Relationship between interleukin-6 levels and ambulatory blood pressure in women with polycystic ovary syndrome

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Objective: To determine 24-hour ambulatory blood pressures (ABP) in patients with polycystic ovary syndrome (PCOS) and its relationship with interleukin-6 (IL-6).
Design: Prospective controlled study.
Setting: University hospital.
Patient(s): Fifty-four PCOS patients.
Intervention(s): Ambulatory blood pressure monitoring was conducted. Anthropometric, hormonal, metabolic, and inflammatory parameters, including plasma IL-6, C-reactive protein (CRP), fibrinogen, and nitric oxide (NO), were measured in each subject.
Main Outcome Measure(s): Ambulatory blood pressure and plasma IL-6, CRP, fibrinogen, and NO.
Result(s): Serum IL-6 levels of PCOS women in the highest systolic blood pressure (SBP) quartile were significantly higher than those of women in the lowest SBP quartile. The high serum IL-6 levels (serum IL-6 level $\geq$5.1 pg/mL) were associated with a higher probability of raised SBP ($\geq$126 mm Hg), with an odds ratio of 2.2 (95% confidence interval 0.8–7.9). The systolic and diastolic (DBP) blood pressures were significantly related to serum IL-6 levels. The IL-6 levels were positively and significantly correlated with serum CRP levels. Interleukin-6 and CRP were negatively and significantly correlated with serum NO levels.
Conlusion(s): The results suggest that raised plasma IL-6 levels may be related to ambulatory SBP and DBP in PCOS. (Fertil Steril® 2010;94:1437–43. ©2010 by American Society for Reproductive Medicine.)

Key Words: PCOS, ABP, IL-6, CRP, NO

Inflammation is considered to play a key role in pathophysiologic mechanisms of atherosclerosis and cardiovascular disease (1, 2). Chronic inflammation is a novel mechanism contributing to increased risk of coronary heart disease (CHD) in women with polycystic ovary syndrome (PCOS) (3). The inflammatory state may induce endothelial dysfunction, which is followed by hypertension and cardiovascular disease (2, 4–6). Interleukin-6 (IL-6), C-reactive protein (CRP), and fibrinogen were found to be significantly increased in PCOS (3, 7–10).

Plasma IL-6 levels were found to be elevated in PCOS independently from obesity or sleep apnea and significantly associated with systolic blood pressure (SBP) and diastolic blood pressure (DBP) (7, 11, 12). Interleukin-6 could act in a proinflammatory and procoagulant way, with implications for atherosclerosis progression and thrombotic complications (13), because IL-6 is a regulator of CRP and has a key role in the initiation of inflammation (13). The increased plasma concentrations of IL-6 and fibrinogen predict an increased risk of CHD (14–19). Nitric oxide (NO) also has a key role in blood pressure regulation, because it inhibits vasoconstriction (20). Interleukin-6 and CRP could play an active role in modulating endothelial nitric oxide synthase (eNOS) bioactivity (21–23). Endothelial dysfunction and impaired NO secretion have been shown in young PCOS patients without metabolic or cardiovascular disease (24). According to these results, serum IL-6 levels may affect the regulation of blood pressure in PCOS.

A few studies have shown either similar or increased office blood pressures in lean PCOS patients compared with lean control subjects (25, 26). More recently, Lugue-Ramirez et al. (27) reported that abnormalities in the regulation of blood pressure are frequent, and obesity is the major determinant of the abnormalities in blood pressure in young PCOS patients. However, the mechanism of abnormalities in blood pressure has not yet been elucidated in PCOS women.

Twenty-four-hour ambulatory measurements are considered to provide more reliable prognostic information and to be a more accurate method than office measurements for revealing labile blood pressure or borderline hypertension (28). The predictive value of 24-hour blood pressure for cardiovascular events is greater than that seen for office blood pressure values in populations (29). Ambulatory blood pressure monitoring provides information not only about 24-hour average blood pressure but also about specific periods such as day, night, or morning (30).

As yet, the relation between ambulatory blood pressures (ABP) and IL-6 has not been specifically determined in PCOS. The principal goal of the present study was to establish the role of IL-6 as a possible determining factor, aside from obesity and other known

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metabolic risk factors, affecting the blood pressure of young women with PCOS, while taking into consideration the influence of CRP, fibrinogen, and NO. To address the above issues, we defined the patient's blood pressure as being in the high blood pressure (SBP $\geq$126 mm Hg and/or DBP and $\geq$80 mm Hg) or normal blood pressure (SBP $<$126 mm Hg and/or DBP $<$80 mm Hg) range according to the 24-hour figures obtained in the initial ABP. Specifically, we tested whether or not the serum IL-6 level was higher in PCOS patients with high blood pressure than those with normal blood pressure, and we investigated the relationship between IL-6 levels and CRP, fibrinogen, and NO, all of which are related to hypertension.

