

Study on Cytomorphological Spectrum of Hashimoto's Thyroiditis and to Evaluate the Correlation among Cytopathological Grading, Serum ATPO Levels and Biochemical Parameters in Tertiary Care Centre

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Abstract:

Introduction: Hashimoto's thyroiditis (HT) is an organ-specific autoimmune disease. Fine needle aspiration cytology is the safe and accurate method for diagnosis. Antithyroid peroxidase (ATPO) antibody titres correlate best with the degree of thyroidal lymphocytic infiltration.

Objectives: This study was aimed to grading the thyroiditis and to correlate with clinical, biochemical and serum anti thyroid peroxidase levels.

Materials and Methods: The present study is a prospective study and is conducted for a period of 1 year in our tertiary care centre. A total of 179 patients were referred for evaluation of thyroid lesions. We have included, newly diagnosed, those confirmed as having HT through FNAC (41 cases). ATPO, TSH were done for this study group.

Results: Out of 70 HT cases ,41 cases met the inclusion criteria. In these 41 cases, 40 were females, age ranged from 11 years to 60 years, with majority of patients were in 4th decade. Majority of patients had cytomorphologically graded, grade 1 thyroiditis (21 cases), followed by grade 2(16 cases) and grade 3 (4 cases). ATPO was elevated in 38 cases. TSH was elevated in 6 cases. There was no obvious correlation between the cytological grades of thyroiditis and levels of anti-thyroid peroxidase antibody and TSH.

Conclusion: Lymphocytic infiltration of the thyroid gland occurs much earlier than serological evidence in Hashimoto's thyroiditis. Although elevated levels of ATPO levels strongly associated with Hashimoto's thyroiditis there is no obvious correlation was observed between cytomorphological grades of thyroiditis and levels of ATPO and TSH. Hashimoto's thyroiditis should be diagnosed by multidisciplinary approach.

Keywords: Hashimoto's thyroiditis, ATPO, TSH, FNAC.

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Introduction

Hashimoto's thyroiditis is a form of an autoimmune thyroiditis and is the second most common lesion of the thyroid next to colloid goitre, and most affected patients are premenopausal women. But nowadays incidence of autoimmune thyroiditis is increasing, and at the same time, it is affecting young women also and resulting in hypothyroidism. Data of various studies on thyroid diseases have shown that an estimated 42 million people in India suffer from thyroid diseases, with a prevalence of 3.9 % hypothyroidism [1]. Haraku Hashimoto first described Hashimoto's thyroiditis in 1912. [2,3]

The mechanism of autoimmune thyroid disease is of both humoral and cellular nature. [4] The leading pathogenetic event is the breakdown of immune tolerance that affects both central and peripheral self-tolerance mechanisms, caused by a combination of immunologic, genetic, and environmental factors. Self-reactive CD4 T cells can stimulate autoreactive B cells to be recruited into the thyroid and to secrete thyroid antibodies.

The three main target antigens for thyroid antibodies are thyroglobulin, the storage protein for thyroid hormones, and thyroid microsomal antigen, which has been identified as thyroid peroxidase. As

per the studies, Anti-thyroid peroxidase antibodies will rise in > 90 % of patients with autoimmune thyroiditis.[5] Fine needle aspiration cytology is the most reliable, more informative, and cost-effective test in diagnosing Hashimoto's thyroiditis.[6,7,8] A cytological grading of Hashimoto's thyroiditis based on the number of lymphocytes infiltrating the gland & the degree of destruction of the gland due to immune mechanism was done for the first time by Bhatia et al where cytological grades of Hashimoto's thyroiditis correlated with clinical and biochemical anti thyroid peroxidase antibody [ATPO] levels.[9]

In this scenario, the present study is taken up to evaluate the correlation between cytomorphological grading and severity of autoimmune thyroiditis with anti-TPO antibody levels and biochemical parameters.

