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Effects of Serotonin, Melatonin, and Zinc Levels on Prolactin Hormone in Hyperprolactinemic Patients in Basrah Governorate, Iraq

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ABSTRACT

Prolactin is a versatile hormone that plays a significant role in the metabolic, mammalian body, impacting reproductive, immunological, sexual, and other functions. It is not surprising, then, that the neuronal regulator of prolactin excretion is complex, including the coordinated actions of numerous hypothalamic nuclei. A plethora of experimental information exists on the hypothalamic control of hormonal secretion under numerous physiological stimuli. The present study was conducted to investigate the effect of serotonin, melatonin, and zinc levels on prolactin hormone in hyperprolinemic patients in Basrah Governorate, Iraq. Thirty healthy subjects (15 males and 15 females) as a control group and 47 patients (25 females and 22 males) with hyperprolactinemia were recruited from the Infertility and *in vitro* Fertilization Centre, in Basrah Governorate, Iraq. Blood samples were collected from the study groups. The levels of the hormone's prolactin, serotonin, and melatonin were estimated by using the enzymatic immunoassay technique, ELISA. Zinc concentration was determined by employing the spectrophotometric method. The results revealed a significant ($p<0.05$) increase in prolactin levels in both male and female patients when compared to the control groups, with the increase being higher in women than in men. Also, a decrease in zinc levels in patients with hyperprolactinemia with a significant difference ($p<0.05$) when compared with the healthy control group was observed. Patients with hyperprolactinemia had a significant ($p<0.05$) increase in serotonin and melatonin hormones when compared to their control groups. The findings of this study suggest that these hormones are considered stimulating factors for the secretion of prolactin.

Keywords: Hyperprolactinemia, Melatonin, Prolactin, Serotonin, Zinc.

Introduction

Prolactin is an excellent hormone that has a lot of implications for sexual behaviour and reproduction.[1] More than 300 biological roles, including osmoregulation, reproduction, immunological activities, and behaviour, have been identified so far, and their pleiotropic structure has been discovered. The secretion of prolactin by lactotrophs in the anterior lobe of the pituitary gland is well-ordered. Ambient lighting conditions may entrain prolactin surges to a given time of day, possibly through the suprachiasmatic nucleus, which is thought to be the master clock in mammals.[2] In mammals, the prolactin hormone stimulates the production of milk. It is not only responsible for milk production but also regulates and maintains the osmotic pressure in fish and preserves the immune function of man.[3–5] The availability of prolactin secretion sites has increased its role. Although prolactin receptors can be found in a variety of tissues, their main function is the production of breast milk.[6,7] Prolactin is a 199-amino-acid protein that circulates as a 22-kDa protein or a 25-kDa glycosylated shape in humans, but it also comes in a few larger biomolecular forms. In humans, the molecule of prolactin is organized as the only chain of amino acids. In between the 6-cysteine remains, there are 3 sulphide bonds. The cysteine remains are Cys^{191}, Cys^{4}, Cys^{199}, CYS^{58}, Cys^{174}, and Cys^{11}.

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Sequence homology varies greatly between rodents and primates, ranging from 56% in rodents to 97% in primates. In mice and rats, pituitary prolactin comprises 197 amino acids, while in pigs, sheep, cattle, and humans, it contains 199 amino acids with a molecular mass of around 23,000 Da. The primary type of prolactin found in the pituitary gland is 23 kDa. In humans and many other mammals, alternatives to prolactin have been found. Proteolytic cleavage, posttranslational variations of the amino acid chain, and alternate splicing of the primary transcript led to the variation in prolactin. Alternative splicing of prolactin mRNA is one source of the variation. There is evidence that a consecutively connected prolactin difference of 137 amino acids exists in the anterior pituitary. Although alternative splicing is not the main cause of prolactin variations. Also, it involves the retention of introns.[8,9] In addition to proteolytic cleavage, further posttranslational processing of mature molecules can create the majority of prolactin variations in the anterior pituitary gland or plasma. It includes dimerization, polymerization, phosphorylation, glycosylation, sulfation, and deamidation. In the hypothalamus, higher levels of prolactin might disrupt reproductive function. Prolactin is developed by pituitary lactotrophs which are also recognized as mammotrophs. In the pituitary of humans, the lactotrophs, consist of approximately 15-25% of the entire number of cells. Males and females have the same number, which does not change much with age. Lactotroph hyperplasia is observed during pregnancy and lactation. It might be the consequence of transdifferentiation of somatotrophs, lactotroph proliferation, and/or expansion of a stem cell population. After several months of delivery, the hyperplastic process involutes. Breastfeeding might slow down or stop the procedure. This stimulating consequence of pregnancy on lactotrophs is also accurate for prolactinomas and might cause pregnancy-induced malignancy.[10–12]

