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Cardiovascular Risk Factors and Metabolic and Endocrine Features in Non-hyperandrogenic and Hyperandrogenic PCOS Phenotypes of Malay Women

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ABSTRACT

Objective: To compare the cardiovascular risk factors and metabolic features between hon-hyperandrogenic and hyperandrogenic Malay women with PCOS.

Design: Cross-sectional clinic based study.

Subjects: 109 Malay women with PCOS based on the Rotterdam criteria divided into non-hyperandrogenic and hyperandrogenic phenotypical groups.

Measurements: Anthropometric, biochemical and endocrinological parameters were compared between the two groups of Malay PCOS women.

Results: Overall, both groups women had very similar metabolic and endocrine characteristics. Cardiovascular risk factors were comparable in both phenotypes of PCOS. Regression analysis indicated that free testosterone and HbA1c were significantly higher in the hyperandrogenic women (p =.002 and p =.021 respectively).

Conclusions: Malay women with non-hyperandrogenic and hyperandrogenic PCOS phenotypes have similar cardiometabolic profile and cardiovascular risk factor prevalences. Therefore, all Malay women with PCOS should be considered as a singular entity with regards to cardiometabolic risk.

Key words: Risk Factors, Metabolic and Endocrine, PCOS Phenotypes.

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder of women of reproductive age, reaching a prevalence of 15% in certain populations (Bozdag G 2016). It is a syndrome characterised by hyperandrogenism, ovulatory dysfunction and polycystic ovarian morphology.

Since it was first described by Stein and Leventhal, PCOS has transformed from a purely reproductive into a mainstream metabolic condition (Stein IF 1935). It is now commonly associated with increased prevalences of cardiovascular and metabolic (cardiometabolic) abnormalities such as obesity, dyslipidemia, hypertension and diabetes mellitus (DM) (Daan NM 2015, Pinola P 2017). Unsurprisingly, this increased prevalence of cardiometabolic factors has been postulated to lead to an increased future risk of cardiovascular disease (Dahlgren E 1992). The evidence for this is inconclusive and long term cardiovascular risk of PCOS requires further study (Schmidt J 2011, Meun C 2018). Nevertheless, there is concern that women with PCOS may start with an adverse cardiovascular profile and this might worsen over time.

Diagnosis of PCOS

The diagnosis of PCOS is based on a combination of clinical, biochemical and ultrasound scan criteria. Although various definitions exist, the Rotterdam consensus criteria are the most commonly accepted diagnostic criteria for PCOS (ESHRE/ASRM 2004). Earlier definitions of PCOS required the presence of ovulatory dysfunction and hyperandrogenism, but the Rotterdam criteria were controversially the first to include polycystic ovarian morphology (Azziz R *et al* 2006). There is also dispute on the precise definition of hyperandrogenism for the diagnosis of PCOS with an argument over the suggestion that PCOS is a purely hyperandrogenic disorder (Azziz R 2006, Franks S 2006).

The inclusion of polycystic ovarian morphology in the diagnostic criteria has 2 implications. Firstly, it has markedly increased the prevalence of PCOS. More importantly, it enables women without overt signs of hyperandrogenism to be diagnosed as PCOS (with just oligoanovulation and polycystic ovarian morphology). The terms "mild" and "classical" have been imposed on the non hyperandrogenic and hyperandrogenic women respectively, although they both share the other phenotypic features of PCOS.

From the Rotterdam criteria, it is possible to subdivide PCOS women into 2 broad categories (and 4 subgroups) solely on the presence or absence of hyperandrogenism. The hyperandrogenic subgroups include those with hyperandrogenism, ovulatory dysfunction and polycystic ovarian morphology together, and hyperandrogenism and ovulatory dysfunction and hyperandrogenism and polycystic ovarian morphology separately. The non hyperandrogenic (the milder PCOS women) subgroup is made up women with just ovulatory dysfunction and polycystic ovarian morphology.

The main concern with the Rotterdam criteria is whether to manage the non hyperandrogenic group as a separate entity from the other PCOS women. It is thought that the lack of hyperandrogenism might manifest a less severe metabolic phenotype. Some studies have indicated that

hyperandrogenic PCOS women have an increased prevalence of metabolic dysfunction (Moran LJ 2010, Wild RA 201, Yildiz BO 2012). Other studies have however not shown any significant metabolic differences between the phenotypes (Kauffman RP 2008).

