Dyslipidemia, Insulin Sensitivity Indices, MetabolicRisk Factors, And Anti Müllerian HormoneLevels In Egyptian Women With Polycystic Ovary Syndrome

1Hanan A. Ghali Md; 2Amal A.Zidan Md, 1Department Of Obstetrics And Gynecology, Faculty Of Medicine, Zagazig University , 2Department Of Clinical Pathology, Faculty Of Medicine, Zagazig University

Abstract

Objective: To determine the levels and relationships between lipoproteins, triglycerides (TG), insulin sensitivity measures, and anti müllerian hormone (AMH) levels in polycystic ovary syndrome (PCOS) women as compared to healthy eumenorrheic controls.

Patients and method

We examined 110 PCOS and 36 controls aged 19-34 years old, body mass index (BMI), waist hip ratio (WHR), were determined, plasma levels of glucose, insulin, TG, total cholesterol(TC), low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C), lipoprotein a LP(a), Apolipoprotein B (Apo B); total testosterone, sex hormone binding globulin(SHBG) and AMH were measured, homeostasis model assessment of insulin resistance (HOMA-IR) and quantitative insulin sensitivity check index (QUICKI) were calculated.

Results

According to BMI level 29.1%, 37.2% and 33.6% were lean, overweight, and obese in PCOS group which did not differ significantly from controls. High LP (a) levels were found in 68.2% followed by low HDL-C in 63.7% of PCOS women. At all BMI levels HDL-C, and QUICKI levels were significantly lower, whereas TG, FAI, HOMA-IR levels were significantly higher in PCOS than normal women. AMH levels were significantly higher in PCOS vs. control women, yet, significantly lower in obese vs. lean PCOS women. HDL-C, and FAI were independently associated with HOMA-IR, (p=0.037), and LP (a)(p=0.23) respectively.

Conclusion

Dyslipidemia is common in PCOS and is positively correlated to FAI, BMI, and HOMA-IR.

Keywords: Dyslipidemia, polycystic ovary syndrome, anti müllerian hormone, HOMA-IR.

Introduction

Polycystic ovary syndrome (PCOS), a condition of chronic anovulation and hyperandrogenism, constitutes the most frequently encountered reproductive endocrinopathy affecting 6-10% of all women (1). Women with PCOS are hyperinsulinemic (2) and fully 40% of all cases will develop type 2 diabetes by age of 50 (30). PCOS increases the risk for cardiovascular disease, a finding consistently reported across several geographic areas and ethnic groups (4). Up to 70% of women with PCOS have dyslipidemia (5), particularly increased levels of low-density lipoprotein cholesterol (LDL-C) and decreased high-density lipoprotein cholesterol (HDL-C) levels (6).

Some studies, however, did not record any differences in lipid or lipoprotein profiles between PCOS patients, and weight matched controls (7), (8). Genetic environmental and ethnic factors may be responsible for the conflicting results. Polycystic ovaries are characterized by greater amounts of primary and preantral follicles than their normal counterparts (9), yet the antral follicle growth is arrested at the 4-7 mm stage due to production of local inhibitors as anti müllerian hormone (AMH) or inhibin (10).

AMH is produced in the granulosa cells of early developing follicles (11), and strongly correlates with the number of antral follicles (12). Women with PCOS display elevated levels of AMH when compared with age and body mass index (BMI) matched normally cycling women (13), (14). As asserted by a number of studies, AMH is a useful marker of ovarian

Correspondence
Hanan A. Ghali Md
Department Of Obstetrics And Gynecology, Faculty Of Medicine, Zagazig University

responsiveness, embryo number or assisted reproductive technology outcomes (15), (16).

This study was conducted to assess the differences between Egyptian PCOS and healthy eumenorrheic women in plasma lipids and lipoproteins, insulin sensitivity index, metabolic risk factors, and AMH serum levels and to determine their relationship to each other.

Subjects and methods

The study group consisted of 110 PCOS patients recruited from women attending the obstetrics and gynecology outpatient clinic of Zagazig University affiliated hospitals from 2007 to 2009. The criteria for PCOS included the Rotterdam consensus (17) with the Androgen Excess Society (AES) modification (18) namely; oligo or anovulation with oligomenorrhea or amenorrhea and/or polycystic ovaries (> 12 follicles 2-9 mm, or ovarian volume >10 ml) on transvaginal ultrasound, clinical or biochemical evidence of hyperandrogenism (Ferriman-Gallway score > 8) (19) free androgen index >8, respectively.

Patients with thyroid dysfunction, hyper-prolactinemia , Cushing syndrome, late onset non –classical 21-hydroxylase deficiency, and androgen secreting tumours were excluded after appropriate testing.

