Section A-Research paper

Prevalence of genetic polymorphisms in *CYP19A1,* gene in patients with polycystic ovarian syndrome in comparison with control and correlation with insulin resistance in Peer Panjal range(J&K)

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ABSTRACT

Background: PCOS is a common problem in youngerly age group which has got a genetic connection with insulin resistance

Objectives: To identify the gene responsible for PCOS.

Materials and Methods: genetic study was conducted using PCR and RFLP methods, Clinical examinations were conducted to correlate the same with the insulin resistance

Results: CYP19A1 gene is not responsible for PCOS however we have found Multiple features which connects the same as aimed.

Conclusion: Insulin resistance features such as Acanthosis nigricans /IFG/IGT or diabetes mellitus occurred in a high proportion of PCOS cases which could be due to excess cortisol.

Keywords; PCOS, Polymorphism, insulin resistance.

INTRODUCTION

Polycystic Ovarian Syndrome (PCOS) is referred to some times as Sclerocystic Ovarian Disease, Stein-Leventhal Syndrome and Polycystic Ovarian Disease (PCOD). PCOS is a complex, heterogeneous, polygenic endocrine disorder in women of reproductive age and is considered as a multifactorial reproductive, cosmetic and metabolic problem. The etiology of PCOS is not well understood and its pathophysiological and molecular basis is still a puzzle. PCOS is likely to be the result of a number of both genetic and environmental factors. Some of the contributing factors to PCOS also include a low level of chronic inflammation in the body and fatal exposure to male hormones. However, androgen excess and insulin resistance leading to hyperinsulinemia are considered to be the basic defects in PCOS that was described way back in 1921 by Archard & Theirs as "diabetes of bearded women"[1].

The world-wide prevalence of PCOS syndrome is 6-10% and in its "classical" form may affect 5 - 7% of women[2]. PCOS is quite common in Asian population. A high prevalence of up to 35% is reported for the Indian women and the incidence and prevalence of PCOS in overweight and obese women is greater than 20%[3]. Women with PCOS are at a higher risk for a number of illnesses, including high blood pressure, diabetes, heart disease and other cardiovascular problems and cancer of the uterus, ovary and breast[4].

Single gene Mendelian model which predicts that there is single gene defect inherited in a recessive or dominant pattern and that woman who inherit this defect develop clinically evident PCOS.

Multifactorial model where PCOS is considered as a multifactorial genetic disorder and women carrying this defect through inheritance or environmental factors will have increased risk of clinical PCOS[5].

Aim:

To identify the frequency of the genetic polymorphisms in *cyp19a1,* gene in patients with polycystic ovarian syndrome in comparison with control.

MATERIAL AND METHOD

Study Setting, design, sampling and period of the study

Out-patients and in-patients of Government Medical College & Hospital, Rajouri, diagnosed with polycystic ovarian syndrome were inducted into the study. The study is a descriptive, cross-sectional type. Convenient sampling of subjects was done for the study. The project began in December 2021 and ended in Jan 23

Institute's Research ethical committee

Institute's Research ethical committee approval was obtained for the study. After obtaining informed consent from each participant, hundred and three (103) patients with a clinical diagnosis of PCOS (where large family trees was known) were included in the study. All patients received a long, careful and simple explanation of the purposes of the study and its pathophysiological basis.

Criteria for the definition of PCOS

The diagnosis of PCOS was made according to the ESHRE/ASRM criteria for the PCOS diagnosis (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004)[6] based on the presence of two of the three following criteria: oligo- and/or anovulation (menstrual dysfunction), clinical and/or biochemical signs of hyperandrogenism and polycystic ovaries (PCOS) at ultrasonogram[7].

Methods

Patients and Participants:

103 female patients with a clinical diagnosis of PCOS, their 371 first and second-degree relatives and 50 age matched controls were included in the study after obtaining informed consent.

All the subjects including patients and their family members were interviewed in detail and examined for anthropometry such as BMI, Hirsutism /excess hair, Acne, Baldism, Acanthosis nigricans, Skin tags, Buffalo humps, Moon face, Double chin.