Phenotype and Ethnicity

The phenotype of PCOS, ovulatory dysfunction, hyperandrogenism and polycystic ovarian morphology, is modified by ethnicity and BMI. Ethnic origin has been noted to affect the prevalence of cardiovascular risks in PCOS women (Zhao Y 2013). Studies from different parts of the world have identified different prevalences of metabolic syndrome and metabolic characteristics in different races. Ethnic origin and culture contribute significantly to the manifestations of PCOS and should be considered when applying thresholds for metabolic characteristics (Fauser BC 2012). In short, in order to fully understand the complexities of PCOS, the prevalence of the various characteristics of this syndrome need to be assessed in populations and races. As yet no data is available on the metabolic features of the different PCOS phenotypes in Malay women.

The extent to which the "milder" PCOS phenotype faces long term health issues compared to the hyperandrogenic PCOS phenotypes remains to be seen. At this point of time, the question is whether hyperandrogenic and non-hyperandrogenic PCOS women experience similar cardiometabolic risks.

Aim

The aim of this study was to assess the cardiometabolic profile and cardiovascular risk factors prevalent within a population of reproductive aged Malay women with PCOS divided between non-hyperandrogenic and hyperandrogenic phenotypes.

MATERIALS AND METHODS

Subjects

The subjects for the study were women aged between 20-40 years previously diagnosed as PCOS and selected from the Malay PCOS Prevalence Study, a clinic based cross-sectional study. Between February 2013 and June 2017, 253 Malay women were recruited from the Reproductive Endocrinology Clinic (REC) and outpatient department (OPD) at the clinical practice of the principal investigator. The women attending the REC were referred from a range of sources, including primary care clinics, obstetrics and gynaecology clinics and self referrals and presented mainly with menstrual problems, an inability to conceive, excessive body hair and other related conditions. The screening and assessment was performed by the principal investigator and trained assistants. Detailed explanation on the study was provided prior to written consent, following which the subjects underwent history taking, physical examination and an ultrasound scan. Once PCOS was confirmed, the women were subjected to further anthropometric and laboratory assessment.

Subjects were excluded from the study if they were found to be pregnant, had any chronic medical conditions, where on any medications known to affect reproductive function, metabolism or liver protein production. All women underwent testing for serum FSH, LH, TSH, 17-hydroxprogesterone and prolactin to rule out hypo- and hypergonadotrophic hypogonadism, thyroid disease, non-classical congenital adrenal hyperplasia and hyperprolactinemia respectively. Cushing's syndrome was ruled out clinically. Undiagnosed vaginal bleeding and other significant genital tract pathology were also excluded.

Measures

Standard anthropometric measurements for all subjects were height, weight, waist circumference, body mass index (BMI) and blood pressure (BP). Height and weight were measured with light clothing on and without shoes. Weight was measured on a calibrated beam scale to the nearest 0.5 kg. Height and waist circumference were measured to the nearest 0.5 cm with a measuring tape. Waist measurements were taken halfway between the lower rib margin and the iliac crest at the end of a gentle expiration. BP was measured with a random-zero sphygmomanometer using an appropriate sized cuff applied to the left arm in a sitting position after at least 5 minutes of rest. Patients with persistently elevated BP were rechecked on another occasion prior to confirmation of hypertension. The modified Ferriman-Gallwey (mFG) visual score was used to assess and quantify hirsutism (Ferriman D 1961). The trained investigator assessed the 9 specified androgen-dependent areas of the body (upper lip, chin, upper and lower back, upper arms, chest, upper and lower abdomen and thighs). Each site was rated on a 5-point Likert scale ranging between 0-4 (from absent to extensive terminal hair). A complete assessment included facial and body hair inspection, oily skin, acne, androgenic alopecia and acanthosis nigricans. Polycystic ovaries were diagnosed via high resolution abdominal or vaginal ultrasound scanning (TAS or TVS, respectively) using a A Mindray DC6 ultrasound machine (Mindray Medical International Limited). TVS was the method of choice unless the subject objected, in which case TAS was carried out. TAS was focused on establishing ovarian volume due to the inability to accurately estimate the number of follicles. The endovaginal probe had a frequency of 5-9 MHz. Apart from the ovaries, the uterine dimensions, presence of growths, ovarian cysts and follicles were also noted.

