

Correlation of clinical findings in PCOS with gene variants – a preliminary study in South Indian population

Tamanna Chauhan¹, Dr. Ramani Devi², Dr. Anchana Devi C¹ and
Dr. Pavithra Amritkumar^{1*}

¹Post graduate and Research Department of Biotechnology, Women's Christian College, College Road,
Chennai-600006, India

²Ramakrishna Medical Center, 20-21, Vivekananda Nagar, Woraiyur, Trichy-620003, India

* Corresponding Author: Dr. Pavithra Amritkumar

Abstract: Polycystic ovary syndrome (PCOS) is an endocrine disorder that affects 1 in 10 women of childbearing age. Women with PCOS have a hormonal imbalance and metabolism problems that may affect their overall health and appearance. The present study aims to correlate the clinical findings in PCOS with two gene variants in FSHR gene and TNF- α gene as a preliminary study in south Indian population. In this case-control study, a total of 68 individuals of South Indian origin (34 women with clinical history of PCOS and 34 case-matched healthy controls subjects) were recruited. Following informed consent, 5ml blood was withdrawn from individuals and subsequently subjected to genomic DNA extraction by standard phenol– chloroform-isoamyl alcohol (PCI) method. Two specific sets of polymerase chain reaction, followed by restriction (PCR-RFLP) relating to the gene variants were carried out using specially designed primer pairs. The distribution of Polymorphism rs6166 (A/G) in exon 10 in FSHR gene among the PCOS subjects in this study was found to be G/G (30%) and A/G (35%). In case of control subjects, the same polymorphism was found to be G/G (18%) and A/G (23%). In case of FSHR gene, the PCOS subjects showed nearly double the homozygosity (30%) of the polymorphism (G/G) in the FSHR gene than control subjects (18%), indicating that this polymorphism has significant role in PCOS in South Indian population. In case of the -1031 polymorphism in the promoter region of TNF- α gene, we observed absence of this polymorphism associated with PCOS in our study. This is the first study to screen for two important gene variants in FSHR and TNF- α genes associated with PCOS in south Indian population.

Keywords -Polycystic ovarian syndrome, PCOS, PCR-RFLP, FSHR gene, TNF- α gene, south India

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I. Introduction

Polycystic ovary syndrome (PCOS) has been characterized as an endocrine disorder by the presence of polycystic ovary along with excessive androgen secretion and ovary dysfunction. It was first 'discovered' in 1935 by Doctor Stein and Leventhal, so for many years it was known as the Stein-Leventhal syndrome. It is one of the most common endocrine disorders affecting 5% to 12% of women of reproductive age worldwide and is considered to be one of the major causes of female infertility in reproductive age [1]. Studies of PCOS in India carried out report a prevalence of 3.7% to 22.5%, with 9.13% to 36% prevalence in adolescents only [2,3].

The main characteristics of this syndrome are anovulation (absence of ovulation), resulting in abnormal menstruation, absence of menstruation, heavy menstruation, irregular menstruation, short and light menstruation, or spotting in menstruation, ovulation-related infertility, amenorrhea, and polycystic ovaries; hirsutism, and insulin resistance, excessive amounts or effects of androgenic hormones, often associated with obesity, overweight, or weight gain, high cholesterol levels, Type 2 diabetes, infertility, dark patches of skin in folds and creases, oily skin, acne issues, depression, inappropriate male features, loss of scalp hair, or presence of unwanted hair [4,5,6]. PCOS is becoming a prevalent disorder among women of reproductive age with lifelong complications. One of the most challenging aspects of this syndrome is its ambiguous diagnostic criteria and vast complexity of characteristics. While the diagnosis is straight forward using the Rotterdam Criteria which include two of the following three criteria are required: oligo/anovulation, hyperandrogenism and polycystic ovaries on ultrasound [7], researchers are trying to search for new ways to treat PCOS. Once other conditions are ruled out, one may be diagnosed with PCOS if one has at least two of the following symptoms: Irregular periods, including periods that come too often, not often enough, or not at all, high levels of androgens, extra hair growth on face, chin, and body (hirsutism), acne, thinning of scalp hair, higher than normal blood levels of androgens and multiple cysts on one or both ovaries

[https://en.wikipedia.org/wiki/Polycystic_ovary_syndrome].