1. Biochemical assay such as; Serum fasting insulin, Cortisol, Testosterone, Dehydroxyepiandostenedione, LH, FSH, TSH were done in all cases, along with fasting lipid profile.
2. Blood pressure was measured for all, an oral 2 hr GTT was performed after 75 gm of glucose for all patients.

Genetic analysis:

Study of genotype and allelic frequencies were done by means of PCR- RFLP In collaboration with TAU, Zambia. DNA was extracted from heparinised or EDTA blood. Ethical clearance was obtained from institutional Ethical Committee and the study was performed.

Collection of blood samples: The blood samples were collected from the patients with PCOS from District Rajouri of state Jammu and Kashmir. Patient’s history was collected and pedigree was drawn.

Isolation of genomic DNA

Reagents employed

WBC lysis buffer		RBC lysis buffer	
Tris-HCl	: 10 mM	Ammonium chloride: 138 mM	
Sodium chloride	: 400 mM	Tris	:17mM
Sodium EDTA	: 2mM		

The chemicals for WBC and RBC lysuis buffer were purchased from Sisco Research Laboratories, India.

Polymerase Chain Reaction (PCR)

Reagents employed

10X PCR buffer: 50 mM KC1, 100 mM Tris HC1 pH 8.3 (Merck, India), 15 mM MgCl2 (Merck, India), Triton X-100 (1%) (Sigma, USA).

dNTP mix (4mM): 4 uL of each of dATP (100 mM), dGTP (lOOmM), dCTP (100 mM), dTTP (100 mM) - (Fermentas, USA), MilliQ water- 84 uL, Total -100 uL.

Procedure

The PCR reagents (Table 1) were purchased from Sigma-Aldrich, USA and Taq polymerase from Fermentas, USA.

Table 1. Composition of PCR reaction mixture.

REAGENTS	VOLUME (µL)
DNA (100-150 ng)	1.5
MQ water	16
10X PCR buffer	2.5
dNTP 4 mM	2.5
Forward Primer (100 ng)	0.75
Reverse Primer (100 ng)	0.75
Taq Polymerase (1U)	1.0

PCR amplification was carried out using Master Cycler Thermocycler (Eppendorf, USA) and documented in a Gel Documentation system (Uvitec, UK).

Agarose gel electrophoresis

The bands were visualized under UV Gel Documentation system (Uvitec, UK)[8].

Restriction Fragment Length Polymorphism (RFLP)

The restriction fragments are separated according to their size by agarose gel electrophoresis. The table below (Table 2) shows the list of restriction enzymes used in the present study.

Table 2. List of restriction enzymes used in RFLP for genotyping. The composition specific to each enzyme is given with the reaction conditions.

Enzyme	Recognition site	Buffer				BSA	Temp	Company
		1	2	3	4			
MspA1	5'..CMG②CKG..3' 3'..GKC GMC..5' □						37	NEB

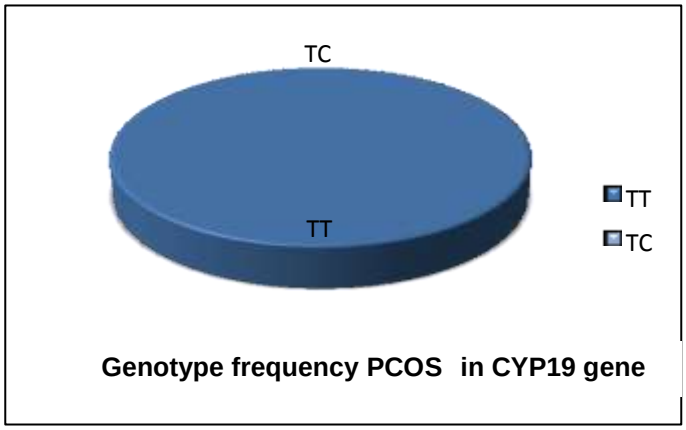
Single letter code: R = G or A; K = G or T; B = C, G or T; Y = C or T; S = C or G; D = A, G or T; W = A or T; H =A, C or T; N = G, A, T or C M = A or C; V = A, CorG; Reaction mixture was prepared to 10 final volumes. All the reagents were thawed before use. 500ng of the PCR product was digested with 1 U of restriction enzyme at appropriate incubation temperature for 16 hours. A master mix of MQ water, buffer and enzyme was prepared, spun down and then distributed into the individual labelled tubes. Then the digested products were loaded on agarose gel of appropriate percentage depending on the fragment size. The gel was run for 30-45 minutes and checked for the digestion.