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Serotonin administration of agonists for the 5HT1A, 5HT2A, and 5HT2C receptors has been shown to increase prolactin secretion in humans. Prolactin secretion is also stimulated by serotonin and 5-HTP, which are precursors to serotonin. When melatonin concentrations are elevated, such as those found at night or under conditions of shortened day length (i.e., short photoperiod), the interaction of the indoleamine with MEL1A receptors in the pars tuberalis leads to decreased prolactin secretion.[13,14] This study was aimed at evaluating the effect of serotonin, melatonin, and zinc levels on prolactin hormone in hyperprolactinemia patients in Basrah Governorate, Iraq.

Materials and Methods

Study groups
Forty-seven adult Iraqi patients with hyperprolactinemia (experimental group) and 30 healthy adults (control group) were selected for the study. Their ages ranged from 13-55 years. Males and females with hyperprolactinemia for unclear reasons were included in the study, but patients with a tumor in the pituitary gland were excluded.

Ethical approval
Ethical approval for this study was obtained from University of Basrah, College of Science, Department of Chemistry (APPROVAL NUMBER/ 7/54/4319 in 15/10/2020).

Collection of blood samples
The study was conducted at the Infertility and *In Vitro* Fertilization Centre in Basra Governorate, Iraq. Five milliliters of venous blood were collected from both males and females with hyperprolactinemia and healthy subjects. The serum was then separated and transferred to new disposable tubes, where it was frozen at -70 °C until analysis.

Measurements of zinc and hormones
Zinc was measured by using Elitech Group Clinical System technology (catalog number E239.20A). The hormones prolactin, serotonin, and melatonin were evaluated using an ELISA kit (Monobindinc, BT LAB, and LTA kit numbers: CA 92630, E1128Hu, E1013Hu, respectively) from Bioassay Technology Laboratory.

Statistical analysis
The statistical package for the social sciences (SPSS; version 26) was used to analyze the data. An independent t-test was employed to compare the patients and healthy control groups for any significant difference. P-value ≤ 0.05 was considered significant.

Results and Discussion

Demographic characteristics of study groups
Seventy-seven blood samples were obtained: 30 control samples (15 males and 15 females), and 47 patients with hyperprolactinemia (22 males and 25 females), which were collected from people aged between 13-55 years. The standard deviations of the age for both the patient and control groups are presented in Table 1.

Prolactin hormone concentrations
The results presented in Table 2 and Figure 1 showed that there was a significant ($p = 0.0001$) increase in prolactin hormone levels in all the patients (39.44 ± 18.48) compared to the control group (11.90 ± 4.27). Females have a higher prolactin hormone concentration of 52.06 ± 17.58 ng/ml when compared to males with a value of 27.39 ± 8.83 ng/ml with p- values of 0.0001 and 0.006 for the female and male patients, respectively. Prolactin is a protein hormone and also cytokine that is produced and secreted from specialized cells of the anterior pituitary gland, called lactotrophs. It can also be produced in other extra pituitary tissues, which include immune system cells such as natural killer cells, T- and B-lymphocytes, and macrophages. The major function of prolactin is to stimulate the secretion of other cytokines and the development of cytokine receptors, indicating that it may have an impact on the immune system.[15–17]

