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Influence of luteinizing hormone receptor gene in the women with PCOS

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ABSTRACT

PCOS – polycystic ovarian syndrome is a reproductive hormonal disorder which is because of the cysts formation in the ovaries after puberty. It is diagnosed by the ultrasound scanning of ovaries. Luteinizing hormone is commonly known as lutropin produced from anterior pituitary which is released by the hypothalamus. The luteinizing hormone receptor gene is responsible for this reproductive syndrome. The main objective of this study is to analyze luteinizing hormone receptor gene in women with PCOS and the case

history of PCOS patients by analysing the biochemical characters of total soluble protein, carbohydrates and cholesterol level with determining the hormonal levels of LH, FSH and PROLACTIN of PCOS patients. The blood from the normal women and the women with PCOS were collected with volunteers concern. The levels of Luteinizing hormone, follicle stimulating hormone and prolactin of the collected samples were analyzed by CLIA method (Chemiluminescene immunoassay). The spectrophotometric quantification of total soluble proteins, carbohydrates, cholesterol showed a increase in its levels in women with PCOS as compared with normal woman. Expression of Luteinizing hormone receptor gene was studied by PCR which showed amplified product for the test samples but which was not expressed in the control sample collected from the normal woman.

Keywords: LH Receptor Gene; PCOS; Gene expression; LH; FSH; Prolactin.

Abbreviations: PCOS - Polycystic Ovary Syndrome; LH - Luteinizing Hormone; FSH - Follicle Stimulating Hormone; LHR - Luteinizing Hormone Receptor gene; BMI - Body Mass Index; IMT - Intimal Medical Thickness; TSH - Thyroid Stimulating Hormone; SHBG - Sex Hormone-Binding Globulin; GnRH - Gonadotropin-Releasing Hormone; HCG - Human Chorionic Gonadotropin; LOS - Laparoscopic techniques of Ovarian Surgery; FAI - Free Androgen Index.

1. INTRODUCTION

PCOS – Polycystic ovary syndrome is a reproductive disorder because of the cysts formation in the ovaries. The increased level of Luteinizing Hormone (LH), Follicle stimulating Hormone (FSH), Prolactin is a main diagnostic cause of this syndrome. The psychological stress of women will be considered as the main symptom of this disorder (Ehrmann et al., 1995). Its main cause is an ovulatory infertility in women of reproductive age (Urbanek et al., 2003). The women with PCOS had higher ovarian volume, Luteinizing Hormone (LH), Follicle stimulating hormone (FSH) ratio, free androgen index (FAI), Concentations of LH, Testosterone, androstenedione, and lower concentrations of FSH and Sex Hormone-binding globulin (SHBG) than the control women.

Luteinizing hormone, commonly known as lutropin is a glycoprotein with two monomeric units, namely alpha and beta subunits, secreted by anterior pituitary gland. The alpha subunit is structurally similar to the Follicle stimulating hormone, Human chorionic gonadotropin, Thyroid stimulating hormone with 92 amino acid in humans and 96 in other vertebrates causing the following disorders such as: Ovarian hyper stimulation, Mammary cancer, Depletion of ovarian follicles and Miscarriage (Mann et al., 1997). The constitutively activating mutation of the LH receptor was identified in a women with gonadotropin independent precocious puberty, had no clinical (or) laboratory evidence of ovarian hyperandrogenism (Rosenthal et al., 1996). Thus increased LH stimulation, even in the presence of FSH, appears to be insufficient to induce the stromal hyperplasia of polycystic ovary syndrome (Ehrmann et al., 1995).

