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*Original Research Article*

**Association of The T45G Polymorphism of Adiponectin Gene with Polycystic Ovary Syndrome in Women of Babylon Province/ Iraq**

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**Abstract**

 Polycystic Ovarian syndrome (PCOS) is being the most frequent cause of anovulatory infertility, Adiponectin is the most abundantadipocytokine and may play a role in the regulation of insulin sensitivity and IR in PCOS and count for 0.01% or 3–30 μg/ml of total plasma proteinthe adiponectin gene contains 3 exons spans 16 kb on chromosome3q27.The aim of the present study was to evaluate the genetic influence of the adiponectin gene polymorphisms in the development of PCOS among women of Babylon Province/ Iraq. sixty three women were studied ,and were classified into two groups of : first group consists of 32 women infected with polycystic ovaries syndrome, the second consists of 31 healthy women to detect the presence of T45G polymorphism within the gene. From all subjects a whole-blood sample was taken and was used for isolation of peripheral blood leukocytes. The adiponectin T45G polymorphism, located in exon 2, was genotyped by amplification of genomic DNA.The present study included study of the relationship of this gene with PCOS women in reproductive age, A statistically significant difference was observed in the frequency of TT,TG and GG genotypes between women with PCOS and controls .

**Key words:** PCOS, T45G polymorphism, Adiponectin gene

**الخلاصة**

 تعد متلازمة تكيس المبايض Polycystic Ovarian syndrome (PCOS) السبب الأكثر شيوعا لغياب الإباضة في العقيمات، اديبونيكتين هو adipocytokine الأكثر شيوعا ويمكن أن يلعب دورا في تنظيم حساسية الأنسولين و مقاومة الانسولين في PCOS ويكون نسبة 0.01٪ أو 3-30 ميكروغرام / مل من البروتين الكلي في البلازما يحتوي جين الاديبونكتين على ثلاثة مناطق للتشفير ( (3 exons يمتد 16 كيلو بايت16 kb على الكروموسوم 3q27. الهدف من هذه الدراسة هو تقييم التأثير الوراثي لتعدد الأشكال في جين اديبونيكتين في تطور متلازمة تكيس المبايض بين النساء في محافظة بابل/العراق. تمت دراسة ثلاث وستين امراة، وصنفت إلى مجموعتين : تتكون المجموعة الأولى من 32 امرأة مصابة بمتلازمة تكيس المبايض، وتتكون المجموعة الثانية من 31 امرأة يتمتعن بصحة جيدة للكشف عن وجود تعدد الأشكال خلال هذا الجين. وتم أخذ عينة دم كامل من جميع عينات الدراسة ولعزل الكريات البيضاء في الدم المحيطي. تم ايجاد تعدد الأشكال جين T45G ، والتي تقع في منطقة التشفير (exon 2)، من خلال تضخيم الحمض النووي الجيني DNA. وشملت هذه الدراسة دراسة العلاقة بين هذا الجين مع النساء PCOS في سن الإنجاب، ولوحظ وجود فروق ذات دلالة إحصائية في الأنماط الجينية TT، TG وGG بين النساء المصابات بمتلازمة تكيس المبايض ومجموعة السيطرة.

**الكلمات المفتاحية:** مبيض متعدد الاكياس ,T45G تعدد الاشكال, اديبونكتين جين.

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**Introduction**

P

olycystic Ovarian syndrome (PCOS) is one of important Hormones' distribution disease which affected fertile women with an estimated prevalence of 4–8 % [1] . The ESHRE/ASRM consensus conference heldin Rotterdam in 2003 defined the syndrome as having two of thefollowing three conditions diagnosed as PCOS:

oligo-ovulation;clinical or biochemical evidence of androgen excess; and multicysticovaries[2]. The clinical symptoms of(PCOS) characterized by hirsutism; Irregular cycle; anovulation and obesity especially in abdominal region as a result of increasing of male hormones (androgen) [3].In addition to its reproductive features, PCOS also has numerous metabolic consequences, includingincreased risk of obesity[4],insulin resistance (IR) [5], type 2 diabetes mellitus (T2DM) [6]and premature arteriosclerosis[7].

