# The Effect Study of Prolactin on Married and Single Women and the Dangers of High and Low Prolactin

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**Abstract**

This study was conducted in a hospital in the city of Tikrit from the first of December 2023 to the first of March 2024. The aim is to study the difference between the high and low concentrations of milk hormone in single and married women and to identify the dangers and benefits of high and low levels of this hormone, as samples were taken from both cases and at different ages and treated with (prolactin kit) from Accu Bind, and the level of milk hormone was measured. The results of the samples of married women suffering from high levels of milk hormone were the first group, and the mean of their ages was 33 years, and the arithmetic mean of the percentage of milk hormone in the blood of the samples was 24. As for the results of the samples of single women suffering from low levels of milk hormone, the mean age of the subjects was 30 years, and the arithmetic mean of the milk hormone ratio in the blood of the samples was 25.47, and the increase in milk hormone levels leads to many of the symptoms that appeared in the studied samples, including Delayed menstruation, the appearance of milk discharge in non-breastfeeding or single women, as well as a marked increase in the appearance of hair in the body areas in general. Significant differences were observed in single women experiencing low levels of the milk hormone, prolactin, associated with increased levels of dopamine—a hormone known to suppress prolactin production. This imbalance was linked to pituitary gland disorders caused by improperly prescribed medications, particularly in cases involving severe headaches.

**Keywords:** Prolactin Hormone, Milk Hormone, Single

## Introduction

Prolactin, sometimes referred to as "milk hormone" or "natural milk hormone," is a hormone that is mainly released from the brain's anterior lobe of the pituitary gland. the most important of which are:

1. The hormone plays a role in reproductive health in both sexes.
2. It is important in regulating individual behavior.
3. It plays an important role in the immune system and metabolism.
4. Controls the regulation of many body fluids.

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The hormones responsible for signaling the pituitary gland to control the secretion of milk hormones are:

1. The role of estrogen increases the secretion of prolactin.
2. The hormone dopamine inhibits its secretion[9]

Normal levels of prolactin vary according to gender and pregnancy status, and are determined by testing the prolactin hormone and its levels are as follows: Normal milk hormone levels for non-pregnant women are less than 25 nanograms per milliliter (ng/mL).

Normal milk hormone levels for pregnant women: It ranges from 34 to 386 ng/mL[6]. One of the functions of the hormone dopamine is to restrict the production of the hormone prolactin, as they have an inverse relationship, as the more dopamine is produced, the less prolactin is produced[11].

**Prolactin**

Also known as milk hormone or lactotropin is a peptide hormone (composed of amino acid chains) that is secreted from the anterior lobe of the pituitary gland and its role is related to the lactation process as it acts as a stimulant for the release of milk from the mammary glands, called the process (lactation)[3].

The hormone also influences endocrine, autocrine, and paracrine behaviors through prolactin receptors and multiple cytokine receptors[13].

**Hormone Structure**

Prolactin is made up of a single polypeptide chain with 199 amino acids and a molecular weight of 24,000 Daltons. The polypeptide structure has three folds since the ends of the chain have equivalent sulfur bonds[8].

**Hormone Production**

The largest amount of prolactin is produced in milk cells located in the anterior lobe of the pituitary gland, and the hormone is also produced in small amounts from both the mammary glands and the endometrium. Dopamine restricts the production of prolactin, as they have an inverse relationship, the more dopamine is produced, the less prolactin is produced.[11]

Prolactin (PRL) is a polypeptide hormone primarily secreted by the anterior pituitary gland, but it is also produced in breast epithelial cells, neural tissue, sebaceous glands, and immune cells. Elevated levels of prolactin (hyperprolactinemia) have been linked to various autoimmune diseases, including systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), Sjögren's syndrome, systemic sclerosis, and type I diabetes mellitus (DM).[14]

The effects of thyroid hormones on impaired reproductive function are thought to be largely due to changes in the level of TSH, whose secretion interferes with FSH, LH and prolactin and may therefore have overlapping function [8]. It releases large amounts of progesterone and small amounts of estrogen, which are essential for implantation and preparation for pregnancy[9].

**The Effect of The Hormone**

The prolactin hormone shows several vital effects in the human body, the most important of which are:

- Stimulating the mammary glands to produce milk.
- Orgasm following sexual activity.
- Stimulate the proliferation of oligodendrocyte precursor cells: Oligodendrocyte precursor cells produce myelin, which is the cortex covering the axon in the central nervous system [12].