Prolactin hormone is found at high concentrations among adult males and females, particularly those affected by hyperprolactinemia among females. Hyperprolactinemia is a condition of elevated serum prolactin. Nonpuerperal hyperprolactinemia is caused by prolactinomas (lactotroph adenomas), which accounts for about 40% of all pituitary tumours. Hyperprolactinemia might also develop because of pathological or pharmacological hypothalamic-pituitary interruption of dopaminergic pathways and is occasionally idiopathic. Hyperprolactinemia can cause hypogonadism, galactorrhoea, and infertility, regardless of the cause, or it can remain asymptomatic. Bone loss occurs as a result of sex steroid attenuation caused by hyperprolactinemia. Spinal bone density is decreased by about 25% in women with hyperprolactinemia and is not unavoidably restored with normalization of prolactin levels. In females with hyperprolactinemia, the level of prolactin was abnormally high, measuring 52.06 ng/ml. This is because females develop breasts faster than males, especially during their reproductive years, and therefore have a higher secretion of prolactin in their bodies when compared to male bodies in a similar health conditions. Male patients in this study had a prolactin level of 27.39 ng/ml, which was significantly lower than that of female patients.

Zinc level
Zinc levels in male and female hyperprolactinemia patients were studied. As controls, healthy male and female samples were used. The zinc concentrations in the total, male, female, and control samples are shown in Table 3 and Figure 2. In the total hyperprolactinemia patients, a value of 55.17 ± 14.07 ng/ml was recorded for zinc, while their corresponding total control group had 81.42 ± 23.23 ng/ml, with a significant p-value of 0.001. The female patients' zinc levels were 57.29±12.22 ng/ml, compared to 90.23±19.26 ng/ml in the female control group, with a significant p-value of 0.0001. Male patients had zinc levels of 53.02±15.90 ng/ml, while male controls had zinc levels of 73.20±24.18 ng/ml, with a p-value of 0.01. The results suggest that all groups of hyperprolactinemia patients had zinc deficiency when compared to the control groups. Gender had a significant effect on the level of zinc, according to the results. The study revealed that female patients with hyperprolactinemia condition had higher zinc levels when compared to their male counterparts. Zinc is a gender-controlling element in the human body. This is evidenced by the concentration obtained from both male and female controls, which showed that females have higher zinc levels in their bodies than males, as seen in Table 3.

Zinc is an essential element that is involved in many biological processes in both plants and animals. Its deficiency can impair T and B cell maturation and alter cytokine secretion.[18,19] Zinc therapy improves sperm quality by increasing density, and motility, and improves pregnancy outcomes. Zinc is an antioxidant and membrane stabilizer that keeps sperm viable. Seminal fluid has a high concentration of sperm. Zinc may play a role in sperm metabolism. Also, it regulates lipid flexibility and membrane stabilization in spermatozoa. Conception and embryonic implantation require an acrosome reaction. It suppresses transcription on the chromatin of human sperm and perhaps other mammalian species. Some zinc and other oligo-elements in the semen diluent may reduce reactive oxygen species (ROS) damage to chromatin.

Table 1: The demographic characteristics of the study groups

Groups		No.	Age (mean ± SD
Total	Control	30	29.57 ± 8.77
	Patients	47	27.79 ± 7.85
Male	Control	15	29.33 ± 7.70
	Patients	22	30.95 ± 8.69
Female	Control	15	29.80 ± 10.00
	Patients	25	25.00 ± 5.90

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Table 2: Prolactin hormone levels of the study groups

Parameters	Total Control	Total Patients	Male		Female	
			Control	Patients	Control	Patients
No.	30	47	15	22	15	25
Prolactin (ng /ml)	11.90 ± 4.27	39.44 ± 18.48	11.49 ± 2.29	27.39 ± 8.83	12.32 ± 5.69	52.06 ± 17.58
p–value	0.0001		0.006		0.0001	

*Significant p-value was < 0.05

Table 3: Zinc levels of the study groups

Parameters	Total		Male		Female	
	Control	Patients	Control	Patients	Control	Patients
NO.	30	47	15	22	15	25
Zinc (ng/ml)	81.42 ± 23.23	55.17 ± 14.07	73.20 ± 24.18	53.02 ± 15.90	90.23± 19.26	57.29 ± 12.22
p-value	0.001		0.011		0.0001	

