Research Article

Serum biomarkers for the early detection of poly cystic ovary in a sample of non-obese Egyptian women.

Attia Mohamed Attia
Department of Obstetrics and Gynecology, Faculty of Medicine, Al-Azhar University, Cairo, Egypt.

Abstract
Background: Polycystic ovary (PCO) is one of the most prevalent endocrinal disturbance among women during their child-bearing period. We aimed to highlight the serum levels of biochemical, endocrinal, and metabolic biomarkers of PCO in non-obese Egyptian women. **Methods:** This cross-sectional study was conducted on 44 non-obese Egyptian women with PCO based on Rotterdam criteria in comparison with 44 healthy control women. The biomarkers levels were detected in the serum using fasting blood samples. **Results:** There was a significant difference between the two groups regarding the waist circumference and ratio between the waist and hip in the favor of the PCO group (P <0.001). Having the biochemical and endocrinal biomarkers, PCO group showed higher levels of glucose, insulin, triglycerides, cholesterol, low-density lipoprotein, the ratio between luteinizing-hormone and follicle-stimulating-hormone, vascular endothelial growth factor, 17β-estradiol, and testosterone (P <0.001 for all). On the other hand, the PCO showed significant lower levels of progesterone, sex hormone-binding globulin, and high-density lipoprotein (P <0.001 for all). There was no difference between the two groups regarding vitamin D and Kisspeptin (P = 0.095 and 0.944, respectively). **Conclusions:** Many biomarkers were associated with the risk of PCO development among non-obese women.
Keywords: Biomarkers; Polycystic ovary; Non-obese women.

Introduction
Polycystic ovary (PCO) is one of the most prevalent endocrinal disturbance among women during their child-bearing period as it presents in 6-12% of these population[1]. Polycystic ovary syndrome (PCOS) is composed of PCO with increase the level of androgens, acne, hirsutism, anovulation, increase the abdominal contour, obesity, insulin resistance, cardiovascular abnormalities, inflammations in different sites, and infertility[2]. Furthermore, PCOS is characterized by increasing the serum level of luteinizing hormone (LH); therefore, the ratio between LH and the follicle-stimulating hormone (FSH) increases which in turn would down-inhibit the gonadotropin-releasing hormone level.

The role of vitamin D in fertility is so important due to its valuable action on the ovary and the Immune modulation. It works through its water-soluble receptors that are present in high concentrations in ovary, endometrium, myometrium, and cervix[3]. Furthermore, vitamin D deficiency was proven to be associated with PCOS which may indicates that it may be a cause of this condition[3-5].

Having the kisspeptin, it is a protein that is secreted in the hypothalamus and hypothesized to be associated with the gonadotropin- releasing hormone secretion. It works on the Kiss-1 receptors which are most abundant in the hypothalamic region which supports its relations with the gonadotropin-releasing hormone[6-7].

Regarding the vascular endothelial growth factor (VEGF), it is a protein that has strong angiogenic ability. It plays an important role in ovarian angiogenesis which is a physiological process. Moreover, It has a cardinal role in the hypervascularity of the theca interna and stroma of the PCO[8,9].

Through this study, we aimed to investigate the association between the serum levels of vitamin D, kisspeptin, and VEGF in addition to other biochemical and hormonal biomarkers and the early detection of PCOS in non-obese Egyptian women.

Methods

Study design and ethical considerations

This study was an observational cross-sectional study in which we included any non-obese women, with or without PCOS, who attended to our clinic in both Al-Hussein University Hospital and Bab-Elsharya University Hospital from May 2019 to June 2019. Written informed consents were obtain from all the participants, and the international review board approval was obtained from the ethical committee of Al-Azhar University. Therefore, this study was performed under the declaration of Helsinki.

Study participants

The include participants were allocated in one of the two study groups. The first group (the case group) which included women with PCOS represented in two of the three criteria that highlighted in Rotterdam criteria[10]. On the other hand, the control group included women without polycystic ovary with normal ovulation but have infertility due to any other cause (tubal, male, or unexplained). These control women should not have endocrinal disturbance and clinical or laboratory signs of androgenesis or increased androgen levels. Furthermore, they should have normal ovulatory cycles and normal ovary morphology as detected by ultrasound.

Any patient with any endocrinal disease, tumor, or cardiovascular disease was excluded from both case and control groups. Furthermore, we omitted women with endometriosis, diabetes mellitus, hypertension, renal disease, oral contraceptive pills or insulin-synthesizers usage six months before the enrollment. Moreover, we excluded smoker women and women with hormonal treatment in the six months before the enrollment.

