

Assessment of Metabolic, Cardiovascular, and Anthropometric Parameters in PCOS Women Attending Gynecology Clinic at Nnewi, Anambra state, Nigeria.

Augustine Chinedu Ihim^{*1}, Charles Chinedum Onyenekwe¹, Nkiruka Nwamaka Eze¹, Patrick Chinedu Obi², Osakue N¹, Awalu JC³, Tochukwu Anthony Ikwelle¹

¹Department of Medical Laboratory Science, Faculty of Health Sciences and Technology,

Nnamdi Azikiwe University, Awka Anambra, Nigeria.

²Department of Internal Medicine, Federal University Teaching Hospital Owerri, Imo State, Nigeria.

³ Department of Medical Laboratory Science, Evangel Akaeze Ebonyi State, Nigeria

* Corresponding Author

DOI: https://doi.org/10.51244/IJRSI.2024.1105044

Received: 30 April 2024; Revised: 09 May 2024; Accepted: 14 May 2024; Published: 11 June 2024

ABSTRACT

Polycystic ovarian syndrome (PCOS) is a common hormonal disorder in reproductive-age women, characterized by irregular periods, excess androgen levels, and ovarian cysts, often leading to infertility. While incurable, symptoms can be managed through lifestyle changes and treatments. Its exact cause is unknown, but genetic and metabolic factors are implicated. A comprehensive evaluation assessed various health markers including insulin resistance, glycemic index, lipid profile, blood pressure, BMI, and waist-tohip ratio in PCOS women at Nnewi Anambra State Nigeria's gynecology clinic, likely aiming to understand metabolic health, cardiovascular risks, and overall well-being. In this cross-sectional study conducted at a hospital, 45 participants diagnosed with PCOS were compared to 45 participants without PCOS, all aged between 18 and 50. Biochemical parameters were assessed using the enzyme-linked immunoassay technique. Data was expressed as Mean ± standard deviation. The differences in parameters studied between the PCOS group (test) and the control group were evaluated using an independent t-test. Statistical significance was set at a p-value of <0.05. Results showed that significantly lower differences exist in the mean serum levels of LDL-C (1.23±0.73) and Insulin resistance (15.13±8.13), in women with PCOS compared with the control (1.96±0.43) and (18.46±10.20)(p<0.05) respectively. A higher significant difference exists in the BMI (32.42±8.72) and WHR (0.71±0.14) of the test group (women with PCOS) compared with the control group (26.79 ± 6.35) and $(0.60\pm.178)$ respectively. The study found that women with PCOS attending the gynecology clinic at Nnamdi Azikiwe University Teaching Hospital in Nnewi, Anambra state exhibited obesity, without dyslipidemia as indicated by notably elevated mean values of the body mass index.

Keywords: PCOS, Insulin resistance, Glycemic index, Blood pressure, Lipid profile, Waist-to-hip ratio, Body mass index (BMI)

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a frequently encountered hormonal imbalance that affects 5-10% of



women in their reproductive years. It's marked by ongoing irregular menstrual cycles, including infrequent or absent periods (oligomenorrhea, amenorrhea), and it can also lead to difficulties in conceiving [1, 2]. The diagnosis of PCOS relies on meeting certain criteria laid out in the Rotterdam 2003 guidelines. To be diagnosed, a person typically needs to have at least two out of three key features: irregular or absent periods (oligo/anovulation), high levels of male hormones either shown through physical signs or blood tests (clinical and/or biochemical hyperandrogenism) and visible cysts on the ovaries seen in an ultrasound [3]. Excessive hair growth (hirsutism) is often the most noticeable among these symptoms. Other skin-related issues like acne, oily skin, and hair loss are less common but can also occur in people with PCOS. Women who have polycystic ovary syndrome (PCOS) are more likely to experience additional health issues and face long-term health risks. Specifically, they have an increased likelihood of developing conditions like type 2 diabetes, obesity, high blood pressure, abnormal cholesterol levels, and heart diseases [4, 5].