Samples and Assays

All blood samples were drawn at one sitting as a measure of convenience to the subjects between 0800-1000 after an overnight fast of at least 8 hours. Sampling was carried out during the early follicular phase of the menstrual cycle (days 1-3) in those with eumenorrhea or mild-to-moderate oligomenorrhea. In amenorrheic or severe oligomenorrheic subjects, samples were taken during the early phase of a withdrawal bleed induced by a 7-day course of oral norethisterone. Sera planned for periodic shipment to the core laboratory for eventual assay was aliquoted into 1.5 cc microfuge tubes and stored at –20 to –70 degrees C. Aliquoting was done to preserve the basal levels of potential analytes in a frozen state, since thawing and refreezing of samples has a deleterious effect. For all measurements, the

inter-assay coefficient of variation was less than 10% while the intra-assay variation was less than 15%. For the biochemical assays, venous blood was collected into serum gel tubes (BD Biosciences, Mountain View, CA) and fluoride oxalate tubes. Samples were separated by centrifugation at 2000 x g for 15 minutes and serum aliquots were stored at -20^0 C within 1 hour of collection. Fasting plasma glucose was measured using an enzymatic colorimetric method with hexokinase, whilst HbA1c was assayed using the HPLC method. Serum insulin was assayed using a competitive chemilum inescent immunoassay performed on the DPC Immulite 2000 analyser (Euro/DPC, Llanberis, UK). The analytical sensitivity of the insulin assay was 2µU/ml, the coefficient of variation was 6% and there was no stated cross reactivity with proinsulin.

Medical assay kits were used for serum total cholesterol (TC), triglycerides (TG) and high-density lipoprotein cholesterol (HDL-C) assessment using Modular Analytics SWA (Roche). TG was assayed with Lipase/glycerol kinase/GPO-PAP and HDL-C and TC both with cholesterol esterase and cholesterol oxidase. Low-density lipoprotein cholesterol (LDL-C) was calculated using an online calculator (merckmanuals.com) based on Friedwald's equation [LDL-C = TC - HDL-C - (TG/2.2)] (Friedewald WT 1972). This equation, however, was not applicable if TG > 4.5 mmol/L.

Steroid hormones were tested in batches within 2-4 weeks of being frozen. All assays were performed in the same laboratories and assays did not change from 2012 to the end of sampling, 2017. Both total testosterone (TT) and SHBG were stored at 4^0 C until analysis. Dehydroepiandrosterone sulphate (DHEAS) was stored at -40^{0}C until analysis which was done on a monthly basis. TT, SHBG, prolactin, TSH, FSH, LH, insulin, DHEAS and 17α-hydroxyprogesterone were measured by enzyme-linked immunoabsorbent assay (ELISA) using kits supplied by IBL International, Germany on a multiplate reader (Multiskan GO, Thermo Scientific). Free androgen index (FAI) was calculated using the formula [TT (nmol/L) x 100/SHBG (nmol/L)]. Calculated free testosterone (cFT) was obtained from an online calculator at issam.ch/freetesto.htm based on the Vermeulen formula.

The research site, located in the town of Lumut in northern Malaysia was a Women and Children medical centre. All blood tests were run at the central laboratory belonging to the Institute of Medical Research (IMR) in Kuala Lumpur, about 250 km away. Sample shipment was coordinated from the research site to this lab without difficulty. The assays all had <10% interassay coefficients of variation.

Definitions

PCOS was diagnosed according to the Rotterdam criteria, requiring the presence of any 2 criteria from androgen excess (clinical or biochemical hyperandrogenism), ovulatory dysfunction and polycystic ovarian morphology (PCOM) as seen by ultrasound scan. Ovulatory dysfunction was defined as oligomenorrhoea (spontaneous intermenstrual cycles of ≥ 45 days or ≤ 8 menstrual cycles per year), or amenorrhoea (absence of menstruation for > 182 days). Based on Southeast Asian cut-offs, clinical hyperandrogenism was defined as hirsutism diagnosed when the subjects scored ≥ 2 on the mFG score, acne or androgenic alopecia (Afifi L 2017). Biochemical hyperandrogenism was diagnosed if FAI and/or cFT were more than 7.1% and 0.035 nmol/L, respectively (based on ROC curve analysis of Malaysian women) (Dineshinee RN 2018). PCOM was at least one ovary with ≥12 antral follicles measuring 2-9 mm in diameter, or an ovarian volume of >10 cm^3 on either ovary, measured in 3 dimensions using the formula for a prolate ellipsoid (length x width x height x 0.5) and excluding any cysts, dominant follicles or corpora lutea (Balen AH 2003).

