T.C.

YEDİTEPE UNIVERSITY

INSTITUTE OF HEALTH SCIENCES

DEPARTMENT OF PHYSIOLOGY

INVESTIGATION OF RELATIONSHIP BETWEEN CARDIOVASCULAR RISK FACTORS INCLUDING OBESITY, CIGARETTE SMOKING, DYSLIPIDEMIA AND ANDROGEN LEVELS IN POLYCYSTIC OVARY SYNDROME PATIENTS

MASTER OF PHYSIOLOGY THESIS

HAUWA AHMED SABO

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APPROVAL

This thesis has been deemed by the jury in accordance with the relevant articles of Yeditepe University Graduate Education and Examinations Regulation and has been approved by Administrative Board of Institute with decision dated ..... and numbered .....

Prof. Dr. Bayram YILMAZ
Director of Institute of Health Science

DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree except where due acknowledgment has been made in the text.

Date: 15/12/2022

Signature:

Name Surname: Hauwa Ahmed Sabo

DEDICATION

To my father, the light that disappeared from my life but is everlasting inside me.

To my mother, the reason I am what I became today.

To my siblings, your support kept me going.

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Completing this thesis was a marathon and I would not have been able to finish without the support and aid of countless people who in one way or the other contributed and extended their valuable assistance.

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LIST OF ABBREVIATIONS

A4 - androstenedione

AE-PCOS - Androgen Excess – PCOS Society

BMI – body mass index

CVD – cardiovascular diseases

DHEAS - dehydroepiandrosterone sulfate

E2 - estradiol

FAI – free androgen index

FSH – follicle stimulating hormone

fT – free testosterone

GC – granulosa cell

HDL – high density lipoprotein

IR – insulin resistance

LDL – low density lipoprotein

LH – luteinizing hormone

PCO - polycystic ovaries

PCOM - polycystic ovarian morphology

PCOS – polycystic ovarian syndrome

SHGB – sex hormone binding globulin

TC – theca cell

tT – total testosterone

WHO – World Health Organisation

ABSTRACT

Sabo, HA. (2023). "Investigation of relationship between cardiovascular risk factors including obesity, cigarette smoking and dyslipidemia and androgen levels in women with polycystic ovarian syndrome". Yeditepe University, Institute of Health Sciences, Department of Physiology, MSc thesis, İstanbul.

Polycystic ovary syndrome (PCOS) is a highly prevalent disorder, representing the single most common endocrine-metabolic disorder in reproductive-aged women. Adolescents and reproductive aged women with PCOS have an increased prevalence of cardiovascular risk factors. These include hypertension, hyperlipidemia, obesity, impaired glucose tolerance, diabetes, and metabolic syndrome. As it is well established by the diagnostic Rotterdam Criteria; hyperandrogenism and hyperandrogenemia are one of the features of PCOS. This retrospective research was conducted on 92 PCOS patients (age 15-45) who attended Yeditepe University Hospital from 2020 until 2022 to check if there is a correlation between the rising androgens; dehydroepiandrosterone sulfate (DHEAS), Androstenedione (A4), total testosterone (tT), free testosterone (fT), free androgen index (FAI) and cardiovascular risk factors. Patients were grouped based on smoking habits, (smokers and non-smokers), obesity (body mass index (BMI) < 23 normal weight and > 23 overweight), and based on both smoking and obesity into four groups; non-smoker nonobese (0), smoker nonobese (1), obese non-smoker (2) and smoker obese (3). Spearman's Rho was conducted to check for monotonic relationship low density lipoprotein (LDL), high density lipoprotein (HDL), triglycerides (TG), total cholesterol with the four androgen groups. P value of 0.05 was considered to be statistically significant and Data was analysed using SPSS version 29.00. The Mann-Whitney U test that was conducted between smokers and non-smokers showed that; fT, tT, and FAI was higher in smokers than non-smokers, however, no difference in DHEAS and A4. The Mann-Whitney U test between obese and non-obese showed that FAI was significantly higher in obese than non-obese patients. Kruskal Wallis showed no difference in fT, tT and FAI across the four groups. Post hoc analysis showed group 1 had higher fT than group 0, group 3 had higher fT than group 0. Group 1 also had higher tT than group 0. Group 1 also had higher FAI than group 0, group 3 also had higher FAI than group 0. There was no significant correlation between rising androgens and lipids.

Keywords: smoking, obesity, androgen, polycystic ovarian syndrome.

ÖZET

Sabo, HA. (2023). "Polikistik over sendromu bulunan kadınlarda obezite, sigara içiciliği ve dislipidemi gibi kardiyovasküler risk faktörleri ile androjen seviyeleri arasındaki ilişkinin incelenmesi". Yeditepe Üniversitesi, Sağlık Bilimleri Enstitüsü, Fizyoloji Anabilim Dalı, Yüksek Lisans Tezi, İstanbul.

Polikistik over sendromu (PKOS), yaygın görülen bir hastalık olmakla beraber, reprodüktif çağdaki kadınlarda en çok izlenen endokrin-metabolik hastalıktır. PKOS tanısı almış adolesanlar ve reprodüktif yaş aralığındaki kadınlar, artmış kardiyovasküler hastalık risk faktörü prevelansına sahiplerdir. Bu faktörler arasında; hipertansiyon, hiperlipidemi, obezite, bozulmuş glukoz toleransı, diyabet ve metabolik sendrom bulunmaktadır. Rotterdam Kriterleri'nce açıkça belirtilmiş olan, hiperandrojenemi ve hiperandrojenizm, PKOS'nun özelliklerinden biridir. Retrospektif olarak gerçekleştirilen bu çalışma, PKOS tanısı konulmuş olan, 2020-2022 yılları arasında, Yeditepe Üniversitesi Hastanesi'ne başvurmuş, 92 hasta (15-45 yaş aralığında) üzerinde gerçekleştirilmiş olup; artan androjen hormon seviyeleri (Dihidroepiandrosteron sülfat (DHEAS), Androstenedion (A4), total testosteron (tT), serbest Testosteron (sT), serbest androjeni indeksi (FAI)) ve kardiyovasküler hastalık risk faktörleri arasındaki korelasyonu değerlendirmektedir. Veriler, SPSS versiyon 29.00 ile analiz edilmiştir. Hastalar; sigara alışkanlığı (sigara içenler ve sigara içmeyenler), obezite (vücut kitle endeksi (VKİ) <23 normal vücut ağırlığı ve >23 yüksek vücut ağırlığı), ve hem sigara alışkanlığı hem de obeziteye göre 4 gruba ayrılmıştır; sigara içmeyen-obez olmayan (0), sigara içen-obez olmayan (1), obez-sigara içmeyen (2), sigara içen-obez (3). Sperman'ın Rho korelasyon katsayısı aracılığıyla; düşük yoğunluklu lipoprotein (LDL), yüksek yoğunluklu lipoprotein (HDL), trigliseritler (TG), total kolesterol ile 4 androjen grubu arasında monotonik ilişki varlığı araştırılmıştır. P değeri 0,05 istatistiki olarak anlamlı kabul edilmiştir. Mann-Whitney U testi, sigara içen ve sigara içmeyene gruplara uygulandığında; sT, tT ve FAI sigara içen grupta daha yüksek izlenilmiştir, ancak DHEAS ve A4 değerlerinde farklılık izlenmemiştir. Obez ve obez olmayan hastalara Mann-Whitney U testi uygulandığında, FAI, obez hastalarda daha yüksek izlenmiştir. Kruskal Wallis testi; sT, tT ve FAI açısından 4 grup arasında anlamlı fark olmadığını göstermiştir. Post hoc analizi uygulandığında; grup 1'in grup 0'a göre daha yüksek sT ve tT değerlerine sahip olduğu izlenilmiştir. Grup 3, grup 0'dan daha yüksek sT değerlerine sahip izlenilmiştir. Grup 1 ve grup 3, grup 0'a göre daha yüksek FAI değerlerine sahiptir. Artan androjenler ile lipidler arasında korelasyon izlenmemiştir.

Anahtar Kelimeler: Sigara içiciliği, obezite, androjen, polikistik over sendromu.

1. INTRODUCTION

1.1 Background of the Study

Polycystic ovary syndrome (PCOS) is a frequently encountered disease, in reproductive aged women it is the most common endocrine-metabolic disorder. (1). For PCOS four different phenotypes have been established in the literature: hyperandrogenism + oligo-anovulation + polycystic ovarian morphology (PCOM), hyperandrogenism + oligo-anovulation; hyperandrogenism + PCOM, and oligo-anovulation + PCOM. All these different phenotypes have different long term metabolic results (2). According to Rotterdam criteria, presence of at least two out of the three features is required for diagnosis of PCOS: ovulatory dysfunction, hyperandrogenism and polycystic ovaries (PCOs) (3,4).

1.1.1 Prevalence of PCOS

PCOS is a clinically heterogenic condition, patients can have insignificant symptoms such as mild acne and ovulatory dysfunction with no menstrual abnormalities, which may go undiagnosed for years, or severe hormonal and metabolic dysfunctions. Studies that compare differences between and within ethnic groups and geographical regions are also lacking. Due to these circumstances, its prevalence cannot be defined accurately.