According to [6], women diagnosed with PCOS have notably higher levels of insulin in their blood compared to healthy women. They also observed that the levels of insulin in the bloodstream were linked to higher levels of male hormones (hyperandrogenism) but were not associated with irregular menstrual cycles (oligo anovulation) or the appearance of ovaries typical of PCOS (PCO morphology) [7]. conducted a study where they analyzed data from multiple studies, and they found that women with polycystic ovary syndrome (PCOS) had a much higher chance of developing endometrial cancer. The odds of developing endometrial cancer for women with PCOS were 2.79 times higher compared to those without PCOS. Insulin resistance is a common occurrence in Polycystic Ovary Syndrome, particularly in overweight women. This suggests that PCOS and obesity together worsen insulin regulation problems. When a person is obese, their body produces more insulin to manage blood sugar levels, leading to high levels of insulin in the bloodstream, known as hyperinsulinemia. This insulin resistance, along with the increased insulin levels, is directly connected to all the symptoms of PCOS, including excess male hormones (hyperandrogenism), menstrual irregularities, acne, excess body hair (hirsutism), and metabolic issues [8, 9]. It is well established that insulin resistance (IR) and compensatory hyperinsulinemia are central aetiological abnormalities in women with PCOS which lead to the overproduction of ovarian and adrenal androgens and an increase in androgen bioavailability through inhibition of sex hormone-binding globulin (SHBG) secretion [10]. The prevalence of IR in women with PCOS is estimated at 50-70% [11], or according to another source, even as high as 75% in lean and 95% in overweight women [12].

The glycemic index (GI) and glycemic load (GL) are key to understanding how carbohydrates affect our bodies. GI tells us how much a carbohydrate-rich meal raises our blood sugar levels after eating. Foods with higher GI values mean they cause blood sugar spikes more quickly. Conversely, GL considers both the GI and the amount of carbohydrates consumed, offering a more precise picture of how food affects blood sugar and insulin levels. So, while GI gives us some insight, GL provides a more comprehensive view of a food's impact on our bodies [13]. High GI and high-GL diets have been associated with several chronic conditions [13]. Eating patterns that include high glycemic index (GI) and glycemic load (GL) have been found to increase the chances of developing type 2 diabetes, cardiovascular disease, and stroke. This risk is especially higher for people who are overweight or obese [14]. Additionally, there is strong evidence suggesting that GI and GL are connected to various hormonal and non-hormonal cancers [15]. Women who have polycystic ovary syndrome (PCOS) experience higher blood pressure, problems with the inner lining of blood vessels, decreased flexibility of arteries, excess fat around the abdomen, abnormal levels of fats in the blood, ongoing low-level inflammation, elevated levels of certain substances like endothelin-1 and homocysteine [16].

MATERIALS AND METHODS

The reagents and kits for the biochemical analysis were commercially obtained and the manufacturer's standard operating procedures were strictly observed. This cross-sectional study was conducted in Nnewi



North, Anambra state, southeast of Nigeria.

Study participants.

This study lasted from January to June 2023 at the Gynecology clinic of Nnamdi Azikiwe Teaching Hospital, Nnewi, Anambra State. Nnamdi Azikiwe University Teaching Hospital is a Premier Tertiary Healthcare Institution that offers accessible and affordable health solutions, medical training, and research. It has its functional accident and emergency services and provides a wide range of medical, surgical, diagnostics, outpatient, rehabilitative, and support services. Nnewi has an estimated population of 1,113,546 people with the city covering over 1,076.9 square miles (2,789 km2) (National Population Commission, 2019. This is a cross-sectional study design in which blood samples were collected from the participants once.

Sample size.

The sample size was calculated using G*power software version 3.0.10 (Universitat Dusseldorf, Germany). Power analysis for the difference between two independent means (two groups) was conducted in G*power to determine a sufficient sample size using an alpha of 0.05, a power of 0.80, and a large effect size of 0.8. Based on these, the calculated total sample size of 42 has 80% power to detect a difference of 0.25 at a significance level of 0.05. A total number of 90 participants were recruited for this study to make room for possible attrition. This comprises 2 major groups. This hospital-based cross-sectional study had 45 participants with PCOS as a test group and 45 participants without PCOS or fibroid as the control group. Already diagnosed PCOS patients attending the Gynaecology clinic of Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State with or without fibroid between the ages of 18-50 were not recruited for this study. PCOS patients not attending the Gynaecology clinic of Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State with or without fibroid between the ages of 18-50 were not recruited for this study. Patients with other chronic diseases aside from PCOS were also excluded from this study.

