

Study on the influence of adiponectin genetic variants and adiponectin levels among Indonesian women with polycystic ovary syndrome

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Abstrak

Latar belakang: Resistensi insulin dan obesitas sentral adalah keadaan yang sering ditemukan pada wanita PCOS dan ditandai dengan abnormalitas penanda biologi yang terkait dengan terjadinya gangguan metabolik. Hubungan antara adiponektin dan resistensi insulin telah banyak diteliti, namun penelitian terhadap pasien PCOS baru sedikit yang dilakukan. Penelitian ini bertujuan untuk menentukan kemungkinan hubungan polimorfisme T45G dengan penanda biologi PCOS dan pengaruhnya terhadap adiponektin serum pada populasi Indonesia.

Metode: Lima puluh dua pasien PCOS dan 52 subjek ovulasi normal tanpa hiperandrogenisme sebagai kontrol disertakan dalam penelitian ini. Sampel darah dikumpulkan antara hari ke 3 dan 5 siklus menstruasi spontan, jam 7 hingga 9 pagi, setelah menjalani puasa. Dilakukan pengukuran kadar serum FSH, LH, testosteron, SHBG, glukosa, insulin, profil lipid dan adiponektin. Resistensi insulin ditentukan dengan HOMA-IR, HOMA- β , dan SHBG. DNA genom dari darah perifer pasien dan subjek kontrol digunakan untuk memeriksa polimorfisme T45G menggunakan metode PCR.

Hasil: Terdapat perbedaan yang signifikan antara kelompok PCOS dan kontrol terhadap IMT, LH, testosteron, SHBG, dan FAI, tetapi tidak signifikan terhadap frekuensi distribusi polimorfisme gen T45G. Kadar adiponektin ditemukan lebih rendah pada kelompok PCOS daripada kontrol, dan terdapat hubungan antara resistensi insulin dengan PCOS. Pada pasien PCOS frekuensi polimorfisme T45G ditemukan lebih tinggi pada wanita dengan adiponektin kadar rendah dari pada kelompok adiponektin kadar tinggi, meskipun tidak bermakna secara statistik. Tidak ditemukan hubungan antara penanda biologi PCOS (LH, testosteron, SHBG, dan FAI) dengan polimorfisme gen T45G.

Kesimpulan: Polimorfisme gen adiponektin (T45G) tidak berhubungan langsung dengan penanda biologi PCOS, namun demikian hubungannya dengan adiponektin perlu penelitian lebih lanjut. (*Med J Indones.* 2012;21:83-91)

Abstract

Background: Insulin resistance and central adiposity are frequent disorders in PCOS women, which are marked by biological marker dysregulation related to this metabolic abnormalities. Association between adiponectin and insulin resistance has been investigated in many studies, while only a few studies were done in PCOS patients. This study is to determine the association of T45G polymorphisms in Indonesian population with PCOS biological markers and their influence to adiponectin serum.

Methods: Fifty-two PCOS patients and 52 normal ovulatory women without hyperandrogenism as control subjects were included. Blood samples were collected between day 3 and 5 of a spontaneous menstrual cycle at 7 to 9 am, after overnight fasting. Serum levels of FSH, LH, testosterone, SHBG, glucose, insulin, lipid profile and adiponectin were measured. Insulin resistance was estimated by HOMA-IR, HOMA- β , and SHBG. T45G gene polymorphisms were determined by PCR after genomic DNA was obtained from peripheral blood of patients and control subjects.

Results: There were significant difference between PCOS and control group in term of BMI, LH, testosterone, SHBG, and FAI, but not significant to T45G gene polymorphisms frequency distribution. Adiponectin levels were lower in PCOS patients than control. There was an association between insulin resistance with PCOS. Among PCOS patients, no association between adiponectin LH, testosterone, SHBG, and FAI with T45G gene polymorphisms. T45G gene polymorphisms were more frequent in PCOS with low adiponectin levels compared to those with high adiponectin levels, although not significant statistically.