PCOS women were classified into one of the four phenotypes: hyperandrogenism and ovulatory dysfunction (AO), hyperandrogenism and polycystic ovarian morphology (AP), hyperandrogenism, ovulatory dysfunction and polycystic ovarian morphology (AOP) and ovulatory dysfunction and polycystic ovarian morphology (OP). All the phenotypes with hyperandrogenism, commonly referred to as the "classical" phenotypes (AO+AP+AOP =) were grouped as hyperandrogenic (HA). The OP phenotype automatically became the non-hyperandrogenic (NHA) group.

Abdominal obesity was defined as waist circumference ≥ 88 cm according to the cut-off point for the South East Asian population (Alberti KGMM 2009). BMI was calculated as weight (kg) divided by height x height (m^2) and overweight and obesity were defined as BMI 25.0-29.9 and ≥ 30.0 kg/m respectively.

Normal levels of HbA1c were set at < 5.6% (38.0 mmol/mol), prediabetes between 5.6-6.2% (38.0-44.0 mmol/mol) and diabetes if > 6.3% (45.0 mmol/mol), with normal fasting plasma glucose levels being < 6.0 mmol/L (Ministry of Health Malaysia Management of T2DM 2015). The homeostatic model assessment for insulin resistance (HOMA-IR) was used to quantify insulin sensitivity using the formula [fasting serum inulin (mIU/L) x fasting plasma glucose (mmol/L)/22.5. A HOMA-IR of < 0.99 in Malay women would indicate normal insulin sensitivity (Al-Mahmood AK 2006).

For Malaysian women, dyslipidemia has been defined as either TG > 1.7 mmol/L, HDL-C < 1.2 mmol/L, LDL-C > 2.6 mmol/L and TC > 5.2 mmol/L (Ministry of Health Malaysia Management of Dyslipidemias 2015).

Hypertension was diagnosed by having a previous diagnosis of hypertension, use of antihypertensive medication or the persistent elevation of systolic BP ≥ 130 mmHG or diastolic BP ≥ 80 mmHg.

At least 3 out of 5 components were required to diagnose metabolic syndrome, based on the Joint Interim Statement of the International Diabetes Federation (IDF) (Alberti KGMM 2009). These were waist circumference > 88 cm (country specific for South Asian population), HDL-C < 1.2 mmol/L, TG > 1.7 mmol/L, BP ≥ 130/80 mmHg and fasting glucose level of ≥ 5.5 mmol/L.

Statistical Analysis

All statistical analyses was performed using The Statistical Packages for Social Sciences (SPSS for Mac, version 22.0 SPSS, Chicago, IL, USA), abbreviated as SPSS. This included sample size calculation, exploratory and descriptive analysis

and hypothesis testing. A 2-sided p <.05 denoted statistical significance.

The Pearson's chi-squared test was used to estimate the sample requirement, allowing for dropouts. This provided a requirement for 106 experimental subjects. Data preparation involved visual checking and exploratory analyses involving variable inspection, missing values (system and user) and outliers. Data trends were visually and statistically evaluated for normality and almost all data was found to be not normally distributed.

The statistical hypotheses were:

- Null hypothesis = Non hyperandrogenic PCOS women have similar cardiometabolic features and cardiovascular risk factors to hyperandrogenic PCOS women.
- Alternative Hypothesis = Non hyperandrogenic PCOS women do not have similar cardiometabolic features and cardiovascular risk factors to hyperandrogenic PCOS women.

After a careful review of the literature, 11 predictor variables were found to have the potential for predicting cardiovascular and metabolic risk. These were IR, hypercholesterolaemia, low HDL-C, high LDL-C, high triglyceride, high fasting blood sugar, hypertension, elevated serum HbA1c, obesity, central obesity and metabolic syndrome. Generally it was planned to qualify the association between these variables and hyperandrogenism through the application of univariate logistic regression (LR) analysis. Variables that seemed to be associated were to be analysed further using MLR analysis with backwards stepwise selection. Logistic regression analysis was also used to estimate the effects of covariates on the prevalence of metabolic syndrome, using the mean of predicted probabilities.