Data collection

Demographic variables, including age, sex, body mass index (BMI), waist circum-ference, hip circumference, and the ratio between waist to hip (RWH), were collected from participants upon enrollment. Blood samples were collected from all participants during the early follicular phase.

After centrifuging, the serum was analyzed as regard the levels of lipid profile, vitamin D, kisspeptin, VEGF, LH, FSH, leptin, ghrelin, glucose, and insulin using the prescribed kits for each substance. Further-more, the levels of testosterone, progesterone, and sex hormone-binding globulin (SHBG) were analyzed from a sample of blood collected on the day 20 from the menstruation using enzyme-linked immune-sorbent assay (ELISA) -based kits.

Statistical analysis

SPSS software version 23 was used for the data analysis after preparation. Normal data were expressed as mean and standard deviation (SD), while non-normal data were expressed as median and range. The diagnostic ability of the selected biomarkers was assessed using the receiver operating characteristic (ROC) curve the area under the curve (AUC). The correlation between vitamin D, kisspeptin, and VEGF and the outcome was assessed using the Pearson correlation co-efficient. Throughout the statistical analysis, the statistical level of significance was set when P value < 0.05.

Results

Through out the study period, 44 women were included in the case (PCO) group and matched 44 women were included in the control group. There was no difference between the two groups as regard the age, BMI, and the hip circumference, while the waist circumference and RWH were higher in the PCO group than the control group ($P < 0.001$) (Figure1 and Table 1).

Having the biochemical biomarkers assessment, there was a significant difference between the two groups as regard the serum levels of cholesterol, triglycerides (TG), low density lipoprotein (LDL), fasting blood sugar and insulin being higher in the PCO group ($P <0.001$) (Figure1 and Table 1). On the other hand, the PCO group showed significant lower levels of progesterone, sex hormone-binding globulin, and high-density lipoprotein ($P <0.001$ for all). There was no difference between the two groups regarding fasting level of leptin and ghrelin ($P = 0.765$ and 0.930, respectively) (Figure1 and Table 1).

Although, there was no difference between the two groups regarding the FSH level, PCO group showed a significant higher level of

serum LH and, subsequently the LH to FSH ratio (*P*<0.001 for both). Similarly, the levels of 17β-estradiol and testosterone were significantly higher in the PCO group (P <0.001 for all). On the other hands, the serum levels of progesterone and SHBG were significantly lower in the PCO group when compared with the control group (P <0.001 for all). Despite there was no significant difference between the two groups regarding the serum level of vitamin D (*P*=0.095) and kisspeptin (*P*= 0.944), PCO group was higher than the control group regarding the serum level of VEGF (P<0.001) (Figure1 and Table 1).

There was positive association between vitamin D and the serum level of testosterone in the PCO group (*P*=0.046); whoever, this positive association was positive between vitamin D level and hip circumference (*P*=0.033), RWH (*P*=0.013), LH to FSH ratio (*P*=0.028), and the level of progesterone (*P*=0.022) (Table 2).

There was positive association between the serum level of kisspeptin and the serum level of HDL in the PCO group (*P*=0.033), while there was negative correlation between the serum level of kisspeptin and TG in the control group (P=0.005) (Table 3). Furthermore, there was a negative association between VEGF and fasting blood insulin (Table 4).

The ROC analysis revealed that serum cholesterol, TG, HDL, and RWH reported very good diagnostic ability (AUC ≤ 0.8). Furthermore, 17β-estradiol, SHBG, serum insulin, and LDL showed strong diagnostic ability with AUCs 0.893, 0.964, 0.861, and 0.974, respectively (Figure 2).