**Normal levels of prolactin**

Normal levels of prolactin vary according to gender and pregnancy status, which is determined by prolactin hormone testing as follows.

1. Normal milk hormone levels for non-pregnant women: ≤ 25 ng/mL.
2. Normal milk hormone level for pregnant women: 34 - 386 ng/mL.
3. Normal milk hormone level for men: ≤ 15 ng/m [14.]

**Prolactin concentrations in the blood**

- The concentration of prolactin in the blood rises during pregnancy in response to the high concentration of estrogen, which prepares the mammary glands for milk production and lactation.

- Differences during the day, prolactin concentration is high in the early morning, and it also rises after eating, exercise, intercourse, and sometimes after surgery [5].

## Materials and Methods

### Study sample

This study included taking samples of 20 married women and 20 single women for patients suffering from infertility and hormonal imbalance who visited Tikrit Teaching Hospital in Salahuddin Governorate between the ages of 18_50 years and conducting hormonal testing for them, which included prolactin hormone & LH & FSH where the two groups were compared and we obtained the test results that we will discuss in the discussion paragraph.

### Blood samples

Blood samples were taken and collected every morning when reviewing the required cases in the hospital's laboratory by drawing 10 ml of venous blood and placing it in special tanks that do not contain blood clotting substances and we separate the blood components using the Centrifuge device to obtain the serum that we will use in the examination process.

### Data collection

When collecting the information, we used a questionnaire that indicated the name, age, gender, and residential address of the case, in addition to the results of the required test, distributed to two groups:

a. The first group consists of 20 samples of married women, aged between 28 and 42 years, including some with one child, some with two children, and some without children. These women were diagnosed with high levels of prolactin (milk hormone) and were selected from patients visiting Salahuddin General Hospital in Tikrit between December 1, 2023, and March 1, 2024. The condition was identified based on elevated hormone levels and the presence of specific symptoms, including:

- Delayed menstruation

- Acne breakouts

- Increased facial and body hair

### The second group: single women

b. The second group consists of 20 samples of single women, defined as unmarried women without children, in accordance with cultural and religious norms where having children outside of marriage is prohibited. These women exhibited low levels of the milk hormone but did not present the symptoms observed in the first group. The majority of these women were unable to produce milk (unmarried women) and were selected from patients referred to Tikrit Teaching Hospital during the same period as the first group. The diagnosis was based on hormone levels falling below the normal range.

### Materials used:

The Prolactin Hormone kit from Accu Bind was used which contains the following reagents [14].

| | | |
|---|---|---|
| PRL calibrator | PRL enzyme reagent | Streptavidin-coated plate |
| Wash solution concentrate | Substrate A &B | Stop solution |

Figure 3 shows the reagents of ELISA kit

### Preparation of materials:

Wash buffer:

In a suitable storage container, 980 milliliters of distilled or deionized water were combined with the concentrated wash buffer, which was then stored at room temperature.

B- Working Substrate Solution:

Pour the contents of the vial labeled "A" into the transparent vial labeled "B" and mix the solution well until used.

### Working Method:

A- Drawing blood samples

3 ml of venous blood is drawn for each person from the above totals and placed in tubes containing Barrier Gel and left for a quarter of an hour to form a blood clot and placed in a centrifuge at 4000 r/min for 5 minutes to separate the serum from the rest of the components, and saved until the time of use.

B- Sample separation method

The part required to measure the milk hormone from the blood is Serum serum, which is free of fibrin

that works to clot the blood this is the main difference between blood plasma and serum and to obtain Serum; the blood sample must be transferred from the syringe to an empty test tube (yellow cap tubes), and left for 10 - 15 minutes at room temperature, with 15 minutes at room temperature.

Then the blood tubes were placed in the Centrifuge and the samples were rotated for 5 minutes at a speed of 4000 rpm, after removing the sample from the machine we will find at the top a liquid of yellow color (serum) and a transparent middle layer representing the gel in the tube and the blood components precipitated and clotted at the bottom in red color.

