the HRP conjugate. The plate was then washed for the second time after which the enzyme substrate was added. A stop solution was then added to stop the enzymatic reaction. The absorbance was read on a microliter plate reader. The intensity of colour developed from the enzymatic reaction was directly proportional to the concentration of LH in the sample. A set of standards was used to design a standard curve from which the amount of LH in subject samples and controls were directly read.

3.8.5 Principles and methods of measuring follicular stimulating hormone (FSH)

The test followed the sandwich type assay which employed the use of two highly specific monoclonal antibodies: A monoclonal antibody specific for FSH restrained onto a microwell plate and an alternative antibody specific for a dissimilar region of FSH conjugated to horse radish peroxidase (HRP). Follicle stimulating hormone (FSH) in samples and standards were allowed to attach to the plate, washed, and subsequently incubated with the HRP conjugate. After incubation, another washing was repeated and enzyme was then added. The enzymatic response was stopped by addition of the stop solution. The absorbance was read on a microtiter plate reader. The concentration of the dye developed by the enzymatic reaction was directly proportional to the concentration of FSH in the sample. A set of standards was used to design a standard curve from which the quantity of

FSH in subject and control samples was directly read.

3.8.6 Principles and methods of measuring estradiol

The E2 Enzyme immunoassay (EIA) was based on the principle of competitive binding between E2 in the test specimen and E2-HRP conjugate for a constant amount of rabbit anti-Estradiol. In the incubation, goat anti-rabbit IgG-coated wells were incubated with 25ul E2 standards, controls, patient samples, 100uL Estradiol-HRP Conjugate Reagent and 50uL rabbit anti-estradiol reagent at room temperature (18-25°C) for 90 minutes. During the incubation, a fixed amount of HRP-labelled E2 competes with the endogenous E2 in the standard, sample, or quality control serum for a fixed number of binding sites of the specific E2 antibody. Thus, the amount of E2 peroxidase conjugate immunologically bound to the well progressively decreases as the concentration of E2 in the specimen increases. Unbound E2 peroxidase conjugate is then removed and the wells washed. Next, a solution of TMB Reagents is added and incubated at room temperature for 20 minutes, resulting in the development of blue colour. The colour development is stopped with the addition of stop solution and the absorbance is measured spectrophotometrically at 450nm. The intensity of the colour formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled E2 in the sample. A standard curve is obtained by plotting the concentration of the standard versus the absorbance. The E2 concentration of the specimens and controls run concurrently with the standards can be calculated from the standard curve.

3.8.7 Principles and methods of measuring prolactin

The prolactin Quantitative Test Kit was based on a solid phase enzyme-linked immunosorbent assay. The assay system utilized one anti-prolactin antibody for solid phase

(microtiter wells) immobilization and another mouse monoclonal anti-prolactin antibody in the antibody-enzyme (horse radish peroxidase) conjugate solution. The test sample was allowed to react simultaneously with the antibodies, resulting in the prolactin molecules being sandwiched between the solid phase and enzyme-linked antibodies. After 60 minutes incubation at room temperature, the wells were washed to remove unbound labeled antibodies. A solution of TMB was added and incubated for 20 minutes, resulting in the development of a blue colour. The colour development was stopped with the addition of 2N HCl and the colour was changed to yellow and measured spectrophotometrically at 45nm. The concentration of prolactin was directly proportional to the colour intensity of the test.

3.9 Data management

The standard safety laboratory protocol for sample collection and handling was strictly followed. All data was entered into Microsoft excel spreadsheet for storage and subsequently analysed. Codes were assigned to research participants. Only the Principal investigator was able to match codes to study subjects. Questionnaires were stored in a safety cabinet. Electronic data was stored under a password. Data was presented in tabular or graphical form. Questionnaires were kept under lock and key and will be shredded after two years.

3.10 Statistical tool and data analysis

The Graphpad prism 6 was used for analysing statistical data and the comparison of means between women with PCOS and controls. Student t-test was used to evaluate significant differences between two means \pm SD. P-values less than 0.05 was considered significant. The Spearman correlation coefficient (r) was used to find the correlation between two

continuous variables and regression analyses was used to determine the risk factors of PCOS.

3.11 Ethical issues

The proposal was submitted to the Ethical and Protocol Review Committee of the University of Ghana, College of Health Sciences for review and approval. All data was handled anonymously and confidentially. Unique identifiers were used for computer based data entry and kept discretely. Subjects were educated on the study. Those who consented were recruited into the study.