On average PCOS prevalence of Europe was 276.4 per 100,000. Even within Europe, the estimates are markedly different across countries and regions, with the highest rates per 100,000 in the Czech Republic (460.6) and the lowest in Sweden (34.10) (5). Other Scandinavian countries, Germany, and the UK had relatively low rates as well in the epidemiologic studies (5). The rates in Central and Eastern Europe were more than three times higher than those in Western countries (5). They were comparable among Eastern countries, ranging from 406.4 in Lithuania to 443.1 in Russia (5). PCOS prevalence was lowest in Turkey and Albania in the Balkan region and central Europe, while in most of the other countries, prevalence ranged between 420 and 440 per 100,000. Between 1990 and 2016, the rates across European regions were relatively the same (5). Overall, the prevalence of PCOS using the Rotterdam criteria is as high as 15% in reproductive aged women. (3,4)

1.1.2 Risk Factors in PCOS

Cardiovascular risk factors for reproductive aged women and adolescents with PCOS are more prevalent compared to healthy patients. These risk factors include obesity, impaired glucose tolerance, diabetes, hypertension, mood disorders and metabolic syndrome (6). Studies, both cross-sectional and longitudinal, show that comorbidities such as hypertension, dyslipidemia, type 2 diabetes mellitus are more frequent in patients with PCOS (obese or non-obese), all of which increases the cardiovascular event risks. There is no defining data regarding these risks occur in all PCOS patients or only in certain subtypes (7). According to the World Health Organization (WHO), cardiovascular diseases (CVDs) are the number one cause of global mortality (8). Approximately 17.9 million people died from CVDs in 2019, representing 32% of all global deaths. Most CVDs can be prevented by addressing behavioural risk factors such as tobacco use, unhealthy diet and obesity, physical inactivity, and harmful use of alcohol. Detection of CVDs as early as possible is of utmost importance, so that management with lifestyle modifications and if necessary, medical therapies, can begin (8).

1.1.3 Statement of Research Problem

PCOS is a multi-layered problem, it results in long term cardiovascular and metabolic consequences. The classic thought of PCOS being only a reproductive disorder with hyperandrogenism, chronic anovulation and infertility has long been abandoned, as the reproductive implications of the disease are only the ‘’tip of the iceberg’’ (9). In all stages of PCOS, patients may have metabolic abnormalities such as dyslipidemia, hypertension, endothelial dysfunction, and low-grade chronic inflammation to a certain extent, all of which contribute to CVD development (9).

Smoking is another CVD risk factor, and several studies show evidence of smoking increases the hyperandrogenic state in PCOS patients. In patients with PCOS who smoke, aromatase enzyme activity, which converts testosterone into estrogen in the ovaries, is inhibited by smoking and nicotine consumption (10). As the aromatase activity is decreased, estrogen production via conversion will decrease and this will result in more androgens and contribute to the hyperandrogenic state (10). Other studies demonstrate higher androgen levels in reproductive age women who smoke (11). Androgens that are secreted from adrenal glands are also affected by smoking. DHEAS an almost purely adrenal androgen and A4 are found to be higher in smokers (12, 13, 14, 15).

Obesity by itself, significantly alters the metabolism, transport, activity, and production of androgens in both females and males. Androgens, both in male and females, interfere in the distribution of body fat. In some studies, it has been shown to be theoretically possible, that lower testosterone in men and higher fT in women serves a function in metabolic syndrome development (16). Although there are implications on the relation between CVD risk factors such as obesity, smoking, dyslipidemia and hyperandrogenaemia/hyperandrogenism of PCOS patients; current data is still not enough to come to any conclusion.

1.2 Justification of the Study

The high prevalence of PCOS and its multifaceted effects that lead to long term comorbidities, and indirectly through cardiovascular events, mortalities; necessitates further research on the relationship between obesity, cigarette smoking, dyslipidemia and hyperandrogenism.

1.3 Significance of Study

This study will elucidate relation between cardiovascular risk factors such as obesity, cigarette smoking, dyslipidemia, and differing androgen levels in women with PCOS, which will improve the understanding, screening, and interventions to prevent long term health outcomes.

1.4 Aim of the Study

To assess women with PCOS in a multidisciplinary perspective; endocrinological, gynaecological and cardiovascular perspective.

1.5 Specific Objectives

i. To determine the effects of smoking on androgen levels in women with PCOS
ii. To determine the effects of obesity on androgen levels in women with PCOS
iii. To determine the correlation between dyslipidemia and androgen levels in women with PCOS

1.6 Hypothesis

Smoking and obesity do not have significant effects on serum androgen levels and lipid profile has no significant correlation with serum androgen levels in PCOS patients.

2. LITERATURE REVIEW

2.1 Overview of PCOS

PCOS is a highly prevalent disorder, representing the single most common endocrine-metabolic disorder in reproductive-aged women (1). For the diagnosis of PCOS, a single criterion such as hyperandrogenism or PCOs is not enough. Studies on PCOS reveal it to be a multigenic disorder with epigenetic and environmental influences (15). The clinical findings can be irregular menses, hyperandrogenism findings such as acne, male type hair growth/hirsutism and obesity. Risk of type 2 diabetes mellitus and CVDs are increased in these patients (17,18).

According to Rotterdam criteria (19), presence of at least two of the three features is required for diagnosis of PCOS: Ovulatory dysfunction, hyperandrogenism and PCOs. In 2006 Androgen Excess – PCOS (AE-PCOS) Society Task Force (20) reviewed the criteria and suggested; hyperandrogenism and/or hyperandrogenaemia, oligoovulation or anovulation and exclusion of other androgen excess disorders. Another definition of PCOS was made by National Institute of Child Health and Human Development, it includes hyperandrogenism and ovulatory dysfunctions without the classic PCO morphology (21). The most severe phenotype of all the PCOS subtypes is this one, which is also included in the Rotterdam Criteria and AE-PCOS review. Differential diagnosis of disorders that cause hyperandrogenism and/or anovulation such as thyroid diseases, hyperprolactinemia, non-classic congenital adrenal hyperplasia, hypercortisolism, androgen-secreting tumour should be excluded, as such disorders have overlapping symptoms and findings. (19, 20, 21)

2.1.1 Rotterdam Criteria

Of all the definitions and diagnostic criteria that has been published, most accepted, and used diagnostic tool for PCOS is the Rotterdam Criteria. (19, 22-25). Two out of three criteria must be met in order to define PCOS; oligoovulation/anovulation, hyperandrogenism/hyperandrogenaemia and PCO (at least 12 follicles, that are 2 to 9 mm in size and/or at least a 10 mL ovarian volume in one or both ovaries) (19). Ovarian ultrasonographic findings were updated after the study of Jonard et al. (26) in 2003. In this study (26) PCOS patients were found to have more follicles that has a size between 2 to 5 mm when compared to the control group who had infertility due to tubal factor or male factor. Follicle size of 2-5 mm did not have more diagnostic power than 2-9 mm with a specificity of 99% and sensitivity of 75% and the cut-off was designated as at least

12 follicles. In the later years, AE PCOS society recommended the cut-off to be updated to at least 25 follicles (26)

2.1.2 PCOS Clinical Manifestation and Subtypes

Figure 1 represents the different clinical subtypes of PCOS and the most severe phenotype is the one with both hyperandrogenism and oligo-ovulation, with or without PCOs. Other phenotypes are ovulatory PCOS with hyperandrogenism + PCOM and non-hyperandrogenic phenotype with oligoovulation + PCOM which is the least severe type (27). According to the statement issued by AE-PCOS, the last phenotype is not considered PCOS (20).

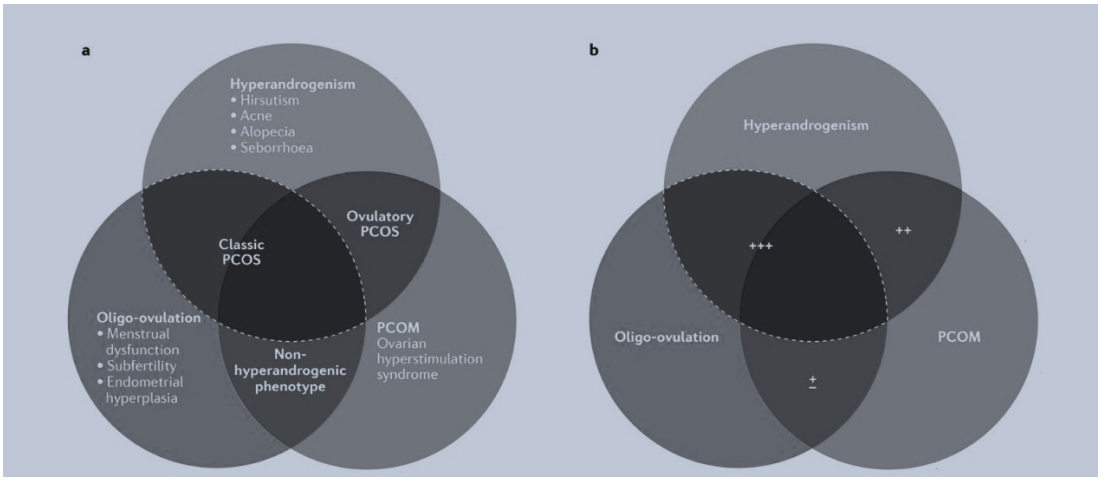

Figure 2.1: PCOS phenotypes and subtypes

PCOS has different phenotypes with intertwined clinical aspects (a) and metabolic outcomes (b). Inside the dashed portion the classic PCOS is outlined, which is the most severe form. It consists of hyperandrogenism findings, including acne, alopecia, hirsutism, and ovulatory dysfunctions including menstrual dysfunction, infertility, endometrial hyperplasia. Comorbidities in this form are more severe, including heavy insulin resistance, diabetes mellitus type 2 and cardiovascular outcomes. Second type that is defined as ovulatory PCOS consists of hyperandrogenism and PCOM. This type has less severe insulin resistance and other comorbidities. The phenotype outlined in the bottom is the non-hyperandrogenic type, with PCOM and ovulatory dysfunctions. It has the least concurrence with comorbidities. (b) ±: weak, ++: moderate, +++: strong (27).