Conclusion: T45G gene polymorphisms has no direct association with PCOS biological markers, but its association with adiponectin needs further study. (*Med J Indones.* 2012;21:83-91)

Keywords: Adiponectin, insulin resistance, polycystic ovary syndrome, T45G adiponectin gene

Polycystic ovary syndrome (PCOS) is a heterogeneous condition that is associated with the following clinical features: oligo/amenorrhea (caused by chronic oligo/anovulation), acne or hirsutism (resulting from hyperandrogenemia), infertility, and obesity. It affects < 10% of women of reproductive age,¹ with approximately 16 - 80% of the affected women being obese,² and is

responsible for 50 - 70% of cases with anovulatory infertility.³

It has long been recognized that insulin resistance is associated with hyperinsulinemia and has an important role in PCOS's pathogenesis, which initiates hyperandrogenism through the increased of ovarian

androgen hormone biosynthesis. Moreover, insulin resistance and hyperinsulinaemia may increase the risk of long-term metabolic disorders, such as impaired glucose tolerance and type 2 diabetes, as well as cardiovascular disease. Insulin resistance is also triggered by other factors such as obesity, sedentary life style and decreasing physical activities.⁴ Obesity is related to adipose tissue that produced many adipocytokines, which have been known to have substantial connection to insulin resistance.⁵

Adiponectin is an adipocytokine that is secreted solely by adipose tissue, and its concentration is inversely related to the degree of adiposity. Decreased adiponectin levels are associated with obesity, coronary artery disease, type 2 diabetes, and insulin resistance.⁶ These findings point to an important role of adiponectin in the pathophysiology of insulin resistance associated metabolic disorders, which may lead to PCOS. Furthermore, it is supposed that genetic variability in the adiponectin gene may be a determinant of the phenotypic expression of PCOS, because the gene of adiponectin is located on the long arm of chromosome 3 (area 3q27), where susceptibility to metabolic syndrome (insulin resistance, obesity, hypertension and coronary disease) and diabetes mellitus type 2 genes were located.⁷ Sequence polymorphisms of the adiponectin gene have been identified in humans and have been examined for possible association with insulin resistance and circulating adiponectin concentrations.⁸

There were many polymorphisms of adiponectin that had been known, one of them is T45G polymorphism, a silent T-to-G substitution in exon 2 (45T>G). This highly prevalent polymorphism has been reported to be associated with the risk of obesity, insulin resistance, and diabetes mellitus type 2.^{9,10} Furthermore, in a recent study the 45T>G polymorphism was also studied in women with PCOS and was related to d-androstenedione concentrations.¹¹ Therefore, the aim of this study was to examine the association of polymorphisms at position +45 of the adiponectin gene with the biological markers of PCOS and their influence on serum adiponectin.

METHODS

This is a case control study on Indonesian reproductive women. The cases were PCOS patients, and the control group was normal ovulatory women. Informed consent was obtained from all participants, and the study was approved by the Institutional Review Board of Hassanuddin University.

Study participants

Inclusion criteria: Indonesian reproductive women aged between 20 to 40 years old. Exclusion criteria: hyperprolactinemia, had a history of disorders of glucose tolerance, hypertension, use of any medications that might interfere with the normal function of hypothalamic-pituitary-gonadal axis, and unmarried.

Cases

The cases were PCOS patients who met the inclusion criteria and were diagnosed using the Rotterdam consensus.¹² The case group patients were outpatients of Yasmin Clinic, the Obstetrics and Gynecology Clinic of Cipto Mangunkusumo Hospital, Jakarta.

Controls

The control group consisted of women selected among staffs of Yasmin Clinic and Prodia Clinical Laboratory who met the inclusion criteria, had non PCOS menstrual disorder, and were Yasmin Clinic patients. Exclusion criteria for controls: hyperandrogenism that was defined by the clinical presence of hirsutism, acne or alopecia, and/or increased androgen concentrations.

Procedures

All the women underwent a complete screening panel at Prodia Clinical Laboratory, including physical examination, weight and height measurement, and ultrasound examination of the ovaries, and calculation of the body mass index (BMI). All the patients were on an unrestricted diet. An oral glucose tolerance test (75 g) was carried out, with glucose and insulin measured at 0 and 120 minutes. Further, the presence of menstrual dysfunction and obesity were determined. Menstrual dysfunction was defined by the presence of oligomenorrhea or amenorrhea.

