THE ASSOCIATION OF GHRELIN AND LEPTIN IN POLYCYSTIC OVARIAN SYNDROME

BY

NURUL JANNAH BINTI ISMAIL

A dissertation submitted in fulfilment of the requirement for the Master of Obstetrics and Gynecology

Kulliyyah of Medicine
International Islamic University Malaysia

OCTOBER 2020

ABSTRACT

The hormone ghrelin has been extensively studied since its discovery in 1999. It is mainly secreted by stomach cells and found in other tissues, such as the hypothalamus, pituitary, pancreas, lung tissue, placenta, and ovaries. Another hormone, leptin, discovered in 1994, is produced by white adipose tissue that sends information to the brain regarding the amount of fat stored in the body. It is also known as the satiety hormone that gives negative energy balance in humans and involves a long list of endocrine functions, including obesity, insulin resistance, hyperandrogenism, and polycystic ovary syndrome (PCOS). Although PCOS is one of the common endocrinopathies that we know affecting women of reproductive age, to date, there are no clear etiology and pathophysiology for it. The term "polycystic ovary syndrome" comprises more than its simple name. It encompasses a broader spectrum of signs and symptoms of ovarian dysfunction. It also forms a heavy socioeconomic burden to the country besides psychological impact. Hence, it is critical to clarify the etiology of PCOS to decrease the burden of this disease. It is disheartening that there is no complete cure for PCOS, and the exact etiology of PCOS has not been fully understood. Although several studies were published concerning the link between ghrelin and leptin with PCOS, the inconsistent findings render the relationship controversial. Thus, in this review, we discussed the connection between PCOS and the hormones and tried to understand their connection. We also highlighted the gaps in previous studies. Current knowledge shows that ghrelin and leptin could have a possible link to PCOS. Further understanding of this relationship may lead to a better understanding of the etiology of PCOS, advancement of its management, and discovery of future pharmacological intervention.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Master of Obstetrics & Gynaecology

……
Muna Khaleel Al- Kubaisi
Supervisor

……
Roslina Abdul Rahim
Co-Supervisor

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Master of Obstetrics & Gynaecology

……
Sirajudeen Kuttulebbai Naina Mohamed Salam
Internal Examiner

This dissertation was submitted to the Department of Obstetrics & Gynaecology and is accepted as a partial fulfilment of the requirements for the degree of Master of Obstetrics & Gynaecology.

……
Hamizah Ismail
Head, Department of Obstetrics and Gynaecology

This dissertation was submitted to the Kulliyyah of Medicine and is accepted as a partial fulfilment of the requirements for the degree of Master of Obstetrics & Gynaecology.

……
Azmi Md Nor
Dean, Kulliyyah of Medicine

DECLARATION

I hereby declare that this thesis is the result of my own investigation, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

Nurul Jannah Ismail

Signature ..... Date .....

INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA

DECLARATION OF COPYRIGHT AND AFFIRMATION OF FAIR USE OF UNPUBLISHED RESEARCH

THE ASSOCIATION OF GHRELIN AND LEPTIN IN POLYCYSTIC OVARIAN SYNDROME

I declare that the copyright holder of this thesis/dissertation is International Islamic University Malaysia.

No part of this unpublished research may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording or otherwise without prior written permission of the copyright holder except as provided below.

1. Any material contained in or derived from this unpublished research may be used by others in their writing with due acknowledgement.

2. IIUM or its library will have the right to make and transmit copies (print or electronic) for institutional and academic purposes.

3. The IIUM library will have the right to make, store in a retrieval system and supply copies of this unpublished research if requested by other universities and research libraries.

By signing this form, I acknowledged that I have read and understand the IIUM Intellectual Property Right and Commercialization policy.

Affirmed by Nurul Jannah Ismail

……..……..…….…… ……..……..

Signature Date

ACKNOWLEDGEMENT

IN THE NAME OF ALLAH, THE MOST GRACIOUS, THE MOST MERCIFUL

Praise and thank you to Allah, the lord Al-Mighty for his blessing and generosity for giving me strength and courage to complete this dissertation.