The adrenal gland expresses the LH receptor (LHR) remains controversial. Recently it was shown that human adrenals express the LHR gene in the Zona fasciculate and Zonareticularis. There is also circumstantial evidence of LH effects an adrenal androgen production that begins to increase when reaches the adult levels during puberty without a concomitant increase in ACTH (adrenocorticotrophic hormone). The adrenal gland function and LHR expression using a TG mouse model with chronically elevated serum LH concentration, namely, bLH beta CTP mice were evaluated. The reduced LH secretion includes an ovulation (or) luteal insufficiency, leading to menstrual disorders and infertility (Sundararaman et al., 2003). But the relationship between the variant LH and infertility, including ovulatory disorders, luteal insufficiency, delayed ovulation, hyper prolactinemia and hyperandrogenemia and premature ovarian failure was also observed (Takahashi et al., 2000). The objective of this study is to analyze luteinizing hormone receptor gene in women with PCOS and the case history of PCOS patients by analysing the biochemical characters of total soluble protein , carbohydrates and cholesterol level with determining the hormonal levels of LH,FSH and PROLACTIN of PCOS patients.

2. MATERIALS AND METHODS

2.1. Sample collection

Blood samples were collected from normal healthy and PCOS affected women by venipuncture, which was separated into two aliquots and one aliquot was used for DNA isolation and other was used to separate serum for biochemical analysis. Samples were collected under fasting.

2.2. Biochemical analysis of control and test blood sample

The estimation of total soluble proteins was carried out using the method developed by Bradford. The method is rapid and sensitive using the principle of protein dye binding. 1 ml of blood was collected from both control and PCOS affected women. The blood sample was centrifuged at 800 rpm for 8 minutes. 220 µl of the supernatant from both the control and PCOS samples were mixed with 1. 5ml Bradford's reagent and the absorbance were read at 595 nm. The concentration of protein from the absorbance reading were calculated and compared.

The estimation of total carbohydrates was performed by phenol sulphuric acid method. 30 µl of plasma was extracted from 1 ml of PCOS and control blood samples. To this 150 µl concentrated sulfuric acid and 30 µl 5% (W/V) phenol was added. The contents were mixed well and kept at 90^{o} C in water bath for 5 minutes. The solution was cooled and absorbance was read at 490 nm.

The serum cholesterol estimation method is sensitive and stable for the determination of serum cholesterol by direct treatment of the serum with a reagent composed of ferric chloride dissolved in a glacial acetic acid-sulphuric acid mixture. Ferric chloride stock solution was prepared by dissolving 60g FeCl2 in 100ml glacial acetic acid. 10 ml stock made up to 100ml with glacial acetic acid and was used as precipitating agent. Diluting agent was prepared by making up 8. 5 ml stock to 100ml with glacial acetic acid. One ml of blood collected from control and test sample was centrifuged at 2000 rpm for 6 minutes and 25 µl serum was collected. 220 µl of precipitating agent was added to both the samples. The contents were mixed well and centrifuged. To the supernatant equal volume of diluting agent and concentrated sulfuric acid was added and mixed well. The absorbance was read at 560 nm.

3. HORMONAL ASSAYS

3.1. Luteinizing hormone (LH), Follicle stimulating hormone (FSH), Prolactin

Table 1 Case History of Normal and PCOS Affected Women

PROFILE	CONTROL	PCOS(1)	PCOS(2)	PCOS(3)
AGE	21	28	24	23
WEIGHT	48kg	56kg	50kg	50kg
HAEMOGLOBIN LEVEL	13.5 gms/dl	13.8 gms/dl	13.6 gms/dl	13.4gms/dl
LOCATION	Coastal area	Coastal area	Coastal area	Coastal area
DIET	Non-veg	Non-veg	Non-veg	Non-veg
OTHER PROBLEMS	-	Hirsutism	Psychological stress	Psychological stress
MENSTRUAL CYCLE	Regular	10-15days	10-20days	Irregular

Luteinizing hormone is produced from the anterior pituitary in response to luteinizing hormone-releasing hormone (LH-RH or Gn-RH), which is released by the hypothalamus. The Luteinizing hormone level was determined by Chemiluminescence Immunoassay (CLIA) method. Chemiluminescence Immunoassay (CLIA) detection using Microplate lumino meter provides a sensitive, high throughput, and economical alternative to conventional colorimetric methodologies, such as Enzyme-linked immunosorbent assays (ELISA). FSH is a glycoprotein secreted by the basophilic cells of the anterior pituitary. Gonadotropin release hormone (GnRH), produced in the hypothalamus, controls the release of FSH from anterior pituitary. Human prolactin (lactogenic hormone) is secreted from the anterior pituitary gland in both men and woman. Human prolactin is a single chain polypeptide hormone with a molecular weight of approximately 23,000 daltons.