Globally, many studies have been done regarding the screening of mutations, its effects and find the accurate cause of the syndrome. Certain studies demonstrated that the genetic risk factors are associated with various genes present in human genome. Several polymorphisms and mutations are of clinical and biological significance and helps in the proper functioning of such candidate genes and their products. In case of India, there have been only few genetic studies done. These studies include Screening of CYP11A1 Gene Polymorphism and to screen inactivation mutation of Exon 1 of *FSHR* Gene from South Indian population [8,9]. In order to decode the underlying molecular mechanisms leading to PCOS, researchers are now investigating candidate genes to understand the inherited causes and its related phenotypes.

Follicle Stimulating Hormone Receptor (*FSHR*) gene encodes a protein that belongs to family 1 of G-protein coupled receptors (NCBI, <https://ghr.nlm.nih.gov/gene/FSHR#resources>). *FSHR* gene, which has cytogenetic Location of 2p16.3, and molecular location of base pairs 48,953,161 to 49,154,527 on chromosome 2, is expressed in Sertoli cells of testis and granulosa cells of the ovary in the gonads of male and female, respectively [10]. Mutations in this receptor gene cause ovarian dysgenesis type 1 and ovarian hyper stimulation syndrome [<https://ghr.nlm.nih.gov/gene/FSHR>].

Tumor necrosis factor-alpha (*TNF- α*) is a major pro-inflammatory cytokine and is expressed mainly in monocytes, macrophages and adipose tissue. It maps to chromosome 6p21.3, spans about 3 kilo bases and contains 4 exons. Several association studies have reported that some polymorphisms of tumor necrosis factor-alpha (*TNF- α*) gene are related with gynecological diseases including pre-eclampsia, endometriosis. *TNF- α* is associated with the clinical, biochemical manifestations of PCOS. A study by Yun et. al., (2011) has indicated that the promoter -1031(T/C) polymorphism of *TNF- α* gene is associated with PCOS in a Korean population [11]. Several other studies have suggested considering *TNF- α* as an immunological and molecular indicator for gynecological-related diseases. [<https://www.ncbi.nlm.nih.gov/gene/7124>].

In this present study, we have focussed on two variants, namely rs6166 (A>G) in exon 10 of Follicle-stimulating hormone receptor (*FSHR*) gene and -1031 (T>C) polymorphism in the promoter region of Tumor necrosis factor-alpha (*TNF- α*) gene to understand the role of genomic variants in clinical PCOS cases. The study of these gene variants comprehensively in clinical cases of PCOS will help in knowing the possible causes of PCOS in south Indian population.

II. Materials and Methods

2.1 Ethical Compliance

The approval for recruiting women subjects of South Indian origin with clinical history of Polycystic Ovarian Syndrome (PCOS) and healthy women control subjects was obtained from the Institute Review Board and Ethics Committee of Women's Christian College, Chennai. An appropriate questionnaire related to the objectives of the study was designed, which was also approved by the ethical committee.

2.2 Subjects and Clinical documentation

34 women of South Indian origin with clinical history of PCOS and 34 case-matched healthy controls subjects were recruited for this study. The samples were collected from the following sources: Ramakrishna Medical Centre, Trichy, Women's Christian College, Chennai and Seethapathy Clinic & Hospital, Chennai. 5ml of blood sample was collected after obtaining written and signed informed consent from all the participating members.

Women with clinical history of oligomenorrhoea, amenorrhoea, clinical hyperandrogenism and or PCOS detected in sonography, based on the revised diagnostic criteria according to the 2003 ASRM/ESHRE Rotterdam consensus [7] and adult subjects of south Indian origin willing to participate in the study were included. Women suffering from Diabetes mellitus, Hypertension, Thyroid disorder, ovarian tumours, premature ovarian tumours and Minor subjects were not included in this study. South Indian origin fertile healthy women having normal menstrual cycle were included as control for this study. Detailed medical history and family history was documented through structured questionnaire. Consanguinity, if married and Parental consanguinity were also noted down from both PCOS and Healthy subjects to relate and check with their family history. Pre-test counseling relating to genetic studies was provided to each of these individuals prior to testing.

2.3 Methodology

2.3.1 Genomic DNA isolation

Five ml of blood was drawn by venipuncture in EDTA-coated vacutainers™ (Becton and Dickinson, USA) and the samples from both subjects and controls were coded systematically and records were maintained appropriately. Genomic DNA was extracted by standard phenol– chloroform-isoamyl alcohol (PCI) method [12].

2.3.2 PCR Amplification- *FSHR* gene

The amplification of a portion of *FSHR* gene (size 520 bp) containing the polymorphism region rs6166 in exon 10, was carried out by hot-start PCR, using specific primer pairs, Forward Primer: 5'-TTT GTG GTC ATC TGT GGC TGC-3' and Reverse Primer: 5'-CAA AGG CAA GGA CTG AAT TAT CAT T-3' [13]. The reaction was carried out in Hi-Media Gradient Thermocycler, India.

