ORIGINAL ARTICLE

COMPARISON OF FREE ANDROGEN INDEX IN POLYCYSTIC OVARY SYNDROME AND NON-POLYCYSTIC OVARY SYNDROME INFERTILE PATIENTS

Madiha Khattak[1], Robina Usman[2], Nuzhat Sultana[3], Almas Khattak[3]

[1]Department of Physiology, Khyber Medical College, Khyber Medical University, Peshawar-Pakistan
[2]Department of Physiology, Peshawar Medical College, Riphah International University, Islamabad-Pakistan
[3]Department of Pathology, Northwest School of Medicine, Khyber Medical University, Peshawar-Pakistan

Background: One of the leading causes of infertility in child bearing age females is polycystic ovary syndrome. It is characterized by altered hormonal profile causing androgen excess and insulin resistance which eventually leads to decreased ovulation rate. **Methods:** This was a cross-sectional study that included 40 polycystic ovary syndrome (PCOS) patients and 40 infertility patients that did not have polycystic ovary syndrome determined by sonography and clinical features through quota sampling technique. Serum Total Testosterone and Sex Hormone Binding Globulin Levels were assayed. Using these two parameters, Free Androgen Index was calculated. Body Mass Index and central obesity was also determined. **Results:** Total Testosterone, Free Androgen Index and Body Mass Index were raised in PCOS group as indicated by *p*-value <0.05. Hirsutism was present in PCOS group (*p*-value <0.05). Sex Hormone Binding Globulin Levels were decreased in PCOS patients (*p*-value <0.05) but were within the lower half of normal range. **Conclusion**: Levels of Sex Hormone Binding Globulin were decreased in PCOS cases and Free Androgen Index can help in better determining hyperandrogenaemia than total testosterone alone.

Keywords: Free Androgen Index (FAI); Polycystic Ovary Syndrome (PCOS); Sex Hormone Binding Globulin (SHBG); Total Testosterone (TT); Hirsutism; Infertility; Insulin Resistance; Hyperandrogenaemia

Citation: Khattak M, Usman R, Sultana N, Khattak A. Comparison of free androgen index in polycystic ovary syndrome and non-polycystic ovary syndrome infertile patients. J Ayub Med Coll Abbottabad 2021;33(4):577–81.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common cause of infertility and endocrine abnormality in women of reproductive age group. The most common criteria for diagnosing PCOS is the Rotterdam 2003 criteria according to which a patient should have two of the following three in order to be diagnosed as having polycystic ovary syndrome. 1) Oligoovulation or anovulation in the form of oligomenorrhoea or amenorrhoea, 2) Evidence of hyperandrogenaemia either clinical or biochemical and 3) polycystic ovary on ultrasound.[1] Hormonal abnormalities include raised total testosterone (TT), elevated ratio of luteinizing hormone to follicle stimulating hormone, metabolic problems like hyperinsulinemia and insulin resistance and abnormal oral glucose tolerance test (OGTT). These patients have central obesity, infertility, irregular periods and male like hair distribution. The aetiology of PCOS includes hyperinsulinemia, hyperandrogenism and genetic predisposition with factors of environment acting upon these genes. However, the exact cause of PCOS is still not known and definitive treatment is still not available. Its treatment includes correcting menstrual irregularities, decreasing weight, treating infertility, promoting insulin sensitivity and managing hyperandrogenemia.[2]

