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Original Article

Biochemistry Section

# Evaluation of Serum Prolactin Level in Patients of Subclinical and Overt Hypothyroidism

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## ABSTRACT

**Background:** Prolactin secretion is controlled by prolactin inhibitor factor that is secreted from hypothalamus; factors like vasoactive inhibitory peptide (VIP) and thyroid releasing hormone (TRH) lead to increase in prolactin secretion. Hyperprolactinemia is a common condition that can result from a number of causes including hypothyroidism. Objective of the study was to determine correlation between serum levels of prolactin and thyroid hormones in euthyroid, subclinical and overt hypothyroid cases.

**Materials and Methods:** Consecutive patients presenting for various thyroid related problems were segregated into two groups subclinical and overt hypothyroidism according to their diagnosis based on history and clinical examination, laboratory reports, inclusion and exclusion criteria. Newly diagnosed 75 patients in each group were finally enrolled. Similar number of age and sex matched controls were selected. All subjects filled a predesigned questionnaire for the evaluation of hypothyroid symptoms. Thyroid profile for $T_3$, $T_4$ (total and free), TSH and prolactin were determined in all the subjects and analyzed.

**Results:** Prolactin elevation was found in 16 patients (21.33 %) with overt hypothyroidism, and in six patients (8%) with subclinical hypothyroidism. The control group and subclinical hypothyroid patients exhibited no significant difference in terms of total and free $T_3$, total and free $T_4$. For TSH and prolactin on the other hand, a statistically significant elevation was found in patients with overt hypothyroidism when compared with subclinical hypothyroidism; and in patients with subclinical hypothyroidism when compared to the controls. A significant statistical difference was observed between the two groups of hypothyroid patients for all hypothyroid symptoms except alopecia and hirsuitism.

**Conclusion:** The incidence of hyperprolactinemia in hypothyroidism was found to be higher when compared with normal controls. Serum prolactin assessment should be performed on all patients with hypothyroidism (overt and subclinical) before performing further tests.

**Keywords:** Bradycardia, Cardiovascular effects, Heart rate, Hypotension

## INTRODUCTION

Hyperprolactinemia is the most prevalent endocrine disorder in hypothalamic-pituitary axis that can result from a number of causes, including medications, hypothyroidism and pituitary disorders. Prolactin secretion is controlled by prolactin inhibitor factor that is secreted from hypothalamus, other factors like vasoactive inhibitory peptide (VIP) and Thyroid releasing hormone (TRH) lead to increase in prolactin secretion [1].

Hyperprolactinaemia may develop in patients with primary hypothyroidism through a variety of mechanisms. In response to the hypothyroid state, a compensatory increase in the discharge of central hypothalamic thyrotropin-releasing hormone occurs, which results in stimulation of prolactin (PRL) secretion. The role of TRH as a hypothalamic hypophysiotrophic hormone releasing TSH from the anterior pituitary gland is well-known but its role in the stimulation of PRL release from the anterior pituitary is still controversial [2-4]. Prolactin elimination from the systemic circulation is reduced in patients with primary hypothyroidism, which contributes to increased prolactin concentrations [5,6]. Many other reasons are speculated for increasing prolactin levels in hypothyroid patients.

In 1988 for the first time, an increase of serum prolactin was reported in a woman with carpal tunnel syndrome and subclinical hypothyroidism [7]. After that, one study showed the relationship between subclinical hypothyroidism, hyperprolactinemia and sterility [8].

While the prevalence of hyperprolactinaemia in overt hypothyroidism has been reported to be as high as 40%, its prevalence and clinical significance in subclinical hypothyroidism has only been reported in case reports and few studies [9,10].

Because of the variable results and low patient numbers in previous studies, we planned the present study with an intent to:

1) Estimate serum prolactin levels and determine the prevalence of hyperprolactinaemia in newly diagnosed subclinical and overt hypothyroid patients and
2) To evaluate the relationship between thyrotropin (TSH) and prolactin levels in subclinical and overt hypothyroid patients.