Thirty six women with menstrual cycles in the normal range (25-34 days) for at least 12 months, who were age and body mass index (BMI)- matched with the PCOS group and having normal androgen and 17- hydroxyprogesterone levels were enrolled as controls. On admission, all subjects had a full medical examination including past medical history and use of medications. Exclusion criteria for all subjects were hormonal therapy including contraceptives, the presence of diabetes mellitus , renal or hepatic diseases , and hypolipidemic drugs. All subjects provided informed consent to participate in this study.

All subjects underwent anthropometric measurements, a blood draw within the first 4 days of menstrual cycle in the control group and after a spontaneous bleeding episode in the PCOS group. Height (without shoes) was measured to the nearest 0.5 cm with a vertical ruler. Weight (in light clothing) was measured to the nearest 0.2 kg with a portable scale. BMI was calculated as weight (kg)/ height (m2) and women were classified as; lean < 25 kg/m2, overweight ≥ 25 kg/m2, and obese > 30 kg/m2. Waist circumference (WC) was measured according to the American Heart Association/National Heart, Lung and Blood institute guidelines (20). At the narrowest circumference between the top of iliac crest and the lower margins of ribs, an inelastic tape was placed in a horizontal plane, around the abdomen. The tape must be snug, not compressing skin, and parallel to the floor. Measurement was made at the end of normal expiration. Hip circumference (HC) was measured at the widest circumference of the buttocks at the area of the greater trochanters, then the waist hip ratio (WHR) was calculated. Blood was collected early in the morning after a 12 hour overnight fast for lipid and hormone assays. Plasma total cholesterol and TG were measured on a Roche Modular System using commercial kits; (Roche Diagnostics, Rotkrenz, Switzerland) with a coefficient of variation of 2.3 and 2.4% respectively.

HDL-C, lipoprotein (a) [Lp(a)], and apolipoprotein B (apo B) were measured using commercial assays (Roche Diagnostics) with a coefficient of variation 4.1, 2.3, 1.2% respectively . LDL-C was calculated using the Friedwald formula (21). Plasma AMH was measured using an Immunotech immunoenzymatic assay (Beckman Coulter, Marseille, France). The detection limit of the assay was 0.1ng/ml. Fasting venous blood samples were taken for the assessment of plasma glucose, insulin, serum testosterone, and sex hormone binding globulin (SHBG). Glucose (mg/dl) was assessed by using enzymatic assay (Yellow Springs Glucose Analyzer), insulin (IU/ml) was measured by a solid-phase enzyme- amplified sensitivity immunoassay (INS-EASIA; Biosource Technologies, Nivelles, Belguim). Total testosterone (T) (ng/dl) was measured by testosterone enzyme immunoassay test kit (LI7603; Linear Chemicals). Dehydroepiandrosterone- sulfate (DHEA-S) (ng/ml) was measured by radioimmunoassay (Cisbio International, France).

Sex hormone binding globulin (SHBG) serum levels (nmol/L) were measured by ELISA (SHBG ELISA, MX 520 11;IBL, Hamburg, Germany). Glucose tolerance was determined by a 75- gram oral glucose tolerance test. The quantitative insulin sensitivity check index (QUICKI) (22), and homeostasis model assessment of insulin resistance (HOMA-IR) (23) were used as surrogate measures of insulin sensitivity. They were calculated as follows; QUICKI= 1/log fasting insulin (μ IU/ml) + log fasting glucose (mg/dl) [mg/d for glucose= mmol/L *18.182], HOMA-IR= fasting blood glucose (mg/dl) * fasting insulin (μIU/ml)/405.

Free androgen index (FAI) was calculated as T (nmol/L)*100/ SHBG (nmol/L). Dyslipidemia was diagnosed using the definitions proposed by the National Cholesterol Eductaion Program-Third Adult Treatment Panel (NCEP ATP III) for the metabolic syndrome (20), (25) viz; TG >1.7 mmol/L (150mg/dl), lowHDL-C: <1.29 mmol/L (50 mg/dl), high LDL-C: >4.1 mmol/L (155 mg/dl), elevated Lp (a) if > 30 mg/dl,and, elevated apo B if > 100 mg/dl.

Statistics

Statistical analysis was performed using SPSS (version 15.0) [SPSS, Chicago, IL, USA]. Continuous variables were presented as means ± SD, normalityl was assessed by Kolmogrov-Smirnov test. Data which are not normally distributed were log transformed and non-parametric tests were employed. Non-parametric Mann-Whitney u test was used for numeric variables' differences, and Chi-square test for categorical variables. Multiple regression was performed for plasma lipids and lipoproteins, as well as, AMH as dependent variables. Independent variables were the group (PCOS vs. control), FAI, BMI, QUICKI, and HOMA-IR. For all analyses two-tailed $p \leq 0.05$ was considered to indicate statistical significance.