Hyperandrogenism has been thought to be the primary cause of PCOS. The hypothalamo-pituitary-ovarian axis is defective, causing increased levels of Luteinizing Hormone (LH) which in turn causes stimulation of LH receptors in ovaries which results in increase in the activity of cytochrome P450c17α. This enzyme has two functions 1) conversion of progesterone to 17α hydroxyprogesterone via 17α hydroxylase activity and 2) conversion of 17α hydroxyprogesterone via 17–20 lyase activity to androstenedione. The ovarian thecal cells convert androstenedione to testosterone via 17β reductase enzyme and is released into the blood.[3] This androstenedione is also converted to oestradiol by FSH dependent aromatase enzyme in the ovarian granulosa cells. As in PCOS, LH levels are high as compared to FSH therefore androstenedione gets accumulated in the ovary.[4] Androstenedione cause growth of the follicles in the early stages but persistence of its high levels retard the further growth of follicles into mature ones and hence ovulation does not occur and the ovary becomes polycystic in appearance.[5] As there is no ovulation so cyclic menstruation is scanty or none at all. Increased levels of androgens in blood cause hirsutism, acne and androgenic alopecia.[6]

There is much debate that insulin resistance and resulting hyperinsulinemia is the primary cause of PCOS. Majority of PCOS women are insulin resistant in part due to genetic predisposition and in part due to obesity and have deranged OGTT and BMI >25 kg/m^2.[7] Post receptor binding defect is thought to be the cause of insulin resistance. Greater than normal levels of insulin are required to give the desired effect thus resulting in hyperinsulinemia. In

insulin resistance there is decreased translocation of Glucose transporters (GLUT 4) into the cell membrane which in combination with increased glycogenolysis causes increased glucose in the blood. This increases insulin secretion by the pancreas. Insulin itself directly stimulates cytP450c17α and also augments the actions of LH on the ovarian theca cells and causes increased androgen biosynthesis.[3]

Sex hormone binding globulin (SHBG), a protein secreted by the liver, transports sex steroids such as testosterone and oestradiol in the blood.[8] Hyperinsulinemia decreases the secretion of sex hormone binding globulin and results in excess of free androgens in the blood. Despite of the presence of hirsutism some patients with PCOS have testosterone in the normal range when checked for. Therefore, there is a need to find some other marker of hyperandrogenaemia. Free Androgen Index (FAI) is also a good marker of hyperandrogenaemia. FAI is calculated by dividing total testosterone level by SHBG levels and multiplying by 100. A value >10 indicates hyperandrogenism. The FAI takes into consideration the SHBG levels as well and thus indicates the free testosterone in blood. Free testosterone is the testosterone that circulates freely in the plasma and is not bound to SHBG. Free testosterone is very difficult to check for and this testosterone is responsible for the hirsutism and other signs of hyperandrogenaemia. SHBG regulates the quantities of non–protein-bound or free sex hormones in the circulation. Low levels of serum SHBG are quite frequently present in women with PCOS and give rise to symptoms of androgen excess like hirsutism and acne.[9] Majority of the PCOS patients are overweight and obese. Obesity is also another cause of hyperandrogenaemia. There is increased peripheral conversion of fats in adipose tissue to androgens. Obesity increases insulin resistance and resulting hyperinsulinemia. Adipokines are hormone-like proteins that are secreted by adipose tissue. These are thought to augment insulin resistance in PCOS.[10]

MATERIAL AND METHODS

The study design was cross-sectional, took place at Jinnahabad Medical Center which is a private hospital in Abbottabad over a time duration of 6 months from January to June 2013. Blood samples were taken from patients attending the gynaecology clinic there. Ethical approval was granted by the Advanced Studies Research Board (ASRB) of Khyber Medical University.

Keeping the significance level at 0.05, beta at 0.20 and at the power of 80, the calculated sample size for PCOS cases was 17[11] and for control group the calculated sample size was 55[12]. However, in the time frame available to complete the research project, and to keep both the groups at equal size, we collected blood samples from 40 cases and 40 controls.

We took 80 patients out of which 40 had PCOS and 40 were non-PCOS through quota sampling technique. Rotterdam 2003 criteria was used to diagnose the cases of PCOS.[1] All patients were suffering from infertility which was defined according to WHO guidelines which state that infertility is "a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse." If a woman has never before given a live birth, then its primary infertility and secondary infertility is "the inability to get pregnant or give a live birth after previously getting pregnant and giving a live birth" (www.who.int/reproductivehealth/topics/infertility/definitions).The patients with history of diabetes, hypertension, use of hormones, smoking or drug abuse were excluded.

