ROLE OF GNRHR GENE RS4986942 SNP WITH POLYCYSTIC OVARIAN SYNDROME AMONG IRAQI WOMEN

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ABSTRACT

Polycystic Ovarian Syndrome (PCOS) is a common endocrine disorder in women during their reproductive age. This study was designed to estimate the variations among biochemical parameters in women with PCOS and correlation with GNRHR polymorphism that may play a vital role in this syndrome. This study included 60 Iraqi women with PCOS. Patients were selected from Kamal al Samarrai Hospital in Baghdad City, Women's Hospital in Kerbala City and Infertility at AlSadr Hospital in Najaf City, all were selected based on the Rotterdam criteria and the healthy control group consists of 30 healthy women of different ages. Fertility hormone measurements showed that the level of the Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) were significant differences in Polycystic ovary syndrome (PCOS) patients when compared to the control, while the mean average of Gonadotropin-releasing hormone (GnRH) and The hormone dehydroepiandrosterone (DHEA) in Polycystic ovary syndrome (PCOS) patients hyper than the control. Molecular analysis of whole GNRHR gene amplified using specific primers showed one known SNPs (rs4986942) was detected in the analyzed population in the investigated exon 1 of the GNRHR gene. The heterozygous (CT) form of the rs4986942 SNP is highly associated with the development of PCOS in the investigated population.

Key words: Polycystic ovary syndrome, gonadotropin-releasing hormone receptor, ELISA, PC R, novel mutation

I. INTRODUCTION

Polycystic ovary syndrome (PCOS) may be a common endocrine disorder in women, particularly in women of procreative age. The prevalence of PCOS was calculable to be 5–10%. The worldwide be diagnosed by physiological condition, acne, amenorrhea, oligomenorrhea, hirsutism, endocrine resistance, obesity, hyperandrogenism, and polycystic ovaries by ultrasonography. Association of PCOS with infertility is well studied and is thought to be answerable for 40% of female infertility. Moreover, it is a number one explanation for carcinoma [1]. Besides reproductive abnormalities, PCOS is additionally powerfully related to a good vary of metabolic disorders, resembling viscus steatosis, aldohexose intolerance, dyslipidemia, polygenic disorder mellitus sort II (T2DM), and cardiovascular disease [2].

The endocrine and organic chemistry disorders, genetic and environmental issue is answerable for the etiology of this condition. Unhealthy lifestyle, diet or any infectious mediators increase the danger of PCOS [3].

There are several hypotheses concerning the pathophysiology of PCOS include; proof against rupture of follicles thanks to shell thickness, gonad hyperandrogenism, luteinizing secretion(LH) hyper secretion, hyper insulinemia, and impaired ovarian cyst development due to inflated follicular development blocker paracrine factors, resembling anti-mullerian hormone (AMH), concerning biochemical disorders the amount of gonadotropin-releasing secretion (GNRH), cyst stimulating hormone (FSH), luteinizing hormone (LH), Dehydroepiandrosterone (DHEA) and lactogenic hormone is additionally disturbed just in case of PCOS. In addition, steroid sequence enzymes are answerable for the synthesis of varied steroid hormones (The steroid hormones regulate several physiological processes, as well as the event and performance of the procreative system), including glucocorticoids, mineralocorticoids, progestin, androgens and estrogens. They incorporates
many specific hemoprotein P450 enzymes (CYPs), group steroid dehydrogenases (HSDs), and steroid reeducates [4].

The genetic factors that are responsible for the etiology of PCOS. Its cause involves candidate factors, SNP’s. In keeping with databases, PCOS etiology involves 241 gene variations [5]. Polymorphism or any ester modification cause a defect within the transcriptional activity of a gene [15]. During this review, we tend to aimed to estimate the variations among organic chemistry parameters in women with PCOS and correlation with GNRHR polymorphism. The human GNRHR (gonadotropin-releasing secretion receptor) gene spans 18.7 kilobyte of genomic sequence on body 4q13.2 and consists of three exons GNRHR gene was the primary gene found to cause chromosome recessive norm osmic upset hypogonadotropic incompetence (IHH) [4]. The GNRHR macromolecule may be a G-protein coupled receptor (GPCR) expressed on the cell surface of pituitary gonadotropins, This GPCR interacts with pulsatile GnRH to initiate the secretion of gonadotropin and LH that stimulate the testes or ovaries to provide sex steroids, and gametes.