Foremost, I would like to convey my deep and sincere gratitude to my inspiring Associate Professor Dato Dr Hamizah Ismail, the Head of Obstetrics & Gynaecology Department International Islamic University Malaysia who gave trust and exposed me to pursue this dissertation work. She is also the one that introduced me with ghrelin and leptin and arranged to reach my lovely research supervisors. I would like to express my deepest gratitude to my research supervisors Associate Professor Dr Muna Khaleel Al-Kubaisi and Associate Professor Dr Roslina Abdul Rahim for their times, support, commitment and guidance throughout this thesis journey. Thank you for your patience, enthusiasm and motivation given to me in my ups and down moments to complete my thesis. It was a great privilege and honour to be under your guidance.

I am extremely grateful and thanks to my husband Abdelmohaimen Sayyid Aly Abdelmaged for his endless support and motivation throughout this journey. Even though with the current COVID 19 pandemic situation we are in now worsened our long-distance relationship due to works, he never failed to give encouragement, endless prayers, love and patience throughout this tough time.

There are no suitable words to describe my gratitude to my father, Ismail bin Haji Omar and my mother, Siti Ruzainah binti Peseri for their sacrifice looking after my daughters, their unconditional love, endless prayers and motivation, also being together with me in Kuantan during this journey. May Allah grand “Jannatul Firdaus”, give His blessing good health, and righteous long life to both of you and my husband.

I would like to convey my love and thanks to my lovely two daughters, Kawtharul Jannah and Salsabilatul Jannah for your patience and sacrifice during this whole process. Thank you for being the apple of my eye and lift up my inner motivation to complete this journey.

Finally, sincere gratitude to all lecturers, colleagues and staff in Obstetrics and Gynaecology Department, International Islamic University Malaysia who had help and contribute a lot throughout my master programme.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ABBREVIATIONS

BMI	Body mass index
DHEAS	Dehydroepiandrosterone sulfate
FSH	Follicular stimulating hormone
GH	Growth Hormone
GHR	Growth hormone receptor
GHRH	Growth hormone releasing hormone
GHSR	Growth hormone secretagogue receptor
GHSR1a	Growth hormone secretagogue receptor 1a
GHRPs	GH releasing peptides
GnRH	Gonadotrophin releasing hormone
GOAT	Ghrelin O-acyltransferase
GPCR	G protein-coupled receptor
LEPRa	Leptin receptor short isoform
LEPRb	Leptin receptor long isoform
LH	Luteinizing hormone
MeSH	Medical Subject Headings
MetS	Metabolic Syndrome
ob	Obese
PCOS	Polycystic Ovarian Syndrome
WAT	White adipose tissue
WHO	World Health Organisation
X/A-like cells	A group of a unique endocrine cells in gastric oxyntic mucosa which secret ghrelin

CHAPTER ONE

INTRODUCTION

The novel ghrelin hormone is a 28 amino acid peptide h ormone initially found in a rat's stomach in 1999 by the reverse pharmacological procedure (Kojima et al., 1999). It is found after the extensive research efforts that aimed to identify the endogenous ligand for the growth hormone secretagogue receptor 1 (GHSR1) and proven as the different mechanisms of regulating growth hormone (GH) release from the usually known pathway (Müller et al., 2015; Kojima et al., 1999). Ghrelin has been identified and known as the most potent endogenous orexigenic peptide and plays a big role in humans, especially in body weight, glucose homeostasis, energy balance, and also in the reproductive area (Delhanty & Van Der Lely, 2011; Diéguez et al., 2010; Tschöp et al., 2001).

On the other hand, leptin is a product of the *ob* gene, mainly expressed and secreted by white adipose tissue (WAT) and acts as a potent endogenous anorexigenic peptide in the body. It is involved in the long-term regulation of body weight, energy homeostasis, and reproductive function (Gale et al., 2004).

Polycystic ovary syndrome (PCOS) is one of the commonest endocrine metabolic disorders in the world. It is estimated that the prevalence of PCOS is about 10% of reproductive women, according to the Rotterdam criteria (Bozdag et al., 2016), and can be as high as 15%–20% (Sirmans & Pate, 2013). The varying numbers of the prevalence are dependent on which criteria are used to diagnose PCOS and places. For example, in the southeastern United States, the overall prevalence of PCOS is 4.6% (Knochenhauer et al., 1998), while Spain reported 6.5% (Asunción et al., 2000).

To date, the association of ghrelin and leptin in PCOS is still unclear. Thus, this study assessed a series of scientific literature for the past ten years to explore and understand the association or relationship between the hormones and PCOS.

