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ORIGINAL ARTICLE

# Kisspeptin, GnRH, Prolactin and Ovarian Hormones Levels in Hyperprolactinemia, Type 1 Diabetes and Obesity Women

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**KEYWORDS**

Women;
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**ABSTRACT:** Kisspeptin is a peptide that plays a crucial function in the regulation of puberty initiation, by sexual immaturity and slowed puberty advancement. Infertility is a result of hyperprolactinemia, which inhibits the pulsatile release of GnRH from the brain and lowers the pulsatile production of LH from the pituitary. In type 1 diabetes, also known as autoimmune diabetes, pancreatic cells die off and the body cannot produce enough insulin, leading to high blood sugar levels. Obese people are more likely to suffer from hyperinsulinemia, hyperlipidemia, hyperleptinemia, chronic inflammation, menstrual abnormalities, pregnancy troubles, and infertility. So, this study aims to know the reproductive status of women by estimating kisspeptin and other hormones. The study was conducted on 92 women, ages (20-40) years, Samples were collected from the AL-Sader Teaching Hospital, Maysan for Child and Birth Hospital, the specialized Centre for Diabetes and Endocrinology and some private clinics and centres, the period June 2022 to February of 2023. The women were divided equally into four groups: control group (with regular menstrual cycles), hyperprolactinemia group (hyper serum prolactin), obesity group (have a BMI over 30 kg.m–2), and type 1 diabetes group. The results showed the values of kisspeptin did not differ significantly ($P>0.05$) in all groups, values of GnRH in hyperprolactinemia, obesity and type 1 DM groups increased significantly ($P\leq0.05$) compared with the control group, Prolactin, estradiol and progesterone in the hyperprolactinemia group increased significantly ($P\leq0.05$) in comparison with other groups. According to the above results, we conclude that high levels of prolactin, also obesity and type 1 DM influence the reproductive hormones in women.

## INTRODUCTION

Kisspeptin is a peptide made up of (145) amino acids, the kisspeptin gene (KISS1 gene), which is responsible for encoding kisspeptin, produces [1]. Kisspeptin-14, kisspeptin-13, and kisspeptin-10 all share the same COOH-terminal part of the kisspeptin precursor. It is important to note that the G protein-coupled receptor GPR54 is the mechanism through which all forms of kisspeptin work together [2].

Kisspeptin plays a crucial function in the regulation of puberty initiation, as shown by sexual immaturity and slowed puberty advancement [3, 4] involves the genetic inactivation of the kisspeptin receptor in people and animals. Kisspeptin neurons in the hypothalamus mediate the inverse relationship between sex hormones and GnRH neurons [5]. Strong evidence suggests that a second population of kisspeptin neurons is required to mediate the positive feedback effects of oestrogen on generating the pre-ovulatory LH spike. Proof of this was

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found in female mice lacking a pre-ovulatory LH spike after receiving a kisspeptin antagonist [6].

Infertility is a result of hyperprolactinemia, which inhibits the pulsatile release of GnRH from the brain and lowers the pulsatile production of LH from the pituitary [7]. The hormone GnRH is suppressed by prolactin. Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are decreased as a result of hyperprolactinemia [8]. Amenorrhoea, infertility, and hypogonadotropic hypogonadism are all related to hyperprolactinemia in females [9]. Prolactin receptors have only been identified in a small number of GnRH neurons while being present in most kisspeptin neurons [10].

Insulin deficiency due to pancreatic cell death characterizes type 1 diabetes mellitus, also known as autoimmune diabetes, a chronic illness that results in hyperglycemia [11]. Compared to women without diabetes, women with T1D are said to experience higher menstrual disruption and reproductive problems. These anomalies have been seen to affect women of all reproductive ages [12 - 14], delayed puberty, irregular periods compared to women of similar age without T1D [15-17], adolescents and young adults with T1D report androgen excess symptoms (acne, hirsutism) more frequently [18,19,20,21]. Less live births and pregnancies occur among women with T1D, and go through menopause earlier than women without diabetes [22, 23].