II. MATERIALS AND METHODS

Sample collection: The study included sixty blood samples from women suffering Poly Cystic Ovary Syndrome (PCOS) during the period "October 2020 to June 2021" at AlQasim Green University / College of Biotechnology for Graduate Studies. collected from from Kamal al Samarrai Hospital in Baghdad City , Women's Hospital in Kerbala City and Infertility at AlSadr Hospital in Najaf City and thirty blood samples from healthy women served as control.

2.1. Measurement of fertility hormones

PCOS patient went through routine examinations, which included a detailed history, physical and pelvic examination, ultrasound, and other laboratory studies as deemed necessary by the attending gynecologists. In addition, blood samples were obtained for hormonal studies and DNA analysis, serum concentrations of follicle stimulating hormone (FSH), luteinizing hormone (LH), Gonadotropin-releasing hormone (GnRH) and The hormone dehydroepiandrosterone (DHEA), were measured with Enzyme-linked Immunosorbbent Assay (ELISA, Bioteck ELx808 Manual 12 mode l0, 1997, Germany) Subjects in the control group were all selected according to the following criteria; Menstrual cycle (26 to 30 days), Normal age 15 to 35 years, No history of endocrine disorders, No medication or oral contraceptive use, Blood samples were taken during the follicular phase (3, 4 or 5 days).

2.2. DNA extraction

Molecular methods performed in Al-Qasim Green University, College of Biotechnology. DNA samples were extracted from blood using HIGENOMB Kit according to manufacturer’s instructions (using a mammalian genomic DNA extraction kit (HIMEDIA, India). Confirms DNA integrity by using standard 0.8% (w/v) agarose gel electrophoresis pre-stained with a higher concentration of SYBR safe staining in TAE buffer (Trisacetate 40 mM; 2 mM EDTA, pH 8.3) a 1 kb ladder as a molecular weight marker (iNtrON Biotechnology, South Korea). The isolated DNA was used as a template to amplify a desired gene region.

2.3 PCR protocols

A conventional PCR technique was used to amplify the specific regions of GNRHR gene, which is exon 1. using specific primers as shown in Table (1) to amplify GMRHR gene using the following program Initial denaturation at 94 °C for 5 min., 30 cycle of denaturation at 94 °C for 1 min, Annealing at 58 °C for primers for 1 min, extension at 72 °C for 1 min., and final extension at 72 °C for 10 min.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primercode</th>
<th>Sequence description (5’-3’)</th>
<th>AmpliconSize bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>GNRHR 10F</td>
<td>TGTATAATGGCTTACCTGTGGTCC</td>
<td>582</td>
<td></td>
</tr>
<tr>
<td>10R</td>
<td>AGATGCACCAGAGACACAAGG</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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2.4 DNA sequencing
The resolved PCR amplicons were commercially sequenced from both directions, forwards and backwards, according to the instructions of the sequencing company (Macrogen Inc. Geumchen, Seoul, South Korea). Only the sequences were analyzed further. Clear chromatographs of ABI (Applied Biosystem) sequence files to ensure annotations and variations are not due to PCR or sequencing artifacts. By comparing the observed DNA sequences of local samples with the obtained DNA sequences, the virtual positions and other details of the obtained PCR fragments were identified. The results of the sequencing of the PCR products of different samples were edited, aligned and analyzed with the corresponding sequences in the reference database with the software BioEdit Sequence Alignment Editor Version 7.1 (DNASTAR, Madison, WI, USA). The sequenced samples were numbered in PCR amplicons and at their corresponding position within the reference. Each variant recognized within the examined gene was annotated by SnapGene Viewer ver. 4.0.4 (https://www.snapgene.com).

