Cytomorphological Spectrum of Hashimoto’s Thyroiditis and Its Correlation with Hormonal Profile and Hematological Parameters

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Abstract

Introduction: Hashimoto’s thyroiditis (HT) is a well-known autoimmune disorder of the thyroid diagnosed on fine needle aspiration cytology (FNAC) and a common cause of hypothyroidism in women. Often serological and hematological parameters are additional investigations aiding the diagnosis of this entity. Aim: To grade HT based on cytomorphology and to correlate the cytological grades with thyroid hormone status and basic hematological parameters. Materials and Methods: During a period of 2.5 years, 1762 patients underwent FNAC of thyroid at our tertiary healthcare center. Cytological evidence of lymphocytic thyroiditis was seen in 102 cases, of which 58 cases in addition had thyroid hormone levels and hematological parameters for correlation. Results: Of the 58 cases, 55 were females. Majority of the patients had grade II thyroiditis (56.9%), followed by grade I (34.5%) and grade III (8.6%). Elevated thyroid-stimulating hormone was seen in 74.2% of cases, with 39.7% of patients presenting with subclinical hypothyroidism and 18.9% being euthyroid. Mean hemoglobin was low in all grades, more so in hypothyroid state, while other hematological parameters were normal when correlated with grade and hormonal status without any significant P value. Conclusion: Cytomorphological grading of HT can explain the pathogenesis of this autoimmune disease. Subclinical hypothyroidism was significantly observed. There was no significant statistical correlation of cytomorphological grades with thyroid status. In this study, most of the hypothyroid cases had low hemoglobin levels while other basic hematological parameters did not show any statistically significant correlation with the thyroid hormonal status.

Keywords: Anemia, anti-TPO, fine needle aspiration cytology, Hashimoto’s thyroiditis, hemoglobin, thyroid hormone profile

INTRODUCTION

Thyroid diseases are among the most common endocrine disorders worldwide. Data on various studies on thyroid diseases have shown that an estimated 42 million people in India suffer from thyroid diseases.[1] This article focuses on Hashimoto’s thyroiditis (HT), a synonym for chronic lymphocytic thyroiditis; an entity that was described for the first time by Hakaru Hashimoto in 1912.[2] It is a well-known autoimmune disorder and the second most common lesion of the thyroid next to colloid goitre, commonly associated with hypothyroidism.

Fine needle aspiration cytology (FNAC) is quick, cost-effective, and highly sensitive in diagnosing HT. The cytomorphological grading of this form of thyroiditis was first done by Bhatia et al.[2] Several studies have also compared the cytological grades with hormonal and biochemical parameters.[3-5] Studies till date have shown the prevalence of anemia among patients with hypothyroid. However, studies on correlation of this form of autoimmune thyroiditis with hematological parameters [hemoglobin (Hb), total count (TC), differential count, and red blood cell count] are sparse. This study was done to categorize the cytological grades as per the classification of Bhatia et al. and to compare the grades with thyroid hormone status along with a study on the correlation of the grades with routinely done hematological parameters.

MATERIALS AND METHODS

During the period from January 2015 to August 2017, a total of 1265 patients were referred to the cytopathology department of our hospital for FNAC of the thyroid gland. An examination of this form of autoimmune thyroiditis with hematological parameters [hemoglobin (Hb), total count (TC), differential count, and red blood cell count] are sparse. This study was done to categorize the cytological grades as per the classification of Bhatia et al. and to compare the grades with thyroid hormone status along with a study on the correlation of the grades with routinely done hematological parameters.

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of the thyroid gland was done and the enlargement was classified as diffuse or nodular, with a note on the presenting complaints and brief history of the patients. FNAC was done using a 24-G needle using primary nonaspiration technique and aspiration technique only when needed under aseptic precautions. A minimum of two passes were made, and the aspirated material was fixed in ethanol. Cases in which smears were unsatisfactory, repeat aspiration was done, with minimum of two good smears selected having features of lymphocytic thyroiditis for the study. Both Papanicouloou and hematoxylin and eosin stains were done on the fixed smears, and Giemsa stain was done on air-dried smears. The patients also had estimation of thyroid hormones done with normal ranges of T3, T4, and thyroid-stimulating hormone (TSH) being 0.52–1.85 ng/mL, 4.4–10.8 μg/dL, and 0.39–6.16 μIU/mL, respectively, using Electro Chemiluminescence in Cobas e411 autoanalyzer from Rosche. An automated analyzer – Sysmex 400 – was used to obtain hematological parameters which were done using anticoagulated venous blood samples. These parameters [Hb, TC, red blood cell (RBC) count, and platelet count] were standardized by routine external and internal quality control checks. Cases with cytological features diagnostic of HT – Hurthle cells showing anisonucleosis with polymorphous lymphocytic population and scanty or absent colloid along with thyroid hormonal profile and hematological parameters tested at the time of FNAC – were taken for the study. Cases previously diagnosed as HT, on any medications, and with associated thyroid disorders were excluded from the study.