3.12 Expected outcome

It was expected that levels of serum kisspeptin will be high in PCOS patients.

3.13 Dissemination of results

Copies of the research findings was submitted for the award of a Master of Philosophy in Chemical Pathology and some copies were given to the various libraries of the University of Ghana to serve as a source of information and suggestions for subsequent research works. In addition, the results will be published in peer review journals and presented at conferences.

CHAPTER FOUR

RESULTS

4.1 Demographic and clinical indices of the study population

There was a total number of eighty-one (81) subjects in the study. These comprised of forty- one (41) PCOS patients undergoing management, age-matched with forty (40) non-PCOS subjects. The mean age between PCOS subjects and the non-PCOS subjects was not significantly different [$p = 0.51$] (Table 4.1). The mean BMI between the PCOS subjects and the non-PCOS subjects was not significantly different ($p = 0.082$).

Table 4.1 Demographic and clinical indices of the study population

| Parameters | PCOS subjects N=41 | Non-PCOS subjects N=40 | 95% C.I. Difference | p-value ($p < 0.05$) |
|-------------------------------|-----------------------------------|---------------------------------------|--------------------------------|---|
| Age (years) | 32.80 \pm 0.90 | 33.55 \pm 1.12 | -2.27 to 3.76 | 0.51 |
| BMI (kg/m²) | 30.91 \pm 0.78 | 28.83 \pm 0.49 | -4.44 to 0.27 | 0.082 |

Table 4.1 showed the demographic parameters (Age and BMI). Values for Age and BMI were specified as mean \pm standard deviation. P-value < 0.05 is significant.

4.2 Body Mass Index Percentage distributions within the study population

The percentage distribution of the body mass index (BMI) between the PCOS and nonPCOS subjects was studied and grouped into four (4) categories: underweight (<18.5 kg/m²), normal (18.5- 24.9 kg/m²), overweight (25.0- 29.9 kg/m²) and obese (>30 kg/m²).

A total of 2% of the PCOS subjects and 0% of the control group were underweight. About 5% each of the PCOS and non-PCOS subjects were normal weight. Overweight group comprised of 42% PCOS subjects and 65% non-PCOS subjects whilst obese group was made up of 51% PCOS subjects and 30% non-PCOS subjects (Fig. 4.1).

Fig. 4.1 BMI Percentage distribution within the study population

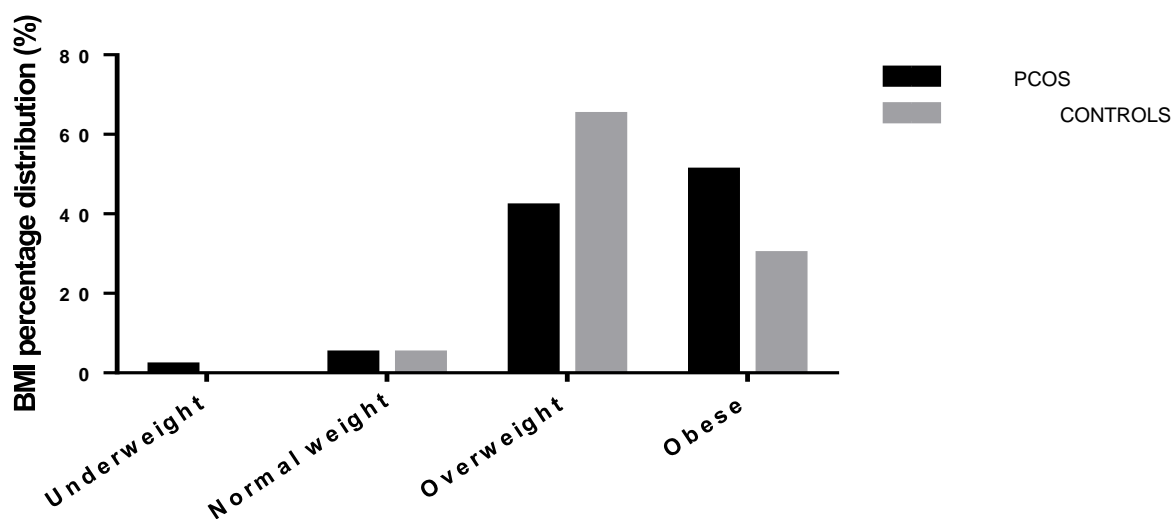


Figure 4.1 showed the percentage distribution of the body mass index for the study subjects. Values expressed as percentages.

