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EXPLORATION OF BRAFV600E AS A DIAGNOSTIC ADJUVANT IN THE NON-INVASIVE FOLLICULAR THYROID NEOPLASM WITH PAPILLARY-LIKE NUCLEAR FEATURES (NIFTP) IN A SET OF IRAQI PATIENTS

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ABSTRACT

Background: Noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) was introduced by the WHO in 2016 as a distinct diagnostic category, characterized by indolent behavior and lack of recurrence or metastasis. While classical papillary thyroid carcinoma (CPTC) is strongly associated with BRAFV600E mutations, follicular-patterned neoplasms, including NIFTP, are usually driven by RAS or RAS-like mutations and rarely harbor BRAFV600E. Recently, reliable immunohistochemistry (IHC) for BRAFV600E has emerged as a useful molecular surrogate in thyroid pathology. **Objective:** To evaluate the utility of BRAFV600E immunohistochemistry as a diagnostic adjuvant in the exclusion of NIFTP. **Methods:** A cross-sectional study was conducted on 61 thyroid tumors, including 30 cases of NIFTP and 31 cases of follicular variant papillary thyroid carcinoma (FVPTC), diagnosed at multiple centers in Karbala, Iraq, between January 2022 and June 2024. All samples were assessed for BRAFV600E expression using IHC. **Results:** Among NIFTP cases, 18 (60%) were negative and 12 (40%) were positive for BRAFV600E. In contrast, 22 of 31 FVPTC cases (71%) were positive and 9 (29%) were negative. BRAFV600E positivity was significantly higher in FVPTC than NIFTP ($p = 0.021$). No significant association was observed between BRAFV600E status and age, gender, tumor size, or focality. Both invasive encapsulated FVPTC (71.4%) and infiltrative FVPTC (70.6%) demonstrated high BRAFV600E positivity with no significant difference between subtypes. **Conclusion:** BRAFV600E IHC serves as a valuable diagnostic adjuvant for excluding NIFTP. Positive staining supports a diagnosis of FVPTC, particularly invasive subtypes, and aids in refining diagnostic accuracy when histologic features are ambiguous.

KEYWORDS: BRAFV600E, Non-Invasive, Follicular, Thyroid, Neoplasm, Papillary-like Nuclear Features (NIFTP).

INTRODUCTION

Noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) was introduced by the WHO in 2016 as a distinct diagnostic entity based on literature demonstrating that tumours meeting the diagnostic criteria for NIFTP, formerly encapsulated or well-circumscribed non-invasive follicular variants of papillary thyroid carcinoma, do not metastasise or recur, and they are cured by resection.[1] Classical-type papillary thyroid carcinomas (CPTC) frequently harbour BRAFV600E mutations, while follicular-patterned thyroid neoplasms, including NIFTP, are often driven by RAS mutations or RAS-like mutations and essentially never BRAFV600E.[2] A reliable clinically validated antibody has been developed for immunohistochemistry (IHC) that consistently correlates with the presence of the BRAFV600E mutation.[3] The diagnostic criteria of NIFTP are: (1) encapsulation or clear demarcation from the adjacent thyroid tissue. (2) Follicular growth pattern with <1% papillae, no psammoma bodies and <30% solid/trabecular/insular growth pattern. (3) Nuclear alterations of papillary carcinoma (with a score of 2–3). (4) No vascular or capsular invasion (after thorough examination of the tumour interface with the surrounding tissues). (5) No tumour necrosis. (6) No high mitotic activity (i.e., <3 mitoses per 10 high-power fields).[2] Complete submission of the tumour to the parenchymal interface for microscopic examination is already widely practised and recommended in order to exclude invasion. However, given the criterion of 1% or absence of papillae for NIFTP, the question arises if complete submission of not just the periphery but also the entire

central portions of the tumours is required. This is an important question, particularly for larger nodules. In this study we will employ BRAFV600E in the investigation of follicular-patterned thyroid neoplasms: noninvasive follicular thyroid neoplasm with papillary-like nuclear features. NIFTP, infiltrative follicular variant papillary thyroid carcinoma (IFVPTC), and invasive encapsulated follicular variant papillary thyroid carcinoma (IEFVPTC) to assess its potential as an effective marker differentiating NIFTP from other follicular patterned thyroid neoplasms. and if it is a helpful surrogate for true papillae in addition to the BRAFV600E mutation If so, BRAFV600E could be an alternative to pursuing complete submission of follicular-patterned tumours for NIFTP exclusion criteria, including papillae, intranuclear pseudo-inclusions, psammoma bodies or aberrant growth patterns. This immunostain might be performed as an alternative to submission of the entire central portion of large, non-incidental tumors.[4] Aim of study: to assess the BRAFV600E as an effective diagnostic adjuvant in the exclusion criteria of NIFTP so this immunostain might be performed as an alternative to submission of the entire central portion of large, non-incidental tumor.