2.1.3 Hyperandrogenism and Hyperandrogenaemia

Clinical hyperandrogenism and increase in serum androgen levels (hyperandrogenemia) are another hallmark of PCOS. It usually starts as early as peripubertal stage of development, and it continues throughout adolescence (28). Clinical hyperandrogenism consists mainly of hirsutism, acne and alopecia. Androgenic hormones have effects on the pilosebaceous unit which in turn result in these clinical findings. (29) Due to individual difference of pilosebaceous unit sensitivity to androgens, serum androgen levels and clinical findings have low correlation. (30,31)

Hirsutism, the most common clinical finding in PCOS patients, is defined by male pattern hair growth in locations on the body that are sensitive to androgen excess and is quantitatively measured by modified Ferriman-Gallwey Score (29). This scoring method uses; legs, thighs, forearms, chin, upper lip, lower abdomen, upper abdomen, chest, arms, lower back, and upper back as sites of androgen sensitive areas (32,33). On these areas a score of zero to four is given and all of them are summed up and a cut-off value of 8 and above is considered as hirsutism in most populations (28,34).

Another common characteristic of clinical hyperandrogenism is acne. Mechanism of action for this feature is stimulation of sebaceous secretions by androgens (35). It's prevalence in PCOS patients ranges greatly in between 15% and 95% and it is not clear whether there is a higher prevalence than the normal population (6% to 55%) (36-42). Unlike hirsutism, there is no method for measuring acne severity (35). It is well known that in most populations' acne is common among adolescents. The difference of isolated acne and acne of PCOS is that they are not related with menstrual disruptions or infertility (43, 44).

The last feature of hyperandrogenism is alopecia, scalp hair loss of women, which is less common than the other manifestations of hyperandrogenism (45, 46, 47). Anagenic phase of hair cycle lasts 2 to 3 years for 90% of the scalp hair. With increased androgens, hair follicles become smaller and thinner during the anagen phase, resulting in alopecia. In a study of 263 patients, 27% of them confirmed thinning of hair and no relationship was seen between hyperandrogenaemia and alopecia (48). Alopecia on milder forms might be much more common in PCOS patients, due to alopecia becoming apparent after loss of 25% of the scalp hair (49). In its most severe forms, alopecia will involve frontal and bitemporal hair (50).

For hyperandrogenaemia, four androgens are known to be related more with the hyperandrogenism of PCOS: fT, tT, A4 and DHEAS (51). These hormones belong to the

steroid hormone family, and they are mainly secreted from ovaries and adrenals (42-53).

Ovarian androgen production is explained by the well-known ‘’two cell-two gonadotropin theory’’, which includes Theca Cells (TCs), Granulosa Cells (GCs), Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) (54). LH induces androgen production inside TCs, by promoting steroidogenic enzyme genes (steroidogenic acute regulatory enzyme (StAR), CYP17A1, CYP11A1 and 3β hydroxysteroid dehydrogenase (3β-HSD)) (55-58). From TCs, androgens are moved into GCs in healthy women. In the GCs androgens are converted into estrogen by P450arom (59). Whereas in PCOS patients, follicles are under androgenic effects, which reduces the P450arom expression (60). Thus, androgen levels increase due to less conversion into estrogen (61).

In the adrenal glands, zona reticularis and zona fasciculate portions are the main areas where the DHEA, DHEAS and A4 are produced (62). In a previous study, hyperandrogenism was explained by excess adrenal androgen secretion causing negative feedback on the hypothalamo-pituitary-ovarian axis, affecting the Gonadotropin Releasing Hormone (GnRH) rhythm, resulting in LH increase and thus finally increase in ovarian androgen production, leading to the hyperandrogenic state (63). Another suggestion on the hyperandrogenism of PCOS is the unusual response to Adrenocorticotropic Hormone (ACTH) stimulation and abnormal processing of adrenal products (64).

Serum fT is the most sensitive for hyperandrogenism out of all four (65, 66). Some data suggests serum A4 levels increase in less than 20% of PCOS patients (66). DHEAS, an almost exclusively adrenal androgen, is considered as the marker of increased adrenal androgens (67, 68, 69). This androgen is usually increased in more than 50% of PCOS patients (70). DHEAS and A4 do not show their androgenic effects directly, in fact, they are converted to testosterone before they show any androgenic activity (29).

2.1.4 Ovarian Morphology in PCOS

As it is stated in its name, the polycystic morphology is one of the three main features that could accompany the patients with PCOS. Ovaries are filled with small follicles and are increased in size and stromal volume. Characteristically follicles are peripherally placed and enlarged stroma is in the center (71). With increasing local androgen levels, aromatase activity on GCs is disrupted, which in turn results in follicular maturation to be suspended. While growth of the follicles is stunted, new follicles

continue to arise. This is due to GCs still being sensitive to the effects of FSH and growth factors (72-75). As the follicles arise, but not able to continue growing, ovary is filled with multiple 2-10 mm sized follicles. These follicular cysts are surrounded by hyperplastic TCs. In time, atresia of these undeveloped follicular cysts results in ovarian stromal tissue to grow and through this process, androgen production is increased even more (76). The PCO is larger than 10 mL^3 in volume and their sizes vary between 2-10 mm. Number of follicles that should be in each ovary is up to debate; while the Rotterdam Criteria states the number to be at least 12, the AE – PCOS Society suggests the number to be increased up to 25 (77, 78, 79). Although polycystic morphology is one of the defining criteria of the PCOS, the fact that polycystic morphology can be seen in 33% of women with normal menstruation and in normal pubertal development (80-85).

2.1.5 Anovulation

A woman having menstruation in regular intervals generally suggests, but does not prove, ovulation in the ovaries (86). The time between one menstrual period to another should not be less than 21 days or more than 35 days. Anything different than these ranges suggests ovulatory dysfunction (87). Approximately 60 to 85% of patients with PCOS have menstrual irregularities which are usually exhibited as oligomenorrhea (>35 days) or amenorrhea (no menstruation), and rarely polymenorrhea (<21 days, seen in less than %2) (88-91). When encountered, chronic anovulation is not easily tied to a specific clinical situation, but rather needs laboratory testing to ascertain a diagnosis. Although for majority of people who have anovulation also have menstrual irregularities, contrary to expectations, women with both hyperandrogenism and anovulation are seen to have more regular cycles than anovulatory patients without hyperandrogenism (92). According to WHO anovulation is characterised into 3 groups; 1) hypogonadotropic hypogonadism, in which there is a failure or suppression of hypothalamus or pituitary gland 2) normogonadotropic normogonadic ovarian dysfunction (PCOS is in this group), 3) hypergonadotropic hypogonadism, which reflects ovarian failure (93).

Anovulation is not only a concern for women with infertility. Women without any desire of conception are affected by it as well. Depending on the cause, these women, due to the unopposed estrogen might have a higher risk of endometrial hyperplasia and cancer. For others there may be a hypoestrogenic environment due to chronic anovulation, which may in turn result in lower bone mass and fractures at an early age. Many of these women

has insulin resistance (IR) as a mechanism of action in their anovulatory state and if prolonged it may lead to type 2 diabetes mellitus and CVDs (94).

2.1.6 Insulin Resistance

IR is as the name itself suggests when insulin in the body has decreased activity in the target tissues (95, 96). Although it is not always the case, increased fasting serum insulin levels may act as a proof of IR. Regardless of women's weight, IR is common in PCOS patients, it is seen as frequently as 50 to 75% (97, 98, 99). If a distinction by weight is applied, up to 80% of obese PCOS patients and 25% of lean PCOS patients have IR (100). There is a 35% decrease in sensitivity to insulin in PCOS patients. (101,102,103). Also impaired glucose tolerance in PCOS patients can be as high as 35% and up to 10% of this patient group has type 2 diabetes, when checked with a 75-gram oral glucose tolerance test (OGTT) (104,105,106). Different ways to establish a diagnosis for IR has been suggested; through laboratory findings; a fasting glucose/insulin ratio <4.5, homeostatic model assessment of IR (HOMA-IR) value over 3.2-3.9 may indicate IR (20, 107).