the adiponectin was identified in 1995 by [8]. It is the most abundant adipocytokine and accounts for 0.01% of total plasma protein[9],studies [9,10] revealed that levels of adiponectin are reduced in obese and type 2 diabetesin comparison with normal individuals, the women with PCOS are also foundto have lower adiponectin levels than the normal controls.A number of studies also suggest that serum adiponectin level correlates on the contrary with insulin resistance[11];low levels of adiponectin are consistently associated with a higher risk of type 2 diabetes [12].

Adiponectin has been shown to have antiatherogenic effects[13,14].

The adiponectin gene composed of three exons and two introns spanning a 17-kb region, and has been located on chromosome 3q27 [8,15,16], this gene coding for a 30-kDa protein that consists of an N terminal collagenous domain and a C-terminal globular domain [17]; Onecommon and two rare genetic polymorphisms in the adiponectin gene have been identified in non-diabetic populations [18,15]. The silent T/G polymorphism in exon 2 of the human adiponectin gene(T45G!Gly15Gly) could somehow affect plasma adiponectin levels [19,20].

Despite the numerous reports studying the association between adiponectin gene polymorphisms and insulin resistance or obesity, until now, few studies have examined adiponectin gene polymorphisms in women with PCOS. In this study, we investigated the possible association of the T45G adiponectin gene polymorphism with PCOS in Iraqi Babylon patients.

**Material and Method**

 Thirty-two PCOS patients and thirty-one healthy controls in reproductive age were collected from Babylon hospital for the period from September 2014 to March 2015 , the sample was collectedand stored in test tube with EDTA and freezing at ( 86 – C) for DNA extraction . Genomic DNA was isolated from peripheral blood leukocytes of women with PCOS and the controls using Reliaprep TM blood gDNAMiniprep System (Promega). The adiponectin T45G polymorphism, located in exon 2, was genotyped by amplification of genomic DNA using the following primers: F50-GAA TGAGACTCTGCTGAGATGG and R50- TATCATGTGAGGAGTGCTTGGATG. [21]. PCR products were obtained using 25ml reactions of Go Tag Green Master Mix (Promega) using 3 ml of templet DNA and 1 ml of (10PM) forward and reverse primer by Verti 96 thermo cycler (Applied Biosystem). The amplification conditions were as follows: 94C for 5 min, followed by 35 cycles of 30 s at 94 C, 30 s at 30 C and 45 s at 72 C, and ending with a single 10 min extension step at 72 C. The resulting fragment was 372 bp in length. The polymorphism was typed with enzyme Smal (Bio Labs Inc., New England). Digestion of the G allele produced two fragments with lengths 216 and 156 bp. The digestion products were resolved after electrophoresis in 2% agarose gel containing ethidium bromide(Figure 1).

**Statistical Analyses**

 Genotype and allele distribution was compared between cases (women with PCOS) and controls using Pearson’s *c*2 test[22].

**Results and Discussion**

 Three pattern of genotype were obtained in current study: TT,TG and GG, The results revealed as shown in Table (1)The percentage of genotype(TT) in PCOS women were (59.4 %) While in control group (74.2 %), the percentage rate of genotype (TG) were( 31.3% ),( 25.8%) in patients and control group respectively, while genotype( GG) absent in the control group and were represented in the group of PCOS women with percentage (9.3 %).