Sampling technique

A random sampling technique was used for recruiting the participants as they were available until the sample size was achieved. Participants were individuals who met inclusion criteria and consented to the study after the purpose of the study had been explained to them.

They were assured of the confidentiality of the information obtained from them during and after the study and were at liberty to quit the study at any time without it affecting their treatment.

Blood Sample Collection and Processing

A tourniquet was wrapped around the arm, 3-4 inches above the collection site (the superficial vein within the elbow pit). The needle cap was removed and held in line with the vein, pulling the skin tight, and the required amount of the blood was collected by pulling the plunger of the syringe out slowly, the tourniquet was removed, cotton wool was placed on the collection site and the needle was removed and the venous blood sampling, five milliliters (5ml) volume was collected from each participant using 5.0ml sterile disposable syringe and dispensed into 5ml plain sample containers all labeled with the participant's name and age while the glucose sample was conducted using a glucometer. The blood in the plain containers was spun for 5 minutes at 3000 revolutions per minute(rpm) after allowing the blood to clot for 30 minutes and the serum was separated from the red cells using a dry clean Pasteur pipette into a dry clean plain specimen container. The sample was stored at -20 degrees Celsius, the analysis consisted of a hormonal assay technique using ELISA technique.



Collection of Samples

Five milliliters (5ml) of venous fasting blood samples were collected aseptically after 10-12 hours by venipuncture method from each subject. Two microliters (2µl) of the fasting blood sample were used for glucose estimation. The remaining blood sample was dispensed into the plain sample bottle, allowed to clot, retract, and centrifuged at 4000rpm for 5 minutes the serum was then extracted into the well-labeled plain tube, and stored in aliquots of three at 4°C until required for the determination of other biochemical parameters. The weight and height of each woman were measured using a standard beam balance scale and stadiometer respectively, while the Body mass index (BMI) was calculated as weight in kilograms divided by height squared in Meters; BMI (Kg/m²) =Weight(kg)/Height(m²). The waist-to-hip ratio was determined by measuring the waist and hip circumferences, and then dividing the waist measurement by the hip measurement. Blood pressure was measured using a stethoscope and a sphygmomanometer.

Laboratory methods

All the reagents were commercially obtained, and the manufacturer's standard operating procedures were strictly observed.

Insulin resistance (IR) was determined by enzyme-linked immunosorbent assay using ACCUBIND ELISA kits as described by [17], while the Total cholesterol, Triglycerides, HDL-C, and LDL-C were determined using enzymatic methods, as described by [18, 19, 20, 21] respectively.

STATISTICAL ANALYSIS

Statistical Package for Social Science (SPSS) (version 26.0) for Windows, SPSS Inc. Chicago, USA, was used to analyze the data. Data was expressed as Mean \pm standard deviation (SD). The differences in parameters studied between the PCOS group (test) and the control groups were evaluated using an independent t-test. Statistical significance was set at p-value < 0.05.

Table 1 Comparison of the mean values of insulin resistance, glycemic index, and lipid profile in women with PCOS and the control(mean±SD).

No significant difference was observed in the mean serum levels of TC (3.21 ± 0.90), TG (0.87 ± 0.41), HDL-C (1.39 ± 0.73), VLDLC (0.41 ± 0.21), and FBG (5.96 ± 1.49) of the test group (women with PCOS) when compared with the control (3.44 ± 0.74 , 0.811 ± 0.38 , 1.09 ± 0.21 , 0.36 ± 0.09 , and 5.80 ± 0.56)(p>0.05) respectively. However, a significantly lower difference exists in the mean serum levels of LDL-C (1.23 ± 0.73) and HOMA-IR (15.13 ± 8.13) of the test group when compared with the control (1.96 ± 0.43 , 18.46 ± 10.20)(p<0.05).

