A PILOT STUDY OF SERUM ANTI-MüLLERIAN HORMONE IN POLYCYSTIC OVARY SYNDROME AND ITS RELATIONSHIP WITH INSULIN RESISTANCE, LIPID PROFILE AND ADIPONECTIN

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Dissertation Submitted In Partial Fulfillment of the Requirement
for the Degree of Master of Pathology
(Chemical Pathology)

UNIVERSITI SAINS MALAYSIA

2018

ACKNOWLEDGEMENTS

It has been an intense learning period for me while completing this dissertation. I would like to express my sincere gratitude and appreciation to people who have supported and helped me throughout this period.

I would first like to express my greatest appreciation to my supervisor, Dr. Aniza Mohammed Jelani for providing all the valuable opportunities, encouragement and guidance for me to conduct this research and successfully complete my dissertation.

Similar, profound gratitude goes to my co-supervisor, Dr. Julia Omar for her professional guidance on this research work. I would also like to thank Dr. Rahimah Binti Abdul Rahim who is also my co-supervisor for her support and help especially in study subject recruitment.

This work would not have been possible without the assistance and help of the laboratory staffs. I am particularly indebted to Mr. Tan Say Koon, medical laboratory technologist, who has been very helpful in samples analyses.

Finally, but by no means least, I would like to thank my loving parents, Alex Thien Joon Huat and Hong Swee Lan, husband, George Wong Pah Kiam, and my son, Ayden Wong Yan Wey, who always there encouraging me and provide unending inspiration.

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LIST OF ABBREVIATIONS AND SYMBOLS

AE-PCOS	: Androgen Excess and PCOS
AMH	: Anti-Müllerian hormone
ASRM	: American Society for Reproductive Medicine
ECLIA	: Electrochemiluminescence immunoassay
ELISA	: Enzyme-linked immunosorbent assay
ESHRE	: European Society of Human Reproduction and Embryology
FSH	: Follicle-stimulating hormone
GnRH	: Gonadotropin-releasing hormone
HDL-C	: High-density lipoprotein cholesterol
HOMA-IR	: Homeostasis model assessment-insulin resistant
HREC	: Human Research Ethics Committee USM
HUSM	: Hospital Universiti Sains Malaysia
IQR	: Interquartile range
IR	: Insulin resistance
LH	: Luteinizing hormone
MetS	: Metabolic syndrome
MIS	: Müllerian-inhibiting substance
NIH	: National Institutes of Health

OGTT	: Oral glucose tolerance test
PCOS	: Polycystic Ovary syndrome
QUICKI	: Quantitative insulin sensitivity check index
SD	: Standard deviation
SHBG	: Sex hormone binding globulin
TG	: Triglycerides

ABSTRAK

Kajian Perintis Hormon Anti-Müllerian Dalam Serum Pesakit Sindrom Polisistik Ovari Dan Hubungkaitnya Dengan Kerintangan Insulin, Profil Lipid dan Adiponektin.

Tujuan: Kajian ini dijalankan untuk menentukan aras hormon anti-Müllerian (AMH) dalam serum pesakit yang mengalami sindrom polisistik ovari (PCOS) dan kaitannya dengan kerintangan insulin, profil lipid dan adiponektin.

Kaedah kajian: Kajian keratan rentas lintang (cross sectional study) dijalankan di Hospital Universiti Sains Malaysia (HUSM), Kampus Kesihatan, Kubang Kerian, Kelantan, Malaysia. Seramai tiga puluh (30) wanita (umur 18-40 tahun) yang didiagnosis PCOS telah dipilih dari klinik ginekologi bermula dari Julai 2016 hingga April 2017. Sampel darah vena dikumpulkan daripada semua subjek dan dianalisis untuk aras serum AMH, insulin, adiponektin, trigliserida, high-density lipoprotein cholesterol (HDL-C) dan plasma glukosa. Kerintangan insulin dikira dengan menggunakan rumus *homeostasis model of assessment-insulin resistance (HOMA-IR).* Aras serum AMH dianggarkan dengan menggunakan analisis deskriptif dan korelasi aras serum AMH dengan parameter metabolik dianalisis dengan menggunakan kaedah statistic korelasi Pearson (Pearson correlation) atau korelasi Spearman (Spearman correlation).

Keputusan: Median serum AMH dalam subjek PCOS adalah 6.8 ng/mL (IQR: 7.38 ng/mL). Terdapat korelasi negatif yang signifikan antara serum AMH dengan HOMA-IR (r = -0.49, p = 0.006) dan serum AMH dengan serum trigliserida (r = -0.55, p = 0.002). Korelasi positif yang signifikan telah diperhatikan antara serum AMH dengan serum HDL-C (r = 0.56, p = 0.001) dan serum AMH dengan serum adiponektin (r = 0.44, p = 0.014) dalam semua subjek.

