DETECTION OF LEVEL AND GENE POLYMORPHISM OF THE PROLACTIN HORMONE AND ITS RELATIONSHIP WITH OTHER HORMONES IN INFERTILE WOMEN WITH HYPERPROLACTINEMIA

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ABSTRACT

The current study was conducted to detection of the role of the genetic polymorphism of the prolactin hormone (rs1341239) -1149 G / T, and its association with some hormones such as luteinizing hormone (LH), follicle-stimulating hormone (FSH), and serotonin for some sterile women in Tikrit city, (60) blood samples were collected from sterile women, (30) samples of healthy women, their ages ranged between (16-46) years, from private clinics. I Chroma was used to measure the level of prolactin hormones, LH, FSH, were used. Enzyme linked immune-sorbent assay (ELISA) to measure the level of serotonin. DNA was extracted from blood samples and the -1149 G/T (rs1341239) polymorphism of the prolactin gene was determined using Tetra-Amplification Refractory Mutation System-Polymerase Chain Reaction (Tetra-ARMS-PCR).The levels of prolactin, LH, FSH, and Serotonin hormones were increased in infertile women compared with healthy women. There are non-significant differences in the allele and genotype frequencyof the -1149 G/T prolactin gene polymorphism in the patient group compared with the control group. The GT (OR value 2.388) and TT (OR value 1.921) genotypewere dominant in the infertile women compared with control group. The T allele is considered a risk factor with OR 1.562 while the G allele is more protective. Infertile womenwith GG genotype have high prolactin levels, while, FSH, LH and serotonin were decreased. There is a relationship between levels and -1149 G/T polymorphism of the prolactin and infertile Iraqi women. This conclude is very important in the diagnosis and follow up the infertility in the women.

Keywords:–1149 G/T prolactin polymorphism, Infertile women, Hyperprolactinemia

I. INTRODUCTION

Infertility is defined as a dysfunction of the reproductive system that leads to a decrease in the rate of pregnancy after one year of marriage and regular unprotected sexual intercourse or is a condition of biological deficiency that occurs in women or men preventing pregnancy, a disease that affects both men and women (Liz et al., 2013). It occurs for a number of reasons, including genetic causes or hormonal disorders such as prolactin hormone or immunological hormone, which divides infertility into three types, including primary infertility, which affects women after a whole year of marriage despite the continuation of the marital relationship without taking any contraceptives. In general, about 50% of couples have secondary infertility after having one child, one pregnancy ends with an abortion or an extraterrestrial pregnancy, unexplained infertility affects women without genetic or hormonal causes and the real causes of this type of infertility are unknown (Thomas et al., 2007). The reproduction of the milk hormone gene is regulated by two separate promoters. In the pituitary gland the reproduction of the milk hormone gene is controlled by the promoter under the influence of pit-1 cloning factor. In areas outside the pituitary gland such as T-lymphocytes, monocytes, monocytes and macrophages, the secretion of milk hormone is regulated by another promoter located at a distance of 5,840 base pairs. This promoter contains a multiplicity of single nucleotide polymorphism (SNP) in the GATA sequence at the site -1149 G/T. The presence of G allele in a person increases the number of copies of mRNA cloned from the milk hormone gene and thus the high concentration of the hormone in the blood (Nore, 2013).

Prolactin hormonediscovered Greuter and Striker in 1928 as a milk-regulating hormone, riddle and his group called this hormone "PRL" Prolactin in 1933 after they confirmed its presence by stimulating the release of the
crop gland in the Pigeons. It is a protein hormone that contains 199 amino acids, has a half-life (20) minutes, and is not associated with plasma (Wie et al, 2017). Prolactin is a neurogenic multipeptides hormone produced mainly by Lactotrophs found in the anterior pituitary gland of man and has also been found in many other sites outside the pituitary gland such as nerve tissues, genital tissues, immunity and other sites such as tear glands, fatty tissues, blood endothelial cells and kidney (Harvey et al., 2015). PRL promotes the synthesis of DNA, milk production in the breast, the spread of epithelial cells and the wide spread of prolactin receptors on human tissue facilitates its role in various physiological processes including cellular growth, vessel formation, blood formation, differentiation, osmosis balance, energy metabolism, reproduction, and lactation, in addition to contributing to the regulation of stress responses by inhibiting the axis of hypothalamic–pituitary–adrenal (HPA) (Wei et al., 2017).