Increase of insulin itself effects the hyperandrogenism in PCOS patients. This is done by two different ways. One is through insulin directly stimulating ovarian TCs (108). These cells are also shown to be extra sensitive to insulin in PCOS patients; even in physiologic levels of insulin is enough to cause androgen synthesis in TCs of these patients. This is not possible in normal patients without PCOS, unless there is an increased level of insulin (109, 110). Insulin also acts with LH and increases its activity to increase androgen production (111). The second way of insulin inducing hyperandrogenism is through inhibition of sex hormone binding globulin (SHBG) production (112, 113). Less SHBG means, more free androgens in the bloodstream and thus another pathway for hyperandrogenism occurs.

2.1.7 Dyslipidemia

PCOS patients are prone to certain metabolic conditions, and perhaps the most common one of them is dyslipidemia, which can be seen in up to 70% of these patients (114). It should also be noted that PCOS patients may have totally normal lipidemic indices (115-120). As it is commonly seen in patients with IR; decrease in high-density lipoproteins (HDL), increase in triglycerides and even low-density lipoprotein (LDL) levels are also seen in majority of PCOS patients (121,122)

2.2 Effects of Smoking on Androgens in PCOS Patients

Any type of tobacco and nicotine consumption has effects on the endocrine system. Thus, the hypothalamic-pituitary-ovarian (HPO) axis is no different. Figure 2.2 represents the results of smoking in the HPO axis and other end organs.

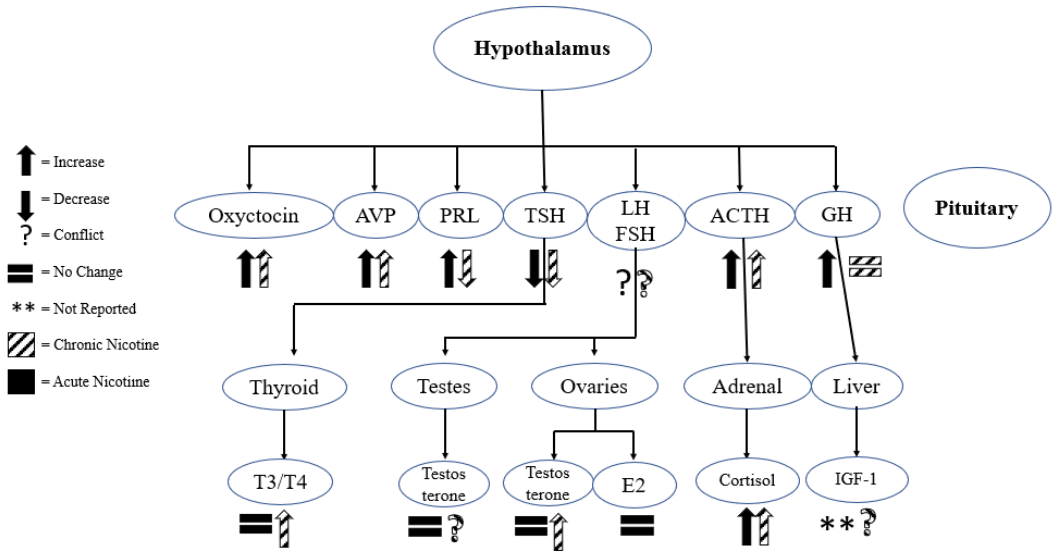

Figure 2.2: Effect of smoking on hypothalamus, pituitary gland, thyroid, testes, ovaries, adrenal gland and liver:

(a) On the hypothalamic level and other brain areas, nicotine changes vasopressin (AVP) and cortisol. (b) Nicotine effects thyroxine (T4) and triiodothyronine (T3) by direct activation of thyroid gland. Arrows and equal signs that are in black imply acute administration of nicotine, which the data is mainly gathered through nicotine studies. Red signs imply chronic exposure; data is gathered from smokers. Action on other hormones are usually on pituitary level. It should be underlined that nicotine's effects on hormones are not perfectly clear, most of the data are gathered through serum hormone level measurements. (Oxytocin and AVP are in posterior pituitary, whereas the other are from anterior pituitary) (123).

Evidence regarding smoking's effects upon androgens are contradictory. Longscope and Johnston's research on smokers and blood androgen levels shows; higher A4 levels in smokers but unchanged testosterone, estrone (E1), estradiol (E2) levels between the smokers and non-smokers (124). They found no difference in androgen and estrogen production or their metabolism in pre and postmenopausal women (124). In the study by Thomas et al. (1993), data presented no difference in length of individual

menstrual cycle phases, gonadotropin production, E2 and progesterone production, E2 metabolism and androgen production in smokers (125).

However, in other studies on smokers and people who ceased smoking imply smoking does indeed increase androgens in all ages (126, 127). Another study on young female smokers, who are either; currently smoking, smoked during adolescence and their relationship with testosterone, carbon monoxide levels and age of onset of puberty. The results showed positive correlation with testosterone levels and number of cigarettes smoked in last 30 days (128). In the study by Manjer et al. (2005) as the number of daily cigarettes smoked increases in female smokers, testosterone concentration increases (128). Whereas E.J. Thomas et al. (129) showed no increase in gonadotropins, E2, progesterone or androgens with smoking. Lastly, research by Cochran et al. (130), compared plasma levels of DHEAS, A4, E2 and progesterone between smokers and non-smokers. Smokers had significantly higher DHEAS, A4 and androgen/estrogen ratio. Progesterone levels were lower in this study.

2.3 Effects of BMI on Androgen Levels in PCOS Patients

Obesity effects the endocrine systems on multiple levels. Hormone concentrations in blood, as well as their secretion, metabolism, transport and action on the end organs (131, 132). Androgens, being hormones themselves, are no different in terms of relation with obesity. With obesity sex hormone metabolism and secretion changes (133). Androgens regulate the distribution of body fat depending on the sex and with obesity it becomes more apparent (134) Increase of androgens in both males and females paves the way for endocrine disorders in both sexes (131, 133). In both sexes infertility risk increases with dysregulated sex hormones (135). Lastly both obesity itself and androgenic imbalance causes the patients to be more susceptible to type 2 diabetes and CVDs (136). With increasing body weight testosterone concentrations drop in obese men, whereas in women a functional hyperandrogenism is seen, as the fat accumulates in the abdominal region (137).

2.4 Relation Between Lipid Levels and Androgen Levels in PCOS Patients

Data collected from different studies show that androgens influence lipid metabolism (138). In men, if the testosterone levels are suppressed, HDL levels were found to increase. Both in men and women, when androgens are administered, HDL

levels drop (139, 140, 141). Men usually have higher cholesterol and TG levels than women, excluding HDL, which is generally lower in men (142).

In hyperandrogenic women with hirsutism, increased fasting androgens and insulin were found in relation with lipoprotein irregularities (138). In other studies, there is evidence of high levels of triglycerides and low HDL in hyperandrogenic women, as the most encountered endocrine comorbidity (143).

Androgen levels and lipoproteins has been found to be negatively correlated in a study on pubertal boys. As the boys reach puberty, their serum testosterone levels had risen, while their HDL levels decreased (144). No significant correlation in LDL was found, as they did not change with puberty. However, in girls, as they reach age of puberty, HDL stays the same or minimally rises, which was positively correlated with increased E2 (145). In postmenopausal women the circumstances change, as the E2 decreases in circulation, HDL minimally decreases, while the LDL rise significantly (146). Data is still conflicting between studies, as other studies suggest lipoprotein levels and hormone levels are found to be weakly correlated. In one study all types of lipids versus androgens and estrogens were analyzed. Correlation was weak to almost none, while BMI was highly correlated with the androgens (147). Another research on serum testosterone and lipids showed negative correlation between testosterone and HDL in middle aged women and men (148). It should not be forgotten that the studies being cross-sectional, difficult to normalize the results (due to difference in hormone measurement techniques), under internal and outside features/effects of individual patients (lifestyle, diet, exercises/activities) make the analyses discrepant (148).

3. MATERIALS AND METHODS

3.1 Study Design, Sample Size and Selection

This was a cross-sectional study on women diagnosed with PCOS based on the Rotterdam criteria. The research was conducted in Yeditepe University Hospital, Koşuyolu. Data was extracted from hospital records of patients between 2020 to 2022. All the patients between this timeline were added to this research. The exclusion criteria were women with cushions syndrome, history of thyroid dysfunction or medication, surgery. Women who have been receiving hormone therapy including oral contraceptives, including steroids within 6 months of their first visit to the hospital. 21-hydroxylase non-classical adrenal hyperplasia patients were excluded. Patients with laboratory tests done at laboratories that are not Yeditepe hospital laboratories. 92 patients who fit this criterion consented to participate in the research.

3.2 Methods

Only laboratory test results done at Yeditepe University Laboratories were used for this research to ensure that hormone measuring techniques was the same. The progesterone level blood test was done on 21st day of the cycle. Blood samples were collected on the second or third day of menstrual cycle to analyze the androgen levels (DHEAS, A4, tT and fT), lipid profile (TG, LDL, HDL, total cholesterol). Trans-vaginal (with 8 MHz frequency) and trans-abdominal (with 2.5 MHz frequency) ultrasonography was conducted to determine the ovarian volumes, antral follicle counts and whether there is polycystic morphology.