3.2. Extraction of genomic DNA from whole blood

1 ml of normal blood sample (control) and PCOS affected blood sample were collected in a separate microfuge tubes. Then 500µl of 70% ethanol was added to both the tubes and vortexed for 1 minute to mix well. Tubes were centrifuged at 10,000 rpm for 8 minutes at 37^{o}C. Pellets were collected and 1 ml of Tris buffer-I (RBC lysis buffer) was added and spun at 2000 rpm for 5minutes at 37^{o}C. The pellets were collected and washed again with 1ml of Tris buffer-I to remove the contamination of RBC and spun it for 2000 rpm for 5 minutes. To that 220 µl of Tris buffer-II (proteinase-K buffer) was added and incubated at 15^{o}C for 15 minutes to degrade the proteins. Meanwhile 100 µl of NaCl was prepared and added to the microfuge tubes after incubation. The tubes were then centrifuged at 8,000 rpm for 8 minutes at 15^{o}C. To the supernatant 1 ml of ethanol is added and centrifuged at 8,000 rpm for 5

minutes at 37°C. pellets were collected and washed with 70% ethanol to precipitate DNA. It was then dried and saturated with TE buffer for nanovue quantification.

3.3. Primer designing

The primer LHR gene used in the PCR is:
Left Primer: TGTGCTTTCACATTGTTTGGAAAAGT.
Right Primer: CTGAAGTCCAAAAGCTCAAATGCT.

3.4. Amplification of DNA:

Polymerase chain reaction (PCR) was carried out for the DNA extracted from the blood samples (control and tests) was assayed by PCR amplification of LHR gene. Amplification of samples was done by preparing the reaction mixture comprising 2 µl of the DNA sample, 2 µl of primer(1 µl of left primer, 1 µl of right primer), 10 µl of PCR super mix (Medox pcr mix) and 26 µl of Nuclease free water. And the PCR program as,

Step1: 95°C for 4minutes.
Step2: 95°C for 1minute.
Step3: 60°C for 1. 30 minute.
Step4: 72°C for 2 minutes.
Step5: Go to step2 for 30 cycles.
Step6: 72°C for 10 minutes.
Step7: End

Then the PCR products were analysed by running the sample in 2% agarose gel electrophoresis. The gel was then viewed under UV transilluminator.

4. RESULTS AND DISCUSSION

4.1. Case study

The present study compares that the women with PCOS having a menstrual cycle between 10-15 days ,10-20days and irregular mensus with a sign of symptom hirsutism and a psychological stress in the south Indian women. In addition, to this information women with PCOS having the signs of acne, mental distress, infertility, infrequent menstrual periods, excess body hair, hormonal imbalance (Table 1). The ultrasound scanning reports also proves that they are having a profile of PCOS.

4.2. Biochemical analysis

The spectrophotometric quantification of total soluble protein shows that increased level in PCOS when compared to the normal and it may be the intake of protein rich seafood. But the carbohydrates level was raised in the control but it was not significant to PCOS. Similarly the cholesterol level also increased in PCOS compared to normal because the seafood contains rich fat (Table 2 and Fig. 1).

