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Sex-Related Differences in p63 and CD56 Expression in Papillary Thyroid Carcinoma

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ABSTRACT

BACKGROUND. Thyroid cancer is one of the most common endocrine malignancies and occurs significantly more frequently in women than in men. Nevertheless, the molecular mechanisms underlying sex-related differences remain incompletely understood. Immunohistochemical markers, including p63 and CD56, play an important role in the diagnosis of thyroid pathology; however, data regarding their sex-specific expression patterns are limited.

OBJECTIVES. The present study aimed to compare the immunohistochemical expression of p63 and CD56 in papillary thyroid carcinoma (PTC) specimens obtained from female and male patients and to explore potential sex-related differences in their expression.

METHODS. Surgical specimens obtained from 26 patients with PTC, including 22 women and 4 men, were investigated. Immunohistochemical evaluation of p63 and CD56 expression was performed using a semi-quantitative scoring system. Statistical analysis was conducted using SPSS version 21.0. Because of the ordinal nature of the immunohistochemical scores and the small sample size, comparisons between female and male patients were performed using the Mann–Whitney U test.

RESULTS. No statistically significant sex-related differences were detected in the expression of p63 or CD56 ($p>0.05$). Because of the limited sample size, particularly the small number of male patients, these findings should be interpreted as preliminary.

CONCLUSIONS. In this exploratory cohort, no statistically significant sex-related differences in p63 or CD56 expression were detected. Given the very small male subgroup, these findings should be interpreted with caution and cannot exclude clinically meaningful sex-related differences. Larger, adequately powered multicenter studies incorporating comprehensive clinicopathological and molecular data are required to further investigate potential sex-related differences in papillary thyroid carcinoma.

KEYWORDS. CD56; Immunohistochemistry; P63; Papillary thyroid carcinoma; Sex differences.

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BACKGROUND

Sexual dimorphism significantly influences the biology and clinical course of numerous neoplastic diseases. Thyroid cancer (TC) occurs approximately three to four times more frequently in women than in men. [1-3] Nevertheless, the molecular and hormonal mechanisms underlying this difference have not yet been fully elucidated.

Papillary thyroid carcinoma (PTC) is the most common malignant tumor of the thyroid gland. Despite its generally favorable prognosis, certain cases are characterized by aggressive behavior, regional and distant metastases, as well as resistance to standard therapeutic approaches. [4,5]

In recent years, considerable attention has been directed toward the influence of sex hormones, the immune microenvironment, and genetic factors on thyroid carcinogenesis. These factors may play an important role in tumor development and progression. [2,6]

Immunohistochemical markers are widely used in the diagnosis of thyroid pathology. p63 is a transcription factor involved in epithelial differentiation and stem cell regulation. [6,7] CD56 is a cell adhesion glycoprotein normally expressed in follicular epithelium and is frequently lost in PTC. [7,8]

Most of the available literature focuses on the diagnostic and prognostic significance of these markers, whereas their sex-related expression characteristics remain largely unexplored. To the best of our knowledge, studies directly evaluating p63 and CD56 expression in PTC according to sex are extremely limited.

This study aimed to compare the immunohistochemical expression of p63 and CD56 in papillary thyroid carcinoma specimens obtained from female and male patients. The study was designed as an exploratory analysis to investigate whether sex-related differences in the expression of these markers could be identified within this cohort.

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VOLUME 4 ISSUE 3. JUL-SEP 2026

METHODS

This retrospective exploratory study included surgical specimens obtained from patients with histologically confirmed papillary thyroid carcinoma (PTC). The study was designed to compare the immunohistochemical expression of p63 and CD56 between female and male patients.

The study included surgical specimens obtained from clinics in Tbilisi and the National Center of Interventional Medicine of Western Georgia (n=26). Patients were divided into two groups according to sex:

- Group 1 - Female patients (n=22)
- Group 2 - Male patients (n=4)

Cases were selected based on the availability of adequate formalin-fixed, paraffin-embedded tissue for immunohistochemical evaluation. Because of the limited number of available male cases during the study period, an unequal sex distribution was unavoidable.

The study was conducted in accordance with the requirements of the bioethics committees of Ivane Javakhishvili Tbilisi State University and Tbilisi State Medical University, based on the ethical principles of the Declaration of Helsinki. [9]

Tissue samples were fixed in 10% buffered formalin. Following dehydration and paraffin embedding, serial sections measuring 5-7 µm were prepared and stained with hematoxylin and eosin (H&E).