Table 1 Comparison of the mean values of insulin resistance, glycemic index, and lipid profile in women with PCOS and the control (mean \pm SD)

Parameter	Control	Test	T-test	P-value
TC (mmol/l)	3.44 ± 0.74	3.21 ± 0.90	-0.846	0.4
TG (mmol/l)	0.811 ± 0.38	0.87 ± 0.41	0.757	0.451
LDL-C (mmol/l)	1.96 ± 0.43	1.23 ± 0.73	-3.748	0.001
HDL-C (mmol/l)	1.09 ± 0.21	1.39 ± 0.73	1.94	0.056
VLDL-C (mmol/l)	0.36 ± 0.09	0.41 ± 0.21	0.709	0.181
HOMA-IR (MIU)	18.46 ± 10.20	15.13 ± 8.13	0.033	0.011



FBG (mmol/L) 5.80 ± 0.56 5.96 ± 1.49 0.001 0.0503

*Statistically significant at p<0.05.

Keys, TC=Total cholesterol, TG=Triglycerides, LDL-C=Low-density lipoprotein cholesterol

HDL-C=High-density lipoprotein cholesterol, HOMA-IR= Homeostatic Model Assessment of Insulin Resistance, FBG=Fasting blood glucose.

Table 2 Comparison of the mean values of systolic blood pressure, diastolic blood pressure, body mass index, and waist-hip ratio of women with PCOS and control (mean±SD).

No significant difference was observed in the mean levels of SBP (125.93 ± 14.75) and DBP (78.38 ± 10.15), of women with PCOS compared with the control group (women without PCOS) (p>0.05). However, there is a significantly higher difference in the mean levels of BMI (kg/m²) (32.42 ± 8.72) and WHR (0.71 ± 0.14) of the test group (women with PCOS) compared with the control group (women without PCOS).

Table 2 Comparison of values of systolic blood pressure, diastolic blood pressure, body mass index, and waist-hip ratio of women with PCOS and control (mean±SD)

Parameter	Control	Test	T-test	P-value
SBP (mmHg)	123.34 ± 14.09	125.93 ± 14.75	0.84	0.403
DBP (mmHg)	79.07 ± 11.76	78.38 ± 10.15	- 0.297	0.767
BMI (kg/m²)	26.79 ± 6.35	32.42 ± 8.72	3.495	0.001
WHR	0.60 ± 0.178	0.71 ± 0.14	3.128	0.002

*Statistically significant at p<0.05.

Keys, SBP=Systolic blood pressure, DBP= Diastolic blood pressure, BMI= body mass index

WHR= Waist hip ratio.

DISCUSSION

Polycystic ovary syndrome (PCOS), also known as hyperandrogenic anovulation (HA) or Stein–Leventhal syndrome [22] is a chronic and diverse disorder that presents with menstrual irregularities, infertility, excessive hair growth (hirsutism), acne, and obesity [23]. This condition is characterized by the presence of at least one ovary with a volume exceeding 10 mL and at least one ovary containing multiple small cysts ranging from 2 to 9 mm in diameter [24]. Diagnosis often occurs when complications arise, significantly impacting the patient's quality of life, such as hair loss, alopecia, acne, and fertility issues [25]. The pathophysiology of PCOS primarily focuses on hormonal imbalance, chronic low-grade inflammation, insulin resistance, and hyperandrogenism. These factors disrupt folliculogenesis and elevate the risk of associated conditions like endometrial cancer and type II diabetes [26].

Women who have polycystic ovary syndrome (PCOS) tend to have a combination of risk factors for cardiovascular diseases (CVD) [27]. A key aspect in the development of CVD is the advancement of atherosclerosis, with dyslipidemia playing a significant role. While LDL-C is typically the focus for reducing CVD risk, the prevalent metabolic syndrome in women with PCOS [28, 29, 30], has shifted the focus of many authors towards alterations in triglycerides and HDL-C, with lesser emphasis on other lipid