METHOD

This retrospective cross-sectional study was carried out in the pathology departments of Imam Hussein Medical City, Safeer Al-Hussain Hospital, Al-Kafeel Hospital, and Al-Sajaad Specialized Laboratory, Karbala, Iraq, between January 2022 and June 2024. The aim was to assess BRAFV600E expression in three types of follicular thyroid neoplasms: noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP), invasive encapsulated follicular variant papillary thyroid carcinoma (IEFVPTC), and infiltrative follicular variant papillary thyroid carcinoma (IFVPTC), along with their clinicopathological correlations. A total of 61 archival cases were included, comprising 30 NIFTP and 31 FVPTC (14 IEFVPTC and 17 IFVPTC). Histopathological slides were reviewed to confirm diagnosis and eligibility. Paraffin-embedded tissue blocks were sectioned at 4 µm thickness for immunohistochemistry (IHC). Inclusion criteria encompassed all cases with available tissue blocks diagnosed within the study period, while samples with technical errors in processing, staining, or storage were excluded. BRAFV600E-positive papillary thyroid carcinoma served as the positive control. The IHC protocol involved deparaffinization with xylene, rehydration through graded ethanol, and antigen retrieval using Tris-EDTA buffer (pH 9.0) at 95.5°C. Endogenous peroxidase activity was blocked, followed by incubation with the primary antibody (BRAFV600E, clone RM8, rabbit monoclonal, Bio SB, USA) for 60 minutes. Detection was performed using a PolyExcel HRP/DAB system, with Mayer's hematoxylin as counterstain. Slides were dehydrated and mounted before evaluation. Image capture was performed using an Olympus DP72 microscope camera. **BRAF immunostain scoring:** Immunohistochemical scoring was done by calculating H-score which is a reliable metric that includes both the proportion (0–100%) and intensity of positive cells (0, absent; 1+, weak; 2+, moderate; 3+, strong staining) and calculated by combining intensity and proportion scores as follows: (1 × percentage of weak staining) + (2 × percentage of moderate staining) + (3 × percentage of strong staining), ranging from 0 to 300.[5] Based on a study using the similar method, where H-score of >10 was considered as positive for mutation, the result was that there was no significant association between cutoff point of H-score against the *BRAF* mutation. There for in the current study, positive H-score (any cytoplasmic expression) was considered as positive immunostaining.[6] Statistical analysis was conducted using SPSS version 30. A p-value < 0.05 was regarded as statistically significant.

RESULTS

The study involved 30 cases of NIFTP and 31 case of FVPTC including 14 case IEFVPTC and 17 case IFVPTC. All were stained for BRAFVE. 18 of 30 (60%) NIFTP case were negative for BRAFVE and 12 of 30 (40%) NIFTP case were positive for BRAFVE. 22 of 31 (71%) FVPTC case were positive for BRAFVE and 9 of 31 (29%) FVPTC case were negative for BRAFVE. The rate of BRAF positivity in FVPTC 71% was statistically significantly higher than in NIFTP 40% (p-value 0.021) as shown in table (1).

Table 1: Rate of BRAFV600E Staining in NIFTP Compared to FVPTC.

Diagnosis	BRAF Status	No.	% within Diagnosis	p-value
NIFTP (n = 30)	Positive	12	40.0%	**0.021**
	Negative	18	60.0%	
FVPTC (n = 31)	Positive	22	71.0%	
	Negative	9	29.0%	
Total (n = 61)	Positive	34	55.7%	
	Negative	27	44.3%	

Ten out of fourteen (71.4%) case of IEFVPTC were positive for BRAFVE and four out of fourteen (28.6%) case of IEFVPTC were negative for BRAFVE. Twelve out of seventeen (70.6%) case of IFVPTC were positive for BRAFVE and five out of seventeen (29.4%) case of IFVPTC were negative for BRAFVE there was no statistically significant difference in rate of BRAF positivity between IEVPTC and IFVPTC (p-value 0.055) as shown in table (2).

Table 2: Rate of BRAFV600E Staining in IEFVPTC and IFVPTC.