4.2 Biochemical and hormonal indices of the study

Fasting blood glucose, plasma kisspeptin, Luteinizing hormone (LH), Follicle stimulating hormone (FSH), prolactin, estradiol and total testosterone levels were investigated in this study. The means of the measured biochemical levels are presented in Table 4.2. The difference in mean concentrations of the fasting blood glucose was significant ($p = 0.0008$). There was also significant differences in the means for plasma kisspeptin ($p = 0.0093$), LH ($p < 0.0001$) and FSH ($p < 0.0001$) between the two study groups. There was significance in the mean testosterone levels ($p = 0.025$) between PCOS subjects and controls.

4.3 The percentage distribution of kisspeptin levels in the study population

In this study the percentage distribution of kisspeptin levels in both PCOS and non-PCOS subjects was analysed. Study subjects were grouped into three (3) categories as shown in figure 4.2: Below the normal range (< 5.8 ng/ml), the normal range ($5.8 - 200$ ng/ml) and above the normal range (> 200 ng/ml). 5% of the PCOS subjects were under the normal range whilst 50% of the non-PCOS patients fell in same category, 95% of the PCOS patients were within the normal reference range whilst 50% of the total number of nonPCOS subjects were in the normal reference range. None of the study subjects had their kisspeptin levels above the normal reference range.

4.4 The percentage distribution of fasting blood glucose levels in the study population

The fasting blood glucose (FBG) levels of the study subjects was analysed and grouped into four (4) categories as shown in figure 4.3: Below the normal range (<3.8 mmol/l), within the normal range (3.8-5.5 mmol/l), pre-diabetes (5.6-6.9mmol/l) and diabetes (>6.9 mmol/l). None of the study subjects had their FBG below the normal range and diabetes category. 27% of the PCOS subjects had their FBG levels within the normal range as 55% of the control subjects fell within the same category. 73% of the PCOS subjects and 45% of the non-PCOS subjects fell within the pre-diabetes category.

4.5 Correlation of kisspeptin levels with biochemical and hormonal parameters in PCOS subjects

The correlation of fasting blood glucose, luteinizing hormone, follicle stimulating hormone, prolactin, estradiol and testosterone with kisspeptin was investigated among the PCOS subjects and results are shown in Table 4.4. The correlations of these parameters with kisspeptin in the PCOS subjects were not significant ($p > 0.05$).

4.4 Correlation of kisspeptin levels with demographic parameters in PCOS subjects of the study

From Table 4.3 below, there was no significant direct correlation in the age and kisspeptin levels of the PCOS subjects ($r = 0.082$, $p = 0.614$). There was also an inverse correlation between the BMI and kisspeptin levels in the PCOS subjects, which is not significant ($r = -0.145$, $p = 0.373$).

Table 4.2 Biochemical and hormonal indices of the study

| Parameters | PCOS subjects N= 41 | NON-PCOS subjects N=40 | 95% C.I. difference | P-Value (p<0.05) |
|---------------------------------|------------------------------------|---------------------------------------|--------------------------------|--------------------------------|
| FBG (mmol/l) | 5.82 ± 0.06 | 5.37 ± 0.14 | -0.72 to -0.20 | 0.0008*** |
| | 20.21 ± 1.74 | 12.15 ± 2.51 | | |
| Kisspeptin (ng/ml) | | | -14.06 to -2.07 | 0.0093** |
| LH (mIU/ml) | 3.94 ± 0.29 | 9.33 ± 1.15 | 3.73 to 7.05 | < 0.0001**** |
| | | | | |
| FSH (mIU/ml) | 6.30 ± 0.65 | 15.76 ± 2.24 | 5.96 to 12.96 | < 0.0001**** |
| | 2.84 ± 0.10 | 2.93 ± 0.09 | | |
| Prolactin (ng/ml) | | | -0.23 to 0.41 | 0.593 |
| | 240.80 ± 13.32 | 275.20 ± 14.95 | | |
| Estradiol (pg/ml) | | | -9.20 to 77.94 | 0.120 |
| | | | | |
| Testosterone (ng/ml) | 12.73 ± 0.37 | 11.27 ± 0.52 | -2.75 to -0.19 | 0.025* |

Table 4.2 showed the fasting blood glucose and hormonal levels in PCOS and non-PCOS subjects. Values were given as mean ± standard deviation. LH = Luteinizing hormone. FSH = Follicle stimulating hormone. *mean difference was significant (p<0.05).

