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Tetrad of Hormonal and Biochemical Manifestations in Phenotypes of Polycystic Ovary Syndrome

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Abstract

A prospective observational case- control study was conducted to evaluate the relationship and prevalence of subclinical hypothyroidism, hyperprolactenemia, impaired glucose metabolism and hyperhomocysteinemia as components of hormonal/biochemical manifestation tetrad amongst four phenotypes of polycystic ovary syndrome (PCOS) with respect to controls. 200 women diagnosed as PCOS as per the ESHRE/ASRM Rotterdam criteria were taken as cases (group I) and 200 as controls (group II).All women recruited came for subfertility treatment to assure homogeneity in study population. Group I was further divided into four phenotypes. Records of demographic (age, BMI, duration of infertility), biochemical(homocysteine,fasting blood sugar,HBA1C)and hormonal (thyroid profile and prolactin)parameters of patients were taken. Both groups were comparable in age, ethnicity and marital status. Percentages of phenotypes A, B, C and D in our Indian population were 25.5%,16%,35.5% and 23 % respectively. BMI was significantly more in PCOS as compared to non-PCOS with highest mean in phenotype B subgroup. All parameters measured were significantly increased in PCOS group compared with non-PCOS group with prolactin levels similar in all phenotypes. Impaired glucose tolerance and diabetes incidence was more in phenotype C and D. Hypothyroidism and hyperhomocystenemia were higher in phenotypes B and C. Phenotypes are affected by hormonal and biochemical manifestations and if followed for long term may be associated with metabolic syndrome in future. Thus non classical phenotypes should also be properly monitored and treated as this hormonal imbalance and biochemical derangements add to the brunt of management of PCOS.

Keywords: Hyperhomocysteinemia, Hyperprolactinemia, Hypothyroidism, Impaired glucose metabolism

Introduction

Polycystic ovarian syndrome (PCOS) is the most common multisystem endocrinopathy with epigenetic and environmental influences affecting early pre-pubertal age to post-menopausal age group with varied presentations in each (1). The variability in etiopathogenesis and clinical presentation has made the appropriate diagnosis of PCOS difficult (2). This endocrine-metabolic disorder affects 5%–10% of women of reproductive age with oligo-anovulation, menstrual disturbances, and/or androgen excess as the main features (3, 4).It has been affirmed by various studies that hypothyroidism is also a state of insulin resistance which is the crux of genesis of PCOS. Individually both hypothyroidism and PCOS have changes in lipid metabolism (increase in total cholesterol and in low-density lipoprotein cholesterol) and endothelial function leading to increased risk of arterial hypertension and other cardiovascular problems(5). Association of thyroid dysfunction and other hormonal and biochemical parameters of PCOS are thus frequently studied to establish a relationship between the two.

PCOS is a constellation of symptoms and signs having significant implications on woman's health and fertility (6). Phenotypes are influenced by genetic, racial, geographic, environmental and lifestyle factors like diet and over-nutrition.This affects them causing more adverse effects in respect to reproductive and metabolic outcome (7). PCOS is characterized by vast diversity in manifestations, necessitating a a multidisciplinary approach to benefit the patient in a holistic manner depending on evidence based medicine practice (8).We have evaluated prevalence of various phenotypes and scrutinized their hormonal and biochemical characteristics compared to non-PCOS women presenting to our center.

For all clinical manifestations, the root cause is hyperandrogenemia and impaired insulin response (9). Hypothyroidism leads to metabolic derangements such as decrease in sex-hormone binding globulin causing increased levels of free androgens in blood and decreased metabolic clearance, defective glucose disposal and hyperlipidemia, thus affecting gonadal function and fertility (10, 11). Increased TSH levels along with hyperprolactenemia lead to deposition of mucopolysaccharides in various organs such as ovaries affecting its function resulting in anovulation and reproductive disruption.

Beta cell dysfunction and insulin resistance can lead to development of type 2 diabetes mellitus. Insulin resistance may lead to increase in homocysteine levels due to altered methionine metabolism (12). High sensitivity C-reactive protein, adiponectin and homocysteine are serum biomarkers of cardiovascular disease and are found to be abnormal in women with PCOS. There is clustering of obesity, impaired glucose tolerance, dyslipidemia and hypertension as risk factors (13).

Measurement of hormonal and biochemical parameters in this study will help in providing early interventions in women with

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PCOS to prevent progression from pre-diabetic state to full blown diabetes and other risk factors.

Materials and methods

Study design and participants

This prospective observational case-control study was conducted at Milann fertility center which is a tertiary care center. Study was conducted from April 2017 to January 2018.Women were divided into two groups according to European Society of Human Reproduction & Embryology/American Society for Reproductive Medicine(ESHRE/ASRM) Rotterdam criteria into cases and controls. Rotterdam criteria for the diagnosis of PCOS includes presence of any two of the following criteria:clinical or biochemical hyperandrogenemia, anovulation/Oligomenorrhea and polycystic ovaries on ultrasonography (14). They were recruited at the time of diagnosis and who have not yet initiated treatment for any biochemical and hormonal derrangements. All participants before allocation signed an informed consent form. Approval was obtained from the Institutional Ethical Committee.

Women of group I were divided into four phenotypes - hyperandrogenism (HA) + oligo-/anovulation (OA) + polycystic ovaries at ultrasound (PCO) (phenotype A,full-blown syndrome); HA + OA (phenotype B, former National Institutes of Health definition); HA + PCO (phenotype C) and OA + PCO (phenotype D). Four different combinations of phenotypes have been deciphered by Rotterdam/AEPCOS society based on clinical and endocrinological findings (15). Consequently records of demographic, hormonal and biochemical parameters of patients were taken.

Patient population

Inclusion criteria:

All PCOS defined as per ESHRE/ASRM Rotterdam criteria of 2003 for group I and non-PCOS in group II, aged 21-35 years, BMI >18 and <30 kg/m2, no symptoms of hypothyroidism and free thyroxine levels (t4) in range of 4.6-12ug/dl, proven subfertility and willingness to participate in study.

Exclusion criteria:

Patients with chronicdiseases like overt hypothyroidism and hyperthyroidism, kidney or liver failure, late-onset adrenal hyperplasia and diabetes, severe endometriosis, severe male factor infertility, multiple fibroids, previous IVF treatment failure and those not willing to participate.

Recruitment and analysis

All patients who came to our center who were unable to conceive were interviewed in detail. History taking comprised of complaints,past treatment history,menstrual history related to age of menarche, regularity, duration, and number of cycles per year,marital history,contraceptive and obstetric history.Questions regarding past medical, surgical history and family history were also asked.Written informed consent was taken from all patients recruited for the study.