Kesimpulan: Aras serum AMH dalam wanita yang mengalami PCOS mempunyai kaitan dengan aras HOMA-IR, trigliserida, HDL-C dan adiponektin. Oleh itu, serum AMH berpotensi digunakan sebagai penanda risiko kardiometabolik (cardiometabolic) dalam golongan ini.

ABSTRACT

A Pilot Study Of Serum Anti-Müllerian Hormone In Polycystic Ovary Syndrome And Its Relationship With Insulin Resistance, Lipid Profile and Adiponectin.

Objectives: This study was done to estimate the level of serum anti-Müllerian hormone (AMH) in patients with polycystic ovary syndrome (PCOS) and to correlate serum AMH level with insulin resistance, lipid profile and adiponectin levels.

Study design: This cross sectional study was conducted at Hospital Universiti Sains Malaysia (HUSM), Health Campus, Kubang Kerian, Kelantan, Malaysia. Total of thirty (30) women (aged 18-40 years old) diagnosed with PCOS were recruited from patients attending gynaecology clinic between July 2016 and April 2017. Fasting venous blood sample were collected from all subjects. Serum AMH, insulin, adiponectin, triglycerides, high-density lipoprotein cholesterol (HDL-C), and plasma glucose levels were measured. Insulin resistance was calculated based on homeostasis model of assessment-insulin resistance (HOMA-IR). The serum AMH level was estimated by using descriptive analysis and the correlation of serum AMH level with the metabolic parameters were analysed by using either Pearson correlation or Spearman correlation.

Results: The median of serum AMH levels in women with PCOS was 6.8 ng/mL (IQR: 7.38 ng/mL). There was a significant negative correlation between serum AMH and

HOMA-IR or triglycerides levels (r = -0.49, p = 0.006 and r = -0.55, p = 0.002, respectively). A significant positive correlation was observed between serum AMH and serum HDL-C or serum adiponectin levels (r = 0.56, p = 0.001 and r = 0.44, p = 0.014, respectively) in all study subjects.

Conclusion: The serum AMH level in patients with PCOS is associated with HOMA-IR, triglycerides, HDL-C and adiponectin levels. Therefore, this pilot study suggested that AMH may be used as a potential cardiometabolic risk marker in women with PCOS.

1. INTRODUCTION

1.1 Polycystic Ovary Syndrome (PCOS)

Polycystic ovary syndrome (PCOS), also known as Stein-Leventhal S yndrome was first described in 1935 by Doctors Stein and Leventhal as amenorrhea associated with bilateral polycystic ovaries (1). PCOS is the most common endocrine disorder among women of reproductive age group. The prevalence of PCOS is variable depending on the diagnostic criteria and population used. The reported overall prevalence of PCOS are 6% according to National Institutes of Health (NIH) diagnostic criteria, 10% for Rotterdam criteria and 10% for Androgen Excess and PCOS Society (AE-PCOS Society) criteria (2). The prevalence based on Rotterdam criteria is higher than NIH criteria for about 2.9 times (3). The overall prevalence of PCOS in South Asian is significantly higher than Caucasian, about 6-fold greater odds (4).

PCOS has been characterised by various criteria including hyperandrogenism (either clinical or biochemical), chronic anovulation and polycystic ovary morphology, which often associated with metabolic disturbances such as insulin resistance, obesity, dyslipidaemia and hypertension (5, 6). The diagnostic criteria for PCOS has been defined by NIH (1990), Rotterdam European Society of Human Reproduction and Embryology (ESHRE) and American Society for Reproductive Medicine (ASRM) (2003), and AE-PCOS Society (2009) as shown in Table 1 (7). NIH (1990) criteria requires simultaneous presence of both hyperandrogenism (clinical and/or biochemical) and menstrual dysfunction (8). Rotterdam ESHRE-ASRM (2003) requires the presence of at least two of three criteria, which are hyperandrogenism (clinical and/or biochemical), ovulatory dysfunction and polycystic ovaries (8). AE-PCOS Society (2009) requires the presence of clinical and/or biochemical hyperandrogenism and ovarian dysfunction

(oligoovulation or anovulation and/or polycystic ovaries) (9). All criteria require exclusion of other disorders that cause similar presentations such as thyroid disorder, hyperprolactinaemia and nonclassic congenital adrenal hyperplasia (8-10).

Table 1: Evolution of Criteria for PCOS

Organization/Year	Criteria
NIH/1990	Hyperandrogenism or hyperandrogenemia Menstrual dysfunction
Rotterdam ESHRE-ASRM/2003	Hyperandrogenism (clinical or biochemical) Oligo- or anovulation Polycystic ovaries*
Androgen Excess and PCOS Society/2009	Hyperandrogenism (clinical or biochemical) Ovarian dysfunction (oligo- or anovulation or polycystic ovaries)
Note:—All criteria require exclusion of other disorders. ASRM = American Society for Reproductive Medicine, ESHRE = European Society of Human Reproduction and Embryology, NIH = National Institutes of Health. *Two of three criteria must be met for the diagnosis of PCOS. Adapted from: Lee TT, Rausch ME, 2012 (7)	

1.2 PCOS Pathophysiology

The pathophysiology of PCOS is complex and continuously evolving. There are several mechanisms that have been thought to play a role in the development of PCOS, which include insulin resistance, genetic inheritance, and hormonal disturbances. The exact aetiology of PCOS remains unknown due to the complexity of its pathophysiology.

