CLINICAL AND BIOCHEMICAL STUDY OF POLYCYSTIC OVARIES SYNDROME IN YOUNG FEMALE POPULATION OF KHYBER PAKHTUNKHWA, PAKISTAN.

ZAHOO AHMED¹, BEENISH GUL², TALAT NAZ³, NOUSHEEN³, SM NAEEM⁴

1. Department of Biochemistry, Khyber Medical College Peshawar, KPK, Pakistan.
2. Institute of Chemical Science University of Peshawar, KPK, Pakistan.
3. Department of Gynae, Khyber Teaching Hospital Peshawar, KPK, Pakistan.
4. Department of Biochemistry, Saidu Medical College Swat, KPK, Pakistan.

ABSTRACT

BACKGROUND: Polycystic ovaries syndrome (PCOS) is one of the commonest endocrine disorder in women. It is associated with characteristic metabolic disturbance, clinically manifested in the form of amenorrhea, oligomenorrhoea. The present study was conducted on young females of district Peshawar and Mardan to investigate PCOS on the basis of clinical and biochemical findings.

OBJECTIVE: To study clinical and biochemical feature regarding polycystic ovaries syndromes in young females of districts Peshawar and Mardan, Khyber Pkhtunkhwkha Pakistan.

PATIENTS & METHOD: It was a cross sectional study of university going female students with age group of 20 to 25 years. A pre-designed, pre-tested questionnaire was used for the collection of data. Total T, SHBG, LH, were measured, each hormone in a single assay, using an automated immunochemiluminescence method (Immulite, Diagnostic Products, Los Angeles, CA). 17-OHP was measured by direct Enzyme-linked immunosorbent Assay (ELISA) method.

RESULTS: A total nine hundred & twenty six (n=926) females were randomly selected. General and clinical features include age, amenorrhea, oligomenorrhoea, acne, hirsutism, obesity and trans-abdominal ultrasound. The biochemical characteristics like hyper LH secretion of the 49.13% (n=455) subject were in a moderate range. In addition to 58.63% (n=543), 37.25% (n=345), 4.10% (n=38) of the women diagnosed with ACTH were in normal, low and high ranges respectively. Four eighty nine (n=489) women (52.80%) showed acyclic estrogen production in normal ranges while 23.97% (n=222) have in low ranges score and 23.21% (n=215) have high ranges of acyclic estrogen production.

CONCLUSIONS: Polycystic ovaries are very common in this age group but are not necessarily associated with other signs and symptoms. The prevalence of polycystic ovary syndrome varies widely according to the definition applied. Sub-group analysis of women with polycystic ovaries according to the presence or absence of features of polycystic ovary syndrome does not reveal an increasing trend for progression of endocrine abnormalities usually associated with polycystic ovary syndrome.

KEYWORDS: Polycystic Ovaries Syndrome (PCOS), Peshawar, Mardan, KPK

INTRODUCTION

Polycystic ovaries syndrome (PCOS) in its most typical form, the association of hyper-androgenism and chronic an-ovulation — is one of the most common endocrine disorders. The clinical and biochemical features are heterogeneous, and there have been many debates to whether it represents a single disorder or several. In recent years, it has become apparent that the polycystic ovary syndrome not

only is the most frequent cause of an-ovulation and of hirsutism, but is also associated with a characteristic metabolic disturbance (resistance to the action of insulin) that may have important implications for long-term health¹. Common clinical features seen in adolescents with PCOS include a menstrual history (amenorrhea, oligomenorrhoea), clinical observation of acne, hirsutism, trans-abdominal pelvic ultrasound and obesity. Biochemical abnormalities include

androstenedione, acyclic estrogen production, LH hyper-secretion, decreased levels of sex hormone binding globin (SHBG), testosterone, adrenocorticotrophic hormone (ACTH), 17-hydroxyprogesterone. Polycystic ovary syndrome is a genetically complex endocrine disorder of women of uncertain etiology and is a common cause of an-ovulatory infertility, menstrual dysfunction and hirsutism^{2,3,4}. PCOS appears to be associated with an increased risk of metabolic aberrations, including insulin resistance and hyper-insulinism, type-2 diabetes mellitus, dyslipidemia, cardiovascular disease and endometrial carcinoma^{5,6,7}.