Oligomenorrhea was defined as an inter-menstrual interval of more than 35 days or a total of eight or fewer menstrual cycles per year. Duration and extent of abnormal hair growth, weight gain, and development of acne or alopecia, along with family history of hirsutism, menstrual disorders, and diabetes mellitus or glucose intolerance was written in record file. Women giving history of any use of hormonal preparation, androgens or any significant drug intake suspected to affect metabolic function were not included in the study.Women with any renal, hepatic, thyroid or cardiac dysfunction,Cushing's syndrome, congenital adrenal hyperplasia and adrenal tumors were excluded. All women had to undergo detailed anthropometric assessment in form of measurement of height,weight,waist and hip circumference,blood pressure and systemic examination .Body mass index (BMI) was calculated by the formula: weight in kilograms/(height in meters)2 .Hirsutism was assessed using the modified Ferriman-Gallwey score, with nine specified body areas counted by a single observer.A score of more than/equal to 8 out of 36 was considered significant. Biochemical hyperandrogenism was defined as a serum total T level of >0.022 nmol/L. Trans-vaginal ultrasonography was done by a single clinician to rule out any inter-observer variation to see presence of more than 12 ovarian follicles 2-9 mm in any ovary and increased ovarian volume > 10 cc(calculated using the formula 0.5 × length × width ×thickness)suggestive of PCOM (polycystic ovary morphology).Women were divided into 2 groups based on ESHRE/ASRM Rotterdam criteria with group I as PCOS women(cases) and group II as non-PCOS women(controls).

Thyroid stimulating hormone (TSH), free thyroxine (FT4), glycosylated hemoglobin (HBA1C), fasting blood glucose (FBS), prolactin (PRL) and plasma homocysteine (PH) were measured in all women recruited in the study. The blood samples were obtained from peripheral vein in early morning after a fasting period of atleast 8 hours. TSH, FT4 and PRL and total T levels were measured by electrochemiluminescence assay (Cobas e411-Hitachi).Plasma homocysteine and fasting blood sugar were measured by enzymatic method through Cobas c311.HBA1C was measured through BioRad DIO method. Intra- and inter assay variations were within the limits permitted by manufacturer company.

Subclinical hypothyroidism is defined as serum TSH levels of >2.5mIU/L with no clinical symptoms and signs having normal FT4 levels according to ASRM guidelines. In our study a TSH cutoff of 2.5 IU/L was taken to define subclinical hypothyroidism to determine association of increased TSH levels in all four phenotypes of PCOS and also in controls. Plasma homocysteine cut off of 8umol/l was taken for hyperhomocystenemia.HBA1C >5.3%, FBS >100 mg/dl and PRL >30 ng/dl cut off were taken for deranged blood sugar levels (marker for insulin resistance) and hyperprolactenemia respectively. The results were described as mean ± standard deviation. Significance level was defined at 5%, and the software used for the analysis was the SAS statistical software package, version 9.1.

Participant flow

The participant flow is shown in Figure I. Out of 221 PCOS patients screened for study, only 200 followed up with blood tests and thus were enrolled for study. Out of 214 NON-PCOS women only 200 followed up with blood tests whowere enrolled for study. Thus 200 women with PCOS in group I and 200 non-PCOS women were included in group II for final analysis.

Statistical analysis

Descriptive statistics were presented as means and standard deviation for continuous variables. Frequencies and proportions were used for categorical variables. Independent sample t test was used for continuous variables which were normally distributed

and Mann-Whitney U test for data not normally distributed. Chi-square test or Fisher's exact test was used for categorical variables where appropriate. Odds ratio (OR) with 95% confidence intervals (CIs) was calculated. For post-hoc analysis one way ANOVA test was used. In addition, receiver operating characteristic analysis was used to evaluate TSH, HBA1C, PRL and PH as associative factors with PCOS infertile women. Histograms have been created to show association between different variables amongst cases and controls.All tests were two-sided with p-value of less than 0.05 considered as statistically significant.

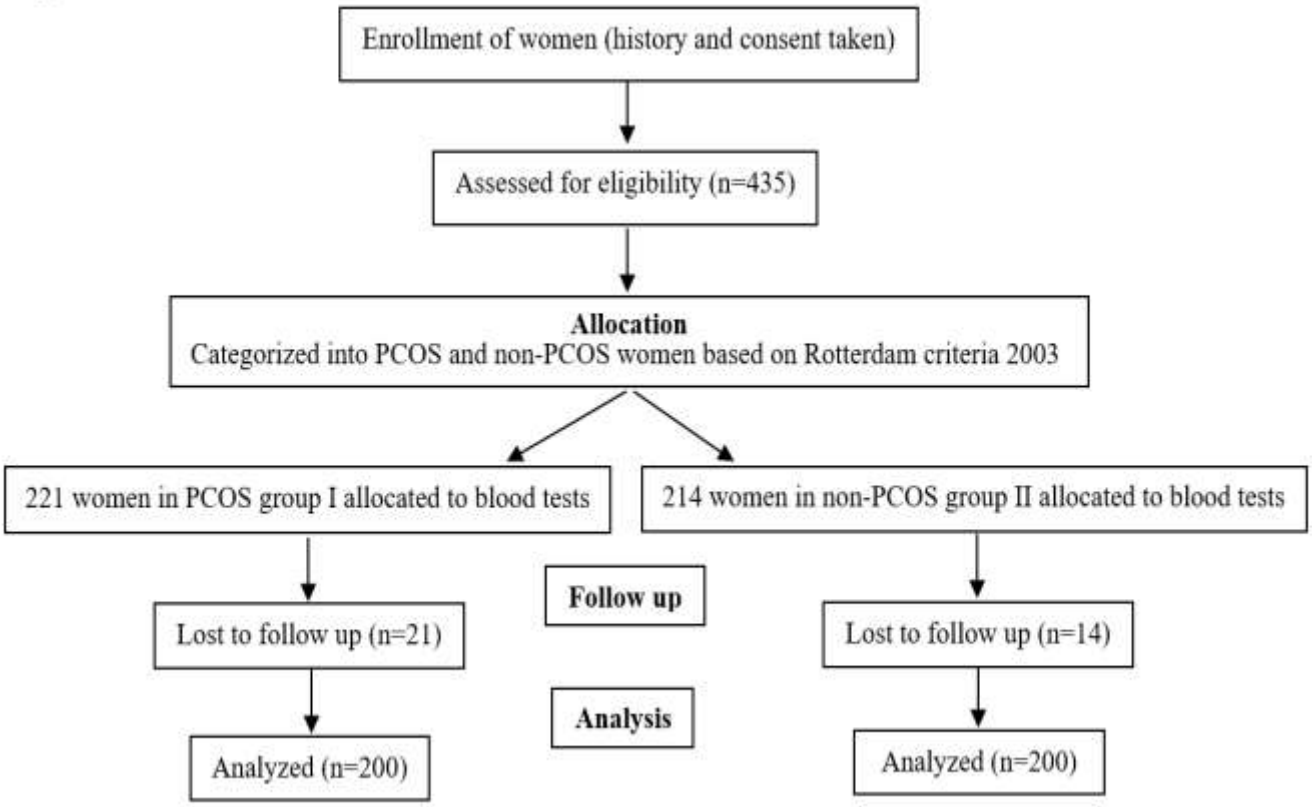

Figure 1. Participant flow chart

Results

Demographic profile of PCOS and non-PCOS women was compared and different phenotypes of PCOS women were also compared through sub-group analysis as illustrated in table 1 and 2.In our study in PCOS group maximum prevalence was of phenotype C (35.5%) followed by phenotype A (25.5%), phenotype D (23%) and least for phenotype B (16%). Mean age of women of phenotype A, B, C and D was 29.5 ±3.7, 28.9±3.7, 29.2±3.8 and 29.4±3.3 years respectively. Mean age of group II women was 30.1±4.1 years. Age was similar in both groups and sub-groups. Women with PCOS had a mean BMI of 25.9±4.3 Kg/m2 and non-PCOS women had BMI of 24.5±3.6 kg/m2. Higher BMI was observed in all phenotypes of PCOS with respect to controls (p=0.001).Obese and overweight women were seen maximum in phenotype B, followed by phenotype D, A and C and were least in controls. Both groups are similar regarding number of women presenting with primary or secondary infertility. Duration of infertility was noticed to be significantly more in cases (group I) with mean of 6.065 ±3.045 as compared to 5.605 ±2.99 in controls (group II). Table 3 illustrates various hormonal and biochemical parameters such as plasma homocysteine, AMH, TSH, PRL, FBS and HBA1C amongst PCOS group and non-PCOS group. In the PCOS group, all hormonal and biochemical parameters were observed to be higher as compared to NON-PCOS group and were statistically significant.