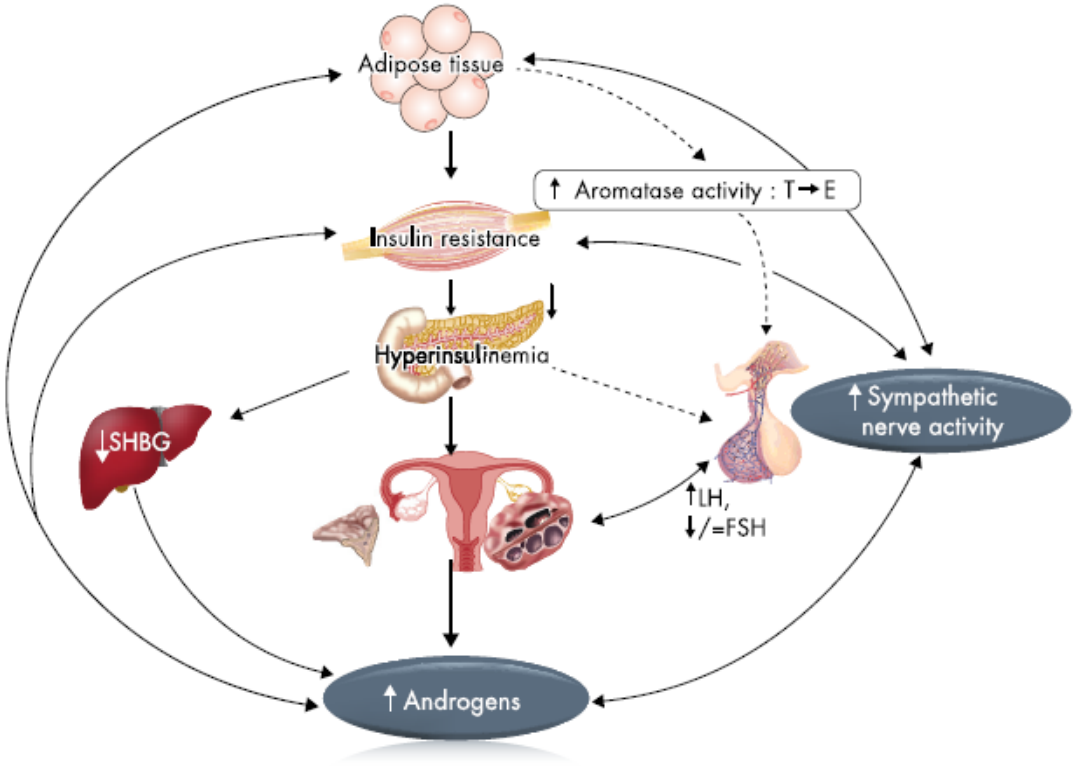

Figure 1.1. Pathophysiology of PCOS—a vicious circle.

Adapted from: Dumesic *et al*., 2015 (11)

Insulin resistance (IR) and hyperinsulinaemia play an important role in PCOS pathophysiology. IR is a condition characterized by inability of target organs to response properly to the action of insulin. As a result, the compensatory hyperinsulinaemia stimulates gonadotropin-releasing hormone (GnRH) gene transcription in hypothalamus,

leads to increase in luteinizing hormone (LH) pulse frequency at the hypophysis and subsequently elevates androgen synthesis by ovary as shown in Figure 1.1 (11, 12). In addition, hyperinsulinaemia also causes increased in androgenic production by directly stimulate ovary to produce androgen and increased free testosterone levels by means of reduction in sex hormone binding globulin (SHBG) synthesis by liver (11-13). Hyperandrogenism may in turn worsen IR, reflect the vicious cycle of IR-hyperinsulinaemia-hyperandrogenaemia (12).

IR may present in up to 50-70% of patient with PCOS and the prevalence varies according to methodology used (14-16). IR can be assessed by several methods, such as homeostasis model assessment-insulin resistant (HOMA-IR), fasting glucose/ insulin ratio, glucose to insulin ratio after 2 hours oral glucose tolerance test (OGTT) using 75g oral glucose, quantitative insulin sensitivity check index (QUICKI), and hyperinsulinaemic-euglycaemic glucose clamp techniques (15, 17, 18). Hyperinsulinaemic-euglycaemic glucose clamp techniques is the gold standard used to determine insulin sensitivity, however it is technically difficult, labour intensive, cost expensive and time consuming (19). HOMA-IR has been the main method for measuring IR in most studies as it is simple and non-invasive (19, 20). HOMA-IR is derived from a simple mathematical nonlinear equation, HOMA-IR = fasting insulin (μU/ml) x fasting glucose (mmol//L)/22.5 (normalizing factor) (20).