Diagnosis	BRAF Status	No.	% within Diagnosis	p-value
IEFVPTC (n = 14)	Positive	10	71.4%	
	Negative	4	28.6%	0.055
IFVPTC (n = 17)	Positive	12	70.6%	
	Negative	5	29.4%	
Total (n = 31)	Positive	22	71.0%	
	Negative	9	29.0%	

Twenty-six out of thirty (26/30) 86.7% of NIFTP were female, 57.1% (8/14) of IEFVPTC were female, 88.2% (15/17) of IFVPTC were female as shown in figure (1).

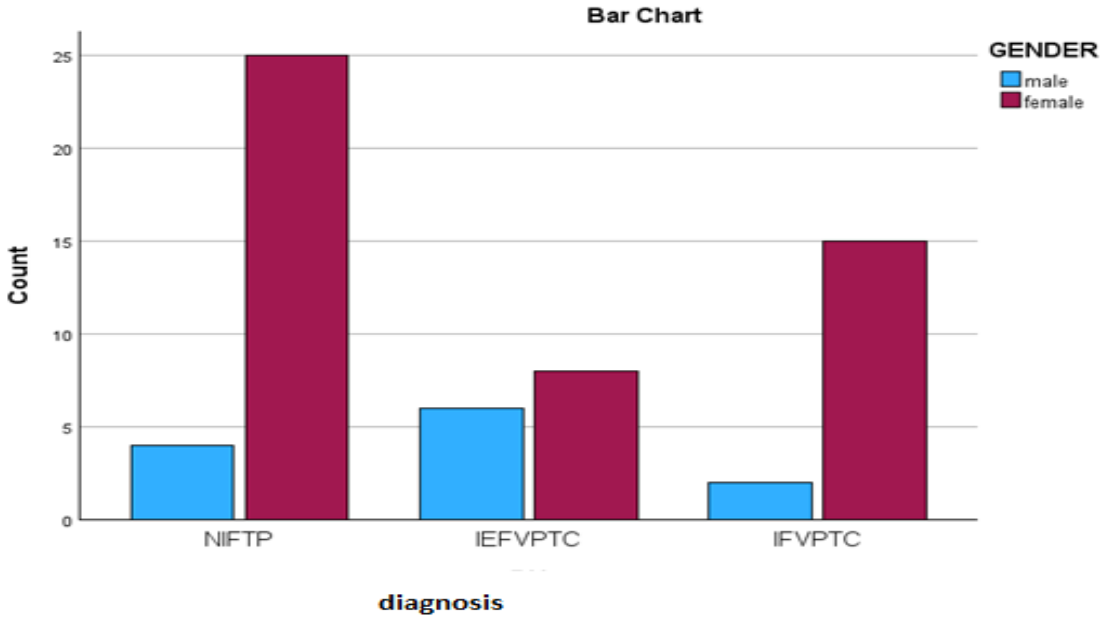

Figure (1): Gender distribution of presented cases of NIFTP, IEFVPTC and IFVPTC.

The age of patients with NIFTP ranged from 15 to 57 years, with a mean of 37 ± 11 years. In comparison, patients with IEFVPTC were between 26 and 57 years of age (mean 41 ± 8 years), while those with IFVPTC ranged from 25 to 60 years (mean 40 ± 10 years). The size of NIFTP tumors varied between 0.5 and 8 cm, with a mean of 2.8 ± 1.8 cm. Among the 12 BRAF-positive NIFTP cases, 9 were not entirely submitted, whereas 3 were completely submitted; of these, 2 were sub-centimeter nodules. Regarding focality, 18 out of 30 NIFTP cases (60%) were solitary. Two cases (6.7%) were multifocal, and another two (6.7%) were bilateral. Additionally, 8 cases (26%) were associated with other thyroid lesions, including IEFVPTC (3 cases), conventional PTC (2 cases), follicular adenoma (2 cases), and Hürthle cell adenoma (1 case), as presented in Table (3).

Table 3: Clinicopathologic Characteristics of NIFTP, IEFVPTC, and IFVPTC Cases.

Parameter	Category	NIFTP	IEFVPTC	IFVPTC	Notes
Age (years)	Mean ± SD	37 ± 11	41 ± 8	40 ± 10	Range: 15–60
Tumor size (cm)	Range/Mean	0.5–8 (2.8 ± 1.8)	-	-	
Submission (BRAF+ NIFTP)	Not entirely submitted	9	-	-	
	Entirely submitted	3 (2 sub-cm, 1 larger)	-	-	
Focality	Solitary	18 (60%)	-	-	
	Multiple	2 (6.7%)	-	-	
	Bilateral	2 (6.7%)	-	-	
	Associated with other lesions	8 (26%)	-	-	IEFVPTC (3), PTC (2), FA (2), Hürthle adenoma (1)

There is no significant difference in the rate of BRAF positivity between sub-cm tumors (NIFTP and FVPTC) and larger counter parts as shown in table (4).