Clinical and anthropological examination

Clinical and anthropometrical variables, BMI and clinical blood pressure were determined in all the subjects and done at Yasmin Clinic. The BMI of each patient was calculated as weight (kg)/height (m)². Obesity criteria were using the Asia Pacific perspective,¹³ defined obese is BMI \geq 25 kg/m² and not obese is BMI < 25 kg/m².

Blood sampling

Blood samples were collected at Prodia Clinical Laboratory, between day-3 and day-5 of a spontaneous

menstrual cycle on 7 to 9 o'clock in the morning, after overnight fasting. Women with amenorrhea within the previous year were categorized as anovulatory without further testing, and blood was taken for hormonal analysis immediately at 7 to 9 o'clock in the morning after overnight fasting. Blood samples were drawn for the measurement of serum gonadotropin levels [follicle stimulating hormone (FSH) and luteinizing hormone (LH)], total testosterone, sex hormone-binding globulin (SHBG), glucose, insulin, lipid profile and adiponectin.

Calculation of free androgen index, HOMA-IR and HOMA- β

The free androgen index (FAI) was calculated using the formula: total testosterone (nmol/L) / SHBG (nmol/L) X 100.

The mathematical models to assess insulin resistance were used:¹⁴

The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the formula: fasting glucose (mmol/L) X fasting insulin (μ IU/mL) / 22.5

The homeostasis model assessment of β -cell function (HOMA- β) was calculated using the formula: [20 X fasting insulin (μ IU/mL)] / [fasting glucose (mmol/L) - 3.5] %.

Assay methods

All assays of plasma glucose, lipid profile, hormonal levels and DNA polymorphism were performed at Prodia Clinical Laboratory.

Clinical chemistry analysis

Glucose serum levels were performed using a glucose oxidase technique with an auto analyzer. Total cholesterol, HDL, LDL and triglyceride were measured by enzymatic methods.

Hormone analysis

LH and FSH were measured with a competitive chemiluminescent enzyme immunoassay technique, using commercial kits (LH[®] Siemens Advia Centaur and FSH[®] Siemens Advia Centaur). Testosterone was measured with an electrochemiluminescent immunoassay technique, using a commercial kit (Testosterone II Cobas, Roche). SHBG was measured with electrochemiluminescent immunoassay technique (SHBG Cobas, Roche), and insulin with a competitive chemiluminescent enzyme immunoassay technique (Immulin[®] 2000 Insulin). Adiponectin levels were measured with a quantitative sandwich enzyme

immunosorbent assay technique, using commercial kits (Human Adiponectin ELISA kit for Total and Multimers, Daiichi Pure Chemicals[®]), and resistin with a sandwich enzyme immunoassay technique (Human Resistin ELISA, BioVendor Laboratory Medicine Inc.).

Genotype analysis

Genomic DNA was extracted and isolated from venous EDTA blood samples (buffy coat) of patients in both groups. The adiponectin T45G polymorphism, was genotyped by amplification of genomic DNA using the following primers: forward, 5'-GAAGTAGACTCTGCTGAGATGG-3'; reverse: 5'-AGATGCAGCAAAGCCAAAGT-3'. PCR products were obtained using 20 μ L reactions (10 μ L genomic DNA, 20 μ M primer forward, 40 μ M primer reverse, 10 μ M dNTP mix (Invitogen. Cat. No.10297-018), 5 U/ μ L Taq DNA polymerase (Invitrogen. Cat. No.10966-018), 25 μ M MgCl₂) in a thermal cycler (Perkin-Elmer 2400). The amplification conditions were as follows: 94°C for 3 minutes, followed by 35 cycles of 30 seconds at 94°C, thirty seconds at 55°C, and 1 minute at 72°C, and ending with a single 5 minutes extension step at 72°C. The resulting fragment was 535 bp in length. The fragment was digested with enzyme *Sma*I (AIT). Digestion of the G allele should produce two fragments of 153 and 382 bp. The digestion products were resolved after electrophoresis in 2% agarose gel.

Data collection and statistical analysis

Data collected were anthropometric, biochemical, metabolic, hormone, and genetic data of adiponectin polymorphism.