Fig. 4.2 The percentage distribution of kisspeptin levels in the study population

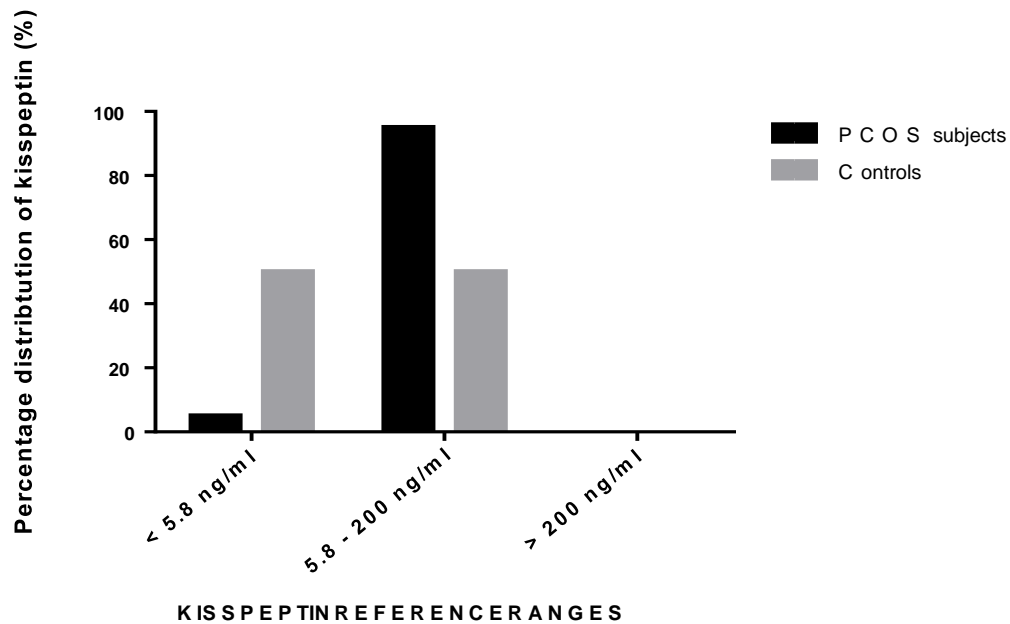


Figure 4.2 shows kisspeptin levels of PCOS and non-PCOS subjects that had been categorised into three groups. None of the study groups had kisspeptin levels above reference range.

Fig 4.3 Percentage distribution of glucose levels in study subjects

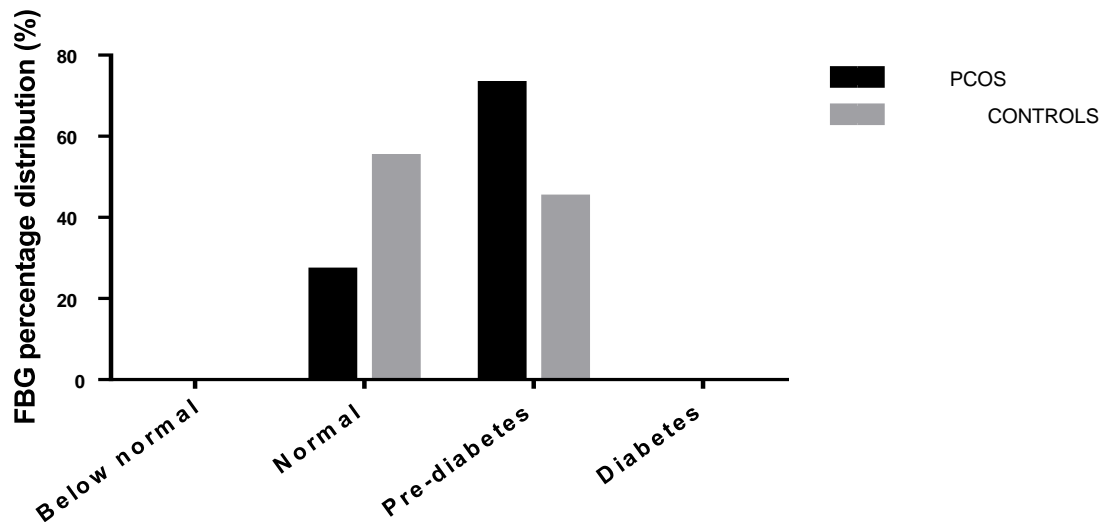


Figure 4.3 shows the fasting blood glucose levels of PCOS and non-PCOS subjects categorised into four groups.