The most widely accepted clinical definition of the polycystic ovary syndrome is the association of hyper-androgenism with chronic an-ovulation in women without specific underlying diseases of the adrenal or pituitary glands⁸. Hyper-androgenism is characterized clinically by hirsutism, acne, and androgen-dependent alopecia and biochemically by elevated serum concentrations of androgens, particularly androstenedione and testosterone. Obesity is common but not universal^{9,10,11}. Typically, these features are associated with hyper-secretion of luteinizing hormone and androgens but with normal or low serum concentrations of follicle-stimulating hormone^{9,11,12,13,14}. Ironically, although the early descriptions of the syndrome were based on ovarian morphology, this has not been considered an essential requirement for the diagnosis^{8,9}. Present work was planned to study and investigate clinical and biochemical feature regarding PCOS in young females of districts Peshawar and Mardan, Khyber Pakhtunkhwa (KPK), Pakistan.

SUBJECTS AND METHOD

Volunteers were recruited from two universities, University of Peshawar and Abdul Wali Khan University Mardan, KPK, Pakistan respectively 926 females aged 18–25 years participated the present study. Information collected included: a menstrual history (amenorrhea, oligomenorrhoea), clinical observation of acne and hirsutism, trans-abdominal pelvic ultrasound, obesity, and biochemical analysis of

a blood sample has been carried out in different laboratories like The Agha Khan University Hospital Karachi, Shaukat Khanam Memorial Cancer Hospital and Research Center Lahore, Institute of Radiotherapy & Nuclear Medicine (IRNUM) Peshawar and Hayatabad Medical Complex Peshawar, Pakistan respectively. The data was collected on a predesigned questionnaire. The blood sample were taken from antecubital vein in supine position were transferred to labeled with ID No. for further analysis. The data were analyzed using SPSS version 16.00 as a soft ware statistical package. Serum samples were analyzed for total testosterone (T), sex hormonebindingglobulin (SHBG), LH hypersecretion, ACTH, androstenedione, acyclic estrogen production. In women who matched the criteria for PCOS, and 17-hydroxyprogesterone (17-OHP) were also measured to exclude hyperprolactinemia, hypothyroidism, and non-classic 21-hydroxylase deficiency, respectively. Total T, SHBG, LH, were measured, each hormone in a single assay, using an automated immunochemiluminescence method (Immulite, Diagnostic Products, Los Angeles, CA). 17-OHP was measured by direct Enzyme-linked immunosorbent assay (ELISA) method. The assay coefficients of variation were as follows: high range of 43% for SHBG, very low 21.59% for hyper LH secretion, moderate 52.80% for Estrogen, and low 49.13%, moderate 42.00% and high 8.85% for 17-OHP.

Clinical Presentation

Hyper-androgenism presents as hirsutism, acne. An-ovulation manifests itself as menstrual disturbance amenorrhea, oligomenorrhoea, trans-abdominal pelvic ultrasound and obesity. Obesity is common but not usually a presenting symptom. In many cases, a history of menstrual disturbance menarche may be delayed and presentation with primary amenorrhea is uncommon but well recognized¹⁷. Hirsutism and obesity may be present in adolescent girls, even before the menarche. At any institution, the relative frequencies of the various presenting symptoms will depend primarily on the particular interests of the referral centers. The

degree of hirsutism can be assessed by the Ferriman–Gallwey score, a simple, semi quantitative method for recording the distribution and severity of excess body hair¹⁸. Examination may also reveal, particularly in obese subjects,^{15-16, 19.}

Diagnose and Differential Diagnosis

The diagnosis of polycystic ovary syndrome is usually made on the basis of a combination of clinical, ultrasonography, and biochemical criteria. A females presenting with oligomenorrhoea is likely to have the polycystic ovary syndrome if she has one or more of these three features: polycystic ovaries on ultrasonography, hyper-androgenemia and hirsutism. Many women with the syndrome have hyper-secretion of luteinizing hormone, although normal serum concentrations of luteinizing hormone do not rule out the diagnosis. The diagnosis of polycystic ovary syndrome in a woman presenting with hirsutism and regular cycles is more contentious, but the finding of polycystic ovaries on ultrasonography in association with moderate hyper-androgenemia (i.e., serum testosterone concentrations of 85 to 150 ng/dL [3 to 5 nmol/L] points to a benign ovarian cause of the hirsutism, whether or not the term “polycystic ovary syndrome” is used. The differential diagnosis of polycystic ovary syndrome includes patients with hirsutism and menstrual disturbances in which the primary diagnosis is of pituitary or adrenal diseases for example, acromegaly, hyperprolactinemia and classic or non-classic congenital adrenal hyperplasia. These “polycysticovary like” syndromes can be identified by the presence of other specific clinical and biochemical features^{15,17.}

