Comparison Of Estradiol To Sex Hormone Binding Globulin Ratio In Polycystic Ovary Syndrome And Non Polycystic Ovary Syndrome Infertile Patients

Madiha Khattak[1], Nuzhat Sultana[2], Almas Fasih Khattak[3], Robina Usman[4], Arshia Khattak[5]

[1]*Department of Physiology, Khyber Medical College, Peshawar, Pakistan*
[2]*Department of Pathology, Northwest School of Medicine, Peshawar, Pakistan*
[3]*Department of Community Medicine, Northwest School of Medicine, Peshawar, Pakistan*
[4]*Department of Physiology, Peshawar Medical College, Riphah International University, Islamabad, Pakistan.*
[5]*Ayub Medical College, Abbottabad, Pakistan.*

ABSTRACT

Objective

To find out the estradiol to Sex Hormone Binding Globulin (SHBG) levels in polycystic ovary syndrome (PCOS) and non PCOS infertile patients.

Methodology

This was a comparative cross-sectional study. It was carried out at Jinnahabad Medical Center Abbottabad from January 2013 to July 2013. Study included 40 PCOS patients and 40 non-PCOS infertile patients. After taking informed consent and detailed history, clinical examination and ultrasound scans were performed. Blood samples were taken in early follicular phase of the menstrual cycle.

Results

The data was analyzed by SPSS software version 19. SHBG was significantly decreased (p=0.008) in PCOS (32.81±32.54 nmol/L) compared to non-PCOS (40.65±21.67nmol/L). Estradiol to SHBG ratio was increased in PCOS infertile group (p=0.006, PCOS=0.03±0.025, Non-PCOS=0.013±0.011). There was no significant difference in Estradiol levels. Body Mass Index was significantly raised in PCOS patients compared to Non PCOS (*p*=0.001).

Conclusion

SHBG levels were significantly decreased in PCOS patients while estradiol to SHBG ratio was increased in PCOS patients.

Key words: Polycystic ovary syndrome, Estradiol, Infertility, SHBG, Body Mass Index.

*For Correspondence.

Dr. Madiha Khattak
Assistant Professor,
Department of Physiology,
Khyber Medical College,
Peshawar Pakistan.

Email:
drmadihakhattak26@gmail.com

INTRODUCTION

Polycystic ovary syndrome (PCOS) was first described by Stein and Leventhal in 1935 when they described seven women with amenorrhea and enlarged ovaries who resumed normal cycles after wedge resection of the ovaries.[1] It was later found out that there is characteristic polycystic appearance of the ovaries in this disease which was subsequently called polycystic ovary syndrome.[2] This syndrome is characterized by hormonal abnormalities which primarily include hyperandrogenemia, hyperinsulinemia, increased luteinizing hormone to follicle stimulating hormone ratio. Because of these the patient presents with oligomenorrhoea or amenorrhoea which leads to oligo-ovulation or anovulation thereby causing infertility. Hirsutism which is male like hair distribution is a common feature of these patients.[3]

Polycystic ovary syndrome appears soon after puberty because of abnormal Gonadotropin Releasing Hormone (GnRH) pulse circuitry and abnormal signaling pathways between hypothalamus, pituitary and ovary as well as adrenal gland.[4] As ovulation is scanty there is uninhibited secretion of estrogens causing proliferation of the endometrium. This excess of estrogens in blood is said to be because of extra glandular conversion of androgens to estrogens and these estrogens are not released from the ovary. In the polycystic ovary the granulosa cells produce very little estrogen because of lack of formation of mature follicle.[5] This may be reason of infertility in PCOS patient because of lack of ovulation.[6]

In PCOS there is increased levels of Luteinizing Hormone (LH) because of defective GnRH signaling. LH acts on its receptors in the ovaries causing increased activity of Cytochrome 450c17α. It's a bifunctional enzyme. The first step of this enzyme is to convert progesterone to 17α-hydroxyprogesterone via 17α-hydroxylase activity.[7] The second step of this enzyme is to convert 17α-hydroxyprogesterone to androstenedione via 17-20 lyase activity.[8] In normal ovary this androstenedione is converted to estradiol via FSH dependent aromatase enzyme in the granulosa cells but in PCOS there is increased LH levels which causes increased production of androstenedione which accumulates in the ovary.[9] In