Table 4: Rate of BRAFV600E Positivity in Relation to Tumor Size.

Diagnosis	Size	BRAF Status	No.	% within Size	p-value
NIFTP (n=30)	Larger	Positive	8	33.3%	
		Negative	16	66.7%	
	Sub-cm	Positive	4	66.7%	
		Negative	2	33.3%	
FVPTC (n=31)	Larger	Positive	19	79.2%	
		Negative	5	20.8%	
	Sub-cm	Positive	3	42.9%	
		Negative	4	57.1%	
Total		Positive	34	55.7%	NIFTP=1.48 FVPTC=1.50
		Negative	27	44.3%	

There is no significant difference in the rate of BRAF positivity in both (NIFTP and FVPTC) between male and female as shown in table (5).

Table 5: BRAFV600E Staining in Relation to Gender.

Diagnosis	Gender	BRAF Status	No.	% within Gender	p-value
NIFTP (n=30)	Male	Positive	1	25.0%	
		Negative	3	75.0%	
	Female	Positive	11	42.3%	
		Negative	15	57.7%	
FVPTC (n=31)	Male	Positive	7	87.5%	
		Negative	1	12.5%	
	Female	Positive	15	65.2%	
		Negative	8	34.8%	
Total		Positive	49	71.0%	NIFTP=0.631 FVPTC=0.379
		Negative	36	29.0%	

There is no significant difference in the rate of BRAF positivity between age groups <35 and ≥35 years old in both NIFTP and FVPTC as shown table (6).

Table 6: BRAFV600E Staining in Relation to Age Group.

Diagnosis	Age Group	BRAF Status	No.	% within Age Group	p-value
NIFTP (n=30)	<35	Positive	3	25.0%	
		Negative	9	75.0%	
	≥35	Positive	9	50.0%	
		Negative	9	50.0%	
FVPTC (n=31)	<35	Positive	7	70.0%	
		Negative	3	30.0%	
	≥35	Positive	15	71.4%	
		Negative	6	28.6%	
Total		Positive	34	55.7%	NIFTP=0.260 FVPTC=1.0
		Negative	27	44.3%	

There is no significant difference in the rate of BRAF positivity between solitary, bilateral, multiple, and associated with other lesion in both NIFTP and FVPTC as shown in table (7).

Table 10: BRAFV600E Staining in Relation to Tumor Focality.

Diagnosis	Focality	BRAF Status	No.	% within Focality	p-value
NIFTP (n=30)	Solitary	Positive	8	44.4%	
		Negative	10	55.6%	
	Multiple	Positive	1	50.0%	NIFTP=0.662 FVPTC=0.598
		Negative	1	50.0%	
	Bilateral	Positive	0	0.0%	
		Negative	2	100.0%	
	+ Other lesion	Positive	3	37.5%	
		Negative	5	62.5%	
FVPTC (n=31)	Solitary	Positive	12	66.7%	
		Negative	6	33.3%	
	Multiple	Positive	1	100.0%	
		Negative	0	0.0%	
	Bilateral	Positive	3	100.0%	
		Negative	0	0.0%	
	+ Other lesion	Positive	6	66.7%	
		Negative	3	33.3%	
Total		Positive	34	55.7%	
		Negative	27	44.3%	

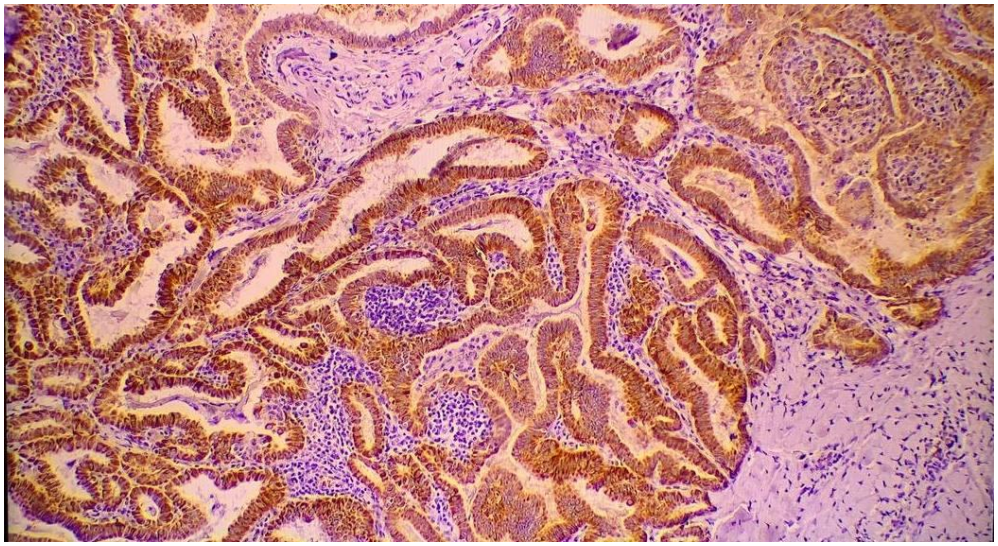

Figure (2): Positive control papillary thyroid carcinoma showing cytoplasmic staining 400x.