2.3.3 PCR Amplification of *TNF- α* gene

The amplification of a portion of *TNF- α* gene (size 264 bp) containing the -1031 polymorphism in the promoter region, was carried out by Polymerase Chain Reaction, using specific primer pairs, Forward Primer: 5'-TAT GTG ATG GAC TCA CCA GG-3' and Reverse Primer: 5'-CCT CTA CAT GGC CCT GTC TT-3'[11]. The reaction was carried out in Hi-Media Gradient Thermocycler, India. **Table-1** indicates the conditions used for the two PCR reactions mentioned in the study.

2.3.4 RFLP analysis for *FSHR* gene polymorphism

The PCR generated 520 bp amplicon was subjected to RFLP analysis using *Bsr*I restriction enzyme as per manufacturer's instruction (Thermo Scientific Labs). The restriction site of *Bsr*I is:

5'... **A C T G G N**↓...3'
3'... **T G A C**↑**C N** ...5'

Concentration: 10 U/ μ L
Source: *Bacillus species* N

2.3.5 RFLP analysis of *TNF- α* gene polymorphism

The PCR generated 264bp amplicon was subjected to RFLP analysis using *Bbs*I restriction enzyme as per manufacturer's instruction (Thermo Scientific Labs). The restriction site of *Bbs*I is:

5'...**G A A G A C** (N)₂↓...3'
3'...**C T T C T G** (N)₆↑...5'

Concentration: 10 U/ μ L
Source: *Bacillus pumillus* Sw 4-3

2.3.6 Agarose gel electrophoresis

The PCR products were checked for amplification with 2% agarose gel and RFLP fragments were checked using 3% agarose gel, both containing ethidium bromide (0.5 μ g/ μ l). The electrophoresis was carried out in 0.5 X TBE buffer for 25 minutes at 120V using 50bp ladder for reference. The DNA bands were visualized and documented using Gel Documentation system (Gelstan Gel documentation system, Mediacare India).

III. Results and Discussion

3.1 Socio-Demographic Data

This study involved adult women of South Indian origin. A total of 68 individuals participated in this study of which 34 were clinically confirmed PCOS subjects and 34 were normal subjects. All the subjects belonged to the 20-35 years age group.

3.1.1 Nativity

In this study, 30 PCOS subjects were from Tamil Nadu (88%), 1 subject was from Andhra Pradesh and 3 of them hailed from Kerala.

3.1.2 Marital status

About 62% of the PCOS subjects were unmarried and 38% of the subjects were married.

3.1.3 Parental Consanguinity

In this study, 14 PCOS subjects were from a consanguineous parental mating and the 20 were from a non-consanguineous parental mating.

3.2 Clinical Data

The clinical data was collected was analyzed and a comparative data between the cases and control subjects is provided in **Table-2**. The hormones screened in the study, Luteinizing hormone (LH), Follicle-Stimulating Hormone (FSH), Thyroid Stimulating Hormone (TSH), Testosterone, Progesterone, Sex Binding Hormone, were in range, except **Prolactin which was (43.50 ng/ml) higher than in previous studies**. In a

study by Liu et al., (2012) on association of the genetic variants of luteinizing hormone [14], luteinizing hormone receptor and polycystic ovary syndrome, the average age at which PCOS was detected was found to be 32.48 ± 3.88 years and the average BMI was reported to be 24.18 ± 3.91 . In our study we found out the average age to be 21 years and the average BMI among PCOS subjects to be 21.59. The average BMI in both the studies is found to be in the same range. The average LH ranges and FSHR range in their study was found to be in the same range as ours (table). Our TSH values were similar to study by Kanwar et al., (2015), while the Prolactin values of our subjects were nearly three times the values reported by them in PCOS subjects [15].

According to study on sex hormone binding globulin (SHBG) by Deswal et al., (2017) lower levels were noted in PCOS subjects [16], but in our study it was in the normal average range comparable with that of the controls. The average insulin values in our study were also in the normal average range ($14.26 \mu\text{IU/ml}$) as in other studies.

3.3 Molecular Data

3.3.1 Genomic DNA Isolation

The DNA was isolated from the blood drawn from the subjects following the PCI protocol. Working concentration of DNA checked on 0.8% Agarose by gel electrophoresis. The gel containing the isolated DNA from the subject samples was observed under a UV Trans-illuminator as shown in **Fig.1**.