This article may be cited as: Khattak *et al.* Comparison Of Estradiol To Sex Hormone Binding Globulin Ratio In Polycystic Ovary Syndrome And Non Polycystic Ovary Syndrome Infertile Patients. Adv Basic Med Sci. 2022; 6(1): 20-23

PCOS there are decreased FSH levels and LH activity predominates resulting in the accumulation of androstenedione. This androstenedione promotes growth of the follicles to the antral stage after which persistence of high levels of androstenedione is damaging to the growing follicle and they become arrested in the antral stage. Hence we see a large number of vesicular follicles in a PCOS ovary.[10] In the thecal cells of the ovary there is an enzyme 17-β reductase which converts excess of androstenedione to testosterone which is the cause of hyperandrogenemia and hirsutism in the PCOS.[11]

Insulin resistance is due to post receptor binding defect leading to defective intracellular signaling leading to increased secretion of insulin from the beta cells of the pancreas and causing deranged Oral Glucose Tolerance Test. PCOS are prone to develop type 2 Diabetes Mellitus later in life.[12] Insulin supplements the actions of LH on the ovary and acts as a co gonadotropin thereby increasing the formation of the androgen androstenedione which is converted to testosterone causing hirsutism.[9]

Sex hormone-binding globulin (SHBG) is a beta globulin synthetized by the liver that binds to 99% testosterone in the circulation. Only 1% of testosterone circulates freely. Decreased SHBG levels leads to decreased SHBG bound testosterone and increased free testosterone leading to oily skin, acne and hirsutism. Hyperinsulinemia seems to be the suppressor of SHBG secretion by the liver in PCOS patients.[13] SHBG also binds to the various estrogens in the blood and decreased SHBG also causes increased free estradiol in the blood leading to hyperestrogenism which presents in the form of increased thickness of the endometrium and scanty menstruation. In an animal study on PCOS induced mice it was found that the implantation related genes are dysregulated and artificial decidualization is also disrupted. The serum estradiol levels were also raised in mice with PCOS. These two factors were thought to be the cause of impaired implantation leading to early miscarriage.[14] Patients with PCOS are also at increased risk of endometrial cancer.[15]

This cross sectional study was undertaken to check for differences in various hormones in infertile patients both with and without polycystic ovary syndrome. In this study we aimed to assess whether estradiol to SHBG ratio was any different among these patients and whether it can be a better marker of hyperestrogenism than estradiol levels alone in this region of South Asia.

METHODOLOGY

This was a comparative cross-sectional study conducted at Jinnahabad Medical Centre, Abbottabad. The research took place over a time period of six months from January 2013 to July 2013. Ethical approval was taken from the Ethical Committee of Khyber Medical University No.DIR/KMU-EB/SII/000018. Informed consent was taken from all the participants in writing. A detailed history was taken with special attention on menstrual history. A general physical examination was done including examination of the reproductive system. Body mass index and waist to hip ratio was also checked.

Cases included females of reproductive age group from 20 to 45 years with polycystic ovary syndrome and suffering from infertility. All the cases taken were meeting the *Rotterdam Criteria* for the diagnosis of PCOS. According to which if two of the following three are present then the patient is suffering from PCOS. (1) Menstrual disturbances in the form of oligomenorrhoea or amenorrhoea, (2) Evidence of hyperandrogenism (clinical features and/or biochemical elevation of testosterone) and/or (3) Polycystic ovary on ultrasound.[8] Controls however included the same reproductive age group women (20 to 45) years without PCOS suffering from infertility which could be primary or secondary infertility as per WHO guidelines.

Ferriman–Gallwey score was used for determining the degree hirsutism. Nine areas of the body were scored which included upper lip, chin, chest, upper back, lower back, upper abdomen, lower abdomen, upper arms and thighs (0=no growth of hair and 4=excessive growth. Hyperandrogenism was taken as a score of eight (8) or more.[16] Abdominal ultrasound scan was performed for examining the ovary. An ovary is considered as polycystic if there are ten or more cysts measuring 2-8 mm in diameter, present peripherally with a dense stroma, enlarged ovaries (>10 cm^3).[17] Blood samples were taken in early follicular phase of the menstrual cycle and were analyzed for estradiol and Sex Hormone Binding Globulin (SHBG) using chemiluminescence assay.

Patients with pregnancy or co-morbidities like diabetes or hypertension or use of medicines especially hormones and smoking or drug abuse were excluded from the study. SPSS software version 19 was used for statistical analysis of the results. The data had a non-normal distribution and Mann-Whitney U test was applied.