In the initial analysis, the prevalence of cardiovascular and metabolic risk factors between NHA and HA were compared. Logistic regression analysis (LR) was used for this inferential statistics. Categorical variables were expressed as percentage and 95% confidence interval (CI). Odds ratio (OR) with 95% confidence intervals (CI) were calculated according to the normal approximation of the binomial distribution. Adjusted p values were obtained after correcting for potential confounding factors (age, BMI and waist circumference).

In the second analysis, cardiometabolic and endocrine parameters were compared between the 2 groups. For significance testing, continuous variables with non-normal distribution were compared using the Mann-Whitney U test and the independent samples t-test used for normally distributed variables. The data is presented as median and interquartile range (IQR) or mean and standard deviation (SD) respectively. Logistic regression analysis was carried out to assess for confounding for age and waist circumference.

The full statistical analysis is included in the supplement.

Ethics Process

This study was performed after obtaining approval from the National Heart Institute of Malaysia Ethics Committee (IJNEC) and the Institutional Review Board of the University of Cyberjaya. It adhered to the tenets of the Helsinki Declaration for biomedical research and carried out guided by the concept of Good Clinical Practice.

Written and informed consent was obtained from all subjects according to the protocol prior to involvement in the research. Accurate information of the purpose, methods, benefits, risks and alternatives to the research was provided with confirmation of the subjects understanding obtained. The subjects were made to understand the diagnostic procedures, their risks and prospective benefits in the study, ensuring the decision to participate was a voluntary process, and withdrawal from the research could be undertaken at any time.

The measures taken to safeguard personal and health data confidentiality was explained, including the storage in a secure area and restricted access to the data. The subjects were impressed on the maintenance of privacy and keeping their information confidential.

RESULTS

Phenotypes

132 women initially qualified for inclusion but 23 were excluded for pregnancy, thyroid disease and dropping out. The remaining 109 Malay women with PCOS were included in this study. The definition of the different PCOS phenotypes is shown in Table 1. All subjects were specifically typed with 14 (13.0%) AO, 24 (22%) as AP, 42 (39%) as AOP and 29 (27%) as OP phenotypes. The HA group made up 73% of the sample and the rest were NHA. This division is summarised in Figure 1.

Regression Analysis

Univariate LR was carried out to ascertain the effects of IR, hypercholesterolaemia, low HDL-C, high LDL-C and central obesity on the likelihood of subjects having hyperandrogenic PCOS. The variables high triglyceride, hyperglycaemia, hypertension, elevated serum HbA1c and metabolic syndrome were excluded from the analysis due to very low counts.

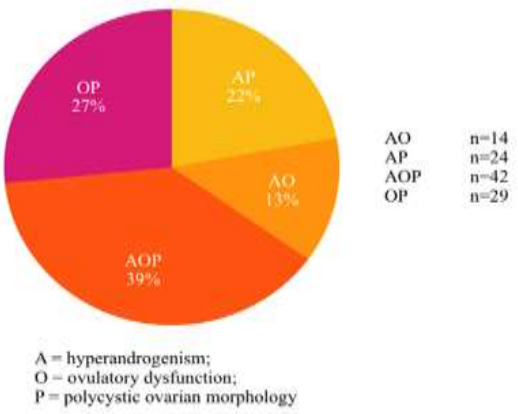

Figure 1: Distribution of PCOS Phenotypes

Table 1: Definition of the four PCOS phenotypes

Phenotype	Hyperandrogenism	Ovulatory dysfunction	Polycystic Ovarian Morphology
AO	Yes	Yes	No
AP	Yes	No	Yes
AOP	Yes	Yes	Yes
OP	No	Yes	Yes

However, the regression analysis model was a poor fit for assessing the prevalence of cardiovascular and metabolic risk factors between non-hyperandrogenic and hyperandrogenic Malay women with PCOS, and there was no significant association for any of the variables on hyperandrogenism (Table 2).

