

Correlation of Thyroid Hormone Levels with Cytological Findings in Thyroid Lesions: A Cross-Sectional Study

Tirthankar Biswas¹, Jyoti Paul²

¹Associate Professor, Department of Pathology, JMN Medical College, Chakdaha, India

²Associate Professor, Department of Biochemistry, JMN Medical College, Chakdaha, India

Received: 14-06-2026 / Revised: 10-07-2026 / Accepted: 25-07-2026

Corresponding Author: Dr. Jyoti Paul

Conflict of interest: Nil

Abstract:

Background: Thyroid lesions are among the most prevalent endocrine disorders worldwide, presenting as diffuse or nodular thyroid enlargement. Thyroid function tests (TFTs — serum T3, T4, and TSH) evaluate the functional status of the thyroid gland, whereas fine needle aspiration cytology (FNAC) provides morphological characterisation of thyroid lesions.

Objective: To evaluate the correlation between thyroid hormone levels and cytological findings in patients with thyroid lesions.

Methods: This observational cross-sectional study was conducted over one year (January–December 2025) in the Departments of Biochemistry and Pathology, JMN Medical College, Chakdaha, India. One hundred and two patients presenting with thyroid swelling who underwent both TFT and FNAC were enrolled. Serum T3, T4, and TSH levels were measured and correlated with FNAC diagnoses. Data were analysed using descriptive statistics and one-way ANOVA; a p-value <0.05 was considered statistically significant.

Results: Nodular goitre was the most frequent cytological diagnosis (n=85; 83.3%). The majority of patients were euthyroid. Serum T4 and T3 levels differed significantly across cytological groups (p=0.035 and p=0.016, respectively), whereas TSH levels did not (p=0.730).

Conclusion: TFTs reflect the functional status of the thyroid gland but cannot independently predict cytological diagnosis. FNAC remains indispensable for the morphological characterisation of thyroid lesions. An integrated approach combining clinical, biochemical, and cytological assessment is essential for accurate diagnosis and appropriate clinical management.

Keywords: Thyroid Function Tests; Fine Needle Aspiration Cytology; Thyroid Lesions; Nodular Goitre; TSH; T3; T4.

DOI: 10.25258/ijpqa.17.7.39

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided original work is properly credited.

Introduction

The thyroid gland is the largest purely endocrine organ in the body and plays a pivotal role in regulating metabolism, thermogenesis, growth, and development through the secretion of two key hormones — triiodothyronine (T3) and thyroxine (T4). The synthesis and release of these hormones are regulated by the hypothalamic–pituitary–thyroid axis via a classic negative feedback loop, with thyroid-stimulating hormone (TSH) serving as the principal regulator at the level of the anterior pituitary [1].

Disruption of thyroid hormone production gives rise to a spectrum of disorders, including hypothyroidism, hyperthyroidism, autoimmune thyroiditis, and nodular or neoplastic thyroid disease [2]. Thyroid nodules are particularly common; palpable nodules are detectable in 4–10% of the general population, whereas ultrasonographic and

autopsy studies report a prevalence as high as 50% [3]. Although the vast majority of nodules are benign, approximately 5–10% harbour malignancy, necessitating timely and accurate diagnostic evaluation [4].

The aetiology of thyroid lesions is multifactorial, encompassing iodine deficiency, autoimmune disorders, genetic predisposition, environmental factors, and hormonal dysregulation [5]. Lesions may be associated with euthyroidism, hyperthyroidism, or hypothyroidism, depending on the extent of functional thyroid tissue involvement. Accordingly, thyroid function assessment — comprising serum T3, T4, and TSH estimation — constitutes a fundamental component of the diagnostic evaluation of patients presenting with thyroid swelling [6].

Serum TSH is regarded as the most sensitive marker of thyroid functional status. Elevated TSH is characteristic of hypothyroidism, whereas suppressed TSH indicates hyperthyroidism; free T3 and T4 measurements serve to confirm the diagnosis and gauge disease severity [6]. However, biochemical assessment alone cannot characterise the morphological nature of a thyroid lesion. Fine needle aspiration cytology (FNAC) has therefore emerged as the gold-standard first-line investigation for the pre-operative evaluation of thyroid nodules, offering a safe, minimally invasive, cost-effective, and highly accurate means of distinguishing benign from malignant disease [7].

FNAC enables classification of thyroid lesions into non-neoplastic categories — such as colloid goitre, nodular goitre, Hashimoto's thyroiditis, and hyperplastic nodules — and neoplastic categories, including follicular neoplasms, papillary carcinoma, medullary carcinoma, and anaplastic carcinoma. The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) provides a standardised framework for cytological reporting and guides clinical decision-making [8]. The functional behaviour of various thyroid lesions may differ according to the extent of parenchymal involvement and the autonomous secretory activity of the lesion [8].

Although both TFTs and FNAC are widely employed in clinical practice, the relationship between biochemical thyroid function and cytological diagnoses remains incompletely characterised. Several studies have suggested that inflammatory and autoimmune lesions are more frequently associated with thyroid hormone abnormalities, whereas malignant lesions are typically identified in euthyroid patients [9]. An integrated clinicopathological approach that combines hormonal and cytological data may therefore enhance diagnostic accuracy and inform clinical management decisions [10].

The present study was undertaken to systematically evaluate the correlation between TFT profiles and FNAC findings across the spectrum of thyroid lesions, with the goal of improving diagnostic interpretation and advancing our understanding of the clinicopathological behaviour of thyroid disease.

Materials and Methods

Study Design and Setting: This observational cross-sectional study was conducted in the Departments of Biochemistry and Pathology, JMN Medical College, Chakdaha, West Bengal, India. The study period extended over 12 months (January 2025 to December 2025).

Study Population: A consecutive sample of 102 patients presenting with thyroid swelling — either nodular or diffuse — who were referred for

concurrent FNAC and TFT evaluation were enrolled. The sample size was determined by the number of eligible patients presenting during the study period.

Inclusion and Exclusion Criteria: Patients were eligible for inclusion if they: (i) presented with clinically detectable thyroid swelling or suspected thyroid lesion; (ii) were referred by the treating clinician for FNAC; (iii) had serum T3, T4, and TSH measured as part of the clinical workup; and (iv) provided written informed consent. Either sex was eligible.

Patients were excluded if they: (i) were receiving treatment for a pre-existing thyroid disorder; (ii) had a history of thyroid surgery or radioiodine therapy; (iii) had inadequate FNAC material for cytological assessment; (iv) had incomplete clinical, biochemical, or cytological records; or (v) were unable or unwilling to provide informed consent.

Thyroid Function Tests: Venous blood (approximately 5 mL) was collected under aseptic conditions in a plain (red-top) vacutainer tube. After allowing the sample to clot, serum was separated by centrifugation at 3000 rpm for 10 minutes. Serum concentrations of T3, T4, and TSH were measured in the Department of Biochemistry using standardised laboratory methods. Reference ranges applied were: T3: 1.12–2.66 nmol/L, T4: 67–163 nmol/L, and TSH: 0.6–4.5 μ IU/mL. Patients were categorised as euthyroid, hyperthyroid, hypothyroid, subclinical hyperthyroid, subclinical hypothyroid, or biochemically euthyroid (defined as TFTs within the normal range in the context of clinical features or borderline hormonal values) on the basis of these reference values.

Fine Needle Aspiration Cytology: FNAC was performed from the thyroid swelling under aseptic precautions without ultrasound guidance, in accordance with routine departmental protocol. Four smears were prepared from the aspirated material: two were stained using the Papanicolaou technique and two using haematoxylin and eosin (H&E). Cytological diagnosis was made by an experienced cytopathologist in the Department of Pathology. Lesions were classified as nodular goitre, thyroiditis, papillary carcinoma, atypia, thyroglossal cyst, or non-diagnostic, consistent with established cytological categories.

Statistical Analysis: All data were entered into Microsoft Excel and analysed using SPSS (IBM, version 25.0). Categorical variables are presented as frequencies and percentages. Continuous variables (serum T3, T4, TSH) are presented as mean $\pm$ standard deviation (SD). Differences in mean hormone levels across FNAC diagnostic groups were assessed using one-way analysis of variance (ANOVA). A p-value of <0.05 was considered

statistically significant, with a 95% confidence interval.

Results

Distribution of FNAC Diagnoses: Table 1 summarises the distribution of FNAC diagnoses among the 102 patients enrolled. Nodular goitre was

the predominant diagnosis, accounting for 85 cases (83.3%). Non-diagnostic samples comprised 7 cases (6.9%). Papillary carcinoma and atypia were each identified in 2 patients (2.0%), and thyroglossal cyst and thyroiditis were each diagnosed in 3 patients (2.9%).

Table 1: Distribution of FNAC diagnoses (n = 102)

FNA Cytology Diagnosis	No. of patients	Percentage (%)
Nodular goitre	85	83.3
Non-diagnostic sample	7	6.9
Papillary carcinoma	2	2.0
Atypia	2	2.0
Thyroglossal cyst	3	2.9
Thyroiditis	3	2.9
Total	102	100.0

FNAC, fine needle aspiration cytology.

Hormonal Profiles by Cytological Diagnosis: The hormonal profiles corresponding to each FNAC diagnostic category are detailed in Table 2. Among patients with nodular goitre (n=85), euthyroidism was the most frequent status, observed in 49 patients (48.0%), with mean T4, T3, and TSH levels of 108.50 ± 27.18 nmol/L, 2.07 ± 0.52 nmol/L, and 1.47 ± 0.91 μ IU/mL, respectively. Hyperthyroidism was identified in 12 patients (11.8%) and biochemical euthyroidism — characterised by elevated T3/T4 with normal TSH — in 17 patients

(16.7%). Subclinical hypothyroidism and subclinical hyperthyroidism were noted in 2 (2.0%) and 5 (4.9%) patients, respectively.

All 7 patients (6.9%) with non-diagnostic samples were euthyroid. Patients with thyroglossal cyst, atypia, and papillary carcinoma were predominantly euthyroid. The 3 patients with thyroiditis demonstrated biochemical euthyroidism with markedly elevated T4 (mean 219.00 nmol/L) and T3 (mean 3.93 nmol/L) values, suggesting subclinical or early hyperthyroidism secondary to thyroid follicular disruption.

Table 2: Hormonal profile by FNAC diagnosis (n = 102)

FNA Cytology	Functional Status	n (%)	T4 (nmol/L) Mean $\pm$ SD	T3 (nmol/L) Mean $\pm$ SD	TSH (μ IU/mL) Mean $\pm$ SD
Nodular goitre	Euthyroidism	49 (48.0)	108.50 ± 27.18	2.07 ± 0.52	1.47 ± 0.91
	Hyperthyroidism	12 (11.8)	214.40 ± 60.61	3.65 ± 1.47	0.11 ± 0.08
	Biochemical euthyroidism	17 (16.7)	178.10 ± 32.80	3.38 ± 0.54	2.18 ± 1.17
	Subclinical hypothyroidism	2 (2.0)	141.00	2.20	15.00
	Subclinical hyperthyroidism	5 (4.9)	90.75 ± 25.81	1.87 ± 0.11	<0.05
Non-diagnostic sample	Euthyroidism	7 (6.9)	87.10 ± 5.00	1.64 ± 0.44	2.46 ± 1.86
Thyroglossal cyst	Euthyroidism	3 (2.9)	129.00	1.85	3.40
Atypia	Euthyroidism	2 (2.0)	128.00	1.70	4.30
Papillary carcinoma	Euthyroidism	2 (2.0)	129.00	2.00	1.90
Thyroiditis	Biochemical euthyroidism	3 (2.9)	219.00	3.93	1.80

T3, triiodothyronine; T4, thyroxine; TSH, thyroid-stimulating hormone; SD, standard deviation; FNAC, fine needle aspiration cytology.

ANOVA of Hormone Levels Across Cytological Groups: One-way ANOVA results for serum T4,

T3, and TSH levels across the six FNAC diagnostic groups are presented in Table 3. Serum T4 levels differed significantly between groups ($F=2.52$, $p=0.035$), as did serum T3 levels ($F=2.94$, $p=0.016$). In contrast, TSH levels did not differ significantly across groups ($F=0.56$, $p=0.730$).

Table 3: One-way ANOVA of serum T4, T3, and TSH levels across FNAC diagnostic groups

Hormone	Source of Variation	df	Mean Square	F-value	p-value
T4 (nmol/L)	Between groups	5	6063.55	2.52	0.035*
	Within groups	96	2410.83	—	—
	Total	101	—	—	—
T3 (nmol/L)	Between groups	5	2.33	2.94	0.016*
	Within groups	96	0.79	—	—
	Total	101	—	—	—
TSH (μ IU/mL)	Between groups	5	3.17	0.56	0.730
	Within groups	96	5.66	—	—
	Total	101	—	—	—

* Statistically significant ($p < 0.05$). df, degrees of freedom; ANOVA, analysis of variance.

Discussion

The present study evaluated the correlation between TFT profiles and cytological findings in a consecutive series of 102 patients undergoing FNAC for thyroid lesions. The key findings are: (i) nodular goitre was by far the most common cytological diagnosis; (ii) the majority of patients across all diagnostic categories were euthyroid; (iii) serum T4 and T3 levels differed significantly between cytological groups, whereas TSH did not; and (iv) normal TFTs were observed across the full spectrum of lesions, including malignancy.

The predominance of nodular goitre (83.3%) in our series is consistent with previously published data from the Indian subcontinent and other iodine-deficient regions, where structural thyroid disease represents the most common cause of thyroid swelling [11]. FNAC is well-established as the investigation of first choice in the evaluation of thyroid nodules, providing a reliable pre-operative distinction between benign and malignant disease without the morbidity of surgical biopsy [7,12].

The finding that the majority of patients with nodular goitre were euthyroid is in agreement with the observations of Hegedüs and Gharib et al., who demonstrated that most benign thyroid nodules do not significantly alter thyroid hormone homeostasis [4,13]. Structural enlargement may occur in the setting of preserved functional parenchyma, particularly in the early stages of iodine deficiency-related goitre. The subset of patients exhibiting hyperthyroidism or subclinical hyperthyroidism likely represents autonomously functioning thyroid nodules (AFTNs), in which hormone secretion is independent of TSH stimulation [6]. Such nodules may progress to overt hyperthyroidism and warrant clinical surveillance [14].

The small proportion of patients with elevated TSH and normal peripheral hormones — consistent with subclinical hypothyroidism — suggests early thyroid failure, most plausibly related to autoimmune thyroiditis or early lymphocytic infiltration [15]. Early identification of subclinical hypothyroidism is clinically important, as untreated disease is associated with an increased risk of overt

hypothyroidism and cardiovascular and metabolic sequelae [16].

In the three patients diagnosed with thyroiditis, biochemical euthyroidism was accompanied by markedly elevated T4 and T3 values. This pattern is consistent with the transient hyperthyroid phase of thyroiditis, during which inflammatory destruction of thyroid follicles results in the release of preformed hormone into the circulation [17,18]. The subsequent hypothyroid phase, observed as the follicular stores are depleted, was not captured in this cross-sectional analysis.

The two patients with papillary carcinoma were euthyroid, consistent with the established finding that differentiated thyroid carcinomas rarely impair thyroid hormone production to a clinically significant degree [19]. This observation underscores a critical limitation of biochemical thyroid assessment: normal TFTs do not exclude malignancy, and cytological evaluation via FNAC remains indispensable for the diagnosis of thyroid neoplasms. Surgery and histopathology remain the gold standard for definitive diagnosis [8].

The significant intergroup differences in serum T4 and T3 (but not TSH) values demonstrated by ANOVA may reflect the heterogeneous functional activity across different thyroid lesion types. The absence of a significant TSH difference across groups may be attributed to the predominantly euthyroid status of patients and the wider biological variability of TSH — which, paradoxically, is the most sensitive functional marker at the individual patient level, yet may show less discriminative power across cytological groups in a cross-sectional design [6]. These findings are broadly consistent with those of Jayaram et al. and Burch et al., who similarly found that TFTs are insufficient to predict the cytological diagnosis of thyroid nodules [9]. Functional assessment and morphological assessment are complementary modalities that provide distinct and non-redundant diagnostic information.

The present study has several limitations. The sample size is relatively modest ($n=102$), and small-group cytological categories (e.g., papillary

carcinoma, n=2) limit the statistical power of subgroup comparisons. Histopathological confirmation was not available for all patients. Free T3 and free T4 — the biologically active fractions — were not measured, nor were thyroid autoantibodies (anti-TPO, anti-Tg), which would have provided additional information on autoimmune aetiology. Ultrasonographic characterisation of nodules and radionuclide scintigraphy were not systematically performed. Ultrasound-guided FNAC, which offers superior diagnostic yield, was not uniformly employed. Future studies with larger, prospectively recruited cohorts, histopathological correlation, measurement of free hormone fractions and autoantibodies, and standardised FNAC reporting using the TBSRTC framework are recommended.

Conclusion

Thyroid function tests are valuable for assessing the functional status of the thyroid gland but cannot independently predict the cytological nature of thyroid lesions. Nodular goitre was the predominant diagnosis in this series, and euthyroidism was the most common hormonal status across all cytological categories. Serum T4 and T3 levels differed significantly between cytological groups, whereas TSH levels did not. Normal TFTs were observed across the full spectrum of lesions, including malignancy, reinforcing the necessity of FNAC in morphological assessment. Accurate diagnosis and optimal clinical management of thyroid lesions require an integrated approach incorporating clinical, biochemical, and cytological evaluation.

Conflicts of Interest: The authors declare no conflicts of interest.

Funding: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Ethical Approval: The study was approved by the Institutional Ethics Committee of JMN Medical College, Chakdaha, India. Informed written consent was obtained from all participants.

References

- Hall JE. Guyton and Hall Textbook of Medical Physiology. 14th ed. Philadelphia: Elsevier; 2020.
- Kumar V, Abbas AK, Aster JC. Robbins and Cotran Pathologic Basis of Disease. 10th ed. Philadelphia: Elsevier; 2020.
- Tunbridge WMG, Evered DC, Hall R, et al. The spectrum of thyroid disease in a community: the Whickham survey. Clin Endocrinol (Oxf). 1977;7(6):481–493.
- Hegedüs L. The thyroid nodule. N Engl J Med. 2004;351(17):1764–1771.
- Vanderpump MPJ. The epidemiology of thyroid disease. Br Med Bull. 2011;99:39–51.
- Bahn RS, Burch HB, Cooper DS, et al. Hyperthyroidism and other causes of thyrotoxicosis: management guidelines of the American Thyroid Association and American Association of Clinical Endocrinologists. Thyroid. 2011;21(6):593–646.
- Gharib H, Goellner JR. Fine-needle aspiration biopsy of the thyroid: an appraisal. Ann Intern Med. 1993;118(4):282–289.
- Cibas ES, Ali SZ. The 2017 Bethesda System for Reporting Thyroid Cytopathology. Thyroid. 2017;27(11):1341–1346.
- Burch HB, Shakir F, Fitzsimmons TR, Jaques DP, Shriver CD. Diagnosis and management of the autonomously functioning thyroid nodule: the Walter Reed Army Medical Center experience, 1975–1996. Thyroid. 1998;8(10):871–880.
- Cooper DS, Doherty GM, Haugen BR, et al. Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. Thyroid. 2009;19(11):1167–1214.
- Handa U, Garg S, Mohan H, Nagarkar N. Role of fine needle aspiration cytology in diagnosis and management of thyroid lesions: a study on 434 patients. J Cytol. 2008;25(1):13–17.
- Gharib H, Papini E. Thyroid nodules: clinical importance, assessment, and treatment. Endocrinol Metab Clin North Am. 2007;36(3):707–735.
- Surks MI, Hollowell JG. Age-specific distribution of serum thyrotropin and antithyroid antibodies in the US population: implications for the prevalence of subclinical hypothyroidism. J Clin Endocrinol Metab. 2007;92(12):4575–4582.
- Vanderpump MPJ. Epidemiology of thyroid disorders. In: The Thyroid and Its Diseases. Cham: Springer; 2019:75–85.
- Pearce EN, Farwell AP, Braverman LE. Thyroiditis. N Engl J Med. 2003;348(26):2646–2655.
- Daniels GH. Management of thyroid nodules. Ann Intern Med. 1993;119(7 Pt 1):634–635.