# Investigate the presence of a SNP in the methylenetetrahydrofolatereductase gene in PCOS patients with Dyslipidemia in Al-Najaf Al-Ashraf province

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**Abstract : Objectives:** Polycystic ovary syndrome (PCOS) is the most predominant heterogeneousendocrine disorder in premenopausal women with anonymous etiology. This study was done to find out if there was association between methylenetetrahydrofolatereductase polymorphism  gene (MTHFR) polymorphism (C677T) in the codon 222 (substitution of alanine tovaline)and PCOS patients with dyslipidemia in Al-Najaf Al-Ashraf province.
**Methods:** This study was carried out during the period from April 2015 till May 2016, and included forty-six PCOS female patients with Dyslipidemia. Control group consists from twenty-five healthy age – matched women; all were without clinical manifestation of any disease. Serumcholesterol, triglyceride, LDL and HDL estimation was done. Polymerase chain reaction-restriction fragment length polymorphism technique was used to estimate genotyping of the C677T methylenetetrahydrofolatereductase gene polymorphism.
**Results:** Allelic distributions of SNP (C677T) in the (MTHFR) gene showed no significant difference (p>0.05) between PCOS patients with dyslipidemia (CC: n= 35, 76.09%; CT: n=9, 19.56%; TT: n=2, 4.35%) and controls (CC: n= 22,88%; CT: n=3, 12%; TT: n=0).
**Conclusion:**TheMTHFR gene C677T polymorphism plays no role in PCOS female patients with Dyslipidemia.
**Keywords :** PCOS, MTHFR, polymorphism, dyslipidemia.

## Introduction

Polycystic ovary syndrome (PCOS) is the most predominant heterogeneousendocrine disorder in premenopausal women with anonymous etiology.[1]

Diagnostic criteria for PCOS was amended by the Rotterdam consensus workshop,theyfound that PCOS women had two of three criteria: anovulation, hyperandrogenism, and sonographic image for polycystic ovaries.[2]

This disease is characterized clinically by irregular menses, Dysglycemia, insulin resistance, diabetes, hyperandrogenism, dyslipidemia, Obesity, and hypertension. All these metabolic disturbances can play essential role in developing the cardiovasculardiseases.[3]

Dyslipidemia is a condition of abnormal lipid profiles, which include elevated levels oftotal cholesterol (TC), triglyceride (TG),andlow-density lipoprotein (LDL)with low levels of high-density lipoproteins (HDL).[4]

Dyslipidemia have been widely reported in PCOS women.[5, 6]This Dyslipidemia may be related with insulin resistant and decreased HDL and increased TG.[7]

A recent study reported that dyslipidemia and insulin resistant incidence were affected by some gene polymorphisms associated with enzymes that involved in folate metabolism, especially methylenetetra-hydrofolatereductase (MTHFR)enzyme, which convert 5,10-methylenetetra-hydrofolateto folate circulating form (5-methyltetrahydrofolate). The last compound will methylate homocysteine to methionine. Methionine then can change to S-adenosyl-methionine, which represent a methyl source to different components, likeDNA, proteins, and lipids. [8]

A study have been reportedassociation between the mutation MTHFR genes and serum lipid levels.[9]

The common functional polymorphism identified in the MTHFR gene was the MTHFR *C677T* polymorphism.[10]

The location ofC677T mutation of MTHFR (the sequence change at nucleotide 677,cytosine to thymine) is affected the enzyme in the region that responsible of catalyzed chemical reaction, which calledcatalytic domain of the protein, and resulted in substitution of an alanine by valine in the222 position (codon) of the MTHFR enzyme, called MTHFR C677T, and that will reduce MTHFR enzyme activity.[11,12,13]

This study was done to find out if there was association between MTHFR polymorphism (C677T) in the codon 222 (substitution of alanine to valine)and PCOS patients with dyslipidemia in Al-Najaf Al-Ashraf province.

## Materials and Methods

This study was carried out during the period from April 2015 till May 2016, in the laboratory of biochemistry in Al-Zahraa Pediatric and Maternal Teaching Hospital in Al-Najaf Al-Ashraf province and laboratory of molecular biology in the Department of Biology/Faculty of Sciences/ Kufa University.

### 1.Subjects

#### a) Study group:

This study was included forty-six PCOS female patients with dyslipidemia. PCOS female were defined according to the Rotterdam ESHRE/ASRM-sponsored PCOS Consensus Workshop Group[2](The diagnosis was under the supervision of gynecologist from the hospital).

#### b) Control group:

Consists from 25 healthy age– matched women,all were without clinical manifestation of any disease.

Height and weight were measured for both groups to calculate body mass index (BMI) according to the standard formula: weight (kg) /height (m) [2].

