EXPRESSION ASSESSMENT FOR PROLACTIN RECEPTOR GENE IN A SAMPLE OF IRAQI WOMEN WITH RECURRENT MISCARRIAGE

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ABSTRACT

Recurrent miscarriage (RM) is one of a very important reproductive problem. The causes were recognized and were included antiphospholipid syndrome, anomalies in uterine, metabolic and irregulary in hormone levels, and abnormalities in cytogenetic parameters. The etiologies of this problem have been planned but are still controversial, for example inherited thrombophilias, chronic endometritis, luteal phase deficiency, and arising in the levels of DNA fragmented sperms. Treatments like surgical of anomalies of uterine or heparin or aspirin for the syndrome of antiphospholipid have enhanced the outcomes for couples with the failure of pregnancy. About 50% of the couples problems still unsolved and are trying to treated by using anticoagulation, immunomodulatory treatments and progesterone supplementation. Despite the cause, the diagnosis (long-term) of couples with frailer pregnancy is high-quality, and finally complete a healthy and live birth. Recent studies showed the linking between RM and the expression of Prolactin receptor gene (PRL-R). This study reporting the evaluation the expression for the (PRL-R) gene in a in 25 samples of Iraqi women with (RM) in comparatively with 5 subject as control. The result showed higher significant differences in patients with (RM) compared with normal women.

Keywords: (PRL-R), gene expression, recurrent miscarriage, Beta-actin


INTRODUCTION

Recurrent Miscarriage (RM), is described as two or more repeated spontaneous miscarriages, generally before the week number twenty of gestation (1). Different causes, including genetic factors, different defects such as endocrine, anatomical defects, have been assumed as being of RM causes, but the causes still unexplained (2,3). Prolactin is define as its function in the development of breast and lactation process. The functions of Prolactin by expression of prolactin receptor, initiating signaling cascades, primarily utilizing activator of transcription (JAK-STAT) and Janus kinase-signal transducer. To understanding of its physiological functions, The disruption pathway has been concerned in the abnormalities in reproduction, diabetes, and tumorigenesis, The secretion of Prolactin is done from extrapituitary sources by adding complexity (4,5). for the duration of pregnancy, the site of prolactin synthesis is the endometrial stroma (6).
women which not pregnant, the prolactin and Prolactin receptor expression in the endometrium during the secretory phase at mid-late was discussed by Jabbour and Jones\(^7\,\,8\). An association between RM and prolactin or Prolactin receptor has been studied by Jin et.al\(^9\). Garzia et al.\(^10\) proved low levels of expression of prolactin in RM women. Bersinger et al.\(^11\) also found the low-regulation for the Prolactin receptor in the women suffering with miscarriage. Many researchers have testing and proving the genetic constituent which causing the RM. The PRL-R in human as a gene located in chromosome number 5, the gene of PRL-R has been found and expressed in the liver( in human), ovary and the breast\(^12\,13\,14\). Hanna et al. (In 2010), tested the genetic polymorphisms concerned in the regulation of the hypothalamic-pituitary-ovarian axis (HPO) would be related with recurrent miscarriage.\(^15\).

Among the thirty-one single-nucleotide polymorphisms (SNPs) and the four short tandem repeat (STR) polymorphisms in twenty candidate genes, the distribution of genotype with reflect on to the PRL-R gene differed between the control and RPL.

This study aimed to evaluate the expression of PRL-R gene in samples of Iraqi women with (RM) in comparatively with apparently healthy women.

**MATERIALS AND METHODS**

**Subjects and blood sample collection**

During the period November 2018 till June 2019, this study was carried out on twenty five Iraqi women patients with (RM), non-smokers and nonalcoholic, aged (25-40 year). The RM samples were taken from the Kamal Al-Samarraee infertility treatment Hospital in Baghdad, as well as five apparently healthy individuals females collected randomly from population living Baghdad as control, aged (25 - 40 year) which are non-smokers, non-alcoholic as control group. Two ml of human peripheral blood from all select subjects were collected and placed into sterile plain tube that contained EDTA. The blood was placed in a cool - box under aseptic conditions and transfer to the laboratory.

