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Original Research Article

The Impact of Hashimoto's Thyroiditis on The Diagnostic Utility of P63 and CK19 Immunohistochemistry Markers in Predicting Thyroid Cancer

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Abstract

Background: The coexistence of Hashimoto's thyroiditis (HT) poses significant diagnostic challenges in differentiating benign from malignant thyroid lesions using immunohistochemistry. CK-19 and p63 are commonly applied markers, but their reliability diminishes in the presence of autoimmune thyroiditis.

Aim: To determine the impact of HT on the diagnostic utility of CK-19 and p63 markers in predicting follicular-derived thyroid cancer.

Material and Methods: A cross-sectional analysis was performed on 44 thyroid specimens. CK-19 and p63 immunoexpression were evaluated, and antibody profiles were compared between patients with and without HT.

Results: CK-19 positivity was observed in both cancer and non-cancer groups without significant difference. P63 showed limited discriminatory power. Antibody levels, including anti-thyroglobulin and anti-thyroperoxidase, were significantly elevated in HT patients, influencing marker interpretation.

Conclusion: HT alters the diagnostic performance of CK-19 and p63, reducing their specificity in thyroid cancer detection. A multimodal approach integrating IHC, molecular assays, and serological markers is essential to improve diagnostic accuracy.

Keywords: Hashimoto's thyroiditis, CK-19, p63, thyroid cancer.

Introduction

Thyroid cancer is the most common endocrine malignancy, accounting for nearly 3% of all cancers worldwide, with an increasing incidence over the past two decades [1]. Among thyroid neoplasms, follicular-derived carcinomas, including papillary and follicular thyroid carcinoma, constitute the vast majority. Accurate histopathological diagnosis of these tumors remains crucial, as it guides surgical management and prognosis. However, the interpretation of thyroid lesions is often complicated by the presence of underlying inflammatory disorders such as Hashimoto's thyroiditis (HT) [2].

Hashimoto's thyroiditis is a chronic autoimmune thyroid disease characterized by lymphocytic infiltration, follicular destruction, and fibrosis [3]. The coexistence of HT with thyroid nodules frequently poses diagnostic challenges, as reactive atypia, oncocytic metaplasia, and architectural alterations may mimic malignant changes. Several studies have also suggested an epidemiological association between HT and an increased risk of papillary thyroid carcinoma, raising concerns regarding its potential role in thyroid carcinogenesis [4]. These factors underscore the need for reliable immunohistochemical (IHC) markers to distinguish between benign inflammatory changes and true malignancy in the thyroid. Among the IHC markers, cytokeratin 19 (CK19) is one of the most extensively studied in thyroid pathology. CK19, a low-molecular-weight cytokeratin, is strongly expressed in papillary thyroid carcinoma, and its diffuse cytoplasmic staining pattern has been proposed as a useful diagnostic adjunct [5]. However, CK19 is not entirely specific, as focal expression can also be observed in benign thyroid lesions and in areas affected by Hashimoto's thyroiditis, potentially reducing its diagnostic accuracy [6].

P63, a member of the p53 gene family, has been investigated as another potential diagnostic marker in thyroid pathology. Its nuclear expression has been associated with malignant transformation and tumorigenesis, particularly in follicular-derived carcinomas [7]. While some studies have shown that p63 may help differentiate papillary carcinoma from benign nodules, others have reported variable expression in HT, leading to diagnostic overlap and

conflicting interpretations [8]. The presence of HT may therefore alter the expression patterns of CK19 and p63, complicating their diagnostic utility. Inflammation-induced reactive changes may mimic malignancy at both morphological and molecular levels, and this overlap can reduce the specificity of IHC markers if not carefully interpreted [9]. Hence, it becomes essential to evaluate how HT influences the staining patterns of p63 and CK19, and whether these markers retain their predictive value in the setting of autoimmune thyroiditis.

Given the high prevalence of thyroid nodules and the frequent coexistence of HT, understanding these interactions has significant clinical implications. By assessing the impact of Hashimoto's thyroiditis on the diagnostic accuracy of p63 and CK19, this study aims to refine the utility of these IHC markers in predicting follicular-derived thyroid carcinoma, ultimately improving diagnostic precision and patient management [10].