MATERIALS AND METHODS

Patients

The study group consisted of 54 PCOS patients. Patients were considered to have elevated or high blood pressure if their 24-hour daytime and nighttime values on ambulatory monitoring were $\geq$126 mm Hg systolic and/or 80 mm Hg diastolic. The reference normal values were identified by the PAMELA study (31). Hypertension is defined as a blood pressure $\geq$140/90 mm Hg. There was no reference data to be used considering ABP in our population. Therefore, we used the PAMELA study as a reference for ABP monitoring. Patients were considered to have normal blood pressure if their 24-hour daytime and nighttime values on ambulatory monitoring were $<$126 mm Hg systolic and/or $<$80 mm Hg diastolic. The PCOS patients were presented to our Gynecology and Obstetrics Department with a chief complaint of irregular menstrual cycles. The diagnosis of PCOS was made when $\geq$2 of the following three criteria existed, as proposed at the Rotterdam Consensus Meeting: oligomenorrhea or amenorrhea, clinical hyperandrogenism and/or hyperandrogenemia, and polycystic ovaries (32). The presence of polycystic ovarian appearance was determined by ultrasonography (33). Oligomenorrhea (cycle intervals $>$35 days), amenorrhea (absence of menstruation for 3 consecutive months), and luteal phase progesterone measurements $<$4 ng/mL in women with regular menstrual cycles were considered to be indicative of oligoovulation. Hirsutism was determined by a modified Ferriman-Gallwey score $>$7 (34). Nonclassic adrenal 21-hyroxylase deficiency, hyperprolactinemia, and androgen-secreting tumors were excluded by appropriate tests before the diagnosis of PCOS was made.

None of the PCOS patients had thyroid dysfunction, disorders of glucose intolerance, pregnancy, delivery, miscarriage, or surgery in the preceding 3 months, hypertension, smoking, cardiovascular events, hepatic or renal dysfunction, or sleep apnea. Any use of heparin or aspirin within 15 days of the test was also an exclusion criterion. None of the cases had received any drugs known to interfere with hormonal levels for at least 3 months before the study. All of the subjects were nonsmokers who did not consume alcoholic beverages on a regular basis. None of the subjects had restricted physical activity because of handicap or other reasons or were encouraged to excercise or to be engaged in work requiring physical activity. The study took place at the University of Ufuk. All subjects gave written informed consent, and the institutional review boards of the hospitals approved the study.

Body mass index (BMI) was calculated as weight (kg)/height $(m)^2$. BMI values of $<$25 kg/m^2 were considered to be lean, 25.1–30 kg/m^2 overweight, and $>$30 kg/m^2 obese (35). Weight and height were measured in light clothing without shoes. Waist circumference was measured at the narrowest level between the costal margin and iliac crest, and the hip circumference was measured at the widest level over the buttocks while the subject was standing and breathing normally. The waist-to-hip artio (WHR) was calculated. A WHR $>$0.72 was considered to be abnormal (36).

Venous blood samples were obtained in the follicular phase of a spontaneous cycle. After a 3-day 300 g carbohydrate diet and 12-hour overnight fasting, serum samples were obtained for the measurements of serum FSH, LH, PRL, total T, DHEAS, and TSH, lipid profile [total cholesterol (C), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG)], and basal insulin levels. Plasma glucose was determined with glucose hexokinase (Cobas Integra 400 Plus; Roche Diagnostics, Mannheim, Germany). The insulin sensitivity index (ISI) was investigated by using basal insulin levels, fasting glucose, and homeostasis model assesment (HOMA-IR). The HOMA-IR was calculated as fasting glucose (mg/dL) $\times$ fasting insulin (μU/mL) $\times$ 0.055/22.5 (37, 38).

Serum levels of FSH, LH, PRL, total T, DHEA-S, insulin, and TSH were measured with specific chemiluminescence assays from Roche Diagnostics (Hitachi Elecsys 2010). Serum levels of 17-OH progesterone (17OH-P) and free T were measured by radioimmunoassay (RIA). Levels of total C, HDL-C, LDL-C, and TG were determined with enzymatic colorimetric assays (Roche Diagnostics). Samples were immediately centrifuged, and serum was separated and frozen at -20°C until assayed. The intra- and interassay coefficients of variation were $<$5% for all of the assays performed.