IR has been critically associated with metabolic complications in patient with PCOS (19, 21). Patient with PCOS are at higher risk for metabolic syndrome, type 2 diabetes mellitus and cardiovascular disease (21-23). Researches are currently working on anti-Müllerian hormone (AMH) as a potential biomarker for cardiometabolic risk in patient with PCOS (24).

1.3 Anti-Müllerian Hormone

Anti-Müllerian hormone (AMH), also known as Müllerian-inhibiting substance (MIS), is a dimeric glycoprotein hormone with a molecular weight of 140 kDa (25). It is a member of the transforming growth factor-β family. The production is expressed by the gene located on the short arm of chromosome 19 (25). The proteolysis of proAMH generates the inactive N-terminal fragment known as "pro-region" and biologically active C-terminal domain known as "mature region", however the C-terminus requires N-terminus to potentiate the full activity (25, 26).

AMH was initially studied for its role as a gonadal factor in males that induces regression of the Müllerian ducts. It is produced by Sertoli cells of the testis. In female, AMH is secreted by granulosa cells of the growing follicles in the ovary from the primary follicle to early antral stages (27). The AMH expression declines in later stages when the size of the follicles are beyond 4mm with further sharp decreases beyond 8mm, there is a change from AMH to estradiol secretion during follicle selection (Figure 1.2) (27-29). AMH expression is sex and age dependant since infancy until adulthood (30). In females, the expression of AMH begins from 36 weeks of pregnancy (25). The serum levels are low and barely detectable during first year of life, increase during late puberty, and decline with aging (30).

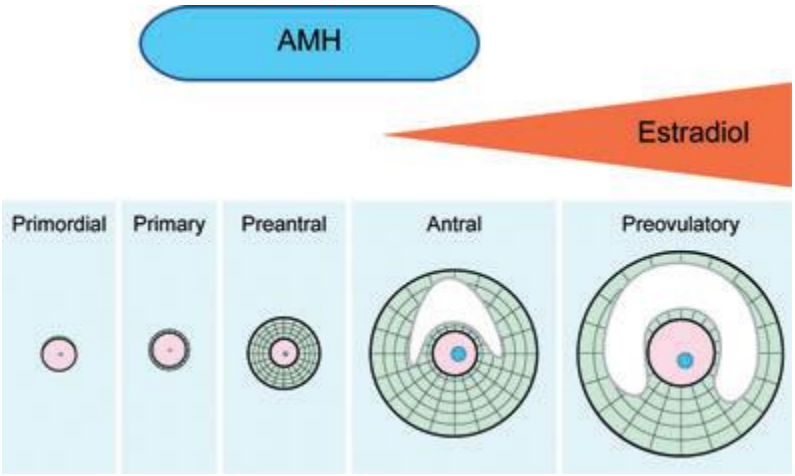

Figure 1.2: Depiction of AMH production by ovarian follicles.

Adapted from: Anderson, 2012 (29)

1.3.1 The role of AMH in female

AMH plays an important role in folliculogenesis to prevent premature depletion of the follicle pool via two mechanisms. First, it exerts inhibitory effect on initial recruitment of primary follicles from the resting primordial follicles pool (31). Second, it also inhibits cyclic recruitment of follicles by reducing their sensitivity to follicle-stimulating hormone (FSH), which control the amount of preantral and small antral follicles entering the preovulatory stage and do not undergo atresia (32, 33).

AMH is well known for its role as a useful marker of ovarian reserve, it reflects the size of the resting primordial follicle pool (34). AMH has few advantages compared to other hormonal markers for ovarian reserve, such as FSH, oestradiol and inhibin B. It shows no significant changes during pregnancy, minimal or no intracycle fluctuation, and not significantly affected by the use of hormonal contraceptives (35-38).

AMH appears to be useful in assisted reproductive method in predicting excessive or poor ovarian response (39, 40). AMH is also a potential tumour marker for the diagnosis and evaluation of treatment efficacy in patient with granulosa cell tumour of ovary (41). Recent studies show AMH plays important roles in PCOS diagnosis and severity (42).

1.3.2 AMH and PCOS

AMH is thought to be a potential surrogate marker for the diagnosis of PCOS particularly in the absent of ultrasound (42, 43). Studies have shown that the level of serum AMH in patient with PCOS is significantly higher than in patient without PCOS (44, 45). This is secondary to an increased number of preantral and antral follicles and increased AMH production by granulosa cell in patient with PCOS (46, 47). However, the level of AMH is significantly varies in different population (48). Thus, the estimation of AMH levels in various populations is important in order to improve the interpretation value once its utility as a maker in PCOS has established.

AMH is also found to correlate well with the severity of the syndrome as indicated by increased LH levels, testosterone levels, number of ovarian follicles and mean ovarian volume by ultrasound (49). Therefore AMH may be utilised as a marker for the extent of the disease (49).