Statistical analyses were carried out using the Statistical Package for the Social Sciences (SPSS software, version 16.0). All data were tabulated and the mean and SD were calculated, unless otherwise stated. Gaussian distribution of continuous variables was tested by the Kolmogorov-Smirnov test. Logarithmic transformations were applied to BMI, LDL, HDL, triglyceride, fasting glucose and insulin, glucose and insulin 2 hours after meal, HOMA-IR, SHBG, FAI, DHEAS, 17OH-progesterone, and adiponectin levels to ensure gaussian distribution of the variables, and the values were presented back-transformed as means and SDs. When the variables did not achieve a normal distribution, the values are presented as means and range.

Biochemical differences between two continuous variables were estimated with t-test or Mann Whitney U-test or chi square test as appropriate. Pearson

coefficients were used to establish associations between SHBG, adiponectin level and polymorphism, with features of PCOS. Sub group analysis was done to establish associations between biological markers of PCOS and adiponectin gene polymorphism with BMI using t-test.

The distributions of genotypes and allele were compared between study groups by using the χ^2 -test. The genotype frequency distributions of T45G polymorphisms were calculated using Hardy–Weinberg equilibrium. For the genotypes present in statistically significant different frequencies, the odds ratios (ORs) and 95% confidence intervals (CIs) were also estimated. $P < 0.05$ was considered statistically significant.

RESULTS

There were 159 patients of reproductive women with menstrual disorders who came to the clinic during the recruitment process. From those patients there were 5 patients who were not willing to participate in this study, twelve subjects did not return for laboratory testing, eighteen subjects were excluded (one patient with hyperprolactinemia, fourteen patients were not married, and 3 patients were out of the age range criteria), and finally we obtained 124 study subjects who met the inclusion and exclusion criteria.

While selecting process to get PCOS patients, based on Rotterdam consensus, we obtained 72 patients of PCOS and the rest of those were non-PCOS patients (52 subjects). By considering the minimum sample size of 25 subjects and the case-control design of this study, it was decided to involve the whole subjects of non-PCOS patients as the control group. Meanwhile for the case group, matching simple random selection method was proceed, which was based on the age range criteria to get 52 patients of PCOS as the case group.

Digestion of the G allele produced two fragments with lengths 153 and 382 bp, while T allele was not digested (535 bp). The digestion products that were resolved after electrophoresis in 2% agarose gel is shown in figure 1.

Association of hormones, adiponectin levels and gene polymorphisms with PCOS

Anthropometric, metabolic and hormones data of women with PCOS and healthy controls are summarized in table 1. BMI values, LH, Testosterone, and FAI levels were significantly higher in the PCOS group compared with controls, whereas SHBG were lower ($p < 0.05$ for BMI, LH and testosterone, $p < 0.005$ for SHBG, and FAI).