Table 2: Biochemical Parameters of study subjects Diagnosed with PCOS

S. No	Hormones	Low	Moderate	High	Total
1	Testosterones(ng/dL)	221 (23%)	400(43%)	305(32.0%)	926
2	Hyper LH Secretion(mIU/mL)	200 (21%)	455 (49%)	271(29.0%)	926
3	ACTH(pg/mL)	345(37%)	543 (58%)	38 (4.00%)	926
4	17-Hydroxyprogesterone(ng/mL)	455 (49%)	389 (42%)	82 (8.80%)	926
5	Acyclic Estrogen (pg/mL)	222 (23%)	489 (52%)	215(23.2%)	926
6	Serum Androstenedione (ng/ml)	200 (21%)	271 (29%)	455(49.0%)	926
7	Sex Hormone Binding Globulin (SHBG) (nmol/L)	305 (32%)	221 (23%)	400(43.1%)	926

RESULTS

A total nine hundred& twenty six (n=926) females were randomly selected. The study was conducted at two Universities of KPK, Pakistan viz University of Peshawar and Abdul Wali Khan University Mardan, KPK, Pakistan. Clinical feature of the study population are depicted in table-1.

Table 1: Clinical Features of subjects suffering from Polycystic Ovary Syndrome.

1	Age	18-25 Years
2	Amenorrhea	30 (03.20%)
3	Oligomenorrhoea	77 (08.32%)
4	Acne	470 (50.70%)
5	Hirsutism	200 (21.50%)
6	Obesity	50 (05.30%)
7	Trans-abdominal Pelvic Ultrasound	99 (10.70%)
	Total	926

These include age, amenorrhea, oligomenorrhoea, acne, hirsutism, obesity and trans- abdominal ultrasound. The table 1 shows that among the study population 3.2% of the study subjects were having amenorrhea, 50.7% and 21.5% of subjects having acne and hirsutism respectively.5.3% having obesity where as those with trans-abdominal pelvic ultrasound were observed to be 10.70% (n=99).

The biochemical parameters of the study population are depicted in table 2. The table shows that majority of subject were having testosterone in moderate range (n=400) followed by high range (n=305). Similar trend of results were noted for ACTH & 17-hydroxyprogesterone, which was found to be (n=543 & 389) respectively. Whereas opposite trend of result was observed for androstenedione and SHBG in which maximum number of subject were found in high level (n=455 & n=400) respectively.

DISCUSSION

Minor differences in the design of the study may explain the slightly higher prevalence of PCOS found in Spain (6.5%) compared to White women from Alabama (4.7%). We defined clinical hyperandrogenism by the presence of hirsutism and/or acne, whereas Knochenhauer,²⁰ only considered hirsutism for the diagnosis of PCOS. Although in none of our PCOS patients did the diagnosis rely solely on acne and oligomenorrhoea, in the study by Knochenhauer, the inclusion of acne as a sign of hyperandrogenism would have increased the prevalence of PCOS in White women to 5.4%. Further, we used less stringent criteria for the definition of oligomenorrhoea, which might be responsible for the 1% increase in the prevalence of PCOS in Spain compared to Alabama. However, we found higher prevalence of hirsutism, as defined by a hirsutism score of 8 or more, and acne in Spanish women (7.1% and 12.3%, respectively) compared to White women from Alabama (2.8% and 5.2%, respectively) suggesting that hyper-androgenic disorders might be more prevalent in Spain²⁰. As reviewed by Knochenhauer,²⁰ Older studies have reported excess "hairiness" in 5–15% of consecutive Caucasian women^{21,22,23,24}. The present study is consistent with the above studies carried out and we found that hirsutism almost above 20 %.