Cytological grading was done as per the criteria proposed by Bhatia et al.,[3] as given in Table 2. Statistical software SPSS v. 20.0 and R environment ver. 3.2.2 were used for the analysis of data, and Microsoft Word and Excel were used to generate graphs and tables. Analysis of variance and Chi-square test were used, with P value less than 0.05 considered as significant. The study was approved by the institutional ethical committee.

RESULTS

Out of the 1265 FNACs of thyroid, 122 were diagnosed as HT. Out of these, only 58 cases were taken up for the study as they met the inclusion criteria. Females constituted an expected high percentage (94.8%), with only three male patients. Age distribution of the cases: A majority of the patients in our study were in the third (48.2%) decade followed by second (25.8%) and fourth decades of life (13.7%).

Cytological grading was done as per the criteria given by Bhatia et al. [Table 1]. Grade II cases constituted the largest category (56.9%) in our study, followed by grade I (34.5%) and grade III (8.6%) [Figure 1a and b].

On examination, at the time of FNAC, the enlargement of the thyroid gland was diffuse in a majority of the cases (45 cases, 77.4%). However, nodular enlargements were seen in the rest (13 cases, 22.6%). Symptoms such as gain of weight, voice change, and visible neck swelling were noticed in 72% of the total cases irrespective of the cytological grades.

Various cytological features were observed on the smears. Hurthle cells – 95.4%, benign follicular cells – 25.3%, anisonucleosis of the Hurthle cells – 87.5%, giant cells – 26%, plasma cells – 54%, centrocytes and centroblasts – 73%, and epitheloid cells – 46% [Figure 2a and b].

Serological correlation

Although hypothyroidism was seen in a majority of the cases in grade II, there was no statistical correlation (P = 0.9) between the cytological grades with hormonal status. Also, subclinical hypothyroidism (SCH) was seen in a significant number of patients (39.6%). A majority of euthyroid cases were seen in grade II, and hyperthyroid cases were seen equally distributed among grades I and II, with none in grade III category [Table 2].

Anti-TPO antibody was available only in 21 cases. Increase in anti-TPO with an increase in TSH was seen in 80.95% of the cases, increase in anti-TPO with normal TSH was seen in 14.2% of the cases, while one case showed increased TSH with normal anti-TPO levels.

Cytological and hematological correlation

When correlated with the basic hematological parameters, it was found that the mean Hb was reduced in all the grades of HT. The lowest Hb was found in grade II (10.9 g%). The total white blood cell (WBC) count and platelet count were

Table 1: Cytological grading and distribution of patients studied

| Grades          | No. of patients | Percentage |
|-----------------|-----------------|------------|
| Grade I         | 20              | 34.5       |
| Grade II        | 33              | 56.9       |
| Grade III       | 5               | 8.6        |
| Total           | 58              | 100.0      |

Table 2: Comparison of the cytological grades with thyroid hormone status

| Grades   | Total cases | Normal       | Hypothyroid | Hyperthyroid |
|----------|-------------|--------------|-------------|--------------|
| Grade I  | 20          | 4 (6.8%)     | 14 (24.0%)  | 2 (3.4%)     |
| Grade II | 33          | 6 (10.3%)    | 25 (43.0%)  | 2 (3.4%)     |
| Grade III| 5           | 1 (1.7%)     | 4 (7.0%)    | 0 (0.0%)     |
| Total    | 58          | 11 (18.9%)   | 43 (74.0%)  | 4 (6.8%)     |
within the normal range in all cytological grades showing no significant statistical correlation [Table 3].

**Hematological and serological correlation**

There was significant correlation only between Hb and thyroid status, with the lowest value of Hb being in grade II hypothyroid cytology cases. The total WBC count, platelet count, and RBC count did not show any statistical correlation with the thyroid hormone status and were within the normal limits in all the three categories (euthyroid, hypothyroid, and hyperthyroid) [Table 4].