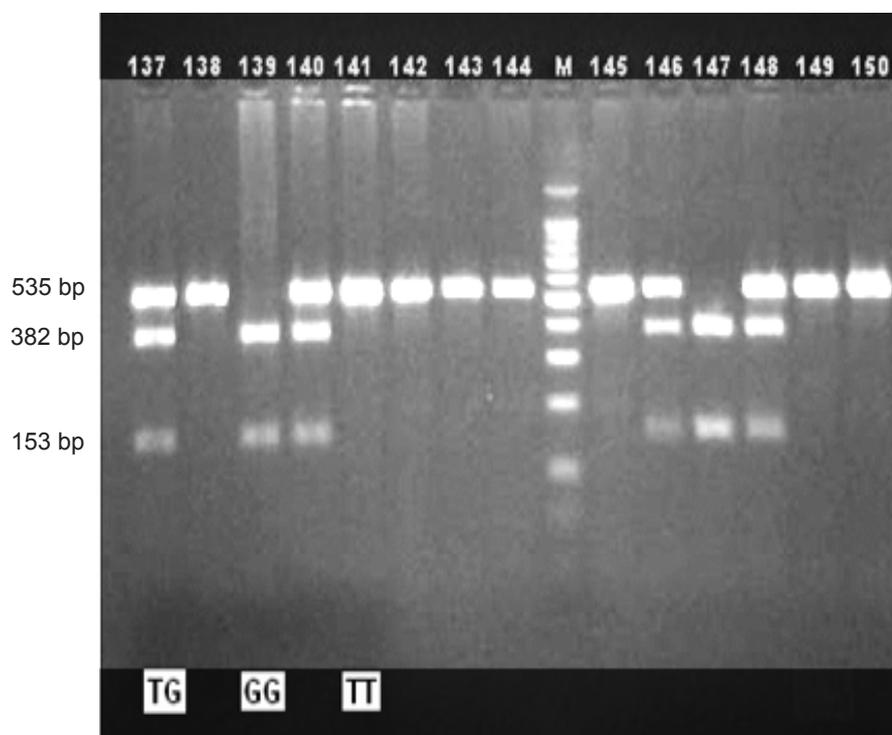


Figure 1. Fourteen representative immunoblot illustrating the main adiponectin fragments of TG, GG and TT genotype, the T allele (535 bp) and G allele (382 and 153 bp)

Table 1. Anthropometric, metabolic, hormone, and adiponectin gen polymorphism profiles of women with PCOS and controls

	PCOS	Controls	p
Number of women	52	52	
Age, years	28.4 (5.0) ^a	29.1 (4.4)	NS ^c
BMI ^d , kg/m ²	26.85 (4.6)	24.8 (4.6)	0.04
Total cholesterol, mg/dL	198.3 (25.3)	198.0 (35.0)	NS
LDL ^d , mg/dL	130.1 (21.9)	132.7 (31.6)	NS
HDL ^d , mg/dL	47.7 (10.1)	49.5 (12.7)	NS
Triglyceride ^d , mg/dL	124.5 (87.6)	99.8 (49.8)	NS
Fasting glucose ^d , mg/dL	90.3 (8.1)	89.7 (6.7)	NS
Fasting insulin ^d , μ IU/mL	10.0 (6.5)	9.5 (8.2)	NS
Glucose 2h ^d , mg/dL	144.5 (29.7)	105.1 (18.9)	NS
Insulin 2h ^d , μ IU/mL	113.0 (74.5)	94.6 (68.0)	NS
HOMA-IR ^d	1.9 (1.3)	2.0 (1.9)	NS
HOMA- β ^d	1.9 (0.4)	1.9 (0.4)	NS
LH, mIU/mL	9.3 (0.9 - 22.4) ^b	7.4 (0.2 - 33.6)	0.005
FSH, mIU/mL	5.1 (1.4 - 7.4)	5.2 (1.2 - 11.3)	NS
Testosterone, nmol/L	1.2 (0.1 - 2.5)	1.0 (0.2 - 4.6)	0.02
SHBG ^d , nmol/L	1056.1 (701.1)	1548.0 (919.4)	0.003
FAI ^d	5.1 (3.3)	3.5 (5.1)	0.001
DHEAS ^d , μ g/dL	242.7 (108.0)	219.3 (86.2)	NS
17OH-progesterone ^d , ng/mL	1.1 (0.9)	1.2 (1.2)	NS
Adiponectin ^d , μ g/mL	3.7 (1.6)	5.0 (2.6)	0.003
Genotypes, n (%)			
TG + GG	24 (52.2)	22 (47.8)	NS
TT	28 (48.3)	30 (51.7)	
Alleles, n (%)			
G	76 (48.4)	81 (51.6)	NS
T	28 (54.9)	23 (45.1)	

^a Values are the mean (SD), ^b Values are the median (range), ^c NS= not significant, ^d Significance was tested on log-transformed values, HOMA-IR= homeostatic model assessment of insulin resistance, HOMA- β = homeostatic model assessment of β cell function, DHEAS= dehydroepiandrosteron

PCOS patients presented with significantly lower serum adiponectin levels than healthy controls (3.7 ± 1.6 versus 5.0 ± 2.6 μ g/mL, $p = 0.003$), which were independent of obesity status (Table 1).

The genotype distributions of the T45G polymorphisms in the adiponectin gene were in Hardy-Weinberg equilibrium ($\chi^2 = 0.07$, $p > 0.05$). Overall, there was no statistically significant difference in the distributions of genotypes and alleles for T45G polymorphisms between PCOS women and controls, indicating that the individual polymorphisms at position +45 of adiponectin gene were not associated with increased risk for PCOS (Table 1).