Results

In this study we found that all PCOS women had menstrual irregularity, 79.7% of them had a Ferriman Gallway score > 8, and 75.8% of them had elevated serum total testosterone levels. As shown in table (1) PCOS women and healthy women had similar age, BMI, and WHR. AMH, total testosterone (TT), FAI; HOMA-IR levels were significantly higher; $p<0.01$ in PCOS women compared to controls. Levels of LDL-C were higher in the PCOS group than healthy women; however, this did not reach statistical significance. Unlike levels of Total Cholesterol TT and ApoB which did not differ between participant groups. HDL-C and Lp (a) levels were significantly lower in PCOS group $p<0.05$ than controls. In the PCOS group BMI categorical distribution was as follows; lean 29.1% (n=32), overweight 37.3% (n=41) and obese 33.6% (n=37). For the controls 30.6% (n=11) were lean 36.2% (n=13) were overweight and 33.3% (n=12) were obese. Both groups were not statistically different for BMI categories. Clinical, hormonal and insulin sensitivity indices according to BMI are listed in table (2).

Total Cholesterol and lipoprotein levels according to BMI are presented in table (3). No significant changes were noted with increasing body weight in total cholesterol (p=0.93), ApoB (p=0.182), LDL-C (p=0.231), DHEA-S (p=0.772). Lp(a),and HDL-C levels increased significantly at each level of increase in

BMI; for Lp(a) obese vs lean; p=0.016, obese vs. overweight; p= 0.027 and overweight vs. lean p=0.033, for HDL-C levels obese vs. lean p=0.021 , obese vs. overweight p=0.043,and overweight vs. lean p= 0.047.

Total testosterone, SHGB, FAI, HOMA-IR were significantly higher in PCOS compared to control group and within the PCOS group they were significantly higher in overweight versus lean,and in obese versus lean women. Obese women in PCOS and control groups had significantly higher fasting blood glucose than overweight and lean women. QUICKI was significantly lower in obese women with PCOS compared to their lean and overweight counterparts. For AMH significantly higher levels were recorded in PCOS than control women for each BMI category. Obese women with PCOS, however, had significantly lower levels than lean PCOS women.

For the control group all variables were not significantly different in different BMI categories except for fasting insulin, HOMA-IR , TG levels which were significantly higher, HDL_C, QUICKI values which were significantly lower in obese vs. lean women p=0.032, p=0.032, p=0.047, p=0.041, p=o.029 and p=0.035 re-

Table (1) :
Anthropometric Characteristics, hormonal and metabolic profiles in polycystic ovary patients and controls

Study Group	POCS	Controls	
	n=110	n=36	
Age (years)	25.8±4.72	26.2±3.83	NS
BMI (kg/m2)	28.7±5.13	29.3±4.35	NS
WHR	0.78±0.15	0.76±0.13	NS
FG score	17.45±7.23	-	
TT (ng/dl)	111.32±25.83	43.29±3.61	<0.001
SHBG (nmol/L)	36.04±0.27	45.12±1.37	NS
DHEA-S (ng/ml)	2753.33±1128.41	2413.44±1215.45	NS
FAI	15.6±12.92	3.89±1.43	<0.001
AMH (ng/ml)	5.81±2.37	2.54±0.95	<0.001
Glucose (mg/dl)	98.41±6.52	85.74±1.89	<0.05
Insulin (µIU/ml)	17.43±6.82	7.41±4.21	<0.01
HOMA-IR	4.87±3.51	1.73±1.26	<0.001
QUICKI	0.333±0.005	0.371±0.003	<0.001
TC (mg/dl)	184.19±9.47	167.73±0.16	NS
LDL-C (mg/dl)	121.48±32.62	118.56±18.74	NS
HDL-C (mg/dl)	42.72±20.13	60.51±6.22	P<0.01
Lp (a) (mg/dl)	37.32±27.15	7.8±4.2	<0.0001
ApoB (mg/dl)	83.45±36.24	80.71±27.25	NS
TG	122.28±69.47	118.39±21.31	<0.05

- Data are mean ± SD, P<0.05 is statistically significant
- BMI= Body Mass Index; WHR= Waist Hip Ratio; FG score= Ferriman Gallway score; TT= Total Testosterone; SHBG= Sex Hormone Binding globulin; DHEA-S= Dehydroepiandrosterone- sulfate; FAI= Free Androgen Index; AMH=Anti Müllerian Hormone; HOMA-IR= Homeostatic Model Assessment Insulin Resistance; and QUICKI= Quantitative Insulin Sensitivity Check Index; TC=Total Cholesterol; LDL-C= Low Density Lipoprotein Cholesterol; HDL-C= High Density Lipoprotein Cholesterol; Lp(a)= Lipoprotein (a); ApoB=Apolipoprotein B; TG=Triglycerides.

spectively. According to NECP III criteria (20),(25) 9 women of the control group (25%) had dyslipidemia. Five women of the dyslipidemic controls (56%) had low HDL-C levels, 4 women (45%) had elevated TG and 2 (22.2%) had high LDL-C levels. Of the dyslipidemic controls 3 women were obese and the remaining was overweight. All obese dyslipidemic control women had high values of TC, TG, LDL-C and low HDL-C levels, 4 of the overweight women had low HDL-C levels, 3 had high TG , 2 women had low LDL-C level.