RESULTS

The mean age of PCOS group was 27.80±5.17 years and of non-PCOS group was 28.10±4.10 years. There was no significant difference in mean age at marriage, duration of infertility among both groups (table 1). The mean estradiol levels in PCOS infertile patients were 130.55±80.55pg/ml while that of infertile non-PCOS group was 111.54±75.02 pg/ml. Eleven out of 40 patients in the PCOS group had raised estradiol levels whereas 4 out of 40 patients in the infertile group had raised estradiol levels. There was one patient in PCOS group with exceptionally raised estradiol levels and was removed from final analysis. The mean SHBG levels in PCOS patients was 32.81±32.54 nmol/L and were significantly lower than non-PCOS infertile group 40.65±21.67 nmol/L (p=0.008). In order to find the estradiol to SHBG ratio we converted the estradiol levels from pg/ml to nmol/L by multiplying estradiol by 0.00367 as given in the instruction manual (table 2).

The waist to hip ratio was determined. In PCOS infertile group the mean waist to hip ratio was 0.86±0.07, 29 out of 40 patients were centrally obese and in non-PCOS infertile group it was 0.85±0.06, 28 out of 40 patients were centrally obese. BMI of PCOS group was greater than the BMI of infertile group without PCOS as indicated by a p value of <0.01. The Ferriman Galeway score was used for hirsutism. The mean score was increased in the PCOS infertile group and was 9.67±5.09 and for non-PCOS infertile patients it was 4.67±1.95.

DISCUSSION

Polycystic ovary syndrome (PCOS) is among the most common endocrine disorders of women of reproductive age group and is also

	PCOS patients (mean±SD)	Non-PCOS patients (mean±SD)	*p*-value
Age at time of sampling (years)	27.80±5.17	28.10±4.11	0.971
Age at marriage (years)	20.05±4.12	19.15±4.28	0.324
Duration of primary infertility (years)	4.79±3.97	5.26±3.31	0.438
Duration of secondary infertility (years)	4.92±2.54	5.76±4.19	0.805
Body Mass Index (kg/m2)	29.36±5.49	23.36±4.06	<0.01
Waist to Hip ratio	0.86±0.07	0.85±0.06	0.546
Hirsutism	9.67±5.09	4.67±1.95	<0.01

Waist to Hip ratio above 0.82 is considered raised and central obesity is present. Ferryman Galewey score for hirsutism was used and a value greater than 8 is considered hirsute

Table 1 Comparison of time duration of infertility, Body Mass Index and waist to hip ratio in PCOS and non-PCOS infertile patients

	PCOS patients (mean±1SD) n=39	Non-PCOS patients (mean±1SD) n=40	*p*-value
Estradiol levels (pg/ml)	130.55±80.55	111.54±75.02	0.405
Sex Hormone Binding Globulin Levels (SHBG) (nmol/L)	32.81±32.54	40.65±21.67	0.008
Estradiol to SHBG ratio	0.03±0.025	0.013±0.011	0.006

Table 2: Comparison of Estradiol levels in PCOS and Non-PCOS infertile patients.

	PCOS Infertile patients (kg/m^2)	Non-PCOS Infertile patients (kg/m^2)
Below 18.4(underweight)	0	5(12.5%)
18.5-24.9(ideal weight)	9(22.5%)	19(47.5)
25-29.9(overweight)	7(17.5%)	13(32.5%)
30-39.9(obese)	23(57.5%)	3(7.5%)
Over 40(very obese)	1(2.5%)	0

Table 3: Percentage of patients falling in different classes of BMI

one of the major causes of infertility. PCOS is present in almost 10% women of reproductive age group. With increased sedentary lifestyle and refined sugars and carbohydrates in the diet along with rapid urbanization the prevalence of this syndrome is increasing.[18] This syndrome is characterized by hormonal derangements but when these patients are assayed for hormonal imbalances they are often in the normal range despite clear signs and symptoms. The reproductive hormones estradiol (98%) and testosterone (99%) are bound to a protein SHBG released from the liver leaving only 2% free estradiol and 1% free testosterone as biologically active in blood. In PCOS there is decreased SHBG secretion from the liver due to hyperinsulinism. Thus free estradiol and free testosterone are raised in the blood. There are various studies which state that Free Androgen Index (Total Testosterone/SHBG x 100) is raised in PCOS. Unlike Free Androgen Index which can be calculated easily by a gynecologist the Free Estradiol Index is not easy because it has albumin added in the formula.[19,20] In this study we checked estradiol to SHBG ratio in PCOS infertile group and non PCOS infertile group. to check if it can detect increased free estrogen activity at tissue level.