Obese people are more likely to suffer from degenerative diseases Hyperinsulinemia, hyperlipidemia, hyperleptinemia, and chronic inflammation are all symptoms of obesity. Menstrual abnormalities, pregnancy troubles, and infertility owing to anovulation in women and reduced testosterone are all examples of reproductive issues. Reduced levels of both gonadotropin hormones are found in obese women [24]. In light of the above, we decided to conduct this study.

## MATERIALS AND METHODS

This study was conducted on 92 women between the ages of (20-and 40) years; Samples were collected from the AL-Sader Teaching Hospital, Maysan for Child and Birth Hospital, the specialized centre for Diabetes and Endocrinology and some private clinics and centres in the Maysan governorate /Iraq, from the period June 2022 to February of 2023. The women were divided into four groups as follows: control group (23healthy women with regular menstrual cycles), hyperprolactinemia group (23 women with hyper serum prolactin), and obesity group (23 women have a BMI over 30 Kg $m^{2-1}$), and type 1 diabetes group (23 women with type 1 diabetes mellitus).

### *Blood sample*

Blood is drawn from a vein 3-5 ml by a syringe at 9-11 am placed in a gel tube and then centrifuged, The serum is kept at a temperature of -20°C, To measure kisspeptin, gonadotropin-releasing hormone (GnRH), prolactin (PRL), estradiol, and progesterone hormones by kit supply from Shanghai company (China) according to the [25, 26] and Roche Cobas, Switzerland according to the [27].

### *Statistical analysis*

The data was analyzed statistically to determine the significance of the different parameters by one-way ANOVA by SPSS version 23, the difference was considered significant at $P<0.05$ the values present as means ± SE.

## RESULTS

The values of kisspeptin did not differ significantly ($P>0.05$) in the control (123.65 ± 2.08), hyperprolactinemia (127.59±1.86), obesity (124.50±2.18) and type one diabetes groups (123.98 ± 2.82) as shown in Figure 1 and Table 1.

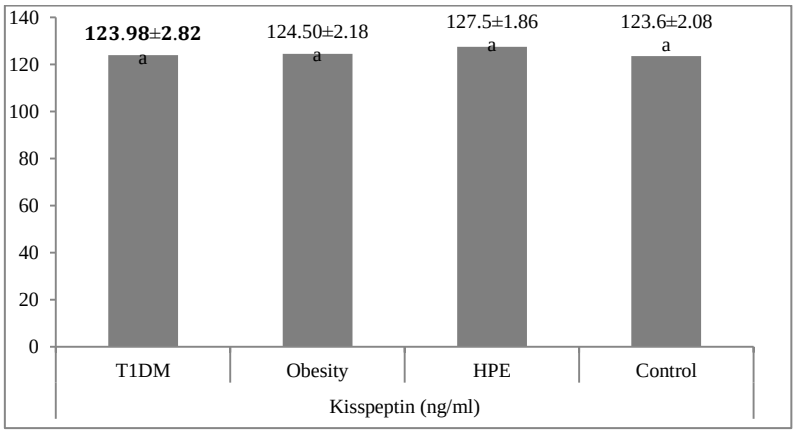


**Figure 1.** The kisspeptin concentration in control, hyperprolactinemia, obesity and type one diabetes in women.

The values of GnRH in hyperprolactinemia (0.69±0.02), obesity (0.66±0.01) and diabetes type one (0.67±0.01) increased significantly (P≤0.05) in comparison with the control group (0.60±0.01). While there are no significant (P>0.05) differences among hyperprolactinemia, obesity and diabetes type one group as shown in Figure 2 and Table 1.