Table 4.3 Correlation of kisspeptin levels with demographic parameters in PCOS subjects of the study

| Kisspeptin levels (ng/ml) in PCOS subjects (N = 41) | | |
|--|---|----------------|
| Parameters | Coefficient of correlation (ρ) | P-Value |
| Age (Years) | 0.082 | 0.614 |
| BMI (kg/m²) | -0.145 | 0.373 |

Table 4.3 shows data presented as Spearman’s correlation coefficient, ρ *Correlation was significant at $p < 0.05$. BMI: body mass index

4.6 Risk factors for high kisspeptin levels in PCOS patients

A further investigation was done to identify the strength of relation of FBG and hormonal parameters with high kisspeptin in the PCOS subjects. It can be observed from table 4.5 that there was a strong association between FSH and high kisspeptin levels in PCOS patients ($p < 0.0001$), as well as a significant association of FBG ($p = 0.0469$) and LH ($p = 0.0130$) with high kisspeptin levels in PCOS patients. The association of high kisspeptin levels in PCOS subjects with prolactin, estradiol and testosterone were however not significant ($p = 0.7829, 0.3914, 0.0899$ respectively).

Table 4.4 Correlation of kisspeptin levels with biochemical and hormonal parameters in PCOS subjects

| Kisspeptin levels (ng/ml) in PCOS subjects (N = 41) | | |
|--|---|----------------|
| Parameters | Coefficient of correlation (ρ) | P-value |
| FBG (mmol/l) | -0.001 | 0.994 |
| LH (mIU/ml) | 0.115 | 0.487 |
| FSH (mIU/ml) | 0.175 | 0.280 |
| Prolactin (ng/ml) | 0.011 | 0.948 |
| Estradiol (pg/ml) | 0.079 | 0.627 |
| Testosterone (ng/ml) | 0.001 | 0.993 |

Table 4.4 showed data presented as Spearman's correlation coefficient, ρ . Correlation was significant at $p < 0.05$

Table 4.5 Risk factors for high kisspeptin levels in PCOS patients

| Risk factors | Odds ratio | 95% C.I. | p-value |
|-----------------------------|-------------------|-----------------|----------------|
| FBG (mmol/l) | 3.333 | 1.088 - 10.21 | 0.047* |
| LH (mIU/ml) | 0.162 | 0.041 - 0.636 | 0.013* |
| FSH (mIU/ml) | 0.025 | 0.003 - 0.219 | < 0.0001**** |
| Prolactin (ng/ml) | 0.782 | 0.265 - 2.308 | 0.783 |
| Estradiol (pg/ml) | 1.920 | 0.583 - 6.318 | 0.391 |
| Testosterone (ng/ml) | 2.954 | 0.975 - 8.951 | 0.099 |

Table 4.5 shows the relationship between correlates (FBG, LH, FSH and testosterone) and high kisspeptin levels in PCOS subjects. Results are given as odd ratio (OR). FBG= Fasting blood glucose, LH=luteinizing hormone and FSH=follicle stimulating hormone
*Odd ratio is significant ($p < 0.05$), CI= Confidence interval.

CHAPTER FIVE

DISCUSSION

Polycystic ovarian syndrome (PCOS) is gradually becoming a major health issue locally and globally. As it has been reported to be an endocrine-related issue with an unclear single cause, the kisspeptin hormone seems to play a very important role hence the need for this research study. The following are discussions of the results of this study.

The difference between the mean ages of the PCOS and non-PCOS subjects was insignificant (Table 4.1). This is because they were age-matched. There was no significant difference in the mean BMI of the study subjects. Most of the subjects in this study were above the normal weight with a greater percentage of the PCOS subjects being obese (Fig. 4.1). This agreed with a study done by Al-Azemi *et al.*, (2004) which reported that 35% to 65% of PCOS patients were obese. Obesity and overweight are clinical features of PCOS (Beydoun *et al.*, 2009). The insignificant difference in the mean BMI of the study subjects cannot be explained as it is contrary to studies done by Madnani *et al.*, (2013), Omokanye *et al.*, (2015) and Umayal *et al.*, (2018), who reported significant difference in the mean BMI of their study subjects.

In this study, the mean differences of fasting blood glucose, luteinizing hormone, follicular stimulating hormone and testosterone were significantly high and were consistent with studies done by Leo *et al.*, (2016), Skorupskaite *et al.*, (2014) and Umayal *et al.*, (2018) who also observed significant increases. The subjects in their respective studies recorded insignificance in their mean age differences and significant difference in their mean BMI.