*Significant p-value was < 0.05

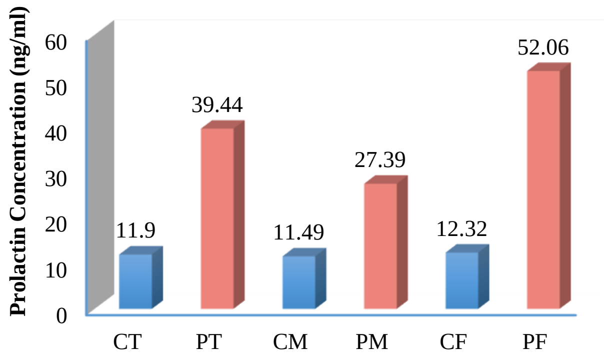

Figure 1: Prolactin concentrations of the study groups.
CT: Control Total; PT: Patients Total; CM: Control Male; PM: Patients Male; CF: Control Female; PF: Patients Female

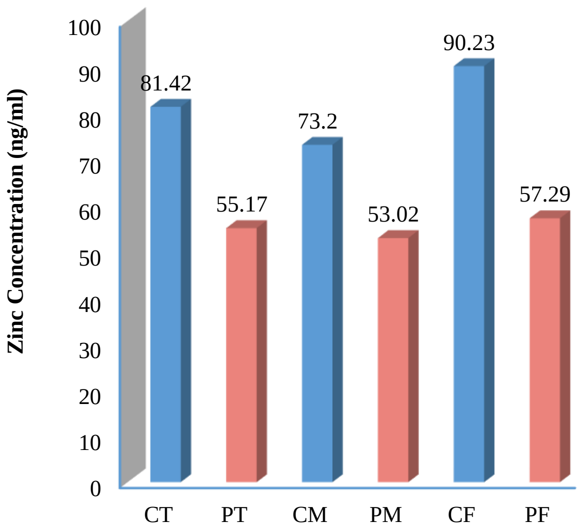

Figure 2: Zinc concentrations of the study groups.
CT: Control Total; PT: Patients Total; CM: Control Male; PM: Patients Male; CF: Control Female; PF: Patients Female

An increase in zinc concentration by 200 ppm decreases the DNA quality of boar sperm. It has to do with sperm DNA fragmentation and zinc levels.[20] However, the results showed a significant difference in zinc concentration between normal and patient females (90.23 vs. 57.29). Male and female hyperprolactinemic patients had similar zinc concentrations, with males recording 53.02 $\pm$ 15.29 (p = 0.011) and females recording 57.29 $\pm$ 12.22 (p = 0.0001). The zinc levels of both male and female hyperprolactinemia patients alter their body systems, according to this study.

Serotonin and melatonin levels

Serotonin and melatonin concentrations were measured in normal males (control), hyperprolactinemic males, normal females (control), and hyperprolactinemic females. As presented in Table 4, the acquired results were utilized to determine the p-values for both patients and controls. The hyperprolactinemic patients had higher mean serotonin levels than the control group, with a p-value of 0.0001. Table 4 shows that both the patients and controls had high levels of serotonin. The male control had a level of 291.81 ng/ml, but the male patients had a level of 760.58 ng/ml, which was significantly above the human limit. The hyperprolactinemia condition increased the serotonin level above the recommended limit. The serotonin level in the female control was 321.35 ng/ml, which was slightly above the recommended limit. Serotonin levels in female patients were the highest, at 778.53 ng/ml. The data also supported gender differences in serotonin levels in people. The results revealed that both males' and females' serotonin levels were affected by hyperprolactinemia.

Melatonin levels were determined in normal (control) and patients with hyperprolactinemic conditions (males and females) by using the Human Melatonin ELISA Kit. The obtained results were used to determine the p-values of both the patient and control groups to determine their statistical significance as presented in Table 4. The total patients with hyperprolactinemia had melatonin concentrations that were 1168.88 ± 173.18 higher than the entire control group of 508.04 ± 235.86, indicating that there was a statistically significant increase in mean melatonin concentrations, with a p-value of 0.0001, less than 0.05. Furthermore, the obtained concentration per gender had a p-value less than 0.05 (P-value 0.0001 for both male and female participants in the study), indicating that statistical significance has increased in both cases, as shown in Table 4. The assessment of melatonin concentrations in tissues and other body fluids based on blood melatonin levels seems insufficient due to the non-homogeneous melatonin distribution. Some studies found that melatonin concentrations in CSF were much higher than in the blood. However, high melatonin concentrations play a vital role in circadian rhythm regulation,[21] blood pressure modification, and sleep promotion. It also acts as a strong antioxidant and potent endogenous free radical scavenger.

