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Research Article

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BRAF V600E Expression in Papillary Thyroid Carcinoma and its Association with Histological Prognostic Factors

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Abstract

Background: *Thyroid cancer is the most common endocrine tumor, accounting for 90% of all endocrine malignancies. Papillary thyroid carcinoma (PTC) is the most common thyroid cancer. Despite its good prognosis, a small number of PTC cases develop lymph node metastasis, extrathyroidal extension, distant metastasis, or recurrent disease. The B-type RAF V600E (BRAF V600E) mutation is the most commonly observed genetic abnormality in PTC and PTC-derived anaplastic carcinoma. This study was done to determine the association between BRAF V600E expression in PTC and tumor size, multifocality, extrathyroidal extension, and chronic lymphocytic thyroiditis.*

Method: *This was a cross-sectional study conducted with 50 cases of papillary thyroid cancer at the Department of Pathology, BSMMU, Dhaka, Bangladesh from March 2022 to February 2024. Tumor size, tumor focality, extrathyroidal extension, chronic lymphocytic thyroiditis, and PTC histologic subtype are among the histological prognostic variables. The BRAF V600E mutation status was examined with immunohistochemistry on the selected paraffin blocks. BRAF V600E expression was determined by scoring based on signal intensity (0-3) and the proportion of tumor cells (0-100%). Demographic data, histological findings and the BRAF V600E expression status of all the cases were compiled, followed by statistical analysis.*

Results: *Out of 50 PTC cases, 23 (46%) cases were BRAF V600E expression-positive. No significant association was found between BRAF V600E expression and tumor size, extrathyroidal extension, tumor focality, or chronic lymphocytic thyroiditis.*

Discussion: *No significant association was found between the expression of BRAF V600E and tumor size, extrathyroidal extension, tumor focality, or chronic lymphocytic thyroiditis in PTC.*

Keywords: Papillary Thyroid Carcinoma, BRAF V600E Immune Expression

1. Introduction

Thyroid cancer is the most common endocrine tumor, accounting for 90% of all endocrine malignancies [1,2]. It is the ninth most common cancer diagnosed globally in 2020, with 5.86 million cases, according to GLOBOCAN. In both adult and pediatric populations, papillary thyroid carcinoma is the most common type of thyroid cancer [3,4]. Papillary thyroid carcinoma was nearly solely responsible for the rise in thyroid cancer incidence. The causes of the rise are complicated, multifactorial, and not fully understood. The most obvious causes include the greater use of diagnostic entities, the changing classification of thyroid neoplasms, environmental risk factors, radiation, obesity, and other issues [5].

Papillary thyroid carcinoma has relatively indolent behavior and a better prognosis compared with other malignant thyroid tumors. But about 25%-30% of PTC cases follow an aggressive clinical course, such as lymph node metastasis, extrathyroidal extension, distant metastasis, or recurrent disease [3,6-8]. Many authors have also reported that the risk of developing PTC increases in those with preexisting Hashimoto's thyroiditis (HT), an autoimmune disease also known as chronic lymphocytic thyroiditis. However, many investigators have reported that PTC with coexistent HT is associated with lower extrathyroidal extension, advanced stage, lymph node metastasis, and recurrence [9-12].

The revised American Thyroid Association guidelines point out that thyroid cancer should be treated according to risk stratification, assessed on the basis of disease stage and genetic testing [13-16]. Studies have reported that the BRAF mutation in PTC is about 50% to 80%. Point mutations of the BRAF gene are the most common genetic alteration known to occur in thyroid papillary carcinomas [3,17-21]. Some researchers claimed that the BRAF V600E mutation in PTC had been associated with more aggressive histological features, such as older age, male sex, tumor size, multifocality, extrathyroidal extension, capsular invasion, lymph node metastasis, advanced TNM stage, and recurrence [22-24].

Conversely, some studies have shown that the BRAF V600E mutation in PTC has not been fully or partially linked to more aggressive histological features, including advanced TNM stage, recurrence, male sex, larger tumors, multifocality, extrathyroidal extension, capsular invasion and lymph node metastasis [25-28]. It has been reported that PTC patients with the BRAF V600E mutation often lose the ability to uptake radioactive iodine, followed by subsequent resistance to radioactive iodine treatment. Currently, BRAF V600E mutation-specific targeted drugs intended for papillary thyroid carcinoma are under clinical trial [29,30]. If these BRAF V600E inhibitors for papillary thyroid carcinoma are approved by the FDA, they can be used as an alternative therapeutic approach for radioactive iodine-resistant patients.