In the PCOS group 71.8% (n=79) were dyslipidemic. Most frequent abnormalities were: high Lp (a) levels (n=75/110) ; 68.2% followed by low HDL-C levels (n=70/110); 63.7%, high TG levels (n=65/110); 59%,high LDL-C levels (n=64/110); 41% , then high total cholesterol levels (n=39/110); 35.4%.

For lean, overweight and obese women low HDL-C levels were reported in 18.5%, 70.7%, 94.6%, high Lp (a) levels in 28.1%, 80.4%, 86.5%; high LDL-C levels in 9.3%, 36.6%, 75.7% ;and high TC levels in 6.3%, 41.5%, 54.1% respectively. The following variables were positively related to BMI, Lp (a) ($r = 0.575$; $p<0.001$), TG ($r = 0.419$; $p=0.001$), LDL-C ($r=0.296$, $p=0.021$), HOMA-IR ($r=0.319$; $p<0.01$). HDL-C, and QUICKI levels were inversely correlated to BMI ($r=0.389$; $p=0.021$),and ($r=-387$; $p<0.01$) respectively. When HOMA-IR was correlated to individual variables it was positively related to Lp (a) ($r =0.423$; $p<0.001$), TG ($r=0.252$;$p<0.05$), AMH ($r=0.268$; $p=0.034$) and inversely correlated to QUICKI ($r=-0.567$; $p<0.001$), and HDL-C ($r =-0.356$; $p =0.005$). FAI was positively correlated to Lp (a), ($r=0.427$; $p<0.001$), AMH($r=0.553$; $p<0.001$), HOMA-IR ($r=0.453$; $p= 0.024$) and inversely related to HDL-C ($r= -0.448$; $p=0.019$) and QUICKI ($r= -0.511$; $p=0.017$). The best predictor of Lp (a) was FAI ($r2=0.244$; $p=0.023$) the model was further improved by adding HOMA-IR ($r2=0.361$; $p=0.017$) and AMH ($r2=0.519$; $p=0.002$). For HDL-C best predictor is HOMA- IR ($r2=0.231$; $p=0.037$) adding FAI ($r2=0.382$; $p=0.022$) and AMH ($r2=0.498$; $p=0.004$) improved the model

Table (2):
Clinical, hormonal, insulin sensitivity indices in different body mass index categories in controls versus polycystic ovary patients

	Lean		Overweight		Obese	
	Control	**PCOS**	**Control**	**PCOS**	**Control**	**PCOS**
Variable	n=11	n=32	n=13	n=41	n=12	n=37
Age (years)	26.9±5.7	24.8±4.7	27.3±2.3	25.9±2.5	23.9±3.4	25.4±3.5
BMI (kg/m²)	22.3±1.7	21.6±2.2	27.5±2.1	28.4±4.3b	32.8±1.8	34.9±3.8c,d
FG score	-	16.52±5.73	-	19.93±6.84	-	18.55±4.71
TT (ng/dl)	43.5±26.2	103.7±18.4a	45.7±22.4	110.8±25.4a,b	44.8±30.17	119.2±32.1a,c,d
SHBG (nmol/L)	47.5±0.9	35.2.7±1.3a	46.8±0.7	34.2±2.2a,b	45.3±1.1	33.6±1.9a,c,d
FAI	4.1±2.2	10.4±3.6a	3.8±1.7	12.8±9.5a,b	4.9±2.5	14.2±6.8a,c,d
DHEA-S (ng/ml)	2255±924	2371±1100	2334±1207	2501±1197	1989±1313	2421±1125
AMH (ng/ml)	2.3±0.90	6.4±2.5a	2.1±1.2	5.2±2.7a	1.8±1.3	4.7±1.1a,c
Glucose (mg/dl)	85.9±4.2	88.1±0.9	89.3±3.8	88.5±5.2	99.7±10.2	101.2±3.3a,c,d
Insulin (µIU/ml)	6.2±2.6	14.8±9.6a	10.8±2.7	19.8±12.9a,b	11.1±1.2	21.3±16.5a,c,d
HOMA-IR	2.1±1.5	3.7±2.1a	1.9±2.2	5.3±3.2a,b	2.9±0.6	6.2±2.8a,c,d
QUICKI	0.375±0.004	0.359±0.006	0.344±0.003	0.2275±0.007a,b	0.335±0.012	0.255±0.0.37a,c,d