changes. The Rotterdam guidelines recommended assessing the metabolic syndrome, hinting at the necessity to measure solely HDL-C and triglycerides, with a lesser focus on other lipid parameters [31]. Recent studies conducted over the past decade have consistently shown an increase in LDL-C levels among women with PCOS [32, 33, 34]. Consequently, leading medical bodies such as the American College of Obstetricians and Gynecologists (ACOG) [35] and the Androgen Excess and PCOS Society [36] now recommend that women diagnosed with PCOS undergo a comprehensive fasting lipid and lipoprotein evaluation to assess their risk of cardiovascular complications. In this study, a significantly lower mean serum level of LDL-C was observed in the test subjects compared to the control, which contradicts a retrospective study on 700 women [37]. This variation could be a result of the difference in the number of subjects studied, compared to this present cross-sectional study. However, the findings on the LDL-C levels from this study would support a recent Korean study, which suggested that non-obese Korean women with PCOS showed no notable quantitative or qualitative alterations in their LDL cholesterol profile [38]. The relationship between PCOS in women and the risks of heart disease and hypertension remains uncertain, as highlighted by previous studies [27, 39]. However, factors such as obesity and insulin resistance are known to worsen these risks [40]. Studies have consistently shown poorer vascular health and higher blood pressure in women with PCOS compared to those without the condition [41]. In a study conducted [42], it was found that high bioavailable testosterone levels, as indicated by a free androgen index of $\geq 19\%$ in women with PCOS increased the risk of elevated blood pressure (SBP \geq 130mmHg and /or DBP \geq 85 mmHg). This suggests that high levels of male hormones in young women with PCOS are associated with increased systolic and diastolic blood pressure, regardless of age, and insulin resistance among the test group. This study showed no significant variance in the average levels of SBP and DBP in the test subjects, compared to the control, and a significantly lower mean level of insulin resistance in the test subjects, compared to the control. The result of a lower mean level of insulin resistance disputes the prospective/case-control study [43], carried out on 271 subjects, which showed insulin resistance among the PCOS subjects, and whose findings are consistent with the study of Carmina and Lobo [44]. This variation could be a result of classic PCOS in the subjects as described by [45]. Approximately 50% of women diagnosed with PCOS are found to be overweight or obese, significantly contributing to insulin resistance (IR) and potentially excessive androgen production in this population [46]. According to [47], their study revealed that women with PCOS often exhibit characteristics of obesity compared to the control group, showing higher weight, Body mass index (BMI), Waist-to-hip ratio (WHR), Body fat percentage (BFP), Trunk fat percentage (TFP), Trunk-toextremity fat ratio, Waist circumference (WC), Hip circumference (HC), total body fat mass, and trunk fat mass. The results of this present study also indicated a significantly higher mean level of BMI and WHR in the test subjects compared with the control, which supports previous findings.

CONCLUSION

The results from this study concluded that women with PCOS exhibited obesity, without dyslipidemia.

Conflicts Of Interest

The authors declare that they have no conflicts of interest.

CONTRIBUTORS

Augustine Chinedu Ihim1, Charles Chinedum Onyenekwe1, Nkiruka Nwamaka Eze1Patrick Chinedu Obi2, Osakue N1, Awalu JC4, Tochukwu Anthony Ikwelle1

ACI, CCO, and NNE conceived and designed the research proposal. ON, PCO, NNE, and ACI performed sample collection, experiments, and data analysis. CCO, ACI, AJC, NNE, and TAI contributed to the final



version of the manuscript. All authors have read and approved the final manuscript.

ACKNOWLEDGMENTS

The authors would like to pay their most profound gratitude to the management and staff of Nnamdi Azikiwe University Teaching Hospital Nnewi, and Reene Medical Diagnostic Laboratory, Awada, Anambra State, for all laboratory analyses of all biochemical parameters

DATA AVAILABILITY

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Funding:

No funding sources.

Conflict of interest:

None declared.

Ethical approval:

The study was approved by the Ethics Committee of the Nnamdi Azikiwe University, Nnewi, Anambra State, Nigeria NAUTH/CS/66/VOL.16/VER.3/2024/034