After written informed consent from the participants, a detailed history with special emphasis on menstrual history was obtained. A general physical examination was done. Ultrasound scan of all the participants was done by a trained lady doctor to check for the presence of polycystic ovaries. Ovary was described as polycystic if there were ten or more cysts (2–8 mm in diameter) presents peripherally, a dense stroma and enlarged ovary size > 10 cm^3.[7]

Hirsutism was checked for by the Ferriman-Gallwey score. According to the Ferriman-Gallwey score, nine areas of the body were looked for hirsutism. These included the chin, chest, upper lip, upper abdomen, lower abdomen, upper back, lower back, upper arms and thighs. Each area was given a score from 0 to 4 so a patient can have a total score ranging from 0 to 36. A patient with a score of >8 was considered to be hirsute.[13]

Body Mass Index was calculated by the following formula:

$$\text{BMI} = \frac{\text{weight in kilogram}}{(\text{height in meter})^2}$$

BMI of less than or equal to 18.4kg/m^2 was classified as underweight, BMI of 18.5–24.9 kg/m^2 was grouped as ideal weight, BMI of 25–29.9 kg/$m^2$was overweight and a BMI of 30–39.9 kg/$m^2$was considered as obese whereas a BMI of over 40 kg/$m^2$was grouped as very obese as per WHO guidelines.[14]

Waist/Hip ratio was calculated according to WHO guidelines. We measured waist circumference half way between the lower margin of the rib cage and the upper margin of the iliac crest usually a little above the umbilicus. The circumference of the hip was measured at the widest part of the buttocks and a non-stretchable tape was used each time (http://en.wikipedia.org). Waist: Hip ratio above 0.82 was considered obese.[15]

Blood samples were taken in the early follicular phase. Five cc blood was drawn from cubital vein and collected in yellow capped gel test

tubes. The sera obtained by centrifuging were collected in aliquots and stored in batches at -20 °C to be used later. The stored sera were analysed by Chemiluminescence assay for total testosterone level in the laboratory of Institute of Basic Medical Sciences (IBMS) in Peshawar. Hormonal assay kits were brought from Musajee enterprises. The machine used was Chemiluminescence Immunoassay (CLIA) strip reader. SHBG was also assayed by Chemiluminescence in the Armed Forces Institute of Pathology at Combined Military Hospital, Rawalpindi. Cold chain was maintained throughout.

We calculated the Free Androgen Index (FAI) by dividing the total testosterone levels in nmol/L by SHBG levels also in nmol/litre multiplied by 100. It has a normal range from 1–10. A value >10 indicates hyperandrogenism.[16] According to the instruction manual, the value of TT in nmol/L was obtained by multiplying ng/ml with 3.47.

RESULTS

There were 40 PCOS patients and 40 non-PCOS infertile patients. We used Mann-Whitney U test and the following parameters were significantly different from each other so the null hypothesis had to be rejected. Total Testosterone was raised in the PCOS group (*p*-value =0.015, U= 548.500), Sex Hormone Binding Globulin Levels (SHBG) were decreased in PCOS group (*p*-value = .013, U=1,057.500), Free Androgen Index (FAI) was raised in PCOS group (*p*-value = 0.001, U= 466.500), Body Mass Index (BMI) was raised in PCOS group as compared to the non-PCOS group (*p*-value = 0.000, U= 314.000). Central obesity was present in both the groups. Waist/hip ratio in both the groups was raised with no significant difference between the two groups (*p*-value= 0.725, U= 763.500). These results are shown in table 1 and 2. Hirsutism was present in most of the PCOS cases but total testosterone was not necessarily raised in each case however free FAI was comparatively raised in more PCOS cases with hirsutism as compared to total testosterone alone as shown in figure-1.