In our study there was no significant difference in the estradiol levels of the PCOS group and non PCOS infertile group. There have been a lot of studies which state that the estradiol levels are not raised in PCOS patients however there is increased levels of estrone in PCOS patient and that is due to peripheral aromatization of androgens in extaglandular tissues. Androgens are converted peripherally to estrone which can result in endometrial abnormalities.[15] Another research also reported that the estradiol level in the serum of infertile PCOS patients were in the normal range.[21] In another study which compared the estradiol level in follicular fluid as well as in serum reported that the serum estradiol levels in infertile patients with PCOS was less than controls with male factor infertility. However, there was no significant difference in the estradiol levels in the follicular fluid between these two groups.[22] In contrast, there are many studies which do say that estradiol levels and Free Androgen Index are raised in PCOS patients and SHBG levels are decreased.[23] The findings of our study were identical to another research conducted by Amato MC *et al.* which also found normal estradiol levels in PCOS patient.[23] They discussed that insulin resistance was a common and universal characteristic of PCOS and that there were lean as well as obese PCOS patients.[21]

Raised insulin levels decrease the production of Sex Hormone Binding Globulin from the liver which is the major globulin that binds to the sex steroids testosterone and estrogen and thereby increase the bioavailability of free forms of these hormones in the blood. The adipose tissue is also a form of an endocrine organ with aromatase activity which converts androgens to estrogens causing abnormal secretion of gonadotropins and excessive estrogenic effects on the endometrium.[24] In this study the BMI of PCOS

infertile group was greater than the BMI of non-PCOS infertile patients as indicated by a p-value less than 0.05. This result was similar to other studies which show the presence of obesity in PCOS.[25] Central obesity was present in both the groups indicating that central obesity has a major role to play in causing hormonal abnormalities. Obesity causes a condition called functional hyperandrogenism and hyperestrogenism that results in anovulation and endometrial dysfunction leading to infertility.[26]

The Estradiol levels of PCOS and non-PCOS infertile patients were similar to each other. The SHBG levels were decreased and estradiol to SHBG ratio was increased in PCOS infertile group compared to non PCOS infertile group.

REFERENCES

1. Parazzini F, La Vecchia C, Franceschi S, Talamini R, Negri E, Crosignani PG. Association of Stein - Leventhal syndrome with the incidence of postmenopausal breast carcinoma in a large prospective study of women in Iowa . Cancer. 1997;80(7):1360–2.

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(1):1–24.

3. Rosenfield RL, Ehrmann DA. The Pathogenesis of Polycystic Ovary Syndrome (PCOS): The hypothesis of PCOS as functional ovarian hyperandrogenism revisited. Vol. 37, Endocrine Reviews. 2016: 467–520.

4. Rosenfield RL, Ehrmann DA. The Pathogenesis of Polycystic Ovary Syndrome (PCOS): The Hypothesis of PCOS as Functional Ovarian Hyperandrogenism Revisited. Endocr Rev. 2016;37(5):467.

5. Toprak S, Yönem A, Çakır B, Güler S, Azal Ö, Özata M, et al. Insulin resistance in nonobese patients with polycystic ovary syndrome. Horm Res Paediatr. 2001;55(2):65–70.

6. Teede H, Deeks A, Moran L. Polycystic ovary syndrome: A complex condition with psychological, reproductive and metabolic manifestations that impacts on health across the lifespan. BMC Med. 2010;8.

7. Khan MJ, Ullah A, Basit S, Almunawwarrah A, Arabia S. Genetic Basis of Polycystic Ovary Syndrome (PCOS): Current Perspectives. 2019(12);249-260.

8. Witchel SF, Oberfield SE, Peña AS. Polycystic Ovary Syndrome: Pathophysiology, Presentation, and Treatment With Emphasis on Adolescent Girls. J Endocr Soc [Internet]. 2019;3(8):1545–73.

9. Mojaverrostami S, Asghari N, Khamisabadi M, Khoei HH. The role of melatonin in polycystic ovary syndrome: A review. Int J Reprod Biomed. 2019 ;17(12):865.

10. Tarumi W, Tsukamoto S, Okutsu Y, Takahashi N, Horiuchi T, Itoh MT, et al. Androstenedione induces abnormalities in morphology and function of developing oocytes, which impairs oocyte meiotic competence. Fertil Steril. 2012 1;97(2):469–76.