### 2. Collection of Blood Samples

Four ml of venous blood was collected from PCOSpatients and control. Three ml was allowed to clot at room temperature then centrifuged at 3000 rpm for 5 minutes the serum was used freshly for the Biochemical tests. One ml of whole blood was collected in EDTA tubes and store at ⁻20ºCuntil used for PCR test.

### 3.BiochemicalTests

Serum cholesterol, triglyceride, LDL and HDL estimation were done by Kits which are products of BIOLABO REAGENT (01260, Maizy, France). LDL level was measured by using Friedewald Formula: LDL = TC - HDL - TG/5.0 (mg/dL). [14]

### 4.DNA isolation and Genotyping by RFLP

Genomic DNA was isolated by using protocol of Genomic DNA Mini Kit, which designed for purifying DNA from frozen blood (Geneaid Biotech. Ltd., Taiwan Company, Cat. No. GB100, LOT. No. TJ21207).

To detect the single-nucleotide polymer-phismin the 677 nucleotide atexon 4 of MTHFR gene (cytosine to thymine)**,** the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method has been used. The sequences of the primer-pair were:

5'- TGAAGGAGAAGGTGTCTGCGGGA-3'

5'- AGGACGGTGCGGTGAGAGTG -3' these primers (synthesized by AccuOligo®Bioneer Corporation .USA) were published previously.[15,16,17]

The PCR amplified 198-bp product in 20 μltube of PCR PreMix Reaction Mixture (PCR PreMix, Bioneer Corporation, USA) containing template DNA (5 μl, 50.3 ng), DNA polymerase (1 unit), reaction buffer (2 μl), stabilizer and loading-dye (2 μl), dNTPs (2 μl), and 2μl of each primer (2 μl forward and 2 μl reverse), and to the final volume of 20 μl distilled water was added.

Amplification was done in a thermal cycler (Cleaver scientific Ltd/UK) programmed for 30 cycles of denaturation at 95°C for 1 min, annealing at 61°C for 1 min, and extension at 72°C for 1 min, preceded by an initial denaturation of 5 min at 95°C. Final extension was for 7 min at 72°C.

The 198-bp PCR product was digested overnight with one unit of Hinfl restriction enzyme (Source: *Haemophilus influenza* Rf.) at 37 °C (synthesized by Promega Corporation, USA, Cat.No. R6201). After the Hinfl digestion, weyieldedone of these results for each sample:

a) Two 198-bpfragments for allele C (Normal allele for the homozygous wild genotype patient,CC).The original PCR fragment remains intact because restriction enzyme not found the cutting position.
b) 175 and 23-bp for allele T(Mutant allele) for homozygous mutant genotype patient, TT.
c) A heterozygous geno type patient(TC)had 198, 175 and 23-bp fragments for both normal and mutant allele.

Finally, the gel electrophoresis method was done according to Sambrook and Russell[18], and 5 μl of each samples was loaded onto 3% agarose gel.

**5. Statistical Analysis:**

Statistical analyses of all results were carried out by the help of SPSS version 17 software statistical package using chi square (P value was considered significant at level less than 0.05).

**Results**

The study population was included 46 PCOS female patients (Diagnosis was under the supervision of gynecologist from hospital).

**Clinical characteristics and lipids parameters:**

The mean age of PCOSpatients was29.07±2.91 years. The results showed a significantly (p<0.05) higher BMI, cholesterol, triglyceride, and LDL levels when compared to controls. While there were no differences between the two groups in serum HDLlevels (Table 1).

**Table1: The results of clinical characteristics and lipids parameters in PCOS patients and controls**.

| Parameter | PCOS patients | Controls |
|---|---|---|
| Age (yrs) | 29.07 ± 2.91 | 28.81±3.16 |
| BMI (kg/m$^2$) | 39.8 ± 3.43* | 26.1 ± 2.83 |
| Cholesterol (mg/dl) | 170.92 ± 4.31* | 152.4±1.42 |
| Triglyceride (mg/dl) | 122.21 ± 3.1* | 95.08±2.17 |
| LDL (mg/d) | 87.41 ± 32.4* | 59.11±8.3 |
| HDL (mg/dl) | 41.11 ± 6.33 | 43.87±11.5 |

**\*p< 0.05 significant,** Values were expressed asmean±SD.

**C677T SNP in the MTHFR alleles:**

a) **Controls:** Among controls (25 healthy women) 22 (88%) had found as homozygous wild(CC, normal alleles), and three (12%) found as heterozygous genotype (with the normal (C) and mutant (T) alleles(CT) (Table 2& Figure 1).

b) **PCOS patients:**Among46 PCOS patients 35 (76.09%) had found as homozygous wild (CC, normal alleles), 9 (19.56%) found as heterozygous genotype (with the normal (C) and mutant (T) alleles (CT), and 2(4.35%) had found as homozygous mutant genotype (TT, mutant alleles) (Table 2 & Figure 1).

