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## Association of Polycystic Ovary Syndrome and Adiponectin Gene Polymorphisms

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### Abstract

**Background:** Polycystic Ovary Syndrome (PCOS) is a common gynecological endocrine disorders; which characterized by hyperandrogenism, and anovulation with increased risks of infertility. Adiponectin is a protein specifically and abundantly expressed in adipocytes. Adiponectin Gene Polymorphisms (SNPs) influence adiponectin levels associated with risk for obesity, Insulin Resistance (IR) and T2DM. We aimed to investigate the relation between PCOS and adiponectin gene polymorphism affecting the metabolic disturbance.

**Materials:** Sixty women (Group 1) with PCOS were selected, had presented at gynecological-department of Minia university hospital with fertility problems. Forty healthy women (Group 2) matched with age and with regular menses and without hyperandrogenemia volunteered as controls. Serum level of F.S.H, L.H, total testosterone and glucose were measured. Adiponectin was measured by commercial immunoassays. AMH was measured by (Human AMH ELIZA kit) and insulin was measured by ELIZA, then the glucose/insulin ratio was calculated. Genomic DNA was isolated from peripheral blood leukocytes. The polymorphism was typed according to the curve obtained by real time PCR.

**Results:** The results showed low significant value of adiponectin and glucose-insulin ratio was with PCOS, whereas FSH, LH, prolactin, total testosterone and AMH were higher in PCOS. Adiponectin, AMH, total testosterone, LH levels and glucose insulin ratio between PCOS and control group showed significant differences. While, there were no significant differences in case of FSH and prolactin. Plasma adiponectin concentration was positively correlated with insulin-stimulated glucose disposal with a significant of 0.003. The genotyping distributions of TG, GG and TT in women with PCOS are 22 (37%), 19 (31.5%) and 19 (31.5%), respectively. The correlation of adiponectin and genotyping is significant  $P = 0.001$ .

**Conclusion:** Prevalence of adiponectin gene polymorphism is higher in PCOS, with significant correlation with the glucose/insulin ratio.

**Keywords:** Receptor; Insulin genetics; Adiponectin genetics; Polycystic ovary syndrome; Polymorphism

### Background

It is known that PCOS is associated with increased risks of infertility, impaired glucose tolerance, type 2 diabetes mellitus, and metabolic syndrome [1]. While 6%-17% of reproductive-age women worldwide suffer PCOS [2,3] some ethnicities, such as South Asian, have higher incidence rates [4]. Etiological studies demonstrated significance of genetic susceptibility for PCOS pathogenesis [5]. Central adiposity play an important role in the insulin resistance of the metabolic syndrome via deregulated production of various adipocyte-derived cytokines and proteins (adipocytokines), including tumor necrosis factor plasminogen activator inhibitor-1, leptin, resistin, and adiponectin [6]. Adiponectin, which is a known protein secreted exclusively by differentiated adipocytes and circulates in large amounts in humans [7]. Adiponectin (ADIPOQ) is the most common gene product in adipose tissue [8,9]. Adiponectin is encoded by Adipocyte, C1q, and Collagen Domain Containing (ACDC), which is located on chromosome 3 at q27. Genome-wide scans have revealed a susceptibility locus at 3q27 for Type 2 Diabetes (T2D), Coronary Artery Disease (CAD), obesity, and metabolic syndrome [10,11]. Several Adiponectin Gene (ADIPOQ) Single Nucleotide Polymorphisms (SNPs) influence adiponectin levels and associated with incidence of obesity, insulin resistance (IR), T2DM, and CVD [12]. The adiponectin gene consists of three exons and two introns spanning a 17-kb region [13]. Sequence polymorphisms identified in humans and investigated for their possible association of insulin resistance indexes and circulating adiponectin concentrations [11,14-16]. Most studies have focused on two polymorphisms, a silent T-to-G substitution in exon 2 (45T-G) and a G-to-T substitution in

intron 2 (276G-T). These polymorphisms associated with obesity, insulin resistance, and the risk of type 2 diabetes [17-20]. Furthermore, these two polymorphisms were selected because of their high frequencies in all investigated population, while other reported polymorphisms were rare. The 45T-G polymorphism was related to 4-androstenedione concentrations in PCOS [21]. Therefore, we have investigated the relation between PCOS and axon polymorphism of the adiponectin gene, and the effect of this relation on metabolic disturbance.

## Materials and Methods

Group 1: Sixty women with PCOS were selected, had all presented at our gynecological department of Minia university hospital from April 2015 to January 2016 with oligomenorrhea or fertility problems. None of the women had galactorrhea, or any systemic disease that could possibly affect their reproductive physiology. Any medication could interfere with the normal function of the hypothalamic–pituitary–gonadal axis. Control group (Group 2) was selected from volunteered healthy forty women, who matched with age, with regular menses and without hyperandrogenemia. All women in the study were genetically unrelated. Before the study, blood samples were drawn from each patient between 08.00 and 08.30 a.m., after an 8 h fast for determination of hormone, adiponectin and glucose levels. A standard 75 g Oral Glucose Tolerance Test (OGTT) and the insulin response to oral glucose loading were performed between 08.30 and 10.30 h, after 10-12 h of fasting. Glucose tolerance was evaluated using the criteria of the American Diabetes Association. For all women, blood samples were collected; part of them were put in EDTA tubes stored at  $-80^{\circ}\text{C}$  for genetic analysis, and the remaining blood centrifuged directly and the serum was withdrawn and stored at  $-80^{\circ}\text{C}$  for estimation of F.S.H, L.H, testosterone, adiponectin and Anti-Mullarian Hormone (AMH).