**DISCUSSION**

Diseases of the thyroid are seen increasingly in today’s world. They have an advantage over other endocrine disorders in that enlargement of the thyroid gland is visible, can be easily subjected to diagnostic aspiration and scanning, and have the accessibility to medical treatment.[1]

HT, a well-known autoimmune disease of the thyroid, is known to be more prevalent in Asians.[6] The pathogenesis of this organ-specific form of thyroiditis is sensitization of the cluster differentiation 4 (CD4+) T-helper cells to the antigens in the thyroid follicular cells. CD 8 + T-cell-mediated cytotoxicity, cell death by the release of cytokines, and antibody-dependent cell-mediated toxicity are caused by binding of the various antibodies (anti-thyroglobulin, anti-TPO) to the cell surface of the follicular cells.[7] This form of autoimmune thyroiditis has also been categorized as having a genetic disease, having combined effects of human leukocyte antigen (HLA) class II genes and non-HLA gene polymorphisms contributing to the pathogenesis of the same.[8]

The peak age incidence of HT in our study was the third to fourth decade of life, which is in accordance with other Indian studies unlike the Western literature (fifth decade of life).[2,9,10] Female predominance was seen in our study with a female: male ratio of 14.7:1, similar to other studies. Diffusely enlarged thyroid is the most common presentation of this form of thyroiditis. Nodular presentation was seen in 22.6% of our cases. Nodules represent early stage of the disease prior to hormonal changes.[2] Higher percentage of nodularity in our study compared with other studies can probably be attributed to the frequent conducting of monthly camps, helping the rural masses access medical treatment at earlier stages.

FNAC is a part of basic investigation done for patients presenting with thyroid enlargements. The classical features of HT on aspirates of FNA are Hurthle cell change with anisonucleosis and varying amount of polymorphous lymphoid infiltrate in a background of scant colloid. Epitheloid granulomas, plasma cells, giant cells, and occasional follicular cells can be seen.[11] The above-mentioned features were all seen in varying proportions in our study.

![Image](image1.png)

**Figure 1:** Cytology of aspirates showing grade I cytology – Hematoxylin and eosin stain × 100 (a) and grade II cytology – MGG stain × 100 (b)

![Image](image2.png)

**Figure 2:** Cytology of aspirates showing dense lymphocytic infiltrate admixed with Hurthle cells (a). Smear showing a granuloma formation with epitheloid cells (b) – Hematoxylin and eosin stain × 100

| Hematological parameter | Cytological grade | No. of cases | Mean | SD | F  | P  |
|-------------------------|------------------|--------------|------|----|----|----|
| Hb                      | Grade 1          | 20           | 11.2 | 1.41 | 0.083 | 0.921 |
|                         | Grade 2          | 33           | 10.9 | 1.35 |      |      |
|                         | Grade 3          | 5            | 11.3 | 0.78 |      |      |
|                         | Grade 1          | 20           | 4.18 | 0.60 | 0.098 | 0.907 |
|                         | Grade 2          | 33           | 4.12 | 0.48 |      |      |
|                         | Grade 3          | 5            | 4.20 | 0.32 |      |      |
| TC                      | Grade 1          | 20           | 6780 | 1241.2 | 4.061 | 0.23 |
|                         | Grade 2          | 33           | 5733 | 1288.3 |      |      |
|                         | Grade 3          | 5            | 6580 | 2019.1 |      |      |
| PLT                     | Grade 1          | 20           | 2.4  | 0.64 | 0.157 | 0.855 |
|                         | Grade 2          | 33           | 2.5  | 0.71 |      |      |
|                         | Grade 3          | 5            | 2.5  | 0.25 |      |      |

SD: Standard deviation; Hb: Hemoglobin; RBC: Red blood cell; TC: Total count; PLT: Platelet
Cytological grading: Grade II type of thyroiditis was the most commonly seen in this study, in agreement with a majority of previous studies.

Although FNAC has a high sensitivity and specificity in diagnosing HT, a few diagnostic pitfalls must be kept in mind. The florid lymphocytic population seen in cytological grade II and grade III disease can be mistaken for non-Hodgkin’s lymphomas. Predominance of Hurthle cells in smears, especially when the enlargements are nodular, must be interpreted carefully. In such cases, the background lymphocytic infiltrate is often assumed to be a part of the hemorrhagic element of the aspirate. In doubtful cases, there must not be hesitancy on the part of the cytopathologist to reaspirate; aspirate from another representative site and if still needed should go for ultrasound-guided FNAC. Differentials of Hurthle cell lesions in particular Hurthle cell neoplasms must always be kept in mind.

Another closely related condition to be considered is subacute thyroiditis, which can be differentiated by the finding of a mixed inflammatory infiltrate and follicular cell degeneration. Also, clinical presentation will often be an acute one with signs of inflammation. A few studies done by Rathi et al. and Ekambaram et al. have shown eosinophilic infiltrate of Hurthle cells as an associated feature of significance in HT. Eosinophils were not significantly associated with any of the cases in this study.

The incidence of malignancy in HT is documented to vary from 0.4% to 28%. In this study, no associated malignancy was documented. The possibilities would be the patients would have probably presented well in advance or would have lost to follow-up.

