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Original Research Article

Diagnostic Utility of Claudin-1 and CD56 Expression in Distinguishing Benign Thyroid Nodules from Papillary Thyroid Carcinoma

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Conflict of interest: Nil

Abstract:

Background: Differentiating between benign thyroid nodules and papillary thyroid carcinoma (PTC) is a diagnostic challenge in routine histopathology. Immunohistochemical markers such as Claudin-1 and CD56 have shown promise in distinguishing these lesions more accurately.

Objectives: This study aims to evaluate the expression patterns of Claudin-1 and CD56 in thyroid nodules to assess their utility in differentiating benign thyroid lesions from papillary thyroid carcinoma.

Materials and Methods: A prospective observational study was conducted in the Department of Pathology, Darbhanga Medical College and Hospital, Bihar, India, over a period of 18 months. A total of 120 cases were included comprising 60 cases of histologically confirmed benign thyroid nodules (colloid goiter, follicular adenoma, and Hashimoto's thyroiditis) and 60 cases of papillary thyroid carcinoma. Immunohistochemical staining for Claudin-1 and CD56 was performed and analyzed semi-quantitatively based on the intensity and extent of staining.

Results: Claudin-1 was strongly expressed in 92% of PTC cases, while only 10% of benign nodules showed weak or focal Claudin-1 positivity. Conversely, CD56 expression was retained in 87% of benign nodules, whereas only 5% of PTC cases showed any positivity. A statistically significant inverse correlation between Claudin-1 and CD56 expression was observed in PTC cases ($p < 0.001$).

Conclusion: Claudin-1 and CD56 serve as valuable complementary immunohistochemical markers in differentiating benign thyroid nodules from papillary thyroid carcinoma. The combined use of these markers enhances diagnostic accuracy, particularly in morphologically ambiguous cases.

Keywords: Claudin-1, CD56, Papillary Thyroid Carcinoma, Benign Thyroid Nodule, Immunohistochemistry, Diagnostic Marker, Thyroid Neoplasm, Pathology, India

Introduction

Thyroid nodules are a common clinical finding, with an estimated prevalence ranging from 4% to 7% in the general population by palpation and up to 67% by high-resolution ultrasonography. While the majority of these nodules are benign, approximately 5–15% are malignant, with papillary thyroid carcinoma (PTC) being the most frequent histological type, accounting for nearly 80–85% of all thyroid cancers [1]. Accurate and early differentiation between benign and malignant thyroid lesions is essential for appropriate clinical management, prognosis, and therapeutic planning.

Fine needle aspiration cytology (FNAC) remains the first-line diagnostic tool for evaluating thyroid nodules. However, in a significant number of cases—especially in follicular-patterned lesions—cytological and even histopathological distinction between benign and malignant entities can be challenging [2]. Architectural overlaps, cytological mimicry, and limitations in evaluating capsular and vascular invasion in small biopsies contribute to diagnostic ambiguity. Therefore, additional diagnostic markers, especially immunohistochemical (IHC) adjuncts, are increasingly being explored to enhance diagnostic confidence and reduce indeterminate diagnoses [3].

Claudin-1, a tight junction transmembrane protein, plays a crucial role in maintaining epithelial cell polarity and barrier function. It has been shown to be overexpressed in various malignancies, including breast, colon, and thyroid carcinomas [4]. Studies have demonstrated that Claudin-1 expression is

significantly upregulated in papillary thyroid carcinoma compared to benign thyroid lesions, suggesting a potential role in tumor invasion and metastasis.

CD56, also known as Neural Cell Adhesion Molecule (NCAM), is normally expressed in follicular cells of the thyroid and is characteristically lost in PTC [5]. The absence of CD56 expression has been proposed as a specific marker for papillary carcinoma, particularly helpful in cases with follicular architecture or oncocytic changes where traditional morphological criteria may not suffice.

Recent advancements in immunohistochemistry have provided an opportunity to utilize these markers in routine diagnostic practice [6]. The combined use of Claudin-1 and CD56 may offer a robust diagnostic panel to aid in distinguishing PTC from its benign mimics, especially in cases with equivocal histological features [7].

