Original Article

Screening of Polymorphisms of Transcription Factor 7-like 2 Gene in Polycystic Ovary Syndrome using Polymerase Chain Reaction-restriction Fragment Length Polymorphism Analysis

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Abstract

Background: Polycystic ovary syndrome (PCOS) is a common endocrine disorder occurring in premenopausal women, with a prevalence rate of 5%–7%. It has been observed in multiple number of studies the coexistence between diabetes mellitus 2 and obesity with this endocrinopathic disorder. Transcription factor 7-like 2 (TCF7L2) gene is shown to be associated with insulin secretion. **Aim:** To screen whether the gene variant of TCF7L2 (formerly TCF4) gene is significantly associated and has susceptibilities with type 2 diabetes in PCOS. This study is essential to uncover diabetogenic association of the TCF7L2 gene variants with PCOS. **Design:** This was a hospital-based study. **Methods:** In this work, blood samples from 43 PCOS patients with age and sex similar to 43 control samples were collected, followed by isolation of DNA. Further genotyping of the TCF7L2 gene was carried out by performing polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). **Statistical Analysis:** Genotype frequencies of the TCF7L2 rs7903146 gene were checked by Hardy–Weinberg equilibrium of genotype in both PCOS and the control group, and also, the frequencies of the genotype were performed accordingly. **Results:** There was no significant allelic variation observed among the patient and the control samples. From the patient details, it was observed that women between the age group of 21 and 25 years are susceptible to PCOS. **Conclusion:** From the PCR-RFLP analysis, it can be stated that there are no expected gene polymorphisms seen in this study, unlike the study carried out on the Chinese population where they observed genotype variations CC, CT, and TT. From this study, we can conclude that TCF7L2 rs7903146 gene cannot be considered as the candidate gene for the occurrence of PCOS.

Keywords: *Polycystic ovary syndrome, polymerase chain reaction-restriction fragment length polymorphism, transcription factor 7-like 2, type 2 diabetes mellitus*

Introduction

Polycystic ovary syndrome (PCOS) is an oligogenic disorder which can also be called as chronic anovulation, oligoovulation, and hyperandrogenism. Females diagnosed with PCOS often show symptoms such as infertility, obesity, and clinical features of hyperandrogenism, such as hirsutism, acne, and alopecia.[1] The purpose of this study is to understand the association of Transcription factor 7-like 2 (TCF7L2) gene in PCOS. The diagnosis of PCOS is based on the Rotterdam criteria, which requires the presence of at least two criteria:

1. Clinical and/or biochemical hyperandrogenism
2. Ovulatory dysfunction

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3. Polycystic ovarian morphology.

Levels of total testosterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH), LH = FSH, prolactin (PRL), and estradiol 2 in the sera were determined by radioimmunoassay.[2] The central distribution of fat, however, is not dependent on body mass index (BMI) and is actually associated with higher insulin concentrations.[3,4] In PCOS, the normal pulsatile secretion of LH is increased by an increased frequency and amplitude of pulses, while that of FSH is unchanged or muted.[5] A high FSH score indicates a low or declining egg supply independent of obesity. It has been well established that the presence of a defect in insulin action amplifies the LH-stimulated androgen secretion from thecal cells.[3] Increased PRL levels in women not breastfeeding may interfere with ovulation and fertility. Thyroid-stimulating hormone stimulates the thyroid gland to produce T4 and T3. Follicles go through the normal maturation process but fail to become eggs due to the hormonal imbalance. PCOS can range from mild to serious and so can the symptoms. Insulin resistance (IR) is one of the major characteristics of PCOS. IR and its associated hyperinsulinemia can be considered as a major etiology of PCOS. These patients have a tendency to develop type 2 diabetes (T2M) due to impaired glucose tolerance.[6] PCOS is associated with IR in majority of cases with or without obesity, but increased IR is observed in obese individuals.[7] There are many gene variants causing T2M mellitus (T2DM) among which only one gene that is clearly identified is transcription factor 7-like 2 (TCF7L2) gene. TCF7L2 gene is present on chromosome 10q25.2.[8] TCF7L2 has been shown to be associated with T2MD in multiple ethnic groups. TCF7L2 is a TCF influencing the transcription of several genes, thereby exerting a large variety of functions within the cell. The most likely candidate is the single nucleotide polymorphism (SNP) rs7903146, which shows the strongest association with diabetes and resides in a noncoding region with no obvious mutational mechanism.[9] Genetic variants in the gene encoding for TCF7L2 have been associated with T2D and impaired β-cell function.[10] PCOS, the most common reproductive endocrine disorder of premenopausal women, is strongly associated with hyperinsulinemia and T2MD. TCF7L2 confers the greatest relative risk for T2DM and significantly predicts conversion to T2D in persons with impaired glucose tolerance. TCF7L2 acts as both a repressor and a transactivator of genes, as directed by the Wnt signaling pathway.[11,12] Stimulation of the pathway leads to association of β-catenin with BCL9, translocation to the nucleus, and association with TCF7L2, which in turn results in the activation of Wnt target genes, specifically repressing proglucagon synthesis in enteroendocrine cells. There are a large number of highly correlated variants, none of which are obvious functional candidates, which show association with diabetes. The most likely candidate is the SNP rs7903146, which shows the strongest association with diabetes and resides in a noncoding region with no obvious mutational mechanism. It is clear, however, that the effect of the TCF7L2 risk allele is a defect in insulin secretion.[13] As there has been evidence of this gene causing T2DM, we tried to investigate if SNP which is a risk factor for T2DM is the susceptible cause for patients with PCOS who are prone to IR.