REFERENCES

- 1. Pasquali, R. and Gambineri, A., 2018. New perspectives on the definition and management of polycystic ovary syndrome. Journal of Endocrinological Investigation, 41, pp.1123-1135.
- 2. Sagvekar, P., Dadachanji, R., Patil, K. and Mukherjee, S., 2018. Pathomechanisms of polycystic ovary syndrome: multidimensional approaches. Front Biosci (Elite Ed), 10(3), pp.384-422.
- 3. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Human reproduction, 19(1), pp.41-47.
- 4. Yanes Cardozo, L.L., Romero, D.G. and Reckelhoff, J.F., 2017. Cardiometabolic features of polycystic ovary syndrome: role of androgens. Physiology, 32(5), pp.357-366.
- 5. Hardiman, P., Pillay, O.S. and Atiomo, W., 2003. Polycystic ovary syndrome and endometrial carcinoma. The Lancet, 361(9371), pp.1810-1812.
- 6. Zhang, L., Fang, X., Li, L., Liu, R., Zhang, C., Liu, H., Tan, M. and Yang, G., 2018. The association between circulating irisin levels and different phenotypes of polycystic ovary syndrome. Journal of Endocrinological Investigation, 41, pp.1401-1407.
- 7. Barry, J.A., Azizia, M.M. and Hardiman, P.J., 2014. Risk of endometrial, ovarian and breast cancer in women with polycystic ovary syndrome: a systematic review and meta-analysis. Human reproduction update, 20(5), pp.748-758.
- Atiomo, W., Khalid, S., Parameshweran, S., Houda, M., and Layfield, R., 2009. Proteomic biomarkers for the diagnosis and risk stratification of polycystic ovary syndrome: a systematic review. BJOG: An International Journal of Obstetrics & Gynaecology, 116(2), pp.137-143.
- 9. Alexiou, E., Hatziagelaki, E., Pergialiotis, V., Chrelias, C., Kassanos, D., Siristatidis, C., Kyrkou, G., Kreatsa, M. and Trakakis, E., 2017. Hyperandrogenemia in women with polycystic ovary syndrome:



prevalence, characteristics, and association with body mass index. Hormone molecular biology and clinical investigation, 29(3), pp.105-111.