Statistical analysis

The results are expressed as the mean ± SD in the text and Tables. Univariate analysis was done by using one-way ANOVA and Kruskal-Wallis test (nonparametric). A statistical software package was used to perform the analyses (SPSS version 17).

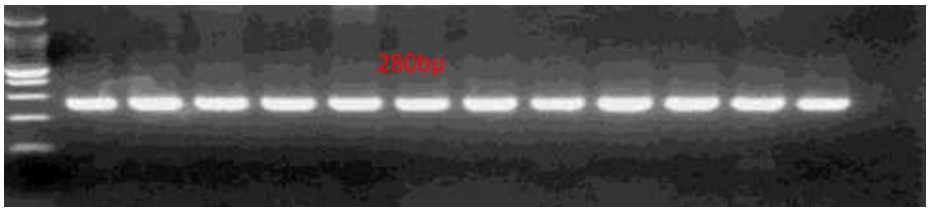
Table3. Genotype frequencies in *CYP19A1* genes in patients with PCOS and controls.

Gene	Polymorphism	Genotype frequency PCOS	Genotype frequency Controls	Chi square (X^2) P value	OR
CYP19A1 (RS-742972)	-34T>C	TT-100 TC-.00 CC-.00	TT-100 TC-.00 CC-.00	-	-



When we screened 103 PCOS patients in *CYP19A* gene for -34T>C polymorphism. The allele of gene *CYP19A* that, contain C instead of T is designated as A2 allele. The unmutated allele with T was designated as A1 allele of *CYP19A* gene. When we screened 103 patients with PCOS and their 291 first-, and second-degree relatives with 50 controls for the presence of polymorphic allele, no patients (0.0%) were heterozygous carrier of polymorphic A2 allele (genotype A1A2). Among 50 patients 0(0%) carried the (A2) allele in the heterozygous state (A1A2). We found no association between -34T>C polymorphism and PCOS patients.

Genetic Study Analysis Gel Picture of PCR- RFLP



CYP – 19A1 Gene

DISCUSSION

Familial clustering of PCOS has been consistently reported, suggesting that genetic factors play a role in the development of the syndrome. Sisters, brothers, fathers, mothers, daughters and now even sons of women with PCOS have been found to have a higher risk for exhibiting either hyperandrogenic or metabolic (hyperinsulinemic) traits of the disorder and thus PCOS has become a 'family affair'[9]. In the present study we examined the prevalence of a polymorphism of gene CYP19 promoter and found no association as A1A2=0.0 and A2A2=0.0 genotype frequencies.

In another study Marszalek *et al* illustrate a similar result in Poland population, while they genotyped 56 PCOS women and concluded the T>C polymorphism of CYP19 gene is not associated with steroid hormone synthesis in PCOS and it is not a primary genetic defect in this disease. In this context, the suggestion that PCOS should be treated as a quantitative trait disorder which does not necessarily imply a truly polygenic aetiology because it would be possible to explain the variable phenotype on the basis of a small number of key causative genes (a so-called oligogenic basis for disease) involved in androgen secretion and insulin secretion/action in conjunction with environmental, particularly nutritional factors[10]. In our study we did not find premature puberty ruling out the possibility of adrenogenital syndrome. It is well recognised that women born with low body weight have tendency to develop increased insulin resistance and increased secretions of dehydroepiandrosterone (DHEA) that leads to the development of PCOS[11].

Conclusion:

1. Our study showed that genetic polymorphism in *cyp19a1* was not associated with PCOS in PeerPanjal Range.
2. PCOS can be considered as metabolic syndrome equivalent
3. Insulin resistance features such as Acanthosis nigricans /IFG/IGT or diabetes mellitus occurred in a high proportion of PCOS cases which could be due to excess cortisol.

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