3.3.2 PCR amplification of *FSHR* gene

The amplification of a portion of *FSHR* gene (size 520 bp) containing the polymorphism region was carried out by Polymerase Chain Reaction using specific primer pairs and viewed in a 2% agarose gel under a UV transilluminator as shown in **Fig.2**.

3.3.3 RFLP analysis for *FSHR* gene polymorphism

The 520bp length amplicon was digested by the *BsrI* enzyme to produce fragments that vary for normal and the variant A > G polymorphism as given below. The presence of polymorphism introduces an additional restriction site which was detected through the characteristic banding pattern in a 3% agarose gel (**Fig.3**).

The various banding patterns observed on restriction digestion:

- **Normal (A/A)** - 520 bp
(Both are normal alleles, visible as single band)
- **Heterozygous (A/G)** - (520 bp + 413bp + 107bp)
(Combination of normal allele and variant allele, visible as three bands)
- **Homozygous (G/G)** - (413 bp + 107 bp)
(Both are variant alleles, visible as two bands)

When subjected to RFLP (**Fig. 3**), 12 out of the 34 PCOS subjects (35%) were found to be having heterozygous condition (A/G), 10 PCOS subjects (30%) had homozygous condition (G/G) and 12 PCOS subjects (35%) were found to have the common variant (A/A). Over all polymorphism among the PCOS subjects in this study is homozygous (30%) and heterozygous (35%), which is higher than the previous reported studies [17,18]. When the PCR products of the control samples were subjected to RFLP, 8 out of the 34 control subjects were found to be having heterozygous condition of A/G (23%), 6 Normal subjects (18%) revealed having homozygous condition (G/G) and remaining 20 (59%) did not have the A>G variant. PCOS subjects showed nearly double the homozygosity (30%) of the polymorphism (G/G) in the *FSHR* gene than control subjects (18%), indicating that this polymorphism has significant role in PCOS. Our study showed higher percentage than previous Indian study [19]. The two polymorphisms, p.Thr307Ala (rs6165) and p.Asn680Ser (rs6166), do not clearly alter receptor function in vitro, but may influence the response to endogenous and exogenous FSH stimulation [20,21].

3.3.4 PCR amplification of *TNF- α* gene

The amplification of a portion of *TNF- α* gene (264 bp) containing the polymorphism region was carried out by Polymerase Chain Reaction using specific primer pairs and viewed in 2% agarose gel under UV transilluminator as shown in **Fig.4**

3.3.5 RFLP analysis of *TNF- α* gene polymorphism

The 264bp length amplicon was digested by the *BbsI* enzyme to produce fragments that vary for

normal and the variant phenotypes as given below.

The various banding patterns on restriction digestion:

1. Normal (T/T) - (251bp + 13bp)

(Both are normal alleles, visible as single band owing to very small size of 13bp band)

2. Heterozygous (T/C) – (251bp + 180 bp + 71bp + 13bp)

(Combination of normal allele and variant allele, visible as 3 bands)

3. Homozygous (C/C) - (180 bp + 71 bp +13bp)

(Both are variant alleles, visible as two bands)

In the absence of polymorphism, the amplicon of 264bp was digested to 251bp and 13bp, which normally is visible only as a single band as 13bp is too small to be visible. In the presence of polymorphism T>C, there is an additional restriction site for *BbsI* enzyme created within the 251bp amplicon, resulting in 180bp and 71bp bands.

All our 34 PCOS subjects and 34 control subjects did not show any variants in the RFLP analysis for the *TNF- α* gene polymorphism (Fig.5). From our preliminary analysis, the variant in the *TNF- α* gene in the promoter -1031, namely C>T, was absent in our population. Yun et al., (2011) have reported that -1031(T/C) polymorphism in the promoter region of *TNF- α* gene is associated with PCOS in a Korean population but from our preliminary analysis [10], this variant in the *TNF- α* gene in the promoter region was absent in our population. This is the first study in south Indian population on the role of this variant in promoter namely, -1031 (C>T) in the *TNF- α* gene, in PCOS.

IV Conclusion

The distribution of Polymorphism rs6166 (A/G) in exon 10 in *FSHR* gene among the PCOS subjects in this study were: G/G homozygous (30%) and G/A heterozygous (35%), which are higher than the previous reported studies in Indian population. In case of control subjects, the same polymorphism was found to be G/G homozygous (18%) and G/A heterozygous (23%). PCOS subjects showed nearly double the homozygosity (30%) of the polymorphism (G/G) in the *FSHR* gene than control subjects (18%), indicating that this polymorphism has significant role in PCOS in our population.