Serum IL-6 levels were measured by ELISA (Human IL-6 ELISA Kit; Medical and Biological Laboratories Co., Nagoyai, Japan), with mean intra- and interassay coefficients of variation of 3.5% and 8.7%, respectively. Serum CRP was measured by latex immunoturbidometric methodology on an automated clinical analyzer system (Cobas Integra, Roche Diagnostics). Plasma fibrinogen levels were measured by fibrometer by the photo-optical technique (MT4C coagulometer; Diagnostic Stage, Asnieres-Sur-Seine, France).

The NO production was assessed by measuring the plasma concentration of NO_3^- and NO_2^- with the nitrate reductase–Griess method, using a commercial kit (Cayman Chemical Co., Ann Arbor, MI). This assay kit has a detection limit of 2.5 μmol/L for nitrate/nitrite. Plasma samples were ultrafiltered through a 10-kDa microfuge ultrafiltration device (Millipore) and NO contents were assessed by a two-step process consisting of nitrate reductase–dependent conversion of nitrate to nitrite. This was followed by spectrophotometric detection (Bio-Rad Benchmark Microplate Reader) of total nitrite after Griess reaction at 540 nm (39).

Blood Pressure Measurements

The ABP measurements were performed using an Accutracker II blood pressure monitor (Suntech Medical Instruments, Raleigh, NC). The method of measurement is oscillatory and uses R-wave gating. Diastolic blood pressure was determined from phase 5 Korotkoff sounds. The blood pressure cuff (12 $\times$ 22 cm for lean patients, 14 $\times$ 30 cm for overweight and obese subjects) was attached to the patient's left arm and chest, and electrocardiogram electrodes were affixed by a skilled technician. Blood pressure was measured every 30 minutes between 6 a.m. and 10 p.m. and every 60 minutes between 10 p.m. and 6 a.m. during a 24-hour study period. Mean SBP and DBP values were calculated as means of the hourly averages. The period 6 a.m. and 10 p.m. was considered to be daytime and from 11 p.m. to 7 a.m. the next day nighttime, reflecting the usual sleeping habits of our population.

Statistical Analysis

Data are shown as mean $\pm$ SD or n (%). Data analysis was performed using SPSS for Windows, version 11.5 (SPSS, Chicago, IL). Shapiro-Wilk test was used to detect whether or not the

TABLE 1

Basic demographic data of the women with polycystic ovary syndrome.

Characteristic	n (%)
Nulliparity	84 (100)
Age (y)	
<20	3 (5.5)
20–29	42 (77.7)
≥30	9 (16.6)
Body mass index (kg/m^2)	
<25	39 (72.2)
≥25	15 (27.7)
Waist-to-hip ratio	
<0.72	11 (20.3)
≥0.72	43 (79.6)
Hirsutism	34 (62.9)

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continuous variables were normally distributed. Groups were compared using Student *t* or Mann Whitney *U* test as appropriate. Correlations between parametric variables and nominal parametric data were assessed by Pearson correlation coefficients.

Multiple linear regression stepwise method was used to determine the independent predictors which mostly affected SBP and DBP: SBP and DBP as the dependent variable and stepwise (probability of F to enter ≤.05; probability of F to remove ≥0.10) introduction of anthropometric, hormonal, inflammatory, and metabolic factors as the independent variables after elimination of nonsignificant predictors from the prognostic model. Logistic regression analysis was performed to assess the association between elevated blood pressure and the categoric data of the IL-6 level (with cutoff value at 5.1 pg/mL, the highest quartile of IL-6 in this study) after adjustment for age, BMI, CRP, NO, fibrinogen, HOMA index, total C, LDL-C, HDL-C, and TG. The IL-6 levels were calculated among the subjects in different SBP or DBP quartiles by using analysis of variance. Statistical significance was defined as $P<.05$.