Within the aforementioned context, the purpose of this study is to investigate the relationship between the BRAFV600E mutation in papillary thyroid carcinoma and histological prognostic factors, such as tumor size, tumor focality, extrathyroidal extension and chronic lymphocytic thyroiditis, that have been linked to aggressive or poor clinical outcomes.

2. Methods

This was a cross-sectional study conducted with 50 cases of papillary thyroid cancer at the Department of Pathology, BSMMU, Dhaka, Bangladesh from March 2022 to February 2024. Tumor size, tumor focality, extrathyroidal extension, chronic lymphocytic thyroiditis, and PTC histologic subtype are among the histological prognostic variables. A total of 50 papillary thyroid carcinoma cases were selected for the study by matching the inclusion and exclusion criteria. After gross and microscopic examination and clinical correlation, histopathological diagnosis of Papillary thyroid carcinoma is confirmed according to WHO classification. Corresponding slides and paraffin blocks were collected from the Department of Pathology, BSMMU, Dhaka, Bangladesh. Representative sections from paraffin block were reviewed and selected for BRAF V600E immunohistochemical stain. Patient demographic data and clinicopathological features were collected from patient and pathology reports.

If the immunoreactivity of the tumor cells is greater than or equal to the surrounding follicular colloid, or diffuse staining, and is observed at a magnification of 40X, the staining intensity is rated as 3+. If tumor cells displayed distinct cytoplasmic smears, but their intensity was less than that of the surrounding follicular colloids, which were clearly visible at 100X magnification, the staining intensity was rated as moderate (2+). If tumor cells displayed hazy or challenging stain detection, the staining intensity was rated as weak (1+). If there was no staining on any of the tumor cells, the score is 0 [23,27]. The percentage of positive cells was assigned a score of 0 for less than 5%, 1 for between 5 and 25%, 2 for between 26 and 50%, 3 for between 51 and 75%, and >75% for 4 points. Ultimately, the staining degree was ascertained by multiplying the scores denoting the percentage of positive cells and staining intensity, as follows: A score of 1-4 denotes a mildly positive (+), 5-8 a moderately positive (+ +), and a score of ≥9 denotes a strongly positive (+++) [28-31].

3. Result

3.1 Age of the Study Participants

The age range of the study participants was 17–65 years, with a mean age of 38.66 ± (12.637) years. The distribution of age groups by decades between 11 and 70 years has been shown in Table 1.

Age (years)	Frequency (n)	Percentage (%)
11-20	10	20
22-30	12	24
31-40	11	22
41-50	10	20
51-60	5	10
61-70	2	4
Total	50	100
Mean age ± (SD) = 38.66 ± (12.637) Minimum=17, Maximum=65		

Table 1: Age of the Study Cases (n = 50)

Table 2 shows the number of patients <55 years of age versus the number of patients ≥55 years of age according to age stratification by AJCC.

Age (years)	Frequency (n)	Percentage (%)
<55	43	86
≥55	7	14
Total	50	100
Mean age ± (SD) = 38.66 ± (12.637) Minimum=17, Maximum=65		

Table-2: Stratification of Study Cases on the Basis of Age (cut off value 55 years) according to AJCC (n= 50)

Sex of the patients

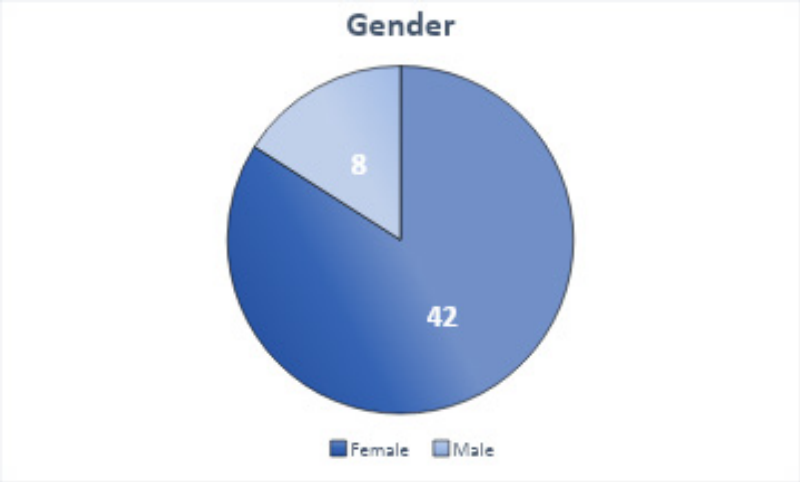

Figure 1: Demography of the Study Cases by Gender (Pie- Chart)

Out of 50 cases, 42 were females and 8 were males, with a male-to-female ratio of 1:5 (figure 1).