Using Pearson s correlate, we found out that Total testosterone and BMI of all the 80 subjects were positively correlated (r = .248, *p*-value < 0.05) and FAI and BMI of all the subjects taken together were positively correlated (r = .340, *p*-value <0.01).

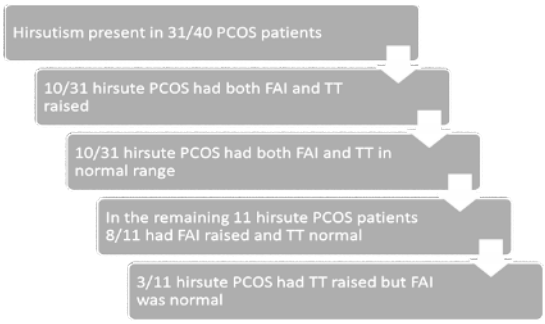

Figure-1: Number of PCOS patients with hirsutism and with raised or normal Total Testosterone (TT) level and also with raised or normal Free Androgen Index (FAI) level.

Table-1: Characteristics of study participants in PCOS and non-PCOS infertile patients.

	PCOS (mean±1SD	INFERTILE (mean±1SD
AGE (mean ±1SD) years	27.80 ± 5.175	28.10 ± 4.106
Body Mass Index (kg/m^2) (mean ±1SD)	29.36±5.49	23.36±4.06
Waist: Hip ratio (mean ±1SD)	0.86±0.07	0.85±0.062
Hirsutism	Present in most patients (31/40)	Present in few patients (4/40)

Table-2: Comparison of total testosterone, sex hormone binding globulin levels and free androgen index in PCOS and non-PCOS infertile patients.

	PCOS Mean±1SD	INFERTILE Mean±1SD	*p*-value (Mann Whitney U test)
Total testosterone (TT) ng/ml	1.09±1.10	0.60±0.33	0.015*
Sex Hormone Binding Globulin Levels (SHBG) (nmol/L)	33.42±32.35	40.65±21.67	0.013*
Free Androgen Index (FAI)	21.18±22.72	7.12±6.84	0.001*

*Significant *p*-value <0.05. Normal level. Total testosterone: 0.2-0.95 ng/ml. Sex Hormone Binding Globulin: 16–120 nmol/L. Free Androgen Index: 1–10, >10 indicates hyperandrogenaemia

DISCUSSION

Polycystic ovary syndrome (PCOS) is among the most common endocrine disorders of women of reproductive age group and is also one of the major causes of infertility. PCOS is present in almost 10% women of reproductive age group.[17]

In patients with PCOS the androgen levels are raised.[4] In this study the total testosterone levels of patients with PCOS were greater than the total testosterone levels of non-PCOS infertile group as indicated by a *p*-value less than 0.05. In our study the FAI of PCOS patients was greater than the FAI of non-PCOS infertile patients as indicated by a *p*-value less than 0.05.

SHBG has a wide range from 16-120 nmol/L. The SHBG levels of PCOS were less than the SHBG levels of infertile patients without PCOS (*p*-value <0.05) however most of the patients in both groups had levels in the lower half of normal range. This was in accordance with another study which stated that insulin resistance and hyperinsulinemia suppressed the production of SHBG from the liver thereby increasing the free testosterone in blood.[18]

Another study by Kim *et al* stated that increased BMI and central obesity is associated with decreased SHBG production which was observed in our study as well.[19] In another research, it was found that decreasing the initial weight by 7% and 150 minutes of moderate exercise increased the SHBG levels.[20] Hyperandrogenism at the ovarian microenvironment level allows and stimulates the follicles to grow to the antral stage and even improves folliculogenesis in patients with diminished ovarian reserve.[21] The growing follicles become arrested because after the antral stage the excess of androgens is detrimental and stops further maturation.[22]