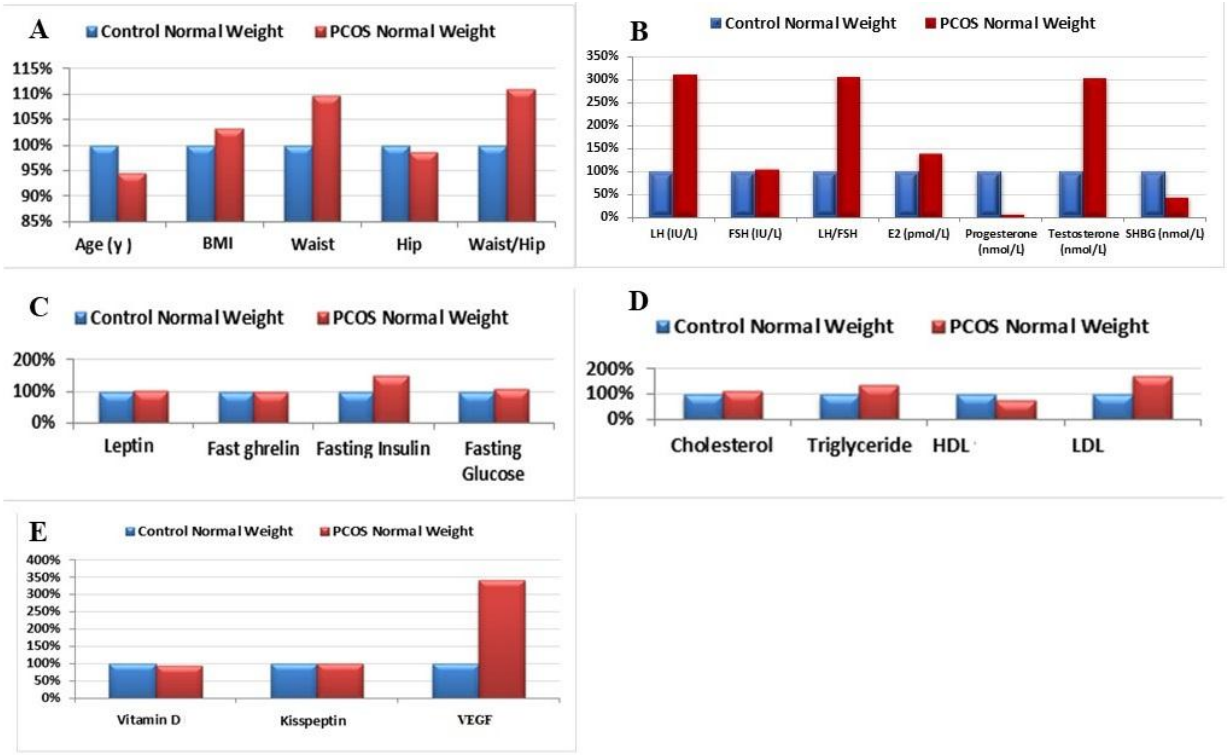

Figure 1. The parameters that were measured in both case and control groups; A, Age, BMI, Waist, Hip, Waist/Hip; B, LH, FSH, LH/FSH, E2, Progesterone, Testosterone, SHBG; C, Leptin, fast ghrelin, Fasting insulin, fasting glucose; D, Cholesterol, HDL, and LDL; E, Vitamin D, Kisspeptin, VEGF.