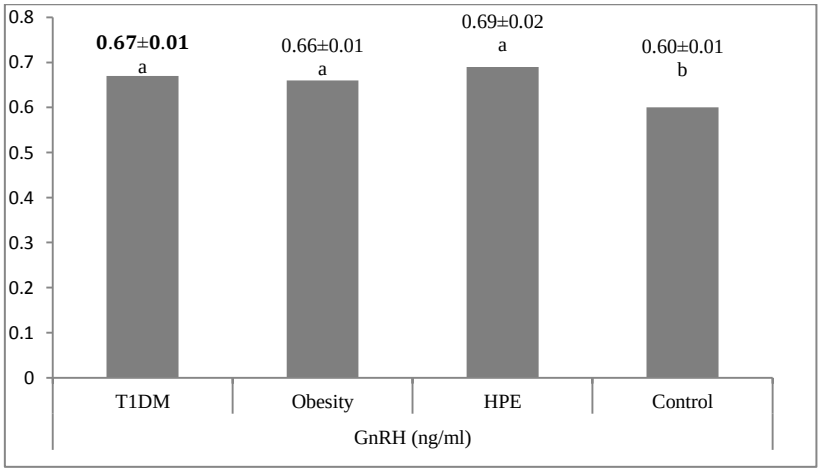


**Figure 2.** The GnRH concentration in control, hyperprolactinemia, obesity and type one diabetes in women.

The values of Prolactin in the hyperprolactinemia group (48.61±4.29) increased significantly (P≤0.05) in comparison with diabetes type one (13.86±1.66), obesity (11.97±0.85) and control groups (11.86±0.97). While there are no differences significantly (P>0.05) among diabetes type one, obesity and control groups as shown in Figure 3 and Table 1.

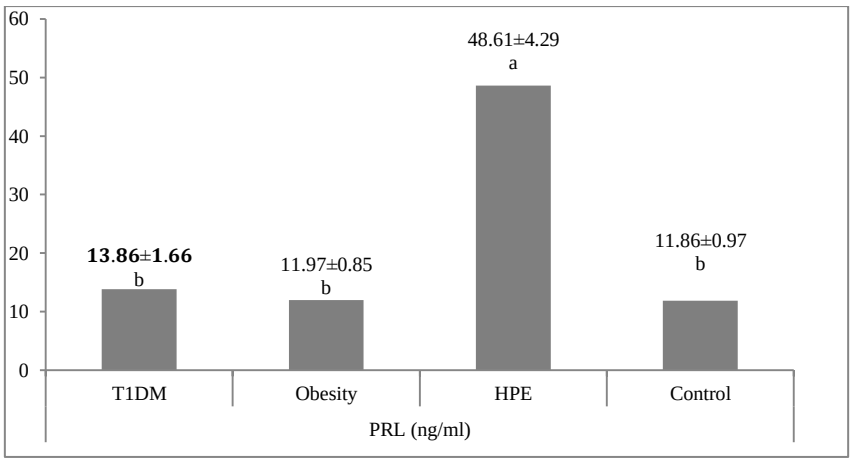


**Figure 3.** The prolactin concentration in control, hyperprolactinemia, obesity and type one diabetes in women.

The value of estradiol in hyperprolactinemia group (54.67±6.32) increased significantly (P< 0.05) in comparison with obesity (39.74 ± 4.18), diabetes type one (23.71±1.98) and control groups (27.45±1.83). Also, the value of estradiol in the obesity group increased significantly (P< 0.05) in comparison with the control and diabetes type one group. While no significant differences (P>0.05) between control and diabetes type one groups as shown in Figure 4 and Table 1.