- Values are mean ± SD, $P<0.05$ is statistically significant
- BMI= Body Mass Index; FG score= Ferriman Gallway score; TT= Total Testosterone; SHBG= Sex Hormone Binding globulin; FAI= Free Androgen Index; DHEA-S= Dehydroepiandrosterone- sulfate; AMH=Anti Müllerian Hormone; HOMA-IR= homeostatic Model Assessment Insulin Resistance; and QUICKI= Quantitative Insulin Sensitivity Check Index.

a $P<0.05$ PCOS vs. Controls.
b $P<0.05$ Overweight vs. lean
c $P<0.05$ Obese vs. lean
d $P<0.05$ Obese vs. overweight

Table (3):
Lipid profile according to body mass index categories in controls vs. polycystic ovary syndrome women

	Lean		Overweight		Obese	
	Control	**PCOS**	**Control**	**PCOS**	**Control**	**PCOS**
Variable	n=11	n=32	n=13	n=41	n=12	n=37
TC (mg/dl)	158.95±27.72	164.89±29.74	169.34±24.61	185.22±27.13	176.22±31.52	188.33±26.42
LDL-C (mg/dl)	97.39±32.06	106.61±25.44	105.28±19.91	111.39±23.05	113.47±18.78	116.99±22.23a,c,d
HDL-C(mg/DL)	65.37±14.26	50.48±10.33a	52.17±16.51	45.96±14.85a,b	49.56±11.26	38.92±12.14c,d
TG (mg/dl)	79.39±43.92	99.71±35.13a	122.74±33.65	131.22±37.19b	132.15±28.27	139.48±36.25a,c,d
Lp (a) (mg/dl)	7.3±6.2	18.8±31.1a	8.5±5.7	27.4±25.9a,b	7.5±5.3	41.2±85.2
Apo B (mg/dl)	82.35±19.7	80.82±20.3	82.62±38.6	86.5±29.4	81.7±42.2	87.3±44.6

- Data are mean ± SD P<0.05 is statistically significant
- TC= Total Cholesterol; LDL-C= Low Density Lipoprotein- cholesterol; HDL-C= High Density Lipoprotein- cholesterol; Lp(a)= Lipoprotein (a); APOB= Apolipoprotein B.

a P<0.05 PCOS vs. Controls.
b P<0.05 Overweight vs. lean
c P<0.05 Obese vs. lean
D P<0.05 Obese vs. overweight

Discussion

It is well-known that healthy women have hormonal protection against cardiovascular diseases delaying their onset by 10-15 years in comparison to men (26). In contrast young women may show increased cardiovascular risk when affected by PCOS (27). In PCOS hyperandrogenemia and chronic anovulation have been associated with metabolic aberrations and metabolic syndrome (28). FAI levels were significantly higher in obese than overweight or lean PCOS women analyzed in this study reflecting the fact that hyperandrogenemia can aggravate visceral obesity in women unlike men (29). Besides, androgen excess is known to lower circulatory HDL-C (30) that androgen excess is arthrogenic, a fact that was further substantiated in our study that demonstrated the inverse relationship between LDL-C and FAI. Moreover FAI was the best predictor of Lp (a) providing another link between androgen excess and cardiovascular risk disease.

Hyperandrogenemia can also perpetuate insulin resistance by inhibiting muscle glycogen synthase activity,and, by increasing the number of less insulin sensitive type IIb skeletal muscle fibers (31), (32), again here , FAI was positively correlated to HOMA-IR and negatively correlated to QUICKI measures of insulin sensitivity. Similar findings were reported in previous studies (33), (34). Lipid alterations are common in women with PCOS (5). Concerning the pattern of dyslipidemia in the PCOS the results of different studies are not entirely congruent in the present study a significant increase only in TG, Lp(a) and a significant decrease in HDL-C in PCOS women compared to healthy women was found , a pattern as that seen with insulin resistance and recorded in previous studies (33), (35), (36). Other authors reported only a single increase in TG (37), (38) or LDL-C (39) or decrease in HDL-C (40) in PCOS women.

When the effect of BMI was illustrated some studies showed decreased HDL-C levels in all PCOS women and an added increased TG levels only in obese PCOS women (41), (42). For all levels of BMI (lean, overweight, or obese) we found that the decrease in HDL-C and increase in TG was significant. Rizzo et al 2009 (4) pointed out that Italian PCOS women had significantly increased TG and LDL-C than healthy controls , however, their concentrations usually remained in the normal range. As we examined Egyptian women with PCOS 59% had TG levels >1.7 nmol/L and 41% had LDL-C levels >4mnol/L were reported which are significantly higher than those of Italian anovulatory women with PCOS 9%, 23% respectively (4). Lp(a) a marker of increased cardiovascular risk was noted to be significantly higher in Egyptian women with PCOS compared to controls with no alterations in ApoB ; in agreement with findings by other authors on Mediterranean PCOS women (4), (43), (44).
Nonetheless, contradictory results were shown for ApoB (45), Lp(a) (45) in PCOS women from different genetic backgrounds.