Both groups were divided into two categories based on their HBA1C and FBS levels into pre-diabetic state and type 2 diabetes mellitus.

CATEGORY 1 (PRE-DIABETES): HBA1C 6-6.4% AND FBS 100-125 MG/DL

CATEGORY 2 (TYPE 2 DIABETES MELLITUS): HBA1C >=6.45 AND FBS >=126 MG/DL

Population histogram was created for each category for both groups.Our study has deciphered prevalence of IGT as 17.5% and type 2 DM as 2% in PCOS women and 5% IGT and 1.5% T2DM prevalence in non-PCOS population as depicted in table 4 which

is quite similar to previous studies done across the world.

Association of pre-diabetes and T2DM was calculated with homocysteine and prolactin levels. High HBA1C and increased fasting glucose level is more prevalent in PCOS women with hyperhomocystenemia.This shows association between these biochemical factors in PCOS resulting in the acquisition of the endocrinological tetrad. There is higher prevalence of hyperprolactenemia in PCOS group as compared to non-PCOS group as illustrated in population histogram in figure 2. 69.5% (n=139) of PCOS women had HBA1C levels of more than /equal to 5.3 % as compared to 44.5% (n=89) of non-PCOS women. With regard to glucose metabolism, mean HBA1C values were found to be higher in women with PCOS with statistically significant difference (p<0.001). Highest HBA1C levels was seen in phenotype D,then phenotype C and B followed by least in phenotype A as depicted in figure 2 (A).Fasting blood sugar was found highest in phenotype D, then phenotype C and B with similar levels and least in phenotype A as shown in figure 2 (B). Measurement of anti-mullerian hormone (AMH) was also assessed in different PCOS women as depicted in figure 2 (C) which shows higher range in phenotype B, then phenotype D and A, followed by phenotype C with least level.

We studied association of subclinical hypothyroidism in different phenotypes of PCOS and histogram was formed for sub-group analysis and main group analysis both. 55 % (n=105) women in group I had TSH>= 2.5 m IU/L and only 39.5% (n=79) of group II had TSH>= 2.5 m IU/L. There was statistically significant difference between the two groups (P=0.041).Our study showed higher prevalence of SCH in women with PCOS compared with non-PCOS women. Bar graph with standard error of mean in figure 3 (C) depicts that TSH levels are highest in women with phenotype B followed by phenotype C and D and least in phenotype A. 6.5%(n=13) women of group I and 5.5%(n=11) women of group II had prolactin levels of more than/equal to 30 ng/ml. There was a statistically significant association of PRL with PCOS (p<0.001). It is portrayed in figure 3 (B) that all four phenotypes of PCOS have similar levels of prolactin. 68 % (n=136) of PCOS women and only 49 %(n-98) of NON-PCOS group of women had plasma homocysteine levels of more than/equal to 8. The mean serum homocysteine concentration was significantly higher in the PCOS group (11.45±6.563 mmol/l) versus in NON-PCOS group (9.72±7.404 mmol/L) with p value <0.001. Our study has outlined higher homocysteine levels in phenotype C followed by phenotype B. Phenotype A and D has similar prevalence with similar homocysteine levels as illustrated in figure 3 (D).

Table 1: demographic data of PCOS and non-PCOS women

Variable	Group I (PCOS women)	Group II (non-PCOS women)	P value
Age (years)	29.3±3.6	30.1±4.1	0.025
BMI(kg/m2)	25.9±4.3	24.5±3.6	0.001
Duration of infertility (years)	6.065±3.045	5.605±2.99	-
Primary infertility(n)	144(72%)	138(69%)	-
Secondary infertility(n)	56(28%)	62(31%)	-

Data are expresses as mean± SD

Table 2: demographic data of different phenotypes of PCOS women (group I)

Variable	Phenotype A	Phenotype B	Phenotype C	Phenotype D
Age (years)	29.5±3.7	28.9±3.7	29.2±3.8	29.4±3.3
BMI(kg/m2)	25.4±3.9	27.3±4.8	24.9±3.5	26.4±4.4
Duration of infertility (years)	6.5±3.5	5.9±3.3	5.6±2.7	6.3±2.8

Data are expresses as mean± SD

Table 3: hormonal and biochemical parameters

Variable	Group I (PCOS women)	Group II (non-PCOS women)	P value
Plasma homocysteine	11.45±6.563	9.72±7.404	<0.001
AMH	6.41±3.699	2.51±2.270	<0.001
TSH	2.74± 1.496	2.57±1.580	0.041
PRL	17.49±6.723	14.59±7.794	<0.001
HBA1C	5.50±0.628	5.31±0.458	<0.001
FBS	87.78±19.02	77.40±13.61	<0.001

Data are expresses as mean± SD

Table 4: prevalence of pre-diabetes and type 2 diabetes melllitus in PCOS and non-PCOS women

Variable	Group I (PCOS women)	Group II (non-PCOS women)
Pre0diabetes	35(17.5%)	10(5%)
Type 2 diabetes mellitus	4(2%)	3(1.5%)