Table 2 Biochemical parameters of Control and PCOS Affected women

S. NO	BIOCHEMICAL ANALYSIS	CONTROL	PCOS 1	PCOS 2	PCOS 3
1.	TOTAL SOLUBLE PROTEIN	0. 100 µg/ml	1. 108 µg/ml	1.114 µg/ml	1.322 µg/ml
2.	CARBOHYDRATES	0. 550 µg/ml	0. 244 µg/ml	0.206 µg/ml	0.374 µg/ml
3.	CHOLESTEROL	0. 107 µg/ml	0. 358 µg/ml	0.269 µg/ml	0.555 µg/ml

Table 3 Hormonal Assay

HORMONAL	CONTROL	PCOS 1	PCOS 2	PCOS 3
LH	10. 15 mIu/dl	22. 40 mIu/dl	15. 34 mIu/dl	22. 38 mIu/dl
FSH	10. 33 mIu/dl	5. 44 mIu/dl	1. 71 mIu/dl	4. 80 mIu/dl
PROLACTIN	18. 52ng/nl	15. 29ng/nl	16. 92ng/nl	20. 14ng/nl

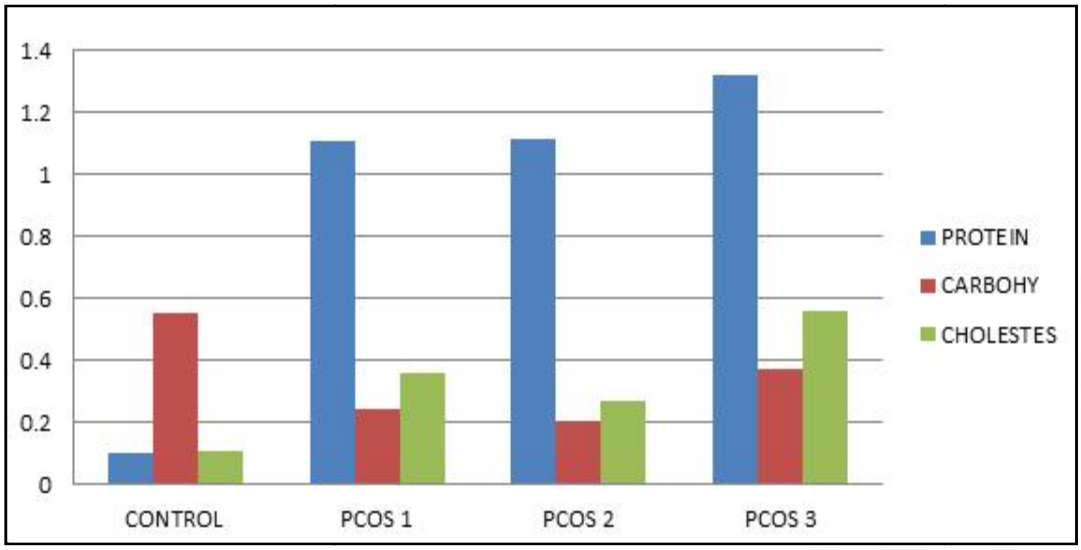

Figure 1 Biochemical analysis of control and PCOS sample

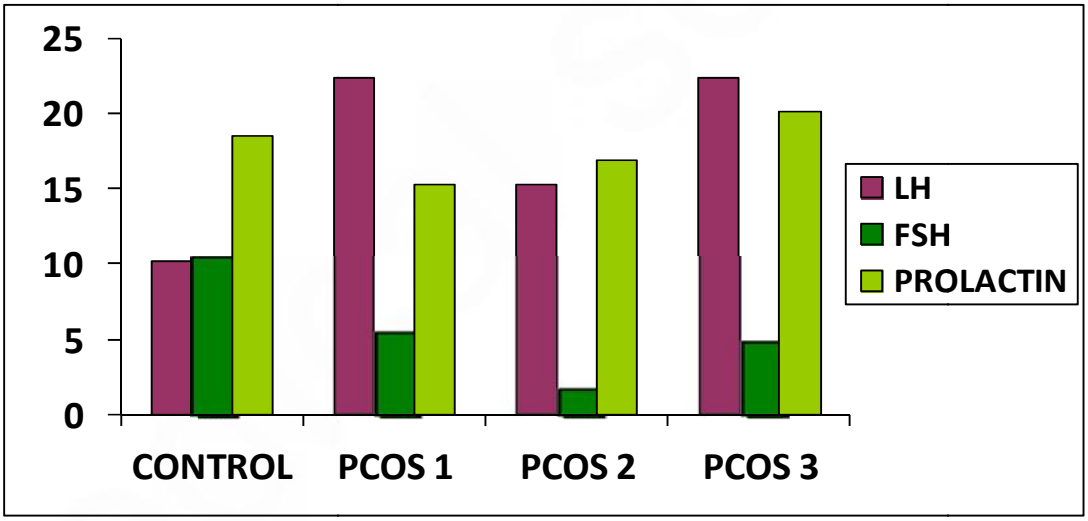

Figure 2 Hormonal assay of control and test samples

4.3. Hormonal assay of LH, FSH and prolactin

The hormonal assay shows that the increased level of Luteinizing hormone in PCOS compared with the normal. Because it is a major hormone which stimulates the non- empty follicles to develop into the corpus luteum, which secretes progesterone during the latter half of the menstrual cycle (Table 3 and Fig. 2). The figure also explains that the LH level of Test-1 and the Test-3 is not having much difference by showing the increased concentration level compared to normal. This increased level is significantly showing that the signal to LH receptor gene is directly involved.