Tumor size (p^T) of PTC in the study cases

In this current study, 21 (42%) of PTC cases had p^T1, whereas 24 (48%) had p^T2 and 5 (10%) had p^T3 (Table 4).

Tumor size (p^T)	Frequency (n)	Percentage (%)
T1	21	42
T2	24	48
T3	5	10
Total	50	100

Table 3: Tumor Size (p^T) of PTC in the Study Cases (n= 50)

3.2 Characteristics of Study Cases by Histologic Prognostic Factors

Table 5 describes the results of the histological prognostic factors of the PTC cases in this study. It is evident that in 90% of cases, the tumor size was ≤ 4 cm, tumour was unifocal in 68% of cases versus multifocal in 32%, chronic lymphocytic thyroiditis was present only in 30% of cases, and extrathyroidal extension was detected only in 8% of cases.

Attributes		Frequency (n)	Percentage (%)
Tumour Size	≤ 4cm	45	90
	>4 cm	5	10
Tumour focality	Unifocal	34	68
	Multifocal	16	32
Chronic lymphocytic thyroiditis	Absent	35	70
	Present	15	30
Extra thyroidal extension	Absent	46	92
	Present	4	8

Lymph node metastasis	Absent	7	14
	Present	18	36
	LN not found	25	50

Table 4: Characteristics of Study Cases by Histologic Prognostic Factors (n=50)

3.3 Association of Age, Sex, Tumor Size, Chronic Lymphocytic Thyroiditis, Extrathyroidal Extension, Tumor Focality And Lymph Node Metastasis of Study Cases With BRAF V600e Expression

The association between age and BRAF V600E expression in the PTC cases in this investigation is shown in Table 8. It is evident that 87% of patients in the age group <55 and 13% in the age group ≥ 55 had BRAF V600E expression-positive results (BRAF V600E mutation). This difference in age between BRAF V600E mutation is not statistically significant.

BRAF V600E expression-positive results were found in 20 (87%) female cases and 3 (13%) male cases. The gender difference in the BRAF V600E mutation was not found to be statistically significant.

11 (47.8%) cases had p^T1, 10 (43.5%) p^T2, and 2 (8.7%) had p^T3 with positive BRAF V600E mutation expression. The tumour size (p^T) and BRAF V600E mutation are statistically non-significant in the Chi-square test (p= 0.183). Chronic lymphocytic thyroiditis is present in 8 (39%) of the BRAF V600E-positive individuals, while it is absent in 15 (65%) of the cases. According to the Chi-square test, the expression of the BRAF V600E mutation in PTC and chronic lymphocytic thyroiditis is statistically not significant (p = 0.496). Extrathyroidal extension was present in four cases. Among the four cases, two show the BRAF V600E mutation. According to the Fisher's exact test, the expression of the BRAF V600E mutation in PTC and extrathyroidal extension is statistically not significant (p= 1.0). Among available 25 cases with lymph nodes, BRAF V600E expression was present in 9 cases. Therefore, it was not possible to assess the association between lymph node metastases and BRAF V600E expression. In 14 (60.9%) unifocal PTC cases and 9 (39.1%) multifocal PTC cases, BRAF V600E expression was positive. The tumor focality and BRAF V600E mutation expression are statistically non-significant in the chi-square test. (p= 0.318).

In this study, 25 of the 50 histologically confirmed cases of PTC have surgical specimens that include lymph nodes. Among these 25 cases, BRAF V600E expression was present in 9 cases. Therefore, it was not possible to assess the association between lymph node metastases and BRAF V600E expression.

Attributes	**BRAF V600E expression**		**Significance (*p*-value)**
	Positive n (%)	**Negative n (%)**	
Age (years)			Fisher's exact=2.759 df=4 *p*= 0.625
<55	20 (86.95)	26 (96.29)	
≥55	3 (13.05)	1 (3.7)	
Sex			Fisher's exact=0.277 df=1 p= 0.711
Female	20 (87)	22 (81.5)	
Male	3 (13)	5 (18.5)	
Tumour size (p^T)			$\chi2$=3.402 df=2 p= 0.183
T1	11 (47.8)	10 (37.0)	
T2	10 (43.5)	14 (45.2)	
T3	2 (8.7)	3 (17.8)	
Chronic lymphocytic thyroiditis			$\chi2$=0.464 df=1 p= 0.496
Absent	15 (65.2%)	20 (74.1%)	
Present	8 (34.8%)	7 (25.9%)	
Extrathyroidal extension			Fisher's exact =0.028 df=1 p= 1.0
Present	2 (8.7)	2 (7.4)	
Absent	21 (91.3)	25 (92.6)	
Tumour focality			$\chi2$=0.995 df=1 p= 0.318
Unifocal	14 (60.9)	20 (74.1)	
Multifocal	9 (39.1)	7 (25.9)	
Total	23 (100)	27 (100)	