Recently, researchers are focusing on the relationship between AMH and metabolic factors such as HOMA-IR, lipid profile, anthropometric parameters, blood pressure and some other markers. This is due to the strong association between PCOS and metabolic complications. However, the results have been mixed.

1.3.3 AMH and HOMA-IR

Studies regarding the correlation between AMH and IR using HOMA-IR have been done but the findings were contradicting. In women without PCOS, there was an independent relationship between AMH and HOMA-IR, which the serum AMH levels were negatively correlated with HOMA-IR (50). However, other study has demonstrated no association between AMH levels and HOMA-IR in women with regular menstrual cycle (51).

In PCOS, inconsistent results have also been reported, there were studies demonstrated positive, negative and even nil association between AMH and IR (52-55). Factors such as ethnic disparity, sample size and anthropometry of the study subjects possibly contribute to the different findings. Whether there is an association between AMH and IR in patients with PCOS is controversial. The examination of such associations have not been done in in our population.

1.3.4 AMH and Lipid Profile

AMH levels were found positively associated with high-density lipoprotein cholesterol (HDL-C) but no relationship with triglycerides (TG) in women with regular menstrual cycle (51). In PCOS, AMH levels were also found to have positively correlated with HDL-C and not significantly related to TG (24).

However, few studies showed different results. An inverse relationship between AMH and HDL-C levels has been described in women with and without PCOS (56). Another study showed no significant relationship between AMH with both TG and HDL-C in Chinese women with and without PCOS (55).

1.3.5 AMH and Adiponectin

Serum adiponectin levels were significantly and independently associated with metabolic syndrome based on a study done in Chinese women with PCOS (57). There was positive correlation between AMH and adiponectin described in women without PCOS (50, 58). However, no such association was noted in patient with PCOS (58). There are limited studies done on relationship between AMH and adiponectin in women with PCOS.

1.4 Rationale of study

This study was carried out to provide knowledge on the distribution of AMH level in patients with PCOS among our population. This is due to the potential utility of this hormone as a marker in various gynaecological disorders including PCOS (59). This knowledge will be valuable in providing reliable interpretation that can be applied in our population once it's utility as a marker in PCOS has established.

Knowledge on the relationship between AMH and metabolic parameters (HOMA-IR, TG, HDL-C and adiponectin) may have significant clinical implications. The metabolic parameters represent the components of metabolic syndrome (MetS) and are known to have a strong linkage to IR, which commonly presents in patients with PCOS (21). IR plays an important role in PCOS pathophysiology and it leads to compensatory increased in insulin level, hyperinsulinemia. AMH level is also found to be increased in patient with PCOS. Insulin and AMH both are believed to have influence on steroidogenesis and folliculogenesis (32, 33, 60, 61). Based on this, there is a possibility that AMH has a relationship with IR and other MetS components including lipid profile particularly TG and HDL-C. AMH might also have a relationship with adiponectin, a novel biomarker for its relationship with IR and other MetS components (62). However, in view of

contradicting results from previous studies done in patient with PCOS, this study was conducted in order to show the type of relationship between AMH and these markers (HOMA-IR, TG, HDL-C, and adiponectin) in our population.

Knowledge on the relationship between AMH and HOMA-IR, TG, HDL-C and adiponectin may contribute to a better understanding regarding the pathophysiology of PCOS and its metabolic complications. Therefore, a knowledge on correlation between AMH and these metabolic markers is useful as AMH may serve as a future marker for monitoring PCOS and its related metabolic consequences as well as a potential treatment agent.

1.5 Objective

1.5.1 General objective

To study the relationship between serum AMH and metabolic parameters in patients with PCOS attending gynaecology clinic in Hospital Universiti Sains Malaysia (HUSM).

1.5.2 Specific objectives

1. To estimate the level of AMH in patients with PCOS attending gynaecology clinic in HUSM.
2. To correlate serum AMH concentration with HOMA-IR, TG and HDL-C in patients with PCOS attending gynaecology clinic in HUSM.
3. To correlate serum AMH concentration with adiponectin in patients with PCOS attending gynaecology clinic in HUSM.

1.6 Hypothesis

There is a relationship between AMH and HOMA-IR, TG, HDL-C and adiponectin.

1.7 Methodology

1.7.1 Study design

Cross sectional study

Study duration: 2 years

Place of Study: HUSM, Health Campus, Kubang Kerian, Kelantan.

1.7.2 Sample Size Calculation

Objective (1)

Estimation of mean value of AMH, using single mean formula:

SD = 5.3

Precision = 1.8

$\alpha = 0.05$

n = 30

n + 10% =33

Objective (2) and (3)

Correlation between AMH and HOMA-IR, TG, HDL-C and adiponectin, calculated using G*Power 3.1.9.2 software:

$\alpha = 0.05$

Power = 0.8

r = 0.5

n = 21

n + 10% = 23

The required sample size: 33 subjects diagnosed with PCOS

1.7.3 Selection of Subjects

Total of 30 subjects diagnosed with PCOS were recruited from patients attending gynaecology clinic in HUSM. The diagnosis of PCOS was according to Rotterdam ESHRE-ASRM (2003) criteria, based on the presence at least two of the following three criteria:

1) Irregular menses due to oligo- or anovulation,

2) Clinical (hirsutism/ acne) and/or biochemical hyperandrogenism (HA) and

3) Typical PCOS ovarian features (≥ 12 follicles with size of 2-9 mm/ovary) present during ultrasound examination.

