University Hospitals Coventry and Warwickshire NHS Trust

The impact of Obstructive Sleep Apnoea in women with Polycystic Ovary Syndrome: A Cross-Sectional Study

PCOS OSA

Protocol version 4.0 (01/08/2017)

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Confidentiality statement

1. Title

1.1 Study Title

- **Full Title:** The impact of Obstructive Sleep Apnoea in women with Polycystic Ovary Syndrome: A Cross-sectional study
- **Short title:** PCOS OSA

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Date 01/08/2017

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3. Contents

4. List of Abbreviations

- AHI	Apnoea/hypopnoea index
- AASM	American Academy of Sleep Medicine
- BMI	Body mass index
- BP	Blood pressure
- CPAP	Continuous positive airway pressure
- CRF	Case Report Form
- DVLA	Driving and Vehicle Licensing Agency
- ESS	Epworth Sleepiness Scale
- FGS	Ferriman-Gallwey score
- FPG	Fasting plasma glucose
- FSH	Follicular stimulating hormone
- GnRH	Gonadotropin releasing hormone
- hCRP	High sensitivity C-reactive protein
- HAD	Hospital Anxiety and Depression scale
- HbA1C	Glycated haemoglobin
- HOMA-IR	Homeostatic model assessment of insulin resistance
- HRA	Health Related Authority
- ICSD-3	International Classification of Sleep Disorders
- IGT	Impaired glucose tolerance
- IR	Insulin resistance
- LH	Luteinising hormone
- MS	Metabolic syndrome
- NAFLD	Non-alcoholic fatty liver disease
- NC-CAH	Non-classic congenital adrenal hyperplasia
- NHS	National Health Service
- OCP	Oral contraceptive pill
- OSA	Obstructive Sleep Apnoea
- PCO	Polycystic ovaries
- PCOS	Polycystic Ovary Syndrome
- PCOSQ	PCOS health-related quality of life questionnaire
- PI	Principle Investigator

- PIS	Patient information sheet
- QoL	Quality of life
- RDI	Respiratory Disturbance index
- SD	Standard deviation
- SDB	Sleep disordered breathing
- SEM	Standard error of the mean
- TT	Total testosterone
- UHCW	University Hospital Coventry and Warwickshire NHS Trust
- WHOQOL-BREF	World Health Organisation QoL-BREF questionnaire
- WHR	Waist-to-hip ratio
- WISDEM	Warwickshire Institute for the Study of Diabetes, Endocrinology and Metabolism

5. Summary

Background: Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age. PCOS is associated with multiple co-morbidities including obesity, insulin resistance, subfertility, impaired quality of life (QoL) and increased risk of type 2 diabetes. Obstructive sleep apnoea (OSA) is a common medical condition that is often undiagnosed, particularly in women. Obesity is a common risk factor for OSA and PCOS and OSA is associated with comorbidities similar to those observed in patients with PCOS such as insulin resistance, increased risk of type 2 diabetes, and impaired QoL. Hence it is not surprising that OSA and PCOS might co-exist.

However, the impact of OSA in women with PCOS remains unclear and understudied. It is plausible that OSA may contribute to the subfertility and impaired QoL observed in women with PCOS by increasing insulin resistance, activation of the sympathetic nervous system, disturbing the hypothalamic/pituitary/ovarian axis, and contributing to excessive daytime sleepiness and reduced mood.

Hypothesis: Women with PCOS and OSA, compared to women with PCOS only, have more severe clinical and biochemical features of PCOS and impaired QoL.

Study design: Observational cross-sectional study.

Setting: A secondary care PCOS clinic in the WISDEM Centre, University Hospital Coventry.

Study outcomes: The primary aim of this study is to examine the relationship between OSA and impaired QoL in women with PCOS. Study secondary outcomes are to examine the relationship between OSA and the clinical and biochemical features in women with PCOS.

Methods: 38 women with PCOS will be recruited from the PCOS clinic. Women with increased risk of OSA, based on the Berlin questionnaire and the Epworth Sleepiness Scale (ESS), will have home-based sleep studies performed. They will also be referred to the Respiratory Physician, as part of routine NHS care.

Study participants will be divided based on the results of the Berlin and ESS questionnaires and sleep studies into two groups: 1) PCOS low risk OSA: women with normal ESS and normal Berlin questionnaire (no sleep studies performed), or women with normal sleep studies; and 2) PCOS OSA: women with OSA proven by sleep studies.

Clinical and biochemical features including reproductive history, depression and anxiety [using the Hospital Anxiety and Depression questionnaire (HAD)], and QoL [using the PCOS health-related quality of life questionnaire (PCOSQ) and the World Health Organisation QoL-BREF (WHOQOL-BREF) questionnaire] will be compared between the two groups.

6. Background

Few studies have suggested that obstructive sleep apnoea (OSA) is more common in women with polycystic ovary syndrome (PCOS) independent of obesity (Fogel et al. 2001; Vgontzas et al. 2001). It has also been suggested that women with PCOS and OSA may show more severe phenotypical and metabolic features of PCOS compared to women with PCOS alone and subsequently represent a different 'higher risk' population (Tasali et al. 2008). However, how much OSA contributes to the clinical and metabolic features of PCOS is not yet clear though important, as OSA is treatable.

6.1 Polycystic Ovary Syndrome

PCOS is a common disorder with a prevalence of 6 – 15% of women of reproductive age (Azziz et al. 2004; Knochenhauer et al. 1998; Diamanti-Kandarakis et al. 1999; Asuncion et al. 2000; Fauser et al. 2012). The Rotterdam criteria (Rotterdam 2004) define PCOS by the presence of two of the following three components: hyperandrogenism, oligomenorrhoea /anovulation, and polycystic ovaries on ultrasound. Oligomenorrhoea is defined as a mean cycle length > 35 days (usually fewer than nine cycles per year), while amenorrhoea is defined as cessation of menses for > 6 months (Broekmans et al. 2006). Hirsutism, excess body hair, is a common feature in women with PCOS. Polycystic ovaries (PCO) on ultrasound are defined as either 12 or more follicles measuring 2–9 mm in diameter in one of the ovaries, or an ovarian volume of >10 cm^3 (Rotterdam 2004). The diagnosis requires the exclusion of conditions with similar presentation including non-classic congenital adrenal hyperplasia (NC-CAH), androgen secreting tumours, Cushing's syndrome; thyroid disorders and prolactinomas.

PCOS is also associated with subfertility (West et al. 2014), obesity (Glueck et al. 2005), insulin resistance (IR) (Dunaif et al. 1989), increased risk of developing type 2 diabetes (Moran et al. 2010), depression and impaired quality of life (QoL) (Barnard et al. 2007; Hahn et al. 2005; Cinar et al. 2011). Although multiple cardiovascular risk markers are increased in in women with PCOS, the link between PCOS and increased cardiovascular morbidity and mortality remains controversial (Randeva et al. 2012).