2.4 Statistical analysis
The statistical analysis of the data was carried out with SAS (Statistical Analysis System Version 9.1). A one-way ANOVA and a post hoc Least Significant Differences Test (LSD) were performed to assess significant differences between the means. Determine whether the population is in Hardy-Weinberg equilibrium. P < 0.05 is considered to be statistically significant.

III. RESULT
The present study included 90 subjects divided into two groups, 60 of Iraqi patients with PCOS and 30 normal healthy controls. Blood samples were collected from both groups for biochemical examinations, and molecular analysis. The statistical analysis of fertility hormone measurements showed that the level of the Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) were significant differences in Polycystic ovary syndrome (PCOS) patients when compared to the control, while the mean average of Gonadotropin-releasing hormone (GnRH) and The hormone dehydroepiandrosterone (DHEA) in Polycystic ovary syndrome (PCOS) patients hyper than the control as shown in table (1).

<table>
<thead>
<tr>
<th>Group</th>
<th>LH</th>
<th>FSH</th>
<th>GNRH</th>
<th>DHEA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>12.97±1.79</td>
<td>5.09±0.51</td>
<td>416.14±124.54</td>
<td>191.14±47.10</td>
</tr>
<tr>
<td>Control</td>
<td>6.62±0.51</td>
<td>11.80±4.97</td>
<td>38.56 ± 0.92</td>
<td>267.82±103.83</td>
</tr>
<tr>
<td>P-value</td>
<td>0.001*</td>
<td>0.02*</td>
<td>0.34**</td>
<td>0.46**</td>
</tr>
</tbody>
</table>

The DNA samples from patients were subjected to molecular analysis after PCR amplification using specific primers of the GNRHR gene and DNA sequencing. The primer amplify exon (1) giving amplicon.
size (582 bp) as shown in Fig 1 methods.

Figure 1: PCR product of amplified 582 bp fragment of exon 1 of GNRHR gene. The PCR product obtained with samples from healthy individuals (lanes order 1-5) and PCR product from PCOS patient samples (lanes 5-13) and (M lane is a 100bp ladder). The PCR product was electrophoresis by a standard 1.5% (w/v) agarose gel that is pre-stained with red safe nucleic acid staining solution (0.7 μg/ml) in TAE buffer, using a 100bp ladder as a molecular weight marker at 70 volts for one and half hour.

DNA sequences of GNRHR gene obtained from patients were subjected to analysis. We detected the presence of genetic change in several main locations at GNRHR gene in individuals with PCOS specifically on exon 1. Total genetic variation is listed in Table 3 and represented in Fig. 2.

DNA sequences of GNRHR gene obtained from patients were subjected to analysis. We detected the presence of genetic change in several main locations at GNRHR gene in individuals with PCOS specifically on exon 1. Total genetic variation is listed in Table 3 and represented in Fig. 2.

![PCR product of amplified 582 bp fragment of exon 1 of GNRHR gene.](image)

Table 3. The patterns of the observed SNP in the 582 bp amplicons that are designed to amplify the exon 1 within the GNRHR gene in comparison with the NCBI referring sequences (GenBank acc. no. NC_000004.12). The symbol “C” refers to the control samples, and the symbol “P” refers to the sample number.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Variation</th>
<th>Zygosity status</th>
<th>Position in the PCR fragment</th>
<th>Position in the reference genome</th>
<th>Amino acid position</th>
<th>SNP type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>S</td>
<td>N</td>
<td>100 bp</td>
<td>300 bp</td>
<td>100</td>
<td>S</td>
</tr>
<tr>
<td>2</td>
<td>N</td>
<td>P</td>
<td>200 bp</td>
<td>400 bp</td>
<td>200</td>
<td>N</td>
</tr>
<tr>
<td>3</td>
<td>P</td>
<td>P</td>
<td>300 bp</td>
<td>600 bp</td>
<td>300</td>
<td>P</td>
</tr>
<tr>
<td>4</td>
<td>P</td>
<td>P</td>
<td>400 bp</td>
<td>800 bp</td>
<td>400</td>
<td>P</td>
</tr>
<tr>
<td>5</td>
<td>S</td>
<td>N</td>
<td>500 bp</td>
<td>1000 bp</td>
<td>500</td>
<td>S</td>
</tr>
</tbody>
</table>