These rates are similar to those observed in the 1999–2000 National Health and Nutrition Examination Survey in similarly aged US

women²⁵. The prevalence of obesity, but not over-weightiness, was higher in PCOS women than in our overall study population (i.e. 42%) and higher than Spanish patients similarly identified (i.e. 30%)²⁶. However, the prevalence of obesity in our PCOS patients was similar to the rate observed in Greek women (i.e. 38%)²⁵. These data further suggested that there are significant ethnic differences in the prevalence of obesity in PCOS and that obesity per se is not a universal feature of the syndrome²⁷. As for as obesity is concerned contrast result (5.2%) were found and were lower when compared with the results of worked carried out in different parts the world^{25,26,27}. This difference may be attributed to the difference in study design, ethnicity, study population and socio-geographical distribution of study subjects. Our findings are in agreement with the majority of above mentioned studies.

Finally, we should note that a recent conference on the subject cosponsored by the European Society for Human Reproduction and Embryology and the American Society for Reproductive Medicine was held May 2003, in Rotterdam, The Netherlands Conference participants suggested that the diagnostic criteria for PCOS be revised such that the disorder would be diagnosable if two of the following three criteria were present, after the exclusion of other etiologies: 1. oligo- and/or an-ovulation, 2. clinical and/or biochemical signs of hyperandrogenism, and 3. polycystic ovaries on ultrasonography^{28,29}. The definition used in the current study is consistent with those proposed in Rotterdam. Nonetheless, per this newer definition, we may have further underestimated the prevalence of PCOS because our subjects did not undergo trans-vaginal sonography and hence, we did not identify two additional potential phenotypes, i.e. women solely with oligo-ovulation and polycystic ovaries, or with hyper-androgenism and polycystic ovaries but normal ovulation. Finally, in an unrelated study we noted that up to 40% of hirsute women who claiming to have regular menses actually have

oligo-ovulation when evaluated more closely²⁹. In the original study by Ferriman and Gallwey a score of 6 or greater was observed in only 5% of the general population³⁰. Furthermore, Lorenzo studied 300 unselected female medical patients, using a modification of the F-G score with lesser areas assessed, and did not observe a score over 5 among them³¹.