Testosterone levels were positively correlated with BMI. In obesity, there is nutrient toxicity which causes hyperinsulinemia. Insulin increases androgen biosynthesis by acting as a cogonadotropin.[23] Increased levels of androgens occur due to increased peripheral conversion of fat in adipose tissue to androgens.[24] Insulin decreases production of SHBG and increases bioavailability of free testosterone thereby leading to hyperandrogenemia.[9]

Waist: Hip ratio of above 0.82 is considered as centrally obese. Central obesity was present in majority of patients with PCOS as well as the non-PCOS infertile group. This was in accordance with another research which found out that central obesity has an important role to play in causing infertility both in patients with PCOS and in those of infertility without PCOS.[25]

CONCLUSION

Patients with PCOS had raised total testosterone and free androgen index as compared to the infertile group without PCOS. SHBG levels of PCOS group were lower than that of non PCOS infertile patients. However total testosterone alone was not raised in every case of PCOS and did not match the presence and extent of hirsutism in each case. FAI calculated from SHBG level and total testosterone level can help in better determining hyperandrogenaemia than total testosterone alone.

Acknowledgements:
I would like to extend my heartfelt obligation to Professor Dr.Khurshid Khattak for allowing me to take blood samples from the patients visiting her clinic. I am grateful to Dr.Shahnaz Khattak who carried out the ultrasound scans of my patients. I wish to express my gratitude to Professor Dr.Muhammad Ayub for his valuable guidance. I am thankful to laboratory technician Safiullah Khattak for his help with the biochemical tests.

AUTHORS' CONTRIBUTION

MK: Research work, sampling, literature search, data collection, data analysis, study design, conceptualization, write-up. RU: Data interpretation, proof reading, data analysis and write-up. NS: Data analysis, data interpretation, proof reading. AK: Data analysis and interpretation.

REFERENCES

1. Kauffman RP, Baker TE, Baker VM, DiMarino P, Castracane VD. Endocrine and metabolic differences among phenotypic expressions of polycystic ovary syndrome according to the 2003 Rotterdam consensus criteria. Am J Obstet Gynecol 2008;198(6):670–7.
2. Costello MF, Misso ML, Balen A, Boyle J, Devoto L, Garad RM, *et al.* Evidence summaries and recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome: assessment and treatment of infertility. Hum Reprod Open 2019;2019:hoy021.
3. Baptiste CG, Battista MC, Trottier A, Baillargeon JP. Insulin and hyperandrogenism in women with polycystic ovary syndrome. J Steroid Biochem Mol Biol 2010;122(1-3):42–52.
4. Blank SK, McCartney CR, Chhabra S, Helm KD, Eagleson CA, Chang RJ, *et al.* Modulation of gonadotropin-releasing hormone pulse generator sensitivity to progesterone inhibition in hyperandrogenic adolescent girls--implications for regulation of pubertal maturation. J Clin Endocrinol Metab 2009;94(7):2360–6.
5. Rosenfield RL, Wroblewski K, Padmanabhan V, Littlejohn E, Mortensen M, Ehrmann DA. Antimüllerian hormone levels are independently related to ovarian hyperandrogenism and polycystic ovaries. Fertil Steril 2012;98(1):242–9.
6. Toprak S, Yonem A, Cakir B, Guler S, Azal O, Ozata M, *et al.* Insulin resistance in nonobese patients with polycystic ovary syndrome. Horm. Res 2001;55(2):65–70.
7. Sirmans SM, Pate KA. Epidemiology, diagnosis, and management of polycystic ovary syndrome. Clin Epidemiol 2014;6:1–13.
8. Martínez-García MÁ, Gambineri A, Alpañés M, Sanchón R, Pasquali R, Escobar-Morreale HF. Common variants in the sex hormone-binding globulin gene (SHBG) and polycystic ovary syndrome (PCOS) in Mediterranean women. Hum Reprod 2012;27(12):3569–76.
9. Hogeveen KN, Cousin P, Pugeat M, Dewailly D, Soudan B, Hammond GL. Human sex hormone–binding globulin variants associated with hyperandrogenism and ovarian dysfunction. J Clin Invest 2002;109(7):973–81.
10. Polak K, Czyzyk A, Simoncini T, Meczekalski B. New markers of insulin resistance in polycystic ovary syndrome. J Endocrinol Invest 2017;40(1):1–8.
11. Orio F Jr, Palomba S, Spinelli L, Cascella T, Tauchmanovà L, Zullo F, *et al*. The cardiovascular risk of young women with polycystic ovary syndrome: an observational, analytical, prospective case-control study. J Clin Endocrinol Metab 2004;89(8):3696–701.
12. Al Kindi MK, Al Essry FS, Al Essry FS, Mula-Abed WA. Validity of serum testosterone, free androgen index, and calculated free testosterone in women with suspected hyperandrogenism. Oman Med J 2012;27(6):471–4.
13. Yildiz BO, Bolour S, Woods K, Moore A, Azziz R. Visually scoring hirsutism. Hum Reprod Update 2010;16(1):51–64.
14. Sui X, LaMonte MJ, Laditka JN, Hardin JW, Chase N, Hooker SP, *et al*. Cardiorespiratory fitness and adiposity as mortality predictors in older adults. JAMA 2007;298(21):2507–16.
15. Ahmad N, Adam SIM, Nawi AM, Hassan MR, Ghazi HF. Abdominal obesity indicators: Waist circumference or waist-to-hip ratio in Malaysian adults population. Int J Prev Med 2016;7:82.
16. Sathyapalan T, Al-Qaissi A, Kilpatrick ES, Dargham SR, Keevil B, Atkin SL. Salivary and serum androgens with anti-Müllerian hormone measurement for the diagnosis of polycystic ovary syndrome. Sci Rep 2018;8(1):3795.
17. Hart R, Hickey M, Franks S. Definitions, prevalence and symptoms of polycystic ovaries and polycystic ovary syndrome. Best Pract Res Clin Obstet Gynaecol 2004;18(5):671–83.
18. Lim SS, Norman RJ, Davies MJ, Moran LJ. The effect of