RESULTS

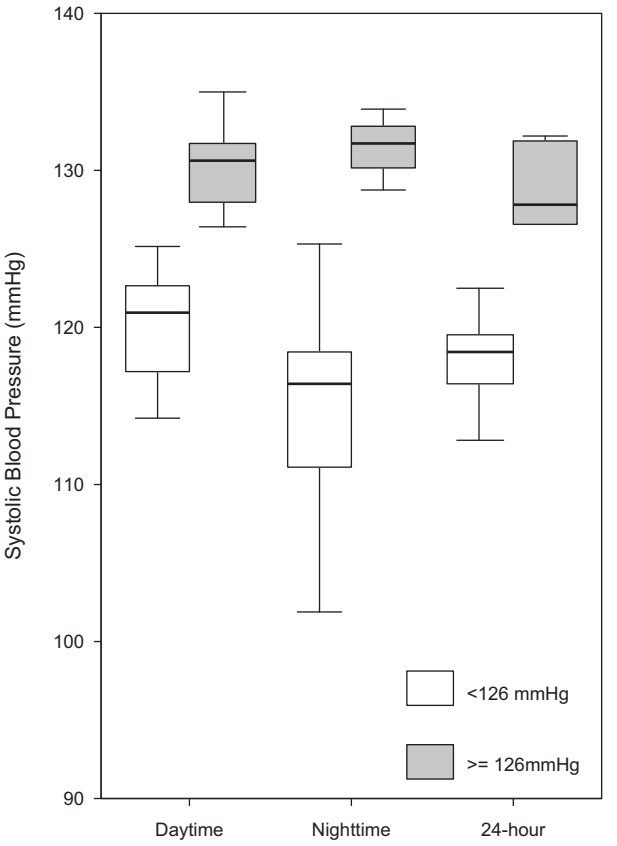
The clinical, endocrine, and biochemical features of the subjects are summarized in Table 1. The majority of the subjects were young (83.2% of the subjects were <30 years of age, with a mean age of 28.8 ± 4.4 years and a range of 19–36 years), and 84% were nulligravidas. None of the patients had hypertension. None of the patients had DBP ≥80 mm Hg. Ambulatory SBP records are shown in Figure 1. During 24 hours, SBP was ≥126 mm Hg in 27.7% of patients. The SBP was ≥126 mm Hg in 38.8% of patients during daytime and in 20.3% of patients during nighttime (Table 2).

The IL-6 and CRP levels were statistically higher in women with SBP ≥126 mm Hg during 24 hours and during daytime than those with SBP <126 mm Hg. Although not statistically significant, the IL-6 and CRP levels were higher in women with SBP ≥126 mm Hg than those with SBP <126 mm Hg during nighttime. Fibrinogen levels were not different in women with SBP ≥126 mm Hg and those with SBP <126 mm Hg during 24 hours, daytime, and nighttime. Serum NO levels in women with SBP ≥126 mm Hg were lower than in women with SBP <126 mm Hg during 24 hours, daytime, and nightime (Table 2).

Based on multiple linear regression analysis, SBP and DBP were significantly related to IL-6 ($P<.001$ and $P<.001$, respectively) as

FIGURE 1

Ambulatory systolic blood pressure during daytime, nighttime, and 24 hours in polycystic ovary syndrome patients with systolic blood pressure <126 mm Hg and ≥126 mm Hg.



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well as age ($P=.01$ and $P=.04$, respectively) (Table 3). Interleukin-6 explained 51% of overall change for 24-hour SBP measurements and 37.8% of overall change for 24-hour DBP measurements. Pure clarification coefficient for age was determined as 5%.

The age- and BMI-adjusted serum IL-6 levels among the subjects were categorized according to SBP or DBP quartiles. PCOS patients with SBP in the top quartile had significantly higher serum IL-6 levels compared with the bottom quartile (Fig. 2). PCOS patients with DBP in the top quartile also had significantly higher serum IL-6 levels compared with the bottom quartile (Fig. 3).

Using logistic regression analysis with adjustment for age, BMI, CRP, NO, fibrinogen, HOMA index, total C, LDL-C, HDL-C, and TG, the high serum IL-6 levels (serum IL-6 level ≥5.1 pg/mL, the highest quartile of IL-6 in this study) was associated with a higher probability of SBP ≥126 mm Hg, with an odds ratio of 2.2 (95% confidence interval 0.8–7.9; $P<.001$).

Correlation Between Variables

The SBP and DBP were positively correlated with IL-6, CRP, age, and BMI, WHR, fasting insulin, HOMA index, LDL-C, and TG. The SBP and DBP were negatively correlated with NO and HDL-C (Table 4). Serum IL-6 levels were positively and significantly

TABLE 2

Inflammatory markers, nitric oxide, and heart rate characteristics between polycystic ovary syndrome patients with systolic blood pressure (SBP) <126 mm Hg and those with SBP ≥126 mm Hg.