Methods

The study includes a total of 43 samples of patients suffering from PCOS whose age and sex are similar to that of the control samples recruited from a hospital, which was approved by the Institute of Ethical Community. PCOS was defined according to the Rotterdam PCOS consensus criteria. The controls were selected by excluding the diagnosis of PCOS according to the 2003 Rotterdam criteria and exhibiting normal menstrual cycles (and exhibiting regular menstrual cycles (21-32 days). All the individuals in the control group were healthy female and without hirsutism. Data collection sheet included information such as age, personal profile including weight, height, and problems (insulin, thyroid, reproductive, and cardio), family history (presence of PCOS in family members), and clinical history. The genomic DNA was extracted from 2 ml venous blood according to the protocol[14] and stored at −20°C. Amplification of DNA was carried using 5`-ATTAGAGAGCTAAGCACTTTTAGGTA-3` as forward primer and 5`- AAGCTTCTCAGTCACACAGG-3` as reverse primer by polymerase chain reaction (PCR) in a thermal cycler (Eppendorf Master Cycler gradient) using a 20 μl PCR mixture containing 10 pmol from each primer, 5 μl water, master mix (amplicon) 10 μl. DNA sample (3 μl) was amplified for 35 cycles with initial denaturation for 5 min at 95°C followed by denaturation at 95°C for 45 s, annealing at 60°C for 50 s, extension for 1 min at 72°C, and final extension for 10 min at 72°C. The PCR products were separated by electrophoresis on 2% agarose gel containing 5 μl ethidium bromide (50 μg/μl) and were visualized using a UV Transilluminator (Medox). The results were evaluated with the gel analysis software. PCR method resulted in a 176 bp product. These products were digested using one unit of restriction enzyme Rsa 1 for 2 h at 37°C which cleaves the C allele to generate DNA fragments of 27 bp and 149 bp, respectively, in size. Digested PCR products were subjected to

electrophoresis on 3% agarose gel, visualized under UV Transilluminator (Medox), and photographed using Gel Dock software.

Statistical analysis

Genotype frequencies of the TCF7L2 rs7903146 gene were checked by Hardy–Weinberg equilibrium of genotype in both PCOS and control group,[8] and the frequencies of the genotype were performed accordingly.

Results

The 43 PCOS women between the age group of 14 and 40 years had a mean and standard deviation of 28.1 ± 4.1 years and a mean BMI of 28.2 ± 6.2 kg/m^2. Major percent of the patient samples obtained for this study belong to the age group between 21 and 25 years [Figure 1a]. Statistical analysis of the patient's clinical report showed that majority of the PCOS patients were observed to be overweight after calculating their BMI [Figure 1b]. Majority of the population within the age group of 21–25 were diagnosed with the symptoms and treatment for PCOS began after marriage due to infertility and patients diagnosed before that would be if any irregularities in periods [Figure 1c and d]. No significant hereditary pass on of the syndrome can be stated with the data obtained from the survey of the patient symptoms of a syndrome such as Insulin problem, cardio problem, obesity, reproductive problems [Figure 2]. The hormone level of the patients involved in the study showed no major variation in the hormone profile from the normal range. The lower FSH value in few patients might be a possibility of ovarian failure. The FSH and LH ratio should be 1:1 for normal functioning as this gives an idea about the normal number of eggs present in the ovaries [Figure 3]. Elevation in the PRL levels is a clear sign of amenorrhea. It is observed that levels are getting elevated with an increase in the age group [Figure 3]. We can also state that the increased levels might be due to stress caused in the patients due to the syndrome outcome as it is a stress hormone too. The elevated FSH levels observed in few might be a cause of decreased thyroid function [Figure 3] From the obtained result, it could be interpreted that the frequencies of the allele obtained in the PCOS population and the healthy female had no significant difference in the allelic frequency among the PCOS patients and the controls were observed to be falling within the same range. After restriction fragment length polymorphism (RFLP), only a single band was obtained in both patients' and control samples [Figure 2]. The restriction enzyme Rsa1 cuts at the C allele forming bands of 149 bp and 27 bp.[8] There was no difference in the pattern of the RFLP product obtained on 3% agarose which shows that there is no homozygous occurrence of the C allele as stated by the