Immunohistochemical staining for p63 and CD56 was performed on formalin-fixed, paraffin-embedded tissue sections using commercially available primary antibodies according to the manufacturer's recommendations. Histological sections were independently evaluated by experienced pathologists using light microscopy. Only adequately preserved tumor areas suitable for microscopic evaluation were included in the analysis, whereas areas with marked tissue artifacts or poor preservation were excluded from scoring.

The following antibodies were used:

- p63 (clone 7JUL, Leica Biosystems)
- CD56 (clone CD564, Leica Biosystems)

The immunohistochemical expression of p63 and CD56 was assessed using a semi-quantitative scoring system based on the percentage of positively stained tumor cells. The applied scoring system was as follows:

- 0 = negative
- 1 = ≤10%
- 2 = 11-50%
- 3 = 51-80%
- 4 = >80%

The present scoring system was intended to provide a standardized semi-quantitative assessment of marker expression. Since only the proportion of positive tumor cells was evaluated, staining intensity was not incorporated into the scoring system. This methodological aspect should be considered when interpreting the results.

Statistical analyses were performed using SPSS Statistics version 21.0. Because immunohistochemical scores represented ordinal variables and the male subgroup consisted of only four patients, non-parametric statistical methods were considered the most appropriate. Differences between female and male patients were analyzed using the Mann–Whitney U test. A two-sided p-value of <0.05 was considered statistically significant.

Because this study was designed as an exploratory pilot investigation with a limited sample size, statistical analyses were intended primarily to identify potential trends rather than to establish definitive sex-related biological differences. Therefore, the results should be interpreted with appropriate caution.

The study represents an exploratory immunohistochemical analysis of sex-related differences in papillary thyroid carcinoma. Owing to the limited sample size, particularly the small number of male patients, the findings should be regarded as preliminary and require confirmation in larger multicenter cohorts.

RESULTS

The median p63 immunohistochemical expression score was 1.0 (IQR: 1.0–2.0) in female patients and 1.5 (IQR: 1.0–2.0) in male patients. The corresponding mean±SD values were 1.36±0.85 and 1.50±0.58, respectively. No statistically significant difference in p63 expression was detected between female and

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male patients (Mann–Whitney U test, p=0.730) (**TAB.1**). Because of the limited sample size, particularly the very small number of male patients, these findings should be interpreted as preliminary. They should not be considered evidence of biological equivalence between the two groups.

TABLE 1. Comparison of p63 and CD56 immunohistochemical expression between female and male patients

Marker	Female (n=22) Median (IQR)	Male (n=4) Median (IQR)	Female Mean±SD	Male Mean±SD	p-value*
p63	1.0 (1.0-2.0)	1.5 (1.0-2.0)	1.36±0.85	1.50±0.58	0.730
CD56	1.0 (0.0-2.0)	0.5 (0.0-1.5)	1.09±0.97	1.00±1.41	0.737

Explanations: *Values are presented as median (interquartile range [IQR]) and mean ± standard deviation (SD). Median (IQR) is presented as the primary descriptive statistic because immunohistochemical scores represent ordinal variables. Group comparisons were performed using the Mann–Whitney U test.

The median CD56 immunohistochemical expression score was 1.0 (IQR: 0.0–2.0) in female patients and 0.5 (IQR: 0.0–1.5) in male patients. The corresponding mean±SD values were 1.09±0.97 and 1.00±1.41, respectively. No statistically significant difference in CD56 expression was detected between female and male patients (Mann–Whitney U test, p=0.737) (**TAB.1**).

An exploratory descriptive analysis of the CD56/p63 expression ratio was additionally performed solely to summarize the relative expression patterns of the two investigated immunohistochemical markers. Cases with undefined ratios due to zero denominator values were excluded from the analysis. The mean CD56/p63 ratio was 0.79±0.79 in female patients and 0.63±0.75 in male patients. No statistically significant difference between the groups was observed (Mann–Whitney U test, p=0.742) (**TAB.2**). Because this index has not been biologically or clinically validated and was derived from semi-quantitative ordinal immunohistochemical scores, it should be interpreted with considerable caution. It should not be considered a validated indicator of tumor differentiation, metastatic potential, or biological aggressiveness.

TABLE 2. Exploratory descriptive comparison of the CD56/p63 immunohistochemical expression ratio between female and male patients

Group	n	Mean ± SD	P-value*
Female	22	0.79 ± 0.79	0.742
Male	4	0.63 ± 0.75	

Explanations: *The CD56/p63 ratio is presented solely as an exploratory descriptive index summarizing the relative expression of the two investigated immunohistochemical markers. Because this index has not been biologically or clinically validated and was derived from semi-quantitative ordinal immunohistochemical scores, it should be interpreted with caution. The ratio should not be considered a validated indicator of tumor differentiation, metastatic potential, or biological aggressiveness. Group comparison was performed using the Mann–Whitney U test.

Overall, statistically significant sex-related differences in p63 expression, CD56 expression, or the CD56/p63 ratio were not identified in this cohort. However, owing to the exploratory nature of the study and the limited number of male patients, these findings should be interpreted with caution and considered hypothesis-generating rather than confirmatory.

Failure to detect statistically significant differences should not be interpreted as evidence of equivalence between the study groups.

DISCUSSION

Sex-related differences in thyroid cancer remain an important topic in contemporary oncological and endocrine research. Although papillary thyroid carcinoma (PTC) occurs substantially more frequently in women than in men, the biological basis of this disparity has not been fully elucidated.

The present study explored the immunohistochemical expression of p63 and CD56 in PTC specimens obtained from female and male patients. p63, a member of the p53 family of transcription factors, is involved in epithelial differentiation, proliferation, apoptosis, and stem-cell regulation. [6,10,11] Previous studies have reported p63 expression in a subset of PTC cases and have suggested that it may reflect alterations in epithelial differentiation pathways. [12-15]

In the current cohort, no statistically significant sex-related difference in p63 expression was

detected. However, because of the limited sample size and the very small number of male patients, these findings should be interpreted as preliminary. Failure to detect a statistically significant difference should not be interpreted as evidence of biological equivalence between female and male patients.

CD56 (NCAM - Neural Cell Adhesion Molecule) is a cell adhesion glycoprotein involved in intercellular communication, adhesion, migration, and regulation of immune responses.16 CD56 is normally expressed in follicular thyroid epithelium, whereas loss or reduction of its expression is frequently observed in PTC and has important diagnostic value. [7,8,17] Previous studies have suggested that reduced CD56 expression may be associated with impaired cell–cell adhesion and may contribute to mechanisms involved in tumor progression. [16-18]

Similarly, no statistically significant sex-related difference in CD56 expression was identified in the present study. These results do not provide evidence of statistically significant sex-related differences in CD56 expression within the present cohort; however, the study was not adequately powered to exclude clinically meaningful differences.

An exploratory descriptive analysis of the CD56/p63 expression ratio was additionally performed solely to summarize the relative expression patterns of the two investigated immunohistochemical markers. Because this index has not been biologically or clinically validated and was derived from semi-quantitative ordinal immunohistochemical scores, it should be interpreted with considerable caution. Therefore, the ratio should not be considered a direct measure of tumor differentiation, metastatic potential, or biological aggressiveness.

Interpretation of the findings should also consider several study limitations. First, the sample size was small, particularly in the male subgroup, resulting in limited statistical power. Second, detailed clinicopathological variables, including tumor size, histologic subtype, lymph-node status, TNM stage, vascular invasion, and Hashimoto thyroiditis, were not systematically available for analysis. Third, the semi-quantitative scoring system was based on the proportion of positive tumor cells and did not incorporate staining intensity. Fourth, interobserver variability was not formally assessed. Finally, normal or benign thyroid tissue was not included as a comparator group.

Despite these limitations, the present exploratory study provides preliminary data regarding sex-related differences in p63 and CD56 expression in papillary thyroid carcinoma. These preliminary findings may contribute to the design of future multicenter studies involving larger, more balanced, and well-characterized patient cohorts, together with comprehensive clinicopathological and molecular analyses.

CONCLUSIONS

In this exploratory cohort of papillary thyroid carcinoma cases, no statistically significant sex-related differences in p63 or CD56 immunohistochemical expression were detected. Because the male subgroup was very small and comprehensive clinicopathological data were not available for analysis, these findings should be interpreted as preliminary. Larger multicenter studies incorporating balanced sex distribution, comprehensive clinicopathological characterization, and molecular or hormonal analyses are required to further investigate potential sex-related differences in papillary thyroid carcinoma. Accordingly, the present findings should be regarded as hypothesis-generating rather than confirmatory.

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