Height and weight measurements were made to calculate the BMI. Lifestyle habits, family history of PCOS, diabetes, thyroid diseases and CVDs were assessed. The scan to determine the ovulatory volume was done with either transvaginal ultrasound sonography (8hz) or transabdominal ultrasound sonography (3hz) depending on the choice of the patient.

The following data was extracted from the hospital record of patients: BMI, Smoking habits, Total cholesterol, LDL, HDL, DHEAS, tT, fT, Sex hormone binding globulin, Menstrual cyclicity, Ferriman Gallwey score, PCOM

3.3 Statistical Analysis

The age was described using Mean and standard deviation. A box plot was drawn to check for outliers. The frequencies of the smoking habits and obesity was shown on tables and with a bar chart. Normality of data was checked using Kolmogorov Smirnov test and Shapiro wilks test.

3.3.1 Smoking Habits and Androgen Levels

Patients were grouped into two groups based on smoking habits.

Group 1: smokers

Group 2: non-smokers

The mean ranks of the four androgens; DHEAS, A4, tT, fT for the two groups were compared by Mann Whitney U test.

3.3.2 Obesity and Androgen Levels

Patients were grouped into two based on BMI. The groups were as follows.

Group 1: patients with BMI from 23 and above

Group 2: Patients with BMI below 23

The mean ranks of the four androgens; DHEAS, A4, tT, fT for the two groups were compared by Mann Whitney U test.

3.3.3 Smoking, Obesity, and Androgen Levels

Patients were grouped based on both smoking and BMI into four groups; non-smoker nonobese (0), smoker nonobese (1), obese non-smoker (2) and smoker obese (3) and the difference in the four androgens across the four groups was compared using Kruskal Wallis test.

3.3.4 Lipid Levels and Androgen Levels

Two correlation analyses were conducted using Spearman's Rho to check for monotonic relationship between LDL, HDL, triglycerides (TG), total cholesterol with the four androgen groups.

P value < 0.05 was statistically significant.

4. RESULTS

4.1 Descriptive Analysis

A total of 92 patients with mean age of 26.13±5.867 (table 4.1) participated in the research. Figure 4.1 shows the age distribution using a box plot. (table 4.3 and fig.4.3). The mean androgens which include fT, tT, DHEAS, A4 is 2.27 ± 1.87, 1.45 ± 0.57, 290.89 ± 111.31, 2.04 ± 1.22 respectively and the mean FAI is 3.88 ± 3.10. The mean HDL, LDL, triglycerides, and total cholesterol is 60.49 ± 13.09, 104.60 ± 34.86, 90.67 ± 56.35 and 182.77 ± 38.74 respectively.). The mean HDL is 60.49± 13.09, the mean LDL is 104.60± 34.86, triglycerides 90.67±56.35 and cholesterol 182.77±38.74.

Table 4.1: Descriptive statistics for age distribution.

Age					
	N	Minimum	Maximum	Mean	Std. Deviation
Age	92	15	40	26.13	5.867
Valid N (Listwise)	92				

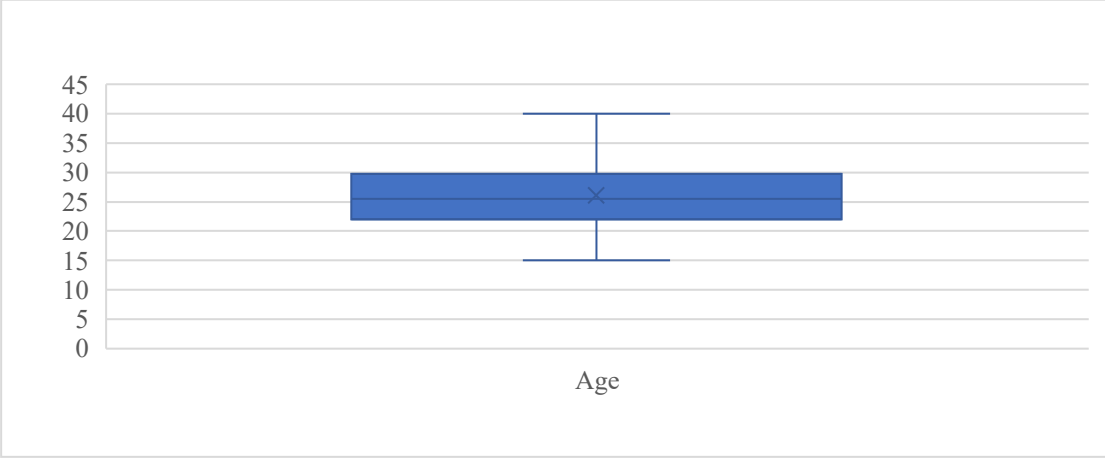

Figure 4.1: Box plot showing the age distribution.

The patients were grouped based on their smoking habits and 37 of them were smokers and 55 were non-smokers (table 4.2 and fig. 4.2).

Table 4.2: Frequency table for smokers and non-smokers.

		Frequency	Percent	Cumulative Percent
Valid	Non-smoker	55	59.8	59.8
	Smoker	37	40.2	100.0
	Total	92	100.0	

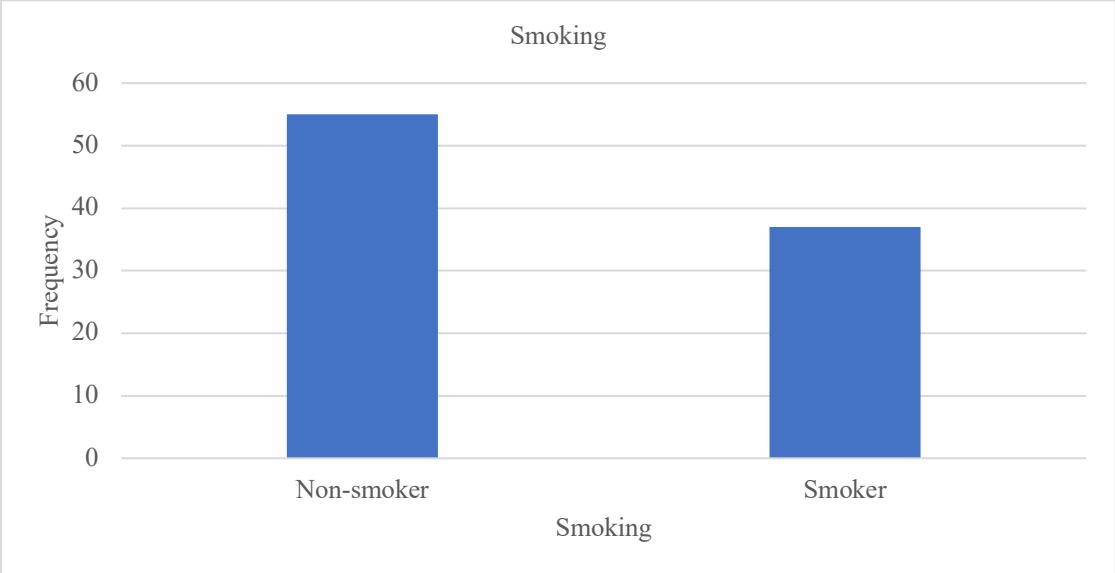

Figure 4.2: Bar chart showing the smoking habits of the sample.

The patients were grouped based on their body mass index and 30 were obese (BMI ≥ 23) and 62 were non-obese (BMI<23).

Table 4.3: Frequency table for obesity. Obese BMI ≥ 23 non obese BMI <23.

		Frequency	Percent	Cumulative Percent
Valid	Non-obese	62	67.4	67.4
	Obese	30	32.6	100.0
	Total	92	100.0	

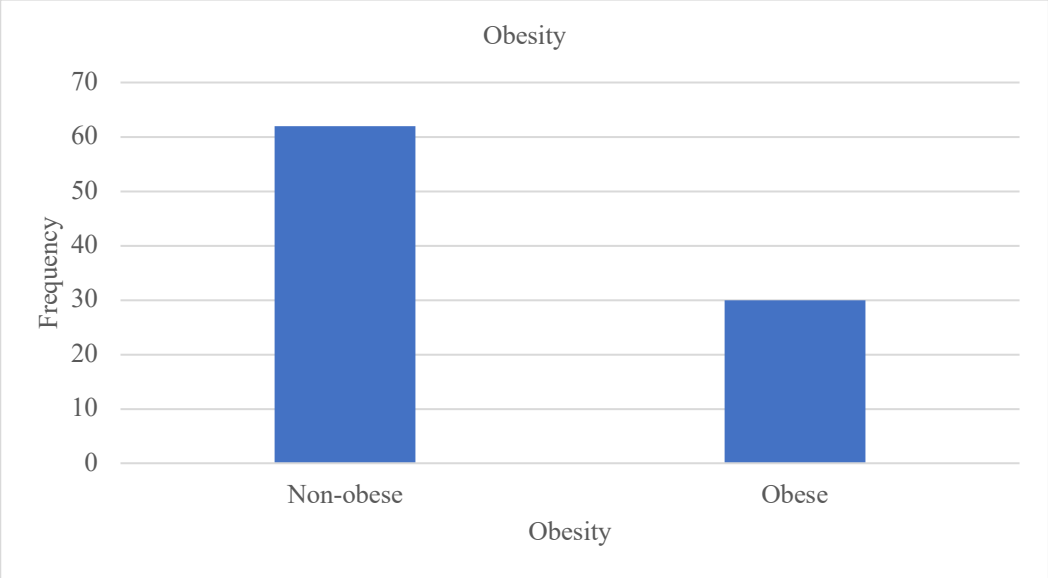

Figure 4.3: Bar chart showing the frequency of obesity in the sample.