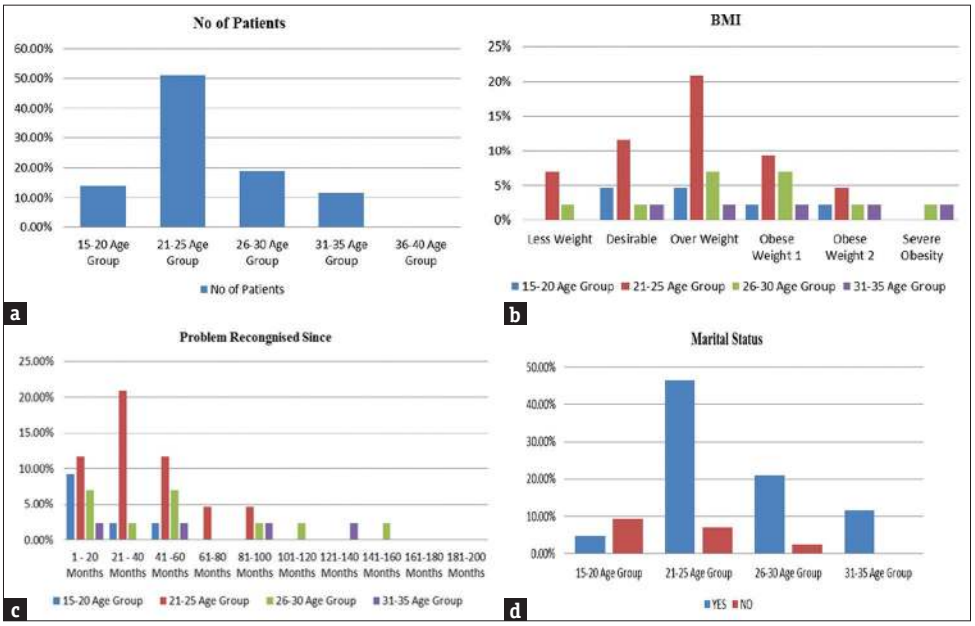

Figure 1: (a) Age group distribution of polycystic ovary syndrome patients chosen for the study. About 50% of the patients belong to the age group between 21 and 25 years. All the remaining age groups samples obtained were just below 20%. (b) Comparison of body mass index of the patients. The maximum patients were observed to be overweight among the age group of between 21 and 25 age groups. There was about 10% difference from the desirable body mass index range. Majority of the patients between age group of 26 and 30 years were 50% overweight or obese. (c) Comparison of the period since the patients were diagnosed with polycystic ovary syndrome. Majority of the patients were diagnosed with polycystic ovary syndrome for the past 21–40 months between the age group of 21 and 25 years. Maximum patients considered in the study were under polycystic ovary syndrome treatment approximately for 60 months. (d) Marital status of the patients when polycystic ovary syndrome diagnosed. About 95% of the patients were diagnosed with polycystic ovary syndrome only after marriage

previous study on the different population on rs7903146. There is no significant difference in the patients' and the control sample.

Discussion

TCF7L2 gene was marked as the gene with the strongest association with T2DM, a major target of Wnt Signaling pathway.[15] This work was based on the idea that the polymorphism variants of the PCOS patient with IR might have the possibility of having the same genotype frequency which can be useful to detect the chance of a PCOS patient to develop a diabetic condition in the future. BMI may possibly have an indirect impact on the action of TCF7L2 gene, probably indirect. It has also been mentioned that a significant IR has also been observed in women who are independent of BMI.[16] In the work performed by Biyasheva *et al*., it was found that there was null association in rs7903146 region and PCOS. It has been stated that PCOS patients have elevated Biyasheva *et al*. Evidence has it that rs7903146 showed allelic frequency of C > T. All the genotype distribution follows Hardy-Weinberg equilibrium rule. Genotypic frequency was observed to be 0.012.[17] A similar work carried out by Xu *et al*. (2010) among the Chinese population came up with the genotypic frequency of 0.795 CC, 0.190 CT, and 0.010 TT rs7903146. Another study showed that the T allele of the TCF7L2 rs7903146 variant was found to be marginally over-represented in Greek patients with PCOS.[18]