Hyperprolactinemia has multiple causes and is the most common endocrine disorder in the pituitary axis (Vilar et al., 2016). Prolactinoma is the most common cause of hyperprolactin, caused by the presence of prolactinoma tumor in the cells that produce prolactin in the front lobe of the pituitary gland, which is the main cause of this condition (60% of cases) and there are other causes Such as dormant pituitary tumors that press on the axis between hypothalamus and pituitary lead to a defect in the effect inhibitor prolactin inhibitor especially drugs used for mental illness as well as pregnancy in addition to the lack of thyroid activity also works on the height of prolactin (Glezer and Bronstein, 2015).

Materials and methods

Included a sample of this study (90) females of ages ranging from 16 to 46 years, representing (30) the control group sample, i.e. healthy women, and (60) samples of women suffering from infertility, inability to have children and attending outpatient clinics to diagnose infertility, The information on each person was recorded according to the search form, which included information such as name, sex, age, weight for each patient, some were married and others were single. Blood samples were drawn from the arm vein with a sterile syringe of 5 ml and divided into two parts: the first included (3.5) ml of each blood sample placed in separate sealed tubes, usually tubes containing gel tubes to separate the blood parts and get serum serum after separation with the centrifuge at a speed of 3000 cycles for 5 minutes, after completing the separation process, keep the serum in freezing at a temperature (-20) until it is used for biochemical tests. The second part included (1.5) ml of blood placed in sealed tubes containing an EDTA-tube clotting blocker, which was well shaken and kept in freezing at a temperature (-20) until used in DNA extraction.

Hormonal tests

Measured the concentration of prolactin, FSH and LH hormones in the serum of the sterile and healthy women following the steps attached to the test kit and as instructed by I Chroma manufacturer BoditechBelyium and Korea, Serotonin was measured according to the steps attached to the ELISA test kit for testing procedures and according to the manufacturer's instructions.

Detection of PRL-1149 G/T Polymorphism

Genomic DNA was extracted from the total blood by method described in a previous study (Ali, et al 2008), then the extrapituitary prolactin –1149 G/T promoter polymorphism was determined by Tetra-ARMS-PCR technique using the following primers supplied by Macrogen company: Outer Forward TTTGTTAGAATTAGTCCATGTTC. Outer Reverse ATTCATCTTTTCTCTCAAACAGCTT. Inner Forward ACCTGGAGAAAGGAGGAAAAAT and Outer Reverse ATTCATCTTTTCTCTCAAACAGCTT. Primary several experiments were carried out for optimization of genomicDNA and primer concentrations to reach the optimum conditions, concentration (100ng) of genomic DNA and (10) picomole showed the best results. Then, the PCR amplification was performed in a total volume of 25 μL including 12.5 μL of 2X Go Taq green master mix supplied by Promega company (USA). For each sample, add 12.5 μL of master mix to a 0.2ml tube, then add the DNA template at 100 ng concentration and primers (forward & reverse(with 10 picomol / microliter and complete the reaction volume with distilled water to 25 μL). Mix the reaction components well and then place the tubes in the thermocycler carefully to perform the reaction according to the following program: one cycle for 5 minutes at 95 °C followed by 35 cycles, each cycle includes 94 °C for 30sec, 57 °C for 30 sec and 72 °C for 30 sec with a final cycle of 5 minutes at 72 ° C for a final extension.

The PCR product was determined using 2% agarose gel electrophoresis stained with redsave stain in the presence of 1Kb DNA ladder (Biolabs-England). Three types of the -1149 G/T prolactin genotypes as GG homozygous
(two bands 342 and 229 bp), GT heterozygous (three bands 342, 229, 165 bp) and TT homozygous (two bands 342 and 165).

**Statistical Analysis**

Statistical analysis of different samples was done using a student’s t-test and ANOVA test. The hormonal tests results were analyzed for the patients and healthy group at a significant level (0.01, 0.05). The alleles and genotypes of the patients and healthy group were also evaluated. The average concentration of prolactin hormone in the patients group was estimated according to the GG, GT and TT genotypes and then compared by using the student’s t-test.