6.2 Obstructive Sleep Apnoea

OSA is a common disorder affecting around 17–26% of men and 9–28% of women (Young et al. 2002). OSA is characterised by recurrent episodes of partial (hypopnoea) or complete (apnoea) upper airway obstructions associated with recurrent oxygen desaturations and cyclical changes in heart rate, blood pressure, intra thoracic pressure and sympathetic activity (Tahrani and Ali 2014). In addition, it results in changes in sleep architecture such as the loss of deep sleep (stages 3 and 4) and/or loss of REM sleep (Tahrani and Ali 2014). Women with OSA seem to have a higher proportion of respiratory events in REM sleep compared to men (O'Connor et al. 2000).

Male gender, obesity and age are major risk factors for OSA (Lee et al. 2008). Large population-based studies suggest that OSA prevalence is 1.5 – 3 times higher in men compared to women and this gender difference decreases after menopause (Lee et al. 2008). Although the exact mechanisms underlying the gender differences in the prevalence of OSA are not clear, it is possible that androgens play a role in the pathogenesis of OSA, while progesterone has a protective role. OSA risk is also influenced by body fat distribution (Ehrmann 2012); visceral fat appears to be more metabolically active and the quantity of visceral fat has been shown to highly correlate with OSA risk (Shinohara et al. 1997). Other predisposing factors to OSA include ethnicity, smoking, alcohol, upper airway anatomy and genetic factors (Lee et al. 2008).

Patients with OSA may present with nocturnal symptoms including snoring, witnessed apnoeas, choking or gasping, insomnia, nocturia, enuresis, frequent arousals, diaphoresis, and impotence (McNicholas 2008). While daytime symptoms may include excessive daytime sleepiness, fatigue, memory impairment, morning headaches, and depression (McNicholas 2008). Despite the high prevalence of OSA, the condition is frequently unrecognized and undiagnosed (McNicholas 2008) particularly in women who may not present with the 'classic ' symptoms of OSA (Lee et al. 2008).

OSA is associated with increased risk of hypertension (Peppard et al. 2000), cardiovascular disease (Arzt et al. 2005; Peker et al. 2002; Marin et al. 2005), mortality (Young et al. 2008; Punjabi et al. 2009), IR and type 2 diabetes (Wang et al. 2013), road traffic accidents (Tregear et al. 2009), depression and impaired QoL (Finn et al. 1998; Akashiba et al. 2002; Engleman and Douglas 2004). Continuous positive airway pressure (CPAP) therapy is the treatment of choice for symptomatic OSA (Greenstone and Hack 2014).

6.3 Proposed mechanisms linking OSA to PCOS

Androgens are thought to play an important role in the pathogenesis of OSA (Kapsimalis and Kryger 2002) through mechanisms including an increase in upper airway collapsibility (Cistulli et al. 1994), and a change in ventilatory chemoreceptors sensitivity and responsiveness (White et al. 1985; Zhou et al. 2003). In contrast, progesterone is believed to play a protective role against the development of OSA in women (Kapsimalis and Kryger 2002) by reducing upper airway resistance (Driver et al. 2005), and improving ventilation and increasing ventilator chemoresponsiveness (Zwillich et al. 1978; Regensteiner et al. 1989). As PCOS is characterised by hyperandrogensim and anovulatory cycles, with subsequent higher oestrogen and lower progesterone levels, it is plausible that PCOS may lead to the development or worsening of OSA symptoms.

The pathophysiology of PCOS is complex and not fully understood but genetic susceptibility, hyperandrogenism, IR, abnormal gonadotropin releasing hormone (GnRH) secretion, and sympathetic overactivity are thought to play a role (Conway et al. 2014; Lansdown and Rees 2012). OSA is associated with IR and women with OSA seem to have a high proportion of respiratory events during the REM sleep (O'Connor et al. 2000). Sleep is thought to have a significant influence on female hormone production (Lee et al. 2014). Sleep deprivation, sleep interruption, and sleep disordered breathing have been suggested to influence GnRH, follicular stimulating hormone (FSH) and luteinising hormone (LH) pulsatility and may cause menstrual disturbances (Baumgartner et al. 1993; Hall et al. 2005). OSA is also associated with sympathetic overactivity (Grassi et al. 2005). Sympathetic overactivity may contribute to IR, alter ovarian function and cause PCO morphology (Lansdown and Rees 2012). Subsequently, OSA may contribute to the development of PCOS or worsening of its symptoms.

6.4 Literature search and review

One of the first studies to suggest a relationship between PCOS and OSA (Fogel et al. 2001) included 18 young [age ±standard error of mean (SEM) 31.1 ±1.3 years], overweight and obese [body mass index (BMI) 36.9 ±1.3 kg/m^2], women with PCOS and 18 age- and BMI-matched controls (age 32.3 ±1.3 years, BMI 36.9 ±1.4 kg/m^2). PCOS was defined by chronic oligomenorrhoea (≤6 periods/year) along with elevated serum total or biologically available testosterone levels. Control women had regular cycles and no evidence of clinical hirsutism or biochemical hyperandrogensim. OSA [defined as apnoea/hypopnoea index (AHI) >10 events/hour] was more prevalent in women with PCOS

(66.67% vs. 16.67%, P<0.05) and AHI strongly correlated with waist-to-hip ratio (WHR), total serum testosterone, and unbound testosterone levels. Insulin levels were not reported. Study limitations include that the PCOS group had higher WHR compared to controls (0.88 ±0.02 vs. 0.82 ±0.01, P<0.01), and this was not adjusted for in the analysis; no information was provided on participants' ethnicities; and small sample size. Although the authors concluded that the high prevalence of OSA in PCOS is likely to be secondary to hyperandrogenism (women with PCOS had significantly higher testosterone levels than controls), they did not consider the role of other risk factors for OSA including WHR, progesterone levels and IR.

Vgontzas at al. (Vgontzas et al. 2001) compared 53 premenopausal women with PCOS, age 30.4 year (range 16 – 45), to 452 controls, age 32.1 year (range 20 – 42). The PCOS group was more overweight than controls, BMI 38.7 kg/m^2 (range 24.3– 67.7) vs. 26.4 kg/m^2 (range 16.1–59.9), P<0.01. PCOS was diagnosed if both oligomenorrhoea (<6 periods/year), and raised total or free testosterone were present. All women with PCOS had PCO on ultrasound examination. Control subjects were selected through random telephone interviews, and those with symptoms suggestive of OSA were oversampled. OSA (defined as AHI ≥10 events/hour plus symptoms) was more common in women with PCOS (11.3% vs. 0.4%; P<0.001) even after adjustment for BMI. Differences between women with PCOS and sleep disordered breathing (SDB) (n=9), defined as either OSA or upper airway resistance syndrome, compared to those with PCOS without SDB (n=44), are summarised in Table 1. In a logistic regression analysis, insulin levels and glucose-to-insulin ratio had a stronger association with SDB than age, BMI, or testosterone levels. Study limitations include that abdominal adiposity was not measured and not adjusted for in the analysis; no information was provided on how women with PCOS were selected to take part in the study; women in the control group were not screened for PCOS; and no information was provided on ethnicities in either group. In addition, the difference in BMI between the PCOS and control groups was very high (12.3 kg/m^2) that statistical adjustment is unlikely to exclude an effect for obesity on the difference in OSA prevalence between the two groups.