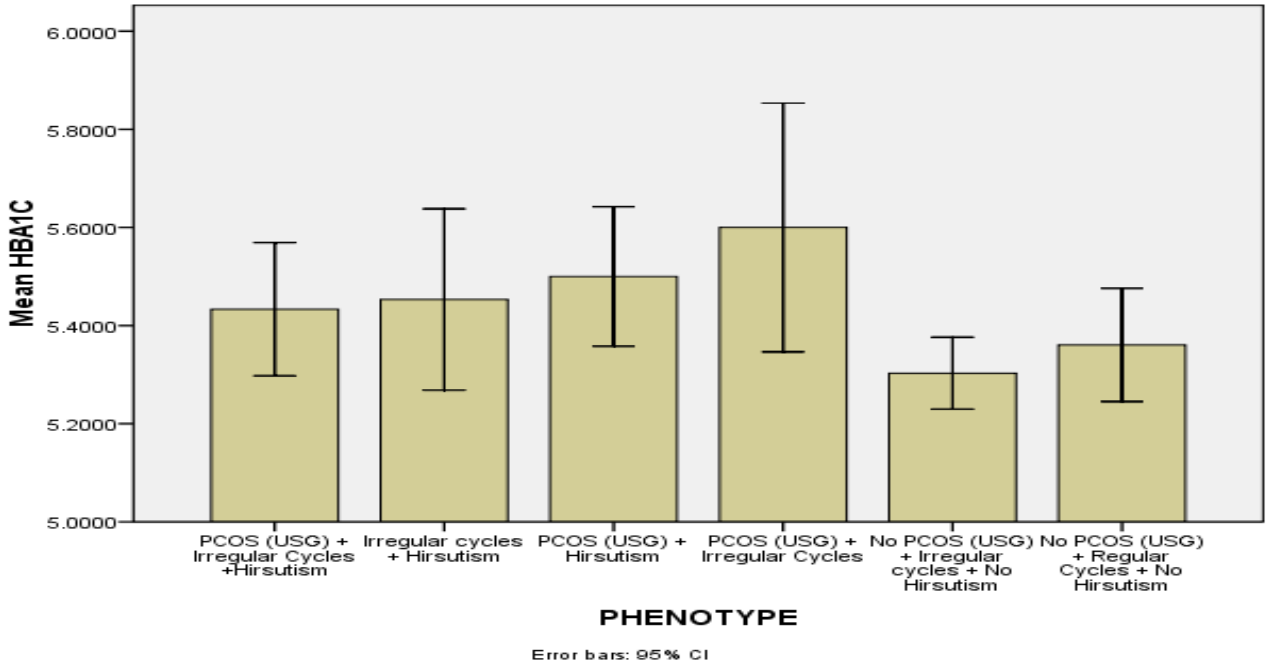

Figure 2 (a). bar graph depicting prevalence of increased hba1c levels in different phenotypes of PCOS

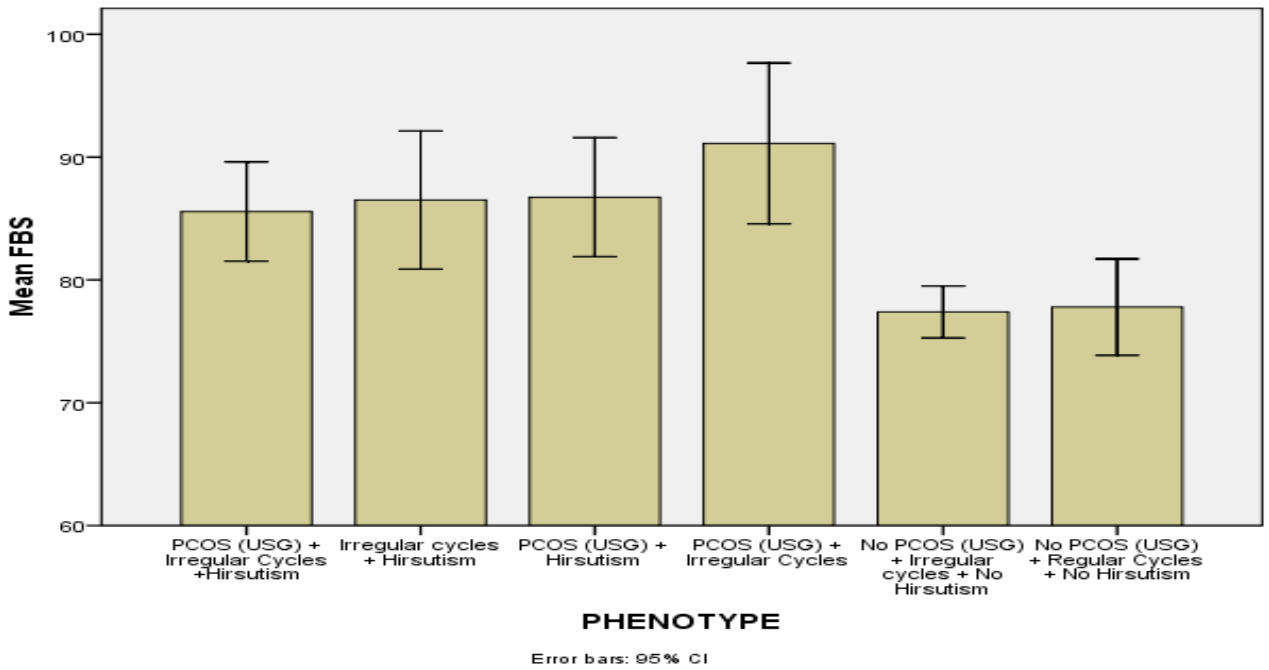

Figure 2 (b). bar graph depicting prevalence of fasting blood sugar in different phenotypes of PCOS

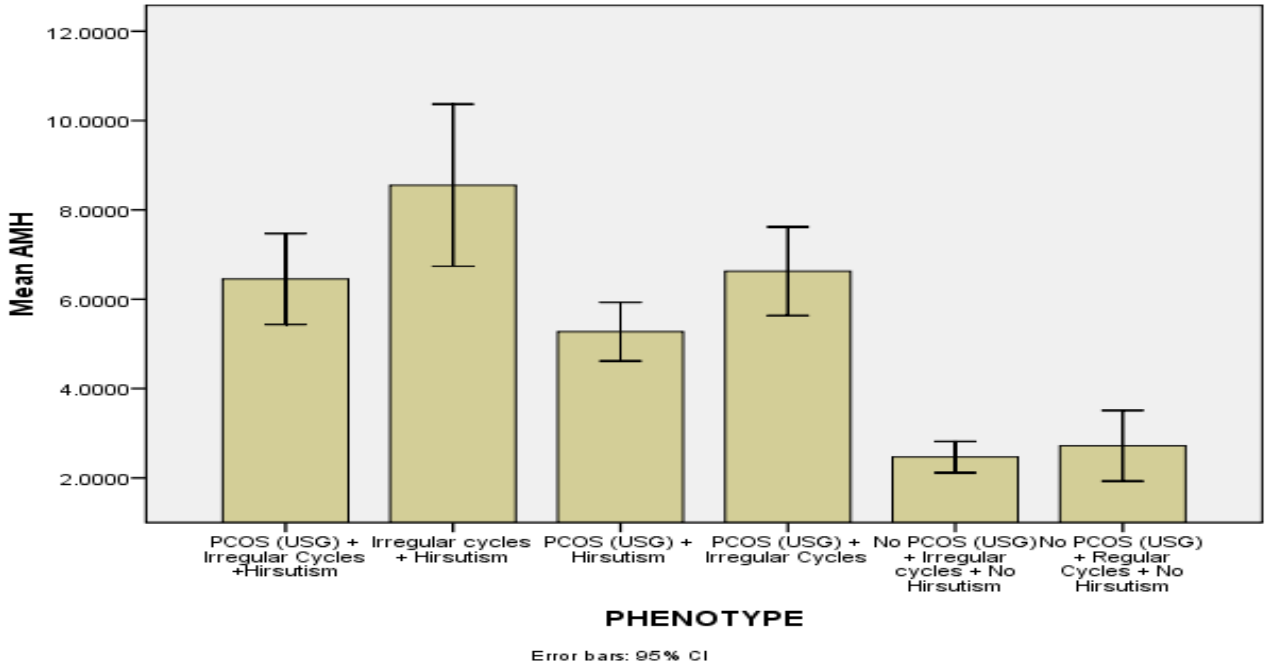

Figure 2 (c). bar graph depicting prevalence of anti mullerian hormone in different phenotypes of PCOS