For all women (Groups 1 and 2), serum level of F.S.H, L.H, total testosterone and glucose were estimated in the laboratory unit in Minia university hospital. Adiponectin was measured by commercial immunoassays (Human Adiponectin RIA Kit, Linco Research, St. Charles, MO, USA) with intra- and inter-assay coefficients of variation below 10%. AMH was measured by commercial immunoassays (Human AMH ELIZA kit, US Biological life sciences, U.S.A) and insulin was measured by ELIZA (Human ELIZA kits, Thermo Fisher Scientific), then a glucose/insulin ratio was calculated.

### Genotype analysis

Isolation of genomic DNA was carried out from peripheral blood leukocytes of women with PCOS (Group 1) and the controls (Group 2). The extraction was done as the followings: Samples were collected in sterile tubes; then DNA was extracted using the QIAamp Min elute kit protocol in automated mode by a Qiacube instrument. Real Time PCR was used according to literature (OD-0002-02) from Life River. The amplification of genomic DNA was done using the following primers: F5-GAATGAGACTCTGCTGAGATGG and R5-

TATCATGTGAGGAG-TGCTTGGATG. PCR products were obtained using 25  $\mu\text{L}$  reactions [5  $\mu\text{L}$  genomic DNA, 12.5  $\mu\text{L}$  Master Mix, 2.5  $\mu\text{L}$  and Primer Probe Mix for wild gene (Green VIC), 2.5  $\mu\text{L}$  Primer Probe Mix for mutant gene (Red FAM) and 2.5  $\mu\text{L}$  of distilled water. The amplification conditions were as follows:  $95^{\circ}\text{C}$  for 10 min, followed by 40 cycles of 15 seconds at  $95^{\circ}\text{C}$ , 60 seconds at  $60^{\circ}\text{C}$  and 90 seconds at  $72^{\circ}\text{C}$ , and ending with a single 10 min extension step at  $72^{\circ}\text{C}$ . The polymorphism was typed according to the curve obtained by the real time PCR (Fast 7500).

### Statistical analysis

Analyses were performed using the Statistical Package for Social Sciences (SPSS version 18.0). Hormonal data were analyzed with durations of disease and with age using Factorial experiment with completely randomized design with three factors, and were analyzed between groups using Factorial experiment with completely randomized design with two factors. Data were represented as mean  $\pm$  SE. Bivariate correlations were performed using the Pearson correlation coefficient (P): a P value of  $<0.05$  was considered statistically significant.

## Results

The demographic data of our case group were as follows. The mean and standard deviation of age were  $24.2167 \pm 3.63641$ . Descriptive analysis in both groups in **Table 1** shows that the adiponectin concentrations and glucose/insulin ratios were significantly lower with PCOS (Group 1), whereas FSH, LH, prolactin, total testosterone and AMH were higher in PCOS. There were significant differences in adiponectin, AMH, total testosterone, glucose insulin ratio between Group 1 and control group (Group 2). On the other hand, there were no significant differences in case of FSH and prolactin. 72% of cases of PCOS came from rural areas, while 28% came from urban area (**Table 2**). Plasma adiponectin concentration was positively correlated with glucose insulin ratio with a significance of 0.003 (**Table 3**). The genotyping distributions of TG, GG and TT in women with Group 1 are 22 (37%), 19 (31.5%) and 19 (31.5%), respectively (**Table 4**). They differ from the same quantities in Group 2: 3 (7.5%), 7 (17.5%) and 30 (75%). Finally, our results show that the correlation of adiponectin and genotyping of both groups is of significant value ( $P = 0.001$ , **Table 5**).

## Discussion

In our study, it was noted that serum adiponectin levels of the phenotypes of PCOS were significantly lower than control women (Group 2). That might be attributed to the difference in hyperandrogenism of PCOS women and the body fat distribution and insulin resistance as well. Accordingly, the decreasing in serum adiponectin levels of PCOS patients may be related to any of these variables. Serum adiponectin plays an important role in the pathogenesis of insulin resistance and consequently it might deduce that hypoadiponectinemia would contribute in insulin resistance of PCOS women [22].

Our results agreed with Spain study of seventy-six PCOS patients and 40 non-hyper androgenic women related to BMI and the degree of obesity. Free testosterone levels, age and abdominal adiposity, irrespective of the degree of obesity, were found as the major determinants of hypoadiponectinemia. Reported results support the hypothesis that hyperandrogenism might indirectly lead to insulin resistance in women, by inducing abdominal adiposity and possibly decreasing adiponectin in PCOS patients [23].