The observed increases in fasting blood glucose, luteinizing hormone, follicular stimulating hormone and testosterone, in this study, is as a result of the excitement of the neurons of the pituitary in the presence of high levels of gonadotropic releasing hormones which subsequently cause an elevated production of LH and FSH (Katagiri *et al.*, 2007). The increased levels of fasting blood glucose is as a result of the characteristic insulin resistance in the PCOS patients (Rojas *et al.*, 2014) caused by serine phosphorylation of the insulin receptors of cells in PCOS patients (Dunaif, 1997). In figure 4.3 which compared the grouped fasting blood glucose levels of the study subjects, it was observed that a greater percentage of the PCOS patients were pre-diabetic. The action of the adrenal glands is another contributing factor to the hyperglycaemia (Marshall *et al.*, 2012). This is because they are an alternate source of kisspeptin production (Katagiri *et al.*, 2007). Their stimulation to produce kisspeptin leads to a simultaneous production of cortisol that ultimately leads to gluconeogenesis to produce glucose from fat and proteins in the body. The pancreas is also another secretory organ for kisspeptin (Katagiri *et al.*, 2007) involved in glucose metabolism. There is a concurrent production of glucagon with kisspeptin secretion in the pancreas and this leads to glycogenolysis in the liver, hence the increase in blood glucose levels. Insulin resistance and high levels of luteinizing hormones stimulate an increased production in testosterone (Rosenfield *et al.*, 2016). Chronic androgen excess causes the formation of fluid-filled sacs in the ovaries called cysts (multiple ovarian cysts) which tend to secrete more testosterone (Diamanti-Kandarakis and Papavassiliou, 2006).

In this study the mean kisspeptin levels in PCOS subjects compared with the non-PCOS subjects was significantly high (Table 4.2) and agreed with studies done by Katulski *et al.*, (2018), Umayal *et al.*, (2019) and Gorkem *et al.*, (2017) who reported relatively high levels of kisspeptin in PCOS subjects when compared to their control subjects. This is because an

overactive KISS1 gene in the hypothalamus, adrenal and pancreas leads to a hyperactive hypothalamus-pituitary-gonadal (HPG) activity which subsequently causes irregular menstrual cycles, excessive testosterone release and other symptoms in PCOS women (Tang *et al.*, 2019).

In this study the insignificant correlation between the age of the PCOS subjects and their kisspeptin levels (Table 4.3) did not agree with a study done by Umayal *et al.*, (2019) who reported an inverse correlation between the age of PCOS patients and their kisspeptin levels. According to Umayal *et al.*, (2019) the symptoms of PCOS and elevated kisspeptin levels were severe in women of younger age, between the ages of 20 years and 30 years. Also there was no significant correlation between the BMI and kisspeptin of the PCOS patients in this study (Table 4.3). This was inconsistent with studies done by Omokanye *et al.*, (2015), Katulski *et al.*, (2018), Umayal *et al.*, (2019) and Beydoun *et al.*, (2009) (Table 4.3) who reported a significant correlation between BMI and kisspeptin. The reason for this inconsistency is not clear and so needs an in-depth study.

The associations of fasting blood glucose (FBG), LH, FSH, prolactin, estradiol and testosterone with kisspeptin levels of the patients were insignificant in this study. These were inconsistent with studies done by Skorupskaite *et al.*, (2014) and Katulski *et al.*, (2018) who reported significant associations between kisspeptin in PCOS patients (Caucasians). This inconsistency is as a result of the difference in race or genetic makeup. Gorkem *et al.*, (2017) however found no significant correlation between kisspeptin and LH levels in PCOS, which agreed with findings in this study.