In case of the -1031 polymorphism in the promoter region of *TNF- α* gene, we observed absence of polymorphism associated with PCOS in our study. This polymorphism has been screened for the first time in Indian PCOS subjects and case matched controls. This is the first study with regard to screening for both *FSHR* gene polymorphism and *TNF- α* gene polymorphism in south Indian population.

This is a preliminary study correlating the molecular data with clinical data in a small subset of PCOS subjects of south Indian origin. Understanding of role of genetic variants in the incidence of PCOS in a population is important and more gene variants should be screened on a larger number of case-controls for a comprehensive understanding of the underlying mechanism of PCOS.

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Conflict of Interest

The authors report no financial or commercial conflicts of interest.

References

- [1]. Moran C, Tena G, Moran S, Ruiz P and Reyna R. Prevalence of polycystic ovary syndrome and related disorders in Mexican women. *Gynecol. Obstet. Invest.* 2010, 69: 274-280.
- [2]. Malik S, Jain K, Talwar P, Prasad S, Dhorepatil B, et al. Management of Polycystic Ovary Syndrome in India. *FertilSci Res.* 2014, 1:23-43
- [3]. Nidhi R, Padmalatha V, Nagarathna R and Amritanshu R. Prevalence of polycystic ovarian syndrome in Indian adolescents. *J*

- PediatrAdolesc Gynecol.* 2011, 24: 223-227.
- [4]. Franks S. Polycystic ovary Syndrome. *New England Journal of Medicine* 1995, 333:853-861.
- [5]. Diamanti-Kandarakis E. Insulin resistance in PCOS. *Endocrine* 2006, 30: 13-17.
- [6]. Ateab M , Nader M, and Aziz. I. Genetic variation in women with insulin resistance. *Int'l Conf. on Medical Genetics, Cellular & Molecular Biology, Pharmaceutical & Food Sciences (GCMBPF-2015)*, June 5-6, 2015, Istanbul.
- [7]. The Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and longterm health risks related to Polycystic ovary syndrome (PCOS). *Human Reproduction* 2004, 19:41-47.
- [8]. Reddy R K, M. L. N. Deepika MLN, Supriya K, PrasannaLatha K, Lakshmana Rao SS, Usha Rani V and Jahan P. Screening Of CYP1A1 Gene Polymorphism in Polycystic Ovary Syndrome Women among the Rural South Indian Population. *J Assist Reprod Genet.* 2014, 31:857–863.
- [9]. Suguna E, Parijatham S, Sai Kumar P, RevathyRamalingam and Jeyachandran P. Screening Of CYP1A1 Gene Polymorphism in Polycystic Ovary Syndrome *Biomedicine* 2016; 36(3): 084-088.
- [10]. Wunsch A, Ahda Y, Banaz-Yaşar F, Sonntag B and Nieschlag E. Single-nucleotide polymorphisms in the promoter region influence the expression of the human follicle-stimulating hormone receptor. *Fertil. Steril.*, 2005, 84: 446-453.
- [11]. Yun J-H, Choi J-W, Kyung-Ju Lee K-J, Shin J-S and Baek K-H. The promoter 1031(T/C) polymorphism in tumor necrosis factor-alpha associated with polycystic ovary syndrome. *Reproductive Biology and Endocrinology* 2011, 9:131.
- [12]. Sambrook JF and Russell DW *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, 2001, 3rd edn, pp. 2100.
- [13]. Sudo S, Kudo M, Wada S, Sato O, Hsueh AJ, Fujimoto S. Genetic and functional analyses of polymorphisms in the human FSH receptor gene. *Mol Hum Reprod.* 2002;8(10):893–899.
- [14]. Liu N, Ma Y, Wang S, Zhang X, Zhang Q, Zhang X, Fu L and Qiao J. Nana. Association of the genetic variants of luteinizing hormone, luteinizing hormone receptor and polycystic ovary syndrome. *Reproductive Biology and Endocrinology* 2012, 10:36.
- [15]. Kanwar G, Jain N, Shekawat M and Sharma N. Estimation of LH, FSHR, Prolactin and TSH Levels In Polycystic Ovarian Syndrome And Correlation of LH And FSHR With Serum TSH Levels. *IOSR Journal of Dental and Medical Sciences* 2015, 14(5):64-68.
- [16]. Deswal R, Yadav AK and Dang AS. Sex hormone binding globulin - an important biomarker for predicting PCOS risk: A systematic review and meta-analysis. *Systems Biology in Reproductive Medicine.* 2017, 64(1):1-13.
- [17]. Hamood QH. Ultrasonographic finding in women with polycystic ovary syndrome in correlation with their FSHR gene polymorphism. *International Journal of PharmTech Research*, 2016, 9(12): 511-517.
- [18]. Branavan U, Muneeswaran K, Wijesundera S, Jayakody S, Chandrasekharan V, Wijeyaratne C. Identification of selected genetic polymorphisms in polycystic ovary syndrome in Sri Lankan women using low cost genotyping techniques. *PLoS ONE* 2018, 13(12): e0209830.
- [19]. SekarN ,Yeole S, Pradeep R , Prabhu Y D, Renu K, Ramgir K S and V G Abilash. To Screen Inactivation Mutation of Exon 1 of FSHR Gene in Polycystic Ovarian Syndrome: A South Indian Cohort Study. *IOP Conf. Series: Materials Science and Engineering*, 2017, 263:022034.
- [20]. Valkenburg O, Uitterlinden AG, Piersma D, Hofman A, Themmen AP, de Jong FH, Fauser BC and Laven JS. Genetic polymorphisms of GnRH and gonadotrophic hormone receptors affect the phenotype of polycystic ovary syndrome. *Hum. Reprod.*, 2009, 24: 2014-2022.
- [21]. Woad KJ, Prendergast D, Winship IM and Shelling AN. FSH receptor gene variants are rarely associated with premature ovarian failure. *Reproductive Biomedicine Online*, 2013, 26: 396–399