Materials and Methods:

The present study is a prospective study and is conducted for a period of 1 year, i.e., from Jan 2020 – Dec 2020 in tertiary care centre. The study was done at Department of Pathology after the clearance was given by the Ethical committee of the institute.

Written informed consent was taken from the patients of the participating subjects. Out of 179 patients with thyroid lesions evaluated, 70 patients were diagnosed as Hashimoto's thyroiditis. Based on cytomorphological features consisting Thyroid follicular cells with lymphocytes and plasma cells in the background, lymphoid cells impinging on follicular cells, Hurthle cell change (variable), lympho – histiocytic collection, multinucleated giant cells and epithelioid cells (variable) [19]

Newly diagnosed cases, those confirmed as having HT through FNAC were included and taken as a study group. Already diagnosed cases that were on medication, HT associated other thyroid lesions excluded from the study. Cytological grading of Hashimoto's thyroiditis was done according to the criteria used by Bhatia et al considering, few lymphoid cells infiltrating the follicles/increased number of lymphocytes in the background as Grade I [Fig 1], moderate lymphocytic infiltration with presence of hurthle cells, giant cells and epithelioid as Grade II [Fig 2], florid lymphocytic infiltration with germinal centre formation as Grade III [Fig 3]. Hormonal assay and serum ATPO levels were done for all selected cases (41 cases) and correlated with quantitative grading of Hashimoto's thyroiditis on cytology smears.

Results

In the 41 cases of the study group, females constituted an expected high percentage (97.5%), with only one male patient (2.5%) aged 15 years presented with diffuse swelling in front of the neck, euthyroid, grade II cytology, and with raised ATPO titre. The age distribution of the cases in this study was only in between the 11 to 60 years. The majority of the patients in our study were in the fourth decade, 12/41 (30%), followed by third and fifth decades of life 9/41 (21%) each equally. In the second and sixth decade, only 7 (17.07%) & 4 (9.70%) cases were reported, respectively. Whereas no cases were found in the first decade. Lowest age patient in the present study is 11 years female child whereas highest age is 60 years [Chart-1].

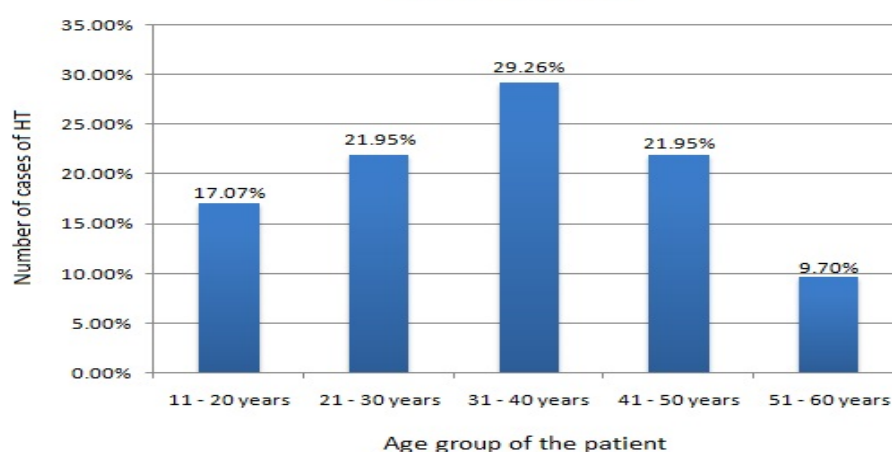


Chart -1: Age distribution among HT

On examination, at the time of FNAC, there was diffuse enlargement of thyroid gland in most cases 35/41 (85.3%), nodular enlargement seen in the rest 6/41 (14.6%).

The majority of patients came with a chief complaint of swelling in front of the neck, other associated symptoms like weight gain, easy fatigability, menstrual irregularities complained

by few patients, but they are euthyroid. Two patients presented with hypothyroid symptoms, i.e., constipation, thick, coarse skin, cold intolerance, hoarseness of voice. Thyroid stimulating hormone elevated, and T3, T4 decreased in both these patients. Thirty-five patients (85.3%) are euthyroid with T3, T4 & TSH levels within normal limits, 4 patients (9.7%) are having normal T3, T4 levels &

raised TSH indicating subclinical hypothyroidism, two patients (4.8%) are hypothyroid with decreased T3, T4 & raised TSH.

Grade I constituted the largest category, 21/41(51.2%), followed by grade II-16/41(39%) and Grade III constituted 4/41 (9.7%) [Table 1]. ATPO levels raised in 38 patients (92.6%), not raised in 3 patients (7.4%) [Chart-2]

Table 1: Cytological grading and distribution of the patients according to Bhatia et al. criteria

Grades	Cytomorphological Features	No. Of Patients	Percentage
Grade I	Few lymphoid cells infiltrating the follicles /increased no of lymphoid cells in the background	21	51.3%
Grade II	Moderate lymphoid infiltration or mild lymphoid infiltration with Hurthle cell clusters /giant cells /anisonucleosis	16	39%
Grade III	Florid lymphoid infiltration with germinal centre formation, very few recognizable follicular cells, Hurthle cells on the smear	4	9.7 %
Total		41	100

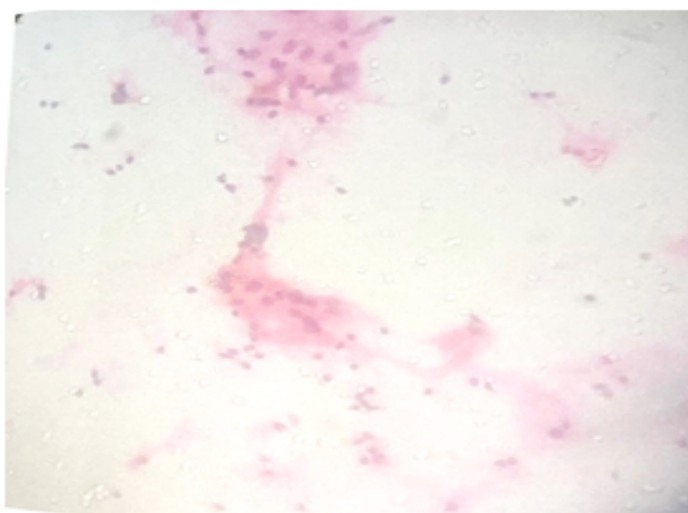


Figure 1: Grade I, Cytology showing few lymphoid cells infiltrating the follicles. (H&E stain 40X)

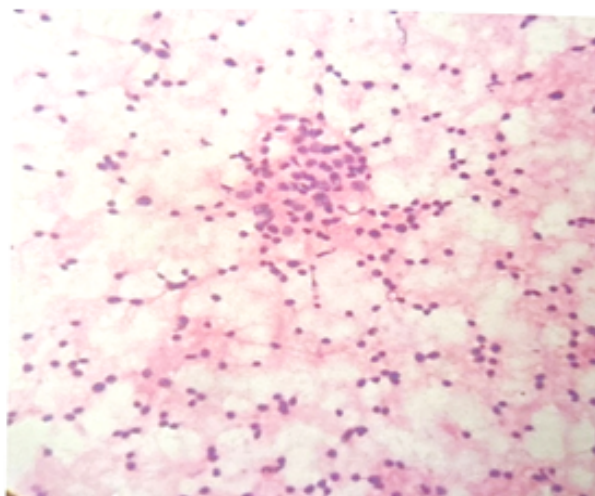


Figure 2: Grade-II, Moderate lymphoid infiltration (H&E stain 40X)

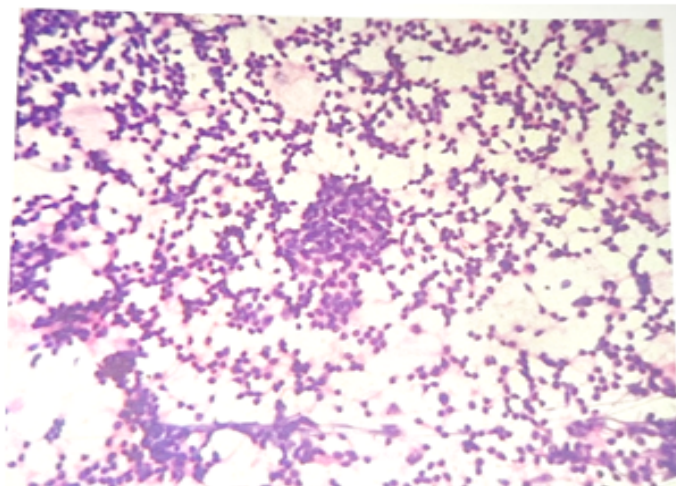


Figure 3: Florid Lymphocytic infiltration, Grade-III (H&E stain 40X)

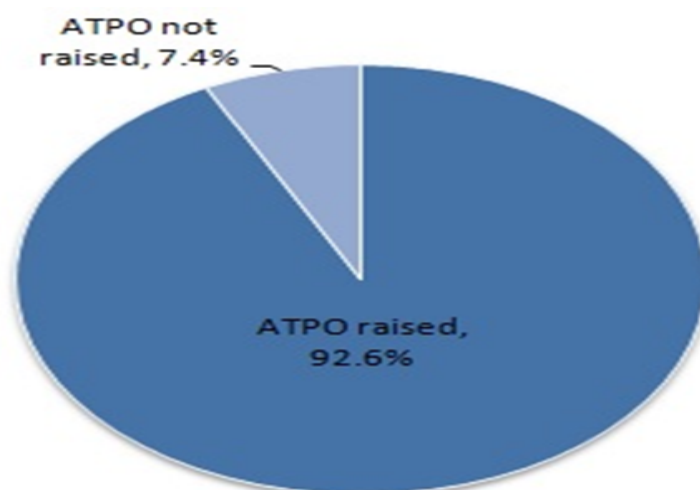


Chart 2: Cases with ATPO antibody titers.

ATPO antibody titer was raised in 38 cases. Only six patients showed hypothyroid state (subclinical & overt hypothyroidism). All these 6 cases showed increased ATPO titer.

The remaining 32 cases are in the euthyroid state. [Table 2] 19 out of 21 (90.4%) patients with grade I thyroiditis, 15 out of 16(93.7%) patients with grade

II thyroiditis and 4 out of 4 (100%) patients with grade III thyroiditis showed raised serum ATPO levels [Chart -3] High titers of ATPO antibodies i.e. > 1000 IU / mL observed predominantly in 3rd decade of life (55.5%), followed by 2 nd decade (42.85%) then in 4th decade (42.66%) and 5th decade (33.33%).[Chart 4]

Table 2: Correlation of the cytomorphological grades with ATPO titre and TSH levels.

ATPO titre	TSH levels	Grade I	Grade II	Grade III	Total
Raised ATPO	Raised TSH	1	2	3	6
Raised ATPO	Normal TSH	18	13	1	32
Normal ATPO	Raised TSH	0	0	0	0
Normal ATPO	Normal TSH	2	1	0	3
Total		21	16	4	41

ATPO correlation with respect to Cytomorphological grading

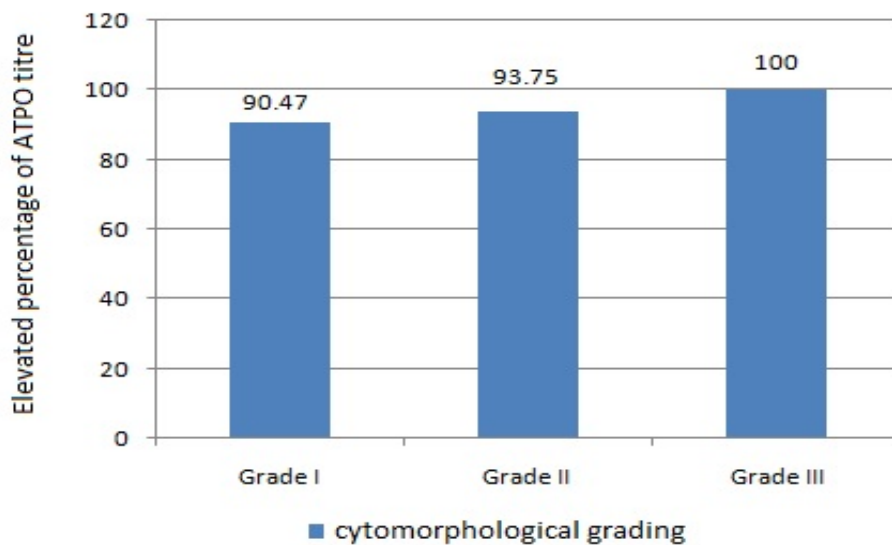


Chart 3: Correlation of ATPO & Cytomorphological grades of thyroiditis.

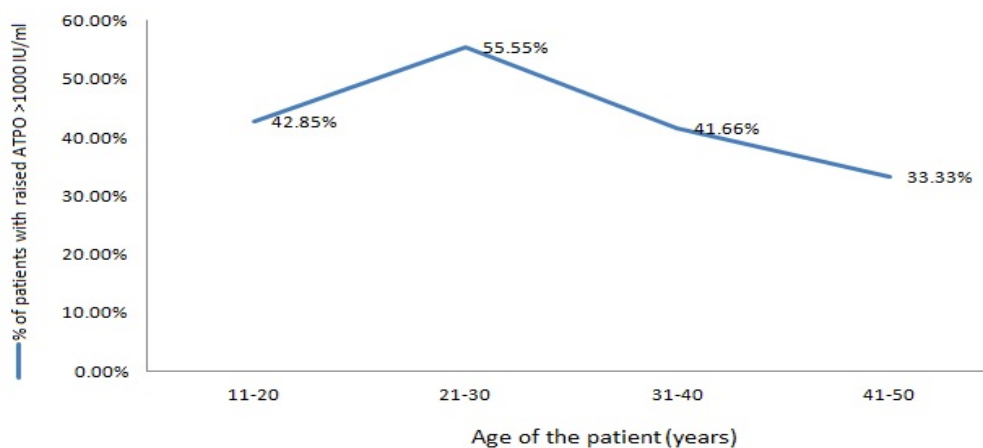


Chart 4: High titer of ATPO with respect to age

Discussion

Hashimoto’s thyroiditis is an autoimmune thyroiditis characterized by infiltration of thyroid gland with lymphocytes and antibody mediated destruction of thyroid follicular cells. Depending on the stage of the disease, the patients present with hyper or hypothyroidism. Chronic phase of the disease leads to hypothyroidism due to few residual and atrophic follicles. In this study out of 179 thyroid cases, 70(39.1%) patients were diagnosed as HT based on cytomorphology features. Our patients were aged between 11years and 60 years. The present study showed mostly females (97.5%) and only one male patient (2.5%), (Chart no.1) This female predominance was observed in most of the studies in the literature.[10] The possible explanation for high female predominance in

thyroid autoimmunity might be associated with the X chromosome containing several immune-related genes crucial to preserving immune tolerance.[11,12] Only one male patient identified in this study with age 15 years presented with the chief complaint of mild swelling in front of the neck for one month, hair loss, and generalized weakness since 15 days. Even though thyroid autoimmune diseases are less common in males, according to studies, there are higher disease progression rates in males who are five times more likely to land up into overt hypothyroidism than females. [13]

The peak age group incidence of HT in this present study was the fourth decade of life, which is in accordance with the study by Bhatia et al. and

another survey by Anila et al. [14] The youngest involved in this study was 11 years female child.

6 out of 41 (14.6%) patients in this study presented with nodular or vaguely nodular or single lobe enlargement in contrast to the study by Bhatia et al. in which only 2.63% of patients with nodular enlargement. This disparity may be due to the early approach of our patients to medical supports. According to a study by Chowdary et al., nodularity is an early feature of HT. [15]

TSH was elevated in 6 out of 41 patients (14.6%), in which 4 (9.8%) are with elevated TSH and normal T3, T4 values indicating subclinical hypothyroidism, and 2 (4.8%) patients had elevated TSH and decreased T3, T4 indicating overt hypothyroidism.

This is in discordance with Bhatia et al. study where elevated TSH observed in 98.68%, Kumar et al.'s study [16], showed 72% with raised TSH, and in the study by Anila et al. 18% of patients presented with raised TSH [14]. This disparity is due to the availability of our tertiary care centre to surrounding people, which helps in regular health check-ups.

38 patients out of 41 (92.6%) of our patients showed raised ATPO titer, which is concordant to Anila et al. study where 95% of patients presented with raised ATPO. ATPO titer not raised in the remaining 3 (7.3%).

Even though ATPO antibodies role in the pathogenesis of Hashimoto's thyroiditis is well documented, a study by Thomas et al. has shown that morphologically there is no difference between anti - TPO antibody positive cases and antibody-negative cases.[17,20] Because the immune destruction inside the thyroid gland occurs much earlier than serological evidence and also, over time cytomorphological features persist, unlike the antibody titers, which may fluctuate, negative serology causes difficulty in diagnosis at times. Since majority of the patients in this study group belong to rural background, they cannot afford for serum ATPO antibody test for many times.

FNAC is a prior and essential investigation for thyromegaly cases. The diagnostic accuracy of FNAC was high, and multiple aspirations being helpful in most of the cases. The advantage of an increased number of aspirations has been stressed by Hamburger et al., who found that as the number of aspirations increases, false-negative results decrease. [18] Cytological lymphocytic grading was done according to Bhatia et al. grading. Grade I cases constituted the largest category with 21/41 (51.2%), followed by Grade II -16/41 (39%) and Grade III 4/41 (9.7%). This data was similar to the data from Anila et al. study in which Grade I constituted 45%, Grade II 36.67%, Grade III

18.3%, and Kumar et al.'s study that constituted 61.90% patients with grade I, 38% with grade II & III combinedly. But in Bhatia et al. study, the largest category was Grade II constituted 44%.

Even though there is a strong association of ATPO towards HT, the present study didn't find a correlation between these two as previous similar studies. That means 10 patients out of 21 patients belong to the Grade I category showed elevated ATPO > 1000 IU/mL. On the other side, half of the patients in grade III, i.e., two patients also had ATPO < 1000 IU/mL (553 IU/mL & 364 IU/mL). But 19/21 (90.47%) patients of Grade I showed raised ATPO titer, 15 / 16 (93.75%) of Grade II had raised ATPO & 4/4 (100%) Grade III showed raised ATPO, which observation was in concordance with Kumar et al. observation. We thought this correlation is limited by a small sample size. Still, in a study by Singh et al. That included a large sample size of 150 cases; it was observed that the grading of thyroiditis and lymphocytic infiltration showed no correlation with the clinical severity of HT.

This present study has limitations like dilution of the sample with blood while needling, improper site selection for aspiration & less number of aspirations causing the lower lymphocytic number may show a picture with downgrading. FNAC cannot represent the entire pathology of the thyroid because needle aspirations yield small samples. Due to the small sample size, the correlation may not appear properly. For better evaluation of correlation, the sample size might be more in number.

Conclusion:

FNAC is the simple and cost-effective diagnostic tool for HT. Lymphocytic infiltration of the thyroid gland occurs much earlier than serological evidence in Hashimoto's thyroiditis. Although elevated levels of ATPO levels strongly associated with Hashimoto's thyroiditis there is no obvious correlation was observed between cytomorphological grades of thyroiditis and levels of ATPO and TSH. Thus multidisciplinary approach is imperative for diagnosis of Hashimoto's thyroiditis.

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