Association of HOMA-IR and HOMA- β with SHBG as predictive marker of insulin resistance

Anthropometric, metabolic and hormones profiles in table 1 shows no significantly difference for HOMA-

IR and HOMA- β as calculation models for diagnosing insulin resistance, but SHBG is lower significantly in PCOS group than controls ($p = 0.003$).

In this study, there was a significant association between adiponectin as well as insulin resistance that was represented by HOMA-IR and HOMA- β and SHBG in women with PCOS group, although this was a weak association (Figure 2).

Association of hormones, insulin resistance, adiponectin level and gene polymorphism in PCOS women according to BMI

As adiponectin levels are known to correlate with BMI, this study stratified the control and PCOS women according to their degree of obesity to obtain a homogeneous BMI distribution between the groups. The characteristic of the major markers related to hyperandrogenism, insulin

resistance, the lipid profile, and adiponectin levels according to BMI in women with PCOS are given in table 2. This table shows that obese PCOS women had lower levels of LH and lower rates of hyperandrogenemia as indicated by the FAI, than non obese PCOS women ($p = 0.04$ and $p =$

0.008 respectively). As expected, obese PCOS women had higher serum adiponectin levels than non obese PCOS women ($p < 0.001$), and SHBG levels, as predictive marker of insulin resistance, was higher in obese than non obese PCOS women ($p = 0.005$).

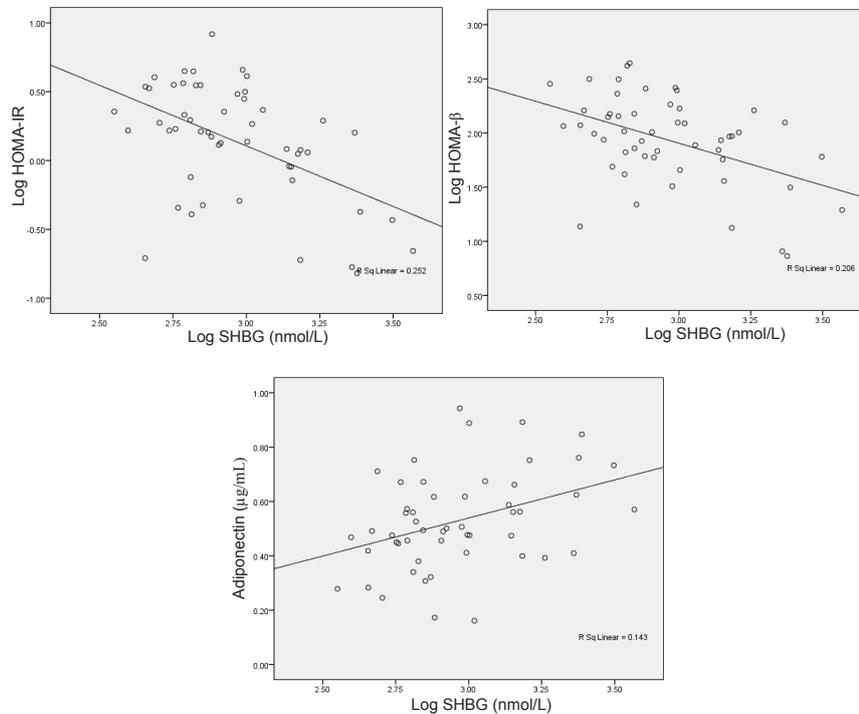


Figure 2. The relationship between sex hormone-binding globulin (SHBG) with HOMA-IR, HOMA- β and adiponectin in PCOS women

Table 2. Metabolic, hormones, and genotypes/ alleles of adiponectin profiles in Group A and Group B in women with PCOS