Transabdominal ultrasound was done in unmarried or virgin subjects while transvaginal ultrasound for married subjects.

Selection criteria

a. Inclusion criteria

- Patient with PCOS
- Age 18 to 40 years old

b. Exclusion criteria

- Suffering from endocrinopathies such as thyroid disease, Cushing syndrome, and diabetes mellitus.

- On medications such as oral contraceptives, hormonal treatment, anti-diabetics, lipid lowering agent, weight reduction therapy.

1.7.4 Sampling method

Patients diagnosed with PCOS from HUSM gynaecology clinic were recruited. Advertisement regarding this study was placed in the gynaecology clinic in HUSM, where doctors and supporting staffs helped in selecting the subjects. All subjects were prospectively recruited in a consecutive manner after informed consent. Patients who were under research team's care was not included in order to ensure vulnerability of subjects were protected.

The subjects was screened for inclusion and exclusion criteria, and consent was obtained by research's team member or assigned staff. An information sheet was given to each of the subject. A copy of consent form was given to subjects after they had agreed to participate in this study.

In handling privacy and confidentiality, subject recruitment and consent taking was done without presence of the treating doctors. Subject was given a comfortable room where details of the study was explained and consent obtained. They were being ensured regarding the data that had been gathered would only be reviewed and processed by the research team.

The subjects were informed and referred to appropriate medical discipline for abnormal result if any, for further management.

1.7.5 Specimen Collection

Fasting venous blood were collected in the morning after an overnight fast of 10-12 hours. The samples were taken at any day of menstrual cycle. Total of 10 ml

of blood were drawn and collected into a plain tube for serum and a tube with oxalate/fluoride for plasma. The serum and plasma were separated by centrifugation at 4000g for 4 minutes at room temperature and stored at -80^{0}C until analysis. The serum was used for AMH, insulin, HDL-C, TG and adiponectin measurement. Plasma was used for glucose measurement. Serum HDL, serum TG, and plasma glucose level were analysed by batch.

1.7.6 Laboratory Measurements

Serum AMH level was measured by electrochemiluminescence immunoassay (ECLIA) sandwich principle using Cobas e 411 analyser by Roche Diagnostic. The limit of detection was 0.010 ng/mL. At serum AMH concentration of 2.44 ng/mL, the intra-assay precision was 1.2% and inter-assay precision was 3.3%. At 12.3 ng/mL, the intra-assay precision was 1.1% and inter-assay precision was 3.7%.

Serum insulin was determined by ECLIA sandwich principle using Cobas e 601 analyser by Roche Diagnostic. The lower detection limit was 0.2 µU/mL. At serum insulin concentration of 21.9 µU/mL, the intra- and inter-assay precision were 3.2% and 4.2% respectively. At 74.3 µU/mL, the intra- and inter-assay precision were 3.7% and 4.6% respectively.

Plasma glucose level was measured by enzymatic method in the presence of glucose oxidase using ARCHITECT C 800 analyser by Abbott Diagnostics. HOMA-IR index was calculated based on fasting plasma glucose concentration and serum insulin with standard formula: HOMA-IR = fasting concentration of insulin (µU/mL) × fasting concentration of glucose (mmol/L) /22.5.

Serum adiponectin level was measured by enzyme-linked immunosorbent assay (ELISA) method using RayBio® Human Acrp30 ELISA Kit by RayBiotech. The limit of detection was 25 pg/mL. The intra-assay precision was <10% and inter-assay precision was <12%.

Serum TG level was measured by quantitative enzymatic colorimetric test "GPO-PAP" using ARCHITECT C 800 analyser by Abbott Diagnostics. The limit of detection was 0.02 mmol/L. At serum TG concentration of 0.62 mmol/L and 2.39 mmol/L, the intra-assay precision were 0.54% and 0.80% respectively. The inter-assay precision at 1.00 mmol/L and 2.65 mmol/L were 0.89% and 1.54% respectively.

Serum HDL-C level was measured by accelerator selective detergent method using ARCHITECT C 800 analyser by Abbott Diagnostics. The limit of detection was 0.06mmol/L. At serum HDL-C concentration of 0.54 mmol/L, the intra- and inter-assay precision were 1.7% and 1.1% respectively. At 2.04 mmol/L, the intra- and inter-assay precision were 1.0% and 0.5% respectively.