Therefore the possible dysfunction of adipose tissue is the main reason of insulin resistance in PCOS [23]. A study by Elbers et al. demonstrated that hyperandrogenism lower adiponectin level and increase insulin resistance in PCOS women [24].

**Table 1** Descriptive analysis of patients with PCOS and controls

		Mean	St. Deviation	P value
Age	Case	24.2167	3.63641	0.001
	Control	30.3750	4.99583	
FSH	Case	14.76	20.9	0.897
	Control	12.87	18.7	
LH	Case	20.0967	29.26833	0.002
	Control	4.6425	2.02090	
LH/ FSH	Case	2.61	0.73	0.001
	Control	0.45	0.16	
Prolactin	Case	14.0983	5.03373	0.914
	Control	12.8950	5.07841	
T.Testosterone	Case	103.9850	116.24456	0.002
	Control	51.4225	82.66094	
G.Insulin ratio	Case	9.9583	2.89115	0.001
	Control	19.2600	2.89321	
AMH	Case	10.2500	2.60173	0.001
	Control	2.1000	0.35301	
Adiponectin	Case	944.5667	290.36782	0.000
	Control	3846.3750	2707.96148	

Several studies have shown the higher incidence of adiponectin gene polymorphisms in PCOS subjects. Possible genetic mechanisms could explain the variations in adiponectin levels in patients with PCOS [25]. On contrary of our results, Mohan et al. revealed a completely similar genotype and allele frequency distribution of SNP in the exon 245 position of the adiponectin gene between the PCOS and control groups. Similarly, a study performed in Greek women, no significant difference was detected between 45T→G polymorphism frequencies in the PCOS and control groups, and this polymorphism at position 45T→G had not been associated with a risk for development of PCOS [26,27]. Escobar-Morreale et al. [28] showed that the adiponectin gene

polymorphism, 45T→G, is not associated with patients with PCOS. In another Greek trial, the TT genotype and T allele at the 45 position was detected at a lower frequency, while the TG genotype and G allele were detected at a higher frequency in obese women with PCOS compared to those of normal weight (agreed with our results); however, these differences were not statistically significant [29].

**Table 2** Residence of patients with PCO and controls

	Rural	Urban
Case	43 (72%)	17 (28%)
Control	25 (62.5%)	15 (37.5%)

**Table 3** Correlation of adiponectin and glucose insulin ratio

	Mean	Std.Deviation	Correlation	Significant
Adiponectin	944.567	290.36782	0.085	0.003
G. I. ratio	9.9583	2.89115		

**Table 4** Genotyping analysis of patients with PCO and control

		N (%)	P value
TG	Case	22 (37%)	0.000
	Control	3 (7.5%)	
GG	Case	19 (31.5%)	0.003
	Control	7 (17.5)	
TT	Case	19 (31.5%)	0.004
	Control	30 (75%)	

**Table 5** Correlation of adiponectin and genotyping

	Mean	Std. Deviation	Std. Error	P
TG	875.5909	147.01625	31.34397	0.001
GG	935.6471	440.00596	106.71712	
TT	2656.8235	2716.96513	380.45122	

Besides that, it was also reported [29] that the SNPs, 45G 15G (T/G) in the ADIPOQ gene are associated with PCOS. Haap et al. [30] found a higher prevalence of 45T→G polymorphism in the adiponectin gene in women with PCOS compared to controls. The 45T→G polymorphism was associated with an increased risk of type 2 DM in a Japanese study [31], which reported a strong association of 45T→G polymorphism with obesity and insulin resistance. Insulin resistance plays a role in type 2 DM pathogenesis. Patients with PCOS have a higher risk of type 2 DM. The commonality between type 2 DM and PCOS pathogenesis is insulin resistance. Some studies suggested that adiponectin gene polymorphisms may be related to type 2 DM [32]. Previous studies examined the association of these and other adiponectin gene variations with type 2 DM and other components of metabolic syndrome. In our study, there was significant statistical difference in genotype distribution and

allele frequencies in PCOS compared to the control group. PCOS subjects were found to have a higher risk of type 2 DM and CVD than healthy women.

## Conclusion

We concluded that there is higher prevalence of adiponectin gene polymorphism in cases of PCOS with significant correlation with glucose insulin ratio.

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## Ethics

The study protocol was approved by the Council of Faculty of Medicine and its Institutional Review Board. Each subject consented before participation in the study. The study protocol was approved by the Ethical Committee of Minia Faculty of Medicine. Written informed consent was obtained formally. The study protocol was approved by the Ethical Committee of Minia Faculty of Medicine. Written informed consent was obtained from all participants prior to participation in the study. The study was conducted in accordance with the ethical guidelines of the 1975 Declaration of Helsinki.

## Consent for Publication

I confirm that all authors agree on publication and give its liability to the corresponding author.

## Competing Interests

The authors declare that they have no competing interests. The authors also declare not to have any financial support. Also the work-done is financially independent.

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