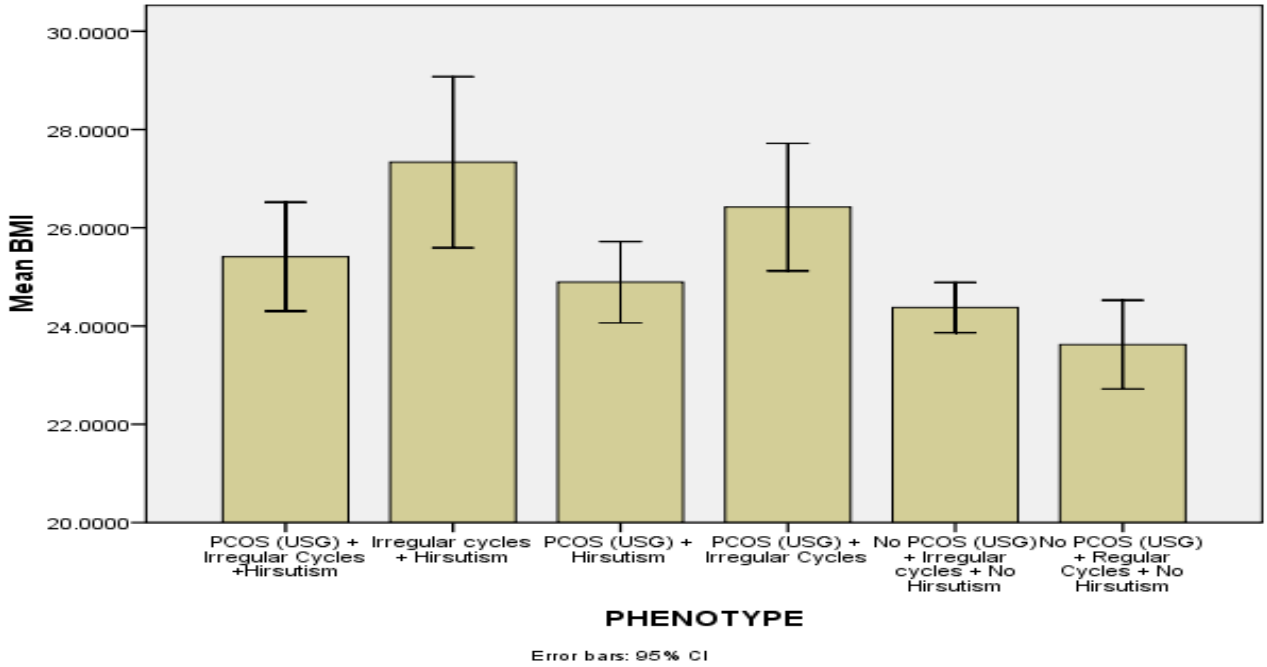

Figure 3 (a). bar graph depicting prevalence of increased BMI in different phenotypes of PCOS

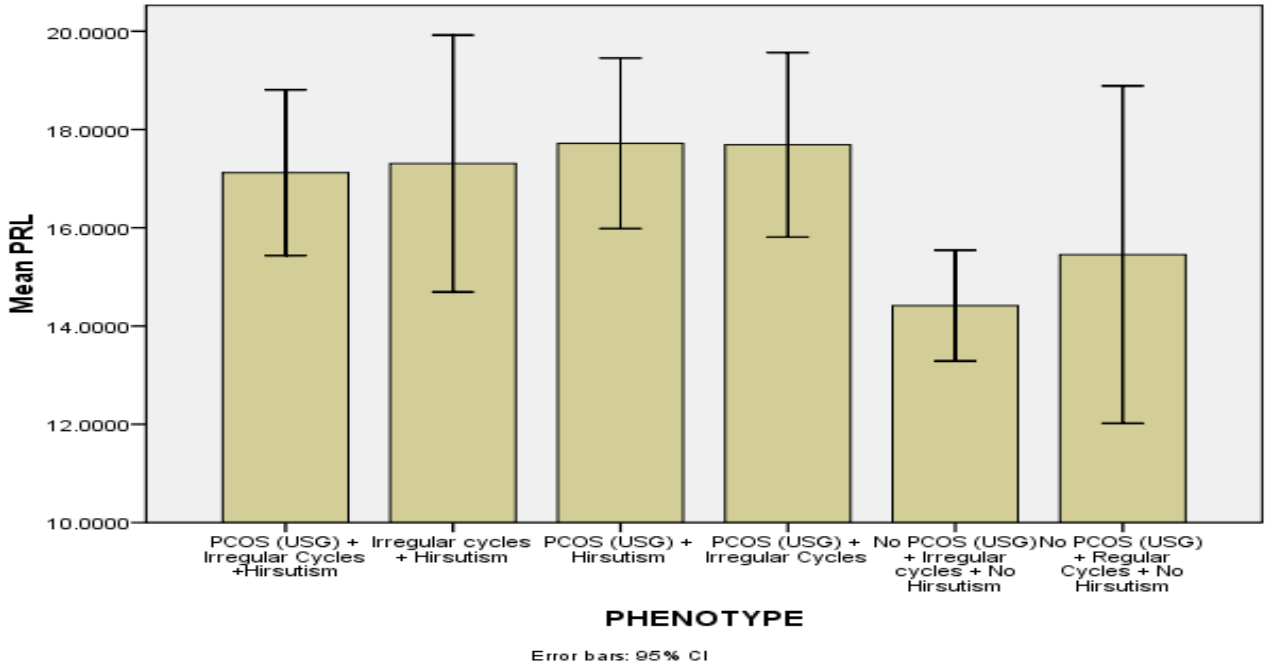

Figure 3 (b). bar graph depicting prevalence of hyperprolactenemia in different phenotypes of PCOS