### II. RESULT AND DISCUSSION

**Comparison between the hormonal levels of patients and control**

The results of the hormone level assessment among infertility and healthy patients indicated a moral increase ($P \leq 0.01$) for the group of sterile women, as prolactin concentration was high and with an average high moral difference in patients ($37.439 \pm 8.788$ ng/ml compared to the control group ($11.950 \pm 4.607$ ng/ml) at moral value (0.001). Also, FSH had a slight increase in concentration in infected women and an average ($6.562 \pm 1.074$) compared to the negatives ($6.075 \pm 0.985$), as well as the hormone LH there was a moral difference, with an average concentration of ($5.185 \pm 0.956$) compared to the average health group ($4.585 \pm 0.490$), Serotonin concentration was low in patients with an average ($28.397 \pm 5.090$ ng/ml compared to the control group ($30.981 \pm 6.376$) at moral value (0.04), as in Table (1).

<table>
<thead>
<tr>
<th>Hormone name</th>
<th>Infertile women No. (60)</th>
<th>Healthy women No. (30)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prolactin (ng/ml)</td>
<td>37.439 ± 8.788</td>
<td>11.950 ± 4.607</td>
<td>≤ 0.001**</td>
</tr>
<tr>
<td>FSH (mμ/ml)</td>
<td>6.562 ± 1.074</td>
<td>6.075 ± 0.985</td>
<td>0.039*</td>
</tr>
<tr>
<td>LH (mμ/ml)</td>
<td>5.185 ± 0.956</td>
<td>4.585 ± 0.490</td>
<td>≤ 0.001**</td>
</tr>
<tr>
<td>Serotonin (ng/ml)</td>
<td>28.397 ± 5.090</td>
<td>30.981 ± 6.376</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Hormones play a key role in fertility in women, including the follicle-stimulating hormone, luteinizing hormone, estrogen, progesterone and milk hormone, with hormonal imbalance in women accounting for about 30% of infertility (Ajibola et al., 2012). The level of prolactin rises by about (25%) from women with PCOS. The high level of prolactin in the blood can stimulate the adrenal gland to release Dehydroepiandrosterone (DHEA-S), a type of androgens that rises in the case of PCOS. These results were agreed with Amiri (2000), the results of this study were compared with AL-Azzawie (2013), Mohammed (2020) in terms of higher prolactin values, FSH, and LH in the infertility patient group compared to the control group. In the Ramanad and his group's study (2012), LH values showed that FSH values were higher in women with PCOS and secondary infertility.

### −1149 G/T Gene Polymorphism

The PCR reaction study of the samples of infected and healthy women using Tetra ARMS technology to detect the presence of a 342bp DNA pack compared to the 100bp volumetric guide after being electrically posted on agarose gel at a concentration of 2%, as in figure (1).
Figure (4-2): PCR extracted piece, agarose 2%, power (70 volts), current (40 mImper), 1.5-hour, and red safe dye.

The results showed the emergence of three genetic models, the natural genetic model GG, which is the 342bp package, the tt-165,229 bp, and the asymmetric genetic model GT of beams (165, 229, 342)bp. The replacement boom of the Nitrogen Base G, which converts it to nitrogen base T at site 1149bp - for prolactin gene has created a cutting site, and when treating PCR output with this enzyme, it will cut the package resulting from a 342bp molecular-sized multiplier into two pieces: the first with a size of 229bp, and the second with a size of 165bp. If a person is not carrying the mutation and in both nights, he or she will have a naturally uniform genetic model GG and one 342bp package will appear when the RFLP-PCR output is posted because there is no sequence required to make the enzyme cut (Yilmaz et al., 2011). If a person has an asymmetrical genetic model GT, one of the two alleles will not be interrupted because they do not have the location of the pieces, while the second night will be cut into two pieces, so three packs will appear when the RFLP-PCR output is posted, the first piece represents the non-floating allele 342bp, and the remaining two packages represent the enzyme-cut allyl (165,229) bp. The results of this study were agreed with Stevens and his group (2001), AL-Azzawie (2013) in terms of the fact that the GT genetic model is the most common among other genetic models of sterile women compared to strains.

