Section: Biochemistry

Original Research Article

A STUDY ON EVALUATION OF THYROID STIMULATING HORMONE (TSH), FREET3, FREET4, PROLACTIN, THYROID PEROXIDASE ANTIBODY AND MACROPROLACTIN IN SUBCLINICAL HYPOTHYROIDISM

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Received *: 17/07/2025*
Received in revised form *: 04/09/2025*
Accepted *: 20/09/2025*

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DOI: 10.70034/ijmedph.2025.3.574

Source of Support: Nil,
Conflict of Interest: None declared

Int J Med Pub Health
2025; 15 (3); 3129-3134

ABSTRACT

Background: Informed consent ensures participants understand study objectives and procedures. Subclinical hypothyroidism (ScH) is characterised by elevated thyroid-stimulating hormone (TSH) with normal FT3 and FT4. This may affect metabolic, cardiovascular, and reproductive health. This study aimed to evaluate TSH, FT3, FT4, prolactin, thyroid peroxidase antibody (ATPO), and macroprolactin in subclinical hypothyroid patients.

Materials and Methods: A case-control study was conducted over 12 months at Karpagam Faculty of Medical Sciences and Research, Coimbatore, including 118 subclinical hypothyroid patients and 118 age- and sex-matched controls aged ≥18 years. Serum TSH, FT3, FT4, ATPO, and prolactin were measured using Enzyme-Linked Fluorescent Assay (ELFA). Macroprolactin was estimated using polyethene glycol precipitation in patients with elevated prolactin. Statistical analyses were performed using SPSS version 13.

Results: The mean age was 38.32±13.34 years in cases and 38.58±14.15 years in controls (P=0.89). Females constituted 92.4%of the cases, and 88.1%of FT3 levels of 2.1–4.48 pg/ml were observed in 102(86.4%) cases and 2 (1.7%) controls (P<0.001). FT4 levels of 0.8–2.7 ng/ml were seen in 105 (89.0%) cases and 81(68.6%) controls (P<0.001). TSH was >4.20 IU/ml in all cases (118;100%) and 112 (94.9%) controls (P=0.020). ATPO positivity was present in 67(56.8%) cases and absent in controls (P<0.001). Prolactin elevation was observed in 7 (5.9%) cases, with 7(10.4%) ATPO-positive cases showing prolactin rise. No macroprolactinemia was detected in cases. Clinical features included cold intolerance 91(77.1%), alopecia 76(64.4%), weight gain 85(72.0%), and constipation 111(94.1%).

Conclusion: ScH is associated with elevated TSH and ATPO, while FT3 and FT4 remain normal. Prolactin elevation is infrequent, and macroprolactin is absent, suggesting a link between thyroid autoimmunity and mild prolactin changes.

Keywords: Subclinical hypothyroidism; TSH; Prolactin; ATPO; Macroprolactin

INTRODUCTION

Subclinical hypothyroidism (ScH) is an endocrine disorder characterised by elevated serum thyroid-stimulating hormone (TSH) levels in the presence of normal free thyroxine (FT4) and free triiodothyronine (FT3) concentrations. It is often asymptomatic and remains undiagnosed unless detected by biochemical screening. The global prevalence of ScH varies from 3% to 15%, with a higher incidence observed in women and elderly populations.[1] The clinical importance of ScH is

increasingly recognised, as studies indicate its association with adverse cardiovascular, metabolic, and reproductive outcomes.[2,3]

Thyroid hormones, primarily thyroxine (T4) and triiodothyronine (T3), play crucial roles in the regulation of metabolism, growth, and development. These hormones exert their effects by entering target cells, binding to nuclear receptors, and modulating gene expression to influence cellular energy expenditure and protein synthesis.[4] The hypothalamic-pituitary-thyroid (HPT) axis tightly regulates thyroid hormone synthesis and secretion. Thyrotropin-releasing hormone (TRH) from the hypothalamus stimulates the anterior pituitary to release TSH, which in turn promotes thyroid hormone production in the thyroid gland.[5]

Hyperprolactinemia, defined as elevated serum prolactin levels above the normal range, is one of the most common endocrine disorders affecting reproductive function.[6] Prolactin secretion is primarily inhibited by dopamine and stimulated by TRH.[7] In hypothyroidism, increased TRH release leads to elevated prolactin secretion, resulting in hyperprolactinemia. This condition has been associated with menstrual irregularities, infertility, galactorrhea, and hypogonadism in both men and women.[8]