1.1 GENERAL OBJECTIVE

The aim of this study is to explore the association of ghrelin and leptin with the polycystic ovarian syndrome.

1.2 SPECIFIC OBJECTIVE

The study aimed to achieve the following objectives:

1. To discuss regarding ghrelin, leptin, PCOS and the relationship or association both hormone towards PCOS.
2. To identify any gaps in previous research on this topic
3. To points the way forward for further research especially in contribution to our local medical knowledge in understanding PCOS and ghrelin & leptin.

CHAPTER TWO

LITERATURE REVIEW

2.1 GHRELIN

Ghrelin is derived from the word ghre, the Proto-Indo-European root of the word for "grow." "GH" and "relin" are abbreviations for "growth-hormone-release," which is the characteristic effect of ghrelin. Ghrelin is a 28 amino acid peptide secreted mainly from X/A-like cells in the stomach (Kojima et al., 1999). It is also expressed widely in other parts of the tissue, giving endocrine and paracrine effects. The des-acyl ghrelin and n-octanoyl-modified ghrelin are the two major forms of ghrelin. For ghrelin to exert metabolic effects in the body, the process of octanoylation or acylation is vital to activate its receptor (Müller et al., 2015). Even though unacylated ghrelin is the predominant form of the peptide, it could not bind to GHSR or influence GH secretion.

2.1.1 Discovery and Physiological Action

Masayasu Kojima, Kenji Kangawa, and colleagues discovered the novel gastrointestinal peptide hormone ghrelin in 1999 using a reverse pharmacological procedure that led to various studies and tremendous research findings. Ghrelin is released by ghrelin cells (X/A-like) of the stomach fundus cells, presenting a unique n-octanoylation modification on its serine in position 3, catalyzed by ghrelin O-acyl transferase (Kojima et al., 1999). It is the first bioactive peptide modified by fatty acid (Nishi et al., 2011). This acylation process is important for ghrelin to bind with GHS-R 1a to exert GH action and other endocrine functions (Kojima & Kangawa, 2005). It might also influence the transport of ghrelin across the blood-brain barrier (Banks et al., 2002).

Ghrelin is the endogenous ligand for the GHSR1a, capable of stimulating GH release from the anterior pituitary (Kojima et al., 1999). GH or somatotropin is a peptide hormone from the anterior pituitary, which participate in the control of several complex physiological processes, including regulating the overall body and cell growth, regulating carbohydrate-protein-lipid metabolism, and maintaining water-electrolyte balance (Argetsinger & Carter-Su, 1996) The regulation of growth hormone is controlled by many factors, particularly two hypothalamic neuropeptides. The Hypothalamic GH-releasing hormone (GHRH) controls GH release, while somatostatin does the opposite (Müller et al., 1999). Initially it was thought that the GH-releasing peptides (GHRPs) promote the release of GH upon reaction on the anterior pituitary. However, it also acted on the hypothalamic arcuate nucleus (ARC), specifically on GHRH neurons (Dickson et al., 1993). It is unknown how these molecules promote GH release, as it is different from the usual GHRH/somatostatin pathway. Thus, in-depth studies of a small synthetic peptide known as growth hormone secretagogues (GHSs) reported potent stimulators of the GH release from the pituitary. This independent pathway of GH through synthetic compound is believed to work through a G protein-coupled receptor (GPCR), the GHS-receptor (GHS-R) type 1a (GHS-R1a) and an endogenous ligand to carry its function. The existence of this endogenous ligand has attracted the attention of many industries and academics, as it will potentially contribute to the treatment of GH deficiency (Kojima & Kangawa, 2005). The search for this mysterious ligand continues and remains elusive until 1999 when Kojima and colleagues discovered it from the rat stomach (Kojima et al., 1999).

2.1.2 Regulation and Tissue Distribution

Ghrelin is initially found in rat's stomach and its purified form comprises 28 amino acid peptides (Kojima et al., 1999). Apart from two amino acids, human ghrelin is homologous to the rat. The ghrelin genes in humans and other mammals or non-mammals is located on a different chromosome. For instance, the human ghrelin gene is located on chromosome 3, while for rats, it is on chromosome 4. The pre-prohormone type, which consists of 117 amino acids, is the primary product of ghrelin. It then undergoes cleavage, resulting in two forms of ghrelin. The first one is the 28- amino acid form (with C-terminal Arg) and the second form is the 27-amino acid form (with C-terminal Pro) generated by alternative splicing of the gene product. The acylation process is crucial to activating ghrelin and allowing it to exert its function. Ghrelin requires the attachment of a fatty acid to its serine 3 residue, which requires ghrelin O-acyltransferase (GOAT) (Gutierrez et al., 2008).