The results of PCR-ARMS analysis of the prolactin gene of the site (rs1341239) -1149 G/T showed three genetic models (GG,GT,TT) and as shown in table (2) the frequency of genetic models as a percentage, the number of sterile and healthy women.

Table (2): Shows the percentage and frequency of genetic mechanisms and models of the prolactin gene of the site (rs1341239) -1149 G/T for Infertile and healthy women

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Infertile women No. (60)</th>
<th>Control No. (30)</th>
<th>P value</th>
<th>OR</th>
<th>(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>17 14.2</td>
<td>14 23.3</td>
<td>Ref.</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>GT</td>
<td>29 24.2</td>
<td>10 16.7</td>
<td>0.09</td>
<td>2.388</td>
<td>0.871 - 6.547</td>
</tr>
<tr>
<td>TT</td>
<td>14 11.4</td>
<td>6 20</td>
<td>0.281</td>
<td>1.921</td>
<td>0.584 - 6.313</td>
</tr>
<tr>
<td>Alleles</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>63 38</td>
<td>38 63.3</td>
<td>0.168</td>
<td>1.562</td>
<td>0.827 - 2.951</td>
</tr>
<tr>
<td>T</td>
<td>57 22</td>
<td>22 36.7</td>
<td>Ref.</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

G allele is the normal allele, T allele is the mutant allele.

The results showed moral differences (P ≤ 0.05) between the percentages of frequency of genetic models and evidence of the prolactin gene of the site (rs1341239) -1149 G/T in the totals of sterile and healthy women. The lowest frequency of the attics was the genetic model TT in proportion (11.4%) to sterile women, genetic models GG, GT was 24.2%14.2%) respectively. There is a lack of morale (P ≤ 0.05) in the frequency of allele T relative to the G-allele in the sterile group compared to the control group. Sterile women who own the TT model (1.921 OR= ,0.584 - 6.313 95% CI=), that the lowest value or for allele T was 1.562 and (2.951=95% CI), the results indicate that the presence of allele G is associated with high concentration of milk hormone, We note the high concentration of milk hormone for the group of sterile women. The basis of milk hormone secretion is similar in both males and females, where it is produced by a single gene located on chromosome No. 6, a size of 10,215 kilos, a base pair consisting of five exons and four electrons. The reproduction of the milk hormone gene is regulated by two separate promoters. In the pituitary gland the reproduction of the milk hormone gene is controlled by the promoter under the influence of pit-1 cloning factor. In areas outside the pituitary gland including immune cells such as T-lymphocytes, monocytes and macrophages, the secretion of milk hormone is regulated by another promoter located at a distance of 5,840 base pairs. This promoter contains a multiplicity of single nucleotide polymorphism (SNP) forms in the GATA sequence at the site -1149 G/T. The presence of allele G in a person leads to an increase in the number of copies of mRNA cloned from the milk gene and thus the high concentration of hormone in the blood (et al., 2010 Nore, 2013; Fojtíková). The results of this study were close to al-Azzawie's findings (2013) that the TT genetic model was the lowest genetic model, while the genetic model GG was the highest among genetic models.

Table (3): Hormonal levels of the Infertilewomen according to site (rs1341239) -1149 G/T prolactin polymorphism

<table>
<thead>
<tr>
<th>Parameter</th>
<th>GG(No. 17)</th>
<th>GT (No. 29)</th>
<th>TT (No. 14)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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The rise of serotonin within the normal range leads to a moderate mood, the feeling of relief and happiness, also the increase in prolactin levels reduces the secretion of hormones gonads through the inhibition of GnRH. The results shown in Table (3) showed the concentration of hormones according to the genotype of the prolactin gene, with significant differences between the concentrations, as the mean of the prolactin hormone for the genotype was $3.6 \pm 87$ for the genotype $3.3 \pm 87$, with the genotype $3.3 \pm 87. 10.210$), and in the TT genotype was the highest ($43.268 \pm 4.088$), while the concentrations of FSH, LH, and serotonin hormones (GG) were the highest among the other genotypes ($43.268 \pm 4.088$), while the concentrations of FSH, LH, and serotonin were the highest among the other genotypes ($29.505 \pm 1.156 \pm 7.056 5.5$ ), respectively, while the TT genotype showed the lowest concentration of dopamine hormone with an average of ($32.065 \pm 3.746$), the GT model ($32.093 \pm 4.752$) and the TT model had the highest ($35.374 \pm 4.738$).