The grouping based on smoking and obesity 44 were non-smokers and non-obese, 18 were smokers and non-obese, 11 were non-smokers and obese while 19 were smokers and obese (table 4.4 and fig 4.4).

Table 4.4: Grouping based on smoking and obesity.

		Frequency	Percent	Cumulative Percent
Valid	Non-smoker & Non-obese	44	47.8	47.8
	Smoker & Non-obese	18	19.6	67.4
	Non-smoker & Obese	11	12.0	79.3
	Smoker & Obese	19	20.7	100.0
	Total	92	100.0	

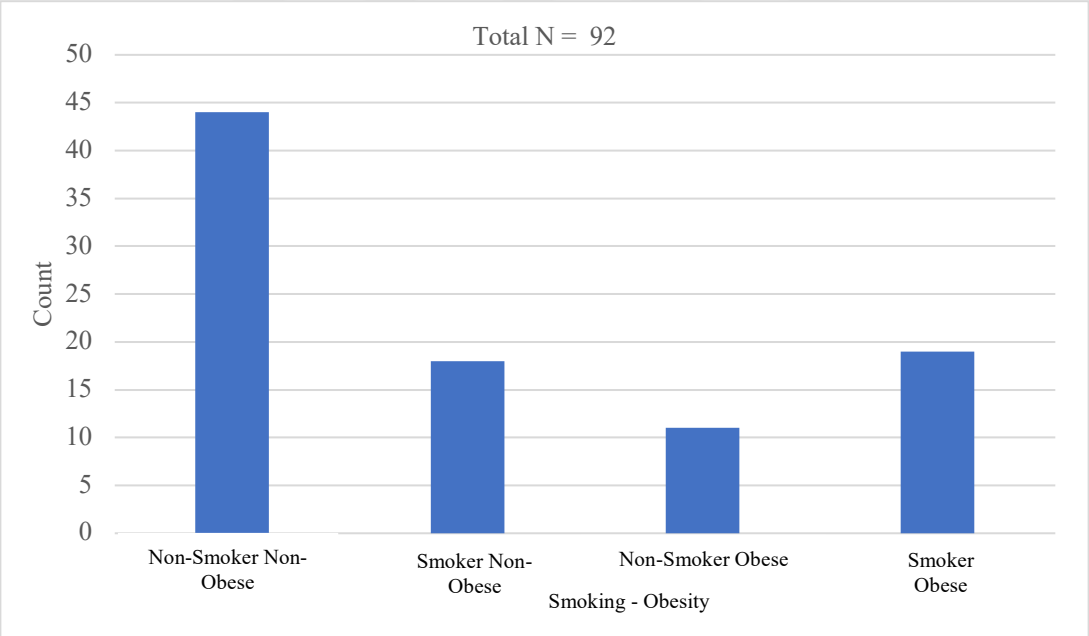

Figure 4.4: Frequency distribution based on smoking and obesity.

4.2 Smoking Habits and Androgen Levels

The Mann-whitney U test between smokers and non-smokers showed that fT, tT, and FAI was higher in smokers than non-smokers with P-value 0.001, 0.028 and <0.001 respectively (table 4.5). Figures 4.5, 4.6, 4.7, 4.8, and 4.9 show the mean ranks of the fT, DHEAS, tT, A4 and FAI respectively between smokers and non-smokers.

Table 4.5: Mann-Whitney U test; between smoker and non-smokers. Statistically significant p<0.05.

	Smoker	Non-smoker	P-value
Free testosterone	58.11	38.69	<0.001
DHEAS	47.92	45.55	0.676
Total Testosterone	53.97	41.47	0.028
Androstenedione	50.46	43.84	0.243
FAI	61.32	36.53	<0.001

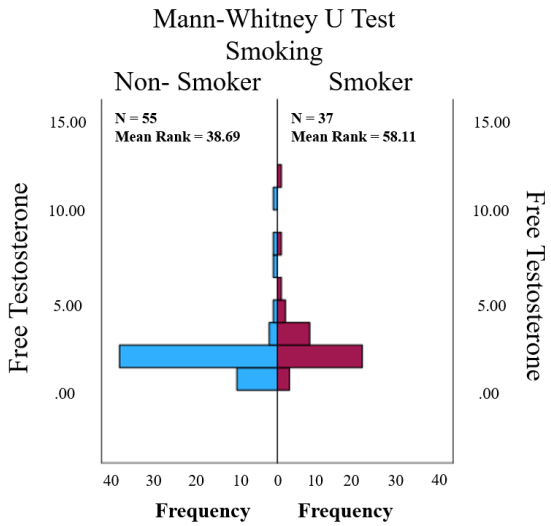

Figure 4.5: Free testosterone mean rank in smokers and non-smokers.

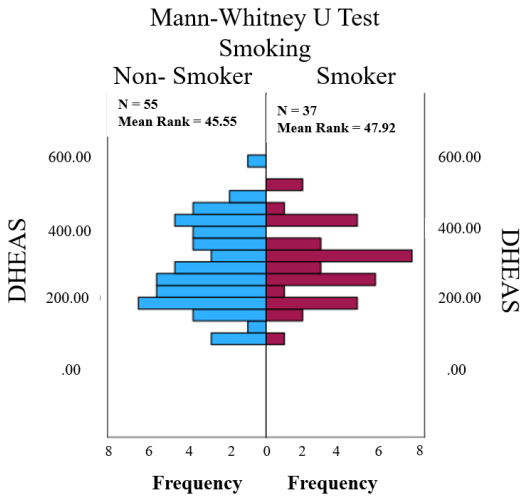

Figure 4.6: DHEAS mean ranks in smokers and non-smokes.

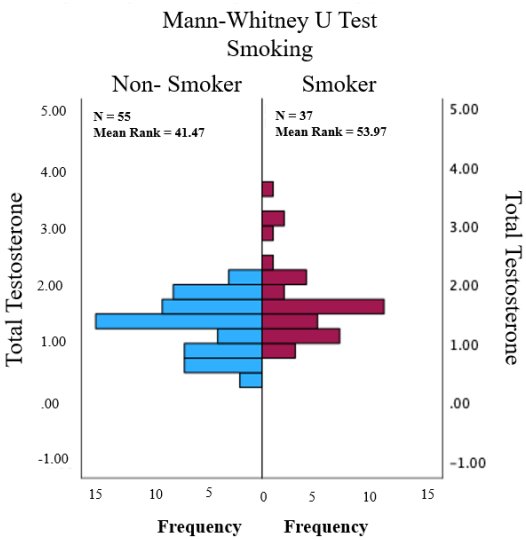

Figure 4.7: Total Testosterone mean ranks in smokers and non-smokers.

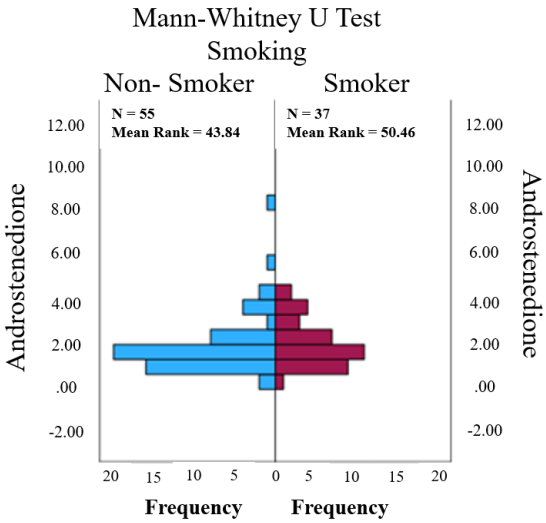

Figure 4.8: Androstenedione mean ranks in smokers and non-smokers.

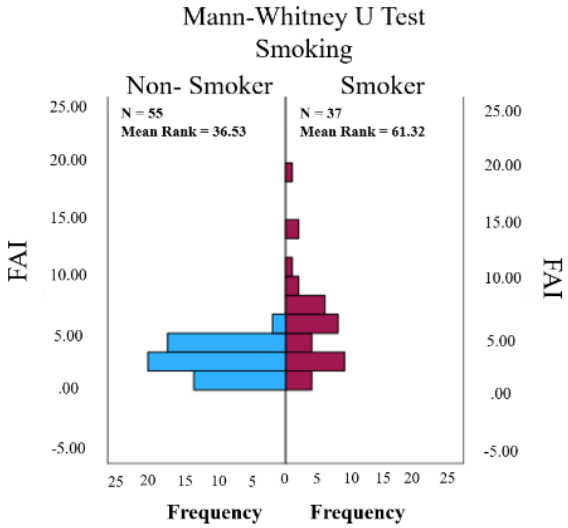

Figure 4.9: FAI mean ranks in smokers and non-smokers.