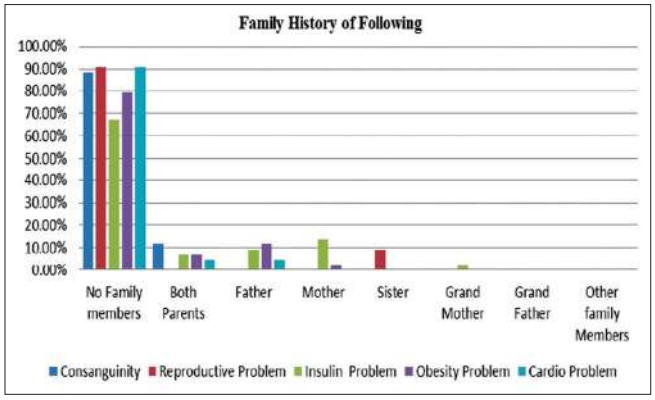

Figure 2: Family history of various symptoms of polycystic ovary syndrome. This figure representation clearly depicts that there is no significant role of hereditary in the clinical symptoms of the syndrome

The study done with the small sample size thus far showed no association what so ever with the variant and development of PCOS. It is a well-researched study that TCF7L2 is a candidate gene in PCOS as there is an overlap in characteristics between PCOS and T2DM.[13] A similar study done by Freathy *et al*. in 2007 with a sample size of 24,053 has demonstrated the effect of genotype on insulin's reaction to oral glucose.[20] Barber *et al*., 2007 has demonstrated strongly that there is no association between the variants of TCF7L2 and development of PCOS.[19]

It is well known that TCF7L2 majorly regulates the pro-glucagon expression through the Wnt pathway and PCOS studies have shown a relationship between the disease and T2DM. Research has proved and disproved any relationship between PCOS and TCF7L2 (a study by Christopoulos *et al*., 2008 in Greek PCOS women showed a relationship, but the studies done by Xu *et al*. 2010 and Kim *et al*. 2012 in Chinese and Korean PCOS women, respectively, showed no such relationship).

The studies in this area so far have shown varied results with varied sample size. Our study shows a negative relationship between PCOS development and TCF7L2

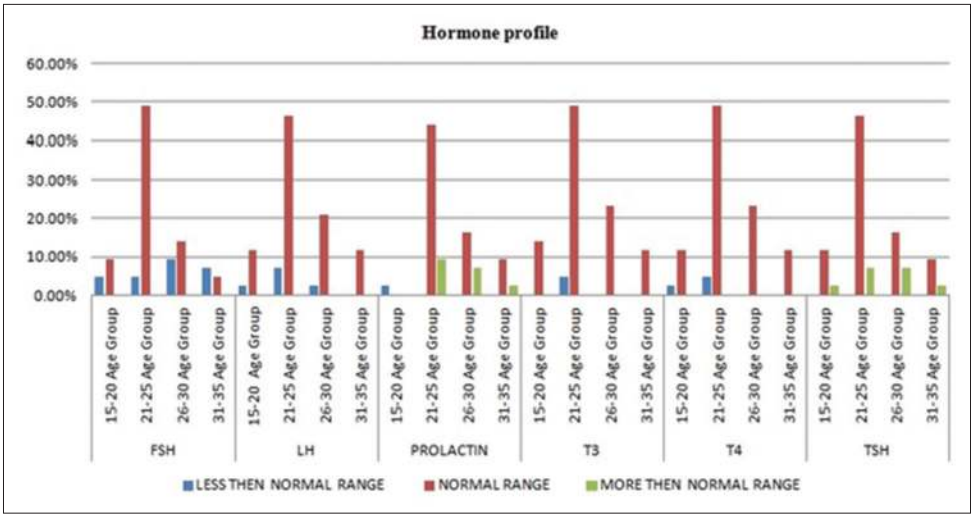

Figure 3: Hormone profile of polycystic ovary syndrome patients. Most of the patients had the hormone profile falling within the normal reference range. Very few patients showed lower than normal range only in case of follicle-stimulating hormone and luteinizing hormone and elevated among prolactin and thyroid-stimulating hormone

variants. However, the sample size being critically low, this study should be considered as a preliminary analysis of this specific cohort which can be further proved strongly with a larger cohort size.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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