	Group A ¹	Group B ²	p
Number of women	20	32	
Total cholesterol, mg/dL	202.3 (24.1)	197.8 (25.8)	NS
LDL cholesterol, mg/dL	131.6 (19.5)	133.1 (23.8)	NS
HDL cholesterol, mg/dL	46.7 (9.7)	46.4 (8.6)	NS
Triglyceride, mg/dL	136.3 (105.6)	125.6 (58.4)	NS
Fasting glucose, mg/dL	91.7 (8.5)	89.3 (8.8)	NS
Fasting insulin ³ , μ IU/mL	9.1 (5.8)	11.6 (7.2)	NS
HOMA-IR	2.1 (1.3)	2.6 (1.9)	NS
LH, mIU/mL	8.0 (4.0)	11.4 (6.0)	0.04
FAI ³	4.4 (2.9)	6.6 (3.6)	0.008
SHBG ³	1216.5 (791.9)	866.3 (514.9)	0.005
DHEAS, μ g/dL	240.5 (121.3)	247.2 (93.9)	NS
17OH-progesterone ³ , ng/mL	0.9 (0.7)	1.2 (1.32)	NS
Adiponectin ³ , μ g/mL	4.5 (1.6)	2.3 (0.5)	< 0.001
Genotypes, n (%)			
TG + GG	8 (38.1)	13 (61.9)	NS
TT	12 (38.7)	19 (61.3)	
Alleles, n (%)			
G	16 (57.1)	12 (42.9)	NS
T	48 (63.2)	28 (36.8)	

¹ Group A, PCOS women with BMI > 25 kg/m², ² Group B, PCOS women with BMI < 25 kg/m², ³ Significance was tested on log-transformed values, Data is presented as mean (SD), NS= not significant

Table 3. Characteristic of various risk factor in women with PCOS according to T45G polymorphisms of the adiponectin gene

	Genotypes		p
	TT	TG + GG	
LH, mIU/mL	10.2 (5.9)	8.3 (3.9)	0.20
Testosterone, nmol/L	1.2 (0.6)	1.1 (0.5)	0.63
SHBG*, nmol/L	1202.0 (857.6)	941.5 (477.9)	0.35
FAI*	5.1 (3.5)	5.1 (3.2)	0.49
Adiponectin*, µg/mL	3.6 (1.3)	3.8 (2.0)	0.93

Data is presented as mean (SD), * Significance was tested on log-transformed values

Table 4. Genotype and allele frequencies of T45G polymorphisms in study participants according to adiponectin levels

	Group A ¹	Group B ²	p
Genotypes, n (%)			
TG + GG	27 (58.7)	19 (41.3)	0,05
TT	23 (39.7)	35 (60.3)	
Alleles, n (%)			
G	23 (44.2)	29 (55.7)	NS
T	85 (54.5)	71 (45.5)	

¹ Group A= women with adiponectin level < 0.7 µg/mL, ² Group B= women with adiponectin level > 0.7 µg/mL, NS= not significant

The genotype and allele frequency of adiponectin polymorphism at position +45 (T45G) in obese and non obese PCOS women are shown in table 2. The frequency of TG + GG genotype were less frequent among obese PCOS women (8/20, 38.1%) than non obese PCOS women (13/32, 61.9%), but the difference was not statistically significant. Allele frequencies for T45G variant in obese PCOS women were 0.75 (T) and 0.25 (G), while 0.70 (T) and 0.30 (G) were in non obese PCOS women. It means that allele G of adiponectin gene (T45G) polymorphism is more frequent in obese than in non obese PCOS women.

In multiple regression analysis between BMI and those parameters to which they were found to correlate significantly in women with PCOS (n = 52 showed that adiponectin level is a significant independent determinant (p < 0.001).

Effect of adiponectin gene polymorphisms on adiponectin serum and insulin resistance in women with PCOS

Considering that PCOS patients and controls differed significantly in BMI values, LH, androgen, SHBG and adiponectin levels, and not significantly different

in genotype and allele of T45G polymorphisms, we further explored these influences. We analyzed the association of genotypes of the T45G polymorphisms with PCOS biological markers in PCOS patients, and the results were shown on table 3. There were no significantly differences of all PCOS biological markers with the genotype of T45G polymorphisms, p > 0.05.

Regarding the T45G polymorphism analysis and the biological markers of PCOS as shown on table 3, the comparison between genotype TT and TG + GG did not yield any significant differences. However, when we performed the analysis in all women of this study participants, which were divided in women with high and low adiponectin levels, genotype frequencies of the T45G polymorphisms between TT and TG + GG showed barely significant difference (p = 0.05), but no significant difference in allele frequency (Table 4).