High concentrations of LH indicate a defect in the secretion process of luteinizing hormone, ovarian failure, the higher the concentration of the hormone, the higher the incidence of infertility in women, and high concentrations of the hormone may be due to a rise in the concentration of the testosterone hormone, while the stimulating hormones FSH. It refers to the weak development of the follicles and later to the lack of ovulation, and this imbalance in the concentrations of hormones causes disturbances in their menstrual cycle and sometimes the absence of ovulation, and this is consistent with what was mentioned by the researcher Fischtach and his group (2000). The researcher Furia (1999) pointed out that the high concentration of LH hormone in sterile females may be due to the infection of a large proportion of sterile women with polycystic ovary syndrome (PCOS), as the results show in a number of studies to an imbalance in the ratio in the secretion of FSH, LH hormone in females. Sterile and infected with this syndrome (PCOS) to reach ((2:1) and this imbalance made the ovaries unable to secrete hormones in the correct way (Klein et al., 1996; Garden, 1998). Also, the high concentration of LH hormone in the sera of patients came as a result of low Estrogen and Progesterone Levels LH is known to be inhibited by elevated estrogen and progesterone levels through a negative feedback mechanism (Warren and Steihl, 1999; Hillier, 2000). Before ovulation, the increase in the LH booster in the middle of the cycle was investigated. If the concentration of these two hormones is low in the majority of patients, it leads to an increase in the LH hormone, and the reason may be the pituitary gland dysfunction, as Furia (1999) indicated that the high concentration of LH hormone Indicates pituitary dysfunction.

The increase in prolactin levels reduces the secretion of hormones gonads through the inhibition of GnRH secretion from the gland hypothalamus hypothalamus may have a direct impact on the ovary, either low gonad hormone levels can cause irregular menstrual cycle, sexual disorders, infertility (2013Crook). The high concentrations of the hormone cause disturbances in the menstrual cycle of women, and cause them infertility through the effect of the hormone on the follicle-stimulating hormone, and thus its impact on the formation of the egg in the early stages of ovulation and its formation in the early stages of ovulation and its formation by the researcher (Gibla et al., 2001). that some women have high concentrations of the hormone without any clinical symptoms of hyperprolactinemia, that the concentrations of prolactin are different during the menstrual cycle where it rises in the second half of the menstrual cycle while its concentration decreases in the first half specifically in the vesicular phase. The increase in prolactin concentration observed in sterile females leads to gradual changes in the deficiency of the luteal phase and to the absence of ovulation and then to amenorrhea due to the action of prolactin that inhibits the pulse secretion of the hormone GnRH. Production of progesterone, and consequently causes infertility (Cavallaro et al., 20 04). Low concentrations of estradiol and progesterone lead to an inappropriate luteinizing phase and consequently to decreased endometrial proliferation in women with elevated prolactin (Ibrahim, 2007).

The rise of serotonin within the normal range leads to a moderate mood, the feeling of relief and happiness, also causes a feeling sleepy as it is a material very calming direct reason to sleep, and low concentration leads to the feeling of depression and bad mood, the feeling of anger and lack of sleep, lack of concentration and migraine headaches continued low concentration Serotonin for a long time leads to stress and fatigue, which exacerbates in the afternoon, as its secretion decreases at sunset, and this explains the feeling of malaise (March 2009). Negri (2006) indicated that the mechanism of serotonin action is focused on the cell membrane, although there are many studies that have been conducted on thousands of volunteers to find out more serotonin inhibitors and the effect of serotonin and enhance the effect of serotonin on the epithelium. Serotonin is concentrated in large amounts in certain areas of the brain and hypothalamus, while the cerebellum contains low concentrations of it.
These results differed with AL-Azzawie (2013), and compared with the study of Navarro-Zarza and co-authors (2019).

**In conclusions**, the average concentrations of reproductive hormones showed moral differences, compared to the control group as there was a rise in prolactin, LH and FSH. The spread of allele G to the prolactin gene among the group of patients, indicating that the genetic factor has a role in infertility cases in women. The effect of allele T of the prolactin gene on the concentration of prolactin suggests that it may be associated with high hormone level.

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