1.7.7 Statistical analysis

Data analysis was performed using SPSS Statistical Package version 22. The distribution of numerical variables were checked using test of normality (Shapiro-Wilk) and histogram with overlap normal curve. Normally distributed numerical variables were described as mean and standard deviation (SD), whereas non-normally distributed numerical variables were described as median and interquartile range (IQR). Bivariate correlation analysis were conducted using either Pearson correlation (if bivariate distribution assumption is met) or Spearman correlation (if bivariate normal distribution assumption is not met).

Bivariate distribution was checked using scatterplot. Scatterplot with elliptical shape indicates bivariate distribution assumption was met. *P* value of <0.05 was used as statistically significant at 95% confidence interval.

The serum AMH level in patient with PCOS was estimated by using descriptive analysis. Correlation of serum AMH level with HOMA-IR, TG, HDL-C, and adiponectin were performed using either Pearson correlation or Spearman correlation.

2. STUDY PROTOCOL

2.1 Literature Review

Polycystic ovary syndrome (PCOS) is a common disorder of reproductive, endocrine and metabolic functions which affect 5%–10% of reproductive age women (1). It is characterised by heterogenous clinical presentations and laboratory features including hyperandrogenism, polycystic ovaries, and chronic anovulation along with metabolic disturbances manifested by hyperinsulinaemia, abdominal obesity, hypertension and dyslipidemia (2).

PCOS predisposes to various significant health implications. Besides menstrual dysfunction, infertility, hirsutism and acne, it increases the long-term risk of type 2 diabetes and cardiovascular diseases (3, 4). Women with PCOS are also thought to be at increased risk for endometrial cancer due to chronic anovulation with unopposed estrogen exposure of the endometrium (5).

Several diagnostic criteria for PCOS have been proposed. The Rotterdam consensus is the most widely accepted criteria and is used in the guidelines for PCOS (4, 8, 9).

The pathophysiology of PCOS is complex and poorly understood. Several mechanisms are thought to be involved in the development of the disorder. These include disturbance of hypothalamic-pituitary axis, dysregulation of ovarian and adrenal steroidogenesis, genetic and insulin resistance (IR). All of these factors cause an imbalance in the reproductive hormones ie FSH, LH and androgens (10).

IR plays a vital role in the mechanism of the disease. It is defined as a metabolic state characterized by failure of target organs to response normally to insulin actions. Consequently it causes an increase in insulin secretion and hyperinsulinaemia. Hyperinsulinaemia contributes to the hormonal disturbances in PCOS. It is due to the

direct action of insulin on ovary. The hormone modulates steroidogenesis and folliculogenesis in ovary. Besides, it promotes follicle growth by its action in the granulosa cells. The hormone also increases the sensitivity of granulosa cells to FSH, and thus increase the number of follicles and ovarian volume. Other than that, its action on theca cells causes increased androgen synthesis (10).

IR has a strong linkage to metabolic syndrome (MetS), a group of factors that is known to increase the risk of cardiovascular disease and type 2 diabetes mellitus, thus strengthen the association between PCOS and MetS (6). IR is measured using Homeostasis Model for Insulin resistance (HOMA-IR) formula (7).

HOMA-IR= Fasting insulin in μU/mL X fasting glucose/22.5

Researchers are currently working on potential biomarkers for PCOS. Anti Mullerian hormone (AMH) is a dimeric glycoprotein hormone with a molecular weight of 140 kDa. It belongs to the family of transforming growth factor-β. The production is expressed by the gene located in the short arm of chromosome 19. Synthesis of AMH in embryonic male testes is important for male sex differentiation through irreversible regression of mullerian duct. In female, AMH is secreted by the granulosa cells of the growing follicles in the ovary particularly from the stage of the primary follicle to the initial formation of antral follicle. AMH is a hormone that is secreted by the granulosa cells of the growing follicles in the ovary. Its concentration increases until puberty and remain stable throughout the reproductive period. Under normal condition AMH inhibits the development of follicles, hence maintaining the ovarian follicle pool. The hormone is well known for its role as a marker for ovarian reserve. It also acts as a predictive marker for the successfulness of assisted reproduction method. Besides, AMH has a role as a potential tumour marker for the diagnosis of ovarian tumour of granulosa cells origin (11,

12). In PCOS, AMH is thought to be a potential surrogate marker for the diagnosis of PCOS particularly in the absence of ultrasound. This is due to its ability to represent the number of follicles in ovary. Previous studies showed that the level of serum AMH in PCOS patients is significantly higher than in non PCOS patients (13, 14). Besides, the level of AMH is also known to be significantly varies in different population (15). This shows the importance of estimating the AMH values in various populations so that once it's utility as a marker in PCOS is established, the value interpretation can be done accurately.

The relationship between AMH and reproductive hormones such as FSH, LH and androgens has been well established (12). Recently, researchers were focusing on the relationship between AMH and metabolic factors such as HOMA-IR, lipid profile, anthropometric parameters, blood pressure and some other markers. It is due to the close association between PCOS and metabolic syndrome (MetS). The components of MetS include dyslipidaemia which is characterised by impaired HDL-C and triglycerides level, impaired fasting glucose level, abdominal obesity and high blood pressure. (6).