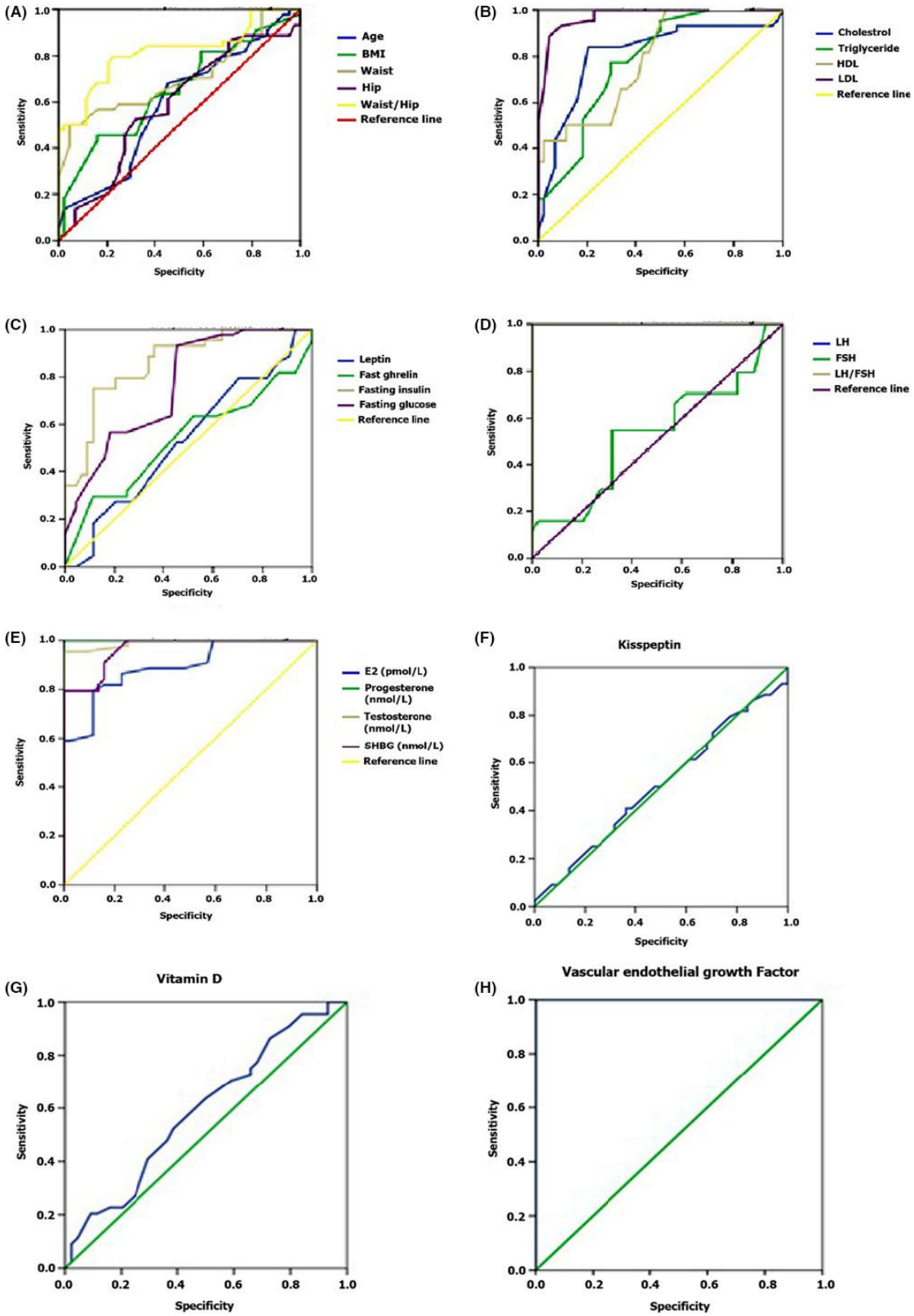

Figure 2. the Receiver operating characteristics of the biomarkers that predict the the PCOS in nonobese women; A, Age, body mass index, waist circumference, hip circumference, and ration between waist and hip; B, Cholesterol, triglycerides, HDL, and LDL; C, Leptin, ghrelin, fasting level of insulin, and fasting blood sugar; D, LH, FSH, and the ratio between LH/FSH ratio (the same AUC which equal 1; so not appeared in the graph); E, estrogen, progesterone, testosterone, and sex-hormone binding globulin (the AUC of progesterone is 1; so not appeared in the graph); F, Vitamin D; G, Kisspeptin; H, Vascular endothelial growth factor.

Table 1: The demographic characteristics of non-obese women in both PCOS and control groups [a]