obesity on polycystic ovary syndrome: a systematic review and meta-analysis. Obes Rev 2013;14(2):95–109.
19. Selva DM, Hogeveen KN, Innis SM, Hammond GL. Monosaccharide-induced lipogenesis regulates the human hepatic sex hormone–binding globulin gene. J Clin Invest 2007;117(12):3979–87.
20. Kim C, Pi-Sunyer X, Barrett-Connor E, Stentz FB, Murphy MB, Kong S, *et al*. Sex hormone binding globulin and sex steroids among premenopausal women in the diabetes prevention program. J Clin Endocrinol Metab 2013;98(7):3049–57.
21. Barad D, Brill H, Gleicher N. Update on the use of dehydroepiandrosterone supplementation among women with diminished ovarian function. J Assist Reprod Genet 2007;24(12):629–34.
22. Qiao J, Feng HL. Extra-and intra-ovarian factors in polycystic ovary syndrome: impact on oocyte maturation and embryo developmental competence. Hum Reprod Update 2011;17(1):17–33.
23. Proietto J. Mechanisms of insulin resistance caused by nutrient toxicity. Hepatol Res 2005;33(2):87–91.
24. Poretsky L, Cataldo NA, Rosenwaks Z, Guidice LC. The insulin-related ovarian regulatory system in health and disease. Endocr Rev 1999;20(4):535–82.
25. Hollmann M, Runnebaum B, Gerhard I. Impact of waist-hip-ratio and body-mass-index on hormonal and metabolic parameters in young, obese women. Int J Obes Relat Metab Disord 1997;21(6):476–83.

Submitted: June 21, 2020	*Revised: May 14, 2021*	*Accepted: August 4, 2021*

Address for Correspondence:

Madiha Khattak, Department of Physiology, Khyber Medical College, Khyber Medical University, Peshawar-Pakistan
Email: drmadihakhattak26@gmail.com