FIGURES

Fig.1: Gel image showing representative samples of working concentration DNA after extraction

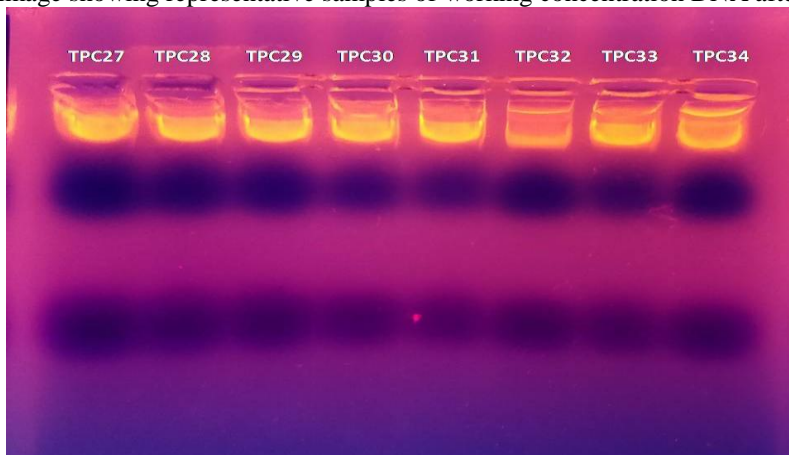


Fig.2: 2% agarose gel image showing PCR amplified products of part of *FSHR* gene