![Fig. 2. The SNP’s rs4986942 checking of GNRHR genetic single nucleotides polymorphisms using the dbSNP server.](image)
IV. DISCUSSION

Polycystic Ovarian Syndrome (PCOS) is the most common endocrinopathy in women of childbearing age and causes not only reproductive but also metabolic abnormalities. Women with PCOS have ovulatory dysfunction, abnormal hormones, hyperandrogenemia, obesity, and hyperinsulinemia [6]. It is a heterogeneous disorder. Which results from the interaction of several genes together with environmental factors. A single gene [14]. The familial accumulation of PCOS cases suggests that genetic factors play an important role in the etiology of PCOS. Although familial case studies of PCOS have produced results that suggest an autosomal dominant trait, the mode of inheritance is not well established [13]. There are many reasons for that. First, genetic heterogeneity makes genetic studies of PCOS difficult to conduct. Second, several avenues are involved in the etiology of PCOS; therefore, several candidate genes may be responsible for this "complex" genetic trait, making it difficult to identify each gene involved. Until recently, the approach to understanding the molecular basis of this complex syndrome has been to study the functions of individual genes. Endocrine and genetic studies of the polymorphisms of the GNRHR gene showed the association with different serum FSH levels and with normal levels and expression of GNRHR transcripts. The results indicated that the heterozygous form (CT) of SNP rs4986942 strongly related to development is associated with SOP. in the population studied and the heterozygous form (AT) of the new SNP A355T tended to be associated with the development of PCOS in the same population studied. These results were consistent with Chen and collagenase. (2017). BMI, LH, LH / FSH and testosterone levels in serum. Roux N and collagenase [10] showed that the patient carries a compound heterozygous mutation of GNRHR p. (Gln106Arg) and p. (Phe309del) demonstrated spontaneous t-breasts, primary amenorrhea, low estradiol levels with low normal gonadotropin levels, and a normal pubertal LH response to GnRH stimulation. Its clinical presentation is consistent with a study showing that p. (Gln106Arg) reduces the binding of GnRH to the receptor, which leads to a partial loss of function. Previous studies on Cai and collagenase [11] have shown that the mutation in the GNRHR that causes abnormalities in the hypothalamic-pituitary or adrenal axis was introduced into the pathophysiology of polycystic ovarian disease. The gonadotropin-releasing hormone (GnRH) leads to a relative increase in the release of LH to FSH. Ovarian estrogen is responsible for creating an abnormal feedback mechanism that causes an increase in the release of LH and FSH. In another study, Francou et al. [12], showed that the functional analysis of mutations in the human GnRH receptor (GnRHR) gene, stored demon, resulted in a statistically significantly higher mean FSH / LH ratio. the relative increase in the release of LH to FSH.

V. CONCLUSIONS

Polycystic ovaries syndrome can be considered as a complex syndrome triggered by the interact effect of genetic factors.

The statistical analysis of fertility hormone measurements showed that the level of the Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) were significant differences in Polycystic ovary syndrome (PCOS) patients when compared to the control, while the mean average of Gonadotropin-releasing hormone (GnRH) and The hormone dehydroepiandrosterone (DHEA) in Polycystic ovary syndrome (PCOS) patients hyper than the control, when Polycystic ovary syndrome (PCOS) showed a high elevation Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH Gonadotropin-releasing hormone (GnRH) and The hormone dehydroepiandrosterone (DHEA). One known SNP (rs4986942) was detected in the analyzed population in the investigated exon 1 of the GNRHR gene.
The heterozygous (CT) form of the rs4986942 SNP is highly associated with the development of PCOS in the investigated population, which entails the importance of this SNP to be used as a potential marker for this disease.

REFERENCES