	SBP (mm Hg)					
	24 h		Daytime		Nighttime	
Variable	<126 (n = 39)	≥126 (n = 15)	<126 (n = 33)	≥126 (n = 21)	<126 (n =43)	≥126 (n = 11)
IL-6 (pg/mL)	3.0 ± 1.7	4.8 ± 0.2[a]	2.8 ± 1.7	4.8 ± 0.4[a]	3.1 ± 1.8	4.0 ± 1.2
CRP (mg/L)	1.8 ± 0.69	3.7 ± 1.52[a]	1.4 ± 0.53	2.9 ± 1.53[a]	1.7 ± 0.69	2.9 ± 1.1
Fibrinogen (μg/dL)	306.0 ± 100.0	404.0 ± 101.1	305.8 ± 107.9	363.4 ± 101.9	318.9 ± 103.4	383.8 ± 121.7
Nitric oxide (μmol/L)	15.9 ± 7.3	8.2 ± 5.3[a]	16.7 ± 7.0	10.3 ± 6.9[b]	14.4 ± 7.5	8.7 ± 4.4[a]
Heart rate (beats/min)	69.7 ± 4.4	78.3 ± 4.1[a]	69.3 ± 4.7	79.0 ± 8.2[a]	68.5 ± 4.5	79.1 ± 9.1[a]

Note: Statistical significance was defined as $P<.05$. CRP = C-reactive protein; IL-6 = interleukin-6.

[a] $P<.01$.

[b] $P<.05$.

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correlated with BMI (r = 0.27; $P<.05$), WHR (r = 0.24; $P<.05$), serum CRP levels (r = 0.47; $P<.001$), fasting insulin (r = 0.32; $P<.05$), and HOMA index (r = 0.41; $P<.01$). Serum IL-6 and CRP levels were negatively and significantly correlated with serum NO levels (r = −0.44; $P<.001$; r = 0.56; $P<.001$, respectively). No correlation was found between serum IL-6 and CRP and fibrinogen. Heart rate was correlated with only serum IL-6 levels (r = 0.41; $P<.01$). No correlation was found for the other parameters.

DISCUSSION

This is the first report showing the relationship between ABP and serum IL-6 levels in women with PCOS. The results of this study suggest that serum levels of IL-6 may be associated with SBP and DBP in PCOS patients. In the multiple linear regression analysis, IL-6 levels were independently associated with SBP and DBP (both $P<.001$). Serum IL-6 levels were significantly related to SBP levels.

The proinflammatory cytokine IL-6, in addition to stimulating the liver to produce acute-phase reactants, takes an active part in the inflammation process along with CRP and fibrinogen, both of which are related to hypertension (7, 11–13, 40). The role it has in the development of atherosclerotic lesions is well known (2, 4, 40). There are few studies reporting a positive association between circulating concentrations of IL-6 and fibrinogen and blood pressure in healthy individuals and hypertensive patients (2, 11–14). Nitric oxide is important vasodilator released from endothelial cells (20).

TABLE 3

Basic characteristics of subjects and the Pearson correlation analysis between blood pressure and anthropometric, hormonal, and metabolic variables.

		SBP		DBP	
Variable	Mean ± SD	r	*P* value	r	*P* value
Age (y)	28.8 ± 4.4	0.27	<.05	0.33	<.05
FSH (IU/L)	4.8 ± 2.4	0.11	NS	0.13	NS
LH (IU/L)	7.3 ± 4.9		NS	0.19	NS
Body mass index (kg/m^2)	24.4 ± 4.1	0.54	<.001	0.46	<.001
Waist-to-hip ratio	0.79 ± 0.07	0.30	.02	0.15	NS
Total cholesterol (mg/dL)	182.4 ± 30.7	0.69	<.001	0.62	<.001
LDL cholesterol (mg/dL)	123.5 ± 28.3	0.19	NS	0.07	NS
HDL cholesterol (mg/dL)	46.9 ± 5.0	0.35	.007	0.37	.004
TG (mg/dL)	102.0 ± 31.3	0.16	NS	0.02	NS
Free T (pg/mL)	1.9 ± 0.66	0.09	NS	0.12	NS
Total T (ng/mL)	0.59 ± 0.06	0.18	NS	0.16	NS
Fasting insulin (μIU·min/mL)	17.6 ± 5.0	0.49	<.001	0.47	<.001
Fasting glucose (mg/dL)	82.9 ± 7.9	0.11	NS	0.13	NS
HOMA-IR	3.3 ± 0.9	0.64	<.001	0.58	<.001
IL-6 (pg/mL)	4.1 ± 0.4	0.71	<.001	0.62	<.001
CRP (mg/L)	3.6 ± 1.6	0.37	<.01	0.29	<.05
Nitric oxide (μmol/L)	9.7 ± 4.2	−0.44	<.001	−0.51	<.001

Note: Statistical significance was defined as $P<.05$. HDL = high-density lipoprotein; HOMA-IR = homeostasis method assessment of insulin resistance; LDL = low-density lipoprotein; TG = triglycerides; other abbreviations as in Table 2.