**Gene Expression study on RM patients**

**Total RNA extraction**

Fresh human bloods were used for isolation of genomic RNA after collection, genomic RNA was extracted using the Trizol method (Direct-zol™ RNA MiniPrep, R2051, ZYMO RESEARCH / USA). according to the manufacturer’s instructions \(^16\). The concentration of RNA and purity were determined spectrophotometrically by measuring their absorbance at 260 (A260) and 280 (A280) by Nano spectrophotometer.

RNA was used for reverse transcription (RT) as total 2μg with the Synthesis of cDNA by the Trans Srt First-Strand Super Mix according to the (PrimeScript™ RTase). The primers were synthesized and designed by the program primer (Table 1). The amplification reaction of QRT-PCR was listed in Table 2.

The amplification conditions of QRT-PCR were: 95°C, 5min; 95°C, 20 sec, 60°C, 20 sec and the extension stage at 72°C, in 20 sec in 40 cycles. The Melt curves can be obtained by increasing the temperature to 95°C for 10 sec, then decreasing the temperature to 25°C for 30 sec. Figure (1).
Table 1: The sequence Primers and molecular weight in QPCR additional expression profiling and validation.

<table>
<thead>
<tr>
<th>Primer</th>
<th>SEQUENCE</th>
<th>GC%</th>
<th>Tm</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRL-R</td>
<td>TGATGTGGCAGACCTTGTCCC</td>
<td>54.5</td>
<td>59.8</td>
</tr>
<tr>
<td></td>
<td>CCATTCAGAAGGCAGGTGTTGAG</td>
<td>52.1</td>
<td>58.1</td>
</tr>
<tr>
<td>Beta-actin</td>
<td>CTGGCACCACACCTTCTACAAT</td>
<td>50</td>
<td>57.2</td>
</tr>
<tr>
<td></td>
<td>AATGTCAAGCAGATTCCCCGC</td>
<td>54.4</td>
<td>60.7</td>
</tr>
</tbody>
</table>

*the gene of β-actin as control (internal).

Table 2: The amplification reaction of QRT-PCR

<table>
<thead>
<tr>
<th>Component</th>
<th>20 μL (Final volume)</th>
<th>Final concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Universal</td>
<td>10 μL</td>
<td>2x</td>
</tr>
<tr>
<td>primer (forward)</td>
<td>0.4 μL</td>
<td>0.2μM</td>
</tr>
<tr>
<td>primer (reverse)</td>
<td>0.4 μL</td>
<td>0.2μM</td>
</tr>
<tr>
<td>Nuclease-free water</td>
<td>Up to 10 μL</td>
<td></td>
</tr>
<tr>
<td>Template DNA Sample Volume</td>
<td>-</td>
<td>1pg-100ng</td>
</tr>
</tbody>
</table>

Figure 1: The melting curves for primers invalidation and additional of QPCR

Data Analysis and Statistics:

The data of this study was compiled into distribution and Statistical description (mean, SE), the computerized data file and frequency, were divided using Statistical software (SPSS). We used least significantly difference (LDS) test and statistical analysis of variance(ANOVA) test by probability of less than 0.01 (p<0.01) according to method which reported by [17].

The quantitative of level for expression of gene was measured using the method (∆∆Ct relative Ct). The values of ∆Ct were evaluated by measuring the Ct of the value of RPL32 for all sample from the target

Ct value for each sample. Then calculate the Fold inductions by using the formula $2^{\Delta \Delta C_t}$, threshold was $\Delta C_t$ as cycle, $\Delta C_t$ as $C_t$ (for housekeeping gene)-$C_t$ (for target gene), $\Delta \Delta C_t = \Delta C_t$ (for treated) − $\Delta C_t$ (for control). The analysis of data was done by computer software to analyze the relative gene expression in all samples. A single sample (T-test) was used to evaluate statistically the difference between the ratios of derived expression of irradiated and the samples which non-irradiated (18).