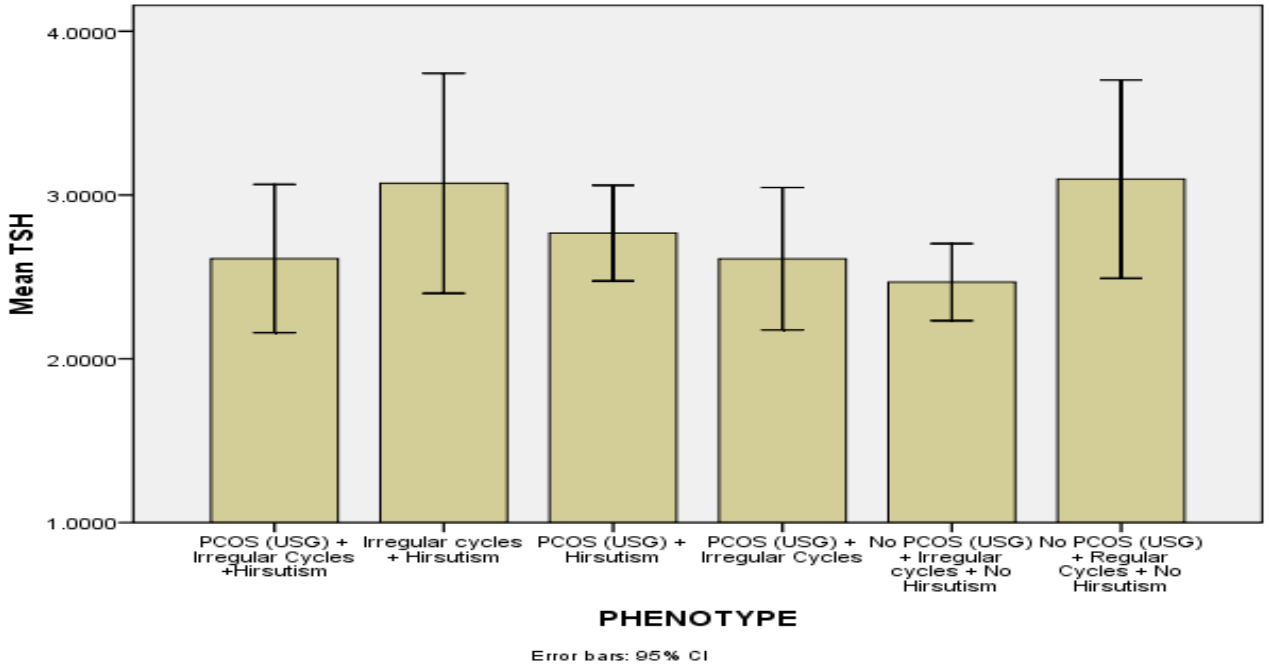

Figure 3 (c). bar graph depicting prevalence of hypothyroidism- increased TSH in different phenotypes of PCOS

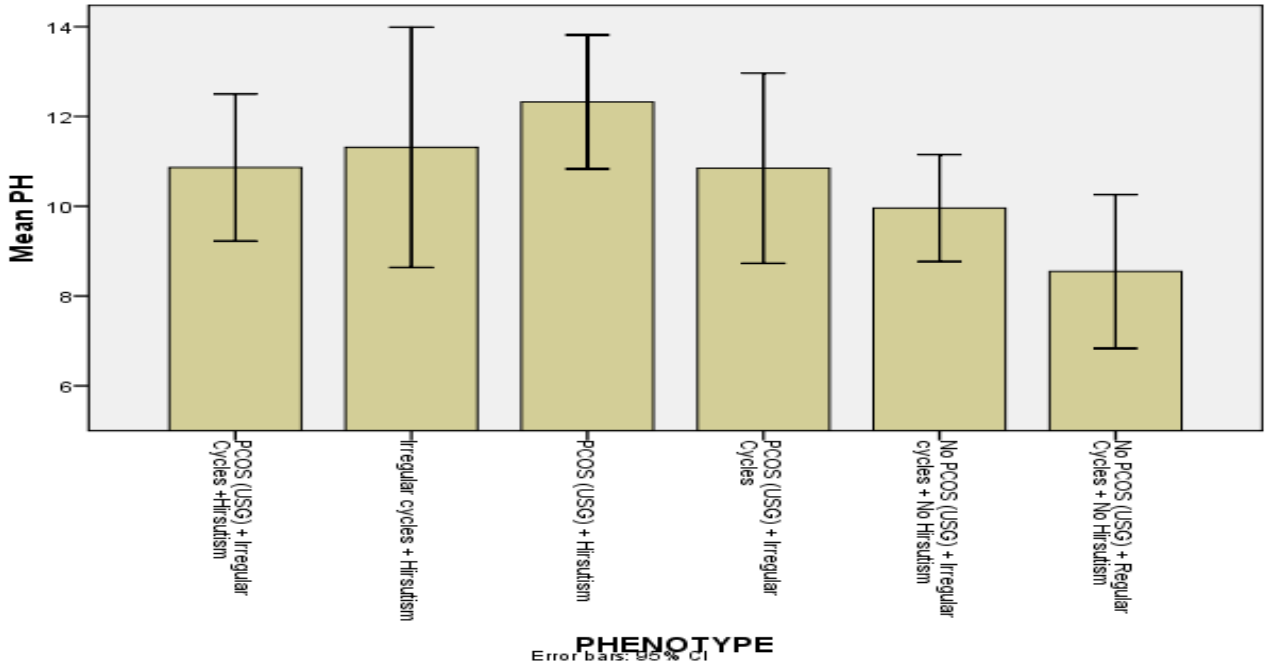

Figure 3 (d): bar graph depicting prevalence of hyperhomocysteinemia in different phenotypes of PCOS

Discussion

The symbiosis hypothyroidism, hyperprolactenemia, hyperhomocystenemia and parameters of imapired glucose metabolism in different phenotypes of PCOS women has been of interest for many decades (16, 17). This study was conducted to calculate and investigate percentage of occurrence of different phenotypes of PCOS and their associated hormonal and biochemical parameters in indian population.

Actual prevalence of PCOS is an ongoing debate due to lack of standard and specific sampling methodologies used in available studies. Nevertheless prevalence is 6.5% according to NIH criteria and 15-25% and 6-15% according to Rotterdam and AE-PCOS society criteria respectively (18). Chauhan et al. observed following prevalence of phenotypes A,B, C and D that is 23.3, 13.3, 52.6 and 19.5 % respectively (19) and Kar et al found it to be 37.04, 11.2, 22.2 and 0.9 % respectively (20). This variation in prevalence of different phenotypes is similar to what is noticed in our study. Similar findings of higher BMI in phenotype B was found in a study done by Welt et al (21).

PCOS characterizes an activation of inflammatory mediators with increase in the levels of adipokines, interleukins and chemokines.Hyperinsulinemia and increased insulin-like growth factor (IGF) play a significant role in disordered folliculogenesis (22). Autocrine,paracrine and endocrine pathways within granulosa and theca cells of ovarian follicle ensures proper steroid bioavailability for optimal folliculogenesis and oocyte maturation (23). Paracrine regulation in theca cells stimulated by granulosa cells further instigate steroidogenesis by increasing theca cell 17a-hydroxylase/17–20 lyase (P450c17) activity and thus enhancing androgen production.Thecal androgen production under LH influence supermounted by hyper-insulinemia is the cornerstone for pathological function of ovaries in PCOS (24).

Paradoxically while peripheral resistance is there towards insulin, ovaries are highly responsive to increase in blood insulin levels. This is due to tissue specific use of different paralogous genes within similar transduction gateway.Insulin receptors on human theca cells surrounded by stroma and granulosa cells help in stimulation of ovarian steroidogenesis (25). Fasting serum insulin and body mass index positively correlate with follicular insulin levels (26). Interactions between insulin and its receptors present on cumulus cells present in follicular fluid help in determination of insulin action on development of oocyte.Lactate formation via glycolysis is an important energy substrate for terminal differentiation and oocyte maturation. In PCOS women,granulosa-luteal cells have decreased glucose uptake and lactate production due to insulin exposure.Glycogen synthesis in theca and granulosa cells is also affected which is responsible for impaired glucose metabolism and abnormal luteinization in follicle (27).

IR exacerbates many manifestations of PCOS significantly .It also puts women at risk of type 2 diabetes mellitus. The prevalence of DM in women with PCOS is 10% approximately and is increasing at a rate of 2-3% annually.Tendency to accumulate body fat increases in PCOS and this is linked to T2DM and cardiovascular diseases (28).

