INTRODUCTION

Numerous females of reproductive age suffer from PCOS, characterized by formation of cysts on the ovaries ranging in size from 2 to 10 mm in antral measurement and posing major public health concerns. PCOS is a symptom of the underlying problems, not a cause. Symptoms include a decrease or increase in menstrual flow (oligomenorrhea or menorrhagia, respectively), increased growth of body hair, thin and patchy skin, acne, and hyperandrogenism 1.

PCOS is a notorious reproductive and metabolic aberration occurring in the cycling ladies. The indicators are pronounced by hyperandrogenism, hindered folliculogenesis and cystic ovaries. It is estimated that between 15 and 20% of the female population worldwide has PCOS 2. It increased the risk of infertility, recurrent and spontaneous abortion, preterm birth and endometrial cancer. PCOS is also firmly connected to massive amounts of metabolic illnesses like hyperlipidemia, hepatic steatosis, hypertension, diabetes mellitus, preeclampsia, gestational diabetes, insulin resistance and glucose intolerance 3. The malfunction of hormones and hormonal receptors in women and mutation in critical hormonal genes like androgen or estrogen receptors, has garnered increasing interest 4,5.

Estrogen affects ovarian folliculogenesis and ovulation by binding to the nuclear estrogen receptor α (ER-α) and estrogen receptor β (ER-β), pertaining to class 1 and influence cellular proliferation, differentiation, and death. In addition, ESR subtypes were designated as ESR1, ESR2 and encoding ER-α, ER-β, have been discovered recently. The locus of ESR1 gene is on chromosome 6q25.1, which is composed of 595 amino acids, 140 kDa of DNA and 8 exons, and features highly conserved introns. The ESR2 gene resides in the 14q23.1 region of chromosome 14, which contains nine exons and 530 amino acids. The loss of ESR1 and ESR2 nonappearance is associated with high early antral follicles and lowered corpora lutea, indicating a partial obstruction of follicular maturation and development 6.

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quantified on RT-PCR amplification of cDNA. Using TriReagent, total RNA was isolated. Before being frozen at 80°C, GC and TC RNA were suspended in 10µl and 20µl (diethyrlpyrocarbonate-treated water), respectively. RNA was transformed to cDNA through incubation of 37 °C in buffer for 30 minutes. Buffer consisted Tris-HCl, KCl, MgCl₂, deoxy-ATP, deoxy-CTP, deoxy-GTP, thymidine 5-triphosphate, oligo dt by 10mM, 50mM, 5mM, 1mM, 1mM, 1mM, 1mM and 5grams, respectively. Then reaction was heated to 95°C before being cooled to 4°C. Then till PCR cycle completion, four micro-liters aliquots of the reverse transcription reaction were frozen at 80 °C. For adjusting the reaction mixture at volume of 100µL, 1 pg of mutant control DNA was added to aliquot of cDNA along with the addition of 2.5 units of Taq DNA polymerase, 50 pmol of all PCR primers and 8µL of PCR buffer (10x).

ER-α and ER-β were amplified separately for 25 thermal cycles. For eliminating control products, amplification products were alienated using 2% agarose gel after being ethanol-precipitated, then were digested using BamHI. We stained DNA by ethidium bromide to extract bands on gel and counted by scintillation counter. To account for variations in the GC and TC content of all samples, the data was further normalized to total cellular DNA. All TC and GC samples were amplified independently using separate protocols.

The supplier of the oligonucleotide primers was Life Technologies, Inc. (Grand Island, NY). Using primers corresponding to the reported sequence at locations 803–822 and 1288–1300, a distinct 505-bp region of ER-α cDNA was amplified. (GenBank Accession# M1674). The ER-β cDNA primers 628–647 and 1052–1073 amplified a 444-bp fragment (GenBank accession number: AF051427).

Statistical Analysis: SPSS version 20 was practiced for statistical analysis of the gathered data. In the statistical analysis comparing the treatment groups, Chi-square test was performed at p-value below 0.05 as the significance threshold. Additionally the data pertaining to continuous variables was evaluated though means, percentage and means ratio with standard deviation.