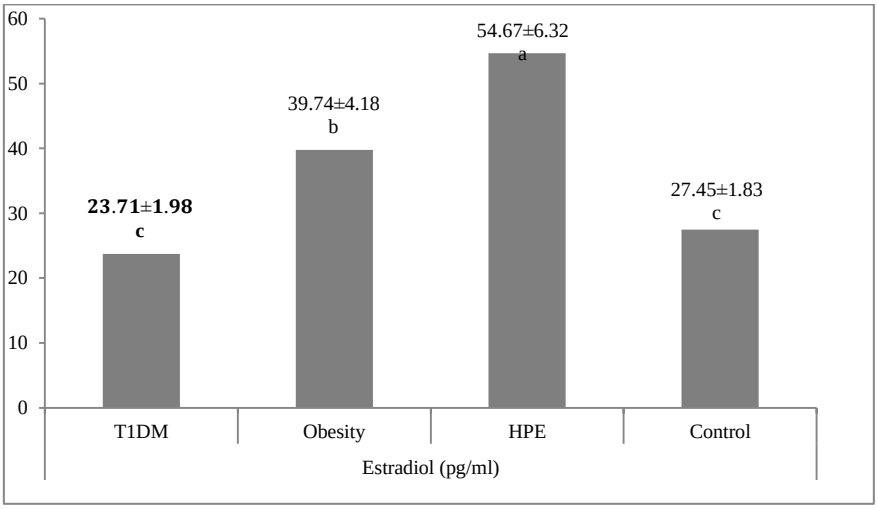


**Figure 4.** The estradiol concentration in control, hyperprolactinemia, obesity and type one diabetes in women.

The value of progesterone in the hyperprolactinemia group (1.22±0.07) increased significantly (P< 0.05) in comparison with diabetes type one (0.96±0.06), obesity (0.93±0.04) and control groups (0.21±0.01). Also, the value of progesterone in the obesity and diabetes type one group increased significantly (P< 0.05) compared to the control group. There were no differences significantly (P>0.05) between diabetes type one and obesity groups as shown in Figure 5 and Table 1.

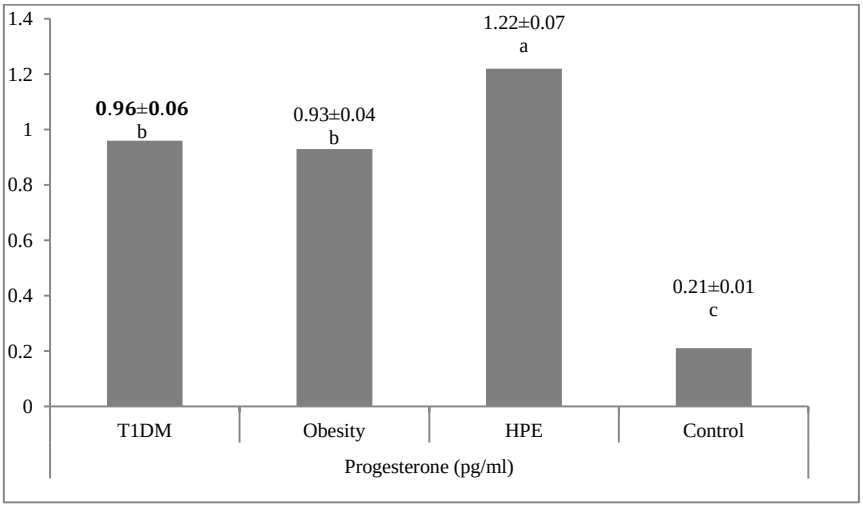


**Figure 5.** The progesterone concentration in control, hyperprolactinemia, obesity and type one diabetes in women.

**Table 1.** Comparing the concentration of kisspeptin and other hormones in women with hyperprolactinemia, obesity and type 1 diabetes.

| Parameters | Control | HOPE | Obesity | T1DM |
|---|---|---|---|---|
| **Kisspeptin (10–6kg $m^{3-1}$)** | $123.6 \pm 2.08^{a}$ | $127.5 \pm 1.86^{a}$ | $124.50 \pm 2.18^{a}$ | $\mathbf{123.98 \pm 2.82^{a}}$ |
| **GnRH (10–6kg $m^{3-1}$)** | $0.60 \pm 0.01^{b}$ | $0.69 \pm 0.02^{a}$ | $0.66 \pm 0.01^{a}$ | $\mathbf{0.67 \pm 0.01^{a}}$ |
| **PRL(10–6kg $m^{3-1}$)** | $11.86 \pm 0.97^{b}$ | $48.61 \pm 4.29^{a}$ | $11.97 \pm 0.85^{b}$ | $\mathbf{13.86 \pm 1.66^{b}}$ |
| **Estradiol (10–9kg $m^{3-1}$)** | $27.45 \pm 1.83^{c}$ | $54.67 \pm 6.32^{a}$ | $39.74 \pm 4.18^{b}$ | $\mathbf{23.71 \pm 1.98^{c}}$ |
| **Progesterone (10–9kg $m^{3-1}$)** | $0.21 \pm 0.01^{c}$ | $1.22 \pm 0.07^{a}$ | $0.93 \pm 0.04^{b}$ | $\mathbf{0.96 \pm 0.06^{b}}$ |

