

Cytomorphological spectrum of Hashimoto’s thyroiditis and significance of eosinophils in its diagnosis

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Abstract

Hashimoto’s thyroiditis is an autoimmune chronic inflammatory condition characterized by destruction of the thyroid gland. It has been observed that there is thyroid tissue eosinophilia in many of the cytology smears that were diagnosed as Hashimoto’s thyroiditis. In this study, we determine whether an eosinophilic infiltration of thyroid gland has higher association with Hashimoto’s thyroiditis than with Colloid goiter. Aim is to study the cytomorphological spectrum of Hashimoto’s thyroiditis and to determine whether an eosinophilic infiltration of thyroid gland has higher association with Hashimoto’s thyroiditis than with Colloid goiter. This was a cross-sectional study which was conducted on 100 cases of enlarged thyroid gland. FNAC of thyroid gland was done and smears were stained with Giemsa stain/Pap stain and divided into the following categories:- Hashimoto’s thyroiditis and Colloid goiter. Out of 100 cases, 43 cases were

diagnosed as Hashimoto’s thyroiditis whereas 57 cases as Colloid goiter. Hashimoto’s thyroiditis (32.56%) was seen in 21-30 years whereas Colloid goiter, (33.33%) in 31-40 years of age. Comparison of average number of eosinophils per HPF were done in which 25 cases (58.13%) showed an average of more than 0.6 whereas only 15 cases (26.31%) in the control group showed an average more than 0.6. Comparison of average ratio of eosinophils to neutrophils per HPF were done in which 5 cases (9.30%) showed an average of more than 0.50 whereas only 1 case (1.75%) in the control group showed an average more than 0.50. Fine needle aspiration cytology is a cost effective and specific technique in evaluation of thyroid lesion. The first step in making an accurate diagnosis of the lesion is the adequacy of aspirates. Proper sampling from the lesion along with cytomorphological features and the presence of tissue eosinophilia can resolve pitfall in diagnosis.

Keywords: Hashimoto's thyroiditis, Colloid goiter, FNAC, Eosinophils

Introduction

A survey by the World Health Organization (WHO) in 2010 revealed that there are around 44,670 new cases and 1,690 deaths caused by thyroid disease every year. [1]

Hashimoto's thyroiditis (HT) frequently involves people between the ages of 30 and 50 years and are 15-20 times more common in women than in men. In 1912, it was described by Dr. Hakuru Hashimoto and is the most common form of thyroiditis with a prevalence rate of 1-4% and incidence of 3-6/10000 population per year. It may be associated with hypothyroidism, euthyroidism or occasionally hyperthyroidism. [2-4]

Literature reports various neoplastic and non-neoplastic lesions in association with Hashimoto's thyroiditis like cellular adenoma, follicular neoplasm, Hurthle cell neoplasm, papillary carcinoma, Non-Hodgkin's lymphoma (NHL) and follicular carcinoma, which emphasizes the need for long-term follow up.

Fine needle aspiration cytology (FNAC) is considered a superior and more cost effective tool in diagnosing Hashimoto's thyroiditis than antibody screening. It helps in preventing unnecessary surgeries in case of thyroiditis. [5]

FNAC can accurately diagnose HT in (92%) of the patients. It is diagnosed on smears by finding lymphoid cells, infiltration of follicular cells by lymphoid cells, Hurthle cells, plasma cells, epithelioid cell granulomas and multinucleated giant cells along with degenerative changes in follicular cells. Eosinophils and neutrophils are most often seen adhering to follicular cells. [6]

Thyroid tissue eosinophilia is seen in many FNAC smears diagnosed as Hashimoto's thyroiditis as reported by few workers. [7]

Material and Methods

Study Design: The study was conducted in the Department of Pathology, SGT Medical College and Hospital, Gurugram referred from the Department of ENT on patient with thyroid swelling during the year 2018-2019. FNAC of enlarged thyroid gland was done and smears were stained with Giemsa stain/Pap stained. The smears were examined and diagnosed by two cytopathologists and divided into the following

Categories: Hashimoto's thyroiditis and Colloid goitre and further evaluated for the following-

- To study the cytomorphological spectrum of Hashimoto's thyroiditis.
- To determine whether an eosinophilic infiltration of thyroid gland has higher association with Hashimoto's thyroiditis than with Colloid goiter.
- To count the number of eosinophils and neutrophils and calculate eosinophils:neutrophils (E:N) ratio per HPF in FNA smears of Hashimoto's thyroiditis and Colloid goitre.