Characteristic	Value	Percentage change	*P* value [b]
Age, y			0.162
Control	25.43 ± 4.80 (19.00–36.00)		
PCOS	24.05 ± 4.41 (18.00–35.00)	–5.4	
BMI			0.264
Control	22.12 ± 1.48 (20.00–24.70)		
PCOS	22.84 ± 1.52 (19.30–25.00)	3.2	
Waist circumference, cm			<0.001
Control	69.34 ± 5.11 (60.00–80.00)		
PCOS	76.11 ± 9.22 (64.00–99.00)	9.7	
Hip circumference, cm			0.355
Control	97.70 ± 6.12 (85.00–108.00)		
PCOS	96.43 ± 6.72 (85.00–116.00)	8.7	
Waist-to-hip ratio			<0.001
Control	0.71 ± 0.04 (0.60–0.77)		
PCOS	0.79 ± 0.07 (0.69–0.91)	11.0	
Cholesterol, mmol/L			<0.001
Control	3.47 ± 0.40 (2.70–4.60)		
PCOS	3.95 ± 0.47 (2.70–4.80)	13.8	
Triglyceride, mmol/L			<0.001
Control	0.68 ± 0.19 (0.40–1.10)		
PCOS	0.94 ± 0.26 (0.60–1.50)	36.6	
High-density lipoprotein, mmol/L			<0.001
Control	1.43 ± 0.29 (0.92–2.00)		
PCOS	1.06 ± 0.27 (0.59–1.40)	–25.3	
Low-density lipoprotein, mmol/L			<0.001
Control	1.28 ± 0.35 (0.78–2.10)		
PCOS	2.22 ± 0.29 (1.60–2.80)	73.2	
Leptin, ng/mL			0.765
Control	13.38 ± 3.69 (6.00–25.00)		
PCOS	13.59 ± 3.02 (9.00–20.00)	1.6	
Fasting ghrelin, ng/mL			0.930
Control	0.53 ± 0.08 (0.40–0.70)		
PCOS	0.52 ± 0.11 (0.40–0.75)	–0.3	
Fasting insulin, pmol/L			<0.001
Control	52.18 ± 15.22 (21.00–75.10)		
PCOS	77.97 ± 21.56 (46.80–149.40)	49.4	
Fasting glucose, mmol/L			<0.001
Control	4.53 ± 0.38 (4.00–5.30)		
PCOS	4.95 ± 0.39 (4.30–5.70)	9.2	
LH, IU/L			<0.001
Control	4.46 ± 1.16 (1.40–6.30)		
PCOS	13.89 ± 3.56 (9.70–21.60)	211.3	
FSH, IU/L			0.355
Control	4.76 ± 1.06 (2.70–6.40)		
PCOS	5.02 ± 1.49 (3.10–8.80)	5.4	
LH/FSH ratio			<0.001
Control	0.96 ± 0.27 (0.42–1.61)		
PCOS	2.93 ± 0.89 (1.95–4.39)	205.3	
17β-estradiol, pmol/L			<0.001

Control	104.93 ± 9.99 (91.00–123.00)		
PCOS	146.11 ± 37.44 (98.00-234.00)	39.2	
Progesterone, nmol/L			<0.001
Control	42.28 ± 12.16 (20.00–68.10)		
PCOS	3.18 ± 0.92 (1.30–4.20)	–7.4	
Testosterone, nmol/L			<0.001
Control	0.88 ± 0.26 (0.43–1.30)		
PCOS	2.67 ± 0.73 (1.17–4.30)	202.3	
Sex hormone-binding globulin, nmol/L			<0.001
Control	55.98 ± 16.59 (32.00–93.00)		
PCOS	25.77 ± 7.36 (18.00–40.00)	–54.0	
Vitamin D, ng/mL			0.095
Control	46.18 ± 8.35 (30.00–67.00)		
PCOS	43.39 ± 7.13 (30.00–59.00)	–6.0	
Kisspeptin, fmol/mL			0.944
Control	0.39 ± 0.07 (0.26–0.51)		
PCOS	0.39 ± 0.08 (0.20–0.59)	0.0	
Vascular endothelial growth factor, nmol/L			<0.001
Control	93.77 ± 17.33 (70.00–126.00)		
PCOS	320.39 ± 94.00 (154.00-488.00)	241.7	
Control	42.28 ± 12.16 (20.00–68.10)		
PCOS	3.18 ± 0.92 (1.30–4.20)	–7.4	
Testosterone, nmol/L			<0.001
Control	0.88 ± 0.26 (0.43–1.30)		
PCOS	2.67 ± 0.73 (1.17–4.30)	202.3	
Sex hormone-binding globulin, nmol/L			<0.001
Control	55.98 ± 16.59 (32.00–93.00)		

Abbreviations: BMI (calculated as weight in kilograms divided by the square of height in meters); FSH, follicle-stimulating hormone; LH, luteinizing hormone; PCOS, polycystic ovary syndrome.
a Values are given as mean±SD (range), unless indicated otherwise.
b Calculated using the independent samples t test.

Table 2: Correlation of vitamin D with PCOS parameters

Parameter	Pearson correlation coefficient	P value [a]
Control group (n=44)		
Hip circumference	–0.322	0.033
Waist-to-hip ratio	0.373	0.013
FSH, IU/L	0.275	0.071
LH/FSH ratio	–0.332	0.028
Progesterone, nmol/L	–0.346	0.022
PCOS group (n=44)		
Testosterone, nmol/L	0.303	0.046

Abbreviations: FSH, follicle-stimulating hormone; LH, luteinizing hormone; PCOS, polycystic ovary syndrome.
a Pearson correlation.