Hemoglobin A1c (HbA1c) is a marker of chronic hyperglycemia which is commonly used nowadays as it reveals average blood glucose for about two to three months.There is no day to day variability and no need of fasting or having any dietary preparations.International expert committee opinion composed of members from European association of diabetes, international diabetes federation and American diabetes association has stated that HBA1C should be used as a screening test and if value <=6

% no further test required and if in between 6-6.5% OGTT test is required for confirmation (ADA, 2011). According to study by Ehrmann et al, impaired glucose tolerance is present in 31-35% and type 2 diabetes mellitus in 7.5-10% of PCOS women in America (29). European women has a slight lesser prevalence of both (IGT 12.4%, T2DM 1.7).Our study showed similar findings. To our knowledge no study has been done on Indian population regarding use of HBA1C or fasting glucose versus oral glucose tolerance test to differentiate between pre-diabetes and type 2 diabetes mellitus.To increase the significance of association between different phenotypes of PCOS and non-PCOS women,fasting blood sugar was also measured. Available data from our study depicts that non classic PCOS with phenotype D has more deranged blood sugar levels as interpreted from increased fasting blood sugar levels and HBA1C.This shows that even in absence of hyper androgenemia manifestations of insulin resistance and impaired glucose metabolism can persist as a root cause of etiopathogenesis of PCOS and various long term effects (30).

Due to this, need of screening all PCOS women using 2-h oral glucose tolerance test was recommended by Androgen Excess Society (AES) in 2007 (31). A more recent consensus statement by the AES (2010) recommends the performance of a 2-h OGTT only in obese women, lean or overweight women, family history of T2DM or age>40 years but this approach seemed time consuming and difficult due to high prevalence of PCOS women (32). Universal screening of PCOS women with OGTT is inconvenient and not recommended. Thus this led to new consensus formulated by American Diabetes Association (ADA-2013) that suggested HbA1C and fasting glucose are equally appropriate as 75 gram 2 hour OGTT for screening. For OGTT patient has to be fasting overnight and attend clinic next day to be assessed. Any strategy which will replace OGTT testing will be beneficial in present scenario. Our study has tried to examine utility of HBA1C and fasting blood sugar in estimation of pre-diabetes (impaired glucose tolerance) and type 2 diabetes mellitus.American diabetes association (2012) has suggested that likewise general population, HBA1C might also be useful screening tool in women with PCOS for diabetes and prediabetes. Study by Vrbikova et al has identified similar prevalence of IGT and T2DM in PCOS women with either OGTT or HBA1C testing suggesting preferred use of latter in view of convenience and no limitations of fasting state (33). The prevalence of glucose intolerance in Indian women with PCOS is reported as 16.3% (adults 19.1%, adolescents 9.7%). The sensitivity and the specificity of HbA1c for the diagnosis of diabetes were 35% (range 14%–55%) and 99% (range 98%–100%), respectively, compared with the diagnosis established by OGTT.Based on similar researches, our study has examined utility of HBA1C and fasting glucose in determining prevalence of pre-diabetes and type 2 diabetes mellitus in PCOS women.

Mueller et al has reported an increase in IR in PCOS women having subclinical hypothyroidism, similar to findings of our study.Increased thyrotropin releasing hormone(TRH) secretion from hypothalamus also stimulates prolactin secretion from pituitary causing hyperprolactenemia (34). Homocysteine is an amino acid which is formed by conversion of methionine to cysteine and is metabolized by re-methylation and trans-sulfuration.Research suggests that non-enzymatic factors such as age,gender,smoking,chronic inflammation, nutrition, caffeine intake and physical activity also influence homocysteine levels in serum. Insulin inhibits cystathione beta synthase activity in liver, so any hyper-insulinemic state or insulin resistance will modulate homocysteine activity. Follicular fluid also contains homocysteine. Disorders of follicular fluid composition can lead to disrupted microenvironment of oocyte.High total homocysteine concentration is detrimental to oocyte quality, fertilization, cleavage, embryo quality, implantation, embryogenesis and fetal outcome. Endometrial blood flow and vascular integrity is also affected negatively thus leading to implantation failure. It has been observed that T2DM, atherogenesis,chronic vascular damage and nephropathy is associated with increased homocysteine levels especially when hyperinsulinemia is present.Framingham Study concludes that hyperhomocysteinemia is associated with hyper-insulinemia and accounts for increased risk of cardiovascular disease.Study by Yarali et al has detected elevated plasma homocysteine levels in women with PCOS which is similar to our study findings (35). Study by Ebisch et al has shown significant negative association between follicular fluid homocysteine levels and quality of oocyte and embryo during ART (36). Berker et al has also concluded similar relevant findings of association of hyper-homocystenemia in PCOS women (37).

Strengths of the study

This prospective study in Indian women is the first study so far. We have assessed prevalence of different clinical manifestations in all four phenotypes of PCOS women. Measurement of both HBA1C and FBS levels led to development of a more powerful association of both hormonal and biochemical parameters in all four phenotypes of PCOS women. Counselling regarding the long term risks associated with hormonal and metabolic derangements can be done with this study.

Limitations

As PCOS was diagnosed according to the Rotterdam criteria, our findings may differ from other PCOS cohorts diagnosed by different criteria (AES or NIH).Reproductive outcome has not been included in this part of the study as it is an ongoing study with a long follow up period. Outcome data will be shared in the next article.

Conclusion

PCOS is a lifelong condition which manifests differently in different phenotypes with definite impact on cardio-metabolic status of a woman (38). Our study has provided evidence that hormonal and biochemical profile is deranged in all phenotypes of PCOS as compared to controls. Thus a careful stringent surveillance and patient-tailored approach needs to be deciphered to follow up such women throughout life. Clinician should understand that proper diagnosis and appropriate identification of phenotype is necessary to counsel these women about their present and future long term health implications (39). Whole disease spectrum should be treated all together and not only orient treatment to problem woman pursued medical assistance for.

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Conflicts of interest

The author declares that there is no conflict of interest.