Inclusion Criteria

Diagnosed cases of Hashimoto's thyroiditis and Colloid goiter.

Exclusion Criteria

Thyroid Neoplasm and Metastatic lesions

Result

The silent features of the results of the present study are as follows

- Age distribution of the cases ranged from 11 to 82 years, (Table I) with female preponderance. (Table II)
- The morphological features of Hashimoto's thyroiditis consisted of lymphocytes impinging on

follicular cells (figure I), Hurthle cells (figure II), germinal center, plasma cells, epithelioid cells (figure III). (Table III)

- Follicular cells and bare nuclei were seen in Colloid goitre mainly. (Table IV)
- Number of eosinophils per high power field was higher in Hashimoto's thyroiditis. (Table V) (figure IV)

- Number of neutrophils per high power field was found to be higher in Hashimoto's thyroiditis. (Table VI)
- Ratio of eosinophils to neutrophils was found significant in Hashimoto's thyroiditis. (Table VII)
- Comparison with other studies also done. (Table VIII)

Table 1: Age Distribution

Age Groups (Years)	Group A		Group B		Total	
	Cases	Percentage	Cases	Percentage	Cases	Percentage
11-20	6	13.95%	6	10.53%	12	12%
21-30	14	32.56%	11	19.30%	25	25%
31-40	6	13.95%	19	33.33%	25	25%
41-50	6	13.95%	11	19.30%	17	17%
51-60	6	13.95%	5	8.77%	11	11%
>60	5	11.63%	5	8.77%	10	10%
TOTAL	43	100%	55	100%	100	100%
Mean±SD	38.79±16.74		39.05±14.96		38.94±15.67	
Median	35.00		40.00		38.00	
Range	16-80		12-82		12-82	

Group A: Hashimoto's Thyroiditis, **Group B:** Colloid Goiter

Table 2: Gender distribution

Gender	Group A		Group B		Total	
	Cases	Percentage	Cases	Percentage	Cases	Percentage
Female	41	95.35%	51	89.47%	92	92%
Male	2	4.65%	6	10.53%	8	8%
TOTAL	43	100%	57	100%	100	100%

Table 3: Morphological features in Hashimoto's thyroiditis

Groups	X ²	p value	Sign.
Group A- Group B	2.01	0.157	NS
	Hashimoto's Thyroiditis		Case Percentage
	Lymphocyte		43 100%
	Lymphocyte Impinging on Follicular Cell		28 65.12%
	Germinal Centre		14 32.56%

Lymphoid to Follicular Ratio	43	100%
Plasma Cells	12	27.91%
Hurthle Cells	23	53.49%
Epithelioid Cells	13	30.23%
Giant Cells	4	9.30%
Macrophages	13	30.23%
Anisonucleosis	9	20.93%

Table 4: Morphological features in Colloid goiter

Colloid Goiter	Case	Percentage
Follicular cells(Monolayered Sheet)	41	71.93%
Follicular cells (Single)	40	70.18%
Bare Nuclei	29	50.88%
Thick colloid	3	5.26%
Thin colloid	54	94.74%
Cystic Macrophages	11	19.30%

Table 5: Eosinophils per high power field in HT and CG

Eosinophils	Hashimoto's Thyroiditis		Colloid Goiter		Total	
	Case	Percentage	Case	Percentage	Case	Percentage
0.0-0.5	18	41.86%	42	73.68%	60	60%
0.6-1.0	20	46.51%	14	24.56%	34	34%
1.1-1.5	5	11.63%	0	0%	5	5%
1.6-2.0	0	0%	1	1.75%	1	1%
>2.0	0	0%	0	0%	0	0%
TOTAL	43	100%	57	100%	100	100%
Mean±SD	0.66±0.30		0.43±0.25		0.53±0.30	
Median	0.60		0.40		0.45	
Range	0.10-1.40		0.10-1.60		0.10-1.60	

Statistical Analysis

Groups	Mean Difference	t-test	p value	Sign.
Group A- Group B	0.23±0.05	4.172	0.001	HS

Table 6: Neutrophils per high power field in HT and CG

Neutrophils	Hashimoto's Thyroiditis		Colloid Goiter		Total	
	Case	Percentage	Case	Percentage	Case	Percentage
0.0-0.5	0	0%	1	1.75%	1	1%
0.6-1.0	0	0%	3	5.26%	3	3%

1.1-1.5	1	2.33%	6	10.53%	7	7%
1.6-2.0	23	53.49%	26	45.61%	49	49%
>2.0	19	44.19%	21	36.84%	40	40%
TOTAL	43	100% _s	57	100% _s	100	100% _s
Mean±SD	2.04±0.30		1.85±0.47		1.93±0.42	
Median	2.00		1.90		2.00	
Range	1.40-3.00		0.40-2.80		0.40-3.00	

Statistical Analysis

Groups	Mean Difference	t-test	p value	Sign.
Group A- Group B	0.21±0.15	2.492	0.014	S

Table 7: eosinophils to neutrophils in HT and CG

Ratio of Eosiniphila to Neutrophils	Hashimoto's Thyroiditis		Colloid Goiter		Total	
	Case	Percentage	Case	Percentage	Case	Percentage
0.0-0.10	2	4.65%	7	12.28%	9	9%
0.11-0.20	8	18.60%	21	36.84%	29	29%
0.21-0.30	9	20.93%	14	24.56%	23	23%
0.31-0.40	14	32.56%	10	17.54%	24	24%
0.41-0.50	5	11.63%	4	7.02%	9	9%
0.51-0.60	4	9.30%	1	1.75%	5	5%
0.61-0.70	1	2.33%	0	0%	1	1%
0.71-0.80	0	0%	0	0%	0	0%
0.81-0.90	0	0%	0	0%	0	0%
0.90-1.00	0	0%	0	0%	0	0%
1.10-2.0	0	0%	0	0%	0	0%
>2.0	0	0%	0	0%	0	0%
TOTAL	43	100%	57	100%	100	100% _s
Mean±SD	0.32±0.14		0.23±0.11		0.27±0.13	
Median	0.32		0.22		0.25	
Range	0.09-0.61		0.08-0.57		0.08-0.61	

Note: On applying unpaired t-test, P-value calculated was <0.0001 with 95% confidence interval, which is extremely significant statistically

Statistical Analysis

Groups	Mean Difference	t-test	p value	Sign.
Group A- Group B	0.09±0.03	3.442	0.001	HS

Table 8: Comparison of findings in HT cases of present study with other literature

Findings	Present Study	Navya et al	Priyadarshini et al	Chandawale SS et al	Rathi et al	Jayaram et al
No. of case studied	43	50	56	52	50	88
Common age group involved	12-82	26-45 yrs	31-50 yrs	21-30 yrs	23-49 yrs	Not recorded
F: M ratio	11.5:1	All females	4:1	16.3:1	6.14:1	Not recorded
Hurthle Cells	23 (53.49%)	40(80%)	41(73.21%)	Not recorded	37(74%)	49(56%)
Lymphocyte Impinging on Follicular Cell	15 (34.88%)	50(100%)	Not recorded	50(96.15%)	36(72%)	61(69%)
Anisokaryosis	9 (20.93%)	Not recorded	12(21.43%)	9(17.3%)	Not recorded	44%
Germinal Centre	14 (32.56%)	Not recorded	Not recorded	Not recorded	27(54%)	59(67%)
Giant Cells	4 (9.30%)	Not recorded	9(16.07%)	Not recorded	Not recorded	39%
Plasma Cells	12 (27.91%)	Not recorded	8(14.29%)	2(3.84%)	9(18%)	21(23%)
Epithelioid Cells	13 (30.23%)	Not recorded	Not recorded	1(1.92%)	Not recorded	16%
Macrophages	11 (30.23%)	Not recorded	Not recorded	Not recorded	Not recorded	Not recorded
Increased eosinophilic infiltration	25(58.13%)*	25(50%)*	Not recorded	1(1.92%)	24(48%)	15(17%)
Neutrophilic infiltration	+	+	+	+	+	+
E/N >0.5	5(11.63%)	26(52%)	16(32%)	Not recorded	Not recorded	Not recorded

*Significant eosinophilic infiltration i.e No. Of Eosinophils/hpf>0.6 is included here

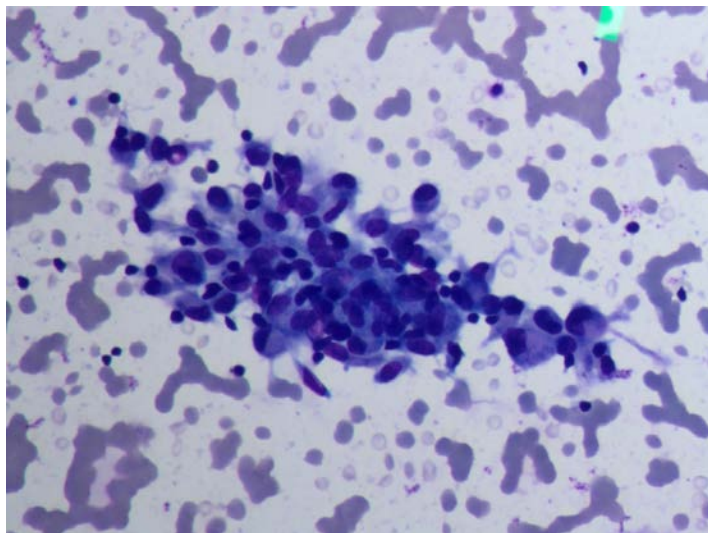


Figure 1: Giemsa stained smear shows lymphocytes impinging on follicular cells (200X)

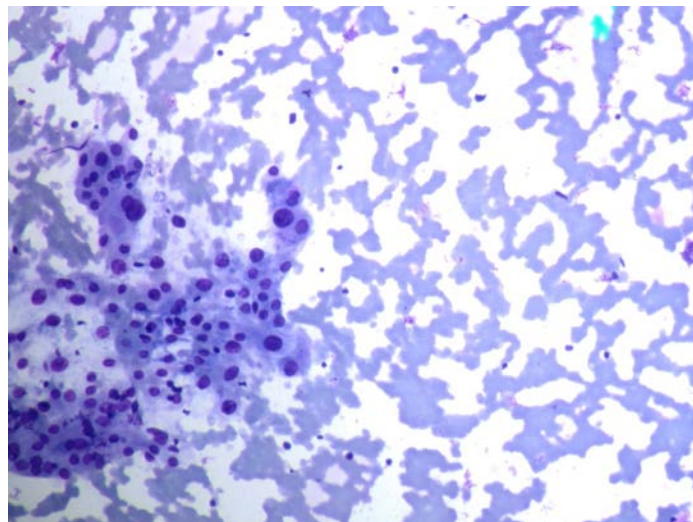


Figure 2: Giemsa stained smear shows Hurthle cells (200X)

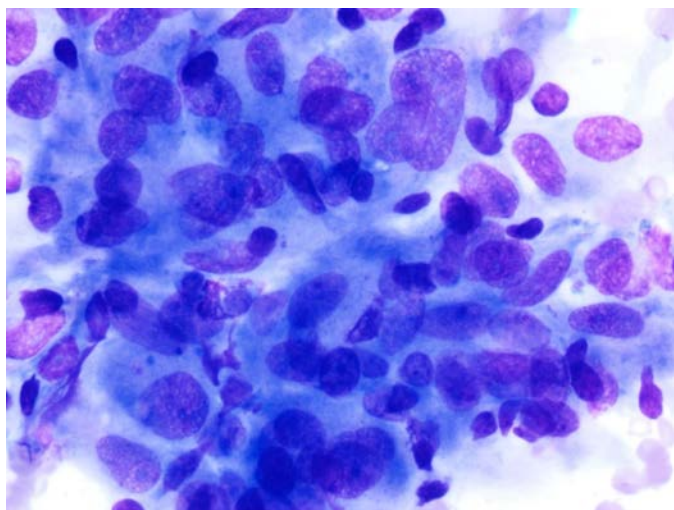


Figure 3: Giemsa stained smear shows epithelioid cells (400X)

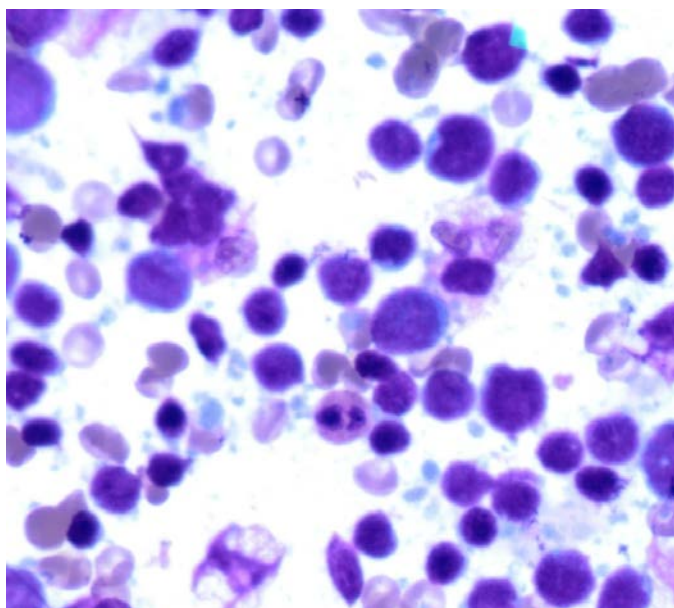


Figure 4: Giemsa stained smear shows eosinophils in thyroid tissue (400X)

Discussion

Hashimoto's thyroiditis (HT) is an autoimmune chronic inflammatory condition characterized by destruction of the thyroid gland. The incidence of HT seems to be increasing in the recent times.²¹ Clinical features of hypothyroidism with painless goiter is seen in majority of patients. The onset is usually insidious and the patient may become aware of symptoms only when euthyroidism is restored. In doubtful cases of goiter

associated with hypothyroidism, FNAC can be used as a confirmatory test.

FNAC is considered as gold standard in diagnosing Hashimoto's thyroiditis beyond its limitation. However a small percentage of cases may be forfeited due to inbuilt constraints of this procedure and cytological diversity of the lesion. Accordingly a thorough cytological interpretation is elementary to arrive at accurate diagnosis in order to avoid surgeries [8,9]

In the present study, 100 cases were included with history, clinical examination and cytological examination.

Age Group

In this study, the age of the cases ranged from 11 to 82 years with the mean age of the patient being 38.94 years and highest frequency of HT (32.56%) was seen in 21-30 years whereas in Colloid goitre, highest frequency (33.33%) was seen in 31-40 years of age.

These findings are in concordance with the studies done by Navya BN et al.[10] and Chandawale et al.[11]

Sex Incidence

In this study, female preponderance (92%) was observed with male-female ratio of 1:11.5. This was in agreement with studies of Chandawale SS. et al.[11], Rathi M et al.[12]

Cytomorphological Diagnosis

Cytomorphological features of all the cases were studied in detail. In our study, out of 100 cases, 43 cases were diagnosed as Hashimoto's thyroiditis whereas 57 cases were diagnosed as Colloid goitre which served as control.

A high prominent Hurthle cell component is said to be one of the major diagnostic pitfalls in differentiating HT from Hurthle cell neoplasm. In this study, out of 43 cases of Hashimoto's thyroiditis, Hurthle cellization of cells was seen in 23 cases (53.49%). This was in agreement with

studies of Navya BN et al.[10], Jayaram G et al.[13], Singh N et al.[14], Jyoti k et al.[15] Rathi M et al.[12], Jayaram G et al.[13] observed out of 88cases, hurthle cells seen in 49cases(56%).

Lymphocytic infiltration of the follicular cell clusters, along with the background cells comprising of mature and reactive lymphoid cells, indicated pre-existing/coexisting HT. In the present study, 28 cases(65.12%) shows lymphocyte impinging on follicular cells out of 43 cases of HT.The result was similar with the study done by Jayaram G et al.[13]whoevaluated 88 cases out of which 61 cases(69%) showed lymphocyte impinging on follicular cells whereas the study done by Singh N et al.[14]showed all cases(150 cases) having lymphocytic infiltration of the follicular cells.

Anisonucleosis is claimed to be especially useful in distinguishing HT from a Hurthle cell neoplasm which paradoxically tends to exhibit a lesser degree of nuclear variability. In our study, out of 43 cases of HT, anisonucleosis was seen only in 9 cases (20.93%). JayaramG et al.[13] evaluated 88 cases in which 44% cases show anisonucleosis. Similar result was seen in a study done by Singh N et al.[14] in which 45.3% cases shows anisonucleosis. Whereas a study done by Jyoti k et al.[15] shows maximum cases 139 cases(92.7%) out of 150 having anisonucleosis.

Lymphoid follicles with germinal centres were noted in 14 cases(32.56%) out of 43 cases of HT. This was in agreement with studies of Jayaram G et al.[13], Singh N et al.[14] and Jyoti k et al.[15]

In the present study, out of 43 cases of HT, giant cells were seen in only 4 cases(9.30%).Similar result was seen by a study done by Jyoti k et al.[15] on 150 cases in which 15 cases(10%) showed giant cells.

In the present study, plasma cells were seen in 12 cases(27.91%). Jayaram G et al.[13]conducted their study on 88 cases in which 21cases (23%) showed plasma cells.Priyadarshiniet al.[16] reported that 8 cases(14.29%) out of 56 cases showed plasma cells.

In the study, 13 cases(30.23%) showedepithelioid cells. In a study conducted by Jyoti k et al.[15]only a minority of cases showed epithelioid cells(14.7%). This was comparable with a study reported by Jayaram G et al.[13]in which 16% cases showed epithelioid cells.

Out of 57 cases of Colloid goitre, follicular cells were arranged in monolayered sheets in 41 cases(71.93%) and scattered singly in 40 cases(70.18%).Bare nuclei were present in 29 cases(50.88%) . Cystic macrophages were present only in 11 cases (19.30%).

In our study, a comparison of average number of eosinophils per HPF between the case and control group were done in which 25 cases (58.13%) showed an average of more than 0.6 whereas only 15 cases (26.31%) in the control group showed an average more than 0.6. The study conducted by Navya et al.[10]in which 25 cases(50%) out of 50 cases showed increased eosinophilic infiltration. A similar study was done by Rathi M. et al.[12]in which 24 cases(48%) out of 50 cases showed increased eosinophilic infiltration. Ekambaram et al.[17]evaluated 50 cases in which 22 cases(44%) showed increased eosinophilic infiltration. Whereas a study conducted by ChandawaleSSet al.[11]showed only 1 case(1.92%) out of 52 cases which had increased eosinophilic infiltration. Jayaram G et al.[13]conducted a study in 88 cases in which increased eosinophilic infiltration seen in 15 cases(17%).

In the present study, average number ofneutrophils per HPF was made in the study group 23 cases (53.49%) showed an average of 1.6-2.0 whereas in 26 cases

(45.61%) in the control group showed an average of 1.6-2.0. 19 cases (44.19%) showed an average of more than 2.0 whereas only 21 cases (36.84%) in the control group showed an average more than 2. Thus, the number of neutrophils per HPF in the smears diagnosed as Hashimoto's thyroiditis was higher than control group (p value 0.014). A similar study was done by Rathi et al.[12] and showed follicular cell infiltration by neutrophils in 26% cases, and Jayaram et al.[13] evaluated in 17% cases of neutrophilic infiltration while Sahoo et al.[18] noted neutrophilic infiltration in 31.37% cases.

In our study, ratio of eosinophils to neutrophils per HPF between the case group and the control were done in which 5 cases (9.30%) showed an average of more than 0.50 whereas only 1 case (1.75%) in the control group showed an average more than 0.50. Manish K et al.[19] evaluated their study on 80 cases. 30% of the cases showed increased E:N ratio with an average of more than 0.5 but none of the cases in the control group showed an average more than 0.5. A study done by Navya et al.[10] showed increased E:N ratio in 52% cases. Ekambaram et al.[17] evaluated in which 44% cases showed increased E:N ratio.

Conclusion

Our study has shown that in cases of Hashimoto's thyroiditis, there is significant infiltration of thyroid gland with eosinophils as compared to colloid goitre. The eosinophilic infiltration was observed in the lymphoid aggregate which was important in ruling out contamination with blood. Presence of eosinophil per HPF increases the sensitivity of cytological diagnosis of Hashimoto's thyroiditis. Larger and independent studies will help us in understanding the significance of eosinophilic infiltration of thyroid gland.

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