C. Prolactin hormone measurement method

The percentage of milk hormone was measured by an Enzyme-Linked Sorbent Assay (ELISA) from BioTek according to the following sequence:

1. The Eppendorf tube is formatted in the microplate wells of the device.
2. Using the Pipette, take 0.025 ml (µ25) of sample serum into the customized Eppendorf tube.
3. Add 0.1 ml (100µ) of PRL enzyme solution to the eppendorf tube.
4. We gently rotate the micro-plate for 20-30 seconds to mix it and cover it, and incubate it for 1 hour at room temperature.
5. The contents of the microplate are emptied by pipetting and the microplate is dried with absorbent paper.
6. Add 0.35mL (350µ) of wash buffer by Pipette, and repeat the process twice, plate washer can be used for up to three washes.
7. Discard the wash solution after completing the process.
8. To reduce reaction time variations, add 0.1 ml (100µ) of working substrate solution to each pit. Additionally, always add the reagents in the same order. After adding the working solution, do not move the microplate, and then incubate for 15 minutes.
9. Add 0.05 mL (50µ) of reaction stop solution to each well and mix gently for 15-20 seconds.
10. Read at a wavelength of 450 nm in the ELISA reader, no later than half an hour.

**Statistical analysis**

The results were statistically analyzed using the statistical program spss version (1999), which includes the calculation of the mean and standard error Mean±SE, through which a comparison was made between the arithmetic means of the first and second groups at the level of probability ($P<0.05$), ($P<0.01$).[1]

## Results

The following are the samples of married and single women, as married women suffer from high levels of milk hormone. The first group had an arithmetic mean age of 33 years and their average calculation of the ratio of milk hormone in the blood of the samples was 24.47, while single women who suffer from low levels of milk hormone had an arithmetic mean age of 30 years and their average calculation of the ratio of milk hormone in the blood of the samples was 25.47. Below table shows the samples of single women with low levels of milk hormone.

**Table 1: Shows the samples of women (married and single) and hormone levels with their ages.**

| 20 | 19 | 18 | 17 | 16 | 15 | 14 | 13 | 12 | 11 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 | NO. Status |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 27.2 | 25.2 | 30.5 | 29.3 | 41.7 | 23.2 | 20.1 | 20.1. | 22.2 | 21.4 | 20.3 | 20.5 | 17.9 | 29.3 | 41.7 | 29.3 | 48.7 | 35.8 | 30.5 | 6.0 mg | Married women |
| 28 | 26 | 25 | 37 | 31 | 42 | 32 | 27 | 44 | 34 | 37 | 35 | 32 | 37 | 31 | 37 | 38 | 34 | 24 | 42 | Age |
| 8.3 | 15.3 | 12.2 | 8.5 | 15.2 | 10.1 | 1.8 | 2 | 14.2 | 9.8 | 10.6 | 15.2 | 1.9 | 13.9 | 3.9 | 13.3 | 4 | 10.8 | 11.9 | 12.4 mg | Single women |

**Table 2: Shows the concentration of milk hormone in married and single women (mean ± standard error).**

| mean ± standard error | | Categories |
|---|---|---|
| Significance level ng/mL | Milk hormone (Prolactin Hormone) | |
| (P<0.05) | 0.15 ± 0.67 | Married women From 28-48 |
| (P<0.01) | 0.51 ± 3.15 | Single women from 20-42 years |

M = Married, S = Single

Two-Sample T-Test and CI: M.Prolact., S.Prolac

Two-sample T for M.Prolact. vs S.Prolac

| | N | Mean | St Dev | SE Mean |
|---|---|---|---|---|
| M.Prolact. | 20 | 28.50 | 4.33 | 1.9 |
| S.Prolac | 20 | 20.51 | 2.69 | 1.3 |