In humans, the hormone is predominantly found and secreted in the stomach and about 30% in the intestine (Date et al., 2000). In the stomach, it is found at the fundus part in the oxyntic gland but not at the pyloric region, as it is secreted by the X/A like cells (Sakata et al., 2006). Other tissues found to express ghrelin are the hypothalamus (Cowley et al., 2003; Kojima et al., 1999). It is present in the group of neurons adjacent to the third ventricle between the dorsal, ventral, para-ventricular, and arcuate hypothalamic nuclei (Cowley et al., 2003). Other than that, it is also expressed at the pituitary gland, lung tissue, immune system, kidneys, ovary, testis (Ishikawa et al., 2006), and even in the placenta (Gualillo et al., 2001). Some studies ghrelin and its receptor in the pancreatic islet (Date et al., 2002). Ghrelin is an orexigenic hormone or known as the hunger hormone. The circulating ghrelin levels vary throughout the day, with a high level during fasting and a low level after food consumption. It plays

important role in the short-term regulation of the appetite by stimulating food intake, which is different from leptin. Ghrelin is a signal of energy deficiency as opposed to leptin. It also acts as a regulator of energy homeostasis, i.e., the most established role of ghrelin and regulates GH secretion (Müller et al., 2015; Delhanty & Van Der Lely, 2011; Baudet & Harvey, 2003;)

Studies in rodents have shown that ghrelin initiated a peripheral signal to the brain to induce adiposity while simultaneously stimulating GH secretion. Ghrelin is reduced in obese people compared to lean people (Tschöp et al., 2001). It may represent a physiological adaptation to the positive energy balance in obesity.

2.1.3 Ghrelin and PCOS

As mentioned above, this neuroendocrine brain-gut hormone has an important role in energy homeostasis, food intake, and weight regulation. It also regulates insulin secretion, glucose homeostasis (Delhanty & Van Der Lely, 2011), and ovarian functions (Gaytan et al., 2003). Apart from its relationship with insulin resistance and obesity, ghrelin also affects reproductive function, the biggest subtopic when discussing PCOS. The presence of ghrelin receptor and its expression in the ovaries (Gaytan et al., 2003), specifically at the granulosa and theca layers of follicular cyst (Komarowska et al., 2006), the deficient activity of corpus luteum possibly by ghrelin (Komarowska et al., 2006), the ability of ghrelin to suppress luteinizing hormone (LH), and its involvement on hypothalamic-pituitary-ovary axis (Kluge et al., 2007) suggests its association with PCOS. On the other hand, PCOS is endocrinopathy that may have major metabolic, reproductive, and psychological consequences. Obesity is one of the main issues in PCOS, even though it is not the characteristic feature of PCOS (Lim et al., 2012). The same goes for insulin resistance and hyperinsulinemia in PCOS, leading to obesity and

metabolic disorders. Therefore, many studies try to find the equation point between those two. Schofl et al. (2002) first reported the low level of ghrelin in PCOS women, which highly correlated with insulin resistance condition despite the unknown (Schöfl et al., 2002). A meta-analysis by Gao et al (2016) supported the previous result, which concluded that ghrelin level is decreased in PCOS women. On the contrary, some studies revealed that ghrelin has no significance difference or displayed a high level in PCOS.

2.2 LEPTIN

Leptin is found in *ob* gene in rats through positional cloning (Zhang et al., 1994). It is a 167 amino acid peptide with a molecular weight of 16 kDa, located at chromosome 6 in rats and chromosome 7 in humans (Münzberg & Morrison, 2015). The name leptin is derived from the Greek word *leptos,* which means thin.