## DISCUSSION

### *Kisspeptin*

The values of kisspeptin did not differ significantly (P>0.05) in the hyperprolactinema, obesity and type one diabetes and control groups. The absence of significant differences between the groups may be due to the patients did not suffer from a functional defect in the kisspeptin system. This belief is confirmed by a study by other researchers, they found kisspeptin levels in the hyperprolactinemia group do not differ from the control group, and this agrees with our results, Where the researcher concluded hyperprolactinemia does not effect circulating kisspeptin levels but they recorded positively correlates between kisspeptin and hyperprolactinemia [28].

Exogenous kisspeptin administration on GnRH pulse generation in instances with hyperprolactinemia was studied throughout two visits, with kisspeptin 112-121 (0.24 nmol $kg^{-1}$) injected every hour for 10 hours. At the 11th hour, a single dosage of GnRH (75 ng $kg^{-1}$) was administered intravenously. There was no discernible difference between baseline and post-administration prolactin levels for the all-patient-in group. Thus, the increase in kisspeptin did not change the level of prolactin. As a result, they thought that the occurrence of hyperprolactinemia was because of kisspeptin stimulating GnRH in the LH pulses [29].

Serum kisspeptin levels in overweight and obese young females do not differ significantly compared with normal-weight females, and there is no correlation between serum kisspeptin and anthropometric indices [30, 31], which is consistent with our study and the findings of [32]. While, other studies found different results, where they observed the relationship between kisspeptin and obesity. Obese women of childbearing age have lower serum kisspeptin concentrations, according to research by [32]. Additionally, an inverse relationship was found between individuals' body mass index and their levels of kisspeptin. Researchers found that class-1 (BMI 30.0-34.9) obese people had lower serum kisspeptin concentrations than the control group. The level of kisspeptin in mothers' blood (MB) from obese and no obese volunteers revealed an increase in this peptide in the obese group and a higher concentration of kisspeptin was found in the cord blood (CB) of obese mothers compared to no obese mothers. Also, a strong positive correlation between the concentrations of kisspeptin in MB and CB [33]. The serum levels of kisspeptin are low in obese females compared to controls. These results indicate that kisspeptin could be involved in the pathophysiology of human obesity [34]. Another study observed that the serum kisspeptin level of obese women was highly significantly increased in comparison to that of normal-weight women [35]. The Obese/overweight girls had higher kisspeptin levels, and there was a positive correlation between kisspeptin and FSH and LH and obesity-related parameters in both genders [36]. The biologically active dose of kisspeptin did not affect self-reported appetite and food intake in women with overweight or obesity [37]. A comparison study was made between patients with T1D, obesity and healthy subjects, they found the Kisspeptin levels were lower in controls compared to the obesity subject but did not reach to statistical significance compared to T1D. Also, no significant difference between subjects with obesity and T1D, this agrees with our results [38]. In another study T1D patients had a different kisspeptin level compared to controls; higher kisspeptin levels were associated with IR [39].

Another research showed no distinction between GDM and healthy control pregnancies in terms of the rate of increase in plasma kisspeptin. Furthermore, plasma kisspeptin levels did not vary between the GDM and healthy pregnancies at any trimester. Neither univariate study nor analysis controlling for mother age, ethnicity, BMI, smoking status, or parity revealed a statistically significant difference in plasma kisspeptin levels between GDM and non-GDM pregnancies [40].

### *Reproductive hormones*

The values of GnRH and progesterone in hyperprolactinemia, obesity and T1DM increased significantly (P≤0.05) in comparison with the group's control group. The values of Prolactin in the hyperprolactinemia group increased significantly

(P≤0.05) in comparison with other groups. The values of estradiol in hyperprolactinemia and obesity increased significantly (P≤0.05) in comparison with groups control group. In this study, the differences in the levels of these hormones in the women with hyperprolactinemia, obesity and T1DM compared to control and compared to each other, probably belonged to the intertwined hormonal interactions. So, in the hyperprolactinemia group, this may be due to the feedback resulting from the increase in prolactin and its connection with dopamine.