## MATERIALS AND METHODS

### Patients and Controls

The 200 consecutive patients presenting to endocrinology clinic of CSS Hospital, Meerut, India, from January 2011 to December 2012 for various thyroid related problems were approached for participation in the present study.

### Inclusion Criteria

The inclusion criteria for the selection of the cases were newly diagnosed cases of primary hypothyroidism. The relevant clinical history was taken from all the patients and the details were recorded in writing. These patients were then segregated into two groups subclinical and overt hypothyroidism according to their diagnosis based on history and clinical examination. Seventy five patients in each group were finally enrolled; all patients provided written informed consent for participation in the study; approved by the Ethics Committee of the hospital.

Similar number of age and sex matched healthy persons (neither having any thyroid problem nor with conditions affecting prolactin levels) were enrolled as controls.

### Exclusion Criteria

The patients excluded from the study were the ones who presented with one of the following criteria:

1. Those with clear medical reasons for hyperprolactinemia, such as lactating and pregnant women, liver or kidney disease, and the ones taking antidepressants, estrogens or antipsychotics.

2. All clinical or pharmacological causes of PRL elevation were ruled out.

A detailed questionnaire was designed for the evaluation of hypothyroid symptoms and all the subjects duly completed it.

**Laboratory Method for Thyroid Function and Prolactin Assessment:** Detailed assessment of thyroid function was done for all the enrolled subjects. Seven ml fasting blood sample was taken from each person, serum separated and preserved at – 20°C and then serum levels of TSH, total and free thyroxine (T4 & fT4), total and free triiodothyronine (T3 & fT3) & prolactin (PRL) were measured in all enrolled subjects by using a enzyme immunoassay competition method with a final fluorescent detector (ELFA) on mini VIDAS from Biomerieux, France.

The normal range for $T_3$ was taken as 0.92-2.33 nmol/l, for $fT_3$ as 4-8.3 pmol/l, for $T_4$ it was 60-120 nmol/l, for $fT_4$ as 9-20 nmol/l. TSH levels ranging between 0.25-5 µU/l was considered normal. These values are in accordance with commercially available kits from Biomerieux, France. Hypothyroidism was defined as subclinical if basal TSH was increased with normal $T_3$, fT3, $T_4$ and $fT_4$ and overt if basal TSH was increased with low $T_3$, fT3, $T_4$ and $fT_4$. The normal ranges of serum prolactin were 5-35 ng/mL for females and 3-25 ng/mL for males. Correlation of PRL levels with the severity of hypothyroidism (overt or subclinical) was performed.

## STATISTICAL ANALYSIS

Statistical analysis was done with Open Epi software, version 2.3 by using through Chi-Square test and oneway ANOVA with Bonferroni test as a post hoc test for pairwise comparison. Spearman's correlation was used to look for association between TSH and prolactin in the study group. A p-value <0.05 was considered statistically significant.

## RESULTS

Out of 200 patients, 50 patients who did not meet with inclusion criteria were excluded from study. Distribution of the subjects in relation to age and sex; in study and control groups is shown in [Table/Fig-1].

Study group (subclinical hypothyroidism and overt hypothyroidism) and control group were similar in regard to age and gender. PRL elevation was found in 16 patients (21.33 %) with overt hypothyroidism, and in six patients (8%) with subclinical hypothyroidism. Laboratory variables ($T_3$, $fT_3$, $T_4$, $fT_4$, TSH, prolactin levels) of the patients and controls are given in [Table/Fig-2].

The control group and subclinical hypothyroid patients exhibited no significant difference in terms of total and free $T_3$, total and free $T_4$. For TSH and prolactin on the other hand, a statistically significant elevation was found in patients with overt hypothyroidism when compared to subclinical hypothyroidism; and in patients with subclinical hypothyroidism when compared to the controls (p<0.001) [Table/Fig-2].

When the TSH levels were compared to PRL levels; in patients with subclinical hypothyroidism, correlation of (r=0.12, p=0.29) was found while in overt hypothyroid patients it was (r=0.02, p=0.85) suggesting no significant correlation between TSH and prolactin.