Table 3: correlation of kisspeptin with PCOS parameters

Group	Parameter	Pearson correlation coefficient	*P* value [a]
Control group (n=44)	Triglyceride, mmol/L	–0.418	0.005
PCOS group (n=44)	High-density lipoprotein, mmol/L	0.322	0.033
Abbreviation: PCOS, polycystic ovary syndrome. **a Pearson correlation.**			

Table 4: correlation of kisspeptin with PCOS parameters

Parameter	Pearson correlation coefficient	*P* value [a]
Control group (n=44)		
Fasting insulin, pmol/L	0.012	0.941
Fasting glucose, mmol/L	0.131	0.396
PCOS group (n=44)		
Fasting insulin, pmol/L	–0.008	0.033
Fasting glucose, mmol/L	0.073	0.759
Abbreviation: PCOS, polycystic ovary syndrome. **a Pearson correlation**		

Discussion

This cross-sectional observational study revealed that the serum levels of SHBG, VEGF, 17β-estradiol, testosterone, LH, fasting glucose, and insulin were significantly higher in non-obese women with PCOS rather than matched women without PCOS. Furthermore, hyperlipidemia, in the form of increased levels of cholesterol, TG and LDL and decreased levels of HDL, was found in patients with PCOS.

Despite there was no difference between the case and the control groups regarding the BMI, the higher waist circumference and the RWH in the PCO group indicates that women with PCOS are vulnerable to develop abdominal adiposity even in non-obese women. Our findings regarding the RWH were concomitant with previous researchers that illustrated its association with the clinical presentation of PCOS[11,12]. Regarding the lipid profile, our results were concomitant with the work that was conducted by Pirwany et al., who revealed association between the increased level Cholesterol, TG, and LDL and decreased level of HDL and the PCOS[13]. This disturbance in lipid profile and the abdominal adiposity in non-obese women with PCOS indicating that these women are more vulnerable to cardiovascular diseases than others. This is supported by a previous stud that illustrated associations between abdominal adiposity and developing cardiovascular diseases like myocardial infarction and stroke[14].

The absence of correlation between the serum levels of leptin and ghrelin and PCO that was revealed in our study was concomitant with previous reports of Daghestani et al., and Glintborg et al., however, Yildizhan et al., revealed high serum level of leptin in non-obese women with PCOS[15-17]. This indicates that the secretion of leptin is associated with the adipose tissue which present in women with PCO.

Having the hormonal levels, our results was in agreement with the previous studies which illustrated that PCO was associated with increased levels of LH, estrogen, and testosterone and decreased levels of proges-terone and SHBG[18,19]. The increased level of 17β-estradiol may be the cause that could explain the absence of change in the FSH level as the 17β-estradiol is elevated as a feedback mechanism[20]. Furthermore, the increased level of testosterone may play an important role in further increase in the abdominal adiposity

and disturbance in the lipid profile in addition to elevation of the oxidative stress substance and subsequently increase the risk of developing cardiovascular diseases[21-23].

Having the vitamin D, its association with the PCO-related parameters was inconsistent. Many studies did not reach a conclusion as regard the association of vitamin D with the sex hormones in women with PCO[24]. The negative association between vitamin D and serum level of testosterone was correlated with a previous research that was done by Zhao et al.,[25].

Having the serum level of kisspeptin, there was discrepancy between our work and the work the was conducted by Yilmaz et al., who revealed a positive association between the serum level of kisspeptin and the PCO[26]. Therefore, the absence of association between the level of kisspeptin and the level of LH that revealed in our study is not going with the hypothesis that state that kisspeptin increase the secretion of LH through the induction of gonadotropin-releasing hormone; therefore, further studies are needed to test this hypothesis. Having the VEGF, its positive association with PCO was in agreement with the literature[27]. This indicates that it has a role in the pathology of the PCOS.

The excellent diagnostic role of LH, the ratio between LH and FSH, testosterone, progesterone, and VEGF in PCOS with AUC =1 and 100% sensitivity and specificity indicates that they are very good and powerful biomarkers that we can rely on in the diagnosis of PCOS without the need for transvaginal ultrasonography which is invasive and hurting. They have a further advantage over the transvaginal ultrasonography as regard the cost.

In conclusion, our work revealed that the serum level of cholesterol, LDL, TG, LH, the ratio between LH and FSH, testosterone, progesterone, SHBG, and VEGF were very efficient biomarkers in the early diagnosis of PCOS in non-obese women. Furthermore, they can be used for prediction the risk of developing PCOS in non-symptomatic women.

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