The relationship between overt hypothyroidism and hyperprolactinemia is well established; however, the association between ScHand hyperprolactinemia remains poorly understood. Several studies have documented a higher prevalence of hyperprolactinemia in overt hypothyroidism, ranging up to 22%, while limited data are available for ScH.[9,10] One study reported hyperprolactinemia in 8% of patients with ScH, suggesting a possible link between mild thyroid dysfunction and elevated prolactin levels. However, other research has shown inconsistent findings, with some studies reporting no significant correlation between TSH and prolactin levels in ScH patients, indicating the need for further investigation.[11]

A critical confounding factor in diagnosing hyperprolactinemia is macroprolactin, a biologically inactive, high-molecular-weight complex of prolactin bound to immunoglobulin G (IgG). Macroprolactin is immunoreactive and detectable by conventional prolactin immunoassays, often causing false-positive hyperprolactinemia diagnoses.[12] The polyethene glycol (PEG) precipitation method is commonly used to distinguish macroprolactin from monomeric prolactin, allowing clinicians to avoid unnecessary diagnostic procedures and inappropriate treatments.[13]

Aim: This study aimed to determine the relationship between serum prolactin and TSH levels, study the prevalence of hyperprolactinemia in ScH, assess the relationship between anti-thyroid peroxidase antibody (ATPO) and prolactin levels in subclinical hypothyroid patients, and estimate the presence of macroprolactin in subclinical hypothyroid cases with elevated prolactin levels.

MATERIALS AND METHODS

This case-control study was conducted with 236 patients at the Karpagam Faculty of Medical Sciences and Research, Coimbatore, over a period of 12 months, from October 2016 to September 2017. The study protocol was approved by the Institutional Ethics Committee, and written informed consent was obtained from all patients before enrolment.

Inclusion criteria

Patients aged ≥ 18 years who were newly diagnosed with ScH (defined by elevated TSH levels with normal free T3 and free T4 levels) were included.

Exclusion criteria

Patients with known hypothyroidism already on thyroid hormone replacement, pregnancy, pituitary tumours, chronic kidney or liver disease, history of psychiatric illness, known macroprolactinemia, or those on medications known to alter prolactin or thyroid hormone levels (such as antipsychotics, dopamine antagonists, or thyrostatic drugs) were excluded.

Methods: Data were collected using a predesigned case-record proforma. A detailed clinical history was obtained, focusing on symptoms of thyroid dysfunction, reproductive health disturbances, and a history of autoimmune diseases. The physical examination included a general assessment, thyroid gland palpation, and signs suggestive of hypothyroidism or hyperprolactinemia.

Fasting blood samples were collected from all the participants. Serum levels of TSH, free T3, free T4, ATPO, and prolactin were measured using Enzyme-Linked Fluorescent Assay (ELFA) on the mini VIDAS automated platform. The reference ranges were as follows: TSH (0.4–4.0 mIU/L), Free T3 (2.0–4.4 pg/mL), Free T4 (0.8–2.0 ng/dL), ATPO (<35 IU/mL), and prolactin (male: 3–25 ng/mL, Female: 5–35 ng/mL).

In patients with elevated prolactin levels, macroprolactin levels were estimated using the PEG precipitation method. A prolactin recovery rate of <40% after PEG precipitation was considered indicative of macroprolactinemia.

Statistical analysis: Data were entered into Microsoft Excel and analysed using SPSS version 13. Continuous variables are expressed as mean±standard deviation, and categorical variables as frequency and percentage. The chi-square test was applied to assess the associations between categorical variables, while the Student's t-test was used to compare the continuous variables between the cases and controls. A p-value of <0.05 was considered significant.

RESULTS

The mean age was 38.32±13.34 years in the subclinical hypothyroid group and 38.58±14.15 years in the control group (P=0.89). Among cases, age distribution was 10–20 yrs: 8 (6.8%), 20–35 yrs: 45 (38.1%), 36–50 yrs: 51 (43.2%), 51–65 yrs: 8 (6.8%),

and >65 yrs: 6 (5.1%). In controls, it was 10–20 yrs: 10 (8.5%), 20–35 yrs: 43 (36.4%), 36–50 yrs: 47 (39.8%), 51–65 yrs: 10 (8.5%), and >65 yrs: 8 (6.8%). The study included 109 (92.4%) female and 104 (88.1%) males were 9 (7.6%) female and 14 (11.9%) male controls [Table 1].

Table 1: Comparison of age and gender between groups

		Subclinical hypothyroid	Controls	p value
Age (years)	10–20	8 (6.8%)	10 (8.5%)	
	20–35	45 (38.1%)	43 (36.4%)	
	36–50	51 (43.2%)	47 (39.8%)	
	51–65	8 (6.8%)	10 (8.5%)	
	>65	6 (5.1%)	8 (6.8%)	
Mean ± SD		38.32 ± 13.34	38.58 ± 14.15	0.89
Gender	Female	109 (92.4%)	104 (88.1%)	-
	Male	9 (7.6%)	14 (11.9%)	

FT3 and FT4 levels differed significantly between groups (P<0.001), with most subclinical hypothyroid cases having FT3 within 2.1–4.48 pg/ml (102, 86.4%) and FT4 within 0.8–2.7 ng/ml (105, 89.0%), whereas most controls had low FT3 (116, 98.3%) and some had high FT4 (32, 27.1%). TSH was elevated (>4.20 IU/ml) in all cases (118, 100%) versus 112 (94.9%) controls (P=0.020). ATPO levels were elevated (>35 IU/ml) in 67 (56.8%) cases, with normal levels in all controls (P<0.001). Prolactin levels were similar (P=0.072), while macroprolactin differed significantly, with most cases normal (102, 99%) and six controls (85.7%) elevated (P<0.001) [Table 2].

Table 2: Comparison of thyroid profile, prolactin, ATPO, and macroprolactin levels between groups

Parameter	Category	Subclinical hypothyroid	Controls	p value
FT3 (pg/ml)	<2.1	14 (11.9%)	116 (98.3%)	<0.001
	2.1–4.48	102 (86.4%)	2 (1.7%)	
	>4.48	2 (1.7%)	0	
FT4 (ng/ml)	<0.8	10 (8.5%)	5 (4.2%)	<0.001
	0.8–2.7	105 (89.0%)	81 (68.6%)	
	>2.7	3 (2.5%)	32 (27.1%)	
TSH (IU/ml)	<0.27	0	2 (1.7%)	0.020
	0.27–4.20	0	4 (3.4%)	
	>4.20	118 (100%)	112 (94.9%)	
Prolactin (ng/dl)	<5	8 (6.8%)	18 (15.3%)	0.072
	5–40	103 (87.3%)	90 (76.3%)	
	>40	7 (5.9%)	10 (8.5%)	
ATPO (IU/ml)	<35	51 (43.2%)	118 (100%)	-
	>35	67 (56.8%)	0	
Macroprolactin (ng/dl)	<5	6 (75%)	2 (25%)	<0.001
	5–40	102 (99%)	1 (1%)	
	>40	1 (14.3%)	6 (85.7%)	

Mean FT3 levels were higher in the subclinical hypothyroid group (2.67±0.65 pg/ml) compared to controls (1.36±0.31 pg/ml, P<0.001). Mean FT4 was also higher in cases (6.05±6.24 ng/ml) versus controls (2.14±0.86 ng/ml, P<0.001). Mean TSH was slightly lower in cases (12.52±10.76 IU/ml) than controls (15.02±10.08 IU/ml, P=0.06). Mean prolactin levels were similar (18.94±21.99 ng/dl vs. 18.46±13.21 ng/dl, P=0.84), while mean ATPO levels were markedly higher in cases (293.69±376.47 IU/ml) than controls (0.00 IU/ml, P<0.001) [Table 3].

Table 3: Comparison of mean hormonal levels between groups

Parameter (Range)	Subclinical hypothyroid	Controls	p value
FT3 (2.1–4.48 pg/ml)	2.67 ± 0.65	1.36 ± 0.31	<0.001
FT4 (0.8–2.7 ng/ml)	6.05 ± 6.24	2.14 ± 0.86	<0.001
TSH (0.27–4.20 IU/ml)	12.52 ± 10.76	15.02 ± 10.08	0.06
Prolactin (5–40 ng/dl)	18.94 ± 21.99	18.46 ± 13.21	0.84
ATPO (<35 IU/ml)	293.69 ± 376.47	0.00 ± 0.00	<0.001

Cold intolerance was reported in 91 (77.1%) cases and 108 (91.5%) controls (P=0.002). Menstrual irregularities occurred in 70 (59.3%) and 116 (98.3%) cases and controls, respectively (P<0.001), whereas dry skin was present in 72 (61.0%) and 104 (88.1%) cases and controls, respectively (P<0.001). Alopecia was seen in 76 (64.4%) cases but absent in controls (P<0.001). Hirsutism was present in 116 (98.3%) cases and absent in the controls (P=0.095). Weight gain occurred in 85 (72.0%) cases and none in the controls (P<0.001). Constipation was observed in 111 (94.1%) patients and none in the controls (P=0.002), and fatigue was reported in 66 (55.9%) patients, with no cases among the controls (P<0.001) [Table 4].

Table 4: Comparison of clinical symptoms between groups

Symptom	Subclinical hypothyroid	Controls	p value
Cold intolerance	91 (77.1%)	108 (91.5%)	0.002
Menstrual irregularities	70 (59.3%)	116 (98.3%)	<0.001
Dry skin	72 (61%)	104 (88.1%)	<0.001
Alopecia	76 (64.4%)	0	<0.001
Hirsutism	116 (98.3%)	0	0.095
Weight gain	85 (72%)	0	<0.001
Constipation	111 (94.1%)	0	0.002
Fatigue	66 (55.9%)	0	<0.001

Anti-TPO positivity was higher in cases (67, 56.8%) than in controls (15, 12.7%). Prolactin positivity was observed in seven (5.9%) cases, and among the 67 anti-TPO-positive cases, seven (10.4%) were prolactin-positive. No macroprolactin was detected in the prolactin-positive cases [Table 5].

Table 5: Comparison of ATPO positivity, prolactin, and macroprolactin status between groups

Parameter	Subclinical hypothyroid	Controls
ATPO positivity	67 (56.8%)	15 (12.7%)
Prolactin-positive cases	7 (5.9%)	–
Prolactin positive among ATPO+ cases (n=67)	7 (10.4%)	–
Macroprolactin in prolactin+ cases	Nil	–

DISCUSSION

In our study, there was no significant difference in the mean age between the subclinical hypothyroid and control groups, with most participants being middle-aged and predominantly female. Similarly, El-Ghany et al. reported mean ages of 30.51±6.74 years for group A, 30.26±6.21 years for group B, and 30.68±7.12 years for group C (P=0.905), with female predominance of 90%, 89%, and 80%.14 Likewise, Goel et al. observed mean ages of 35.32±7.43 years in controls, 35.07±7.08 years in subclinical, and 35.08±4.8 years in overt hypothyroid (P=0.96), with female predominance (63/12, 64/11, 62/13).15 In addition, Sahu et al. reported mean ages of 36.24±8.02 years in overt, 36.14±7.06 years in subclinical, and 36.16±7.1 years in controls (P=0.32), with consistent female predominance (48/8, 48/7, 48/10).[16] Similarly, Hekimsoy et al. found mean ages of 42.9±12.6 years in subclinical, 45.3±12.2 years in overt, and 43.9±11.4 years in controls (P=0.4), with female percentages of 89.1%, 84.9%, and 85.9% (P=0.6).[10] Age distribution was similar between subclinical hypothyroid and control groups, with most participants being middle-aged females, consistent with findings from previous studies.

In our study, FT3, FT4, and TSH levels differed significantly between the groups, with cases showing higher TSH and ATPO positivity than the controls. Macroprolactin levels also differed, with most cases being normal and many controls being elevated. Similarly, El-Ghany et al. reported higher TSH in group B (8.80±0.75) versus group A (5.84±0.87) and controls (2.07±1.04, P<0.001), lower FT4 in cases (1.09±0.12) than controls (1.27±0.17, P<0.001), and slightly lower FT3 in cases (1.14±0.12) versus controls (1.25±0.15, P<0.001), with Anti-TPO positivity 45% in cases and 15% in controls (P<0.001).[14]

Likewise, Hekimsoy et al. reported lower FT3 in overt hypothyroidism (1.8±0.9 pg/ml) than subclinical (3.0±0.5 pg/ml) and controls (3.1±0.3 pg/ml, P<0.001), reduced FT4 in overt cases (0.4±0.3 ng/dL) versus subclinical (1.0±0.2 ng/dL) and controls (0.9±0.2 ng/dL, P<0.001), highest TSH in overt cases (85.2±64.2 µIU/ml) followed by subclinical (9.5±5.9 µIU/ml) and controls (1.4±0.9 µIU/ml, P<0.001), and Anti-TPO positivity of 63.9% in subclinical, 77.4% in overt, and absent in controls (P=0.001).10 Thyroid function tests and Anti-TPO positivity were significantly altered in subclinical hypothyroid cases, showing higher TSH and ATPO levels and lower FT3/FT4 compared to controls, consistent with previous studies.

In our study, the cases showed higher FT3, FT4, and ATPO levels than the controls, indicating thyroid dysfunction and autoimmune involvement in ScH, whereas TSH and prolactin levels were similar. Similarly, Yoo et al. reported a median basal TSH of 5.53 µIU/ml overall, rising across subgroups and exceeding 8.0 µIU/ml in Group 6, where 80% showed exaggerated TRH responses; ΔTSH correlated positively with basal TSH (r=0.320, P<0.001).[17] Likewise, Hekimsoy et al. reported FT3 of 3.0±0.5 pg/ml in subclinical, 1.8±0.9 pg/ml in overt, and 3.1±0.3 pg/ml in controls, FT4 of 1.0±0.2, 0.4±0.3, and 0.9±0.2 ng/dL respectively, with TSH highest in overt cases (85.2±64.2 µIU/ml) versus subclinical (9.5±5.9 µIU/ml) and controls (1.4±0.9 µIU/ml).[10] Subclinical hypothyroid cases showed evidence of thyroid dysfunction and autoimmune activity with higher FT3, FT4, and ATPO levels, while TSH and prolactin remained similar to controls, aligning with previous research findings.

In our study, cases commonly reported alopecia, weight gain, constipation, and fatigue, while controls did not; cold intolerance, menstrual irregularities, and dry skin were more frequent among the controls. Similarly, El-Ghany et al. reported high prevalence of menstrual irregularities (53%), hair loss (50%), fatigue and laziness (47%), bowel disturbance (34%), and alopecia (13%), along with puffiness (29%),

pedal edema (20%), and infertility (34%) in cases.[14] And, Goel et al. observed increasing symptoms with hypothyroidism severity: subclinical cases had fatigue (6.67%), dry skin (5.33%), cold intolerance (5.33%), constipation (6.67%), weight gain (4%), alopecia (6.67%), muscle cramps (4%), and menstrual irregularities (8%), while overt cases had higher rates: fatigue (17.33%), dry skin (18.67%), cold intolerance (20%), weight gain (21.33%), constipation (14.67%), and menstrual irregularities (22.67%).[15]

Similarly, Priya et al. reported fatigue (84.8%), cold intolerance (76%), constipation (65.2%), and depression (59.8%) as the most frequent symptoms in SCH+ patients, with much lower prevalence in controls.18 Subclinical hypothyroid cases commonly experienced alopecia, weight gain, constipation, and fatigue, while controls showed fewer such symptoms, consistent with prior studies reporting higher symptom prevalence in cases.

In our study, ATPO positivity was higher in cases than in controls, with a small proportion of ATPO-positive cases showing elevated prolactin levels and no macroprolactin detected. Similarly, El-Ghany et al. observed hyperprolactinemia in 30% of hypothyroid and 15% of subclinical cases (P<0.001), with a significant association between positive anti-TPO and hyperprolactinemia (71.1% vs.31%, P<0.001) and positive correlations with TSH, anti-TPO, and BMI.[14] And, Goel et al. reported higher prolactin in subclinical (14.1±8.1 ng/ml) and overt cases (27.9±5.5 ng/ml) versus controls (8.2±5.4 ng/ml, P<0.01), with hyperprolactinemia in 8% and 21.33%, respectively (P=0.014).[15]

Similarly, Hekimsoy et al. found Anti-TPO positivity in 63.9% of subclinical and 77.4% of overt cases, with mean prolactin of 17.6±10.5 ng/ml, 21.8±13.7 ng/ml, and 12.4±4.8 ng/ml in subclinical, overt, and controls, respectively (P<0.001), and hyperprolactinemia in 22% and 36%.[10] Similarly, Yoo et al. reported overall TPOAb positivity of 16%, increasing with basal TSH, reaching 42.4% in Group 6 (TSH>9.01 µIU/ml), with thyroid autoimmunity in 54.5% of high-TSH subjects (P=0.004).[17] ATPO positivity was higher in subclinical hypothyroid cases, with a small proportion showing elevated prolactin and no macroprolactin, reflecting a link between thyroid autoimmunity and mild hyperprolactinemia, consistent with previous studies.

Limitations

This was a single-centre study with a relatively small sample size, which may limit the generalisability of the findings. Additionally, the cross-sectional design did not allow long-term follow-up to assess changes over time.

CONCLUSION

TSH and anti-TPO antibody levels were significantly elevated in patients with ScH compared to those in the controls, whereas FT3 and FT4 levels remained within the normal range. Prolactin elevation was observed in a small proportion of cases; however, macroprolactinemia was absent. These findings suggest a possible link between thyroid dysfunction, autoimmunity, and prolactin levels in ScH. Future multicentre studies with larger populations and longitudinal follow-ups are needed to better understand the relationship between thyroid dysfunction, autoimmunity, and prolactin changes.

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