CHAPTER V

Luteinizing hormone beta ($LH\beta$) gene SNPs and its association with PCOS
5.0. Introduction

Luteinizing hormone (LH), a heterodimeric glycoprotein hormone secreted from anterior pituitary gonadotropes and acts primarily at ovary to regulate ovarian folliculogenesis and luteinization. It is made up of two peptides (α and β) and have structural similarities with FSH and TSH; however its bioactivity is conferred by the LH beta (LHβ) subunit gene. LHβ originates from an ancestral cluster genes sharing >98% sequence similarity with human chorianic gonadotropin genes (hCGB) and other pseudogenes located on human chromosome 19. The LH performs its biological functions by binding with its receptor expressed on the granulosa and luteal cells of the ovary. Although LH-receptors are expressed on human follicles already from the start of the cycle, the synergizing effect of LH and FSH appears to be most prominent from the mid-follicular phase and onwards. In addition to follicular growth, LH stimulates the androgen secretion by ovarian theca cells [1, 2].

Abnormal endocrine profile in specific altered LH:FSH ratio (>1.0) is consistently seen in PCOS women [3,4,5]. High serum levels of LH are found to be associated with increased ovarian androgen production, hyperinsulinemia, and polycystic ovaries, as a result of arrested follicular development in PCOS women [6, 7, 8]. Even though LH levels are correlated with reproductive and metabolic phenotypes, role of LHβ variants is unclear in the pathogenesis of reproductive disorders. However LHβ variations are found to be associated with infertility and premature ovarian failure in Japanese women [9, 10, 11] and slow down the progression of puberty in boys [12].

In light of the clinical significance of LH in ovarian steroid synthesis and ovarian folliculogenesis, reproductive physiology, the role of LHβ SNPs rs1800447 (T/C)
(Exon 2; Trp28Arg), rs34349826 (T/C) (Exon 2; Ile35Thr) and SNP rs5030774 (G/A) (exon 3; Gly122Ser) were examined in the study subjects.

5.1. Methodology

The study population consists of PCOS women (n=97) and healthy women (n=101) with normal reproductive physiology were recruited in the study by adopting the criteria as explained in the chapter 2.1. & 2.2. About 3mL of intravenous blood sample was collected from the study subjects and DNA was extracted as phenol chloroform method as mentioned in the methodology section 2.5.1. The PCR reactions were performed for SNPs rs1800447, rs34349826 and rs5030774 as explained in methodology section 2.5.6. SNP genotyping was performed by RFLP using the restriction endonucleases NcoI, BseGI and EcoOI09I respectively as described in the methodology section 2.5.8. Confirmation of PCR-RFLP results were carried out by DNA sequencing of PCR products (section 2.5.9). Suitable statistical tools were applied to draw inference as explained in the methodology section 2.5.10.

5.2. Results

Genotyping of the LHβ SNPs rs1800447, rs34349826 and rs5030774 was done by PCR-RFLP. PCR-RFLP showed the presence of only homozygous wild genotypes in LHβ SNP [Figure 2.3. A, B and C]. The present study heterozygous and homozygous variants are completely absent in both PCOS and control subjects Table 5.1; (Figure 5.1). All the reactions were repeated to confirm the genotypes obtained.
Table 5.1. The allele and genotype frequencies of the \( LH\beta \) SNP in the study subjects

<table>
<thead>
<tr>
<th>SNP</th>
<th>Subjects</th>
<th>Genotypes (n)</th>
<th>Minor allele</th>
<th>Major allele</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C/C</td>
<td>C/T</td>
<td>T/T</td>
</tr>
<tr>
<td>rs1800447</td>
<td>PCOS (97)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>97 (100)</td>
</tr>
<tr>
<td></td>
<td>Controls (101)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>97 (100)</td>
</tr>
<tr>
<td>rs34349826</td>
<td>PCOS (97)</td>
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<td>0 (0)</td>
<td>97 (100)</td>
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<tr>
<td>rs5030774</td>
<td>PCOS (97)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>97 (100)</td>
</tr>
<tr>
<td></td>
<td>Controls (101)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>97 (100)</td>
</tr>
</tbody>
</table>

Values within the parentheses represent the percentage of genotype.

Figure 5.1 Genotype distribution of \( LH\beta \) SNPs in PCOS and controls

The homozygous wild allele only present in both the cases and controls. Concordant results obtained by PCR-RFLP were further confirmed by DNA sequencing (Figure 2.8, 2.9 and 2.10).

5.3. Discussion
Genetic variants in \( LH\beta \) are found to be associated with impaired reproductive functions and can lead to infertility in both sexes. In the present study, PCR-RFLP analysis of \( LH\beta \) SNPs showed the presence of only homozygous wild type genotypes such as T/T for rs1800447, T/T for rs34349826 and G/G for SNPs rs5030774 in both PCOS and control subjects. SNP rs1800447 and rs34349826 exists in complete linkage and their frequency distribution varies among different ethnic populations ranging from 0% in Kota tribe from South India to 54% in Australian aboriginals [13].

Dasgupta et al. [14], conducted a large scale study in Indian women comprising PCOS (n=250) and controls (n=299), reported a prevalence of SNP rs1800447/ rs34349826 less than 2% and complete absence of SNP rs5030774 in PCOS and controls. Similarly, the other SNP rs5030774 was reported only in Chinese woman in Singapore, however did not found in Indian and Malaysian population [15], Korean women [16], Finland, Denmark, Bengali/North-East India and Rwanda population [17].

The variant genotypes of SNP rs1800447/ rs34349826 are proposed as an ancestral allele found to have increased serum bioactivity and shorter half life, however, found to have more potent action at the receptor level compared to wild-type genotypes [18]. SNP rs1800447/rs34349826 has high sensitivity towards GnRH stimulation in comparison to homozygotes [19]. Studies on hormonal levels in Brazilian PCOS women showed an association of variant genotypes with increased testosterone levels [20]. In controlled ovarian stimulation, women with \( LH\beta \) SNP rs1800447/rs34349826 variant genotypes are found to be hyposensitive for exogenous FSH supplementation [21]. Association of variant \( LH\beta \) with female infertility has not been established.
clearly, however, an association with reproductive disorders in Japanese women was widely reported [9, 10, 11]. On the other hand, there is evidence that \( LH_\beta \) SNP rs1800447/rs34349826 protects obese women from developing symptomatic polycystic ovarian syndrome [8], however Indian women did not show any effect between obese and non-obese PCOS [14]. SNP rs1800447/rs34349826 was found to be associated with higher testosterone levels in developing hyperandrogenism in PCOS in Brazilian women [20].

Functional studies on SNP rs5030774 in HEK293 cell lines did not show any affect on biological activity and receptor binding properties [17]. SNP rs5030774 found to be associated with endometriosis in Japanese [11], European [22] and Brazilian women with PCOS [23]. Biochemical studies showed an association between SNP rs5030774 A/A genotype low levels of LH and higher fasting glucose levels in PCOS women [24].

Though excess serum LH levels are involved in the androgen synthesis and recruitment of primordial follicles from the growing follicles in ovarian folliculogenesis, a key etiological factor in the pathogenesis of PCOS, the study results did not find any prevalence of \( LH_\beta \) variants. Concordant results obtained by RFLP and then with direct sequencing confirm the genotypes were not an artifact due to experiment and data analysis.

References

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