Obese women with PCOS from our locality had significantly higher levels of TG than overweight or lean PCOS women as stated in previous reports (41), (42). HDL-C levels decreased significantly with each level of increase in BMI, Rocha and coworkers (33), however, did not notice a difference between overweight and obese Brazilian women with PCOS. Insulin resistance was postulated to be the connecting factor between dyslipidemia

and PCOS (5),indeed, the best predictor for HDL-C was HOMA-IR in this study.

The present study confirmed previous results on increased serum AMH levels in women with PCOS (47), (48) which is due to increased number of preantral follicles and its increased production per granulosa cell in PCOS patients(49). As mentioned in previous studies (50),(51) a positive correlation between AMH and FAI was demonstrated in Egyptian PCOS women, as well. Our PCOS study women displayed a positive correlation between their AMH levels and HOMA-IR. Previous publications on the relationship between AMH levels and high surrogate measures were conflicting; some reported no association (14), (52) or positive correlation (50).

AMH levels were significantly lower in obese PCOS compared to lean PCOS sub group, this inverse relationship was consistently found in previous reports (54), (55). This can be explained by the fact that Insulin resistance can occur independent of obesity in PCOS women (53),and,surprisingly weight loss results in improvement in insulin resistance but not AMH probably because it has a greater effect on metabolic rather than gonadotropic presentation of PCOS and so far LH levels were found to be the most important independent determinant of AMH (48).

References:

1- Aziz R, Woods KS, Reyna R, et al. 2004, the prevalence and features of polycystic ovary syndrome in an unselected population. J Clin Endocrinol Metabolism, 89:2754-9.
2- Burghen GA, Givens JR, Kitabchi AE, 1980, Correlation of hyperandrogensim with hyperinsulinism in polycystic ovarian disease, J Clin Endocrinol Metab ; 50:113-116.
3- Ehrmann DA, Barnes RB, Rosenfield RL, et al , 1999, Prevalence of impaired glucose tolerance and diabetes in women with polycystic ovary syndrome. Diabetes Care, 22:141-6.
4- Rizzo M, Berneis K, Hersberger M, pepe I, et al , 2009, Midler forms of atherogenic dyslipidemia in ovulatory versus anovulatory polycystic ovary syndrome phenotype, Hum Reprod; 9: 2286-2292.
5- legro RS, Kunselman AR, Dunaif A. 2001, A prevalence and predictors of dyslipidemia in women with polycystic ovary syndrome, AM J Med ; 111:607-613.
6- Diamanti-Kandarakis E, Papavassiliou AG, Stylianos A, Kandarakis AS, Chorousos GP, 2007, Pathophysiology and types of dyslipidemiain PCOS, Trends MOL Endocrinol; 18:208-215.
7- Holte J, Bergh T, Berne C, Lithell H. 1994, Serum lipoprotein lipid profile in women with the polycystic ovary syndrome: relation to anthropometric , endocrine and metabolic varialbles, Clin Endocrinol (Oxf); 41:463-471.
8- Cibula D, Cifkova R, Fanta M, Poledne R, et al. 2000, increased risk of non-insulin dependent diabetes mellitus, arterial hypertension and coronary artery disease in perimenopausal women with history of the polycystic ovary syndrome, Hum Reprod; 15:785-789.
9- Webber LJ, Stubbs S, Stark J, Trew GH, et al. 2003, Formation and early development of follicles in the polycystic ovary, Lancet;84:1470-1474.
10- Lockwood GM, Mutukrishina S, Groome NP, Mathews DR, et al. 1998, Mid-follicular phase pulses of inhibin B are absent in polycystic ovarian syndrome and are initiated by successful laparoscopic ovarian diathermy: a possible mechanism regulating emergenc of dominant follicle, J Clin Endocrinol Metab;83:1730-1735.
11- Barrends WM, Ulienbroek JT, Karnmer P, Hoogerbrugge JW , et al. 1995, Anti müllerian hormone and anti- müllerian hormone II receptor messenger ribonucleic acid expression in rat ovaries during postnatal development , the estrous cycle, and gonado-tropin include follicle growth, Endocrinology; 136:4951-4962.
12- van Rooji IA, Broekmans FJ, te Velde ER, Fauser BC, et al. 2002, serum Anti- Müllerian hormone levels: a novel measure of ovarian reserve, Hum Reprod; 17:3065-3071.
13- Somunkiran A, Yavuz T, Yucel O, Ozdemir I. 2007, Anti- Müllerian hormone levels during hormonal contraception in women with polycystic ovary syndrome, Eur J Obest Gynecol Repro Biol.
14- Bayrak A, Terbell H, Urwitz-Lane R, Mor E, et al. 2007, acute effects of metformin therapy include improvement of insulin resistance and ovarian morphology, Fertil Steril;87:870-875.
15- Muttukrishna S, McGarrige H, Wakim R, Khadum I, et al. 2005, Antral follicle count , anti- müllerian hormone of inhibin B: predictors of ovarian response in assisted reproductive technology?, BJOG;112:1384-1390. .
16- McIlveen M, Skull JD, LedgerWL. 2007, Evaluation of the utility of multiple endocrine and ultrasound measures of ovarian reserve in the prediction of cycle cancellation in a high-risk IVF population, Hum Reprod;22:778:785.
17- The Rotterdam ESHRE/ASREM-sponsored PCOS consensus workshop group. 2004, Revised 2003 consensus on diagnostic and long-term health risks related to polycystic ovary syndrome (PCOS), Hum Reprod; 19:41-47.
18- Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, et al. 2006, positions statement: criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome : an androgen excess society guidline, J Clin Endocrinol Metab; 91:37-45.
19- Ferriman D, Galwey G. 1961, clinical assessment of body hair growth in women, J Clin Endocrinol Metab; 21:1440-1447.
20- Grundy SM, Cleeman JI, Daniels SR, Donato KA, et al. 2005, American Heart Association; National Heart, Lung, and blood institute. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and blood institute Scientific Statement, circulation; 112:2735-1752.
21- Friedewald WT, Levy RI, Fredrickson DS. 1972, Etsimation of the concentration of low- density lipoprotein cholesterol in plasma, without the use of the preparative ultracentrifuge, Clin Chem; 18:499-502.
22- Katz A, Sridhar S. Nambi, Mather K, Baron A, et al , 2000, Quantitative Insulin sensitivity check index: A simple accurate method for assessing insulin sensitivity in human, J Clin Endocrinol Metab.
23- Mattews DR, Hosker JP, Rudenski AS, Naylor BA, et al. 1985, Homeostasis model assessment :insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man, Diabetologis;28:412-419.
24- Morley JE, Patrick O, Perry HM III 2002, Evaluation of assays available to measure free testosterone, Metabolism;5:554-559.
25- Executive summary of the third report of the National Cholesterol Education Program (NCEP) 2001 Expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adults treatment panel III), JAMA;285:2486-2497 .
26- National Cholesterol Education Program (NCEP) 2002, Expert panel on detection, evaluation, and treatment of high blood cholesterol in adult (Adult Treatment III). Third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment III) final report, Circulation;106:3134-3701.
27- Lobo RA, Carmina E. 2000, The importance of diagnosing the polycystic ovary syndrome, Ann InternMed;132:989-993.
28- Sam S and Dunaif A. 2003, the Polycystic ovary syndrome: syndrome XX? Trends, Endrocrinol Metab; 12:365-370.
29- Marian P, Holmang S, Gustafsson C, Jonsson L, et al. 1993, Androgen treatment of abdominally obese men, Obes Res; 1:245-251.
30- Diamanti-Kandarakis E, Papavassiliou AG, Kandarakis SA, et al. 2007, pathophysiology and types of dyslipidemia in PCOS, Trends Endocrinol Metab; 18:280-285.
31- Rincon J, Holmang A, Washlstrom EO, Lonnroth P, et al. 1996, Mechanisms behind insulin resistance in rat skeletal muscle after oophorectomy and additional testosterone treatment, Diabetes;45:615-621.
32- Corbould A. 2007, Chronic testosterone treatment induces selective insulin resistance in subcutaneous adipocytes of women, J Endocrinol; 192:585-594..