In addition, the number of TG and GG genotypes were significantly more frequent in women with low adiponectin level than in women with normal to high adiponectin level, and the adiponectin levels were higher in TG + GG genotypes than TT genotype both in healthy controls and in PCOS women (Table 4).

DISCUSSION

The role of adiponectin is far from being completely understood. In particular, in some studies, which associated low levels of adiponectin with obesity, insulin resistance, and PCOS do not permit the causality between the lowering of adiponectin and dysregulated metabolism to be established.¹⁵ Recent studies showed that the serum adiponectin level in PCOS patients was significantly lower than in normal individual,¹⁶ and some studies indicated that low adiponectin level in PCOS women was closely correlated with obesity.^{4,17}

This study demonstrated that low levels of adiponectin are associated significantly with PCOS, also in PCOS women the adiponectin levels were found to correlate significantly with the degree of obesity. Furthermore the multivariate analysis found that adiponectin appeared to have a direct link with PCOS and BMI. These results suggest that adiponectin is involved in the pathogenesis of PCOS and is dependently associated with the degree of obesity.

Jayagopal et al¹⁸ and Kajaia et al¹⁹ found SHBG may serve as predictive marker of insulin resistant in hyperandrogenic women. In this study, in PCOS women group there were negative correlations between SHBG with HOMA-IR and HOMA- β , with *r* values are -0.502 ($P < 0.001$) and -0.454 ($p = 0.001$) respectively. These results suggest that SHBG may serve as a marker for hyperinsulinemia in women with PCOS.

PCOS is associated with insulin resistance accompanied by compensatory hyperinsulinemia. Hyperinsulinemia is thought to result in increased androgen biosynthesis²⁰ and decreased levels of SHBG¹⁸ as surrogate marker of insulin resistance, which play a major role in the of pathogenesis of hyperandrogenism. PCOS-associated hyperandrogenemia may modulate plasma adiponectin,²¹ which could provide a potential mechanism whereby PCOS-related hyperandrogenemia enhances the susceptibility of PCOS women to the insulin resistance.¹⁵

Regarding hypoadiponectinemia in women with PCOS that were found in this study, there must be a contribution of adiponectin genetic variant to the decreased adiponectin levels. In this study, T45G polymorphisms as one of adiponectin genetic variants, were not associated with the anthropometric parameters, hyperandrogenism and adiponectin levels of PCOS women. Furthermore, there were higher frequency of TG + GG genotypes in women with PCOS compared with healthy control, though not significantly different. This finding is in concordance with recent studies, where no association was found between T45G polymorphism

with PCOS or obesity, insulin resistance and adiponectin levels in women with PCOS.^{11,22} However, in this study the T45G polymorphisms were associated with higher adiponectin levels in both healthy controls and PCOS women.

These findings support the hypothesis that the T45G polymorphism of the adiponectin gene is not directly linked to obesity, metabolic disturbances related to insulin resistance, and biological markers of PCOS. Otherwise the T45G polymorphism of adiponectin gene is directly linked to adiponectin levels, as demonstrated in this population studied.

Diamanti-Kandaraki²³ found insulin resistance and hyperinsulinemia are established pathogenic mechanisms for hyperandrogenism in PCOS, and the facilitation of insulin resistance by androgen excess through the induction of adipokines produced by abdominal adiposity, hypoadiponectinemia may contribute to insulin resistance, hyperinsulinism and hyperandrogenism in PCOS women.²⁴ According to this present results, there was a significantly lower insulin resistance and hypoadiponectinemia in PCOS women, whereas adiponectin levels were associated with the degree of obesity. These findings and the significant difference between PCOS and non-PCOS patients suggest that in PCOS women, adiponectin might be weakly associated with insulin resistance.

In conclusion, adiponectin gene polymorphisms (T45G) has no direct association with PCOS biological markers (LH, testosterone, SHBG, FAI), but its association with adiponectin needs further study.

Acknowledgments

The authors thank dr. Andon Hestiantoro, SpOG, dr. Budi Wiweko, SpOG, for assisting in patients participation, and to Ms. Jessica, Mr. Miswar Fattah, Mrs. Wiwik Rositawati and Mr. Mulyana, Research Laboratory, Prodia Clinical Laboratories, for excellent technical assistances.

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