The control group and subclinical hypothyroid patients exhibited no significant difference in terms of clinical presentation while a significant statistical difference was observed between the two groups of hypothyroid patients for all hypothyroid symptoms except alopecia and hirsuitism. Comparison of symptoms between two groups of patients (subclinical and overt hypothyroid cases) and controls is shown in [Table/Fig-3] (p< 0.01).

## DISCUSSION

Hyperprolactinemia of variable magnitude (39% to 57%) has been reported in overt hypothyroidism in several studies; but research on

| Symptoms | Control (n=75) | Subclinical hypothyroidism (n=75) | Overt hypothyroidism (n=75) | p-value |
|---|---|---|---|---|
| Age (years) Mean±SD | 35.32 ± 7.43 | 35.07 ± 7.08 | 35.08 ± 4.8 | p= 0.96 (one way ANOVA) |
| Distribution (Female/Male) | 63/12 | 64/11 | 62/13 | |

**[Table/Fig-1]:** Showing distribution of subjects

| Lab parameters | Controls Mean ± SD | Subclinical hypothyroid Mean ± SD | Overt Hypothyroid Mean ± SD | p-value |
|---|---|---|---|---|
| $T_3$ | 1.37 ± 0.25 | 1.30 ± 0.21 | 0.62 ± 0.15 | Control = subclinical < overt <0.01* |
| $fT_3$ | 6.07 ± 1.08 | 5.8 ± 0.75 | 2.66 ± 0.51 | Control = subclinical < overt <0.01* |
| $T_4$ | 82.3 ± 12.7 | 78.7 ± 10.1 | 35.1 ± 7.8 | Control = subclinical < overt <0.01* |
| $fT_4$ | 14.5 ± 3.4 | 13.8 ± 1.8 | 5.8 ± 1.17 | Control = subclinical < overt <0.01* |
| TSH | 2.5 ± 1.42 | 7.5 ± 1.00 | 16.3 ± 7.08 | Control < subclinical < overt <0.01* |
| Prolactin | 8.2 ± 5.4 | 14.1 ± 8.1 | 27.9 ± 5.5 | Control < subclinical < overt <0.01* |
| Hyperprolactinemia (Female/Male) | 0 | 6 (8%) (5/1) | 16 (21.33%) (11/5) | p= 0.014 (chi-square test) |

**[Table/Fig-2]:** Showing correlation of Hyperprolactinemia with hypothyroidism
Normal values: As per manufacturer's [Biomeriux ] recommendations (T3: 0.92- 2.33 nmol/l; fT3: 4-8.3 pmol/l; T4: 60-120 nmol/l; fT4: 9-20 nmol/l; TSH: 0.25-5 µ unit/l; and Prolactin: F: 5-35 ng/ml; M: 3-25 ng/ml)
*One-way-ANOVA with Bonferroni test as a post hoc test for pairwise comparison was performed

| Symptoms | Control n (%) | Subclinical hypothyroidism n (%) | Overt hypothyroidism n (%) | p-value* |
|---|---|---|---|---|
| Fatigue | 3 (4 %) | 5 (6.67%) | 13 (17.33%) | Control = subclinical < overt = 0.02 |
| Dry skin | 2(2.67%) | 4 (5.33%) | 14 (18.67%) | Control = subclinical < overt = 0.005 |
| Cold intolerance | 3 (4 %) | 4 (5.33%) | 15 (20%) | Control = subclinical < overt = 0.003 |
| Constipation | 3 (4 %) | 5 (6.67%) | 11 (14.67 %) | Control = subclinical < overt = 0.05 |
| Weight gain | 2(2.67%) | 3 (4 %) | 16 (21.33%) | Control = subclinical < overt = 0.0007 |
| Alopecia | 3 (4 %) | 5 (6.67%) | 10 (13.33%) | Control = subclinical = overt |
| Hirsuitism | 2 (2.67%) | 3 (4 %) | 07 (9.33%) | Control = subclinical = overt |
| Muscle Cramps | 2 (2.67%) | 3 (4 %) | 10 (13.33%) | Control = subclinical < overt = 0.02 |
| Menstrual irregularities | 4 (5.33%) | 6 (8%) | 17 (22.67%) | Control = subclinical < overt = 0.006 |

**[Table/Fig-3]:** Showing comparison symptom wise
*p-value is based on chi-square test

the prevalence and extent of hyperprolactinemia in SCH are few and has different results [9-12].