4.4. DNA in agarose gel electrophoresis

The electrophoresis of isolated DNA shows a clear pink-violet band formation when compared with the marker. The pink-violet band is because of metachromatic effect. Proteomic approaches of this specific band will provide the insight of molecular nature and functional role in PCOS affected women (Fig. 3).

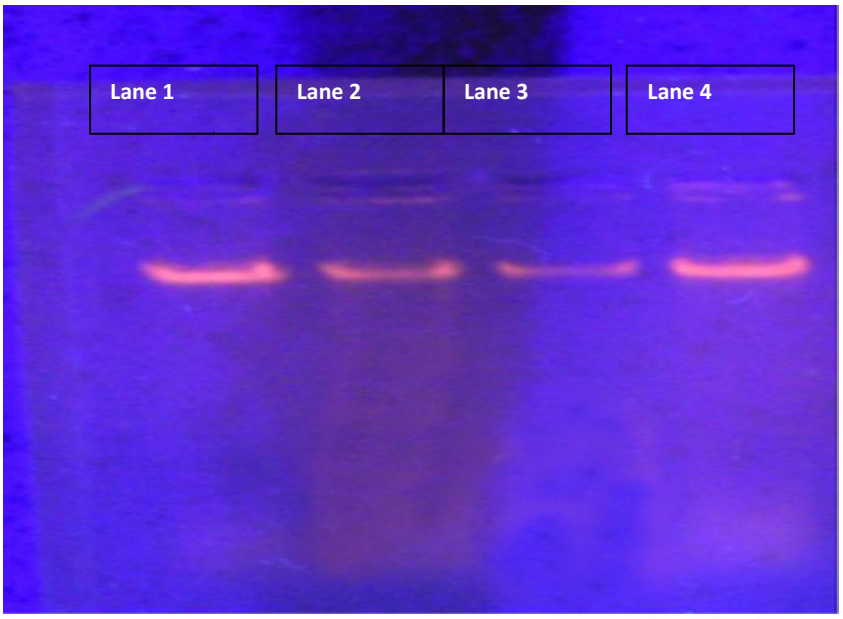

Figure 3 DNA in agarose gel electrophoresis, Lane 1 – DNA from Control sample, Lane 2, 3 and 4 - DNA from Test samples

4.5. Expression of luteinizing hormone receptor gene

The hormone may be ovary-dependent factor that enhances the LHR expression by elevated LH. The high circulating LH concentrations together with a factor (s) associated with the dysfunctional TG ovaries with a polycystic phenotype (possibly estrogen-stimulated PRL secretion), induce LHR expression in female. Expression of luteinizing hormone receptor gene was observed in the women with PCOS but not expressed in normal (Fig. 4). Because the luteinizing hormone level was increased in the blood serum which was proved by hormonal assays (Fig. 2).