Tasali et al. (Tasali et al. 2008) compared 52 premenopausal women with PCOS [age (±SEM) 29.7 ±0.7 year] to 21 controls (age 30.7 ±1.1 year). Women with PCOS were recruited consecutively from the endocrine clinic over a 3.5 year period, while controls were selected through advertisement in the community. Sleep complaints or symptoms of OSA were not used as selection criteria for the study. PCOS was diagnosed based on the presence of oligo/amenorrhoea and elevated free testosterone levels and either infertility, or the presence of clinical hyperandrogensim. The control group had a higher proportion of women of African-American or Hispanic descent (86 vs. 62%; P = 0.054), while the PCOS group was heavier [BMI 39.2 kg/m^2 (range 23.2 – 58.8) vs. 36.0 kg/m^2 (range 27.7 – 48.8)]. OSA (defined as AHI ≥5 events/hour) was more prevalent in women with PCOS (56% vs. 19%) even after adjustment for BMI and ethnicity (P=0.01). The authors concluded that OSA is highly prevalent in women with PCOS independent of age and BMI. Differences between women with PCOS and OSA to those with PCOS without OSA are summarised in Table 1. AHI strongly correlated with IR, and impaired glucose tolerance. Study limitations include small study sample; and that abdominal adiposity was not measured and not adjusted for in the analysis. In addition, the high BMI of study participants, particularly the PCOS group and using two different methods to recruit participants (clinic vs. advertisement) raise suspicion of selection bias. The authors concluded that women with PCOS and OSA represent a metabolically different population 'higher risk' compared to

those with PCOS without OSA. However, this conclusion is limited by the small study sample and the lack of clinical outcomes measured.

In a retrospective study (Nandalike et al. 2012), 28 adolescent girls with PCOS, [age ±standard deviation (SD) 16.7 ±1.9 year and BMI 44.8 ±8.8 kg/m^2] were compared to 28 girls without PCOS (age 17.1 ±1.8 year and BMI 40.2 ±4.7 kg/m^2) and 28 boys (age 16.6 ±1.6 year and BMI 38.9 ±6.6 kg/m^2). BMI Z-scores were similar in the three groups. All study participants had polysomnography performed as they had sleep related complaints such as snoring, trouble breathing at night or excessive daytime sleepiness. All the girls with PCOS had oligo/amenorrhoea plus clinical or biochemical evidence of hyperandrogenism. The female control group had a higher proportion of African Americans (46.4%) compared to the male (21.4%) and PCOS (17.9%) groups. A higher proportion of girls in the PCOS group (32%) and male control group (25%) had a history of adenotonsillectomy, treatment of choice for OSA in children, prior to polysomnography compared to the female control group (10.7%). OSA (defined as AHI was > 5 events/hour or apnoea index > 1 event/hour) was diagnosed in 57% of girls with PCOS, in 14.3% girls without PCOS, and in 75% boys. Differences between girls with PCOS and OSA to those with PCOS without OSA are summarised in Table 1. Study limitations include small sample size; retrospective design; PCOS was not formally excluded in the control group; only adolescents with symptoms suggestive of OSA had polysomnography performed; and abdominal adiposity was not measured and adjusted for in the analysis. Although there was no statistical difference in BMI Z-scores between the study groups, the PCOS group had significantly higher BMI compared to the male and female control groups. In addition, while the authors reported that girls with PCOS and OSA had a trend to be more obese compared to girls with PCOS without OSA, the exact BMIs for those two subgroups (PCOS +/- OSA) were not provided. Subsequently, it is difficult to rule out an effect for BMI, abdominal adiposity or ethnicity on OSA prevalence in this study. In addition, the high BMI of study participants, in particular the PCOS group; and the fact that more girls in the PCOS group had previous adenotonsillectomy performed raise suspicion of selection bias.

Interestingly, De Sousa et al. (de Sousa et al. 2010) compared 22 adolescent girls with PCOS [age (±SD) 15.2 ±1.3 year, BMI 31.7 ±6.2 kg/m^2] to 18 normal-weight girls without PCOS (age 15.0 ±0.9 year, BMI 20.6 ±2.3 kg/m^2) and 11 obese girls without PCOS (age 15.0 ±1.0 year, BMI 34.8 ±8.7 kg/m^2). None of the girls in the PCOS group were found to have OSA. PCOS was diagnosed based on the presence of hyperandrogensim and oligomenorrhoea/anovulation. Girls with PCOS were recruited from the obesity and endocrine outpatient clinics. Study limitations include small study sample, and girls in the control groups were only included if they had a polysomnography that ruled out OSA. The authors concluded that PCOS precedes the development of OSA.

Yang et al. (Yang et al. 2009) compared 18 premenopausal slim [age (±SEM) 29.1 ±1.43 year, BMI 21.7 ±0.57 kg/m^2] women with PCOS to 10 BMI- and age-matched controls (age 31.6 ±3.9 year, BMI 20.9 ±0.58 kg/m^2). None of the participants in either group had an AHI ≥ 5 suggestive of OSA. Women with PCOS were recruited from the Obstetrics and Gynaecology clinic. PCOS was diagnosed based on the presence of oligomenorrhoea, biochemical hyperandrogensim and PCO on ultrasound. The PCOS group had significantly higher testosterone levels, larger waist circumference and WHR but there was no difference in homeostatic model assessment of insulin resistance (HOMA-IR). Study limitations include small number of participants.

In another study (Gopal et al. 2002), 23 premenopausal women with PCOS (BMI 42.7 kg/m^2, range 31.4 – 67.0), had polysomnography performed, regardless of symptoms, and sixteen women (69%) were found to have OSA. OSA was defined as respiratory distress index (RDI) ≥5 events/hour plus symptoms. Women with PCOS were consecutively selected from the Reproductive Endocrinology and Gynaecology clinics. There was no correlation between obesity and the severity of OSA. Study limitations include small study sample; and no information was provided on study participants' age, ethnicity, abdominal adiposity, testosterone levels, or insulin resistance levels. In addition, the high BMI of study participants; and recruiting participants from outpatient clinics raise suspicion of selection bias. The authors concluded that obesity is not the only cause of the increased prevalence of OSA in women with PCOS.

Tock et al. (Tock et al. 2014) screened 38 premenopausal women with PCOS [age (±SD) 28.3 ±6.8 year and BMI 32.9 ±7.7 kg/m^2] for OSA (defined as AHI ≥5 events/hour, regardless of symptoms), and 12 (32%) were found to have the condition. PCOS was diagnosed based on the Rotterdam criteria. Study participants were recruited from the Endocrinology clinic. Women with PCOS and OSA compared to those without OSA had higher prevalence of non-alcoholic fatty liver disease (NAFLD) (83.3% vs. 26.9%); other differences are summarised in Table 1. After adjusting for obesity in multivariate logistic regression, raised free testosterone levels ≥1.07 ng/dL increased the risk of OSA in women with PCOS by 8.2 fold. In a subsequent multiple logistic regression analysis, OSA was a stronger predictor than BMI and IR for the presence of NAFLD. Study limitations include small study sample; and that testosterone was measured by immunoassay rather than tandem mass spectrometry. The authors concluded that hyperandrogenism may be a predisposing factor for OSA in PCOS.