- 10. Diamanti-Kandarakis, E. and Dunaif, A., 2012. Insulin resistance and the polycystic ovary syndrome revisited: an update on mechanisms and implications. Endocrine Reviews, 33(6), pp.981-1030.
- 11. Legro, R.S., Castracane, V.D. and Kauffman, R.P., 2004. Detecting insulin resistance in polycystic ovary syndrome: purposes and pitfalls. Obstetrical & gynecological survey, 59(2), pp.141-154.
- 12. Stepto, N.K., Cassar, S., Joham, A.E., Hutchison, S.K., Harrison, C.L., Goldstein, R.F. and Teede, H.J., 2013. Women with polycystic ovary syndrome have intrinsic insulin resistance on euglycaemic–hyperinsulinaemic clamp. Human reproduction, 28(3), pp.777-784.
- 13. Jayedi, A., Soltani, S., Jenkins, D., Sievenpiper, J. and Shab-Bidar, S., 2022. Dietary glycemic index, glycemic load, and chronic disease: an umbrella review of meta-analyses of prospective cohort studies. Critical reviews in food science and nutrition, 62(9), pp.2460-2469.
- 14. Hardy, D.S., Garvin, J.T. and Xu, H., 2020. Carbohydrate quality, glycemic index, glycemic load and cardiometabolic risks in the US, Europe, and Asia: A dose-response meta-analysis. Nutrition, Metabolism and Cardiovascular Diseases, 30(6), pp.853-871.
- 15. Sieri, S. and Krogh, V., 2017. Dietary glycemic index, glycemic load, and cancer: An overview of the literature. Nutrition, Metabolism and Cardiovascular Diseases, 27(1), pp.18-31.
- Patel, S.S., Truong, U., King, M., Ferland, A., Moreau, K.L., Dorosz, J., Hokanson, J.E., Wang, H., Kinney, G.L., Maahs, D.M. and Eckel, R.H., 2017. Obese adolescents with polycystic ovarian syndrome have elevated cardiovascular disease risk markers. Vascular Medicine, 22(2), pp.85-95.
- 17. Nozal, P., Garrido, S., Alba-Domínguez, M., Espinosa, L., Pena, A., de Córdoba, S.R., Sánchez-Corral, P. and López-Trascasa, M., 2014. An ELISA assay with two monoclonal antibodies allows the estimation of free factor H and identifies patients with acquired deficiency of this complement regulator. Molecular immunology, 58(2), pp.194-200.
- 18. Allain, C.C., Poon, L.S., Chan, C.S., Richmond, W.F.P.C. and Fu, P.C., 1974. Enzymatic determination of total serum cholesterol. Clinical chemistry, 20(4), pp.470-475.
- 19. Bucolo, G. and David, H., 1973. Quantitative determination of serum triglycerides by the use of enzymes. Clinical chemistry, 19(5), pp.476-482.
- 20. Bachorik, P.S., Walker, R., Brownell, K.D., Stunkard, A.J. and Kwiterovich, P.O., 1980. Determination of high-density lipoprotein-cholesterol in stored human plasma. Journal of lipid research, 21(5), pp.608-616.
- 21. Schade, D.S., Cavanaugh, B., Ramo, B. and Eaton, R.P., 2016. The application of the LDL principle. World Journal of Cardiovascular Diseases, 6(5), pp.109-125.
- 22. El Hayek, S., Bitar, L., Hamdar, L.H., Mirza, F.G. and Daoud, G., 2016. Polycystic ovarian syndrome: an updated overview. Frontiers in physiology, 7, p.124.
- 23. Motlagh Asghari, K., Nejadghaderi, S.A., Alizadeh, M., Sanaie, S., Sullman, M.J., Kolahi, A.A., Avery, J. and Safiri, S., 2022. Burden of polycystic ovary syndrome in the Middle East and North Africa region, 1990–2019. Scientific Reports, 12(1), p.7039.
- 24. Balen, A.H., Tan, S.L., MacDougall, J. and Jacobs, H.S., 1993. Miscarriage rates following in-vitro fertilization are increased in women with polycystic ovaries and reduced by pituitary desensitization with buserelin. Human Reproduction, 8(6), pp.959-964.
- 25. Azziz, R., Woods, K.S., Reyna, R., Key, T.J., Knochenhauer, E.S. and Yildiz, B.O., 2004. The prevalence and features of polycystic ovary syndrome in an unselected population. The Journal of Clinical Endocrinology & Metabolism, 89(6), pp.2745-2749.
- Singh, S., Pal, N., Shubham, S., Sarma, D.K., Verma, V., Marotta, F. and Kumar, M., 2023. Polycystic ovary syndrome: etiology, current management, and future therapeutics. Journal of Clinical Medicine, 12(4), p.1454.
- 27. Wild, R.A., 2002. Long-term health consequences of PCOS. Human reproduction update, 8(3), pp.231-241.
- 28. Apridonidze, T., Essah, P.A., Iuorno, M.J. and Nestler, J.E., 2005. Prevalence and characteristics of the metabolic syndrome in women with polycystic ovary syndrome. The Journal of Clinical

Endocrinology & Metabolism, 90(4), pp.1929-1935.