4.3 Obesity and Androgen Levels

The Mann-whitney U test between obese and non-obese showed that FAI was significantly higher in obese than non-obese patients with p-value 0.009 (table 4.6). Figures 4.10, 4.11, 4.12, 4.13, and 4.14 show the mean ranks of the fT, DHEAS, tT, A4 and FAI respectively between obese and non-obese.

Table 4.6: Mann-Whitney U test; between obese and non-obese. Statistically significant p<0.05.

	Obese	Non-obese	P-value
Free testosterone	52.98	43.36	0.105
DHEAS	48.30	45.63	0.653
Total Testosterone	48.18	45.69	0.674
Androstenedione	47.10	46.21	0.881
FAI	56.90	41.47	0.009*

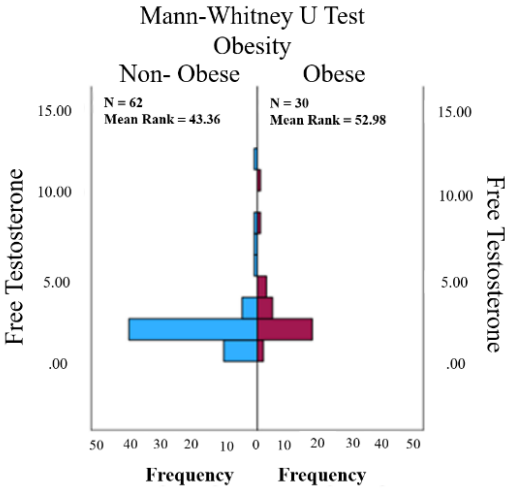

Figure 4.10: Free testosterone mean rank in obese and non-obese.

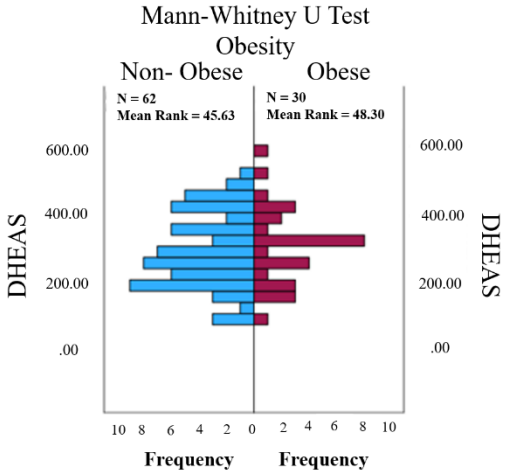

Figure 4.11: DHEAS mean rank in obese and non-obese.

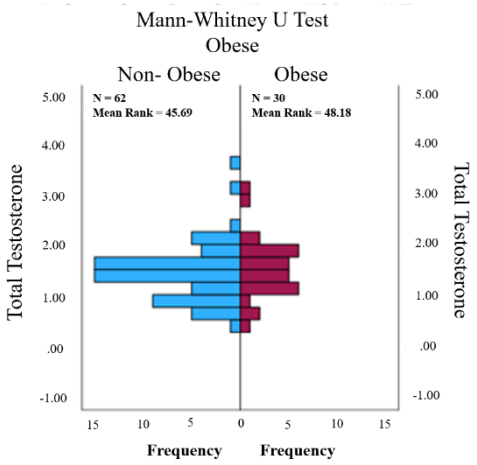

Figure 4.12: Total testosterone mean rank in obese and non-obese.

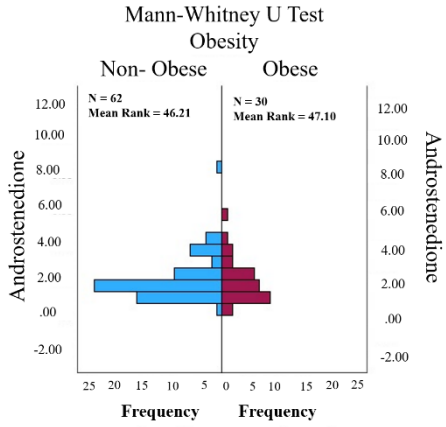

Figure 4.13: Androstenedione mean rank in obese and non-obese.

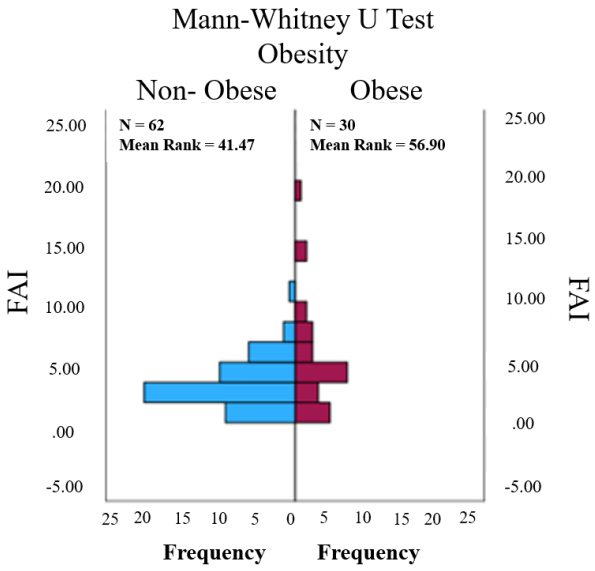

Figure 4.14: FAI mean rank in obese and non-obese.

4.4 Smoking, Obesity and Androgen Levels

Kruskal wallis test rejected the null hypothesis for equality for the fT, tT, and FAI across non-smoker nonobese (0), smoker nonobese (1), obese non-smoker (2) and smoker obese (3). Post hoc analysis was conducted, smoker non-obese had higher fT non-smoker non-obese with p value <0.001, smoker obese had higher fT than non-smoker non-obese with p value 0.011. smoker non-obese also had higher tT than non-smoker non-obese with p value 0.034. smoker non-obese also had higher FAI than non-smoker non-obese with p value 0.001, smoker obese also had higher FAI than non-smoker non-obese with p value <0.001 (table 4.7)

Table 4.7: Post hoc analysis for Kruskal Wallis Test across non-smoker nonobese (0), smoker nonobese (1), obese non-smoker (2) and smoker obese (0). * Statistically significant with p<0.05.

	0 AND 1	0 AND 2	0 AND 3
Free testosterone	<0.001*	0.101	0.011*
Total Testosterone	0.034*	0.832	0.168
FAI	0.001*	0.250	<0.001*

4.5 Lipid Levels and Androgen Levels

There was no significant correlation between the lipid levels and androgen levels.

5. DISCUSSION AND CONCLUSION

5.1 Discussion

In this study, the PCOS patients were analysed based on 2 categories: i) lifestyle (smoking habit), ii) anthropometry (BMI). The result suggests that there is significant increase in the fT, tT, and FAI in smokers, however, DHEAS and A4 were not statistically different among the two groups, however, they were slightly higher in smokers. This provides further evidence that hyperandrogenism is associated with smoking in adult females. Smoking has been believed to affect the endocrine system. Several studies show evidence of smoking increasing the hyperandrogenic state in PCOS patients (149,150). A group of other studies on healthy women also showed that smoking has significant effect on the androgen levels (151, 152). Susanne et al., found that smoking significantly increases fT and FAI in PCOS patients (149), the smokers in this study tended to have a higher BMI and reduce SHGB. In our study, the smokers had same pattern of higher BMI and lower SHGB. This automatically results in higher free testosterone and increased FAI. Also, over 60% of the obese patients were smokers. Another influence of smoking in PCOS patient is on aromatase enzyme activity, which converts testosterone into estrogen in the ovaries, is inhibited by smoking and nicotine consumption (10). As the aromatase activity is decreased, estrogen production via conversion will decrease and this will result in more androgens and contribute to the hyperandrogenic state (10). Furthermore, the association between nicotine and androgen levels may be related to the metabolic pathway involved, as nicotine metabolism is mediated primarily by cytochrome P450 (153). The cytochrome P450 family is also involved in the biosynthesis of testosterone in the liver (154). This can also explain the finding that testosterone is elevated in hyperandrogenic women who are smokers while there is no change in DHEAS. A study by Manjer et al. (128) also showed that as the number of daily cigarettes smoked increases in female smokers, testosterone concentration increases in postmenopausal women. Another study on young female smokers, who are either; currently smoking, smoked during adolescence and their relationship with testosterone, carbon monoxide levels and age of onset of puberty showed positive correlation with testosterone levels and number of cigarettes smoked in last 30 days (127). Both studies (127, 128) were based on a large sample size and potential cofounders were eliminated just the way we used exclusion criteria to limit bias. Furthermore, women exposed to smoking compared to unexposed women, had significantly higher serum tT and FAI and lower SHBG (155). The women in high-exposed group had significantly higher tT, fT,

FAI and T/E2 ratio and lower SHBG than those in non-exposed group (155). However, the evidence of smoking effects on the androgens are contradictory. Contrary to our findings, some studies have shown that smoking does not have significant effect on androgens in women (129). Thomas et al. (129) found no difference in the androgen levels of smokers and non-smokers. Although this study used the same statistical methods of Mann Whitney U test, the sample size and population characteristics varied greatly with our study. The women analysed were non-PCOS women and a small sample size was used. Other studies have also shown no significant effect of smoking on androgens (156); however, this study differed in methodology as it compared PCOS patients to control. Androgens that are secreted from adrenal glands are also affected by smoking, DHEAS, an almost purely adrenal androgen, and A4 are found to be higher in smokers (13, 14, 15), however, these studies (13,14) were on postmenopausal women not women with PCOS in their reproductive age. Also, one of the studies was on male subjects (15) not women with PCOS. Hence, the difference in the methodology and sample characteristics in these previous studies and our studies can be an explanation of the contradictory results.