In another study (Chatterjee et al. 2014), 50 women with PCOS (BMI 27.1 kg/m^2) were screened for OSA and 33 (66%) were found to have sleep disordered breathing (SDB). Women with PCOS were randomly selected from the gynaecology and reproductive endocrinology clinics. PCOS was diagnosed based on the Rotterdam criteria. SDB was defined as an RDI of ≥5 events/hour along with symptoms or an RDI of >15 events/hour with or without associated symptoms. Differences between women with PCOS and OSA to women with PCOS without OSA are summarised in Table 1. On logistic regression analysis which adjusted for BMI, only the associations between fasting plasma glucose and diastolic blood pressure with SDB remained significant. Study limitations include that the age of study participants was not provided and small study sample.

Finally, in an interventional study (Tasali et al. 2011) 19 obese women with PCOS and OSA [age (±SEM) 31.2 ±1.2 year, BMI 46.4 ±2.4 kg/m^2] were treated with CPAP for 8 weeks with subsequent improvement in insulin sensitivity (relative increase of nearly 7%), and reduction in diastolic blood pressure (around 2.3mmHg). Day-time and night-time norepinephrine levels also reduced after therapy. Study limitations include that the authors only performed 'per protocol' analysis including only 9 women, and data from 10 participants were excluded from the analysis as their average use of CPAP was less than 4 hours per night. In addition, the study did not include a control group. It is also not clear if the changes in insulin sensitivity and blood pressured observed in the study will translate into meaningful clinical outcomes.

Study	Notes	n	OSA		Women with PCOS and OSA compared to women with PCOS without OSA										
			Diagnosis	%	Weight or BMI	Waist or WHR	IR	Hyperandrogenism	BP	FPG	IGT	MS	FSH / LH	Clinical features	QoL/ Depression
(Vgontzas et al. 2001)	USA	53	AHI ≥10 + symptoms	11–17%	↑	NA	Insulin ↑*	Free and TT ↔	NA	↔	NA	NA	NA	NA	NA
(Fogel et al. 2001)	USA	18	AHI >10	66.8%	NA	↑	NA	NA	NA	NA	NA	NA	NA	NA	NA
(Gopal et al. 2002)	USA	23	RDI ≥5 + symptoms	69.6%	↔	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
(Tasali et al. 2008)	USA	52	AHI ≥5	55.8%	↑	NA	HOMA-IR ↑*	Free and TT ↔	NA	↔	↑	NA	NA	NA	NA
(Yang et al. 2009)	Taiwan, slim women	18	AHI ≥ 5	0%											
(de Sousa et al. 2010)	Germany, adolescents	22	Not stated	0%											
(Nandalike et al. 2012)	USA, adolescents, retrospective	28	AHI > 5 or apnoea index > 1	57.2%	↔	NA	HOMA-IR ↑	Free and TT ↔	↑	↔	NA	↑	NA	NA	NA
(Tock et al. 2014)	Brazil	38	AHI ≥5	31.6%	↑	↑	HOMA-IR ↑	Free testosterone ↑*	NA	↔	↑	NA	NA	NA	NA
(Chatterjee et al. 2014)	India	50	RDI ≥5 + symptoms or RDI >15	66%	↑	↑	HOMA-IR ↔	Free testosterone ↔	↑	↑*	NA	↑	NA	↑ FGS	NA

Table 1 Differences between women with PCOS and OSA compared to women with PCOS only in published studies. n, number of participants; OSA, obstructive sleep apnoea; PCOS, polycystic ovary syndrome; BMI, body mass index; WHR, waist-hip-ration; IR, insulin resistance; HOMA-IR, homeostatic model assessment of insulin resistance; TT, total testosterone; BP, blood pressure; FPG, fasting plasma glucose; IGT, impaired glucose tolerance; MS, metabolic syndrome; FSH, follicular stimulating hormone; LH, luteinising hormone; QoL, quality of life; ↑ statistically significant increase; NA, not available; ↔ equal; FGS, Ferriman-Gallwey score; *adjusted for weight.

6.5 Summary of the literature

1. The majority of studies investigating OSA in women with PCOS were conducted in the USA. The prevalence of OSA in women with PCOS in the reported studies ranged from 0 – 69% (Median 55.8%, Mean 39.8%). The reason for this wide range in OSA prevalence is probably a combination of using different cut-offs and different methods to diagnose OSA; small studies sample size; and selection bias.

2. The data available suggest that the risk for OSA in women with PCOS is, as expected, increased with age and obesity. However, while it is probable that PCOS precedes the development of OSA, it is also possible that OSA precedes and increases the risk of developing PCOS in some women. Observational long-term studies are needed to assess how frequent is the development of OSA in women with PCOS and vice versa.

3. It seems plausible that OSA is a marker of severity in PCOS; however, it is difficult to conclude that from the studies conducted so far as abdominal adiposity and ethnicity have often not been accounted for in these studies. In addition, the relationship between OSA and hyperandrogenism in PCOS seems controversial. It is also not known if the presence of OSA in women with PCOS is associated with worse clinical outcomes including oligomenorrhoea, hirsutism, subfertility, depression, impaired quality of life, type 2 diabetes or cardiovascular disease. Subsequently, well conducted observational studies are needed to examine the effects of OSA in women with PCOS.

4. While one study suggested that CPAP therapy in women with PCOS and OSA improves insulin sensitivity and reduces blood pressure, it is not clear if this will translate into long-term meaningful clinical outcomes. Interventional, randomised-controlled trials with long-term follow up are needed to assess the effect of CPAP therapy on clinical and metabolic function in women with PCOS and OSA.

6.6 Justification for the study

PCOS and OSA are common conditions that are associated with obesity. PCOS is associated with subfertility, depression and anxiety, impaired QoL, increased risk of type 2 diabetes, and high prevalence of OSA. Hyperandrogensim and low progesterone levels are commonly seen in women with PCOS and may explain the increased prevalence of OSA. OSA is associated with disturbed sleep pattern, increased IR, disturbed hypothalamic/pituitary/ovarian axis, and sympathetic overactivity and subsequently may lead to a more severe phenotype of PCOS in affected women. Data from studies investigating the relationship between OSA and PCOS are limited by small sample size; not accounting for important confounders; and being at risk of selection bias. In addition, the effects of OSA on clinical outcomes in women with PCOS have not been investigated. Subsequently well conducted large observational studies are needed to investigate the relationship between OSA and clinical outcomes in women with PCOS. This is particularly important as OSA is a treatable condition.

7. Hypothesis

Women with PCOS and OSA, compared to women with PCOS only, have more severe clinical and biochemical features of PCOS and impaired QoL.

8. Study aims

Primary outcome:

- To examine if OSA is associated with impaired QoL in women with PCOS.

Secondary outcomes:

- To assess the relationship between OSA and body composition, hirsutism (modified Ferriman-Gallwey score), period cyclicity, reproductive outcomes, insulin resistance (HOMA-IR), hyperandrogenism, depression and anxiety, FPG, glycated haemoglobin (HbA1C), reproductive hormone profile, lipids, and inflammation (high sensitivity C-reactive protein, hCRP) in women with PCOS.