11. Xu Y, Qiao J. Association of Insulin Resistance and Elevated Androgen Levels with Polycystic Ovarian Syndrome (PCOS): A Review of Literature. J Healthc Eng. 2022;2022.

12. E C, G I, C V, P L, N S, T SP, et al. Hormonal profile in women with polycystic ovarian syndrome with or without type 1 diabetes mellitus. J Clin Endocrinol Metab. 2007;92(12):4742–6.

13. Qu X, Donnelly R. Sex Hormone-Binding Globulin (SHBG) as an Early Biomarker and Therapeutic Target in Polycystic Ovary Syndrome. Int J Mol Sci. 2020;21(21):1–17.

14. Li SY, Song Z, Song MJ, Qin JW, Zhao ML, Yang ZM. Impaired receptivity and decidualization in DHEA-induced PCOS mice. Sci Rep. 2016;6. 38134.

15. Ding DC, Chen W, Wang JH, Lin SZ. Association between polycystic ovarian syndrome and endometrial, ovarian, and breast cancer: A population-based cohort study in Taiwan. Medicine (Baltimore). 2018;97(39). 12608

16. Lumezi BG, Berisha VL, Pupovci HL, Goçi A, Hajrushi AB. Grading of hirsutism based on the Ferriman-Gallwey scoring system in Kosovar women. Adv Dermatology Allergol Dermatologii i Alergol. 2018;35(6):631-635.

17. Teede HJ, Misso ML, Costello MF, Dokras A, Laven J, Moran L, et al. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. Hum Reprod. 2018 1;33(9):1602–18.

18. Boyle JA, Cunningham J, O'Dea K, Dunbar T, Norman RJ. Prevalence of polycystic ovary syndrome in a sample of Indigenous women in Darwin, Australia. Med J Aust [Internet]. 2012 Jan 16 [cited 2022 Aug 24];196(1):62–6. Available from: https://www.mja.com.au/journal/2012/196/1/prevalence-polycystic-ovary-syndrome-sample-indigenous-women-darwin-australia

19. Duggan C, De Dieu Tapsoba J, Stanczyk F, Wang CY, Schubert KF, Mctiernan A. Long-term Weight Loss Maintenance, Sex Steroid Hormones and Sex Hormone Binding Globulin HHS Public Access. Menopause. 2019;206(4):417–22.

20. Chadid S, Barber JR, Rohrmann S, Nelson WG, Yager JD, Kanarek NF, et al. Age-Specific Serum Total and Free Estradiol Concentrations in Healthy Men in US Nationally Representative Samples. J Endocr Soc [Internet]. 2019;3(10):1825–36.

21. Amato, M.C., Guarnotta, V., Forti, D., Donatelli, M., Dolcimascolo, S. and Giordano, C. Metabolically healthy polycystic ovary syndrome (MH-PCOS) and metabolically unhealthy polycystic ovary syndrome (MU-PCOS): a comparative analysis of four simple methods useful for metabolic assessment. Hum Reprod. 2013;28(7):1919–28.

22. Amato G, Conte M, Mazziotti G, Lalli E, Vitolo G, Tucker AT, et al. Serum and Follicular Fluid Cytokines in Polycystic Ovary Syndrome During Stimulated Cycles. Obs Gynecol. 2003;101(6):1177–82.

23. Zarei E, Binabaj MM, Fatemeh ·, Zadeh M, Sepideh ·, Nosrat B, et al. Kisspeptin levels in relation to sex hormone profile among PCOS patients. Irish J Med Sci. 1971;1:3.

24. Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale F, Futterweit W, et al. The Androgen Excess and PCOS Society criteria for the polycystic ovary syndrome: the complete task force reportTask Force on the Phenotype of the Polycystic Ovary Syndrome of The Androgen Excess and PCOS Society. Fertil Steril. 91:456–88.

25. Neubronner SA, Indran IR, Chan YH, Thu AWP, Yong EL. Effect of body mass index (BMI) on phenotypic features of polycystic ovary syndrome (PCOS) in Singapore women: a prospective cross-sectional study. BMC Womens Health. 2021 1;21(1).

26. Gambineri A, Laudisio D, Marocco C, Radellini S, Colao A, Savastano S, et al. Female infertility: which role for obesity? Int J Obes Suppl. 2019 ;9(1):65-75.