** T-Test of difference = 0 (vs ≠): T-Value = 8.92 P-Value = 0.0003

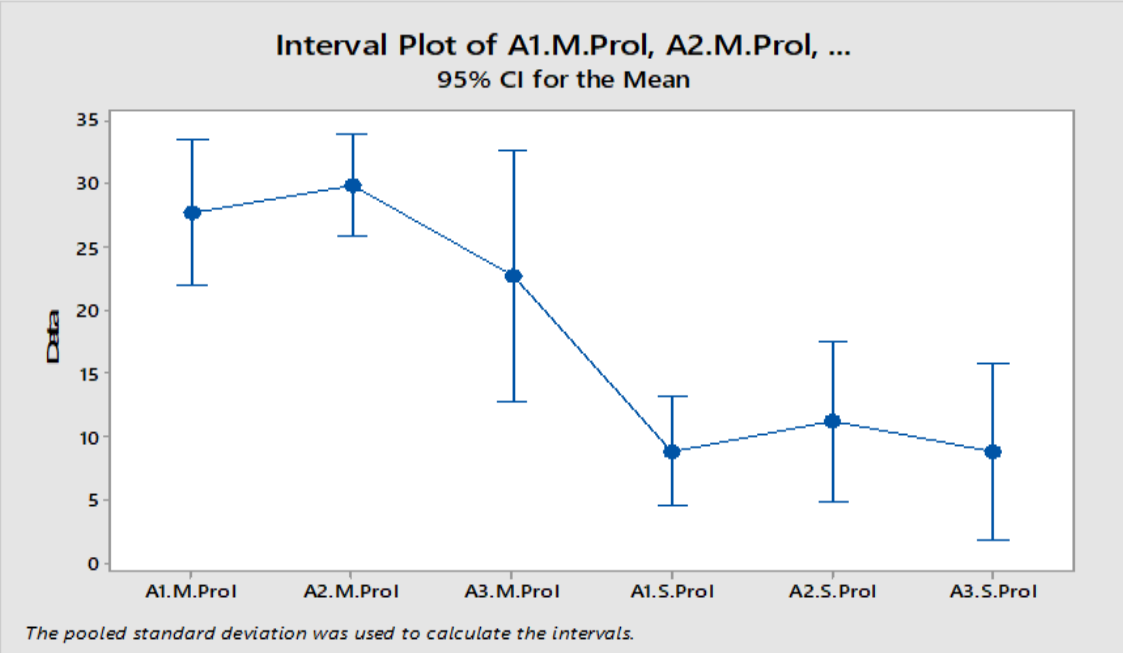


**Figure 1: Shows the analysis by age.**

AGE

A1=20 - 30 YEARS

A2=31 - 40 YEARS

A3=41 - 50 YEARS

PROLACTIN

One-way ANOVA: A1.M.Prol, A2.M.Prol, A3.M.Prol, A1.S.Prol, A2.S.Prol, A3.S.Prol

**Analysis of Variance

| Source | DF | Adj SS | Adj MS | F-Value | P-Value |
|---|---|---|---|---|---|
| Factor | 5 | 3746 | 749.21 | 15.70 | 0.0007 |
| Error | 34 | 1623 | 47.72 | | |
| Total | 39 | 5369 | | | |

Means

| Factor | N | Mean | StDev | 95% CI |
|---|---|---|---|---|
| A1.M.Prol | 6 | 27.75 a | 4.65 | (22.02, 33.48) |
| A2.M.Prol | 12 | 29.83 a | 5.08 | (25.78, 33.89) |
| A3.M.Prol | 2 | 22.70 b | 0.71 | (12.773, 32.627) |
| A1.S.Prol | 11 | 8.85 c | 2.07 | (4.61, 13.08) |
| A2.S.Prol | 5 | 11.22 c | 3.61 | (4.94, 17.50) |
| A3.S.Prol | 4 | 8.82 c | 3.95 | (1.81, 15.84) |

Pooled St Dev = 6.90810

In the figure 2 The mean age of married women was 23.81 years, while the mean age of single women was 20.51 years.