2.2.1 Discovery and Physiological Function

The discovery of leptin in 1994 gives hope to obese patients all over the world. There is a long journey in discovering the leptin hormone, starting way back in 1950. During that time, they had found a recessive mutation called *ob* gene in house mice kept in captivity, where the mice were three times heavier than the normal mice (Ingalls et al., 1950). Subsequently, in 1953, Kennedy proposed the lipostatic theory, where the adipocytes produced a circulating factor or determinant to interact with the hypothalamus to regulate food intake and body weight of the experimental rats (King, 1976). Although other researchers confirmed the circulating factor found on the *ob* gene, they failed to identify it. Finally, about 40 years later, Friedmann and his team

discovered the gene in 1994 through positional cloning of mouse *ob* gene that has been shown to encode a 4.5 kb mRNA transcript, with a highly conserved 167 amino acid chain (Zhang et al., 1994). The discovery of leptin provides an impetus and has attracted many researchers to explore and identify a wide spectrum of physiological and potential effects elicited by this adipose-derived hormone in humans.

2.2.2 Regulation and Tissue Distribution

Leptin is a peptide hormone encoded by the *ob* gene and primarily secreted by white adipocytes tissue (WAT). Other than adipocytes, leptin can be found and expressed in the hypothalamus, heart, skeletal muscle, stomach, ovary, testis (Cioffi et al., 1996) mammary glands (Smith-Kirwin et al., 2014), and even at the placental tissue (Sagawa et al., 1999).

It plays a key role in the feedback system between the adipocytes and the hypothalamus, particularly at the ventromedial nucleus, i.e., the satiety center. Upon synthesis by the adipocytes, leptin is secreted to the bloodstream and signals critical information to the brain about the peripheral energy storage and the availability in the body. Leptin has two forms, i.e., the long form (LEPRb) and the short form (LEPRa). The LEPRb mRNA has been identified in the brain, specifically at the arcuate nucleus, dorsomedial nucleus, ventromedial nucleus, and ventral premammillary nuclei (Burguera et al., 2000). Meanwhile, the LEPRa mRNA has been identified in the choroid plexus, vascular endothelium, liver, lung, gonads, and kidney. Thus, after secreted into the bloodstream, the LEPRa receptors help transport leptin across membranes, particularly the blood-brain barrier and the kidney. Subsequently, it will be bound to the long form of leptin receptor in the thalamus and exert an anorexigenic

effect on the body, e.g., suppressing appetite and feeding, increased autonomic activity, and thermogenesis (Zhou, 2013; Pénicaud et al., 2012;). This will promote a weight loss effect on the body. Thus, any disturbance of the leptin mechanism pathway will lead to the loss of appetite suppression, and the person will eat more and subsequently becomes obese. The high level of leptin in the obese population concerning body fat mass contradicts its action, whereby it does not act as a satiety hormone and promote weight loss. Hence, leptin resistance is possible due to several factors, like a mutation in the leptin receptor, which causes hyperleptinemia but fails to exert its physiological function (Friedman, 2014; Zhou, 2013). Leptin resistance is a complex issue as it may occur at any point in the regulatory feeding circuit or impaired leptin transportation.

2.2.3 Leptin and PCOS

Leptin has an important relationship with obesity and fertility. It is involved in the reproductive system considering its influence on the gonadal function, specifically the ovary and its action on the hypothalamic-pituitary-ovary (Celik et al., 2015; Vázquez et al., 2015). In the reproductive field, follicular fluid leptin is an optimistic marker for a successful assisted reproduction treatment, especially in PCOS (Mantzoros et al., 2000). Besides that, it is also involved in insulin action and fat metabolism. The said factors may contribute to the hypothesis of leptin involvement in PCOS. Raised leptin level in PCOS, particularly in obese PCOS women, has been reported in several studies, while some studies reported no significant difference (Zheng et al., 2016).

2.3 POLYCYSTIC OVARIAN SYNDROME

Stein and Leventhal described the characteristics of polycystic ovaries back in 1935. They observed seven women with a menstrual problem (amenorrhea/irregular menses),

and most of them had subfertility issues. After proceeding with surgery, they noticed that all patients had bilateral polycystic ovaries. PCOS characteristics include amenorrhea, hirsutism, and enlarged polycystic ovaries (Stein & Leventhal, 1935). Since then, studies on PCOS have grown tremendously all over the world. Despite the various biochemical, histological findings, sonographic, and clinical appearances associated with PCOS, up until now, there is no universally accepted definition for it.

It predominantly affects women of reproductive age, with diverse clinical manifestations. Even though the pathophysiology behind the PCOS is not established, the patients shared similar manifestations. PCOS clinical manifestations include hyperandrogenism, anovulation, infertility, and increased risk of metabolic morbidities, e.g., insulin resistance, glucose intolerance, and cardiovascular disease risk (Alexander et al., 2009).