Table 4: Correlation between haematological parameters and thyroid hormonal status

| Haematological parameter | Thyroid status | No. of cases | Mean | SD  | F   | P       |
|--------------------------|---------------|-------------|------|-----|-----|---------|
| HB                       | Normal        | 11          | 10.5 | 1.44| 5.11| 0.009***|
|                          | Hypo          | 43          | 10.2 | 1.12|     |         |
|                          | Hyper         | 4           | 10.8 | 1.83|     |         |
| RBC                      | Normal        | 11          | 3.91 | 0.60| 2.98| 0.059   |
|                          | Hypo          | 43          | 4.04 | 0.44|     |         |
|                          | Hyper         | 4           | 3.80 | 0.66|     |         |
| TC                       | Normal        | 11          | 6200 | 997.9| 0.04| 0.996   |
|                          | Hypo          | 43          | 6158 | 1510.6|    |         |
|                          | Hyper         | 4           | 6175 | 1575.5|    |         |
| PLT                      | Normal        | 11          | 2.79 | 0.50| 2.08| 0.134   |
|                          | Hypo          | 43          | 2.41 | 0.67|     |         |
|                          | Hyper         | 4           | 2.12 | 0.68|     |         |

***P<0.05 significant. HB=Hemoglobin RBC Red blood cell count TC=Total count PLT – Platelet count SD=Standard deviation

Early phase of hormonal imbalance. In this study, 39.6% of the cases presented with SCH, similar to a study of Bhatia et al. However, Bagchi et al. found subclinical disease in 8%–17% of their subjects, which is lower compared with our study. The higher rates again could be attributed to the early access of the patients to medical testing. A Majority of the cases were associated with hypothyroidism. Euthyroid cases were seen mainly in grade II category followed by grade I.

Hashitoxicosis refers to a transient state of hyperthyroidism seen in HT, representing the aggravated destruction of the follicles. Four patients of cytologically diagnosed HT in our study presented with serological hyperthyroidism. However, when calculations were done, there was no statistical significance between the cytological grades and the thyroid status of the individuals. This finding is in conclusion with similar studies done till date.

The role of anti-TPO antibodies in the pathogenesis of HT is well-documented. A study by Thomas et al. has shown that morphologically the anti-TPO antibody–positive cases are indistinguishable from the antibody-negative cases. Although negative serology in this form of thyroiditis causes a diagnostic dilemma at times, it is a well-established fact that the intrathyroid immune destruction occurs much earlier than the serological changes. Also, over time cytomorphological features persist unlike the antibody titers, which may fluctuate. In addition, a majority of the population in the surroundings of our hospital being from a rural background cannot afford antibody testing many a times. The fact that only 21 among the 102 cases had anti-TPO testing done in this study further supports this fact.

Thyroid hormones have a well-documented role in the metabolism and proliferation of red cells. Studies have postulated that hypothyroidism induces hypoplasia and hyperthyroidism hyperplasia in all myeloid cell lineages. Also, for WBCs like lymphocytes, T3 is a precursor substance for normal B-cell formation in bone marrow by mediating pro-B-cell proliferation.
of hematological parameters with thyroid hormones have been done, proving a strong association between the two by the virtue of affecting hematopoiesis. A slightly depressed total leucocyte count (TLC), neutropenia, and thrombocytopenia have been observed in hypothyroid patient. A study by Idhad et al. have shown HB and TLC to be low and platelet count to be high for subjects suffering from autoimmune thyroid disease. Meanwhile, studies have also showed no significant correlation between the TLC and platelet count, but significant correlation for HB and RBC count between hyper- and hypothyroid. In this study, the only hematological parameter that showed significant correlation with thyroid hormones was HB level, which was low in hypothyroid compared with normal and euthyroid cases. Previous studies have shown both microcytic anemia and macrocytic anemia to be associated with the hypothyroid state of autoimmune thyroiditis. In addition, our study also correlated the cytological grades of HT with the basic hematological parameters. HB was mildly low in all the three cytological grades, with no other hematological parameter showing significant correlation with the cytological grades. The platelet count, TC, and RBC count did not show significant difference between the grades. Similar studies with more stringent criteria done in a larger group would help shed more light on this issue.

**Conclusion**

This study reaffirms that cytology still remains the gold standard for the diagnosis of HT. A serological state of SCH with nodular enlargement of thyroid possibly indicates early presentation. The cytomorphological grading showed no correlation with thyroid hormonal status and hematological parameters. Also, most of the hypothyroid cases had low HB levels, in agreement with previous studies, with no other hematological parameters showing any statistically significant correlation.

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**Conflicts of interest**

There are no conflicts of interest.

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