Table 2: LR analysis showing the predictive association of several clinical variables and the presence of hyperandrogenism

	Variable	*p*-value	OR	ROC-AUC
Univariate analysis	HOMA-IR	.646	0.754	0.519
	High Cholesterol	.411	1.430	0.545
	Low HDL-C	.895	1.059	0.507
	High LDL-C	.750	0.864	0.516
	High waist circumference	.275	0.662	0.559

Prevalence of Risk Factors

Table 3 shows the prevalence of cardiovascular and metabolic risk factors in the two groups of PCOS women. Overall, there were no statistically significant differences between non hyperandrogenic and hyperandrogenic women.

Whether hyperandrogenic or not, women with PCOS had similar prevalences of insulin resistance as measured by HOMA-IR (86.2 vs 82.5%, p =.645, non hyperandrogenic and hyperandrogenic respectively).

Table 3: Summary of the prevalence of cardiometabolic risk factors in non-hyperandrogenic (NHA) and hyperandrogenic (HA) women with PCOS

Risk factor	NHA PCOS (n=29)	HA PCOS (n=80)	*p* value
Age (years)	15 (51.7)	48 (60.0)	.439
Abdominal obesity (waist circumference ≥ 80 cm)[2]	10 (34.5)	37 (46.3)	.273
Overweight & Obese (BMI > 25 kg/m^2)[1]	13 (44.8)	50 (62.5)	.099
Hypertension (DBP ≥ 80, SBP ≥ 130 mmHg)	1 (3.4)	6 (7.5)	.446
Insulin resistance (HOMA-IR) (< 0.99)	25 (86.2)	66 (82.5)	.645
Fasting Plasma Glucose (> 6.0 mmol/L)	1 (3.4)	14 (17.5)	.060
HbA1c (> 5.6%)	8 (27.6)	21 (26.3)	.889
Cholesterol (≥ 5.2 mmol/L)	16 (55.2)	37 (46.3)	.410
HDL-C (< 1.2 mmol/L)	16 (55.2)	43 (53.8)	.895
LDL-C (> 2.6 mmol/L)	19 (65.5)	55 (68.8)	.749
Serum Triglycerides (> 1.7 mmol/L)	9 (31.0)	26 (32.5)	.885
Metabolic syndrome	3 (10.3)	19 (23.8)	.123
Note: Values presented as number of subjects (percentage). HOMA-IR = homeostatic model assessment of insulin resistance; HDL-C = high density lipoprotein cholesterol; LDL-C = low density lipoprotein cholesterol; HbA1c = Haemoglobin A1c; BMI = body mass index; DBP = diastolic blood pressure; SBP = systolic blood pressure. [1] = adjusted only for age; [2]=adjusted only for age and BMI			

Among the lipids, the prevalences of LDL-C, total cholesterol and HDL-C were all elevated above 50% in both groups of patients, being quite similar in their respective percentages.

More women in the hyperandrogenic group had elevated plasma glucose than in the non hyperandrogenic group (14 vs 1, respectively) but this difference was not significant. The number of subjects with hypertension in the sample was too low to allow for any meaningful comparisons.

There were 19 women with hyperandrogenism (23.8%) who had metabolic syndrome compared to only 3 non hyperandrogenic women PCOS (10.3%), but the difference was not statistically significant.

The incidence of metabolic syndrome was higher in the hyperandrogenic group (23.8% vs 10.3%) which seemed markedly different, but was not statistically significant. The prevalence of high BMI and high waist circumference were similarly increased in hyperandrogenic PCOS women, but not enough to gain significance.

Mostly though, the cardiometabolic risk factors were similarly prevalent in both the non-hyperandrogenic and hyperandrogenic women (no statistical significance).

Metabolic and Endocrine Characteristics

Univariate LR was carried out to ascertain the effects of the various metabolic and endocrine parameters on the likelihood the subjects had hyperandrogenism. The regression analysis model was not statistically significant for all the parameters.

Table 4 summarises the metabolic and endocrine characteristics of non-hyperandrogenic and hyperandrogenic Malay women with PCOS. P-values are shown as crude (non adjusted) and after adjustment for free testosterone. Both groups of women had similar ages (median 30 years, 20-40).

Table 4: Comparison of baseline and endocrine characteristics of non-hyperandrogenic (NHA) and hyperandrogenic (HA) Malay women with PCOS.