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FIGURE 2

Serum interleukin-6 (IL-6) levels after adjustment for age and body mass index (BMI) among polycystic ovary syndrome women in different systolic blood pressure (SBP) quartiles. The SBP quartiles were as follows: 1st: <106 mm Hg; 2nd: 106–112 mm Hg; 3rd: 113–125 mm Hg; and 4th: ≥126 mm Hg. The age- and BMI-adjusted serum IL-6 levels were found to be statistically significant ($P<.01$ by ANOVA) between the two lower and two higher SBP quartiles. IL-6 levels were calculated among subjects in different SBP quartiles by using ANOVA as overall P value. The difference was found to be statistically significant ($P<.001$) between the 1st and 4th quartiles.

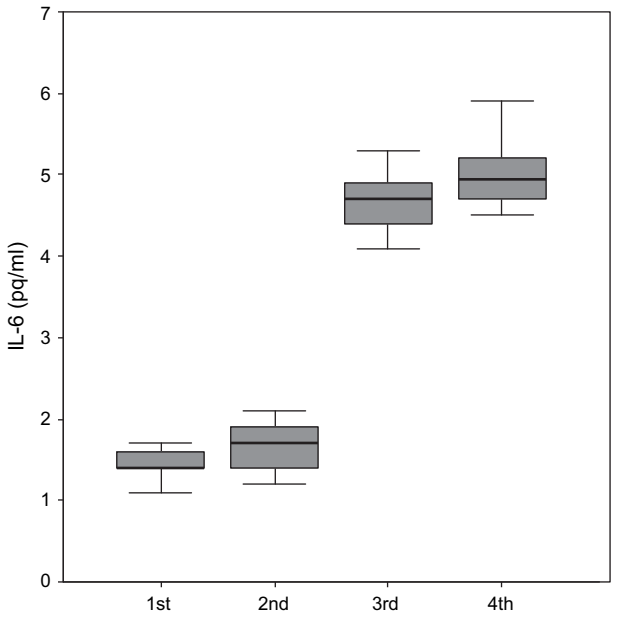

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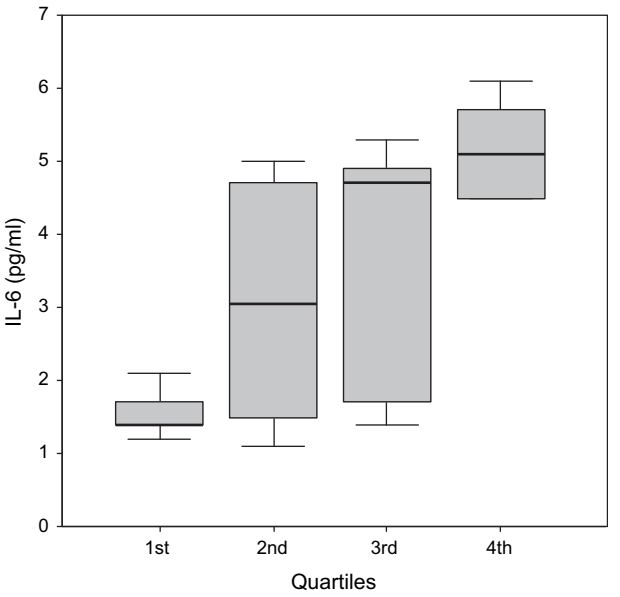
FIGURE 3

Serum interleukin-6 (IL-6) levels after adjustment for age and body mass index (BMI) among polycystic ovary syndrome women in different diastolic blood pressure (SBP) quartiles. The DBP quartiles were as follows: 1st: <65 mm Hg; 2nd 65–70 mm Hg; 3rd: 71:76 mm Hg; and 4th: ≥77 mm Hg. The age- and BMI-adjusted serum IL-6 levels were found to be statistically significant ($P<.01$ by ANOVA) between the 1st and 3rd and between the 2nd and 4th SBP quartiles. IL-6 levels were calculated among subjects in different SBP quartiles by using ANOVA as overall P value. The difference was found to be statistically significant ($P<.001$) between the 1st and 4th quartiles.



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Polycystic ovary syndrome is associated with endothelial dysfunction (20). Interleukin-6 and CRP could play an active role in modulating eNOS bioactivity (20–23, 41).