- 29. Dokras, A., Bochner, M., Hollinrake, E., Markham, S., VanVoorhis, B. and Jagasia, D.H., 2005. Screening women with polycystic ovary syndrome for metabolic syndrome. Obstetrics & Gynecology, 106(1), pp.131-137.
- 30. Moran, L.J., Misso, M.L., Wild, R.A. and Norman, R.J., 2010. Impaired glucose tolerance, type 2 diabetes and metabolic syndrome in polycystic ovary syndrome: a systematic review and metaanalysis. Human reproduction update, 16(4), pp.347-363.
- 31. Fauser, B.C., Tarlatzis, B.C., Rebar, R.W., Legro, R.S., Balen, A.H., Lobo, R., Carmina, E., Chang, J., Yildiz, B.O., Laven, J.S. and Boivin, J., 2012. Consensus on women's health aspects of polycystic ovary syndrome (PCOS): the Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. Fertility and sterility, 97(1), pp.28-38.
- 32. Wild, R.A., Alaupovic, P. and Parker, I.J., 1992. Lipid and apolipoprotein abnormalities in hirsute women: I. The association with insulin resistance. American Journal of Obstetrics and Gynecology, 166(4), pp.1191-1197.
- 33. Chekir, C., Nakatsuka, M., Kamada, Y., Noguchi, S., Sasaki, A. and Hiramatsu, Y., 2005. Impaired uterine perfusion associated with metabolic disorders in women with polycystic ovary syndrome. Acta obstetricia et gynecologica Scandinavica, 84(2), pp.189-195.
- Berneis, K., Rizzo, M., Hersberger, M., Rini, G.B., Di Fede, G., Pepe, I., Spinas, G.A. and Carmina, E., 2009. Atherogenic forms of dyslipidemia in women with polycystic ovary syndrome. International Journal of Clinical Practice, 63(1), pp.56-62.
- 35. Dokras, A., Saini, S., Gibson-Helm, M., Schulkin, J., Cooney, L. and Teede, H., 2017. Gaps in knowledge among physicians regarding diagnostic criteria and management of polycystic ovary syndrome. Fertility and sterility, 107(6), pp.1380-1386.
- 36. Wild, R.A., Carmina, E., Diamanti-Kandarakis, E., Dokras, A., Escobar-Morreale, H.F., Futterweit, W., Lobo, R., Norman, R.J., Talbott, E. and Dumesic, D.A., 2010. Assessment of cardiovascular risk and prevention of cardiovascular disease in women with the polycystic ovary syndrome: a consensus statement by the Androgen Excess and Polycystic Ovary Syndrome (AE-PCOS) Society. The Journal of Clinical Endocrinology & Metabolism, 95(5), pp.2038-2049.
- 37. Guo, F., Gong, Z., Fernando, T., Zhang, L., Zhu, X. and Shi, Y., 2022. The lipid profiles in different characteristics of women with PCOS and the interaction between dyslipidemia and metabolic disorder states: a retrospective study in Chinese population. Frontiers in Endocrinology, 13, p.892125.
- 38. Kim, J.J. and Choi, Y.M., 2013. Dyslipidemia in women with polycystic ovary syndrome. Obstetrics & gynecology science, 56(3), p.137.
- 39. Legro, R.S., 2003. Polycystic ovary syndrome and cardiovascular disease: a premature association. Endocrine Reviews, 24(3), pp.302-312.
- 40. Ehrmann, D.A., Liljenquist, D.R., Kasza, K., Azziz, R., Legro, R.S., Ghazzi, M.N. and PCOS/Troglitazone Study Group, 2006. Prevalence and predictors of the metabolic syndrome in women with polycystic ovary syndrome. The Journal of Clinical Endocrinology & Metabolism, 91(1), pp.48-53.
- 41. Paradisi, G., Steinberg, H.O., Hempfling, A., Cronin, J., Hook, G., Shepard, M.K. and Baron, A.D., 2001. Polycystic ovary syndrome is associated with endothelial dysfunction. Circulation, 103(10), pp.1410-1415.
- 42. Chen, M.J., Yang, W.S., Yang, J.H., Chen, C.L., Ho, H.N. and Yang, Y.S., 2007. Relationship between androgen levels and blood pressure in young women with polycystic ovary syndrome. Hypertension, 49(6), pp.1442-1447.
- 43. DeUgarte, C.M., Bartolucci, A.A. and Azziz, R., 2005. Prevalence of insulin resistance in the polycystic ovary syndrome using the homeostasis model assessment. Fertility and sterility, 83(5), pp.1454-1460.
- 44. Carmina, E. and Lobo, R.A., 2004. Use of fasting blood to assess the prevalence of insulin resistance in women with polycystic ovary syndrome. Fertility and sterility, 82(3), pp.661-665.
- 45. Baptiste, C.G., Battista, M.C., Trottier, A. and Baillargeon, J.P., 2010. Insulin and hyperandrogenism



in women with polycystic ovary syndrome. The Journal of Steroid Biochemistry and Molecular Biology, 122(1-3), pp.42-52.

- 46. Glueck, C.J. and Goldenberg, N., 2019. Characteristics of obesity in polycystic ovary syndrome: Etiology, treatment, and genetics. Metabolism, 92, pp.108-120.
- 47. Zhang, H., Wang, W., Zhao, J., Jiao, P., Zeng, L., Zhang, H., Zhao, Y., Shi, L., Hu, H., Luo, L. and Fukuzawa, I., 2023. Relationship between body composition, insulin resistance, and hormonal profiles in women with polycystic ovary syndrome. Frontiers in Endocrinology, 13, p.1085656.