REFERENCES

1. Stephen. Frank,MD. Polycystic Ovary Syndrome.*J The New England Journal of Medicine*.1995; 333: 853-861
2. Ricaedo, A. Keslie, S. Woods. The Prevalence and Features of the Polycystic Ovary Syndrome in an Unselected Population.*The Journal of Clinical Endocrinology & Metabolism*. 2004; 89: 2745–2749.
3. Hull, MG. Epidemiology of infertility and polycystic ovarian disease: endocrinological and demographic studies.*J Gynecol Endocrinol*.1987; 1: 235–245.
4. Azziz, R. The evaluation and management of hirsutism. *J Obstetric Gynecol*.2003; 101: 995–1007.
5. Ovalle, F. Azziz, R. Insulin resistance, polycystic ovary syndrome, and type-2 diabetes mellitus.*J Fertil Steril*.2002;77:1095–1105.
6. Wild, RA. Long-term health consequences of PCOS.*J Hum Reprod Update*. 2002;8: 231–24.
7. Legro, RS. Polycystic ovary syndrome and cardiovascular disease: a premature association. *J Endocr Rev*.2003; 24:302–312.
8. Zawadzki, JK. Dunaif, A. Diagnostic criteria for polycystic ovary syndrome: towards a rational approach. In: Dunaif A, Givens JR, Haseltine FP, Merriam GR, eds. Polycystic ovary syndrome. Oxford, England: Blackwell Scientific. 1992:377-84.
9. Stei, IF. Leventhal, ML. Amenorrhea associated with bilateral polycystic ovaries. *Am J Obstet Gynecol*. 1935; 29:181-91.
10. Goldzieher, JW. Green, JA. The polycystic ovary. 1. Clinical and histological features. *J Clin Endocrinol Metab*. 1962; 22:325-38.
11. Yen, SSC. Vela, P. Rankin, J. Inappropriate secretion of follicle-stimulating hormone and luteinizing hormone in polycystic ovarian disease. *J Clin Endocrinol Metab*.1970; 30:435-42.
12. McArthur, JW. Ingersoll, FM. Worcester, J. The urinary excretion of interstitial-cell and follicle-stimulating hormone activity by women with diseases of the reproductive system. *J Clin Endocrinol Metab*.1958; 18:1202-15.
13. Gambrell, Jr. Greenblatt, RB. Mahesh, VB. Inappropriate secretion of LH in the Stein-Leventhal syndrome. *J Obstet Gynecol*. 1973; 42:429-40.
14. Holte, J. Bergh, T. Gennarelli, G. Wide, L. The independent effects of polycystic ovary syndrome and obesity on serum concentrations of gonadotrophins and sex steroids in premenopausal women *J Clin Endocrinol*.1994; 41:473-81.
15. Franks, S. Polycystic ovary syndrome: a changing perspective. *J Clin Endocrinol*. 1989; 31:87-120.
16. Conway, GS. Honour, JW. Jacobs, HS. Heterogeneity of the polycystic ovary syndrome: clinical, endocrine and ultrasound features in 556 patients. *J Clin Endocrinol*.1989; 30:459-70.
17. Yen, SSC. The polycystic ovary syndrome. *J Clin Endocrinol*.1980; 12:177-207.
18. Ferriman, D. Gallwey, JD. Clinical assessment of body hair growth in women. *J Clin Endocrinol Metab*.1961; 21:1440-7.
19. Dunaif, A. Graf, M. Mandeli, J. Laumas, V. Dobrjansky, A. Characterization of groups of hyperandrogenic women with acanthosis nigricans, impaired glucose tolerance, and/or hyperinsulinemia. *J Clin Endocrinol Metab*.1987; 65:499-507.
20. Knochauer ES, Key TJ, Kashar-Miller M, Waggoner W, Boots LR, Azzi R. 1998 Prevalence of the polycystic ovary syndrome in unselected black and white women of the Southeastern United States: a prospective study. *J Clin Endocrinol Metab*.1998; 83:3078–3082
21. Abraham, GE. Ovarian and adrenal contribution to peripheral androgens during the menstrual cycle. *J Clin Endocrinol Metab*.1974; 39:240–246.
22. Goldzieher, JW. Green, JA. The polycystic ovary. I. Clinical and histologic features. *J Clin Endocrinol Metab*.1962; 22:325–338.
23. Hartz, AJ. Barboriak, PN. Wong, A. Katayama, KP. Rimm, AA. The association of obesity with infertility and related menstrual abnormalities in women. *Int J Obes*. 1979;3:57–73.
24. Raj, SG. Thompson, IE. Berger, MJ. Taymor, ML. Clinical aspects of the polycystic ovary syndrome. *J Obstet Gynecol*.1977; 49:552–556.
25. Diamanti, KE. Kouli, CR. Bergiele, AT, Filandra, FA. Tsianateli, TC. Spina, GG. Zapanti, ED. Bartzis, MI. A survey of the polycystic ovary syndrome in the Greek island of Lesbos: hormonal and metabolic profile. *J Clin Endocrinol Metab*.1999; 84: 4006–4011.
26. Asuncion, M. Calvo, RM. San Millan, JL. Sancho, J. Avila, S. A prospective study of the prevalence of the polycystic ovary syndrome in unselected Caucasian women from Spain *J Clin Endocrinol Metab*.2000; 85:2434–2438.
27. Flegal, KM. Carroll MD. Ogden, CL. Johnson, CL. Prevalence and trends in obesity among US adults, *JAMA*. 2002; 288:1723–1727.
28. The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2004 Revised consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *J Fertil Steril*. 2003-4; 81:19–25.

29. The 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. *J Hum Reprod.*2003-4; 19:41–47.
30. Ferriman, D. Gallwey, JD. Clinical assessment of body hair growth in women. *J Clin Endocrinol Metab.*1961; 21:1440–1447.
31. Lorenzo, EM. Familial study of hirsutism. *J Clin Endocrinol Metab.*1970; 31:556–564.

CORRESPONDENCES ADDRESS

Name: Dr. Zahoor Ahmed

Address: Associate Professor,

Department of Biochemistry

Khyber Medical College, Peshawar.

E-mail: zakhan70@yahoo.co.uk