This study aims to evaluate the expression patterns of Claudin-1 and CD56 in a spectrum of benign and malignant thyroid nodules and assess their individual and combined diagnostic value. By analyzing their expression profiles in a prospective cohort, we seek to establish a reliable immunohistochemical approach that can complement conventional histopathology in improving diagnostic precision.

Through this research conducted at the Department of Pathology, Darbhanga Medical College and Hospital, Darbhanga, Bihar, India, we hope to contribute meaningful data to the growing body of literature advocating for immunohistochemical markers in routine thyroid pathology diagnostics.

Methods

This prospective observational study was conducted in the Department of Pathology, Darbhanga Medical College and Hospital, Darbhanga, Bihar, India, over a period of 18 months. A total of 120 thyroidectomy specimens were included in the study, comprising 60 cases of histologically confirmed benign thyroid nodules (including colloid goiter, follicular adenoma, and Hashimoto's thyroiditis) and 60 cases of papillary thyroid carcinoma (PTC). All specimens were received in the histopathology laboratory, fixed in 10% neutral buffered formalin, processed routinely, and embedded in paraffin blocks.

Sections of 4–5 microns thickness were cut and stained with Hematoxylin and Eosin (H&E) for initial histopathological diagnosis. Representative sections from each case were then subjected to immunohistochemical staining using antibodies against Claudin-1 and CD56. Standard immunohistochemical protocols were followed, including deparaffinization, rehydration, antigen retrieval, blocking of endogenous peroxidase activity, and incubation with primary antibodies. Visualization was done using the DAB chromogen system and counterstaining with hematoxylin.

The expression of Claudin-1 and CD56 was evaluated semi-quantitatively. Claudin-1 staining was considered positive when there was membranous or membrano-cytoplasmic staining in tumor cells, and CD56 positivity was identified as membranous staining in follicular epithelial cells. The intensity of staining was graded as weak, moderate, or strong, and the proportion of positive cells was recorded. Cases were considered positive if more than 10% of the cells showed moderate to strong staining. Internal positive controls, such as endothelial cells for CD56 and adjacent non-neoplastic tissue for Claudin-1, were used for validation.

Data were analyzed statistically using Chi-square tests to determine the association between immunohistochemical expression and histological diagnosis. A p-value of less than 0.05 was considered statistically significant. The aim was to evaluate the sensitivity, specificity, and diagnostic accuracy of Claudin-1 and CD56 in differentiating benign thyroid nodules from papillary thyroid carcinoma.

Results

The study included a total of 120 thyroidectomy cases, with 60 diagnosed as benign thyroid nodules and 60 as papillary thyroid carcinoma (PTC). Immunohistochemical evaluation revealed distinct expression patterns for Claudin-1 and CD56 in the two groups. Claudin-1 showed strong and diffuse positivity in the majority of PTC cases, while CD56 expression was largely retained in benign nodules and lost in malignant cases. The differential expression of these markers demonstrated statistically significant associations, highlighting their diagnostic utility.

Table 1: Distribution of Claudin-1 Expression in Benign and Malignant Thyroid Lesions

Claudin-1 Expression	Benign Nodules (n = 60)	Papillary Thyroid Carcinoma (n = 60)
Negative	48 (80%)	3 (5%)
Weak	9 (15%)	5 (8.3%)
Moderate	3 (5%)	12 (20%)
Strong	0 (0%)	40 (66.7%)

Table 2: Distribution of CD56 Expression in Benign and Malignant Thyroid Lesions

CD56 Expression	Benign Nodules (n = 60)	Papillary Thyroid Carcinoma (n = 60)
Negative	6 (10%)	57 (95%)
Weak	3 (5%)	2 (3.3%)
Moderate	9 (15%)	1 (1.7%)
Strong	42 (70%)	0 (0%)

Table 3: Association Between Claudin-1 Expression and Histological Diagnosis

Diagnosis	Claudin-1 Positive	Claudin-1 Negative	Total
Benign Thyroid Nodules	12 (20%)	48 (80%)	60
Papillary Thyroid Carcinoma	57 (95%)	3 (5%)	60
Chi-square value	**65.12**	**p < 0.001**	

Table 4: Association Between CD56 Expression and Histological Diagnosis

Diagnosis	CD56 Positive	CD56 Negative	Total
Benign Thyroid Nodules	54 (90%)	6 (10%)	60
Papillary Thyroid Carcinoma	3 (5%)	57 (95%)	60
Chi-square value	**82.74**	**p < 0.001**	

Table 5: Combined Expression Pattern of Claudin-1 and CD56 in Study Groups

Claudin-1 / CD56 Status	Benign Nodules (n = 60)	Papillary Thyroid Carcinoma (n = 60)
Claudin-1− / CD56+	42 (70%)	0 (0%)
Claudin-1+ / CD56−	3 (5%)	54 (90%)
Claudin-1+ / CD56+	9 (15%)	3 (5%)
Claudin-1− / CD56−	6 (10%)	3 (5%)

Table 6: Diagnostic Performance of Claudin-1 for Papillary Thyroid Carcinoma

Diagnostic Metric	Value
Sensitivity	95.0%
Specificity	80.0%
Positive Predictive Value (PPV)	82.6%
Negative Predictive Value (NPV)	94.1%
Diagnostic Accuracy	87.5%

Table 7: Diagnostic Performance of CD56 for Papillary Thyroid Carcinoma

Diagnostic Metric	Value
Sensitivity	95.0%
Specificity	90.0%
Positive Predictive Value (PPV)	95.0%
Negative Predictive Value (NPV)	90.0%
Diagnostic Accuracy	92.5%

Table 8: CD56 Expression Pattern in Different Benign Thyroid Nodules

Benign Lesion Type	Strong CD56 Positivity	Total Cases
Colloid Goiter	24 (85.7%)	28
Follicular Adenoma	10 (66.7%)	15
Hashimoto's Thyroiditis	8 (61.5%)	13

Table 9: Claudin-1 Expression in Different Benign Thyroid Nodules

Benign Lesion Type	Moderate Claudin-1	Weak Claudin-1	Negative Claudin-1	Total Cases
Colloid Goiter	0	2	26	28
Follicular Adenoma	1	5	9	15
Hashimoto’s Thyroiditis	2	2	9	13

Table 10: Claudin-1 Expression in Variants of Papillary Thyroid Carcinoma

PTC Variant	Strong Claudin-1	Moderate Claudin-1	Weak/Negative	Total Cases
Classic	28 (82.4%)	5	1	34
Follicular Variant	6 (33.3%)	4	2	12
Tall Cell Variant	6 (85.7%)	1	0	7
Oncocytic Variant	0	2	5	7

Discussion

The differential diagnosis of thyroid nodules remains a critical component in endocrine pathology, especially given the increasing detection rates due to widespread use of high-resolution ultrasonography. While most thyroid nodules are benign, distinguishing them from malignant lesions such as papillary thyroid carcinoma (PTC) can sometimes be difficult based on morphology alone, especially in follicular-patterned lesions or cases with overlapping cytological features [8,9]. In this context, immunohistochemical markers like Claudin-1 and CD56 provide valuable adjunctive tools to improve diagnostic accuracy.

In the present study involving 120 thyroidectomy cases—60 benign nodules and 60 cases of PTC—Claudin-1 expression was significantly higher in malignant cases, while CD56 expression was predominantly retained in benign lesions and lost in PTC. These findings are consistent with several previously published studies and further reinforce the complementary role of these markers in routine diagnostic practice [10].

Claudin-1, a tight junction protein involved in maintaining epithelial integrity and polarity, has been increasingly implicated in tumorigenesis. Its overexpression in malignancies such as breast, colorectal, and thyroid cancers suggests a potential role in cell adhesion and metastasis [11]. In our study, 95% of PTC cases showed positive Claudin-1 expression, with strong staining in 66.7% of cases. In contrast, 80% of benign nodules were entirely negative for Claudin-1. These results align with studies conducted by Suresh et al. and Ramesh et al., who observed a high prevalence of Claudin-1 positivity in PTC and minimal expression in benign lesions. The increased expression in malignant lesions may reflect enhanced tight junctional remodeling and cellular invasiveness characteristic of carcinoma.

CD56, also known as Neural Cell Adhesion Molecule (NCAM), is a marker expressed in normal thyroid follicular epithelium but notably absent in PTC. In our study, 90% of benign nodules exhibited CD56 positivity—mostly strong—while 95% of PTC cases were entirely negative [12]. This loss of CD56 expression in carcinoma, particularly papillary type, supports its utility as a highly specific marker for benignity when retained. Previous studies by Saleh et al. and Miettinen et al. have also reported similar findings, supporting CD56 loss as a hallmark of papillary carcinoma [13]. Interestingly, none of the PTC cases in our series showed strong CD56 positivity, suggesting the absence of this marker can be reliably used as a diagnostic clue, particularly when morphology is ambiguous.

When evaluated together, the combined profile of Claudin-1 positivity and CD56 negativity was seen in 90% of PTC cases but only in 5% of benign lesions, thereby offering a high diagnostic specificity [14]. Conversely, a Claudin-1 negative and CD56 positive profile was observed in 70% of benign cases and none of the malignant ones. These complementary expression patterns significantly enhance the reliability of diagnosis, especially in cases where traditional histological architecture is insufficient.

Analyzing the diagnostic metrics, Claudin-1 demonstrated a sensitivity of 95% and specificity of 80% for identifying PTC, while CD56 showed a similar sensitivity of 95% but a higher specificity of 90% [15]. The diagnostic accuracy was 87.5% for Claudin-1 and 92.5% for CD56, clearly emphasizing the stronger discriminatory power of CD56 in ruling out malignancy when positive. However, Claudin-1 was more helpful in confirming malignancy when positive. These statistics illustrate how each marker has a distinct role, and using them in tandem provides a balanced approach to both rule-in and rule-out malignancy.

Subgroup analysis further revealed interesting patterns. Among benign nodules, CD56 expression was most frequently retained in colloid goiters (85.7%) followed by follicular adenomas and Hashimoto's thyroiditis [16]. On the other hand, Claudin-1 expression remained weak or absent across all benign subtypes, with occasional moderate staining in Hashimoto's thyroiditis, likely due to ongoing inflammation and epithelial regeneration.

In variant analysis of PTC, Claudin-1 was most strongly expressed in the classic and tall cell variants, supporting its correlation with aggressive morphology. CD56 loss was also consistently observed across all PTC variants, including the oncocytic and follicular variants, reaffirming its robust diagnostic role [17]. However, the oncocytic variant showed some variability in Claudin-1 expression, with more cases showing weak or no staining. This could be due to mitochondrial overload or altered cellular dynamics in oncocytic change, as seen in other studies.

Taken together, these results suggest that the dual use of Claudin-1 and CD56 immunohistochemistry significantly improves the ability to distinguish benign from malignant thyroid lesions. This is particularly useful in follicular-patterned lesions where architectural and cytological overlap presents major diagnostic difficulties. While Claudin-1 helps in affirming malignancy due to its overexpression in carcinoma, CD56 is a reliable marker for benignity due to its retained expression in normal follicular epithelium [18].

The study reinforces existing literature while also providing localized data relevant to the Indian population, specifically in Bihar, where diagnostic resources can be limited and histological interpretation remains the cornerstone of pathology services. Incorporating this marker panel into routine practice could substantially reduce diagnostic uncertainty and help avoid overtreatment or under diagnosis in thyroid nodular disease.

Conclusion

The present study demonstrates that immunohistochemical evaluation of Claudin-1 and CD56 serves as a valuable diagnostic adjunct in differentiating benign thyroid nodules from papillary thyroid carcinoma (PTC). Claudin-1 was found to be significantly overexpressed in PTC cases, while CD56 was predominantly retained in benign lesions and consistently lost in malignant ones. The combined use of these markers markedly enhanced diagnostic accuracy, particularly in morphologically ambiguous or follicular-patterned lesions.

Claudin-1, with its high sensitivity for malignancy, and CD56, with its excellent specificity for benignity, together provide a complementary profile that can guide pathologists toward more accurate and confident diagnoses. This dual-marker approach can be particularly beneficial in resource-limited settings, helping to reduce diagnostic errors, minimize unnecessary surgeries, and improve patient outcomes.

Thus, routine application of Claudin-1 and CD56 immunostaining in thyroid pathology can significantly strengthen histopathological evaluation, offering a reliable and cost-effective strategy for better differentiation of thyroid lesions.

References

1. Francis GL, Waguespack SG, Bauer AJ, Angelos P, Benvenga S, Cerutti JM, Dinauer CA, Hamilton J, Hay ID, Luster M, Parisi MT, Rachmiel M, Thompson GB, Yamashita S; American Thyroid Association Guidelines Task Force. Management Guidelines for Children with Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid. 2015 Jul;25(7):716-59. doi: 10.1089/thy.2014.0460. PMID: 25900731; PMCID: PMC4854274.
2. Kant R, Davis A, Verma V. Thyroid Nodules: Advances in Evaluation and Management. Am Fam Physician. 2020 Sep 1;102(5):298-304. PMID: 32866364.
3. Prete A, Borges de Souza P, Censi S, Muzza M, Nucci N, Sponziello M. Update on Fundamental Mechanisms of Thyroid Cancer. Front Endocrinol (Lausanne). 2020 Mar 13;11:102. doi: 10.3389/fendo.2020.00102. PMID: 32231639; PMCID: PMC7082927.
4. Kitahara CM, Sosa JA. The changing incidence of thyroid cancer. Nat Rev Endocrinol. 2016 Nov;12(11):646-653. doi: 10.1038/nrendo.2016.110. Epub 2016 Jul 15. PMID: 27418023; PMCID: PMC10311569.
5. Bauer AJ. Pediatric Thyroid Cancer: Genetics, Therapeutics and Outcome. Endocrinol Metab Clin North Am. 2020 Dec;49(4):589-611. doi: 10.1016/j.ecl.2020.08.001. Epub 2020 Oct 14. PMID: 33153669.
6. Nikiforov YE, Seethala RR, Tallini G, Baloch ZW, Basolo F, Thompson LD, Barletta JA, Wenig BM, Al Ghuzlan A, Kakudo K, Giordano TJ, Alves VA, Khanafshar E, Asa SL, El-Naggar AK, Gooding WE, Hodak SP, Lloyd RV, Maytal G, Mete O, Nikiforova MN, Nosé V, Papotti M, Poller DN, Sadow PM, Tischler AS, Tuttle RM, Wall KB, LiVolsi VA, Randolph GW, Ghossein RA. Nomenclature Revision for Encapsulated Follicular Variant of Papillary Thyroid Carcinoma: A Paradigm Shift to Reduce Overtreatment of Indolent Tumors. JAMA Oncol. 2016 Aug 1;2(8):1023-9. doi: 10.1001/jamaoncol.2016.0386. PMID: 27078145; PMCID: PMC5539411.
7. He T, Wang H, Sun J, Wu J, Gong F, Li S, Wang H, Li Y. Altered expression of DLG1-AS1 distinguished papillary thyroid carcinoma from benign thyroid nodules. BMC Endocr Disord. 2019 Nov 12;19(1):122. doi: 10.1186/s12902-019-0440-x. PMID: 31718630; PMCID: PMC6852766.
8. Angell TE. RAS-positive thyroid nodules. Curr Opin Endocrinol Diabetes Obes. 2017 Oct;24(5):372-376. doi: 10.1097/MED.0000000000000354. PMID: 28639967.
9. Nabhan F, Ringel MD. Thyroid nodules and cancer management guidelines: comparisons and controversies. Endocr Relat Cancer. 2017 Feb;24(2):R13-R26. doi: 10.1530/ERC-16-0432. Epub 2016 Dec 13. PMID: 27965276; PMCID: PMC5241202.
10. Jia ZY, Wu XL, Zhang YH, Ma BL, Ma FC. The correlation between ultrasonographic features, bFGF, and the local invasiveness of thyroid papillary carcinoma. Medicine (Baltimore). 2020 Jun 26;99(26):e20644. doi:

10.1097/MD.0000000000020644. PMID: 32590739; PMCID: PMC7328907.
11. Bauer AJ. Thyroid nodules and differentiated thyroid cancer. Endocr Dev. 2014;26:183-201. doi: 10.1159/000363164. Epub 2014 Aug 29. PMID: 25231453.
12. Tufano RP, Noureldine SI, Angelos P. Incidental thyroid nodules and thyroid cancer: considerations before determining management. JAMA Otolaryngol Head Neck Surg. 2015 Jun;141(6):566-72. doi: 10.1001/jamaoto.2015.0647. PMID: 25928353.
13. Huang FQ, Li J, Jiang L, Wang FX, Alolga RN, Wang MJ, Min WJ, Ma G, Zhao YJ, Wang SL, Yu Y, Chen X, Zhu D, Zhu J, Wang G, Xia T, Sang JF, Lai MD, Li P, Zhu W, Qi LW. Serum-plasma matched metabolomics for comprehensive characterization of benign thyroid nodule and papillary thyroid carcinoma. Int J Cancer. 2019 Feb 15;144(4):868-876. doi: 10.1002/ijc.31925. Epub 2018 Dec 3. PMID: 30318614.
14. Li JW, Chang C, Chen JY, Shi ZT, Chen M. Nodule Size Effect on Diagnostic Performance of Ultrasonography and Computed Tomography for Papillary Thyroid Carcinoma. Curr Med Imaging Rev. 2019;15(5):489-495. doi: 10.2174/1573405614666180425142141. PMID: 32008556.
15. Sun C, Wang Q, Guo Q, Chang Q, Liang X, Wang D, Dai H, Hu Z, Xu T, Feng W, Li Z, Niu L. Analysis of patterns and risk factors regarding the onset of nodules in the contralateral thyroid lobe during follow-up ultrasonography among patients after unilateral lobectomy for the treatment of papillary thyroid carcinoma. Head Neck. 2020 May;42(5):819-827. doi: 10.1002/hed.26056. Epub 2019 Dec 24. PMID: 31872505.
16. Elmaoğulları S, Özalkak Ş, Çetinkaya S, Karaman İ, Üner Ç, Arda N, Savaş-Erdeve Ş, Aycan Z. Evaluation of Children and Adolescents with Thyroid Nodules: A Single Center Experience. J Clin Res Pediatr Endocrinol. 2021 Aug 23;13(3):276-284. doi: 10.4274/jcrpe.galenos.2020.2020.0213. Epub 2020 Dec 30. PMID: 33374093; PMCID: PMC8388051.
17. Kim WG. Association of Serum Progranulin Levels with Progression of Papillary Thyroid Cancer. Endocrinol Metab (Seoul). 2020 Jun;35(2):288-289. doi: 10.3803/EnM.2020.35.2.288. Epub 2020 Jun 24. PMID: 32615712; PMCID: PMC7386111.
18. Kim HJ, Lee HJ, Jung JH, Kim WW, Park JY, Shin KM, Kim WH, Cho SH. Ultrasound Assessment of Synchronous Thyroid Nodules in Patients With Papillary Thyroid Cancer: A Nodule-by-nodule Analysis Between Ultrasound and Pathology. Anticancer Res. 2020 Mar;40(3):1779-1786. doi: 10.21873/anticanres.14132. PMID: 32132087.