RESULTS

Three hundred and twenty-two infertile females were enrolled in this study and their clinicopathological features, comprising patients affected with PCOS and the control groups were measured. It was found that the mean age of PCOS and non-PCOS patients were 34.42±1.30 and 31.01±2.14 years, respectively. Both groups had comparable body mass index but the fasting glyemic conditions were elevated in the PCOS patients. There was a significant difference between the total cholesterol level of PCOS (p<0.05) and control patients in which the PCOS patients revealed elevated levels of total cholesterol. Sixty-eight out of one hundred eighty PCOS patients (p<0.05) were hypertensive but the genetic predisposition and gynecological or obstetric history of the control group patients (p<0.05) were significantly elevated than PCOS-affected women. Significantly higher values (p<0.05) for androgenism, serum testosterone and estrogen were prominent in the PCOS-affected women as compared to the other infertile females (Table 1).

For ER-α and ER-β mRNA expressions, RT-PCR revealed linear augment in amplified cDNA. In the GC and TC of women with regular menstrual cycles and PCOS, both ER-α and ER-β mRNAs were detected at comparable levels and expression pattern of ER-β mRNA were observed between GC and TC (Figure 1). ER-α mRNA expression did not differ between size-matched normal and PCOS follicles and the concentration of both kinds of nitrogenous bases was matching i.e. GC and TC (Figure 2). But, ER-β mRNA expression was significantly higher (p<0.05) in TC content as compared to the GC. The concentration of GC and TC was significantly higher in expression in SA control follicles (p<0.05) in dominant follicles. In PCOS, GC, ER-β mRNA expression was significantly lower than (p<0.05) in size-matched control follicles, but comparable with dominant follicles (Figure 3).

Table 1: Clinico-pathological examination of PCOS and Non-PCOS study population

<table>
<thead>
<tr>
<th>Parameters</th>
<th>PCOS women</th>
<th>Non-PCOS women</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Y)</td>
<td>34.42±2.30</td>
<td>31.01±2.14</td>
<td>-</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>29.78±1.76</td>
<td>27.56±1.65</td>
<td>-</td>
</tr>
<tr>
<td>Fasting Glycemia (mmol/L)</td>
<td>7.85±2.13</td>
<td>5.28±1.66</td>
<td>-</td>
</tr>
<tr>
<td>Total cholesterols level (mg/dL)</td>
<td>232.98±17.98</td>
<td>198.12±14.32</td>
<td>-</td>
</tr>
<tr>
<td>Hypertension (n%)</td>
<td>68 (37.77)</td>
<td>49 (35.0)</td>
<td>0.759</td>
</tr>
<tr>
<td>Genetic predisposition (n%)</td>
<td>34 (18.88)</td>
<td>62 (43.66)</td>
<td>0.00065*</td>
</tr>
<tr>
<td>Obstetric or gynecological history (n%)</td>
<td>14 (7.77)</td>
<td>17 (11.91)</td>
<td>0.3376</td>
</tr>
<tr>
<td>Hyperandrogenism</td>
<td>82 (46.55)</td>
<td>07 (4.90)</td>
<td>0.00001*</td>
</tr>
<tr>
<td>Serum testosterone level (ng/dl)</td>
<td>129.08±2.39</td>
<td>43.77±2.10</td>
<td>-</td>
</tr>
<tr>
<td>Serum estrogen level (pg/ml)</td>
<td>88.79±3.87</td>
<td>31.56±2.98</td>
<td>-</td>
</tr>
</tbody>
</table>

* indicated the value is statistically significant at (p<0.05)
In TC from PCOS, ER-β mRNA expression was significantly lesser (p<0.05) compared to size-matched control follicles and higher than (p<0.05) dominant follicles. While, in control group, GC ER-α protein concentrations were significantly greater than TC concentrations (p<0.05). PCOS follicles had lower (p<0.05) GC ER-β protein quantities than size-matched (control follicles) but higher quantities (p<0.05) than dominant follicles. In TC, ER-β protein concentrations in dominant follicles were somewhat lower than in SA control follicles, although quantities in PCOS follicles were equivalent to those in dominant follicles.