**Table2:ThePCRresults with genotypic distribution of patients and controls.**

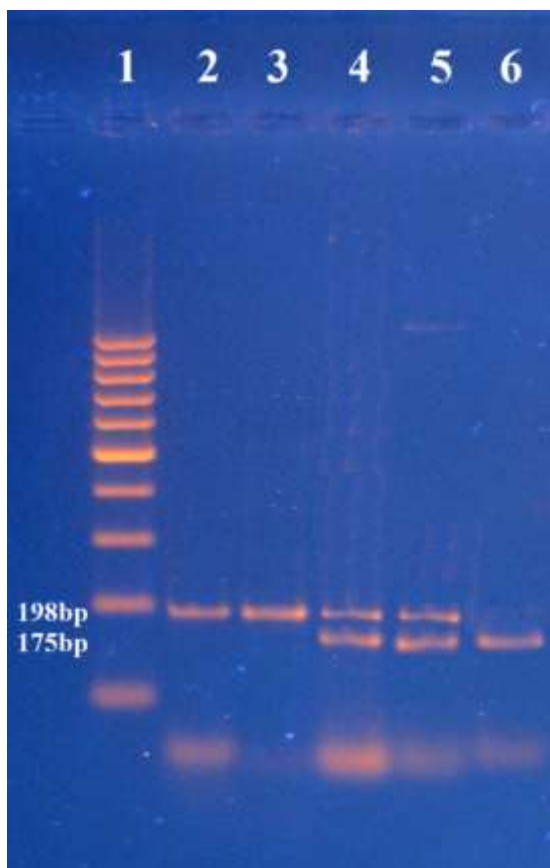| Subjects | PCRresults | | | Total |
|---|---|---|---|---|
| | **Homozygous wild CC** | **Homozygous mutant TT** | **Heterozygous TC** | |
| **Healthy controls** | 22 88% | 0 | 3 12% | 25 35.21% |
| **PCOS group** | 35 76.09% | 2 4.35% | 9 19.56% | 46 64.79% |
| **Total** | **57 80.28%** | **2 2.82%** | **12 16.9%** | **71 100%** |



**Figure 1: The electrophoresis image of PCR-RFLP analysis of MTHFR C677T SNP (Lane 1: molecular weight marker (GeneRuler™ 100bp DNA Ladder, SM0243-Fermentas);Lane 2&3: homozygous wild genotype(CC;198-bp); Lane 4&5: Heterozygous genotype (CT; 198-bp, 175-bp,and 23-bp which undetectable ongel because of small size); Lane 6 homozygous mutant genotype (TT; 175-bpand 23-bp).**

## Discussion

PCOS is the most predominant endocrine disorder in premenopausal women with anonymous etiology.This study was done to find out if there was association between methylenetetrahydrofolatereductase gene (MTHFR) polymorphism (C677T) in the codon 222 (substitution of alanine to valine)and PCOS patients with dyslipidemia in Al-Najaf Al-Ashraf province.

Dyslipidemia have been widely reported in PCOS women, in this study the patients had significantly higher cholesterol, triglyceride, and LDL levels when comparedto controls.

These results in accord with six Iraqi studies[19-24] found that there were significant increases in cholesterol, triglyceride, and LDL levels in PCOS patients with decreased HDL levels as compared with control groups.

Many studies reported that folate deficiency due to the SNP in the MTHFR gene will destroy the lipid metabolism in the liver.[25,26,27]

Allelic distributions of SNP (C677T)ofMTHFR gene in this study showed no significant difference (p>0.05) between PCOS patients with dyslipidemia (CC: n= 35, 76.09%; CT: n=9, 19.56%; TT: n=2, 4.35%) and controls (CC: n= 22, 88%; CT: n=3, 12%; TT: n=0).

These results agreed with other study[28]that concluded the following "Although not statistically significant, there is a slightly higher prevalence of heterozygous (CT) genotype in women with PCOS.MTHFRC677T polymorphism when present may confer an increased susceptibility to develop hyperlipidemia in women with PCOS".

Another study reported that the C wild allele of MTHFR has a protective effect on serum lipids while the T mutant allele has a harmful effect.[29]

That may be explained why the mutant allele in the heterozygous genotype occurred more frequently in women with PCOS and dyslipidemia.

Yet other studies found that no significant association between MTHFR C677T polymorphism in PCOS patients and controls.[30-33]

While other study[34] confirmed that C677T polymorphism of MTHFR gene was related with PCOS, and the two genotypes, CT and TT may be increase the risk of PCOS.

Also, increased the incidence of dyslipidemia and high levels of serum lipid profiles, that resulting from MTHFR gene polymorphism, had been reported in other studies.[35-38]

Recently, Wang *et al*. study[39]explainedthatthe frequency distributions of MTHFR polymorphisms, especially C677T, vary substantially between different regional and ethnic groups.

## Conclusion

In this study, The MTHFR gene C677T polymorphism plays no role in PCOS female patients with Dyslipidemia.

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