9. Study design

Observational cross-sectional study

10. Study groups

10.1 Source of subjects

Women with PCOS will be recruited consecutively from the PCOS clinic at the WISDEM centre, University Hospital Coventry. In addition, patient information sheet (PIS) and a study invitation letter will be sent to women with PCOS who attended the PCOS clinic in the last 12 months.

10.2 Inclusion criteria

- Women with PCOS, defined by the Rotterdam criteria as 2 out of 3:

1. Oligo/anovulation
2. Clinical or biochemical evidence of hirsutism
3. Polycystic ovaries on ultrasound.

And the exclusion of other disorders with similar presentation including NC-CAH, androgen secreting tumours, Cushing's syndrome; thyroid disorders and prolactinomas.

- Age ≥ 18 years.
- Able to provide written consent.
- Able to adequately understand English.
- Patients with unknown diagnosis of OSA, or who were investigated in the past and either found not to have OSA, or found to have OSA and are not on CPAP therapy.

10.3 Exclusion criteria

- Pregnancy or breastfeeding women.
- Patients who are unable to give consent.
- Patients known to have OSA treated with CPAP.

- Anyone under the age of 18 years.
- Unable to adequately understand or speak English.

10.4 Expected number of eligible subjects

In the PCOS clinic we usually see around 8 - 10 women with suspected PCOS per week. We expect 5 – 6 of them will fulfil the inclusion and exclusion criteria and of those we expect 3 – 4 to agree to take part in the study.

11. Recruitment and choosing participants

- **Method of recruitment**

Patients will be given the PIS by the study investigator or clinic nurse while sitting in the waiting area before being seen in the PCOS clinic. They will be advised to take the PIS home and call back if they are happy to take part in the study or if they have any question related to the study. In addition, an invitation letter and study PIS will be sent to patients who attended the PCOS clinic in the last 12 months. A list of patients who attended the PCOS clinic within the last year will be generated and from this list we will identify patients with confirmed diagnosis of PCOS and we will send the PIS and invitation letter to them by post.

Patients will also be recruited from the Weight Management Clinic and the reproductive endocrinology clinic where the PIS will be given to patients with a history of PCOS by the clinic doctor. Patients will be advised to take the PIS home and call back if they are happy to take part in the study or if they have any questions related to the study.

A poster inviting women with a history of PCOS to take part in the study will be displayed at University Hospital Coventry and an e-poster will be displayed at the hospital intranet. Women who answer the advert will be sent the PIS and advised to call back if they would like to take part or if they have any question about the study.

- **Procedures to assess study suitability**

Initial biochemical and clinical screening to confirm the diagnosis of PCOS and investigate the likelihood of OSA will be performed as part of routine clinical care in the National Health Service (NHS).

For patients who self-refer through the advertisement, the study doctor will clarify the diagnosis of PCOS with them on the phone and if the diagnosis is not clear, they will be offered the chance to be seen in the PCOS clinic if they wish.

- **Consent**

We expect patients to have the PIS for at least 24 hours before making a decision about taking part in the study. Patients who agree to take part in the study and have a confirmed diagnosis of PCOS will be invited for a study visit. Consent will be obtained by a study doctor/investigator from all study participants before taking part in any study related activity.

- **Participants**

38 women will be recruited after fulfilling the inclusion and exclusion criteria. Study participants will be screened for OSA using Berlin and Epworth Sleepiness Scale (ESS) questionnaires and if both were normal then no further testing for OSA is required. If the score on either of the questionnaires was abnormal then home-based sleep studies will be arranged and the patient will be referred to the Respiratory Physician as per routine clinical care.

OSA will be assessed by a single overnight home-based cardiorespiratory sleep study using a portable multichannel device (Alice PDx, Philips, US) and scored in accordance with the International Classification of Sleep Disorders (ICSD-3) (American Academy of Sleep Medicine 2014). The scoring of the sleep studies will be performed by Dr Asad Ali, Respiratory Physician, or a sleep technician. Sleep studies with <4 hours of adequate recordings will be repeated and excluded if the quality remained poor. OSA is defined by either an AHI of ≥5 with daytime sleepiness as well as gasping, choking or snoring; or an AHI of ≥15 in the absence of symptoms (American Academy of Sleep Medicine 2014).

Participants will be divided into two groups depending on the results of the ESS and Berlin questionnaires and sleep studies:

1. PCOS low risk OSA: women with PCOS with either normal scores on both the ESS and Berlin questionnaires, or who had normal sleep studies.
2. PCOS OSA: women with PCOS who had sleep studies confirming the presence of OSA.

12. Overview of study visit

Patients who agree to take part in the study will be asked to fast for 10 hours, where only water is allowed, before the study visit. Patients will also be asked to avoid exercise, caffeine (for example coffee, tea, and caffeine rich drinks), and chocolate in the 24 hours prior to the study visit; a clear instruction on which foods or drinks to avoid will be provided in the PIS.

On the study visit and after obtaining consent from study participants, their eligibility criteria to take part in the study will be assessed by the study investigator. Participants who fulfil the study inclusion/exclusion criteria will have full medical history obtained, and physical examination performed. If risk of OSA has not already been assessed in the clinic using the Berlin and the ESS questionnaires, these questionnaires will be completed. Participants with a high score on either the Berlin or ESS questionnaires will be provided with a portable sleep machine to take home; and a referral to the respiratory clinic will be arranged as per routine clinical practice. Information on how to use the sleep machine will be explained to the study participant by study doctor. Participants will be asked to return the machine the next day. Participants will have fasting bloods and early morning urine samples collected and stored. They will fill QoL and depression questionnaires. They will also be asked detailed questions about their fertility history. For women with regular periods, this visit will occur at the follicular phase of the cycle (day 1 – 7); while for oligomenorrhoic women a random blood sample will be collected. The duration of the visit is approximately 1.5 hours.

A summary of patient activity during the study visit is provided in Table 2.

Visit	1
Participants	All
Duration	Approximately 1.5 hours
Informed consent	X
Medical history	X
Reproductive history	X
Anthropometry	X
Physical examination	X
Screening for OSA	X
Early morning urine sample	X
Blood tests	X
QoL and HAD questionnaires	X

Table 2 Description of study visit.

13. Data

Data will be collected by the study investigator and/or a research nurse.

13.1 Anthropometric measurements

BMI is calculated as weight in kilograms divided by the square of height in meters. Waist circumference is defined as the widest circumference at the midpoint between the lateral iliac crest and the lowest rib margin at the end of normal expiration. Hips circumference is defined as the widest circumference over the femoral heads.

13.2 Urine sample

Each participant will be asked to provide an early morning urine sample. Urine samples will be frozen at -20°C until analysed. Samples will be stored at University Hospital Coventry. Urine samples will be used to measure markers relating to PCOS, OSA, obesity and components of the metabolic syndrome, such as urinary isoprostanes which are markers of oxidative stress (Patrono and FitzGerald 1997). Urinary isoprostanes, 8-iso $PGF_{2\alpha}$, will be measured by ELISA using urinary isoprostane EIA kit (Oxford Biomedical Research, Oxford, USA) as per manufacturer's protocol by an operator who is blinded to study group of participants.