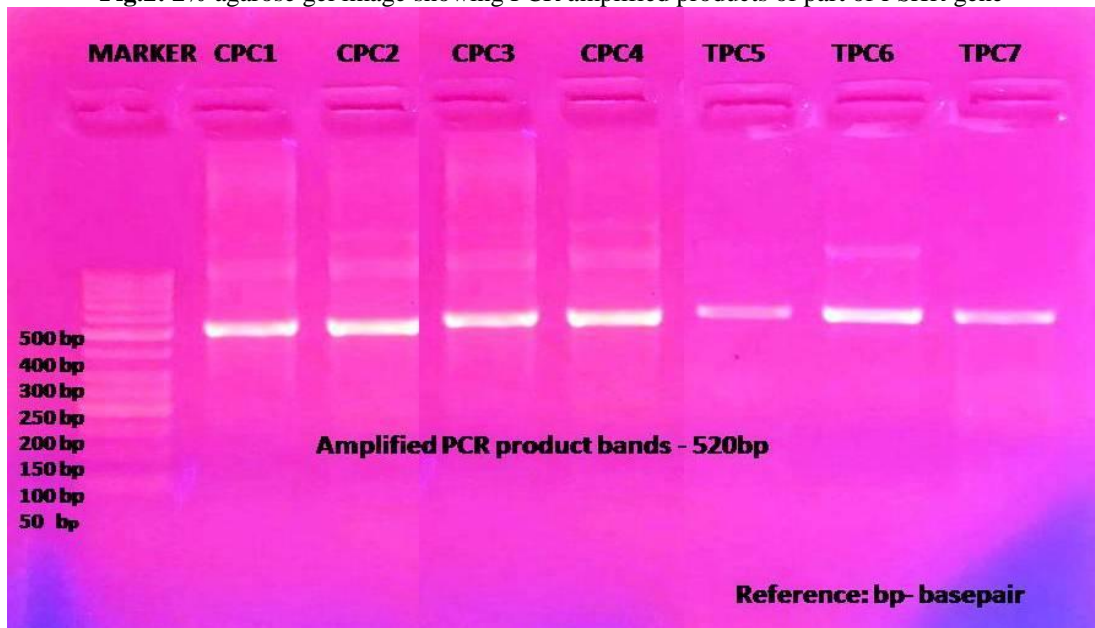


Fig.3: 3% agarose gel image showing PCR-RFLP analysis for the detection of *FSHR* gene polymorphism

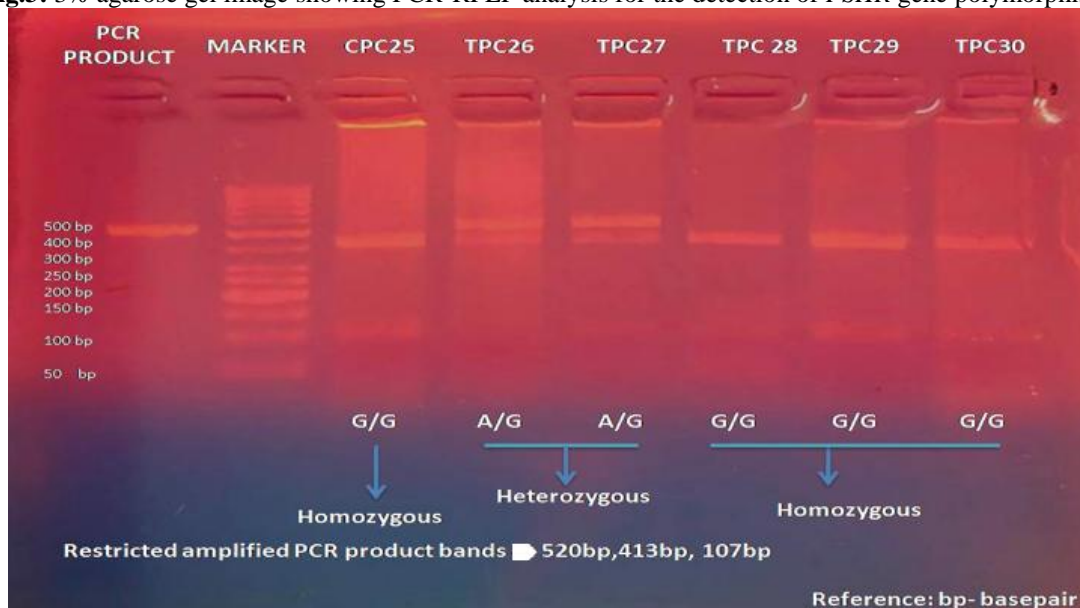


Fig.4: 2% agarose gel image showing PCR amplified products of part of *TNF- α* gene