Parameter	NHA (n = 29)	HA (n = 80)	Significance Nonadjusted U, *p* values	Significance Adjusted *p* values
Age (years)	30 (20-40)	30 (20-40)	1205.0, .757	.757
Waist circumference (cm)[1]	80.0 (29.0-99.0)	86.5 (31.6-125.0)	1350.0, .191	.399
Weight (kg)	61.0 (36.5-94.0)	65.0 (42.5-121.0)	1403.5, . 095	.132
SBP (mm Hg)	110.0 (100.0-164.0)	111.0 (100.0-140.0)	1213.5, .700	.722
DBP (mm Hg)	70.0 (60.0-100.0)	70.0 (60.0-90.0)	1300.5, . 312	.417
HOMA-IR	2.4 (0.5-16.3)	2.6 (0.4-62.3)	1311.0, .300	.095
Serum Insulin (μU/mL)	12.3 (2.5-69.0)	12.3 (1.8-138.8)	1282.0, .403	.128
Fasting Glucose (mmol/L)	5.00 (3.8-6.1)	5.2 (3.8-14.8)	1473.5, .032	.036
HbA1c	5.3 (4.8-6.2)	5.3 (3.8-8.1)	1086.0, .610	.547
Total Cholesterol (mmol/L)	5.3 (3.6-7.8)	5.1 (0.1-9.1)	1086.5, .614	.691
HDL-C (mmol/L)	1.2 (0.2-2.1)	1.2 (0.1-2.3)	1181.5, .883	.685
LDL-C (mmol/L)	3.1 (1.3-5.3)	3.2 (1.3-7.0)	1205.0, .758	.481
Serum TG (mmol/L)	1.4 (0.6-4.5)	1.3 (0.4-5.8)	1062.5, .504	.685
DHEAS (μg/dL)	123.1 (50.4-238.4)	117.0 (10.5-603.0)	1094.5, .935	.809
SHBG (nmol/L)	70.3 (1.0-234.0)	47.2 (0.1-226.1)	819.5, .020	.057
Testosterone (nmol/L)	1.5 (0.6-3.4)	1.8 (0.6-7.3)	1475.5, .030	.662
Free androgen index	2.4 (0.4-134.6)	3.3 (0.9-26666.7)	1551.0, .005	.796
Free testosterone (pmol/L)	14.9 (4.3-34.0)	34.5 (2.2-158.0)	1813.0, <.001	-

Note: Values presented as median (interquartile range). HOMA-IR = homeostatic model assessment of insulin resistance; HDL-C = high density lipoprotein cholesterol; LDL-C = low density lipoprotein cholesterol; TG = triglycerides; DHEAS = dehydroepiandrosterone sulphate; SHBG = sex hormone binding globulin; HbA1c = Haemoglobin A1c; BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure;.

[1] = adjusted only for age

Hyperandrogenic women had greater waist circumference than non hyperandrogenic women (*Mdn* 86.5, IQR 31.6-125.0 vs *Mdn* 80.0, IQR 29.0-99.0) a difference that wan not significant (p =.123). There was no difference in age between these two phenotypes.

There were no statistically significant differences in most of the cardiometabolic and endocrine features. However, the fasting plasma glucose was higher in hyperandrogenic women (*Mdn* 5.2, IQR 3.8-14.8 vs *Mdn* 5.0, IQR 3.8-6.1, p =.032).

As was expected, women with hyperandrogenic PCOS had significantly higher levels of serum total testosterone (*Mdn* 1.8, IQR 0.6-7.3 vs *Mdn* 1.5, IQR 0.6-3.4, p =.030), FAI (*Mdn*13.3, IQR 0.9-2666.7 vs *Mdn* 2.4, IQR 0.4-134.6, p

=.005) and free testosterone (*Mdn* 34.5, IQR 2.2-158.0 vs *Mdn* 14.9, IQR 4.3-34.0 *p* <.001). Their levels of SHBG were also lower than non hyperandrogenic women (*Mdn* 47.2, IQR 0.1-226.1 vs *Mdn* 70.3, IQR 1.0-234.0 respectively, *p* =.020).

DISCUSSION

This study demonstrates that the presence of hyperandrogenism in Malay PCOS women does not overtly influence the prevalence of cardiometabolic derangements.