Several mechanisms have been proposed for the increase in prolactin levels in primary hypothyroidism. First, elevated prolactin levels can be attributed to TRH which is a physiologic mediator of both PRL and TSH release [13]. A higher prevalence of hyperprolactinemia in hypothyroid females than males as observed in our study is because hypothyroidism *per se* is not sufficient to cause hyperprolactinemia and other stimulus, such as estrogen, is required for this effect [14,15]. Second, prolactin clearance may be decreased in hypothyroid patients [16]. Third, reduction in senstivity of prolactin production to the inhibitory action of dopamine and dopamine agonists as suggested by Foord et al., in their study on cultured anterior pituitary cells from hypothyroid rats [17]. Fourth, thyroid hormone itself may also play an important role in the cause of hyperprolactinaemia. Davis et al., noticed that 3,5,3'-triiodothyronine reduces prolactin messenger RNA levels in rodent pituitary cells [18]. Thus, decreased circulating thyroid hormone levels result in increased prolactin synthesis.

While peripheral hypothyroidism is a classical cause of hyperprolactinemia, hyperprolactinemia is rarely the presenting symptom of hypothyroidism. In the study by Raber et al., 84 out of 1003 (8%) hypothyroid patients presented with hyperprolactinemia [19]. Meier et al., in their study on 66 patients of subclinical hypothyroidism reported the prevalence of hyperprolactinemia as 19%. Further when these patients were randomly treated with placebo or L-thyroxine; prolactin levels returned to normal in the ones treated with L-thyroxine while PRL levels remained high in those who received placebo [20]. Several case series also report normalization of serum PRL levels after L-thyroxine treatment in patients with overt hypothyroidism but similar studies in patients with subclinical hypothyroidism are few [19-21].

Notsu et al., measured prolactin levels in 15 healthy controls and in 74 Hashimoto's thyroiditis patients of which 42 were euthyroid, 18 had subclinical and 14 had overt hypothyroidism and prolactin was found to be elevated in 42% of the overt hypothyroid patients, 11% in the subclinical hypothyroid patients, and 14% of the euthyroid patients [21].

In our study hyperprolactinaemia was more common in overt hypothyroidism patients (21%) than in those patients with subclinical hypothyroidism (8%). The serum PRL levels in subclinical hypothyroid group were significantly higher than those in controls and in overt hypothyroid patients when compared with subclinical hypothyroid individuals. When the TSH levels were compared to PRL levels in patients with subclinical hypothyroidism, correlation of (r=0.12, p=0.29) was found while in overt hypothyroid patients it was (r=0.02, p=0.85) suggesting no significant correlation between TSH and prolactin. Similar findings have been observed by Hekimsoy et al., in their study on 200 hypothyroid individuals [22].

Bahar A et al., in their study on subclinical hypothyroid patients for clinical related symptoms observed 23.5% menstrual disorders in hyperprolactinemic patients and 21.8% in normal prolactin groups [23]. According to Binita Goswami's study, amenorrhoea occurs in hypothyroidism due to hyperprolactinaemia which results from a defect in the positive feedback of oestrogen on LH, and because of LH and FSH suppression [24]. Supporting this view is the study by Turankar et al., who reported increase in the serum prolactin levels in infertile women as compared to those in the fertile ones in the control group [25]. In our study as well we observed raised PRL concentration in 4 out of 6 subclinically hypothyroid women with menstrual disorders and 10 out of 17 females with overt hypothyroidism.

The increase in the incidence of hyperprolactinemia even in patients with subclinical hypothyroidism emphasizes the significance of prolactin screening in all hypothyroid cases. If thyroid tests are normal in a patient with high PRL levels, further tests should be done to determine the aetiology of the hyperprolactinaemia.

## CONCLUSION

PRL regulation is altered, not just in patients with overt hypothyroidism, but in those with subclinical hypothyroidism as well. Thyroid function tests should be performed in patients with hyperprolactinaemia before performing further tests.

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