13.3 Blood sampling

The study visit and blood sampling will be performed at the follicular phase of the cycle, unless women are oligomenorrhoic where a random blood sample will be collected. Around 40ml of blood will be collected after an overnight fast. Blood samples will be centrifuged immediately and the serum and plasma aliquots are stored at -80^{o}C till batch analysis. Blood samples will be stored at University Hospital Coventry. Samples will be used to measure markers relating to PCOS, OSA, obesity and components of the metabolic syndrome, such as markers of inflammation (hCRP), IR (FPG, insulin and HOMA-IR), hyperandrogenism (TT, sex hormone binding globulin, free androgen index, androstenedione, and dehydroepiandrosterone sulphate), diabetes (FPG and HbA1C), lipids (total cholesterol, low density lipoprotein, high density lipoprotein, and triglycerides), and reproductive hormones (FSH, LH, oestradiol, Anti-Mullerian Hormone). Participants will be given a

blood form to have their day 21 progesterone measured, unless if oligomenorrhoic where a random sample will be measured. Participants on the combined oral contraception pill (OCP) will not have their reproductive hormones measured.

13.4 Reproductive history

Participants will be asked if they are currently trying for children. Infertility will be defined as failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse (Zegers-Hochschild et al. 2009).

Participants will be asked if they have tried for children in the past, and if so:

- Number of pregnancies
- Number of children
- Number of miscarriages
- Have they been seen in the fertility clinic?
- Have they required any fertility treatment?
- Have they developed gestational diabetes?
- Have they developed pre-eclampsia or any pregnancy related complications?

13.5 Clinical history

Period cyclicity: Women will be asked to estimate how many periods they had in the four months preceding the study visit. We accept that there is a risk of recollection bias with this method. For participants using hormonal contraception, this information will not be collected.

Hirsutism: This will be assessed using the modified Ferriman-Gallwey score (Appendix). The modified Ferriman-Gallwey scoring system (Ferriman and Gallwey 1961) is a useful clinical tool, though subjective, in assessing hirsutism. Women are asked to assess the degree of their body hair using pictures of nine body areas and to give a score ranging from 0 (absence of terminal hair growth) to 4 (heavy terminal hair growth) for each area. A total score of 8 or more on the modified Ferriman-Gallwey scoring system is widely considered suggestive of hirsutism (DeUgarte et al. 2006).

Risk of OSA: The risk of OSA will be assessed as per clinical practice using the Berlin and ESS questionnaires. Although the ESS (Appendix) is commonly used, it mainly assesses sleepiness (Johns 1991). A score of more than 10 on the ESS is consistent with excessive daytime sleepiness. The Berlin questionnaire (Appendix) assesses the risk of OSA and has a sensitivity of around 85% (Netzer et al. 1999).

13.6 Quality of life

QoL will be assessed using the PCOS health-related quality of life questionnaire (PCOSQ), and the World Health Organisation QoL questionnaire (WHOQOL-BREF). We will not be using an OSA disease-specific QoL questionnaire as only a proportion of study participants will have OSA and subsequently such a questionnaire will not reflect the QoL in controls well.

The PCOSQ (Cronin et al. 1998) is a validated method of assessing QoL in women with PCOS. It is the only PCOS condition-specific measure of QoL. The PCOSQ includes 26 items (questions) to cover 5 domains which were found to be of most importance to women with PCOS: emotions (8 items),

body hair (5 items), weight problems (5 items), menstrual problems (4 items) and infertility (4 items). Each item is scored out of 7, in which a score of 7 denotes no problems or difficulties and a score of 1 indicates maximum impairment. Each domain score is calculated by dividing the total score of all items within a domain by the number of items in that domain. The PCOSQ takes 10–15 minutes to complete and lower scores suggest impaired QoL. The PCOSQ has been found to demonstrate acceptable internal consistency (0.70–0.97) and test-retest reliability (Malik-Aslam et al. 2010). A difference of 0.5 on the 1 to 7 scale has been suggested to represent the minimal important difference in the questionnaire score, i.e. the smallest change in score that women feel is important in their daily lives (Cronin et al. 1998).

The WHOQOL-BREF (Skevington and McCrate 2012) is a validated generic measure of QoL. It includes 26 questions to assess four major domains (subscales): physical, psychological, social and environment. Scores are given out of a hundred and higher scores are better; a score of more than 50% indicates an acceptable to good QoL. The WHOQOL-BREF has been shown to display high internal consistency (0.92); good test-retest reliability (0.66 – 0.72); discriminant validity (discriminating sick from healthy people), and content validity (correlation with the Short Form 36 Health Survey (SF-36)) (Skevington and McCrate 2012). WHOQOL-BREF provides a very good holistic assessment of QoL in the general population and its validation study included a group of women with PCOS, and a group of people with sleep disorders (Skevington and McCrate 2012).

13.7 Depression and anxiety

Depression and anxiety will be assessed using the Hospital Anxiety and Depression (HAD) scale. Participants on antidepressant medications will be considered as having 'clinical depression'.

The HAD scale (Zigmond and Snaith 1983) is an internationally validated (Herrmann 1997) method of assessing patients' mood. It consists of 14 items (questions), seven reflecting anxiety and seven reflecting depression. Each question answered will give the patient a four point (0 – 3) response category with the possible scores range from 0 to 21 for anxiety and 0 to 21 for depression. A score of 0 to 7 in each sub-scale is regarded as normal while a score of 11 or higher indicates probable presence of mood disorder. The HAD scale takes 3 – 5 minutes to answer and the patient is asked to choose the answer which best reflects how they felt in the last week.

13.8. Sleep studies

The diagnosis of OSA will be made using portable home-based sleep machines (Aice PDx, Philips, US). Alice PDx is a small, portable, easy to use and validated device for the diagnosis of OSA. The device is connected to a nasal cannula to measure patient airflow; pulse oximeter to measure oxygen saturation; and two belts applied around the chest and abdomen to measure chest and abdominal wall movements. The device also has an indicator which displays how many hours of good quality data were obtained. The study doctor will explain to the patient how to use the device and the patient needs to wear the device before going to bed. The analysis of the sleep study data will be performed by Dr. Asad Ali or a sleep technician. Patients with confirmed diagnosis of OSA on sleep studies will be followed up in the chest clinic as part of NHS routine care.

14. Statistical considerations

14.1 Sample size calculation

The QoL between the two groups will be compared based on the PCOSQ and the WHOQOL-BREF questionnaires.

The validation study for the WHOQOL-BREF (Skevington and McCrate 2012) included a group of people with sleep disorders and a group of healthy controls. Based on this study a total sample size of 38 participants will give us 80% power, and 5% significance (two-tailed), to detect a mean difference of 17.8 on one item of the WHOQOL-BREF (large effect size 0.98) between cases (PCOS OSA) and controls (PCOS low risk of OSA), **Table 3**. We used an allocation ratio for cases/controls of 0.67, 40% of participants in the PCOS OSA group, and 60% in the PCOS low risk OSA group. Sample size was calculated using GPower 3.1.