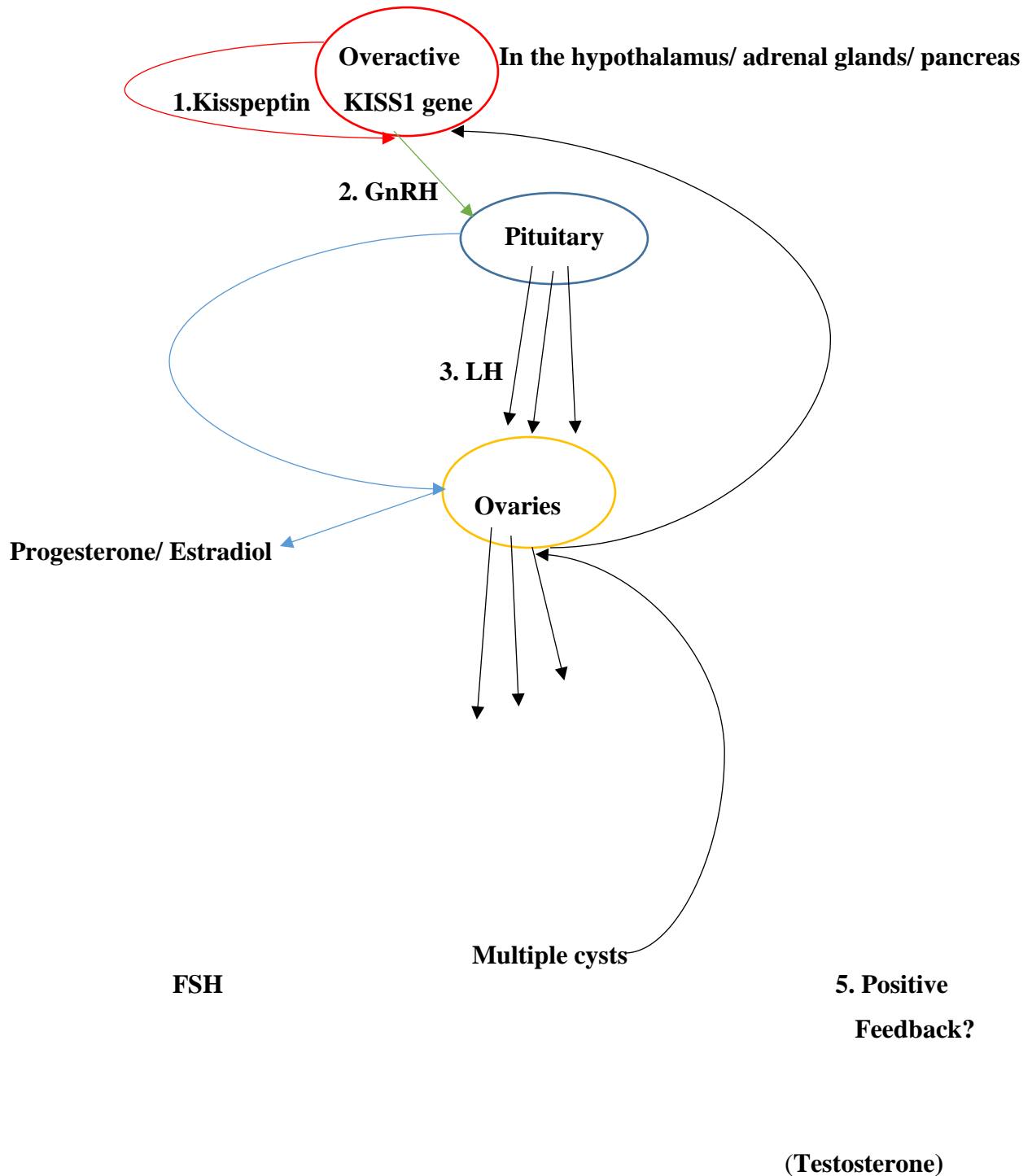
Multivariate regression analysis in the PCOS patients showed that the PCOS patients were 3.33 times more likely to develop high blood glucose (Table 4.5) as their kisspeptin levels

rose. This made high blood glucose a strong predictive marker for high kisspeptin levels in PCOS patients. Same analysis done for FSH as well as LH showed significantly strong associations, where PCOS patients were 2.5 more likely to develop increased FSH and 16.2 more likely to develop elevated levels of LH. This made them good predictive markers for high kisspeptin in PCOS patients. These agreed with studies done by Omokanye *et al.*, (2015), Katulski *et al.*, (2018), Umayal *et al.*, (2019) and Beydoun *et al.*, (2009) who reported that LH, FSH, testosterone and fasting blood glucose are predictive markers for high kisspeptin levels in PCOS patients. The odds ratio of prolactin and estradiol of the PCOS subjects in this study were not significant. Some studies have shown that the levels of prolactin and estradiol in PCOS vary. They are either slightly high or are within the normal range (Laven *et al.*, 2002; Bartolone *et al.*, 2000).