The FAI which is a marker of excessively biologically active testosterone in the blood is seen to be significantly higher in obese than non-obese patients. Some findings have also shown that obesity affects the endocrine systems on multiple levels hormone concentrations in blood, as well as their secretion, metabolism, transport, and action on the end organs (21,22). Androgens, being hormones themselves, are no different in terms of relation with obesity. With obesity sex hormone metabolism and secretion changes (23). With increasing body weight in women, a functional hyperandrogenism is seen, as the fat accumulates in the abdominal region (24). However, there was no statistically significant change in the four androgens among the obese and non-obese patients although the androgens were slightly higher in the obese patients in our study. Obese women have increased androgen production associated with an enhanced metabolic clearance rate. The balance of these alterations allows the maintenance of normal circulating androgen levels (19,20). The lack of difference in androgen across the different BMI groups could be because of increase in the metabolic clearance rate. The significant increase in FAI in the obese patients shows that there is in fact an increase in the fT of the obese patients or due to significant decrease in SHBG. Obese patients in our study had a lower SHBG than the non-obese patients. This could be why the FAI was significantly higher in the obese patients. Many women with PCOS (between 38% and

88%) are overweight or obese (157,158). Another explanation is 32.6% of our sample were overweight which not even up the lower limit of percentage of obesity in PCOS patients. Even modest weight loss of 5% body weight has been shown to result in significant improvements in both symptoms of hyperandrogenism and ovulatory function in women with PCOS (159,160). Obesity per se probably also contributes to features of hyperandrogenism even in women with normal ovaries (161). These previous studies differed from our study in sample characteristics and methodology.

The androgens and FAI were compared across the nonsmokers non-obese, smokers and non-obese, nonsmokers and obese and smokers and obese. Kruskal wallis test which showed there is statistically significant difference in at least one of the groups for fT, tT, FAI, however, no statistically significant in DHEAS and A4. After posthoc analysis, the difference was observed in non-smokers non obese and smokers non obese for the fT (p=<0.001), non-smokers non obese and smokers obese (p=0.011). For tT, there was significant difference in non-smokers non obese and smokers non obese (p=0.034). With regards to FAI, there was significant difference between non-smokers non obese and smokers non obese (p=0.001), also with non-smokers non obese and smokers obese (p=<0.001) group of patients. This confirms that smoking has significant effect on androgen levels in PCOS patients, likewise obesity. However, with obesity the difference is not significant in any of the androgen levels except for the FAI. Also, with the Kruskal wallis test across the four groups with respect to smoking and obesity, the difference was seen more when non-smokers were compared to smokers.

Our result suggests that each of the lipid's LDL, HDL, TG, total cholesterol has no significant positive or negative correlation with DHEAS, A4, tT, and fT levels in PCOS patients. In a previous study, all types of lipids versus androgens and oestrogens were analyzed and the correlation was weak to almost none (147). However, results collected from different studies mostly show that androgens influence lipid metabolism. There is evidence of high levels of triglycerides and low HDL in hyperandrogenic women, as the most encountered endocrine comorbidity (143). Another study (138) has shown that androgens influence lipid metabolism, however this study was on a small sample size and on women with hyperandrogenism. Serum testosterone and lipids showed negative correlation between testosterone and HDL in middle aged women and men (148). Our research however, was conducted on confirmed PCOS patients based on Rotterdam criteria. Furthermore, it should not be forgotten that the studies being cross-

sectional, difficult to normalize the results (due to difference in hormone measurement techniques), under internal and outside features/effects of individual patients (lifestyle, diet, exercises/activities) make the analyses discrepant.

5.2 Conclusion

This paper provides the effect of smoking on androgen levels, BMI and androgen levels, and correlation of lipids and androgens aiming to provide us a better understanding of the cardiovascular risk factors in relation to PCOS. Our study concludes that smoking has significant effects on free testosterone, total testosterone and FAI and increase in BMI has significant effects on FAI. Also, there was no significant correlation between lipids and androgen levels. At the same time, it opens the need for more retrospective and prospective studies on a larger group of data to clear the contradictions of the cardiovascular risk factors and PCOS. This will help in creating preventive strategies to reduce the cardiovascular risk factors in women with PCOS. Our analysis was restricted to Turkish PCOS patients, the conclusions may not necessarily apply to other populations. However, this fact also greatly reduced the potential impacts of population stratification bias.

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YEDITEPE UNIVERSITY

No : E.83321821-805.02.03-45
Subject : Non Interventional Clinical Research Ethics Board Decision

To Prof. Dr. Bayram Yılmaz

Yeditepe University Non-Interventional Clinical Research Ethics Board has reviewed the research proposal placed upon ethical approval for the project as the research title, the researchers, the application number, the ethics board meeting details, and the provided documents have been outlined below. This research proposal has been evaluated by the members of the ethics board and full ethical **APPROVAL** has been granted.

Research Title:	**Investigation of relationship between cardiovascular risk factors including obesity, cigarette smoking, dyslipidemia, hypertension, impaired glucose tolerance and androgen levels, ovarian morphology and ovulatory state in Polycystic Ovary Syndrome patients.**
Researchers:	**Hauwa Ahmed Sabo, Prof. Dr. Bayram Yılmaz, Prof. Dr. Fahrettin Keleştemur, Prof. Dr. Erkut Attar, Dr. Umut Aziz Göksel**
Application Number:	**202206Y0262**

ETHICS BOARD MEETING DETAILS			
Meeting Date:	8th July 2022	**Meeting Place:**	Online (Google Meet)

PROVIDED DOCUMENTS
Wet signed application file, file uploaded to a CD or USB media, and electronic submission
Title of research and the names of the researchers
Letter of application
Application Form
Nature of the Research;
• Type
• Significance and distinctive value
• Aims and objectives
• Method
• Management of the research
• Widespread impact
• Pertinence of the budget and reason (if applicable)
• Timeframe and convenience
• References
Informed consent form (uniquely prepared for the research)
Letter of Undertaking-1

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A commitment to undertake the responsibility of obtaining institutions permission from where the data collection will be made
Letter of Undertaking-2 A commitment to read the latest version of the World Medical Association Declaration of Helsinki and all relevant guidelines of the Ministry of Health
Letter of Undertaking-3 A commitment on whether there are previous ethical board applications
Letter of Undertaking-4 A commitment that no action will be taken during the research that is not included in the research budget and that will bring additional burden to the volunteer or the Social Security Institution
Letter of Undertaking-5 A commitment to reading the circular on treatment approaches and scientific research in COVID-19 patients
Letter of Undertaking-6 A commitment to reading the document of Research Application Permissions that is published by Ministry of Education
'Resume Forms' filled separately by all the researchers and signed
Additional documents (Scale permissions used, if any, etc.)

Prof. Dr. Didem ÖZDEMİR ÖZENEN
Chair

Assoc. Prof. Dr. Gökhan ERTAŞ
Deputy Chair

Assoc. Prof. Dr. Elif SUNGURTEKİN EKÇİ
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Doç. Dr. Binnur OKAN BAKIR
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Personal Informations

Name	Hauwa Ahmed	**Surname**	Sabo

Education

Degree	Department	The name of the Institution Graduated From	Graduation year
University	Human Physiology	**Bayero University Kano**	2019
High school	O levels	Saint Louis Secondary School	2014

[#] All the grades must be listed if there is more than one (KPDS, ÜDS, TOEFL; EELTS vs),

Languages	Grades ([#])
English	TOEFL-105
Hausa	Native speaker

Work Experience (Sort from present to past)

Position	Institute	Duration (Year - Year)
Administrative assistant	E-health Africa	Jan - March 2021
Data collector	DFID	Oct – Dec 2020
Data collector	Malaria consortium	Jul - Sept 2020
Administrative assistant	E-health Africa	Jan - Sept 2020
Secretary/Assistant	Population service International	Jun – Dec 2019

Computer Skills

Program	Level
MS office	Excellent
SPSS	Excellent

***Excellent , good, average or basic**

Scientific works

The articles published in the journals indexed by SCI, SSCI, AHCI

Articles published in other journals

Proceedings presented in international scientific meetings and published in proceedings book.

Journals in the proceedings book of the refereed conference / symposium

Others (Projects / Certificates / Rewards)