	Well n=141		Sleep disorders group n=45		Pooled SD	Mean difference	Effect size	n/ PCOS OSA group	n/PCOS low risk OSA group	Total sample
Item	Mean	SD	Mean		SD					
Physical Health	75.41	18.72	57.65	17.67	18.2	17.8	0.98	15	23	38

Table 3. Difference in WHOQoL-Bref scores on the physical health item between people with sleep disorders breathing and healthy controls (Skevington and McCrate 2012).

14.2 Statistical analysis

Continuous baseline characteristics for each of the PCOS OSA and PCOS low risk OSA groups will be summarised using the mean (±SD) and the median (interquartile range). If the data are approximately normally distributed, the means for the two groups will be compared using the independent t-test. If the data are not normally distributed, the two groups will be compared using the Mann-Whitney U test. Data will be checked for normality using the Shapiro-Wilk test (Razali and Wah 2011). Categorical baseline characteristics for each of the PCOS OSA and PCOS low risk OSA groups will be summarised by reporting the count and percentages in each category. The two groups will be compared using the chi-squared test.

To compare quality of life for the PCOS OSA and PCOS low risk OSA groups, we will fit linear models for each of the primary outcomes of interest (domain scores on the PCOSQ and WHOQOL-BREF questionnaires). The dependent variable in a linear model will be a primary outcome and independent (predictor) variable of interest will be whether a woman is from the PCOS OSA or PCOS low risk OSA group. As the groups may be imbalanced in other predictors for the primary outcomes, the models will adjust for potential confounders such as age, BMI, WHR, use of oral contraceptive pill, ethnicity, education level, marital status, other ongoing illnesses, and parity. Data will be assessed if they are approximately normally distributed; and if they are not, they will be transformed, for example, by taking the logarithm of the outcome variables.

Correlations will be evaluated using Pearson's coefficient (or Spearman's coefficient for non-normally distributed data). A two tailed P value of <0.05 will be considered statistically significant. Statistical analysis will performed using the SPSS statistics package (SPSS Inc., Chicago, USA).

15. Ethical Considerations

The study will be performed subject to favourable opinion from NHS Research Ethics Committee (REC), and the NHS Health Related Authority (HRA), and approvals from University Hospitals Coventry and Warwickshire NHS Trust Research and Development Department. Patients will be offered the PIS by a nurse while they are sitting in the waiting area before being seen in the PCOS clinic. The PIS and a study invitation letter will also be sent to patients who attended the PCOS clinic in the last 12 months.

We excluded women who do not speak adequate English from the study. However, almost all the women attending the PCOS clinic at University Hospital Coventry speak and understand English adequately.

We are asking study participants to fast before obtaining their written consent. However, these women will already have a confirmed diagnosis of PCOS as they will be recruited from the PCOS clinic. They will also have had enough time to read the PIS to consider whether they would like to take part in the study. When a patient calls back to express interest in the study and book a study visit, the study doctor will run through the inclusion/exclusion criteria with the patient and obtain her verbal consent on the phone to ensure she is happy to fast before attending the study visit and signing the written consent form. This will save the study participant time and effort, particularly as we are not paying for participants' transport or car parking fees.

Depending on the scores from the HAD scale, this study may result in the identification of women with PCOS who may suffer from depression or anxiety. Women with high scores on the HAD questionnaire will be advised to attend their GP for further evaluation of their mood.

Study participants who are sleepy during the daytime will be advised not to drive if feeling sleepy. Those with a confirmed diagnosis of OSA will be seen in the respiratory clinic and issues related to driving and DVLA regulations will be discussed with them by the Respiratory physician as per routine clinical care; this information will also be included in the PIS.

Women with high risk of OSA will be offered a portable sleep study machine to take home. Studies with less than 4 hours of good quality recordings will be repeated and if the quality remained poor they will be excluded from the study. However, this should not affect women's clinical care as all women with high risk of OSA will be referred to the Chest clinic.

16. Side effects

There are no serious side effects to any of the study procedures. Taking blood samples may well cause discomfort and risks of inflammation/ infection/ bruising at the needle site. The procedure will be explained to the study participant before it is done to minimise the anxiety. The blood taking will be performed by an experienced study nurse or doctor in accordance with the local guidelines.

17. Insurance

UHCW has agreed to act as sponsor for this trial and will undertake the responsibilities of sponsor as defined by the Research Governance Framework and ICH Good Clinical Practice. An authorised representative of the Sponsor has approved the final version of this protocol with respect to the trial design, conduct, data analysis and interpretation and plans for publication and dissemination of results. As sponsor, UHCW provides indemnity for this trial and, as such, will be responsible for

claims for any non-negligent harm suffered by anyone as a result of participating in this trial. The indemnity is renewed on an annual basis and will continue for the duration of this trial.

18. Funding

Dr. Hassan Kahal is an NIHR clinical lecturer in Diabetes and Endocrinology and 50% of his time is allocated to research and funded by NIHR.

19. Public and Patient Involvement

We discussed the study with seven patients attending the PCOS clinic at University Hospital Coventry. All women felt the study is important particularly as it may help women with PCOS to understand their condition better and may help explain some of their symptoms. They also felt the study may help to identify women with PCOS who suffer with OSA and may benefit from treatment. Five out of the seven women said they will be happy to take part in a similar study and one woman said she will consider it.

20. Reporting and dissemination

Results from the study will be presented in the British and/or International Endocrine Society Meetings, and we aim to publish the results of the study in high impact peer-reviewed Endocrinology journals. Study participants will be asked if they want to be informed about study results and if they do a summary of the research findings will be sent to them.

21. Annual Progress Reports

An annual progress report will be submitted to the main REC which gave the favourable opinion 12 months after the date on which the favourable opinion was given and thereafter until the end of the study.

22. Data handling and record keeping

All information that is collected about you during the course of the research will be kept strictly confidential. Paper study documents will be retained in a secure (kept locked when not in use) office located at the WISDEM centre, University Hospital Coventry, during and after the study has finished. Electronic data will be stored on University Hospitals Coventry and Warwickshire NHS Trust computers, which are password protected and user-access restricted to authorised persons in adherence to Trust information Governance Policy. The authorised persons who may have access to the study data include study researchers, regulatory authorities, and people monitoring the quality of the research at University Hospital Coventry. If data is transferred electronically, for example to analyse on a University computer, the data will be encrypted and linked anonymised, i.e. it will have participant's name, date of birth, and address removed and replaced with participant's study ID number. Only the study researchers will be able to link the study ID and participant's personal details. Data will be collected and retained in accordance with the Data Protection Act 1998. Data (paper and electronic) will be held for a total of 25 years.

23. Monitoring and Auditing

The study will be monitored by the Research, Development and Innovation Department at UHCW as representatives of the Sponsor, to ensure that the study is being conducted as per protocol, adhering to Research Governance and GCP. The approach to, and extent of, monitoring will be

specified in a study monitoring plan determined by the risk assessment undertaken prior to the start of the study.

24. End of study

End of the study is the date of the visit of the last study participant. An end of study declaration form will be sent to the main REC and sponsor within 90 days of the end of the study or within 15 days if the study is terminated early.