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Table 4: Serotonin and melatonin concentrations of the study groups

Parameters	Total		Male		Female	
	Control	Patients	Control	Patients	Control	Patients
NO.	30	47	15	22	15	25
Serotonin (ng/ml)	300.14 ± 155.97	771.67 ± 141.69	291.81 ± 130.92	760.58 ± 171.35	312.35 ± 171.60	778.53 ± 124.01
p-value	0.0001		0.0001		0.0001	
Melatonin (ng/L)	508.04 ± 235.86	1168.88 ± 173.18	373.76 ± 127.07	1198.85 ± 173.14	633.36 ± 247.81	1151.53 ± 175.49
P - value	0.0001		0.0001		0.0001	

*Significant p-value was < 0.05

The increase in melatonin levels in hyperprolactinemia patients' bodies affects both females and males,[22] and that gender influences prolactin hormone concentration. All the patients have hyperprolactinemia (high prolactin levels).

Researchers studied the relationship between melatonin and prolactin levels in a group of adolescent boys throughout a 24-hour cycle on three consecutive occasions. Melatonin and prolactin had a significant positive relationship ($P \leq 0.001$) throughout the 24 hours, with concentrations during the overnight period, when both hormones were increased, contributing the most. There was a positive association between night-time concentrations and temperature ($p \leq 0.01$) in February and March, but it was most significant in June ($p \leq 0.001$). Concentrations of melatonin and prolactin in blood samples collected at 15-minute intervals during the morning (8 AM–12 PM) and evening (8 PM–12 AM) were shown to be not significantly linked with one another. Melatonin levels rose in the evening before prolactin levels increased, and they declined in the morning before prolactin levels decreased. In both the morning ($p \leq 0.05$) and evening ($p \leq 0.05$) periods, oral administration of 6 mg melatonin significantly increased prolactin secretion above levels evaluated after placebo administration, with the prolactin response being greater in the evening. According to these observations, the ability of melatonin to trigger prolactin secretion is what regulates the nightly increase in prolactin levels.[23] On the morning of proestrus, rats were also given a single intravenous injection of tryptophan, 5-hydroxytryptophan, serotonin, or melatonin, as well as hypophysectomized, pituitary grafted female rats. When compared to control values, 5-hydroxytryptophan increased serum prolactin levels by approximately 9-fold, 30 minutes after injection and by approximately 6-fold, 1 hour later. It also increased serum prolactin levels in hypophysectomised rats with an anterior pituitary graft by approximately 2-fold. By 30 minutes and 2 hours after injection, tryptophane had nearly doubled serum prolactin levels when compared to control levels, although the increases were not statistically significant at the time. Serotonin did not affect serum prolactin levels as compared to control levels.

Melatonin, on the other hand, boosted serum prolactin levels by 1 and 2 hours, respectively, following injection. According to these observations, serotonin and its precursors in the brain, as well as melatonin, may have a role in boosting prolactin production, thereby counteracting the inhibitory effects of hypothalamic catecholamines. Compared to control levels, serotonin did not influence serum prolactin levels. Melatonin, on the other hand, increased serum prolactin levels by 1 and 2 hours after injection, respectively. According to these results, serotonin and its precursors in the brain, as well as melatonin, may have a role in boosting prolactin production, thereby counteracting the inhibitory effects of hypothalamic catecholamines.[24]

Conclusion

The findings of this study reveal that all participants (males and females) had elevated prolactin hormone levels above normal, with a significant difference compared to healthy subjects (control groups). There was a decrease in zinc levels in patients with hyperprolactinemia, compared with the control groups for males and females, with a significant difference ($p \leq 0.05$). The levels of serotonin and melatonin hormones in hyperprolactinemia patients increased significantly ($p \leq 0.05$) compared to the control groups in both males and females. High prolactin hormone levels in patients may be caused by zinc deficiency and an increase in melatonin and serotonin hormones.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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