



A RETROPROSPECTIVE STUDY ON DIAGNOSTIC EFFICACY OF ULTRASONOGRAPHY AND THYROID AUTOANTIBODIES IN COMPARISON TO HISTOPATHOLOGY IN DETECTION OF HASHIMOTOS THYROIDITIS.

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Abstract:

Introduction: Chronic thyroiditis, commonly referred to as Hashimoto's thyroiditis, is an autoimmune disorder characterized by lymphocytic infiltration of the thyroid gland, often leading to hypothyroidism. While histopathology remains the gold standard it can be done only post-surgery, hence serological testing for anti-thyroid antibodies (anti-TPO and anti-Tg) remains the easily preferred. Recently ultrasound (USG) has gained increasing prominence due to its non-invasive nature, wide availability, and ability to detect structural gland changes that may precede or accompany autoimmune activity. Hence this study was undertaken to compare diagnostic efficacy of ultrasound in comparison to biochemical parameters in patients with Hashimotos thyroiditis with histopathology considered as gold standard diagnosis.

Methodology: A retrospective multicentre study was done in 100 patients diagnosed with hashimotos thyroiditis. Study period was 3 years (). Data was collected from electronic medical records after obtaining ethical committee and institutes approval. Data collected using questionnaire which consists of items on patient characteristics, Biochemical parameters, Sonography assessment and histopathology finding. Sensitivity, specificity, disease prevalence, positive and negative predictive value as well as accuracy are expressed as percentages with 95% confidence intervals. ROC, to predict the best diagnostic test and AUC, to estimate the performance of the test. SPSS version 22 is used with $P < 0.05$ considered as statistically significant.

Results: Mean $\pm$ SD of age was 38 ± 11.3 . Females were 71% with female: male ration of 71:29. Diagnostic accuracy of ultrasound, antithyroid antibodies and both combined together were 86%, 84% and 95% respectively. Though sensitivity of ultrasound and antithyroid antibodies were comparable, specificity of ultrasound is less than antithyroid antibodies. But together combined diagnostic is an outstanding predictor of hashimotos thyroiditis. AUC shows that all the three assessment methods are best predictors of hashimotos with combined method being an outstanding predictor.

Conclusions: In combination with serological tests, ultrasound significantly enhances diagnostic accuracy and aids in early identification of subclinical disease.

Key words: Biochemical parameters, ultrasound, histopathology, diagnostic accuracy, hashimotos thyroiditis, sensitivity, specificity.

Introduction

Chronic thyroiditis, commonly referred to as Hashimoto's thyroiditis, is an autoimmune disorder characterized by lymphocytic infiltration of the thyroid gland, often leading to hypothyroidism. It is the most prevalent cause of hypothyroidism in iodine-sufficient regions and results from an autoimmune attack against thyroid antigens, primarily thyroid peroxidase (TPO) and thyroglobulin (Tg) [1].

In early or active stages, the thyroid may be enlarged and lobulated. In late or burnt-out Hashimoto's, glandular atrophy and volume reduction occur due to fibrosis and destruction of follicular cells [2].

The reported prevalence of subclinical and overt hypothyroidism due to autoimmune thyroiditis in India ranges from 10.95% to 13.13% among adults, with women being disproportionately affected [3,4]. A nationwide epidemiological study in eight Indian cities showed that one in ten adults suffers from hypothyroidism, with chronic thyroiditis being a major contributor [5]. The disease places a significant burden on the healthcare system due to long-term management, reduced productivity, and associated comorbidities such as dyslipidemia and cardiovascular diseases [6].

Diagnosis typically relies on a combination of clinical features, serological markers, and ultrasonographic findings. Clinical symptoms include fatigue, weight gain, cold intolerance, constipation, depression, and menstrual irregularities. A painless, diffusely enlarged thyroid (goitre) is often noted. As the disease progresses, features of hypothyroidism become more prominent [7]. Symptoms are variable and nonspecific hence lack diagnostic significance.