RESULTS AND DISCUSSION

Gene Expression study on RM patients:

The total RNA was extracted from the Peripheral blood from each RM patients and control groups. The RNA concentration and purity were determined spectrophotometrically by measuring their absorbance at 260 (A260) and 280 (A280) by Nano spectrophotometer.

Relative quantitative gene expression levels for PRL-R gene in peripheral lymphocytes for RM patients regarding to their occupation using $\Delta C_t$ method. This gene showed higher significant differences between PRL-R gene folding in RM patients and control groups (p < 0.02). The PRL-R gene was analyzed by RT PCR. The range of folding value for PRL-R gene in the RM Patients group is shown in Table (3) was 4.88± 1.13 while it was 1.0 ± 0.18. Figure (2)

Table 3: The folding value. The range of folding value for PRL-R gene in the group of RM Patients and In control group

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean</th>
<th>SE</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM Patients</td>
<td>4.88</td>
<td>1.13</td>
<td>0.002</td>
</tr>
<tr>
<td>Control</td>
<td>1.0</td>
<td>0.18</td>
<td></td>
</tr>
</tbody>
</table>

Figure 3: The folding value. The range of folding value for PRL-R gene in the group of RM Patients and In control group
The studies on the molecular basis of transcriptional regulation of the prolactin receptor gene showed the higher Significant PRL levels were found in the infertile women than in controls.\(^{(19)}\)

Different etiological factors dependable for RM such as endocrinological, anatomical, genetic, infectious and immunological. The abnormalities of endocrinology may be polycystic ovaray syndrome (PCOS), hyperprolactinemia, hyperandrogenism, diabetes, thyrodism, or luteal phase defect, causative to RM\(^{(20)}\), the endocrinological disorders play an important role in patients with RM has been reported. Studies in vitro have been shown that prolactin plays a key role in progesterone production in rodents and corpus luteum maintenance, not in humans.\(^{(21)}\)

Moreover, the secretion of progesterone by culturing of granulose cells which obtained from human ovarian follicles is inhibited by increasing concentrations of prolactin about (100 ng/ml), but not by low concentrations of prolactin about (10–20 ng/ml). Therefore, those high levels of prolactin in early follicular growth inhibit progesterone secretion, which results defects in luteal phase.\(^{(22)}\) Many researches on rodents have revealed that prolactin receptors are involved not only in generating, but also in prolonged pregnancy.

It has been reported that hyperprolactinemia may occur in a temporary manner, around the phase of preovulatory. There is high levels more than levels of mid follicular baseline at the peak follicular maturity indicated hyperprolactinemia, associated with RM and unexplained infertility.\(^{(23)}\). On the other hand, one study revealed that low levels in concentration of serum prolactin is associated with an increased risk of MR in a consequent pregnancy in women with unexplained MR\(^{(24)}\). Rate of successful pregnancy is higher in hyperprolactinemic women with RSA who are treated with bromocriptine during randomized control trial (4.6–15.5 ng/ml, \(P < 0.01 \) or \(P < 0.05\)).\(^{(25)}\).

Other study showed that the \(PRLR\) gene mutation, \(PRLR\), resulting change in histidine to arginine amino acid at position of codon number 188. This substitution caused disruption the high-affinity and ligand-binding interface of the \(PRLR\), and this result in a defect in downstream signaling by signal transducer (Janus kinase 2) and the transcription activator of 5 (STAT5). For that, the familial hyperprolactinemia is a germline, losing function or mutation in \(PRLR\), causing the prolactin inattentiveness.\(^{(26)}\).

**ETHICAL CLEARANCE**

The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**CONFLICT OF INTEREST**

The authors declare that they have no conflict of interest.

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REFERENCES


