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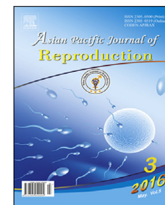

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Peroxisome proliferator activated receptor gamma polymorphism Pro12Ala in polycystic ovary syndrome (PCOS) of South Indian Population

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ABSTRACT

Objective: To investigate the frequency of Pro12Ala polymorphism in PPAR gamma gene among PCOS of South Indian Population. Understanding this polymorphism may help us in better diagnosis prevention and therapeutic approaches towards management of PCOS.

Method: A total of 75 women with the diagnosis of PCOS and 75 healthy controls were included in this study. The Pro12Ala variant in the PPAR gamma gene was analysed by PCR-RFLP.

Results: Genotypic frequencies for PPAR gamma gene Pro12Ala polymorphism showed the frequency of Pro/Pro genotypes was 77% in PCOS and 81% in controls. The odds ratio was 0.71 (95% *CI* 0.31–1.63) and 2.66 (95% *CI* 0.21–32.9) and alleles expressed 0.87 (95% *CI* 0.42–1.79). Regarding hormone levels, there were significant differences between PCOS and non PCOS.

Conclusion: PPARγ2 gene Pro12Ala polymorphism was supposed to be susceptible genes in PCOS. The present study demonstrated that there is a statistical difference between the distributions of PPAR gamma Pro12Ala polymorphism in South Indian Population.

1. Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder affecting up to 7% of women of reproductive age [1]. The disorder is characterised mainly by menstrual irregularities acne, hyperandrogenism central obesity, type 2 diabetes [2]. The pathogenesis of PCOS is unknown. Recent reports show that it remains as a complex multigenic disorder characterised by abnormal gonadotropin release and dysregulation of steroidogenesis [3]. PCOS is a familial condition with autosomal dominant mode of inheritance [4]. It is a combination of genetic abnormalities combined with environmental factors such as nutrition and body weight, which mainly affect the PCOS [5].

Recent studies show that initiation of pathological mechanisms occurs mainly during the foetal life, with high maternal serum levels of androgen/insulin may influence the genetic programme which result in both reproductive and metabolic consequences of PCOS [6]. The peroxisome proliferators activated receptor gamma PPAR gamma gene is located at chromosomal region 3p25 and is mainly expressed in adipose tissue which promotes the differentiation of pre-adipocytes into adipocytes and belong to nuclear hormone receptor family [7]. Peroxisome proliferators-activated receptor-gamma (PPARγ) is a candidate gene involved in glucose homoeostasis, lipid metabolism and adipocyte differentiation also implicated in the pathogenesis of PCOS [8].

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PPARγ is a nuclear receptor which combines with the retinoid X receptors to form heterodimers which controls transcription of genes involved in free fatty acid uptake and lipogenesis, and plays an important role in regulation of insulin sensitivity and adipose tissue metabolism, carcinogenesis and inflammation [9]. A functional polymorphism in exon 2 of PPARγ2 gene produces a Pro–Ala substitution at the codon 12 causing the Pro12Ala polymorphism (rs1801282). This CCA-GCA change causes a conformational change in the protein thus affecting the activity [10]. The Pro12Ala polymorphism of PPAR gamma gene has been associated with reduced transcriptional activity of PPAR gamma and presence of Ala isoform has been linked to higher insulin sensitivity and lower body mass index [11].

According to reports of Zargar *et al.* [12] significant number of PCOS patients show impaired glucose tolerance and are in potential risk of developing type 2 diabetes. The objective of the study was to determine the genetic frequency of Pro12Ala polymorphism in PPAR gamma gene in South Indian PCOS population. The association between the Pro12Ala polymorphism and increased insulin sensitivity in PCOS has been observed in reports of Hahn *et al.* [13]. PPAR gamma gene associated with the risk of colorectal adenoma was reported in a study [14]. PCOS were diagnosed based on the Rotterdam criteria 2003 [15] which includes polycystic ovarian with >10 small peripheral cysts, hyperandrogenism, anovulation and hyperinsulinemia. Subjects were considered to have oligomenorrhea if they had less than 8 cycles per year and amenorrhea in absence of menses for6 months or more [16]. Clinical hyperandrogenism was diagnosed by hirsutism assessment using F–G scoring if seen with 8 or more [17]. Biochemical hyperandrogenism was diagnosed by elevated levels of serum free testosterone as it is considered the single most predictive evidence of hyperandrogenemia [18].

2. Materials and methods

2.1. Collection of blood samples

Peripheral blood samples of 3 mL was collected from the subjects using a heparinised needle and transported to the laboratory with the help of portable coolers. The subjects were recruited in the study from the women population attending the gynaecology clinic of a tertiary care hospital (n = 75) were served as experimental and were included in the study. Anthropometric details and general clinical characteristics and history of disease were obtained by a standardized health questionnaire. Age matched healthy controls were included in the study. The inclusion criteria of the PCOS were based on the Rotterdam diagnostic criteria. The subjects have not received hormonal therapy for at least 3 months before hormone assays. All the study evaluations and procedures were conducted in accordance with the guidelines of Helsinki Declaration on human experimentation. An ethical committee approval was obtained for the study and written informed consent was obtained from the subjects involved in the study.

2.2. Hormone assays

The level of serum follicle stimulating hormone FSH luteinizing hormone LH total testosterone T were determined using commercially available human Elisa kits. The intra and inter assay coefficient of variation of all the assays were less than 10%.

2.3. Genotyping

Genomic DNA was isolated from peripheral blood leucocytes by using the standard Phenol-chloroform extraction method [19]. Pro12Ala polymorphism was determined using polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) method. The PCR reaction mixture contained 50 ng of genomic DNA 0.5 μmol/L of each primer of forward 5′CCA ATT CAA GCC CAG TCC TTT C3′ and reverse 5′GCA GAC AGT GTA TCA GTG AAG GAA TCG CTT TCC G and master mix (Promega) USA in a final volume of 25 μL. The reaction mixture was subjected to denaturation at 95 °C for 2 min followed by 35 cycles at 95 °C for 30 s, 52 °C for 30 s, 72 °C for 45 s then by a final extension of 72 °C for 7 min. The PCR products were 237 bp and were digested with BstUI restriction enzyme at 37 °C for 18 h. After the digested products were electrophoresed on a 2.5% agarose gel and visualised by ethidium bromide staining, the C and the G alleles could be distinguished as bands 217 bp and 20 bp respectively.

2.4. Statistical analysis

The standard χ^2 tests were used to compare genotype frequencies among PCOS and control groups. The results were in Hardy–Weinberg equilibrium of genotype frequencies. The results of continuous data were reported as mean and ±SD. Differences in serum hormone levels between PCOS and control groups were assessed by students t test $P < 0.05$ was considered significant for all tests. Pearson bivariate correlation was studied for hormones among controls and PCOS. Statistical analysis was performed using the SPSS Version 16 (Statistical Package for the Social Sciences, USA).

3. Results

Table 1 depicts the anthropometric data and hormone levels between PCOS and control groups. There was a significant difference in age, FSH, LH, testosterone hormone levels between the two groups. The subjects were grouped into three based on their age as G I, G II and G III and they were significant at $P < 0.05$ levels. However, the level of testosterone hormone was significantly higher in the PCOS group when compared to the control subjects. Pearson bivariate correlation was studied among hormones. Correlation was observed among the Group I as $r = 0.459$, $P = 0.006$ significant at $P < 0.001$ level. A negative correlation was observed among Group II and Group III individuals with ($r = 0.39$ $P = 0.19$, $r = 0.43$ $P = 0.15$).

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