Hyperandrogenism is one of the diagnosis criteria and one of PCOS characteristics caused by excessive androgen production and secretion by ovarian theca cell (Nelson et al., 2001). Generally, apart from hyperandrogenism, the cardinal features of PCOS are chronic anovulation or polycystic morphology (Laven et al., 2002).

The Rotterdam Consensus Workshop group in 2003 has replaced the National Institutes of Health (NIH) diagnostic criteria after they integrated the ultrasound findings of ovaries as one of the diagnostic criteria. Three diagnostic criteria of PCOS are available provided other possible morbidities with similar clinical presentations have been excluded (Fauser, 2004). The first criterion is oligo-ovulation or anovulation, which is usually manifested as oligomenorrhea or amenorrhea. The second criterion is hyperandrogenism, which can be clinical manifestations or biochemical confirmation. Clinical manifestations of hyperandrogenism include hirsutism, even though the assessment of it is relatively subjective. Thus, some physicians used a standard visual

clinical scoring of hirsutism, such as the Ferriman-Gallwey hirsutism. However, it was criticized as unsuitable for all populations and other parts of the body that are not included but greatly contributed to the total hirsutism score (Hassa et al., 2005). For biochemical hyperandrogenism, free testosterone or free testosterone index, or dehydroepiandrosterone sulfate (DHEAS) may be useful for diagnosis (Fauser, 2004). The last criterion is the ultrasound findings of polycystic ovaries, which includes 12 or more follicles in each ovary measuring 2–9 mm in diameter and/or increased ovarian volume of >10 mL (Balen et al., 2003). If the presence of follicles is more than 10mm, a repeated ultrasound needs to be scheduled in the next cycle, provided it is not pathological ovary, which warrants further investigation (Fauser., 2004). At least two out of three criteria need to be fulfilled to diagnose the patient with PCOS.

2.3.1 Obesity and PCOS

Obesity is intricately linked to polycystic ovarian disease. According to the World Health Organization (WHO), obesity is defined as abnormal or excessive fat accumulation that presents a health risk. The usual body mass index (BMI) for an obese person is ≥30kg/m2 and overweight is ≥25kg/m2. However, for Asian people, the cut-off value for overweight and obesity is different, as Asians and Western population has different in mean body mass, for instance overweight is ≥23kg/m^2 while obese is ≥28kg/m^2.

Women have more body fat compared to men in general. In Malaysia, the National Health and Morbidity Survey 2011 reported that in adults over 18, 33% were classified as pre-obese, and 27.2% were obese. Interestingly, due to the multi ethnics, a study conducted in Malaysia reported that the obesity rates of the women population were higher in the Indian and Malay women than the Chinese (Ismail et al., 2002).

Obesity is considered the new epidemic crisis worldwide, and it is crucial to take care of it as it can lead to serious morbidities and mortalities. It is associated with an increased risk of developing metabolic syndrome (MetS), impaired glucose tolerance, cardiovascular event, and many more.

Although obesity and polycystic ovarian disease are strongly correlated and common, the etiology of this association remains unclear. The presence of obesity in PCOS women could be between 30%–70%, depending on the setting of the study and the ethnic of the subjects (Vrbikova & Hainer, 2009). Similarly, Escobar-Morreale et al. (2005) reported that about 35% of morbidly obese women have PCOS. Nevertheless, obesity is not a diagnostic criterion for the polycystic ovarian disease.

As obesity and PCOS are intricately linked together, they shared similar pathophysiological changes, i.e., insulin resistance and secondary hyperandrogenism. Even though we know that not all PCOS women are obese, the rising number of obese populations around the world, especially the adolescents (Stamatakis et al., 2005), might contribute to the incline of PCOS, as well as endocrinopathies and metabolic disease, cardiovascular risk, and reproductive health issues.

2.3.2 Insulin Resistance and Hyperandrogenism in PCOS

In the absence of obesity, PCOS women have a high prevalence of impaired glucose tolerance and type 2 diabetes mellitus. It is estimated that approximately 30 – 40% of PCOS women have impaired glucose tolerance, and by the fourth decade, 7.5%–10% have type 2 diabetes mellitus (Legro et al., 1999; Ehrmann et al., 1999). Leptin and insulin resistance increase ovarian sensitivity to the luteinizing hormone (LH) and contribute to hyperandrogenism progression and anovulation in PCOS patients.