Omental adipose tissue produces threefold more IL-6 than subcutaneous adipose tissue (42). The IL-6 levels were positively and significantly correlated with BMI, WHR, fasting insulin, and HOMA index. These data confirm that obesity and insulin resistance are influential on the elevated IL-6 levels in PCOS patients. According to multiple linear regression results, IL-6 counted for 51% of overall change for 24-hour SBP measurements. The IL-6 levels were

TABLE 4

Multivariate analysis for the association between SBP and DBP and selected variables in PCOS patients.

				95% CI		
Dependent variable	**Independent variable**	**Coefficient of regression (β)**	***P* value**	**Lower bound**	**Upper bound**	**Adjusted R^2**
SBP	IL-6	2.5	<.001	1.63	3.38	51.0%
	IL-6	0.53	<.001	0.24	0.81	56.0%
	Age	0.41	.010	0.10	0.72	
DBP	IL-6	1.73	<.001	1.14	2.33	37.8%
	IL-6	1.38	<.001	0.81	1.95	48.8%
	Age	0.7	.04	0.02	1.45	

Note: Statistical significance was defined as $P<.05$. CI = confidence interval; DBP = diastolic blood pressure; IL-6 = interleukin-6; SBP = systolic blood pressure.

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statistically higher in women with SBP ≥126 mm Hg than in those with SBP <126 mm Hg during 24 hours and daytime. The PCOS patients with SBP in the top quartile (SBP ≥126 mm Hg) had significantly higher serum IL-6 levels than those in the bottom quartile. These findings suggest that elevated serum IL-6 levels in women with PCOS are associated with the level of the SBP. Therefore, our data suggests that the elevated levels of IL-6 initiate and/or mediate an increase in SBP in young PCOS women.

In addition to this, in a multiple linear regression analysis, IL-6 levels remained independently and positively associated with DBP. PCOS patients with DBP in the top quartile also had significantly higher serum IL-6 levels than those in the bottom quartile. Interleukin-6 explained 37.8% of overall change for 24-hour DBP measurements. None of the patients had DBP ≥80 mm Hg. These findings reveal that serum IL-6 levels may also affect DBP even if within normal limits in PCOS patients.

The CRP levels were statistically higher in women with SBP ≥126 mm Hg than those with SBP <126 mm Hg during 24 hours and daytime. Therefore, CRP levels may be involved in the blood pressure regulation in women with PCOS. The SBP and DBP were positively and significantly correlated with serum CRP levels. However, during nighttime, IL-6 and CRP levels were higher in women with SBP ≥126 mm Hg than those with SBP <126 mm Hg. Perhaps our sample size was too small to detect a significant difference in IL-6 and CRP between women with SBP ≥126 mm Hg and women with SBP <126 mm Hg during nighttime. Earlier data in the literature confirm that subjects with high CRP levels had higher mean SBP and DBP (2, 4, 6, 43). Because IL-6 is known to stimulate production of CRP, one might predict that both inflammatory markers affect blood pressure through the same pathway (13, 22, 23). In the present study, serum IL-6 levels were positively and significantly correlated with CRP levels. As levels of serum IL-6 increased, linear increase in CRP levels was also observed. These findings reveal that there is a direct relationship between increase in CRP levels and IL-6 in patients with PCOS. The effect of IL-6 and CRP on blood pressure may be mediated through endothelial dysfunction. This suggests that IL-6 and CRP affect blood pressure through the same mechanism and that their effects may be additive rather than interdependent in PCOS patients.

Nitric oxide also has a key role in blood pressure regulation, because it inhibits vasoconstriction (20). The NO levels were lower in women with SBP ≥126 mm Hg than in those with SBP <126 mm Hg during 24 hours, daytime, and nighttime. The SBP and DBP were significantly and negatively correlated with NO levels. Inflammation has been associated with decreased endothelium-dependent relaxation, a process related to an alteration in the bioavailability of NO (2, 6, 44). There is considerable evidence that links endothelial dysfunction with both essential and pregnancy-induced hypertension (5, 6, 19, 22, 44). Previously, Paradisi et al. (24) demonstrated a lower endothelium-dependent vasodilatation response to metacholine in PCOS patients compared with a control group as a possible effect of impaired NO secretion. In the present study, the reduced NO might be responsible for the increase of SBP in PCOS patients with SBP ≥126 mm Hg. According to our results, IL-6 and CRP may promote increase in the SBP by modulating NO concentration in PCOS patients. An association between IL-6 and CRP levels and NO has not been reported previously in PCOS patients. A recent study in pregnant rats showed that infusion of IL-6 led to increased blood pressure, through inhibition of an endothelium-dependent NO-cGMP–mediated relaxation pathway in systemic vessels (44). Recently, two studies reported that CRP could play an active role in modulating eNOS bioactivity, and endothelial cells incubated with CRP decreased eNOS expression and NO release (45–47). In the present study, both IL-6 and CRP were negatively and significantly correlated with serum NO levels. The decreased serum NO levels accompanied with the increased IL-6 and CRP levels in the group with SBP ≥126 mm Hg, and the negative correlation between CRP and NO, made us think that decrease in NO levels was related to the increase in IL-6 and CRP. Interleukin-6 and CRP, either separately or by potentializing each other's effects, may lead to impairment of endothelial NO release and thus may cause decrease in levels of serum NO. In the present study, as levels of IL-6 and CRP increase, values of SBP rise and levels of NO decrease. Therefore, IL-6 and CRP may increase SBP through inhibiting NO-mediated vasodilatation in women with PCOS. Further studies are needed to verify possible associations between IL-6 and CRP and NO in PCOS patients.