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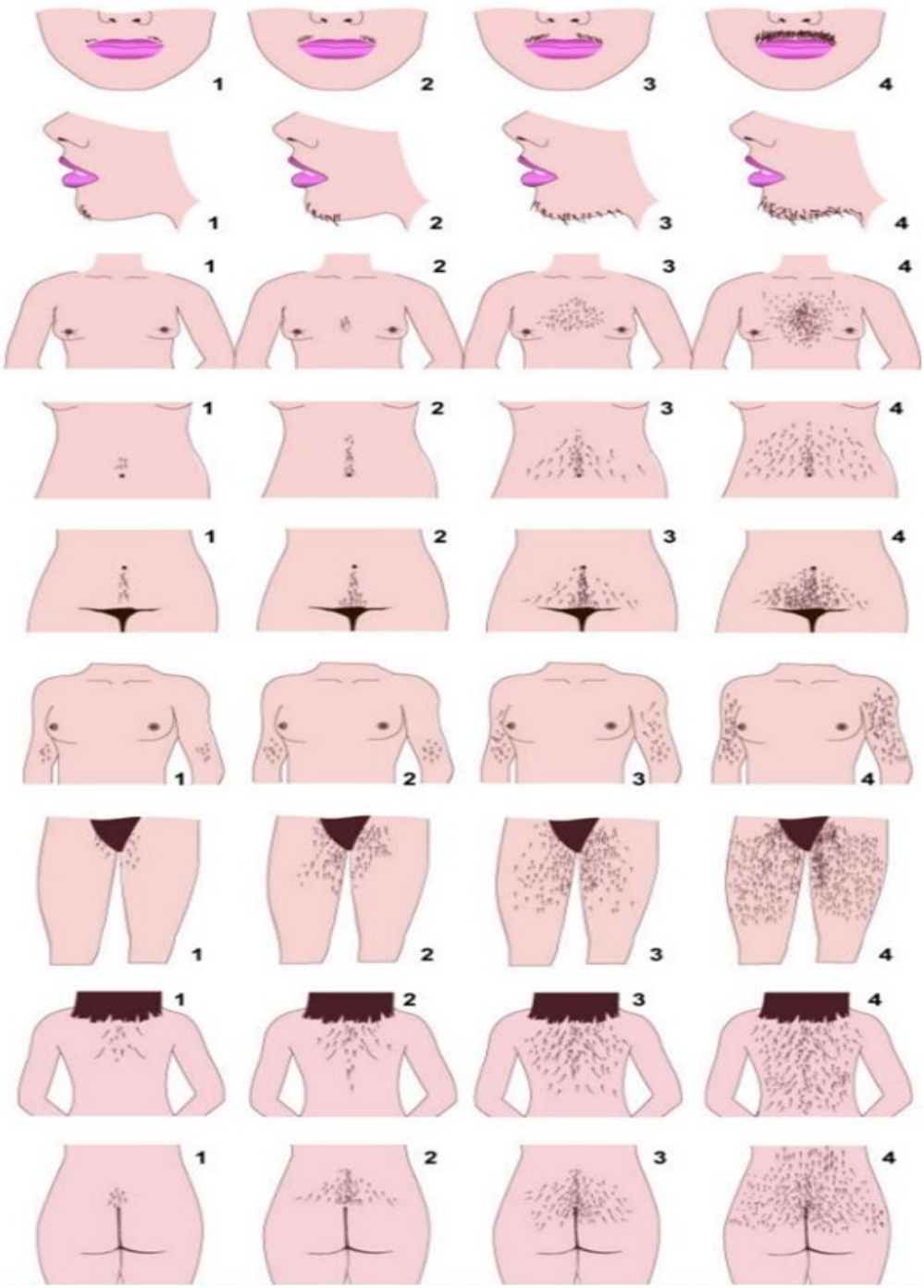
Appendix:

1. Modified Ferriman-Gallwey Score.

2. Epworth Sleepiness Scale (ESS).

3. Berlin Questionnaire.

1. Modified Ferriman-Gallwey Score



Each of the nine body areas is rated from 0 (absence of terminal hairs) to 4 (extensive terminal hair growth) and the numbers in each area are added to obtain the total score. A total score ≥ 8 generally defines hirsutism (Ferriman and Gallwey 1961; DeUgarte et al. 2006).

2. Epworth Sleepiness Scale (ESS)

Epworth Sleepiness Scale

Name: _____ Today's date: _____

Your age (Yrs): _____ Your sex (Male = M, Female = F): _____

How likely are you to doze off or fall asleep in the following situations, in contrast to feeling just tired?

This refers to your usual way of life in recent times.

Even if you haven't done some of these things recently try to work out how they would have affected you.

Use the following scale to choose the **most appropriate number** for each situation:

0 = would never doze
1 = slight chance of dozing
2 = moderate chance of dozing
3 = high chance of dozing

It is important that you answer each question as best you can.

Situation	Chance of dozing (0-3)
Sitting and reading	
Watching TV	
Sitting, inactive in a public place (e.g. a theatre or a meeting)	
As a passenger in a car for an hour without a break	
Lying down to rest in the afternoon when circumstances permit	
Sitting and talking to someone	
Sitting quietly after a lunch without alcohol	
In a car, while stopped for a few minutes in the traffic	

A score more than 10 on the ESS suggests an increased risk of OSA (Johns 1991; Greenstone and Hack 2014).

3. Berlin Questionnaire

Berlin Questionnaire©
Sleep Apnea

Height (m) _____ Weight (kg) _____ Age _____ Male / Female

Please choose the correct response to each question.

Category 1

1. Do you snore?
□ a. Yes
□ b. No
□ c. Don't know

If you answered 'yes':

2. You snoring is:
□ a. Slightly louder than breathing
□ b. As loud as talking
□ c. Louder than talking
□ d. Very loud. Could be heard in adjacent rooms.

3. How often do you snore?
□ a. Almost every day
□ b. 3-4 times per week
□ c. 1-2 times per week
□ d. 1-2 times per month
□ e. Rarely or never

4. Has your snoring ever bothered other people?
□ a. Yes
□ b. No
□ c. Don't know

5. Has anyone noticed that you stop breathing during your sleep?
□ a. Almost every day
□ b. 3-4 times per week
□ c. 1-2 times per week
□ d. 1-2 times per month
□□ e. Rarely or never

Category 2

6. How often do you feel tired or fatigued after your sleep?
□ a. Almost every day
□ b. 3-4 times per week
□ c. 1-2 times per week
□ d. 1-2 times per month
□ e. Rarely or never

7. During your waking time, do you feel tired, fatigued or not up to par?
□ a. Almost every day
□ b. 3-4 times per week
□ c. 1-2 times per week
□ d. 1-2 times per month
□ e. Rarely or never

8. Have you ever nodded off or fallen asleep while driving a vehicle?
□ a. Yes
□ b. No

If you answered 'yes':

9. How often does this occur?
□ a. Almost every day
□ b. 3-4 times per week
□ c. 1-2 times per week
□ d. 1-2 times per month
□ e. Rarely or never

Category 3

10. Do you have high blood pressure?
□ Yes
□ No
□ Don't know

The test is suggestive of OSA if two or more categories were positive. Category 1 is positive if 2 or more responses were positive (shaded areas). Category 2 is positive if 2 or more responses were positive. Category 3 is positive if the patient had a history of hypertension or BMI>30kg/m^2 (Netzer et al. 1999).