Studies regarding the correlation between AMH and the metabolic parameters mainly HOMA-IR have been done but the findings were contradicting (13, 14, 16). Factors such as ethnic disparity, sample size and anthropometry of the study subjects possibly contribute to the different findings. However, no such studies have been done in our population. Besides, there are also possible correlation between AMH and other components of MetS ie. Triglycerides and HDL-C due to a strong relationship between these markers and IR.

Other than that, adiponectin is another potential metabolic biomarker that has a possible correlation with AMH. Adiponectin, a novel biomarker produced by adipose tissue. This

hormone regulates many metabolic processes in the body and is known to have an inverse association with components of MetS. It can suppress the development of serious metabolic diseases such as type 2 diabetes, obesity and cardiovascular diseases by having insulin sensitizing, anti-atherogenic and anti-inflammatory properties (17). In reproductive aspect, the hormone has been shown to have direct effects on folliculogenesis and steroidogenesis (18).

2.2 Rationale

This study is carried out to provide a knowledge on the distribution of AMH in PCOS patients in our population. This is due to the fact regarding potential utility of the hormone as a marker in various gynaecological disorders including PCOS. Knowledge on AMH values among PCOS patients in our population is important. The reason is that, once it's utility as a marker in PCOS is established, the value interpretation can be done accurately. Knowledge on the relationship between AMH and metabolic parameters (HOMA-IR, triglycerides, HDL-C and adiponectin) may have significant clinical implications. The metabolic parameters represent the components of MetS and are known to have a strong linkage to IR, which commonly presents in PCOS patients. IR is an important pathophysiological component of PCOS. Other than its role in causing metabolic complications in PCOS, IR leads to hyperinsulinemia. The hormone modulates steroidogenesis and promotes follicle growth by its action in the granulosa cells. AMH, which is produced in granulosa cells is also known to have a relationship with ovarian steroid hormone and has influence on follicle growth. On this basis, there is a possibility that AMH has a relationship with IR and other MetS components including lipid profile particularly triglycerides, HDL-C. AMH might also have a relationship with adiponectin, a novel biomarker which is established for its relationship with IR and other MetS components. Knowledge on the relationship between AMH with HOMA-IR, triglycerides, HDL-C and adiponectin may contribute to a better understanding regarding the pathophysiology of PCOS and its complications. Other than that, PCOS is known as a risk factor to multiple metabolic health consequences. Therefore, a knowledge on correlation between AMH and those metabolic markers which are also related to metabolic syndrome is useful as AMH might serve as a future marker for monitoring of the disorder and its related metabolic consequences as well as a potential treatment agent.

2.3 Objective

i. General Objective

To study the relationship between serum AMH and metabolic parameters in PCOS patients attending gynaecology clinic in HUSM

ii. Specific Objectives

4. To estimate the level of AMH in PCOS patients attending gynaecology clinic in HUSM
5. To correlate serum AMH concentration with HOMA-IR, triglycerides and HDL-C in PCOS patients attending gynaecology clinic in HUSM
6. To correlate serum AMH concentration with adiponectin in PCOS patients attending gynaecology clinic in HUSM

2.4 Hypothesis

There is a relationship between AMH and HOMA-IR, triglycerides, HDL-C and adiponectin.

2.5 Methodology

i. Study design

Cross sectional study

Study duration: 2 years

Place of Study: Hospital Universiti Sains Malaysia, Health Campus, Kubang Kerian, Kelantan.

ii. Calculation of sample size

iia. Objective (1)

Estimation of mean value of AMH:

SD = 5.3

Precision = 1.8

$\alpha = 0.05$

n = 30

n + 10% =33

iib. Objective (2) and (3)

Correlation between AMH with HOMA-IR, triglycerides, HDL-C and adiponectin:

$\alpha = 0.05$

Power = 0.8

r = 0.5

n = 21

n + 10% = 23

33 subjects diagnosed with PCOS will be recruited for this study

iii. Subjects

PCOS patients attending gynaecology clinic in HUSM. PCOS is defined when at least two of the following three symptoms were exhibited, according to the Rotterdam criteria 2003:

1) Irregular menses due to oligo- or anovulation, 2) clinical (hirsutism/ acne) and/or biochemical hyperandrogenism (HA) and 3) typical ovarian features (≥ 12 follicles with size of 2-9 mm/ovary) present during ultrasound examination. Biochemical HA is defined as serum total testosterone > 2.7 nmol/L (14). Transabdominal ultrasound will be done for unmarried or virgin subjects while transvaginal ultrasound for married subjects.

iv. Selection Criteria

c. Inclusion criteria

- PCOS patient
- Age 18 to 40 years old

d. Exclusion criteria

- Suffering from other medical illnesses including endocrinopathies such as thyroid disease, Cushing syndrome, and diabetes mellitus.
- On medications such as oral contraceptives, hormonal treatment, anti-diabetics, lipid lowering agent, weight reduction therapy.