Interleukin-6 has been shown to stimulate the central nervous system leading to activation of the hypothalamus-pituiatary-adrenal axis and the sympathetic nervous system, which may result in the increase of SBP (48). The heart rate during 24 hours, daytime, and nighttime was higher in PCOS women with SBP ≥126 mm Hg than in those with SBP <126 mm Hg. An increase in the heart rate was associated with elevated serum IL-6 and CRP and decreased serum NO levels. However, in correlation tests, heart rate was only positively correlated with serum IL-6. This leads us to consider that increased heart rate is related to IL-6 rather than changes in the levels of CRP and NO.

The increased plasma concentrations of IL-6 and fibrinogen independently predict increased CHD (14–17, 19). In healthy individuals and hypertensive patients a positive association between serum levels of IL-6 and fibrinogen and blood pressure is present (18, 19, 42). Fibrinogen, the major determinant of plasma viscosity, stimulates red blood cell aggregation and thus increases whole-blood viscosity (18, 19). These increases also lead to increased total peripheral resistance and systemic arterial pressure (15). Interleukin-6 increases the hepatic synthesis of fibrinogen (17–19). Fibrinogen levels in PCOS patients were reported as either increased or similar compared with control groups (9, 10, 49). In the present study, fibrinogen levels were not different between PCOS women with SBP ≥126 mm Hg and PCOS women with SBP <126 mm Hg. In the correlation analysis, no correlation was found between serum IL-6 and fibrinogen. These data suggest that increase in SBP is not related to fibrinogen levels in PCOS women.

Our results suggest that raised plasma IL-6 levels in young PCOS women are associated with elevated SBP (>126 mm Hg). Plasma IL-6 levels, independent of age, insulin resistance, obesity, or dyslipidemia, are a risk factor for SBP and DBP in PCOS patients. Women with PCOS have been reported to have reduced vascular compliance, vascular endothelial dysfunction, and a higher mean arterial blood pressure (3, 8, 26). Furthermore, the degree of impairment in vascular compliance and endothelial function, as well as the increased blood pressure, persist after adjusting for obesity and insulin resistance (3, 8, 24, 26, 50). In the present study, using logistic regression analysis with an adjustment for age, BMI, CRP, NO, fibrinogen, HOMA index, total C, LDL-C, HDL-C, and TG, high serum IL-6 levels (serum IL-6 level ≥5.1 pg/mL) were associated with a higher probability of SBP ≥126 mm Hg, with an odds ratio of 2.2 (95% confidence interval 0.8–7.9; $P<.001$). These findings may have important implications in the long-term consequences of elevated IL-6 in PCOS women. Thus we speculate that serum IL-6 may possibly contribute to the risks of hypertension and cardiovascular disease that may occur later in life in PCOS patients. Therefore, it is reasonable to speculate that the increased cardiovascular risk and hypertension in women with PCOS are due in part to increased IL-6 levels in PCOS patients.

An association between IL-6 and ABP has not been reported previously in PCOS patients. Because of the cross-sectional nature of

the present data, no speculation can be made about whether high IL-6 levels preceded or followed the development of increased SBP or hypertension. Therefore, these findings should be confirmed in prospective cohort studies aimed toward elucidating the role of IL-6 in blood pressure in PCOS patients.

In conclusion, the present results suggest that elevated serum IL-6 levels will probably initiate an increased SBP by inhibiting the endothelium-dependent NO-cGMP pathway or by stimulating the production of CRP or via sympathetic nervous system activation in young PCOS patients.

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