Original Article

Immunohistochemical Expression of bcl 2 and Ki-67 in Thyroid Neoplasms

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Abstract

Background: Thyroid cancer is the most common endocrine cancer today. Its incidence has increased all over the world in the last three decades. An accurate diagnosis of thyroid neoplasm is therefore crucial as it has a major impact on the treatment modality as well as the patient's prognosis. This study was being done to determine utility expression of bcl 2 and Ki-67 in thyroid neoplasms.
Methods: This cross sectional study was conducted in the Department of Pathology at Bhagat Phool Singh Government Medical College for Women, Khnapur Kalan, Sonipat, Haryana. A total of 55 specimens were subjected to IHC (bcl 2 and Ki-67).
Results: Out of 55 thyroid neoplasm cases, 27 were benign and 28 were malignant. All the neoplastic pathologies showed varying degree of bcl 2 positive expression. Ki-67 was negative in all benign neoplasms. Varying degree of positive expression was seen in malignant neoplasms: 100% positivity in follicular thyroid carcinoma (04/04), followed by 50% cases of invasive encapsulated follicular variant of papillary thyroid carcinoma (03/06), followed by 33.33% cases of papillary thyroid carcinoma (05/15). The expression of bcl 2 was found to have 50.00% sensitivity and 33.33% specificity in thyroid neoplasms with diagnostic accuracy of 49.09%. The expression of Ki-67 was found to have 98% sensitivity and 67.50% specificity in detecting malignant thyroid neoplasms with diagnostic accuracy of 76.36%.
Conclusion: Ki-67 proved to be useful marker in differentiating malignant thyroid neoplasms from the benign one as the Ki-67 labelling index more in the malignant neoplasms, it was statistically significant. The other IHC marker studied in the present study was bcl 2 which acted as marker of proliferation for thyroid tissue, can help in detecting the metastatic foci in other organs and to diagnose the cases of poorly differentiated thyroid carcinomas.

Keywords: Immunohistochemistry; bcl 2; Ki-67; thyroid neoplasms

Introduction

Thyroid neoplasms ranged from benign adenomas to malignant carcinomas, with follicular thyroid adenomas (FTA) and follicular thyroid carcinoma (FCa) being particularly challenging to distinguish.[1] While FTAs were derived from follicular epithelium, accounting for discrete masses, malignant carcinomas comprised 1.5% of all cancers. Diagnostic tests that can aid in differentiation have been proved crucial in clinical practice.[2]

Thyroid swelling may appear as a solitary thyroid nodule (STN) or diffuse gland enlargement. It often presents as a painless lump of varying sizes, sometimes accompanied by hoarseness, dysphagia, coughing, and dyspnoea.[3] Differentiating benign from malignant lesions clinically is challenging, with preoperative aspiration cytology and radiography providing some guidance. Ultimately, histopathological examination has been the definitive method for distinguishing neoplastic from non-neoplastic tumors.[4]

According to GLOBOCAN 2022, there were 821,214 new cases of thyroid cancer globally, making it one of the top ten malignancies. Females accounted for 614,729 cases, and males for 206,485, highlighting a female predominance. Thyroid cancer caused 47,507 deaths worldwide, with a projected 5% rise in cases and 7.4% increase in deaths by 2025.[5]

While histopathology is crucial for diagnosing thyroid neoplastic lesions, immunohistochemistry (IHC) aids in challenging cases. Biomarkers like cytokeratin, p-63, Ki-67, bcl 2, CK 19, and calcitonin provide insights into tissue molecular processes, though no single marker suffices for definitive diagnosis. A combined IHC panel is often needed.[6] Histopathologic criteria allow accurate differentiation between benign and malignant lesions, ensuring proper classification of thyroid tumors. Advancement in the knowledge of molecular diagnosis and targeted therapy has made ancillary techniques essential to balance avoiding overtreatment with effective management of aggressive cases at early, curable stages.[7]

Among potential markers, B cell lymphoma 2 (bcl-2) and Ki-67 are of particular interest. Bcl-2, an anti-apoptotic protein, plays a role in tumor progression by promoting cell survival. Its altered expression has been linked with dedifferentiation, reduced survival, and poor prognosis in several malignancies, including thyroid tumors.[8] Conversely, Ki-67, a nuclear proliferation marker, correlates closely with cell growth and is widely used as a prognostic indicator across cancers.[9] Despite their recognized importance individually, there is still limited consensus regarding the combined diagnostic and prognostic significance of bcl-2 and Ki-67 in thyroid neoplasms, particularly in distinguishing benign from malignant follicular lesions.