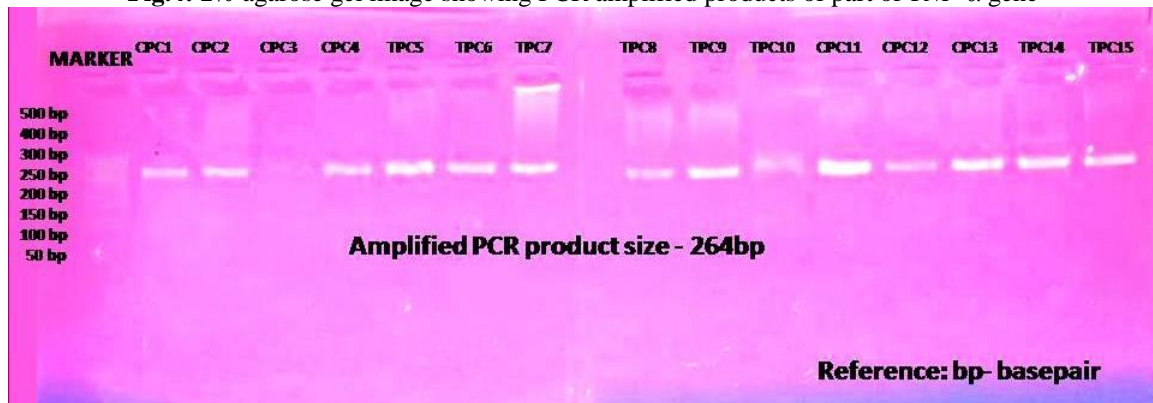
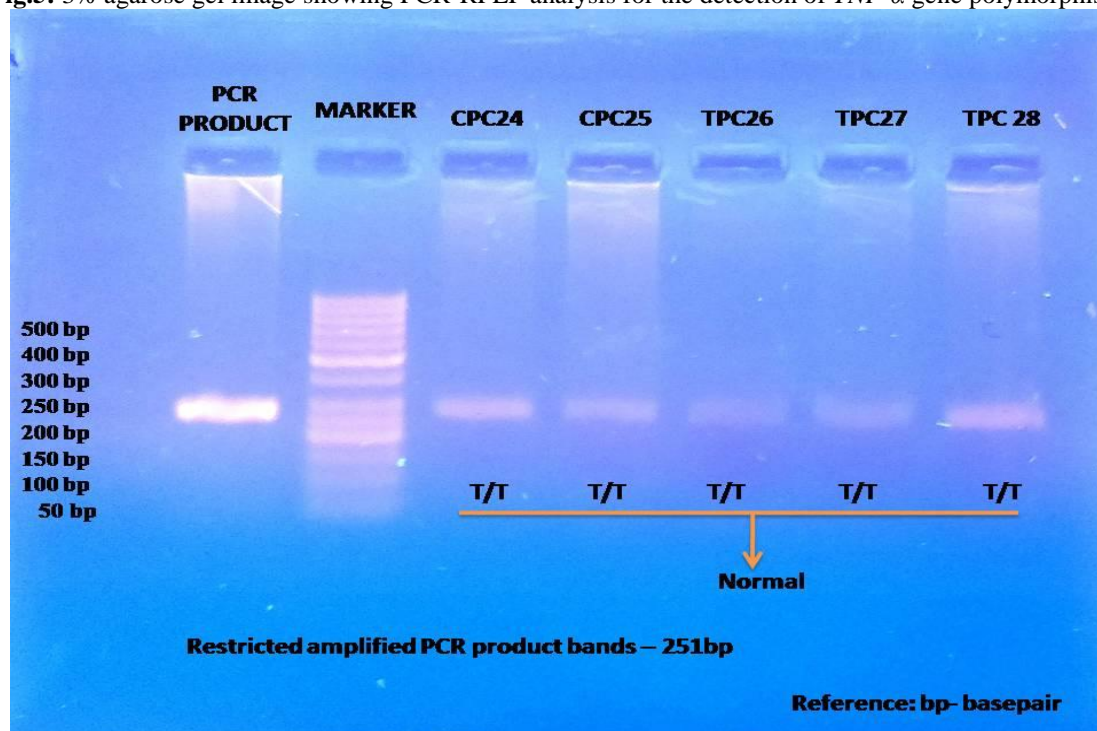


Fig.5: 3% agarose gel image showing PCR-RFLP analysis for the detection of *TNF-α* gene polymorphism



TABLES

Table-1: PCR cycling conditions used in this study

Conditions for Hot-Start PCR reaction		
PCR STEPS	<i>FSHR</i> GENE	<i>TNF-α</i> GENE
	TEMPERATURE/TIME	
Initial denaturation	95°C/ 5 min	95°C/ 5 min
Cycle denaturation	95°C/ 30 sec	95°C/ 30 sec
Annealing	53°C/ 40 sec	63°C/ 40 sec
Cycle extension	72°C/ 45 sec	72°C/ 45 sec
Final extension	72°C/ 5 min	72°C/ 5 min
Hold	4°C/ ∞	4°C/ ∞
No. of cycles	35	35

Table-2: Clinical and endocrine parameters of PCOS subjects and Controls

Parameters	Range observed in Normal subjects	Average Values PCOS subjects
PUBERTY ONSET AGE	12-15 years	13 years
PCOS DETECTED AGE	13-28 years	20.5 years
BMI	18.5-24.5	21.59
FSHR	1.4-9.9mIU/ml	5.44 9mIU/ml
LH	1.7-15.0IU/ml	7.90 mIU/ml
TSH	0.4-4.2 μIU/ml	3.62 μIU/ml
PROLACTIN	3.8-23.2 ng/ml	43.50 ng/ml
SEX HORMONE BINDING GLOBUMIN (SHBG)	20-130 nmol/L	59.14 nmol/L
TESTOSTERONE	15-70 ng/dl	43.50 ng/dl
INSULIN	2.0-23.0μIU/ml	14.26 μIU/ml
BLOOD SUGAR	70-110mg/dl	97.25 mg/dl

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