Dopamine is the inhibitory factor for prolactin. Dopamine is important in maintaining a healthy level of prolactin. In the event of an increase in prolactin secretion, dopamine flows from the hypothalamus into the circulatory system and thus leads to a decrease in prolactin secretion and its return to the normal level in the healthy state. Thus, any imbalance in this dopamine balance will affect most of the hormones that have feedback with prolactin [41]. In the obese group, the reason may be the outputs of adipose tissue and its effect, Adipose tissue has proven its primary role as an endocrine gland, as it is considered an active and important endocrine tissue. It has soluble products called adipocytokines or adipose tissue hormones such as leptin, which has been shown to affect raising the estrogen hormone, especially in women, and therefore the rise in estrogen may lead to a change in the levels of other reproductive hormones [42].

While the differences in the hormones in the T1D group may be due to the absence, decrease or increase in insulin, especially, since all patients in our study were treated with insulin low insulin levels affect progesterone, and therefore may lead to a change in the levels of other reproductive hormones [43]. Prolactin level was found to be significantly higher in the hyperprolactinemia group. Estradiol levels were decreased significantly (don't agree with our results) in the hyperprolactinemia group compartmented with control 25. After 10 hours of injecting 0.24 nmol $kg^{-1}$ of kisspeptin 112-121 every hour for 10 hours. At hour 11, women with hyperprolactinemia received a single intravenous dose of GnRH (75 ng $kg^{-1}$), which resulted in a statistically significant increase in the total number of LH pulses. While prolactin levels remained stable, estrogen levels rose significantly [[29]. The prolactin concentration significantly heightened, while as value of E2 was no significant difference (don't agree with our results) between women with hyperprolactinemia and women with normal prolactin [44]. The levels of estrogen are lower in females with secondary infertility due to hyperprolactinemia and these results are not consistent with our results [45].

Women with prolactinoma had significantly higher levels of prolactin this, agrees with our results. And lower estradiol in Women with prolactinoma all premenopausal age 35-45 years in their study [46]. High levels of Prolactin were observed, estrogen significantly increased, while progesterone decreased significantly in the infertility (hyperpolactinemia women) group compared with the controls [47]. Median blood progesterone levels in normal-weight infertile women were considerably greater than those in both weight extremes of obese individuals [48]. A reduced ESR1:ESR2 ratio may account for the observed shift in glucose consumption observed after menopause [49]. A high BMI is associated with decreased estradiol levels in all phases of the ovarian cycle and hurts pregnancy outcomes in obese women [50]. Serum Estradiol level in obese women was highly significantly increased in comparison to the normal weight women and this agrees with our results.

Positive correlations were found between lowering overall SHBG and increased BMI in obesity. Also, there were no significant changes to levels of free androgens, and estradiol [51]. A significant reduction was shown in the concentration of AMH hormones, while the concentration of estradiol and leptin hormones were significantly higher in obese women compared with the control group, while there was no significant difference in progesterone hormone concentration, this result doesn't agree with our results [52]. Women who develop type 1 diabetes before menarche tend to have shorter reproductive spans overall, with delayed menarche and earlier natural menopause compared to non-diabetic women. Therefore, women with type 1 diabetes had 2.5 fewer reproductive years than those without diabetes [53]. The metabolic imbalance plays an important role in reproductive functions, such as the duration and irregularity of the menstrual cycle, the increase in HbA1c increases the duration of the menstrual cycle in women

of childbearing age who suffer from type 1 diabetes [54].

## CONCLUSIONS

The presence of low fertility in these different groups, women with hyperprolactinemia, obesity, and type 1 diabetes had elevated levels of GnRH and high concentrations of prolactin in hyperprolactinemia while the other group was normal, and women with hyperprolactinemia and obesity with elevated levels of estradiol and women with hyperprolactinemia, obesity and type 1diabetes had high levels of progesterone. This results due to disorders in hormone levels in women in different groups.

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### *Conflict of interests*

Authors declare no conflicts of interest.

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