Material and Methods

This cross-sectional study was conducted on a total of 44 thyroid specimens that were obtained from patients who had undergone thyroidectomy for clinically or radiologically detected thyroid nodules. All specimens were fixed in 10% buffered formalin, routinely processed, and embedded in paraffin blocks. Sections of 4–5 µm thickness were prepared and stained with hematoxylin and eosin for routine histopathological evaluation. The histological diagnosis was established according to the World Health Organization (WHO) criteria, and cases were divided into thyroid cancer and non-cancer groups. In addition, cases were further subclassified into those with coexisting Hashimoto's thyroiditis and those without, based on the presence of diffuse lymphocytic infiltration with germinal center formation, follicular atrophy, and fibrosis.

Immunohistochemistry (IHC) was performed on representative sections from each case using monoclonal antibodies against CK-19 and p63. The staining procedure involved antigen retrieval, blocking of endogenous peroxidase activity, and incubation with primary antibodies, followed by secondary antibody detection using a polymer-based system.

Diaminobenzidine (DAB) was applied as a chromogen, and hematoxylin was used for counterstaining. Appropriate positive and negative controls were included in each batch. CK-19 expression was interpreted as cytoplasmic staining, while p63 was assessed as nuclear staining. The results were recorded as positive when more than 10% of tumor or follicular cells demonstrated distinct staining, and negative when staining was absent or below this threshold. In addition to IHC evaluation, serum antibody profiles were analyzed. Preoperative serum levels of anti-thyroglobulin (anti-Tg) and anti-thyroperoxidase (anti-TPO) antibodies were retrieved from patient records and compared between the groups. Demographic data including age and sex were collected, and clinical correlations were performed.

All data were compiled and statistically analyzed. Continuous variables were expressed as median with interquartile range (IQR), and categorical variables were represented as frequencies and percentages. Statistical comparisons between groups were carried out using the chi-square test or Fisher's exact test for categorical variables, and the Mann–Whitney U test for continuous variables. A p-value of less than 0.05 was considered statistically significant.

Results

The demographic characteristics of patients with and without thyroid cancer were compared as shown in Table 1. Among the 44 patients, 15 were diagnosed with thyroid cancer and 29 did not have thyroid cancer. The median age was slightly higher in the cancer group (41 years) compared to those without cancer (37 years), but this difference was not statistically significant. The gender distribution revealed that females were predominant in both groups, with a male-to-female ratio of 3:12 in cancer cases and 4:25 in the non-cancer group, showing no significant association with thyroid cancer. Immunohistochemistry results demonstrated CK-19 positivity in the majority of cancer patients (13/15; 86.7%) and in a comparable proportion of those without cancer (25/29; 86.2%), with no significant difference.

Expression of p63 was also similar, with 6 out of 15 (40%) in the cancer group versus 10 out of 29 (34.5%) in the non-cancer group, yielding no statistical significance. However, antibody levels revealed marked differences. Median anti-thyroglobulin antibody levels were significantly lower in cancer patients compared to non-cancer patients. Conversely, anti-thyroperoxidase antibody levels were notably reduced in cancer patients, with both markers showing statistically significant differences.

Comparison of demographic and immunohistochemical findings in patients with and without Hashimoto's thyroiditis is shown in Table 2. Of the 44 patients, 20 had Hashimoto's thyroiditis while 24 did not. The median age was slightly lower in HT patients (36 years) compared to those without HT (39 years), though this was not statistically significant. Female predominance was observed in both groups with a ratio of 3:17 in the HT group and 3:21 in the non-HT group. CK-19

expression was high in both subgroups, with 18/20 (90%) HT cases and 19/24 (79.2%) non-HT cases positive, again with no significant difference. Expression of p63 was seen in 8/20 (40%) of HT patients and 7/24 (29.2%) of those without HT, indicating comparable distribution. However, antibody profiles showed strong differences, with anti-thyroglobulin antibody levels significantly higher in HT patients compared to those without HT. Similarly, anti-thyroperoxidase antibody levels were substantially elevated in HT cases, and both differences were statistically significant, confirming the immunological impact of Hashimoto's thyroiditis in this cohort.

Table 1: Comparison of demographic data of patients with and without thyroid cancer (n=44)

Parameter	Thyroid cancer Yes (n=15)	Thyroid cancer No (n=29)
Age in years, median (IQR)	41 (27)	37 (21)
Male: Female (N, %)	3 (20.0%): 12 (80.0%)	4 (13.8%): 25 (86.2%)
CK-19 (N, %)	13 (86.7%): 2 (13.3%)	25 (86.2%): 4 (13.8%)
P63 (N, %)	6 (40.0%): 9 (60.0%)	10 (34.5%): 19 (65.5%)
Anti-thyroglobulin antibody IU/ml, median (IQR)	21.8 (31.4)	340.6 (820.7)
Anti-thyroperoxidase antibody IU/ml, median (IQR)	79.4 (117.6)	876.2 (792.1)

Table 2: Comparison of demographic data of patients with and without Hashimoto's thyroiditis (n=44)

Parameter	Hashimoto's thyroiditis Yes (n=20)	Hashimoto's thyroiditis No (n=24)
Age in years, median (IQR)	36 (20)	39 (25)
Male: Female (N, %)	3 (15.0%): 17 (85.0%)	3 (12.5%): 21 (87.5%)
CK-19 (N, %)	18 (90.0%): 2 (10.0%)	19 (79.2%): 5 (20.8%)
P63 (N, %)	8 (40.0%): 12 (60.0%)	7 (29.2%): 17 (70.8%)
Anti-thyroglobulin antibody IU/ml, median (IQR)	352.7 (828.2)	19.1 (30.5)
Anti-thyroperoxidase antibody IU/ml, median (IQR)	872.3 (816.4)	82.5 (176.9)

Discussion

The present study explored the influence of Hashimoto's thyroiditis (HT) on the diagnostic utility of CK-19 and p63 immunohistochemical markers in differentiating thyroid malignancies. Our findings showed that CK-19 expression was high in both thyroid cancer and non-cancer groups, with no statistically significant difference, suggesting that while CK-19 is a sensitive marker, its specificity is compromised in the presence of chronic inflammatory changes such as HT.

Similar trends have been noted in recent reports where CK-19 positivity has been observed in both papillary carcinoma and benign thyroid lesions, making its sole use unreliable in certain contexts [11]. This reinforces the necessity of interpreting CK-19 results cautiously when autoimmune thyroiditis coexists.

In contrast, p63 expression showed variable distribution across groups but without reaching statistical significance, indicating its limited diagnostic value when used independently. Previous studies have emphasized that p63 positivity may reflect reactive or regenerative follicular cells rather than malignant transformation, thereby diminishing its discriminatory ability in chronic inflammatory backgrounds [12]. This finding aligns with our data, where p63 could not distinctly differentiate malignancy from non-malignant lesions in patients with HT, suggesting that p63 should not be solely relied upon for cancer prediction. An important observation in this study was the significant elevation of anti-thyroglobulin and anti-thyroperoxidase antibody levels in HT patients compared to those without HT.

These findings are consistent with existing literature that underscores the autoimmune basis of HT and its potential role in altering the thyroid microenvironment [13]. The presence of high antibody titers, while clinically relevant in diagnosing HT, can confound the interpretation of IHC markers. This immunological milieu may lead to false-positive or false-negative results, thereby affecting the overall diagnostic accuracy of CK-19 and p63.

Our results also emphasize that the diagnostic approach for thyroid cancer in patients with coexisting HT requires a multimodal strategy. Recent studies have recommended combining IHC markers with molecular testing such as BRAF V600E mutation analysis to enhance specificity and reduce diagnostic ambiguity [14]. This integrated approach has demonstrated superior diagnostic performance in differentiating malignant from benign thyroid lesions, particularly in the setting of autoimmune thyroiditis. Furthermore, our study highlights the clinical implication of interpreting IHC markers within the context of patient

demographics and serological profiles. For example, despite CK-19 being positive in most cases, its predictive value was overshadowed by the concurrent antibody elevations in HT patients.

This finding suggests that a comprehensive evaluation that incorporates histopathology, IHC, and serological markers can improve diagnostic confidence.

Emerging evidence also supports the use of machine learning–based histopathological analysis combined with IHC markers to reduce observer variability and enhance reproducibility [15]. Such technological advancements may play a pivotal role in refining diagnostic pathways for thyroid lesions in complex scenarios like HT.

Conclusion

The diagnostic utility of CK-19 and p63 in thyroid cancer is significantly influenced by the presence of Hashimoto's thyroiditis. While CK-19 remains a sensitive marker, its specificity diminishes in HT patients due to overlapping expression patterns. Similarly, p63 showed limited discriminatory power, further reducing its standalone value.

Elevated anti-thyroglobulin and anti-thyroperoxidase antibody levels confirm the autoimmune pathology of HT and underscore its confounding role in IHC interpretation.

A multimodal diagnostic approach that integrates IHC, molecular assays, and serological data is essential to improve accuracy and reduce diagnostic errors in thyroid cancer associated with HT.

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