Among serological markers, Laboratory findings in chronic thyroiditis often include elevated serum TSH levels, with low free T4 in overt hypothyroidism. Anti-TPO antibodies are the most sensitive marker and are elevated in approximately 90–95% of patients. Anti-thyroglobulin antibodies may also be present [8,9]. Subclinical hypothyroidism, characterized by elevated TSH with normal T4 levels, is a common early manifestation. While highly specific, antibody levels do not always correlate with the degree of thyroid tissue destruction or symptom severity [10].

Characteristic sonographic features of HT include diffuse hypoechogenicity, heterogeneous echotexture, micronodulation or pseudonodules, lobulated gland contours, variable vascularity (increased or decreased) and atrophy in advanced stages. These features result from lymphocytic infiltration, fibrosis, and glandular remodeling [3]. Increasingly, thyroid ultrasonography is being recognized not only as a structural assessment tool but also for its diagnostic utility in autoimmune thyroiditis.

While histopathology remains the gold standard it can be done only post-surgery, hence serological testing for anti-thyroid antibodies (anti-TPO and anti-Tg) remains the easily preferred. Recently ultrasound (USG) has gained increasing prominence due to its non-invasive nature, wide availability, and ability to detect structural gland changes that may precede or accompany autoimmune activity. Hence this study was undertaken to compare diagnostic efficacy of ultrasound

in comparison to biochemical parameters in patients with Hashimotos thyroiditis with histopathology considered as gold standard diagnosis.

Methodology: A retrospective multicentre study was done in 100 patients diagnosed with hashimotos thyroiditis. Study period was 3 years ()

Data was collected from electronic medical records after obtaining ethical committee and institutes approval.

The study included thyroid function test results obtained from electronic records 1 week prior to surgery, specifically levels of free triiodothyronine (FT3), free thyroxine (FT4), and thyrotropin (TSH), along with thyroid peroxidase antibodies (TPOAb), thyroglobulin antibodies (TgAb), thyroid ultrasound findings, and postoperative histopathology (HP) data.

Inclusion Criteria:

Patients who underwent surgery for thyroid nodules, regardless of age or gender

Patients with complete preoperative data for FT3, FT4, TSH, TPOAb, TgAb, high resolution ultrasound findings and Histopathology findings

Exclusion Criteria:

Patients with unclear or indeterminate HP reports

Patients diagnosed with immune or hematological system diseases

Patients taking medications known to affect thyroid function

Procedure: data from electronic data base was entered in to a pretested structured questionnaire. The questionnaire consists of items on patient characteristics like age of patient, gender of patient, duration of thyroid disorder.

Biochemical parameters section includes FT3, FT4, TSH levels, Thyroid autoantibodies status (TPOAb, TgAb). The reference ranges for thyroid function indicators followed were as follows: FT3: 3.1–6.8 pmol/L; FT4: 12–22 pmol/L; TSH: 0.27–4.2 mU/L; TPOAb: 0–34 kU/L; and TgAb: 0–115 kU/L. TPOAb levels exceeding 34 kU/L and TgAb levels above 115 kU/L were considered positive.

Sonography assessment includes high resolution ultrasound findings whether suggestive of hashimotos thyroiditis or not and. The ultrasound features of Hashimoto's thyroiditis (HT) typically appear as a diffusely enlarged thyroid gland with a hypoechoic and heterogeneous texture, and in some cases, may display a grid-like or septated pattern [11,12,13].

Pathological assessment includes finding suggestive of hashimotos thyroiditis or not. Hashimoto's thyroiditis (HT) is characterized by diffuse lymphocytic infiltration of the thyroid gland, eosinophilic changes in thyroid cells, numerous lymphoid follicles, the presence of germinal centers, and widespread fibrosis. In contrast, non-HT cases show diffuse infiltration by lymphocytes and other inflammatory cells but lack the hallmark pathological features of HT.[14]

Operational definitions:

Definitions

- Sensitivity: probability that a test result will be positive when the disease is present (true positive rate).

$$= a / (a+b)$$

- Specificity: probability that a test result will be negative when the disease is not present (true negative rate).

$$= d / (c+d)$$

- Positive likelihood ratio: ratio between the probability of a positive test result given the presence of the disease and the probability of a positive test result given the absence of the disease, i.e.

$$= \text{True positive rate} / \text{False positive rate} = \text{Sensitivity} / (1-\text{Specificity})$$

- Negative likelihood ratio: ratio between the probability of a negative test result given the presence of the disease and the probability of a negative test result given the absence of the disease, i.e.

= False negative rate / True negative rate = (1-Sensitivity) / Specificity

- Positive predictive value: probability that the disease is present when the test is positive.

$$PPV = \frac{\text{sensitivity} \times \text{prevalence}}{\text{sensitivity} \times \text{prevalence} + (1 - \text{specificity}) \times (1 - \text{prevalence})}$$

- Negative predictive value: probability that the disease is not present when the test is negative.

$$NPV = \frac{\text{specificity} \times (1 - \text{prevalence})}{(1 - \text{sensitivity}) \times \text{prevalence} + \text{specificity} \times (1 - \text{prevalence})}$$

- Accuracy: overall probability that a patient is correctly classified.

$$= \text{Sensitivity} \times \text{Prevalence} + \text{Specificity} \times (1 - \text{Prevalence})$$

Sensitivity, specificity, disease prevalence, positive and negative predictive value as well as accuracy are expressed as percentages with 95% confidence intervals. [15]

Statistical Analysis

Data entered in SPSS version 22 package. Data represented in frequencies, mean and standard deviation (SD) and median for non-normal distributed parameters. Chi-square test was used for statistical analysis. $P < 0.05$ was considered to be significant statistically. Sensitivity and specificity, positive and negative predictive value as well as accuracy of antithyroid antibodies and sonographic findings in comparison with histopathology as gold standard was estimated using MEDcalc online calculator. ROC to predict the best diagnostic test and AUC to estimate the performance of the test.

Results:

Out of 100 patients medical records retrieved age range was 15- 78 years with 61% of patients in the age group of 15-40 years followed by 25% and 14% in 41-60 years and >60 years respectively. Mean $\pm$ SD of age was 38 ± 11.3 . Females were 71% with female: male ratio of 71:29. (table 1)

Table 1: Distribution by Patient's characteristics

Patient characteristic	Group	Frequency
Age	15-40 Years	61 (61%)
	41-60 Years	25 (25%)
	>60 years	14 (14%)
	Mean $\pm$ SD	38 ± 11.3
Sex	Male	29 (29%)
	Female	71 (71%)
Duration of thyroid disorder	< 5 years	79 (79%)
	>5 years	21(21%)

Based on histopathology, 88 patients have pathology of hashimotos thyroiditis and 12 patients do not show characteristics of hashimotos thyroiditis. Based on ultrasound 82 showed characteristics of hashimotos thyroiditis and 18 patients do not show characteristics of hashimotos thyroiditis. Based on antithyroid antibody profile 78 showed characteristics of hashimotos thyroiditis and 22 patients do not show characteristics of hashimotos thyroiditis. Based on Combined assessment (ultrasound + biochemical) 87 showed characteristics of hashimotos thyroiditis and 13 patients do not show characteristics of hashimotos thyroiditis. (table 2)

Table 2: Assessment Methods versus pathological finding.

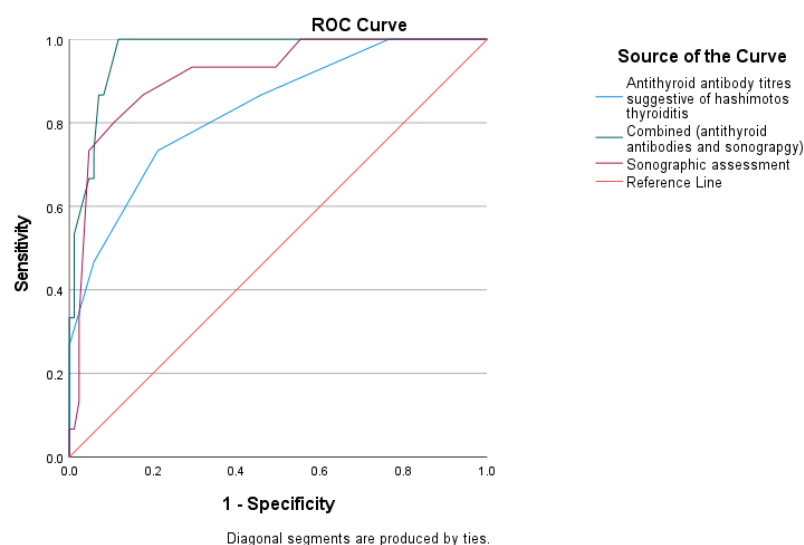
Assessment Method		Pathological Finding		Total
		Positive (88)	Negative (12)	
Ultrasound Finding	Positive	78 True Positive	4 False positive	82
	Negative	10 False Negative	8 True Negative	18
Antithyroid antibodies	Positive	75 True Positive	3 False positive	78
	Negative	13 False Negative	9 True Negative	22
Combined (ultrasound + biochemical)	Positive	85 True Positive	2 False positive	87
	Negative	3 False Negative	10 True Negative	13

Sensitivity and specificity of ultrasound over histopathology with 95% CI, was 88.64% (80.09% to 94.41%) and 66.67% (34.89 % to 90.08 %). Sensitivity and specificity of antithyroid antibodies over histopathology with 95% CI, was 85.23% (76.06% to 91.89%) and 75.00% (42.81% to 94.51%). Sensitivity and specificity of both ultrasound and antithyroid antibodies over histopathology with 95% CI, was 96.59% (90.36% to 99.29%) and 83.33% (51.59% to 97.91%). Diagnostic accuracy of ultrasound, antithyroid antibodies and both combined together were 86%, 84% and 95% respectively. Though sensitivity of ultrasound and antithyroid antibodies were comparable, specificity of ultrasound is less than antithyroid antibodies. But together combined diagnostic is an outstanding predictor of hashimotos thyroiditis. (table 3)

Table 3: Diagnostic accuracy of assessment methods versus histopathology

Statistic	ultrasound Finding		Antithyroid antibodies		Combined (ultrasound + Antithyroid antibodies)	
	Value	95% CI	Value	95% CI	Value	95% CI
Sensitivity	88.64%	80.09% to 94.41%	85.23%	76.06% to 91.89%	96.59%	90.36% to 99.29%
Specificity	66.67%	34.89% to 90.08%	75.00%	42.81% to 94.51%	83.33%	51.59% to 97.91%
Positive Likelihood Ratio	2.66	1.19 to 5.94	3.41	1.27 to 9.12	5.80	1.63 to 20.55
Negative Likelihood Ratio	0.17	0.08 to 0.35	0.20	0.11 to 0.36	0.04	0.01 to 0.13
Disease prevalence	88.00%	79.98% to 93.64%	88.00%	79.98% to 93.64%	88.00%	79.98% to 93.64%
Positive Predictive Value	95.12%	89.72% to 97.76%	96.15%	90.34% to 98.53%	97.70%	92.30% to 99.34%
Negative Predictive Value	44.44%	28.28% to 61.88%	40.91%	27.56% to 55.75%	76.92%	51.58% to 91.25%
Accuracy	86.00%	77.63% to 92.13%	84.00%	75.32% to 90.57%	95.00%	88.72% to 98.36%

Figure: ROC of assessment methods in hashimotos thyroiditis



ROC curve and AUC shows combined antithyroid antibodies and ultrasound (AUC= 0.968) is a significantly better predictor when compared to only ultrasound (AUC= 0.915) and antithyroid antibodies (AUC= 0.832) alone. AUC shows that all the three assessment methods are best predictors of hashimotos with combined method being an outstanding predictor. (figure 1 and table 4)

Table 4: AUC for the assessment methods, in predicting hashimotos thyroiditis.

Area Under the Curve					
Test Result Variable(s)	Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
Antithyroid antibody titres suggestive of hashimotos thyroiditis	.832	.058	.000	.718	.945
Combined (antithyroid antibodies and sonography)	.968	.016	.000	.937	.998
Sonographic assessment	.915	.038	.000	.840	.990

Discussion:

This study compared ultrasound findings and biochemical parameters of hashimotos thyroiditis with histopathology as gold standard.

In the current study out of 100 patients enrolled age range was 15- 72 years with 61% of patients in the age group of 15-40 years followed by 24% and 16% in 41-60 years and >60 years respectively. Mean age in the present study was 38 ±11.3 years, similarly in study by Eftekharian F, et al, mean age was of 38.6 (SD = ±8.03) years.[16] In study by Hu Y L the median and inter quartile range (Q1,Q3)of patients' age was 41(32,52)years and the range was 16-75 years.[17] Which was similar to present study, thus it is seen more in middle aged.

In the current study, Females were 71% with female: male ration of 71:29. In study by HU YL data of patients showed 217 males and 575 females, thus more common in females compared to males.[17]

In the current study sensitivity and specificity of ultrasound over histopathology with 95% CI, was 88.64% (80.09% to 94.41%) and 66.67% (34.89 % to 90.08 %). Sensitivity and specificity of antithyroid antibodies over histopathology with 95% CI, was 85.23% (76.06% to 91.89%) and

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In study by HU YL results were similar to our study, with HP as the diagnostic gold standard, TPOAb exhibited similar sensitivity (59.3% vs 61.2%, $P=0.752$), accuracy (85.0% vs 83.6%, $P=0.379$), area under the receiver operating characteristic curve (AUC) (0.767 vs 0.764, $P=0.886$) and higher specificity (94.2% vs 91.6%, $P=0.033$) when compared with TgAb in diagnosing euthyroid HT. They both exhibited a higher sensitivity (59.3% vs 44.5%, $P=0.002$; 61.2% vs 44.5%, $P<0.001$), accuracy (85.0% vs 79.7%, $P=0.001$; 83.6% vs 79.7%, $P=0.013$) and AUC (0.767 vs 0.684, $P<0.001$; 0.764 vs 0.684, $P<0.001$) than HRUS. Compared with each method alone, the sensitivity and AUC of TPOAb combined with TgAb or HRUS were improved. The combination of three methods showed the greatest sensitivity.

Study by Tan HL stated that the overall sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), false positive rate (FPR), false negative rate (FNR), and accuracy of ultrasound for diagnosing HT were found to be 56.3%, 92.1%, 71.1%, 86.0%, 28.9%, 14.0%, and 83.0%, respectively. The overall sensitivity, specificity, PPV, NPV, FPR, FNR, and accuracy of the TAb for diagnosing HT were 78.2%, 85.7%, 66.0%, 91.7%, 34.0%, 8.3%, 83.7%, respectively. Their study also showed that the sequential diagnostic specificity, PPV, FPR, and FNR of serum thyroid autoantibodies combined with ultrasound for HT was significantly superior to parallel diagnostic methods (specificity: 98.4% vs. 82.6%; PPV: 88.1% vs. 50.5%; FPR: 11.9% vs. 49.5%; FNR: 19.1% vs. 82.6%; all $P<0.001$), while the sensitivity, and NPV were relatively lower (sensitivity: 33.6% vs. 74.8%; NPV: 80.9% vs. 93.3%). [17]

Thus as per this ultrasound and antithyroid antibodies combined together increases the diagnostic accuracy.

Conclusions: Ultrasound is a highly sensitive, non-invasive, and accessible tool for the diagnosis and monitoring of Hashimoto's thyroiditis. While not as specific as serology, its ability to detect structural changes and correlate with disease progression makes it an indispensable adjunct. In combination with serological tests, ultrasound significantly enhances diagnostic accuracy and aids in early identification of subclinical disease.

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