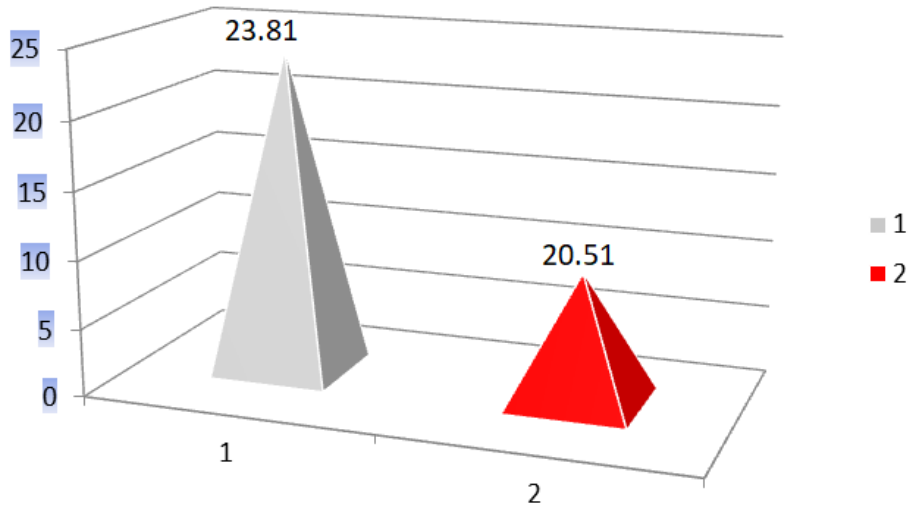


**Figure 2: Shows the different between the single and married women**

## Discussion

Many studies[14] support our findings. Samples from the married group exceeded the typical ranges for the hormone prolactin, which will not align with some of our study's findings , and it is known that the normal levels of the hormone for single women are less than 25 ng/ml. Where it is noted in sample No. 4 in table (4-1) that the prolactin ratio is 48 ng/ml and this ratio is very high from normal values because this woman was not pregnant at the beginning of her third decade of life, as well as with sample No. (6 and 16) for two women in the middle of their third decade, we note that their prolactin ratio is 41. 7 ng/ml and this percentage is much higher than the normal limit and the reason for this high percentage is due to the increased secretion of prolactin leads to a decrease in sex hormone levels, which may lead to infertility or weakness in libido and a decrease in bone density in both sexes. As a result, women, in particular, may experience symptoms such as interrupted or irregular periods, vaginal dryness, excessive body hair, and milky breast discharge despite not being pregnant or breastfeeding. Adolescent girls may also experience menstrual irregularities and breast discharge.

As for the second group of single women, we note that the samples (No. 8 and No. 14) of two women at the beginning of their fourth decade suffer from very low levels of the hormone, reaching 1.9 and 1.8 ng/ml, as well as sample No. 13 of a 30-year-old woman with a prolactin level of 2.0 ng/ml, which is very low, and the effect of these levels is the inability to produce milk, menstrual disorders, infertility and sexual dysfunction. Symptoms begin to appear as you get older or gradually appear months or even years later.

Some of the results in the first group (married women) matched some of the results of the researcher's study[5]. The symptoms that accompanied high milk hormone levels in women, such as acne, milky discharge from the breast in non-breastfeeding or single women, delayed menstruation, and other symptoms, were very clear, as well as for married women, where many of the symptoms of high milk hormone mentioned above appeared. However, some of the group's results were contrary to a study conducted by the researcher[5], as some symptoms did not appear in all women in the samples used, such as sudden attacks of headaches, visual impairment, and the sensation of hot flashes, as the percentage of the appearance of these symptoms amounted to 25% of the total samples in the first group.

As for the second group (single women) who suffer from low milk hormone levels, some of the results of the samples agreed with the symptoms reported in[14]. As low milk hormone levels led to a decrease or absence of adequate milk production in women after production, some of the results were also consistent with[9]. In his research on conditions associated with low milk hormone levels, some of the samples were suffering from pituitary dysfunction or dopamine overproduction according to the study[14].

## Conclusions

After completing the work and continuous research, it was concluded that:

1. The increase in the levels of the milk hormone Prolactin leads to a number of symptoms that appeared in the study samples, including delayed menstruation and the appearance of milk discharge in non-lactating or single women, as well as a noticeable increase in the appearance of hair in the body areas in general.
2. The increase in milk hormone affects blood pressure, as it has been observed that there is an increase in blood pressure for patients with high levels of milk hormone.
3. It was observed in patients from single women who suffer from low milk hormone in the presence of high dopamine hormone, which is the hormone responsible for reducing the proportion of milk hormone, and this imbalance is the result of a disorder in the pituitary gland due to some drugs that are prescribed incorrectly, especially in cases of severe headaches.

**Recommendations**

For this research, we recommend the following:

1. Conduct extensive studies on prolactin hormone, its long-term effect, and its relationship with some of the body's vital functions.
2. Study the relationship between the milk hormone and some blood components such as cholesterol, triglycerides, and diabetes.
3. Conducting a more extensive study to investigate the effect of milk hormone on the elderly and identify side effects
4. We recommend increasing health awareness and not using medications without consulting a doctor, especially with regard to medications that affect the pituitary gland or those that affect the hormone
5. Exercise and increase physical activity.

**Ethical clearance**

This study has been approved by the ethics commission for University of Tikrit, College of Science, Department of Biology .Tikrit, Iraq. The reference letter (NO.0/) dated: 03/12/2024.

**Conflicts of interest:** no conflict.

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