Table 5: Association of Age, Sex, Tumour Size, Chronic Lymphocytic Thyroiditis, Extra Thyroidal Extension and Tumour Focality Of Study Cases with BRAF V600E Expression (n=50)

Lymph nodes	Metastasis	BRAF V600E expression		Significance (*p*-value)
		Positive n (%)	Negativen (%)	
Absent	7	4	3	Not applicable
Present	18	9	9	
Total	25	13	12	

Table 6: Histopathological Association of Lymph Node Metastasis with BRAF V600E Expression (n= 25)

4. Discussion

The BRAF V600E mutant in papillary thyroid cancer has been associated with increased age, extra-thyroidal extension, TNM stage, lymph node metastases, recurrence, and a short lifespan [6,7]. Conversely, conflicting findings were found in multiple studies regarding the relationship between the BRAF V600E mutant and unfavorable clinical characteristics in PTC [32]. Several studies showed that there was no significant association between the BRAF V600E mutation and age, which is similar to my study [23,25,33,34]. Many researchers revealed in their studies that most of the patients who were diagnosed with papillary thyroid carcinoma were female. But they did not find any statistically significant association between the BRAF V600E mutation and gender [18,33,34,35]. This result is consistent with my study's findings. Sun et al. (2015) from China, Fraser et al. (2016) from Australia, and Kristiani et al. (2021) from Indonesia have also reported no significant differences in tumor size between positive and negative BRAF V600E expression in cases of papillary thyroid carcinoma, which is consistent with this study [27,33,34,36].

According to several investigations, including comprehensive evaluations, multifocality was not linked to BRAF V600E expression in PTC patients [23,37,38,39,40]. My study's findings are consistent with these outcomes. On the other hand, several studies showed no association between BRAF V600E expression and extrathyroidal extension [41-43]. These results are consistent with the findings of the current study.

There have been investigations reporting that PTCs that coexist with CLT have a lower frequency of the BRAF V600E mutation [25,38,44,45]. This study found only 15 PTC with coexistent lymphocytic thyroiditis, which might have been the reason for not getting any statistically significant association with the BRAF V600E mutation. Some researchers reported that there was no significant association between lymph node metastasis and the BRAF V600E mutation, similar to this study [40,46]. Several researchers reported that there was no association between the BRAF V600E mutation in PTC and tumor size (p^T) [47]. This finding is consistent with my study as well.

5. Conclusion

The results of this current study revealed that 23 (46%) of the PTC cases had the BRAF V600E mutation. However, no significant association was found between the expression of BRAF V600E and tumor size, extrathyroidal extension, tumor focality, or chronic lymphocytic thyroiditis. In the study cases, the BRAF V600E mutant did not exhibit a statistically significant correlation with age or sex; however, the presence of the mutation was six times higher in those under the age of fifty-five than in those beyond the age of fifty-five.

Illustrations

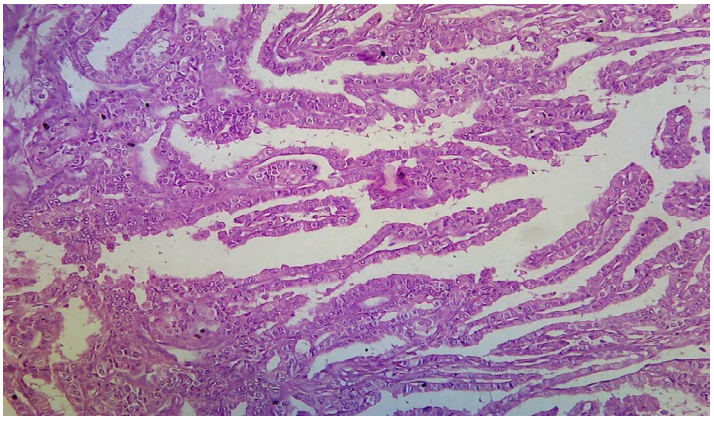

Figure 2: Photomicrograph Showing Papillary Thyroid Carcinoma, H&E, 100X (case no: 21)