33- Michelle P. Rocha, Jose A. M. Marcondes, Cristiano R. G., et al. 2010, Dyslipidemia in women with polycystic ovary syndrome: incidence, pattern and predictors; Gynecol Endocrinol.
34- Roa Barrios M, Arata-Bellabarba G, Valeri L, et al. 2009, Relationship between triglyceride /high- density lipoprotein cholesterol ratio, insulin resistance index and cardiometabolic risk factors in women with polycystic ovary Syndrome, Endocrinol Nutr;56(2):59-65
35- Wild RA, Painter PC, Coulson PB, Carruth BK, et al. 1985, Lipoprotein lipid concentration and cardiovascular risk in women with polycystic ovary syndrome, J Clin Endocrinol Metab;61:946-951.
36- Strowitzki T, Halser B, Demant T. 2002, Body fat distribution, insulin sensitivity , ovarian dysfunction and serum lipoproteins in patients with polycystic ovary syndrome , Gynecol Endicrinol; 16:45-51.
37- Slowinska- Srzendnicka J, Zglicznski W, Soszynski P, et al. 1991 The role hyperinsulinemia in development of lipid disturbances in nonobeseand obese women with polycystic ovary Syndrome, J Endocrinol; 14:569-575.
38- Pirwany IR, Fleming R, Greer IA, Packard CJ, et al. 2008, Lipids and lipoprotein subfractions in women with PCOS: relationship metabolic and endocrine parameters, Clin Endocrinol (Oxf);54:447-453.
39- Legro RS, Kunselman MA, Dunaif. 2001, A. Prevalence and predictors of dyslipidemia in women in women with polycystic ovary syndrome , Am J Med;111:607-613.
40- Yilmaz M, Biri A, Bukan N, Karakoc A. Sancal B, et al. 2005, levels of lipoprotein and homocysteine in non-obese and obese patients with polycystic ovary syndrome, Gynecol Endocrinol;20:256-263.
41- Conway RS, Agrawal R, Betteridge DJ, Jacobs HS. 1992, Risk factors for coronary artery disease in lean and obese women with the polycystic ovary syndrome, Clin Endocrinol (Oxf);37:119-125.
42- Slowinska-Srzendicka J, Zgliczynski S, Wierbicki M, et al. 1991, The role hyperinsulinemia in the development of lipid disturbances in nonobese and obese women with the polycystic ovary syndrome, J Endocrinol Invest; 14:596-575.
43- berneis K, Rizzo M, Fruzzetti F, Lazzarini, V, et al. 2007, Atherogenic lipoprotein phenotype and LDL size and subclasses in women with polycystic ovary syndrome, J Clin Endocrinol Metab; 186-189.
44- Berneis K, Rizzo M, Hersberger M, Rini FG, et al. Atherogenic forms of dyslipidemia in women with polycystic ovary syndrome, Int J Clin Pract;63:56-62.
45- Demirel F, Bideci A, Cianz P, Camurdan MO, et al. 2007, Serum leptin , oxidized low density lipoprotein and plasma asymmetric dimethylargine levels and their relationship with dyslipidemia in adolescent girls with polycystic ovary syndrome, Clin Endocrinol (Oxf);67:129-134.
46- Sahin Y, Unluhizarci K, Yilmazsoy A, Aygen E, et al. The effects of metformin on metabolic and cardiovascular risk factors in non-obese women with polycystic ovary syndrome, Clin Endocrinol (Oxf);67:904-908.
47- Pigny P, Jonard S, Robert Y, DeWailly D. 2006, Serum anti- müllerian hormone as a surrogate for antral follicle count for definition of the polycystic ovary syndrome, J Clin Endocrinol Metab;91:941-945
48- Athanasia Piouka, Dimitrios Farmakiotis, Ilias Katsikis, Dijuo Macut, et al. 2008, Anti- Müllerian hormone levels reflect severity of PCOS but are negatively influenced by obesity: relationship with increased luteinizing hormone levels, Physiol Endocrimol Metab, 296:E238-E243.
49- Pellatt L, Hanna L, Brincat M, Galea R, et al. 2007 Granulosa cell production of anti- Müllerian hormone is increased in polycystic ovaries , J Clin Endrocinol Metab;98:3393-3397
50- Eldar-Geva T, Margolioth EJ, GAL M, Ben-Chertrit A, et al. 2005, Serum anti- Mullerian harmone levels during controlled ovarian hyperstimulation in women with polycystic ovaries with and without hyperandrogensim , Hum Reprod;20:1814-1819.
51- La Maraca A, Oriveto R, Gilulini R, Jasonni VM, et al. 2004, Müllerian- inhibiting substance in women with polycystic ovary syndrome: relationship with hormonal and metabolic characteristics , Fertil Steril;82:970-972.
52- Fleming R, Harborne L, Maclaighlin DT, et al. 2005, Metaformin reduces serum mullerian-inhibiting substance levels in women with polycystic ovary syndrome after protracted treatment , Fertil Steril ;83:130-136.
53- Dunaif A, Segal KR, Futterweit W, et al. 1989, Profound peripheral insulin resistance, independent of obesity , in polycystic ovary syndrome, Diabetes; 38:1165-1174.
54- Freeman EW. Garcia CR, Sammel MD, Lin H, et al. 2007, Association of anti- müllerian. Hormone levels with obesity in late reproductive age women, Fertil Steril; 87:101-106.
55- Steiner AZ, Stanczyk FZ, Patel S,et al. 2009, Antimullerian hormone and obesity: insights in oral contraceptive users, Contraception; 81(3):245-248.