Hyperandrogenism is one of the cardinal characteristics in PCOS, as agreed by many experts. While the most hyperandrogenism is about 70% etiologically coming from PCOS (Escobar-Morreale et al., 2012), other diseases exhibit hyperandrogenism, e.g., congenital adrenal hyperplasia and idiopathic hirsutism. A subset of PCOS exists, known as HAIR-AN syndrome (hyperandrogenism, insulin resistance, and acanthosis nigricans), found in 1%–3% of women with hyperandrogenism.

However, the pathophysiological behind hyperandrogenism is still complex and often debatable. In a normal hypothalamus-pituitary-ovarian axis, gonadotrophin-releasing hormone (GnRH) is secreted by the hypothalamus in a pulsatile manner and act on the anterior pituitary to secrete LH. During the follicular phase, LH then acts on the ovarian theca cells and causes an influx in androgenic precursor output. Meanwhile, the follicular stimulating hormone (FSH) increases and acts on the ovarian granulosa cell to convert these androgens into estrogens (estradiol), which functions in follicular development. Unfortunately, this cycle does not occur in PCOS. The absence of PCOS pulsatility, high LH concentration, and normal or low-level of FSH cause the sex steroid to be higher than usual. Negative feedback is noted in the pituitary due to a high-level estrogen level, which finally, an affects FSH secretion. Thus, theca cells undergo hyperplasia and subsequently increases androgen level, causing the patient in a hyperandrogenic state and present with a menstrual problem, chronic anovulation, and hirsutism.

CHAPTER THREE

METHODOLOGY

3.1 DATA BASE

A comprehensive literature search from the PubMed database was conducted from 2010 to September 2020 for references concerning the topic of interest. The articles were filtered in the English language only.

3.2 SEARCH KEY WORDS:

The medical subject heading (MeSH) terms and keywords used are ghrelin, leptin, and PCOS. We notice that Due to the absence of standard PCOS nomination, alternative nominations were used, i.e., polycystic ovarian disease, polycystic ovary, functional ovarian hyperandrogenism, ovarian hyperthecosis, sclerocystic ovary syndrome, and Stein-Leventhal syndrome.

3.3 TYPES OF STUDIES USED

Studies were included if the criteria were met, including human studies, women in their reproductive age group (15 – 49 years old), the diagnosis of PCOS must follow the Rotterdam Criteria 2003, the level of leptin and ghrelin must be taken from the blood, the subject must not be on hormonal treatment, and the studies must have a comparison between PCOS and healthy controls. Studies will be excluded if the full article could not be retrieved or if they involved animal studies. Other exclusion criteria were subjects in both groups have a systemic illness, including thyroid disorder, Cushing syndrome, congenital adrenal hyperplasia, systemic inflammatory disease, acromegaly, hyperprolactinaemia, congenital adrenal hyperplasia, systemic inflammatory diseases,

acromegaly, functional hypothalamic amenorrhea, diabetes mellitus, using oral contraceptive pills, or any other hormonal medication, were excluded from the study.

3.4 KEY WORDS

The literature search using the keywords mentioned above came up with 6,549 articles using the keyword ghrelin and 19,590 articles using the keyword leptin. Meanwhile, the keyword PCOS and its synonyms result in 10,028. The combination of ghrelin and PCOS yielded 49 articles, while the combination of leptin and PCOS resulted in 217 articles. The merged ghrelin or leptin with PCOS, resulted in 240 articles.

3.5 LITERATURES

A total of 240 related articles came up in the PubMed database according to the keywords applied. Subsequently, after reviewing the articles and the abstracts, 75 articles matched the topic of interest. The exclusion criteria were applied during the screening of articles, which includes pregnant women, patient having systemic illness such as thyroid disorder, Cushing syndrome, congenital adrenal hyperplasia, systemic inflammatory disease, acromegaly, hyperprolactinemia, functional hypothalamic amenorrhea, congenital adrenal hyperplasia, diabetes mellitus, using oral contraceptive pills, or any other hormonal medication at least three months prior to the study. After applying the inclusion and exclusion criteria, 27 articles suited the topic discussed. Other articles, journals, letters to the editor, and peer reviews were also excluded from the search. Moreover, the references of involved articles were manually searched, and additional information obtained for subtopics was obtained through search engines.