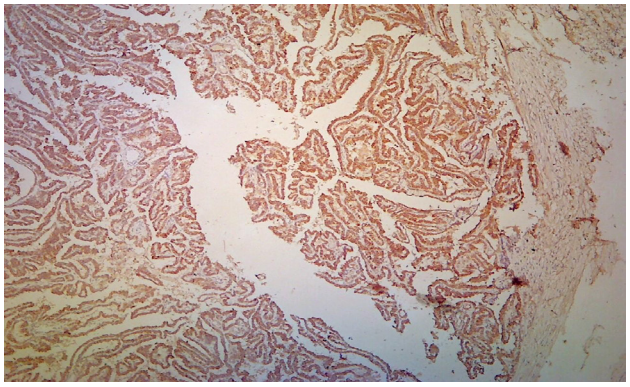

Figure 3: Photomicrograph Showing Papillary Thyroid Carcinoma with Strong and Diffuse Cytoplasm Positivity for BRAF V600E (case no: 21)

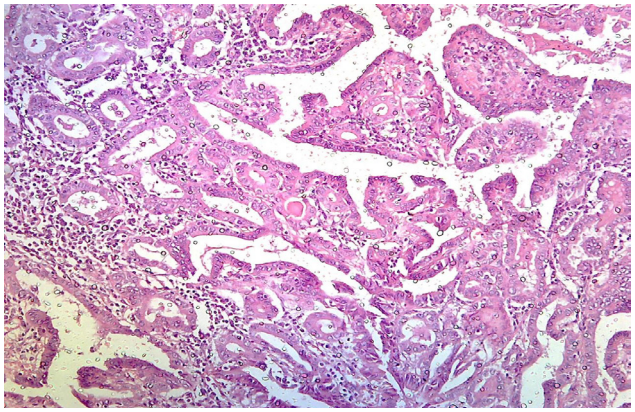

Figure 4: Photomicrograph Showing Papillary Thyroid Carcinoma, H&E, 100X (case no: 3)

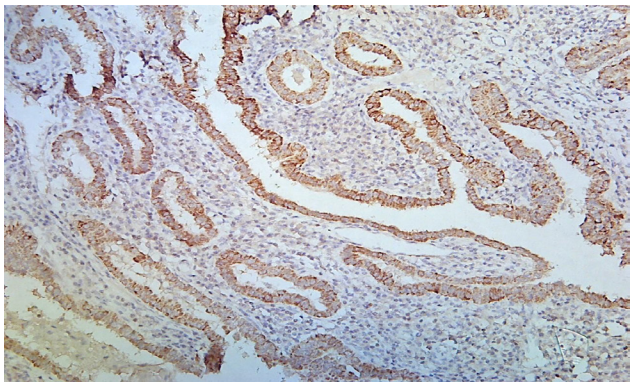

Figure 5: Photomicrograph Showing Papillary Thyroid Carcinoma with Moderate and Diffuse Cytoplasm Positivity for BRAF V600E (case no: 3)

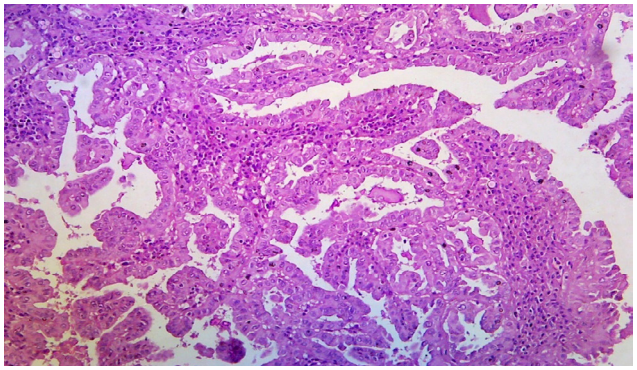

Figure 6: Photomicrograph Showing Papillary Thyroid Carcinoma, H&E, 100X (case no: 50)