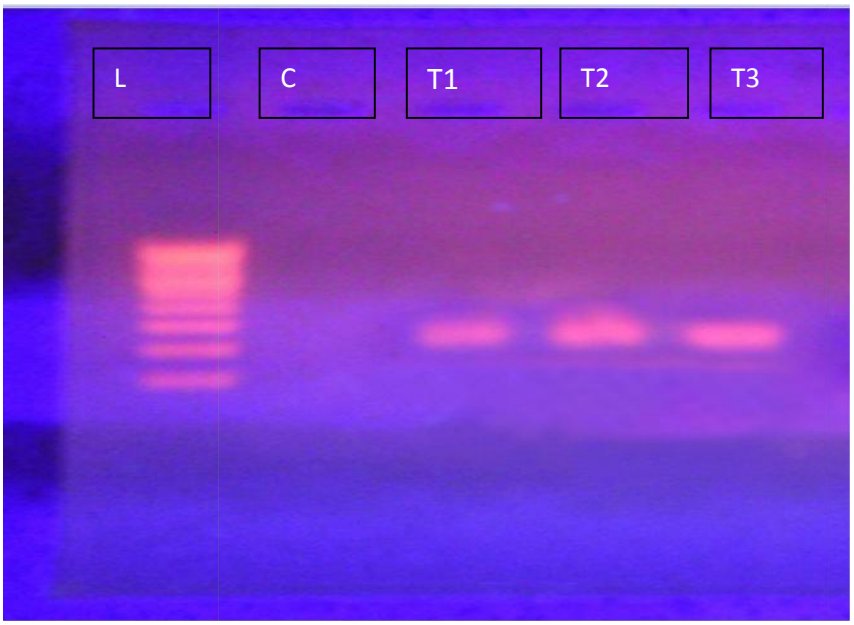

Figure 4 Amplified Product of DNA in Agarose Gel Electrophoresis, L – Ladder, C- Control, T1, 2 and 3 – Test samples

The present study made an attempt that the Luteinizing hormonal level was increased in the blood serum of women with PCOS. The amplified DNA shows the expression of Luteinizing hormone receptor gene, because the receptors are major factor to express

this gene in women with PCOS. The Luteinizing hormone receptor genes are preferred because many investigations are only concentrated with Follicle stimulating hormone compared with LHR genes.

5. CONCLUSION

In the present study, PCOS blood was collected from Manoj clinic with the help of Gynaecologist and normal blood from volunteers concern. In this study we analysed the influence of Luteinizing Hormone Receptor Gene in PCOS and normal woman .In this work we undergone the case study of normal and PCOS women for identifying the LHR gene. The results revealed that an increase in Luteinizing hormonal level is a precautious signal for PCOS.

SUMMARY OF RESEARCH

- This study has done to analyse the influence of LHR gene which cause PCOS.
- The biochemical analysis of total soluble protein, carbohydrates, cholesterol shows that the significant increase in the cholesterol level in PCOS women when compared to the normal .This may be because of the more intake of sea food in the coastal area.
- The increased level of cholesterol and the expression of LHR gene confirm this syndrome.
- The hormonal assays also revealed the increased level of LH is a factor of PCOS.
- Reduction in carbohydrate level indicates that the impairment of carbohydrate synthetic machinery and this recommends more intake of carbohydrate rich food in the daily dietary menu of PCOS patients and reduce more sea food intake to control the cholesterol level.

FUTURE ISSUES

- Whether LHR gene is only more prominent to this PCOS.
- Further confirmation studies of restricted site in PCOS patients and LHR gene was continued with RFLP to obtain more insights.
- Polymorphism of restricted sites and the gene mapping can be done.

DISCLOSURE STATEMENT

There is no financial support for the proposed research work.

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