Figure 3: ER-β mRNA expression comparison of GC and TC contents of PCOS and control

**DISCUSSION**

PCOS was usually considered to result from genetic and hormonal factors. Over 70 possible genes have been discovered as being associated with various PCOS genotypes, namely in reproductive and endocrine metabolic dysfunctions. Therefore this study was conducted to correlate ER-α and ER-β genes of estrogen receptors to PCOS susceptibility. Even while there were non-significant changes in BMI, fasting insulin or total cholesterol levels, there was high prevalence of obesity in ladies with PCOS. These characteristics were consistent with research indicating that PCOS was associated with more severe obesity than the general population. Although age variations were observed, it was doubtful that this factor contributed to PCOS genetic findings.

In the present investigation, mRNA and protein levels of ER-α and ER-β expression were determined in GC and TC from control ovaries with typical follicular cycles and PCOS in humans. Expression of ER-α mRNA did not differ between size-matched normal and PCOS follicles, and the concentration of both types of estrogen receptors was consistent. Nevertheless, ER-β mRNA expression was substantially higher (p<0.05) in TC than in GC. Expression of GC and TC was equivalent in control follicles (p<0.05) in comparison to dominant follicles. Immunohistochemical examinations conducted before the identification of ER-α with antibodies that perceived ER-β showed the occurrence of ERβ only in GC region in antral follicles. Recent discoveries revealed that ER-β occurrence in GC of human follicles during all developmental stages, while, it was deficient in GC but existed in TC. An observational study revealed that PCOS women's endometrial tissue exhibited significantly elevated ESR1 expression and elevated ESR1/ESR2 ratio than corresponding groups (controls). By extracting follicles and granulosa cells from PCOS versus regular-cycling women, another study revealed similar results: expression of ESR1 was seen high in follicles, while the expression of ESR2 was found low in granulosa cells, in comparison controls. In addition, these three SNPs seemed connected with a variety of estrogen-dependent illnesses, including venous miscarriage, menopause onset, and fracture risk after menopause. Our findings coincided with research revelations, that reported ER-α and ER-β mRNA expression levels as elevated in proliferative eutopic-endometrium, which were significantly lowered in secretory-endometrium (p<0.05). In contrast, both proliferative as well as secretory segments of ovarian endometriotic cysts displayed decreased intensities of ER-α and ER-β mRNA. These findings suggest that cyclical fluctuations in ovarian hormones have a distinct effect on expression of ER-α and ER-β mRNA in endometriotic cysts and eutopic endometrium.

It was asserted in a study that PCOS caused by the aberrant expression of estrogen and ERs in the ovaries and uterus, and the therapeutic application of associated small-molecule targeted medications. Another study evaluated the concern of ethnicity and presented the meta-analysis demonstrating non-significant (p>0.05) relationships between the ESR1 rs2234693, ESR1 rs9340799, and ESR2 rs4936938 variations and individual with PCOS susceptibility.

**CONCLUSION**

PCOS is the major cause of infertility among Pakistani women, accounting for 63.20 percent of cases, according to the findings of our study. PCOS is mostly an endocrine condition that is curable if identified accurately. On the basis of ER and haplotype data, ER-α and ER-β encoding genes for mRNA served as good markers for the determination of the association of estrogen receptors with PCOS in Pakistan, particularly for metabolic features; yet, additional study is indispensable to wholly enlighten their critical role in PCOS and provide clinically useful indicators.

**Authors’ Contribution:** All the authors bear uniform contribution to this research.

**Conflict of Interest:** The authors affirmed no conflict of interest.

**Recommendation:** This study recommended the diagnosis of PCOS based on the estrogen receptors and haplotype data because ER-α and ER-β encoding genes for mRNA served as good markers for the determination of the association of estrogen receptors with PCOS in Pakistan.

**Funding:** No funding or sponsorship.

**Ethical Approval:** The procedures were conducted in compliance with the ethical criteria of the committee responsible for human testing and the most recent (2008) version of the Helsinki Declaration of 1975. All tissue samples were acquired from the subjects with their informed consent.

**REFERENCES**

Association of Estrogen Receptors with Polycystic Ovary Syndrome and Subsequent Infertility in Affected Patients