The PCOS phenotypes in this study were divided into non-hyperandrogenic and hyperandrogenic types. The non-hyperandrogenic PCOS phenotype of PCOS (ovulatory dysfunction and polycystic ovarian morphology) women are those generally considered without hyperandrogenism and referred to as the milder form of PCOS. The hyperandrogenic PCOS women have usually been associated with a higher prevalence of cardiovascular risk factors even after correction for age and obesity. Here, it has been shown that most of the cardiometabolic risk factors are quite similar in both the non-hyperandrogenic and hyperandrogenic Malay women with PCOS. The implication of this finding is that Malay women with the milder form of PCOS may be missed in clinical practice and this might affect their long term morbidity and mortality.

Although similar studies have previously been carried out, this is the first one known to study the different PCOS phenotypes in Malay women. Overall, the cardiovascular and metabolic risk factors within the phenotypes divided by the presence or absence of hyperandrogenism were similar. However, these results could be influenced by the small sample size in the subgroup of non-hyperandrogenic women.

The difference in the prevalence of metabolic syndrome between the two groups of subjects provides intriguing insight. Metabolic syndrome occurred in 23.8% of the hyperandrogenic PCOS group of subjects, which is similar to previous reports. The 1992 NIH criteria for PCOS only includes hyperandrogenic women in its diagnosis (before the advent of widespread use of ultrasound scanning for diagnosing polycystic ovarian morphology) (Zawadsi JK 1992). Based on this criteria, the prevalences of metabolic syndrome have been previously reported as being between 20-46% (Moran LJ 2010).

Apart from hyperandrogenism, IR is more prevalent in PCOS compared to normal women. It would therefore be interesting to not the differences in IR between the non-hyperandrogenic and hyperandrogenic PCOS women. In this study, there was no significant difference in IR between the two groups. This lack of difference also applied to weight and waist circumference.

On general inspection, the prevalence of hypertension in the non-hyperandrogenic group were very low compared to the hyperandrogenic group (3.4% vs 7.5%). Not much can be analysed from this due to the very low numbers of

Hyperandrogenism is considered an important component of PCOS and has been shown to be closely related to metabolic abnormalities. Rates of hyperandrogenemia, dyslipidemia and insulin resistance (IR) have been noted to be higher in hyperandrogenic than non-hyperandrogenic PCOS women (O'Reilly MW 2014). Other studies have found no differences in the metabolic features of the different PCOS phenotypes, similar to our study (Hosseinpanah 2014). This discrepancy can be explained by many reasons, not least genetic and ethnic variation. Environmental factors, specifically lifestyle, economic factors and obesity would be strong influences in the expression of PCOS phenotypes. Of equal importance would be the effects of study design and inclusion criteria. The method of recruitment of PCOS subjects can severely influence the estimates of cardiometabolic parameters. Subjects recruited from the population would be expected to have less sever from of PCOS as opposed to this recruited from the clinic as in our study. Clinic based subjects would probably have a longer duration of the syndrome and present with more extreme features. This in turn would mean that the health concerns of referred or clinic based subjects would be more severe.

This study has several strengths that make it an addition to existing literature. This is one of the very few studies of Malay women with PCOS, especially the assessment of cardiovascular, metabolic and endocrine features of the different PCOS phenotypes. The cut-off points for HOMA-IR, lipids and habit were based on local values for Malay women.

Several limitations have to be considered when interpreting these results. The sample size obtained from a single centre was not large enough to allow for a more general evaluation of the predictors of cardiometabolic status and lacked power. The subjects wee also recruited from a specialist reproductive and endocrine clinic. These clinic-based subjects may have more severe or advanced degrees of metabolic abnormalities, making the interpretation and comparison of data difficult. With regards to the laboratory assessment, two important points need to be addressed. Free testosterone was obtained from calculation and not measurement due to the lack of proper equipment, which might cause some inaccuracy. The assessment of insulin sensitivity was based on the use of HOMA-IR, a surrogate measure which has limited validity in women with PCOS (Diamanti-Kandarakis 2004).

CONCLUSION

Malay women with non-hyperandrogenic and hyperandrogenic PCOS phenotypes have similar cardiometabolic profile and cardiovascular risk factor prevalences. Therefore, all Malay women with PCOS should be considered as a singular entity with regards to cardiometabolic risk.

CONFLICT OF INTEREST

None

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