**Figure 1 :** Gel electrophoresis of PCR products of adiponectin gene treated with SmaI FOR 3Hours at 25° C IN 2% agarose gel at 100 volt/cm2 for 30 min. then 50 volt for 90 min. visualized by U.V.Lane 1,13 DNA ladder (100-1000), Lane (2-5,8,10,11) samples with TT genotype Lane (6,7,12) samples with TG genotype Lane (9) sample with GG genotype

**Table 1 :** Genotyping in the gene with polycystic ovary syndrome (PCOS) women

|  |  |  |
| --- | --- | --- |
| **CONTROL** | **(PCOS)** | **Genotyping**  |
| **%** | NO. | % | NO. |  |
| **74.2** | 23 | 59.4 | 19 | TT |
| **25.8** | 8 | 31.3 | 10 | TG |
| **0** | 0 | 9.3 | 3 | GG |
| 31 | 32 | TOTAL |

Although no difference was observed when X2-test was performed on the distributions of the threegenotypes separately, a statistically significant difference

(x2 = 108, P < 0.05) was observed in the frequency of TT, TG and GGgenotypes between women with PCOS. figure (2).

**Figure 2 :**Frequencyof genotype TT, TG & GG in (PCOS) women and control, show signification difference under (P < 0.05)

It's interesting that this study was first recorded presence of statically signification in the frequency of genotype ( TT), (TG)and (GG) for T45G polymorphism of adiponectin gene in Iraqi PCOS patients compared with control , also The study report for a first time the lack of (GG) genotype of the control group and his presence in Iraqi patients with PCOS .the disappear of GG genotype from the control group may reflect the importance of this gene in the incidence of the disease since the meeting of the two mutant alleles(G) in the same woman leads to the disease in addition to increasing the percentage genotype (TT) in the control group compared to women with PCOS.

Our findings conceptual agreement with a previous studyon a differentathinic group (Greek women) with PCOS, which found that the TG and GG genotypes of the 45TG polymorphism were more frequent in women with PCOS than in controls, and these particular genotypes were associated with higher 4-androstenedione concentrations[21]. The current study also correspond with another study Conducted on Han Chinese women were also found asignificant difference between the genotypes of this polymorphisms and showed decrease the presence of TG and GG genotype in the control group compared withPCOS women group and this consistent with present study ,so it seems that this genotypes are a risk factor for PCOS, because it increases the bioavailability of androgens in women with PCOS [23], While the results of the current study did not conform toa Greek study[24] conducted on women with PCOS which found nodifferences in the distributions of T45G polymorphism between women with PCOS, and control And they also refer to the emergence of GG genotype in controls group and absence it in patients with PCOSthey suggest these genomic variants may influence production of adiponectin and the metabolic variables related to insulin resistance/metabolic syndrome in patients with PCOS.On the other hand, an Italian study, show a significant associated between this polymorphism , obesity and features of insulin resistance [25],Several studies have reported an association between This highly prevalent polymorphism and the risk of obesity, insulin resistance, DM2 and high levels of low-density lipoprotein cholesterol [20,26, 27]. While other studies have not documented any association for particular locus with obesity or DM2[28,29,30].

The silent T/Gpolymorphism in exon 2 of the human adiponectin gene(T45G ' Gly15Gly) can affect one way or another plasma adiponectinlevels [19,20].

As well as [25] shows that this polymorphisms might be in linkage disequilibrium, and the G/G haplotype has been strongly associated with the metabolic disturbances of PCOS, as well as lower plasma adiponectin concentration .

Therefore, although the number of women homozygous for the G allele in our study was relatively small to drawfirm conclusions, we postulate that there is a complex relationship, possibly a negative feedback loop, betweenadiponectin and the hypothalamic–pituitary–gonadal axis, specifically steroid synthesis or action. Indeed, in vitrostudies have shown that both glucocorticoids and androgens [31] down-regulate the expression of adiponectin, and there is substantial evidence suggestive of a complex interaction between this hormone and gonadal function[32].

In conclusion, the number of genomic variants associated with PCOS is growing rapidly, suggesting that PCOS may result from the interaction of multiple genomic variants and environmental factors such as obesity and a sedentary lifestylealthough the physiological significance of such a relationshipremains obscure at present.Since the T45G polymorphism is a synonymousmutation, the exact molecular mechanisms responsible forthe biological effects of this variation are not known atpresent. It is plausible that this polymorphism is in linkagedisequilibrium with some other functional genetic alterations.More data are needed to specify the systemic and local function of the newly identified ‘adipocytokines’ in the pathophysiology of PCOS.

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