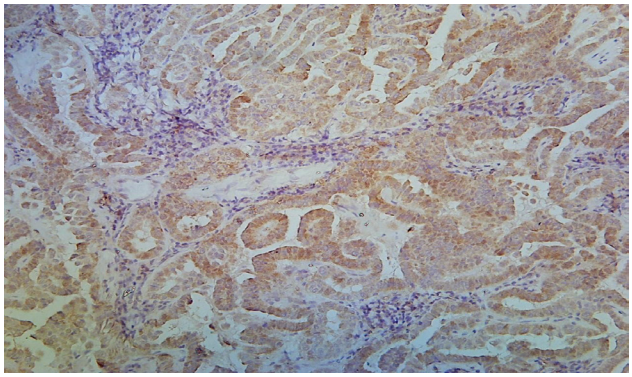

Figure 7: Photomicrograph Showing Papillary Thyroid Carcinoma with Moderate and Diffuse Cytoplasm Positivity for BRAF V600E (case no: 50)

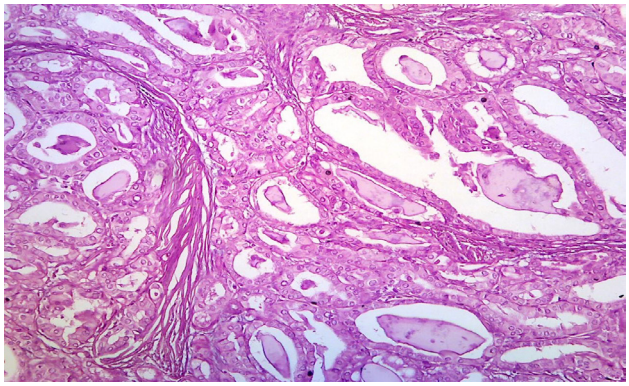

Figure 8: Photomicrograph Showing Papillary Thyroid Carcinoma, H&E, 100X (case no: 20)

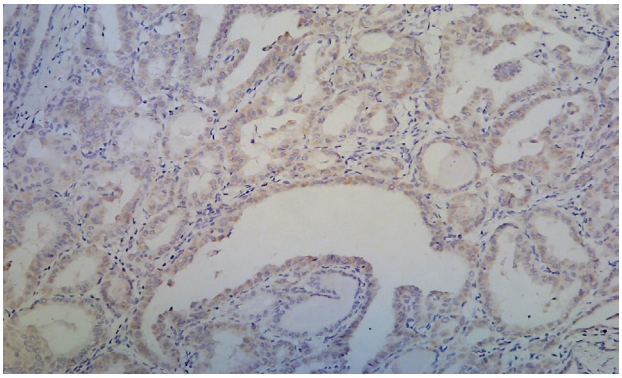

Figure 9: Photomicrograph Showing Papillary Thyroid Carcinoma with Weak and Diffuse Cytoplasm Positivity for BRAF V600E (case no: 20)

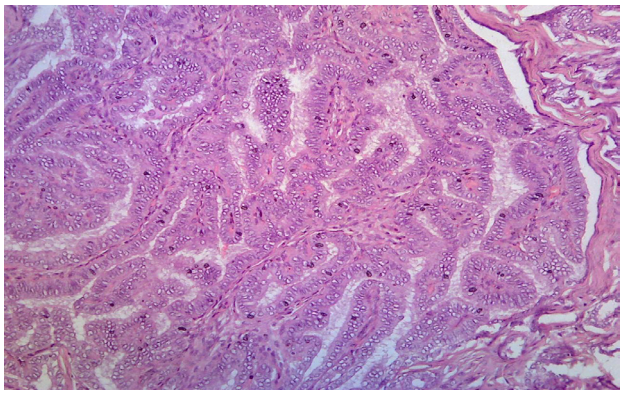

Figure 10: Photomicrograph Showing Papillary Thyroid Carcinoma, H&E, 100X (case no: 31)

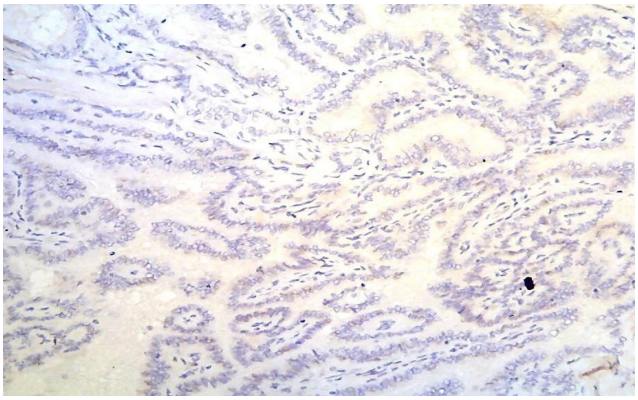

Figure 11: Photomicrograph Showing Papillary Thyroid Carcinoma with No Cytoplasm Positivity for BRAF V600E (case no: 31)

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