Although several studies have evaluated bcl-2 and Ki-67 independently, there is a lack of systematic assessment of their co-expression in thyroid neoplasms, especially in the context of differentiating benign from malignant lesions and predicting tumor behavior. This gap in knowledge has practical implications, as more reliable biomarkers could reduce diagnostic ambiguity, prevent overtreatment of benign lesions, and enable early, targeted management of aggressive tumors.

This study was undertaken to evaluate the expression of bcl-2 and Ki-67 in thyroid neoplasms, with the objective of determining their combined diagnostic and prognostic utility, thereby addressing a critical gap in the literature.

Materials and Methods

This cross sectional study was conducted in the Department of Pathology at Bhagat Phool Singh Government Medical College for Women, Khnapur Kalan, Sonipat, after obtaining approval from the Institutional Ethical Committee (refrence: BPSGMCW/RC892/IEC/23 dated 26/05/2023). It was a retrospective study that included 55 cases of thyroid neoplasms. Cases with history of chemotherapy and radiotherapy and whose blocks could not be retrieved were excluded.

Blocks of known cases of thyroid neoplasms, along with requisition forms, were retrieved. Patient confidentiality was ensured by deidentifying the data, and patients were assigned a unique numerical code. Additional clinical information and relevant investigations were collected from the case sheets and requisition forms.

Tissue sections were processed in automated tissue processing unit and Hematoxylin and Eosin (H&E) sections were prepared for microscopy. A total of 55 specimens, including 27 benign neoplasms and 28 malignant neoplasms were subjected to IHC bcl 2 of Bio-Genex clone bcl-2/100 and Ki-67 of Bio-Genex MIB-1 clone for comparison of two biomarker expression. The diagnosis and typing of thyroid pathology was done as per the World Health Organisation (WHO) classification, 2022 guidelines of thyroid neoplasms.

IHC staining scores were given based on the proportion of cells staining positive and on the intensity of staining. The determination of bcl 2 (cytoplasmic expression) involved counting a total of 500 cells, and the percentage of positive cells was calculated. Positive staining was graded based on intensity as follows: 0-negative, 1+-weakly positive, 2+-moderately positive, 3+-strongly positive. The staining intensity score was calculated by multiplying the percentage of tumor cells staining positive with the intensity of bcl 2 staining. A score ranging from 0 to 6 was assigned as follows: 0-1: Negative, 2: Weakly positive, 3-4: Moderately positive, 5-6: Strongly positive.[8]

Ki-67 (nuclear expression) was determined using the Beesley classification, where no staining or weak staining was considered negative; <25% of cells with buffy staining were classified as weakly positive; and 25-50% of cells with buffy staining were classified as mid-range positive. Positive cells were identified by nuclear immunoreactivity, with 10 high-power visual fields randomly selected under a microscope, and 100 tumor cells per field were counted to calculate the average expression intensity. The Ki-67 labeling index (LI) was calculated as the percentage of cells showing nuclear positivity out of 1,000 cells in a hot area at 400x magnification. Hot areas are the tumor areas with maximum intensity of Ki-67 positive nuclei and devoid of hemorrhage, necrosis, inflammation and crush artefact. Cases with zero or weak positive staining, or a Ki-67 LI below 5%, were classified as negative.[10]

Statistical Methodology The collected data was entered in Microsoft excel spread sheet. Mean and standard deviation were calculated for a quantitative data. Percentage and proportion were calculated for qualitative data. Chi square test was used for association between bcl 2 and Ki-67 expression with classification of thyroid neoplasm using Statistical package for the social sciences (SPSS) software version 26. A p value <0.05 was considered as statistically significant.

Results

This study was conducted on 55 cases of thyroid neoplasms. Gender wise distribution of cases in this study showed an overall predominance of females (48, 87.4%) in thyroid neoplasms with male to female ratio of 6.8 times. Herein the present study, out of 55 cases, the mean age of the study subjects is 36.42±13.56 years, with a minimum and maximum age of 9 years and 68 years, respectively. Remarkably, most of the cases (n=21; 38.2%) involved young people, with the majority being under 21-30 years of age group. The number of cases in benign (27 cases) and malignant neoplasms (28 cases) were almost same with a ratio of 1:1.04.

Out of 27 cases belonged to benign type of tumors, 23 cases were of follicular thyroid adenoma and 4 cases belonged to oncocytic adenoma. The malignant type neoplasm included 28 cases, which was further divided into 15 cases of PTC, 6 cases of follicular variant of papillary thyroid carcinoma (FVPTC), 4 cases of follicular thyroid carcinoma and 1 case of oncocytic carcinoma. Out of 15 PTC cases, 13 were classical PTC, one was columnar cell variant of PTC and one was warthin tumor like variant of PTC. Two cases were observed to be of thyroid c cell derived neoplasm belonging to subtype of medullary thyroid carcinoma.

IHC bcl 2 was positive in 100% (28/28) of malignant cases with similar findings in benign neoplasms where 100% (27/27) cases were positive for bcl 2. Hence there is no utility of bcl 2 in differentiating benign from malignant neoplasms. Various studies in literature states that bcl 2 is important in assessing the degree of differentiation in thyroid neoplasms as there is loss of bcl 2 expression in anaplastic carcinoma and poorly differentiated carcinoma. Hence it also plays a major role in prognosis of these entities. But this property of bcl 2 could not be assessed in our study as we did not encounter any case of anaplastic carcinoma and poorly differentiated carcinoma.

Ki-67 showed negative expression in all the cases of benign thyroid neoplasm as we had considered weak positive expression as negative, like the other previous studies. Ki-67 was expressed positively in 53.60% (15/28) cases in malignant neoplasms. Mean Ki-67 labelling index in benign and malignant neoplasm was 1.93 and 9.18 respectively. The difference in mean Ki-67 labelling index in benign and malignant neoplasm was found to be statistically significant (p value- 0.001). Thus, Ki-67 labelling index is an important tool in assessing nature of the neoplasm and the prognosis.

The expression of bcl 2 was found to have 50% sensitivity and 33.33% specificity at 95% confidence interval with p value of 0.57. The Ki-67 expression was found to have 98% sensitivity and 67.5% specificity in detecting malignant thyroid neoplasms with diagnostic accuracy of 76.36% at 95% confidence interval with p value of 0.97.

Table 1: Gender wise distribution of the cases according to 5th edition WHO classification of thyroid neoplasm 2022 (n=55)

S No	Category	Family	Type	Frequency		Male		Female	
				n	(%)	n	(%)	n	(%)
1	Follicular cell derived neoplasm	Benign tumours	Thyroid follicular nodular disease	00	(0.00%)	00	0.00%	00	0.00%
			Follicular thyroid adenoma	23	(42.00%)	04	17.40%	19	82.60%
			Follicular thyroid adenoma with papillary architecture	00	(0.00%)	00	0.00%	00	0.005
			Oncocytic adenoma	04	(7.20%)	00	0.00%	04	100%
		Low risk neoplasms	Non-invasive thyroid neoplasm with papillary like nuclear feature	00	(0.00%)	00	0.00%	00	0.00%
			Thyroid tumour of uncertain malignant potential	00	(0.00%)	00	0.00%	00	0.00%
			Hyalinizing trabecular tumour	00	(0.00%)	00	0.00%	00	0.00%
		Malignant neoplasms	Follicular thyroid carcinoma	04	(7.20%)	00	0.00%	04	100%
			Papillary thyroid carcinoma (PTC)	15	(27.20%)	02	13.30%	13	86.60%
			Invasive encapsulated follicular variant of PTC	06	(11.00%)	00	0.00%	06	100%
			Oncocytic carcinoma	1	(1.80%)	00	0.00%	01	100%
			”””Follicular derived carcinomas, high grade”””	00	(0.00%)	00	0.00%	00	0.00%
			Anaplastic follicular cell derived carcinomas	00	(0.00%)	00	0.00%	00	0.00%
2.	Thyroid C cell derived carcinoma		Medullary Thyroid Carcinoma	02	(3.60%)	00	0.00%	02	100%

Discussion

Histopathological examination is the gold standard diagnostic tool for determining the behaviour of thyroid nodules. For decades, the diagnosis of PTC has relied on identifying nuclear features and papillary architecture. However, these features alone are sometimes insufficient for a definitive diagnosis, as certain benign lesions can exhibit nuclear characteristics similar

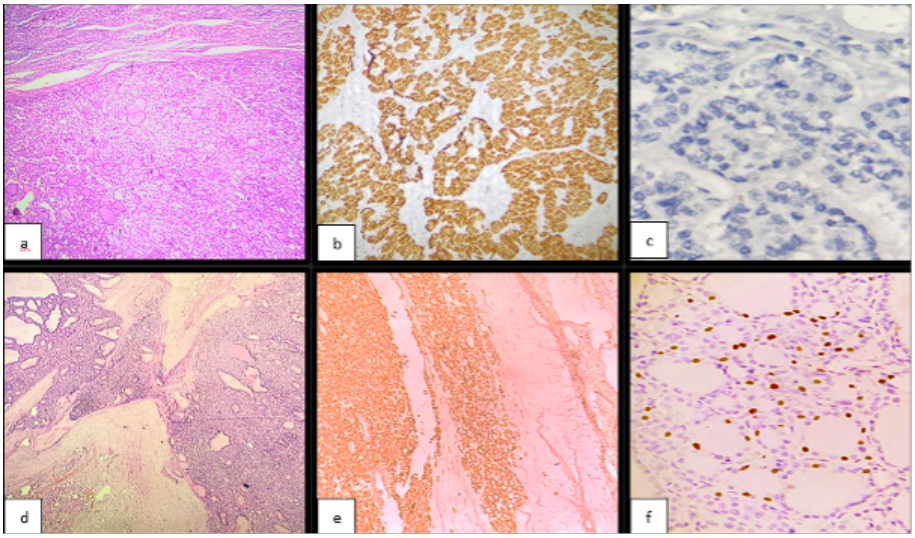

Figure 1: a) Showing Follicular adenoma with intact capsule (H&E, 100X). b) Showing strong positive bcl 2 expression in follicular adenoma (IHC, 100X). c) Showing negative Ki-67 expression in follicular adenoma (IHC, 400X). d) Follicular carcinoma showing transgression of capsule as shown by yellow arrow. (H&E, 100X). e) Follicular carcinoma: showing strong positive staining of bcl 2 (cytoplasmic) in follicular epithelial cells. (IHC, 100X). f) Case of Follicular carcinoma showing positive Ki-67 (nuclear) immunostain. (IHC, 400X)

Table 2: Case distribution of Ki-67 expression in histological type of thyroid neoplasm (n=55)

Histological type	**Negative**		**Weak positive**		**Moderate positive**		**Strong positive**	
(n=55)	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Follicular thyroid adenoma (23)	10	43.50%	13	56.50%	00	0.00%	00	0.00%
Papillary carcinoma thyroid (PTC) (15)	07	46.67%	03	20.00%	05	33.33%	00	0.00%
Invasive encapsulated follicular variant PTC (6)	02	33.33%	01	16.67%	02	33.33%	01	16.67%
Follicular thyroid carcinoma (4)	00	0.00%	00	0.00%	00	0.00%	04	100%
Oncocytic adenoma of thyroid (4)	04	100%	00	0.00%	00	0.00%	00	0.00%
Medullary carcinoma thyroid(2)	00	0.00%	00	0.00%	02	100%	00	0.00%
Oncocytic carcinoma of thyroid (1)	00	0.00%	00	0.00%	01	100%	00	0.00%

to PTC. Additionally, follicular-patterned thyroid tumors often pose diagnostic challenges, primarily due to the difficulties pathologists face in evaluating these structures. Key challenges include determining the presence or absence of capsular invasion and differentiating lesions with PTC-like nuclear features but without papillary architecture, which complicates distinguishing FVPTC from adenomatous goiter. As a result, application of IHC markers in the diagnosis of PTC and its mimics is a great yield in the field of diagnostic pathology.

Gender wise distribution of cases in this study showed an overall predominance of females in thyroid neoplasms with male to female ratio of 6.8 times. Studies from various regions worldwide, Pujani et al[1], Yorukoglu et al[11], Bakshi et al[12], Goyal et al[13], Bhandari et al[14] and Chowdhury et al[15] have consistently shown a higher prevalence of thyroid neoplasm in females compared to males.

IHC bcl 2 was positive in 100% (28/28) of malignant cases and 100% (27/27) of benign cases. The findings of present study are consistent with studies done by Aksoy et al[16], Gharrawi et al[17], Akgun et al[18] showing 100% positivity in both benign and malignant cases.

No Ki-67 positive expression was seen in benign thyroid neoplasms and 54% positive expression of IHC Ki-67 was seen in malignant neoplasm in the present study. With regard to Ki-67 expression in benign cases the present study is consistent with study done by Sharma et al[19], Khogaly et al[20], and Zhou et al[21]. Considering malignant cases, the present study is consistent with study done by Zhou et al[21]. However, in present study there was significant difference in Ki-67 positive expression among benign and malignant neoplasm. This finding is consistent with Song et al[22], Zhou et al[21], Khogaly et al[20], and Sharma et al[19].

In the present study the Ki-67 labelling index (LI) in histological types was as follows – the maximum Ki-67 LI was in follicular carcinoma thyroid (25.00 $\pm$ 0.82%) followed by oncocytic carcinoma thyroid (12%), medullary carcinoma thyroid (10%), papillary carcinoma thyroid (7.59$\pm$10.33%) and minimum in follicular thyroid adenoma (1.91$\pm$1.86%).The value of Ki-67 LI in follicular adenoma in our study is consistent with the values calculated by studies conducted by Pujani et al[1],

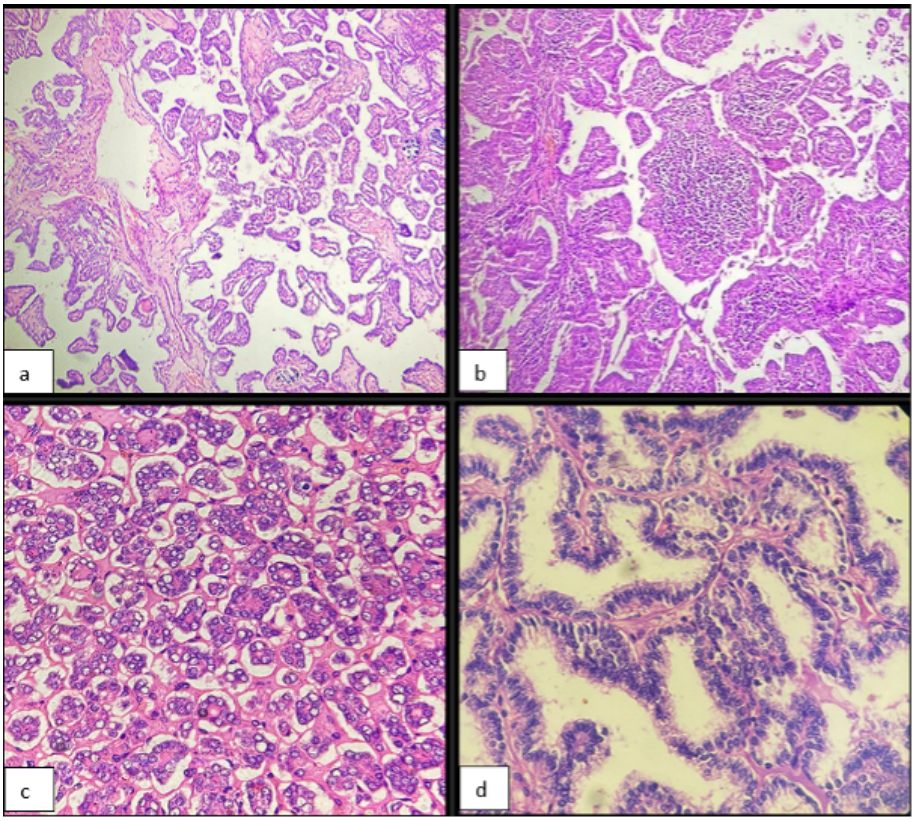

Figure 2: Variants of PTC: a) Classical variant of PTC showing true papillae with areas of calcification (H&E, 100X). b) Warthin like variant or Papillary thyroid carcinoma (H&E, 100X). c) - Invasive encapsulated follicular variant papillary thyroid carcinoma showing epithelial cells arranged in follicular pattern. (H&E, 400X). d) Columnar variant or Papillary thyroid carcinoma (H&E, 400X)

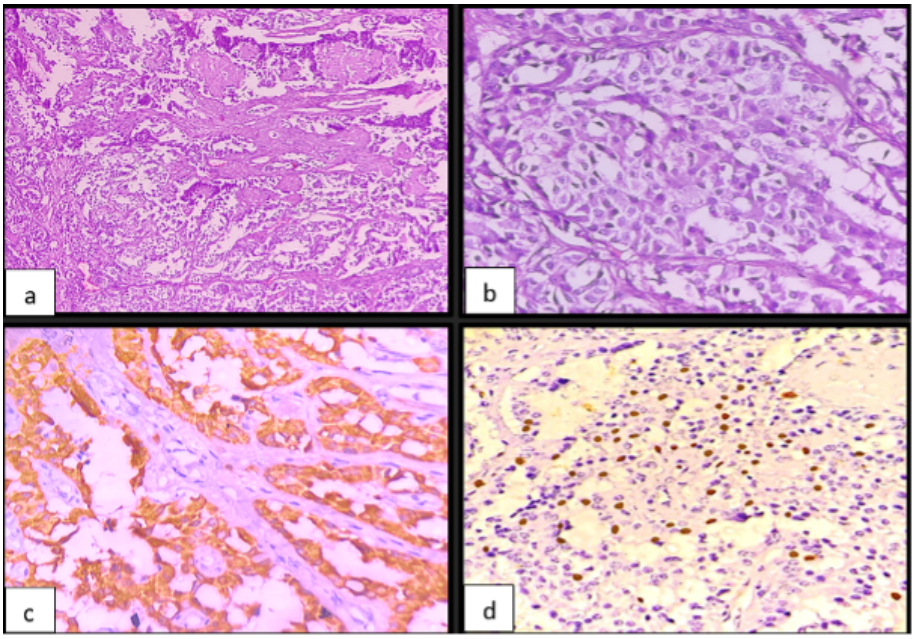

Figure 3: a) Medullary carcinoma thyroid showing sheets and nests of atypical cells, and abundant extracellular amorphous eosinophilic material (H&E, 100X). b) Medullary carcinoma thyroid showing plasmacytoid cells with salt and pepper chromatin (H&E, 400X). c) Strong positive bcl 2 expresssion in medullary carcinoma (IHC, 400X). d) Positive Ki-67 expression in medullary carcinoma (IHC, 400X)

Dwivedi et al[23], and Hellgren at al[24]. In the present study the value for Ki-67 LI for follicular thyroid carcinomas was higher and is not in concordance with the studies conducted by Dwivedi et al[23], and Hellgren at al[24].

Table 3: Comparative analysis of mean Ki-67 Labelling Index in histological types with previous studies

Tumor Type	Study	Ki-67 Labeling Index (%)
Follicular Thyroid Adenoma	Pujani et al, 2010	1.01%
	Aiad et al, 2013	34.90 ± 3.49%
	Zhou et al, 2015	0.46 ± 0.46%
	Dwivedi et al, 2016	2.23 ± 1.05%
	Sharma et al, 2018	0.375 ± 1.05%
	Hellgren et al, 2022	2.60%
	Present study, 2024	1.91 ± 1.86%
Papillary Carcinoma Thyroid	Pujani et al, 2010	3.66%
	Aiad et al, 2013	14.12 ± 2.29%
	Zhou et al, 2015	1.45 ± 1.83%
	Dwivedi et al, 2016	3.45 ± 2.40%
	Sharma et al, 2018	1.38 ± 1.87%
	Present study, 2024	7.59 ± 10.33%
Follicular Carcinoma Thyroid	Pujani et al, 2010	6.00%
	Aiad et al, 2013	61.42 ± 3.38%
	Dwivedi et al, 2016	3.71 ± 1.48%
	Hellgren et al, 2022	5.80%
	Present study, 2024	25.00 ± 0.82%
Oncocytic Adenoma	Present study, 2024	2.00 ± 0.82%
Medullary Carcinoma Thyroid	Pujani et al, 2010	7.00%
	Present study, 2024	10.00%
Oncocytic Carcinoma	Present study, 2024	12.00%
Others	Pujani et al, 2010	9.00%
	Aiad et al, 2013	18.60 ± 1.96%
	Dwivedi et al, 2016	1.07 ± 0.66%

Conclusion

The research highlights the importance of ancillary study-Immunohistochemistry along with histomorphology. Immunohistochemistry has played an important role in differentiating benign from malignant thyroid neoplasm, which can be diagnostically challenging due to variability in histopathological features. The IHC marker studied in the present study was bcl 2, which was not useful in differentiating the benign neoplasms from the malignant; but being a marker of proliferation for thyroid tissue it can help in detecting the metastatic foci in other organs and to diagnose the cases of poorly differentiated thyroid carcinomas as explained and proved in the past literatures. The other IHC marker which we studied was Ki-67. It proved to be useful in differentiating malignant thyroid neoplasms from the benign one as the Ki-67 labelling index more in the malignant neoplasms, it was statistically significant.

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