References

1. Strauss JF. Some new thoughts on the pathophysiology and genetics of polycystic ovarian syndrome. Annals of the New York Academy of Sciences. 2003;997(1):42-48.
2. Zhang HY, Zhu FF, Xiong J, Shi XB, Fu SX. Characteristics of different phenotypes of polycystic ovary syndrome based on the Rotterdam criteria in a large-scale Chinese population. British Journal of Obstetrics and Gynecology. 2009;116:1633-1639.
3. Dokras A, Bochner M, Hollinrake E, et al. Screening women with polycystic ovary syndrome for metabolic syndrome. Obstetetrics Gynecology. 2005;106:1317.
4. Alaee S, Talaiekhozani A, Ziaei GR, Lohrasbi P. Evaluation of Iranian college students' awareness about infertility risk factors. Jundishapur Journal of Health Sciences. 2016; 8 (2).
5. Orio F Jr, Palomba S, Cascella T, et al. The increase of leukocytes as a new putative marker of low-grade chronic inflammation and early cardiovascular risk in polycystic ovary syndrome. Journal of Clinical Endocrinology and Metabolism. 2005;90(1):2-5.
6. Livadas S, Diamanti E. Polycystic ovarian syndrome: definitions, phenotypes & diagnostic approach. Frontiers of Hormone Research. 2013;40:1-21.
7. The Rotterdam ESRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long term health risks related to polycystic ovarian syndrome. Fertility and Sterility. 2003;81:19-25.
8. 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. Biomedical Central Medicine. 2010;30(8):41.
9. De Leo V, Musacchio MC, Palermo V, et al. Polycystic ovary syndrome and metabolic co-morbidities: therapeutic options. Drugs Today. 2009;45:763-775.
10. Nishida C, Ko GT, Kumanyika S. Body fat distribution and noncommunicable diseases in populations: Overview of the 2008 WHO expert consultation on waist circumference and waist-hip ratio. European Journal of Clinical Nutrition. 2010;64:2-5.
11. DeUgarte CM, Bartolucci AA, Azziz R. Prevalence of insulin resistance in the polycystic ovary syndrome using the homeostasis model assessment. Fertility and Sterility. 2005;83:1454-1460.
12. Diamanti-Kandarakis E, Kouli C, Tsianateli T, Bergiele A. Therapeutic effects of metformin on insulin resistance and hyperandrogenism in polycystic ovary syndrome. European Journal Of Endocrinology. 1998;138:269-274.
13. National Cholesterol Education Program Expert Panel on Detection E, Treatment of High Blood Cholesterol in A. Third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III) final report. Circulation. 2002;106:3143-421.
14. Rotterdam EA-SPCWG. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertility and Sterility. 2004;81:19-25.
15. Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W; Task Force on the Phenotype of the Polycystic Ovary Syndrome of The Androgen E, Society P. The Androgen Excess and PCOS Society criteria for the polycystic ovary syndrome: The complete task force report. Fertility and Sterility. 2009;91:456-488.
16. Alaee S, Bagheri MJ, Ataabadi MS, Koohpeyma F. Capacity of Mentha spicata (spearmint) extracts in alleviating hormonal and folliculogenesis disturbances in a polycystic ovarian syndrome rat model. World's Veterinary Journal. 2020(3):451-6.
17. Lizneva D, Suturina L, Walker W, et al. Criteria, prevalence, and phenotypes of polycystic ovary syndrome. Fertility and Sterility. 2016;106:6-15.
18. Yildiz BO, Bozdag G, Yapici Z, et al. Prevalence, phenotype and cardiometabolic risk of polycystic ovary syndrome under different diagnostic criteria. Human Reproduction. 2012;27:3067-3073.
19. Chauhan S, Joshi B, Mukherjee S, et al. A cross-sectional study of polycystic ovarian syndrome among adolescent and young girls in Mumbai, India. Indian Journal of Endocrinology and Metabolism. 2014;18(3):317-324.
20. Kar S. Anthropometric, clinical & metabolic comparison of four Rotterdam PCOS phenotypes-a prospective study. Journal Of Human Reproductive Sciences. 2013;6(3):194-200.
21. 19. Welt CK, Gudmundsson JA, Arason G, et al. Characterizing discrete subsets of polycystic ovary syndrome as defined by the rotterdam criteria: the impact of weight on phenotype and metabolic features. Journal of Clinical Endocrinology and Metabolism.2006;91(12):4842-4828.
22. 20. Diamanti-Kandarakis E, Dunaif A. Insulin resistance and the polycystic ovary syndrome revisited: An update on mechanisms and implications. Endocrine Reviews. 2012;33:981-1030.
23. 21. Panidis D, Tziomalos K, Misichronis G, et al. Insulin resistance and endocrine characteristics of the different phenotypes of polycystic ovary syndrome: a prospective study. Human Reproduction. 2012;27(2):541-549.
24. 22. Gambineri A, Patton L, Altieri P, et al. Polycystic ovary syndrome is a risk factor for type 2 diabetes: Results from a long-term prospective study. Diabetes. 2012;61:2369-2374.
25. 23. Georgopoulos NA, Papadakis E, Armeni AK, et al. Elevated serum androstenedione is associated with a more severe phenotype in women with polycystic ovary syndrome (PCOS). Hormones (Athens) 2014;13:213-221.
26. 24. Legro RS, Finegood D, Dunaif A. A fasting glucose to insulin ratio is a useful measure of insulin sensitivity in women with polycystic ovary syndrome. Journal of Clinical Endocrinology and Metabolism. 1998;83:2694-2698.
27. 25. Salley KE,Wickham EP, Cheang KI, et al. Glucose intolerance in polycystic ovary syndrome: a position statement

of the Androgen Excess Society. Journal of Clinical Endocrinology and Metabolism. 2007;92:4546-4556.

28.26. Williams CM. Lipid metabolism in women. Proceedings Of Nutrition Society. 2004;63:153-160

29.27. Ehrmann DA, Barnes RB, Rosenfield RL, et al. Prevalence of impaired glucose tolerance and diabetes in women with polycystic ovary syndrome. Diabetes Care. 1999;22(1):141-146.

30.28. Saxena P, Prakash A, Nigam A, et al. Polycystic ovary syndrome: is obesity a sine qua non? A clinical, hormonal, and metabolic assessment in relation to body mass index. Indian Journal of Endocrinology and Metabolism. 2012;16(6):996-999.

31.29. Lerchbaum V, Schwetz A, Giuliani B, Pietsch O. Assessment of glucose metabolism in polycystic ovary syndrome: HbA1c or fasting glucose compared with the oral glucose tolerance test as a screening method. Human Reproduction. 2013;28(9):2537-2544.

32.30. Saxena P, Prakash A, Nigam A. Efficacy of 2-hour post glucose insulin levels in predicting insulin resistance in polycystic ovarian syndrome with infertility. Journal Of Human Reproductive Sciences. 2011;4(1):20-22.

33.31. Vrbikova J, Dvorakova K, Grimmichova T, et al. Prevalence of insulin resistance and prediction of glucose intolerance and type 2 diabetes mellitus in women with polycystic ovary syndrome. Clinical Chemistry and Laboratory Medicine. 2007;45(5):639-644.

34.32. Mueller A, Schöfl C, Dittrich RS. et al. Thyroid-stimulating hormone is associated with insulin resistance independently of body mass index and age in women with polycystic ovary syndrome, Human Reproduction, 2009;24(11):2924-2930.

35.33. Yarali H, Yildirir A, Aybar F, et al. Diastolic dysfunction and increased serum homocysteine concentrations may contribute to increased cardiovascular risk in patients with polycystic ovary syndrome. Fertility and Sterility. 2001;76:511-516.

36.34. Ebisch IMW,Peters WH,Thomas,Wetzels AM, Peer PG,Steegers-Theunissen. Homocysteine, glutathione and related thiols affect fertility parameters in the (sub) fertile couple. Human Reproduction. 2006;21:1725-1733.

37.35. Berker B, Kaya C, Aytac R, Satıroglu H. Homocysteine concentrations in follicular fluid are associated with poor oocyte and embryo qualities in polycystic ovary syndrome patients undergoing assisted reproduction, Human Reproduction. 2009;24(9):2293-2302

38.36. Gluszak O, Stopinska-Gluszak U, Glinicki P, Kapuscinska R, Snochowska H, Zgliczynski W, *et al*. Phenotype and metabolic disorders in polycystic ovary syndrome. Endocrinology. 2012:569862.

39.37. Franks S, McCarthy MI, Hardy K. Development of polycystic ovary syndrome: Involvement of genetic and environmental factors. International Journal of Andrology. 2006;29:278-285.