Fig. 4.4 shows a summary of the possible mechanism that controls the increased production of kisspeptin based on this study. A hyperactive KISS1 gene stimulates the hypothalamus (and other organs like the adrenal glands and pancreas) to produce excessive amounts of kisspeptin which cause an increased production of gonadotropic releasing hormones, subsequently exciting the neurons of the pituitary to produce the gonadotropins (LH and FSH). Since kisspeptin has been reported to cause increased pulsatility in the production of LH, the elevated LH levels stimulate the ovaries of PCOS patients to produce more testosterone. The excess testosterone makes the subject resistant to insulin and causes the formation of cysts in the ovaries. The cysts in the ovary secrete more testosterone, contributing to the testosterone excess, a well-known characteristic of PCOS. In such a proposed mechanism, there should be a negative feedback to stop this cascade. According to Kwao-Zigah, *et al.*, (2017), kisspeptin production was reduced as testosterone reduced.

This means that the KISS1 system is made overactive when there is excess testosterone and less overactive when there are lesser amounts of testosterone (Fig. 4.4).

Fig. 4.4 A possible mechanism of control of kisspeptin production in PCOS from this study





The diagram in Fig. 4.3 proposes a possible pathway for the mechanism of kisspeptin control in PCOS from this study. It outlines the possible role that excess testosterone may play in hyperactivity of the KISS1 gene.

CHAPTER SIX

CONCLUSION

Plasma kisspeptin levels were relatively high in Ghanaian PCOS patients. The hypothesis is, however, rejected for this study. This study showed no significant association of age, BMI, prolactin, estradiol and testosterone with kisspeptin in PCOS patients. There were, however, significant association of FBG, LH and FSH with kisspeptin levels in PCOS patients in Ghana.

6.1 Recommendations

It is recommended that genotypic and phenotypic studies of KISS1 gene to help establish the reasons behind the different presentations of the syndrome be done as well as the role of testosterone in the increased production of kisspeptin.

6.2 Limitations

Number of participants was low due to low numbers of women diagnosed with polycystic ovarian syndrome (PCOS).

Due to financial constraints, the measurements of sex hormone binding globulin (SHBG) could not be done as it would have given further insight into the study.

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APPENDICES

APPENDIX I

RESEARCH PARTICIPATION INFORMATION SHEET *(to be explained in a language understood by the participant).*

NAME OF SUPERVISORS: PROFESSOR HENRY ASARE-ANANE AND DR. BEN ANNAN

INSTITUTION: SCHOOL OF BIOMEDICAL AND ALLIED HEALTH SCIENCES, UNIVERSITY OF GHANA.

Dear Respondent,

I am Audrey Anim-Ankumah, a master of philosophy student of the School of Biomedical and Allied Health Sciences, University of Ghana. I am conducting a study currently on the topic, “**THE ASSOCIATION OF KISSPEPTIN WITH POLYCYSTIC OVARIAN SYNDROME**” as part of the requirements for the award of master of philosophy (Mphil) in chemical pathology.

The research seeks to investigate the association of the kisspeptin hormone with the polycystic ovarian syndrome. You are therefore to answer all questions sincerely and to the best of your knowledge and understanding. Information obtained from this study will be kept confidential and used for research only.

It should be noted that the study is voluntary and so you are at liberty to withdraw your participation without giving explanations. For questions concerning this study please do not hesitate to contact the researcher by e-mail via aaankumah@gmail.com or by phone on 0271723490 / 0245726685.

APPENDIX II

RESEARCH PARTICIPATION CONSENT FORM

I have been invited to take part in this study. The purpose of this study has been made clear to me and I have been provided the opportunity to ask questions. I have also been given adequate time to rethink my decision to participate in the study. I have not been pressurized in any way to partake in this study. I have been made to understand that my participation is voluntary and that I can withdraw any time without giving reasons. I understand that this study will not influence the regular care and treatment I receive from my healthcare providers. I know that this study has been approved by the Ethical and Protocol Review Committee of the University of Ghana, College of Health Sciences. I also know that the information obtained from this research will be confidential and also for scientific purposes only. I agree to this, provided my privacy is guaranteed.

I hereby consent to participate in this study.

Signature of Participant..... Thumb print..... Date:/...../2018

Statement of Researcher

I provided verbal and/or written information regarding this study.

I agree to answer any future questions concerning the study as best as I am able.

I will adhere to the approved protocol.

Signature of Researcher.....Date:/...../2018

THANK YOU

APPENDIX III

QUESTIONNAIRE *(to be explained in a language understood by the participants)*

DEMOGRAPHICS (PATIENT CHARACTERISTICS)

1. NAME:
2. PATIENT CODE:
3. AGE: I do not know[.....] (Please tick if applicable)
4. WEIGHT (kg): 5. HEIGHT (metres):
6. BMI: