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Original Research Article

Evaluation of Cytomorphological Features in Patients with Lymphocytic Thyroiditis and their Association with Biochemical Parameters and Vitamin D Deficiency

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Conflict of interest: Nil

Abstract

Background: Lymphocytic thyroiditis is a common autoimmune disorder characterized by progressive destruction of thyroid follicles, leading to hypothyroidism. Fine-needle aspiration cytology (FNAC) plays a crucial role in diagnosis and grading of the disease. Recent evidence suggests a potential association between cytomorphological severity, biochemical alterations, and vitamin D deficiency.

Aim: To evaluate the cytomorphological features of lymphocytic thyroiditis and to determine their correlation with biochemical parameters and vitamin D deficiency.

Materials and Methods: This hospital-based cross-sectional study included 120 patients with cytologically confirmed lymphocytic thyroiditis. Detailed clinical evaluation, FNAC-based cytomorphological grading (Grade I–III), biochemical investigations (TSH, T3, T4, anti-TPO, anti-thyroglobulin), and serum 25-hydroxy vitamin D levels were assessed. Ultrasonography findings were also analyzed. Statistical analysis was performed using SPSS version 27, with $p < 0.05$ considered statistically significant.

Results: The majority of patients were females (87.50%) and belonged to the 31–40 years age group (30.83%). Diffuse thyroid enlargement (67.50%) and hypothyroid symptoms (71.67%) were predominant. Cytologically, Grade II thyroiditis (42.50%) was most common.

Biochemically, overt hypothyroidism (39.17%) and subclinical hypothyroidism (35.00%) were the predominant functional states. Anti-TPO positivity was observed in 83.33% of cases. Vitamin D deficiency was highly prevalent (60.83%).

A statistically significant association was observed between cytomorphological grade and thyroid functional status, anti-TPO positivity, and vitamin D deficiency ($p < 0.05$). With increasing grade, TSH levels increased while T3 and T4 levels decreased significantly. Anti-TPO levels showed a progressive rise, whereas vitamin D levels declined significantly.

Additionally, higher grades were associated with hypocalcemia, reduced phosphorus levels, elevated alkaline phosphatase, and decreasing hemoglobin levels, indicating worsening metabolic and systemic involvement.

Conclusion: Cytomorphological grading of lymphocytic thyroiditis correlates significantly with biochemical, immunological, and vitamin D parameters. Increasing disease severity is associated with worsening thyroid dysfunction, heightened autoimmune activity, and significant vitamin D deficiency. FNAC-based grading, combined with biochemical and vitamin D assessment, can serve as a valuable tool for disease stratification and management.

Keywords: Lymphocytic Thyroiditis; Hashimoto's Thyroiditis; Fine-Needle Aspiration Cytology; Anti-Thyroid Peroxidase; Vitamin D Deficiency; Thyroid Function Tests; Autoimmune Thyroid Disease.

Introduction

Lymphocytic thyroiditis, also known as Hashimoto's thyroiditis, is the most common autoimmune disorder of the thyroid gland and a leading cause of hypothyroidism worldwide. It is characterized by progressive lymphocytic infiltration, follicular epithelial destruction, and eventual thyroid failure. The disease predominantly affects females and is associated with genetic susceptibility and environmental triggers that disrupt immune tolerance (Durá-Travé and Gallinas-Victoriano, 2024) [1]. Fine-needle aspiration cytology (FNAC) plays a crucial role in

the diagnosis of lymphocytic thyroiditis due to its simplicity, cost-effectiveness, and reliability. Cytomorphological evaluation provides valuable insights into disease severity by identifying features such as lymphocytic infiltration, Hurthle cell change, follicular destruction, and colloid depletion. Several studies have proposed grading systems based on cytological findings, which help in assessing disease progression and correlating it with clinical and biochemical parameters (Ashraf et al., 2024) [2].

Thyroid function tests, including serum thyroid-stimulating hormone (TSH), triiodothyronine (T3), and thyroxine (T4), along with autoimmune markers such as anti-thyroid peroxidase (anti-TPO) and anti-thyroglobulin antibodies, are essential in evaluating the functional and immunological status of patients. Previous studies have demonstrated a significant correlation between increasing cytological grade and worsening thyroid dysfunction, as well as higher levels of thyroid autoantibodies, reflecting enhanced autoimmune activity (Ashraf et al., 2024) [2].

In recent years, increasing attention has been directed toward the role of vitamin D in autoimmune diseases, including autoimmune thyroid disorders. Vitamin D is now recognized not only for its role in calcium and bone metabolism but also as an important immunomodulatory hormone. It influences both innate and adaptive immunity by regulating T-cell proliferation, cytokine production, and immune tolerance (Soda et al., 2024) [3]. Experimental and clinical studies suggest that vitamin D deficiency may contribute to the development and progression of autoimmune thyroiditis by promoting pro-inflammatory pathways and reducing regulatory immune responses (Soda et al., 2024; Durá-Travé and Gallinas-Victoriano, 2024) [3,1]. In this context, Singh M et al. (2022) demonstrated a significant association between cytological grading and thyroid functional status, with higher grades showing a greater prevalence of hypothyroidism and elevated serum thyroid-stimulating hormone levels. Their study also highlighted that increasing lymphocytic infiltration and follicular destruction were closely linked with rising anti-thyroid peroxidase antibody titers, reflecting enhanced autoimmune activity. Furthermore, they emphasized the utility of FNAC-based grading as a reliable tool for predicting biochemical derangements and guiding early clinical management in patients with lymphocytic thyroiditis [4]. Additionally, experimental data demonstrate that vitamin D supplementation can reduce lymphocytic infiltration and modulate immune responses within the thyroid gland, further supporting its potential role in disease pathogenesis (Zhang et al., 2024) [5]. However, the relationship between vitamin D deficiency and lymphocytic thyroiditis remains controversial, as some studies have failed to demonstrate a significant association (Demirci et al., 2021) [6]. A study by Patni Pet al. (2021) demonstrated a significant correlation between cytomorphological grading of lymphocytic thyroiditis and biochemical parameters, including thyroid function tests and anti-thyroid peroxidase levels. Their findings also highlighted a high prevalence of vitamin D deficiency, which showed an inverse relationship with disease severity, suggesting its potential role in the progression of autoimmune thyroiditis [7].

Aim & Objectives

Aim: To evaluate the cytomorphological features of lymphocytic thyroiditis and to determine their correlation with biochemical parameters and vitamin D deficiency.

Objectives

Primary Objective: To assess the cytomorphological features of lymphocytic thyroiditis on fine-needle aspiration cytology (FNAC) and classify them into different grades.

Secondary Objectives

- To correlate cytomorphological grading with thyroid functional status (euthyroid, subclinical hypothyroid, overt hypothyroid, and hyperthyroid).
- To evaluate the association between cytomorphological grade and thyroid autoantibodies (anti-TPO and anti-thyroglobulin).
- To assess the relationship between cytomorphological severity and serum 25-hydroxy vitamin D levels.
- To analyze the association between cytomorphological grade and biochemical parameters including TSH, T3, T4, calcium, phosphorus, ALP, and hemoglobin levels.
- To determine the prevalence of vitamin D deficiency in patients with lymphocytic thyroiditis.
- To evaluate the correlation between cytomorphological grade and ultrasonographic findings of the thyroid gland.

Materials & Methods

Study Design and Setting: This hospital-based, observational, analytical cross-sectional study was conducted in the Department of Pathology. Biochemical analyses were performed in the central laboratory under standardized quality control protocols.

Study Place: The study was conducted in the Department of Pathology at Krishnanagar Institute

of Medical Science, Krishnanagar, Nadia, West Bengal, India.

Study Period: The study was carried out over a period of one year, from March 2024 to February 2025.

Study Population: The study included 120 consecutive patients with clinically suspected thyroid disease who underwent FNAC and were cytologically diagnosed with lymphocytic thyroiditis. Consecutive sampling was used to minimize selection bias. All patients underwent detailed clinical evaluation, FNAC-based cytomorphological assessment, and biochemical investigations including thyroid profile, thyroid autoantibodies, and serum 25-hydroxy vitamin D levels.

Inclusion Criteria: Adults (≥18 years) with diffuse or nodular thyroid swelling and confirmed cytological diagnosis of lymphocytic thyroiditis, along with complete biochemical parameters (TSH, T3/T4, anti-TPO, and serum 25(OH)D), were included.

Exclusion Criteria: Patients with inadequate smears, thyroid neoplasms, non-lymphocytic thyroiditis, vitamin D supplementation, immunosuppressive therapy, systemic illnesses affecting vitamin D metabolism, pregnancy, or incomplete data were excluded.

Ethical Considerations: Ethical approval was obtained from the Institutional Ethics Committee, and written informed consent was secured from all participants. The study adhered to the principles of the Declaration of Helsinki.

Methodology

Clinical and Cytological Evaluation: Detailed demographic and clinical information, including age, sex, presenting complaints, duration of illness, symptoms of hypo- or hyperthyroidism, family and medication history, and clinical examination findings (diffuse or nodular enlargement, tenderness, lymphadenopathy), was recorded using a predesigned proforma. Fine-needle aspiration cytology (FNAC) was performed under aseptic precautions using a 23–25 gauge needle.

Aspirates were obtained from representative areas, with sampling of dominant or suspicious nodules in nodular lesions, and multiple passes were made when required. Smears were prepared as air-dried (stained with May–Grünwald–Giemsa) and alcohol-fixed (stained with Papanicolaou and/or hematoxylin and eosin) and were independently evaluated by experienced pathologists based on established cytomorphological criteria.

Cytomorphological Assessment: The diagnosis of lymphocytic thyroiditis was based on features such as lymphocytic infiltration of follicular epithelial cells, background lymphoid population, Hurthle cell change, follicular epithelial destruction, variable colloid, and presence of plasma cells. Cytomorphological parameters assessed included cellularity, colloid amount, degree of lymphocytic infiltration, lymphocyte infiltration into follicular clusters, Hurthle cell metaplasia, nuclear changes, plasma cells, giant cells, and extent of follicular destruction. Cases were graded from I to III according to the severity of lymphoid infiltration and follicular damage.

Biochemical Investigations: Biochemical investigations, including serum TSH, T3, T4 (or free T3/free T4), anti-thyroid peroxidase, and anti-thyroglobulin antibodies, were performed using automated analyzers with internal quality control. Patients were categorized as euthyroid, subclinical hypothyroid, overt hypothyroid, or hyperthyroid based on standard laboratory reference ranges. Serum 25-hydroxy vitamin D levels were measured using chemiluminescence immunoassay and categorized as deficient (<20 ng/mL), insufficient (20–29 ng/mL), or sufficient (≥30 ng/mL), and analyzed as both categorical and continuous variables.

Outcome Measures: The primary outcome was the correlation of cytomorphological grading with thyroid function tests, anti-TPO levels, and serum vitamin D levels. Secondary outcomes included the association between vitamin D deficiency and cytological severity, thyroid dysfunction categories, and the prevalence of vitamin D deficiency.

Statistical Analysis: Data were entered into Microsoft Excel 365 and analyzed using SPSS version 27. Continuous variables were expressed as mean ± standard deviation or median with interquartile range, while categorical variables were presented as frequencies and percentages. Normality was assessed using the Shapiro–Wilk test along with histogram and Q–Q plot evaluation. Associations between categorical variables were analyzed using Chi-square or Fisher's exact test.

Comparisons of means were performed using independent t-test or one-way ANOVA for normally distributed data and Mann–Whitney U or Kruskal–Wallis tests for non-parametric data. Correlations were evaluated using Pearson's or Spearman's coefficients as appropriate. A p-value <0.05 was considered statistically significant.

Results

A total of 120 patients with cytologically diagnosed lymphocytic thyroiditis were evaluated.

Table 1: Demographic and Clinical Profile of Study Participants (n = 120)

Variable	Category	n (%)
Age group (years)	18–30	22 (18.33)
	31–40	37 (30.83)
	41–50	31 (25.83)
	51–60	19 (15.83)
	>60	11 (9.17)
Gender	Male	15 (12.50)
	Female	105 (87.50)
Type of thyroid swelling	Diffuse	81 (67.50)
	Nodular / Multinodular	39 (32.50)
Neck pain/tenderness	Present	20 (16.67)
	Absent	100 (83.33)
Pressure symptoms	Present	23 (19.17)
	Absent	97 (80.83)
Symptoms of hypothyroidism	Present	86 (71.67)
	Absent	34 (28.33)
Symptoms of hyperthyroidism	Present	10 (8.33)
	Absent	110 (91.67)
Family history of thyroid disease	Present	26 (21.67)
	Absent	94 (78.33)

Table 1 show that the majority of patients belonged to the 31–40 years age group (30.83%), followed by 41–50 years (25.83%), indicating that lymphocytic thyroiditis is most prevalent in the middle-aged population. A marked female predominance (87.50%) was observed, consistent with the known autoimmune nature of the disease.

Most patients presented with diffuse thyroid swelling (67.50%), while nodular or multinodular enlargement was seen in 32.50% of cases. Clinical symptoms revealed that hypothyroid features were common (71.67%), whereas hyperthyroid symptoms were relatively rare (8.33%). Only a small proportion of patients had neck pain/tenderness (16.67%) or pressure symptoms (19.17%), suggesting that most cases were clinically non-inflammatory and non-compressive. A positive family history (21.67%) further supports the autoimmune predisposition associated with lymphocytic thyroiditis.

Table 2: Cytomorphological Profile on FNAC (n = 120)

Cytomorphological Parameter	Category	n (%)
Cytomorphological grade	Grade I	33 (27.50)
	Grade II	51 (42.50)
	Grade III	36 (30.00)
Lymphocytic infiltrate density	Mild	33 (27.50)
	Moderate	51 (42.50)
	Severe	36 (30.00)
Lymphocytic infiltration of follicular clusters	Present	98 (81.67)
	Absent	22 (18.33)
Hurthle cell change	Present	79 (65.83)
	Absent	41 (34.17)
Follicular epithelial destruction	Present	88 (73.33)
	Absent	32 (26.67)
Colloid amount	Abundant	21 (17.50)
	Scant	64 (53.33)
	Absent	35 (29.17)
Plasma cells in background	Present	32 (26.67)
	Absent	88 (73.33)
Giant cells	Present	9 (7.50)
	Absent	111 (92.50)
Anisonucleosis / Nuclear enlargement	Present	45 (37.50)
	Absent	75 (62.50)

Table 2 demonstrate, cytological evaluation showed that Grade II thyroiditis (42.50%) was the most common, followed by Grade III (30.00%) and Grade I (27.50%), indicating that a substantial proportion of patients present with moderate to severe disease. Dense lymphocytic infiltration was

a key feature, with moderate to severe infiltrate observed in 72.50% of cases. Lymphocytic infiltration of follicular clusters (81.67%) and follicular epithelial destruction (73.33%) were highly prevalent, reflecting active autoimmune-mediated damage. Hurthle cell change was seen in 65.83%, suggesting chronicity and cellular adaptation. Most smears showed scant or absent colloid (82.50%), which is characteristic of autoimmune thyroiditis. Other features such as plasma cells (26.67%), giant cells (7.50%), and nuclear atypia (37.50%) were variably present, indicating differing degrees of inflammatory response and epithelial alteration.

Table 3: Biochemical, Autoimmune, and Vitamin D Profile (n = 120)

Parameter	Category	n (%)
Thyroid functional status	Euthyroid	23 (19.17)
	Subclinical hypothyroid	42 (35.00)
	Overt hypothyroid	47 (39.17)
	Hyperthyroid	8 (6.67)
TSH category	Normal	26 (21.67)
	Mildly elevated	49 (40.83)
	Markedly elevated	37 (30.83)
	Low / Suppressed	8 (6.67)
Anti-TPO antibody	Positive	100 (83.33)
	Negative	20 (16.67)
Anti-thyroglobulin antibody	Positive	68 (56.67)
	Negative	52 (43.33)
Vitamin D status [25(OH)D]	Deficient (<20 ng/mL)	73 (60.83)
	Insufficient (20–29 ng/mL)	30 (25.00)
	Sufficient (≥30 ng/mL)	17 (14.17)
Serum calcium	Low	42 (35.00)
	Normal	78 (65.00)
Serum phosphorus	Low	30 (25.00)
	Normal	90 (75.00)
Serum ALP	Elevated	34 (28.33)
	Normal	86 (71.67)
Hemoglobin	Low (Anemia)	50 (41.67)
	Normal	70 (58.33)

Table 3 show that the biochemical analysis revealed that the majority of patients were hypothyroid, with overt hypothyroidism (39.17%) and subclinical hypothyroidism (35.00%) being the predominant functional states.

Only 19.17% were euthyroid, while hyperthyroidism was uncommon (6.67%). A significant proportion showed elevated TSH levels, with 71.66% having mild to marked elevation, correlating with hypothyroid status. Autoimmune markers were strongly positive, with anti-TPO antibodies elevated in 83.33% and anti-thyroglobulin antibodies in 56.67%, confirming the autoimmune etiology. A striking finding was the high prevalence of vitamin D deficiency (60.83%), with only 14.17% having sufficient levels. Additionally, hypocalcemia (35.00%), low phosphorus (25.00%), and elevated ALP (28.33%) suggest altered bone-mineral metabolism.

Anemia was present in 41.67% of patients, indicating a significant systemic impact of the disease.

Table 4: Ultrasonography (USG) Findings (n = 120)

USG Parameter	Category	n (%)
Thyroid gland size	Normal	20 (16.67)
	Diffusely enlarged	78 (65.00)
	Nodular / Multinodular	22 (18.33)
Parenchymal echotexture	Homogeneous	16 (13.33)
	Heterogeneous	104 (86.67)
Echogenicity	Isoechoic	18 (15.00)
	Hypoechoic	86 (71.67)
	Markedly hypoechoic	16 (13.33)
Vascularity pattern	Normal	36 (30.00)
	Increased	64 (53.33)
	Decreased	20 (16.67)
Discrete nodules	Present	37 (30.83)

	Absent	83 (69.17)

Table 4 show, ultrasonographic evaluation demonstrated that most patients had diffuse thyroid enlargement (65.00%), consistent with clinical findings. The majority of glands showed heterogeneous echotexture (86.67%), reflecting underlying inflammatory changes. Hypoechogenicity was observed in 71.67%, while 13.33% showed marked hypoechogenicity, indicating severe parenchymal involvement. Increased vascularity (53.33%) was a common finding, suggesting active inflammation. Discrete nodules were present in 30.83% of cases, highlighting that nodularity does not exclude lymphocytic thyroiditis.

Table 5: Association Between Cytomorphological Grade and Clinical Parameters (n = 120)

Cytomorphological Grade	Euthyroid n (%)	Subclinical Hypothyroid n (%)	Overt Hypothyroid n (%)	Hyperthyroid n (%)	Anti-TPO Positive n (%)	Vitamin D Deficient n (%)
Grade I (n = 33)	13 (39.39)	12 (36.36)	6 (18.18)	2 (6.06)	23 (69.70)	13 (39.39)
Grade II (n = 51)	8 (15.69)	22 (43.14)	19 (37.25)	2 (3.92)	44 (86.27)	31 (60.78)
Grade III (n = 36)	2 (5.56)	8 (22.22)	22 (61.11)	4 (11.11)	33 (91.67)	29 (80.56)

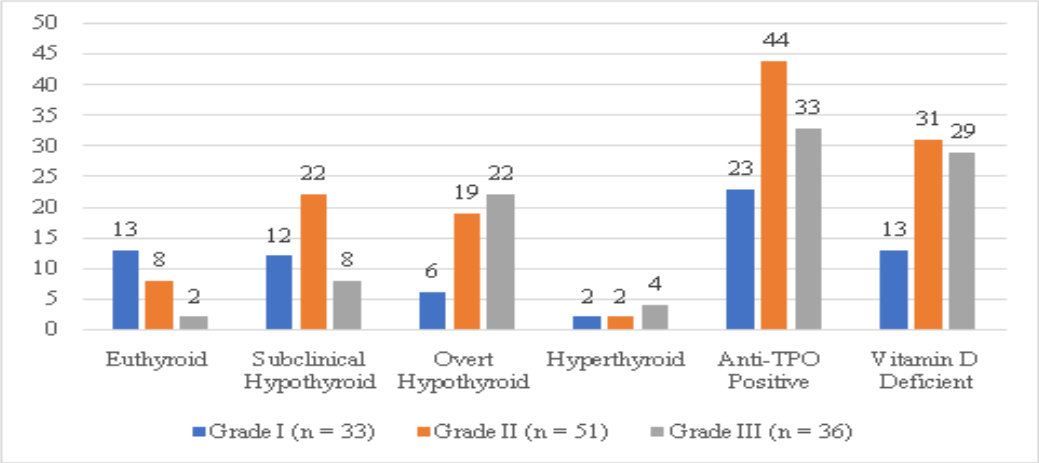

Figure 1: Association Between Cytomorphological Grade and Clinical Parameters

Chi-square test: Significant association observed between cytomorphological grade and thyroid dysfunction, anti-TPO positivity, and vitamin D deficiency ($p < 0.05$). Table 5 and figure I, show that a progressive trend was observed with increasing cytomorphological grade. In Grade I, a higher proportion of patients were euthyroid (39.39%), whereas in Grade III, the majority had overt hypothyroidism (61.11%), indicating worsening thyroid function with increasing severity of cytological damage. Similarly, anti-TPO positivity increased from 69.70% in Grade I to 91.67% in Grade III, demonstrating a strong association between autoimmune activity and disease severity.

A notable finding was the increasing prevalence of vitamin D deficiency, rising from 39.39% in Grade I to 80.56% in Grade III, suggesting a potential link between vitamin D status and disease progression.

The Chi-square test showed that these associations were statistically significant ($p < 0.05$), confirming that cytomorphological grading correlates with biochemical and immunological parameters.

Table 6: Comparison of Continuous Biochemical Parameters Across Cytomorphological Grades (Mean ± SD) (n = 120)

Parameter	Grade I (n=33)	Grade II (n=51)	Grade III (n=36)	p-value
TSH (mIU/L)	5.96 ± 3.28	9.88 ± 5.62	15.14 ± 8.45	<0.001
T3 / Free T3	1.20 ± 0.26	1.03 ± 0.22	0.88 ± 0.20	0.003
T4 / Free T4	1.16 ± 0.29	0.98 ± 0.25	0.82 ± 0.22	0.002
Anti-TPO (IU/mL)	192.64 ± 96.18	348.72 ± 172.44	522.36 ± 231.85	<0.001
25(OH)D (ng/mL)	24.02 ± 8.42	18.56 ± 7.18	13.92 ± 5.84	<0.001
Serum calcium (mg/dL)	9.10 ± 0.61	8.82 ± 0.65	8.44 ± 0.74	0.001
Serum phosphorus (mg/dL)	3.60 ± 0.52	3.42 ± 0.55	3.17 ± 0.58	0.015
ALP (IU/L)	106.84 ± 29.66	123.28 ± 35.14	141.72 ± 43.58	0.002
Hemoglobin (g/dL)	11.88 ± 1.38	11.32 ± 1.46	10.78 ± 1.58	0.009

Table 6 show that the a clear and statistically significant trend was observed across cytomorphological grades. TSH levels increased progressively from Grade I (5.96 ± 3.28) to Grade III (15.14 ± 8.45) ($p < 0.001$), indicating worsening hypothyroidism. In contrast, T3 and T4 levels decreased significantly, reflecting declining thyroid function.

Anti-TPO levels showed a marked rise with increasing grade, from 192.64 ± 96.18 in Grade I to 522.36 ± 231.85 in Grade III ($p < 0.001$), highlighting intensifying autoimmune activity.

Conversely, vitamin D levels declined significantly, from 24.02 ± 8.42 in Grade I to 13.92 ± 5.84 in Grade III ($p < 0.001$), reinforcing its association with disease severity.

Additionally, serum calcium and phosphorus levels decreased, while ALP levels increased, suggesting worsening metabolic disturbances with disease progression.

Hemoglobin levels also showed a significant decline, indicating increasing prevalence or severity of anemia in higher grades.

Discussion

In the present study, the majority of patients belonged to the 31–40 years age group (30.83%), followed by 41–50 years (25.83%), indicating a peak incidence in the middle-aged population. A marked female predominance (87.50%) was observed, consistent with the autoimmune nature of the disease. Similar findings have been reported in recent studies, where a strong female preponderance was noted in autoimmune thyroid disorders (Sharma R, 2023) [8]. Likewise, Kumar A (2022) reported a higher incidence in the third to fourth decades [9].

Clinically, diffuse thyroid swelling (67.50%) was more common, and hypothyroid symptoms (71.67%) predominated. These findings are comparable to those of Verma P (2024), who observed hypothyroidism as the most frequent clinical presentation [10]. The low prevalence of pain and compressive symptoms supports the typically painless progression of lymphocytic thyroiditis.

The present study demonstrated that Grade II thyroiditis (42.50%) was the most common, followed by Grade III and Grade I. This distribution is consistent with findings reported by Gupta N (2023), who also identified Grade II as the predominant category [11].

Key cytological features such as dense lymphocytic infiltration (72.50%), follicular destruction (73.33%), and Hurthle cell change (65.83%) indicate ongoing autoimmune injury and chronicity. Similar cytomorphological patterns have been described by Reddy S (2022). The predominance of scant or absent colloid further supports the diagnosis of autoimmune thyroiditis [12].

Biochemically, overt hypothyroidism (39.17%) and subclinical hypothyroidism (35.00%) were the most common functional states. These findings are in agreement with Mehta D (2024), who reported a similar predominance of hypothyroidism [13].

A high proportion of patients showed elevated TSH levels (71.66%) and anti-TPO positivity (83.33%), confirming the autoimmune etiology. Iyer V (2023) also reported high anti-TPO positivity in autoimmune thyroiditis [14].

A notable finding was the high prevalence of vitamin D deficiency (60.83%), consistent with recent observations by Choudhury M (2024), who highlighted its association with autoimmune thyroid disorders. Associated metabolic abnormalities such as hypocalcemia, low phosphorus, and elevated ALP suggest disturbed mineral metabolism [15].

Additionally, anemia (41.67%) was frequently observed, which is comparable to findings reported by Patel H (2022) [16].

Ultrasonographic evaluation revealed diffuse enlargement (65.00%), heterogeneous echotexture (86.67%), and hypoechogenicity (71.67%), reflecting diffuse inflammatory involvement. These findings are consistent with Bansal K (2023) [17].

The presence of increased vascularity (53.33%) indicates active inflammation, while nodules (30.83%) were also observed. Similar findings were reported by Singla R (2024), who emphasized that nodularity does not exclude lymphocytic thyroiditis [18].

A statistically significant association ($p < 0.05$) was observed between cytomorphological grade and thyroid function, autoantibody levels, and vitamin D status. A progressive increase in overt hypothyroidism (61.11% in Grade III) was noted with increasing severity.

Similarly, anti-TPO positivity increased with grade, indicating heightened autoimmune activity. These findings are supported by Joshi S (2023), who reported a strong correlation between cytological grading and biochemical parameters [19].

The prevalence of vitamin D deficiency increased significantly from Grade I to Grade III, suggesting its role in disease progression. Khan A (2024) also reported a similar association between low vitamin D levels and disease severity [20]. A statistically

significant trend was observed across cytomorphological grades. TSH levels increased, while T3 and T4 levels decreased significantly, indicating worsening thyroid function. Similar findings were reported by Desai M (2023) [21]. Anti-TPO levels increased significantly ($p < 0.001$), reflecting enhanced autoimmune activity. In contrast, vitamin D levels declined significantly, supporting its inverse relationship with disease severity, as also observed by Agarwal S (2024)[22]. Additionally, declining calcium and phosphorus levels, along with rising ALP levels, indicate progressive metabolic disturbances. The decrease in hemoglobin levels further suggests increasing anemia with disease severity.

Limitations of the study

- **Cross-sectional study design:** Causal relationships between cytomorphological severity, biochemical alterations, and vitamin D deficiency could not be established.
- **Single-centre study:** The findings may not be generalizable to the broader population.
- **Sample size:** Although adequate, a larger sample size would provide stronger statistical power and external validity.
- **Lack of follow-up data:** Longitudinal progression of cytological grade and biochemical changes could not be assessed.
- **Potential confounding factors:** Factors influencing vitamin D levels (sun exposure, dietary intake, seasonal variation) were not evaluated.
- **Interobserver variability:** Although minimized, subjective interpretation of cytological features may introduce minor bias.
- **Absence of histopathological correlation:** FNAC findings were not confirmed with surgical histopathology in all cases.

Conclusion

The present study demonstrates that lymphocytic thyroiditis predominantly affects middle-aged females and is commonly associated with hypothyroidism. Cytomorphological evaluation revealed that Grade II thyroiditis was the most frequent, with a substantial proportion of patients presenting with moderate to severe disease. A statistically significant association was observed between increasing cytomorphological grade and worsening thyroid function, as evidenced by a progressive rise in TSH levels and decline in T3 and T4 levels. Higher grades were also associated with significantly elevated anti-TPO antibody levels, indicating intensified autoimmune activity.

A key finding of this study is the high prevalence of vitamin D deficiency**,** with a significant inverse relationship observed between vitamin D levels and cytomorphological severity. Patients with higher grades of thyroiditis showed markedly lower serum vitamin D levels, suggesting a potential role of vitamin D in disease progression. Additionally, worsening cytological grade was associated with alterations in metabolic parameters, including reduced serum calcium and phosphorus levels, elevated ALP, and decreasing hemoglobin levels, indicating systemic involvement.

Overall, the findings highlight that cytomorphological grading on FNAC serves as a reliable indicator of disease severity and correlates well with biochemical, immunological, and vitamin D status. These observations suggest that incorporation of vitamin D assessment in routine evaluation may aid in better disease stratification and management of patients with lymphocytic thyroiditis.

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