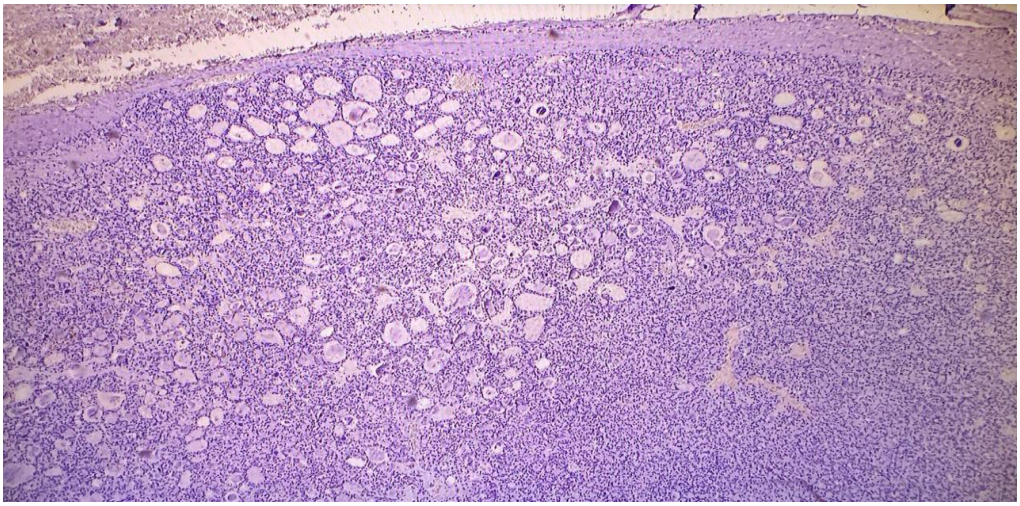

Figure (3) NIFTP negative for BRAFV600E 100x.

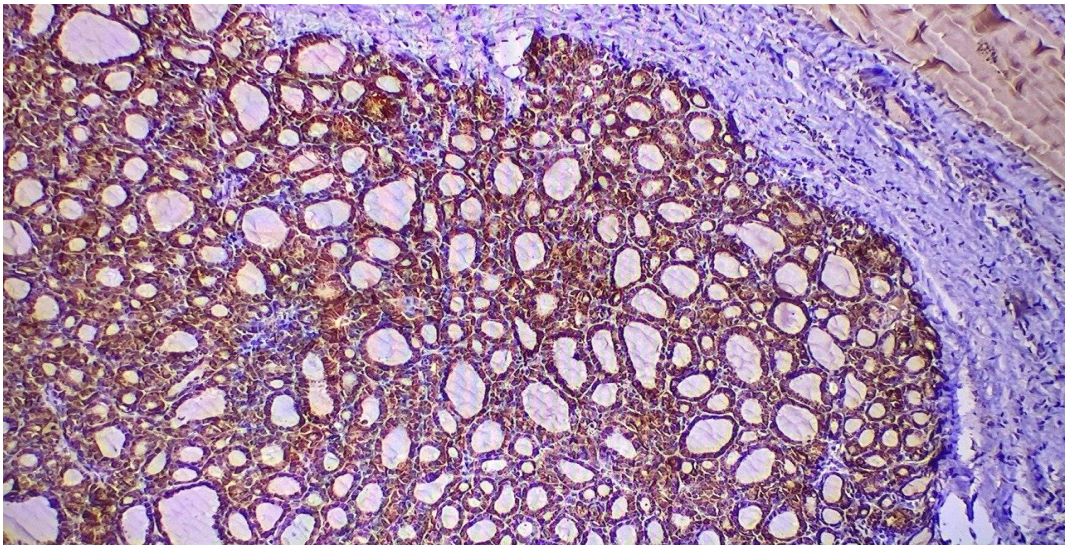

Figure (4): NIFTP diffusely positive cytoplasmic staining for BRAFV600E 400x.

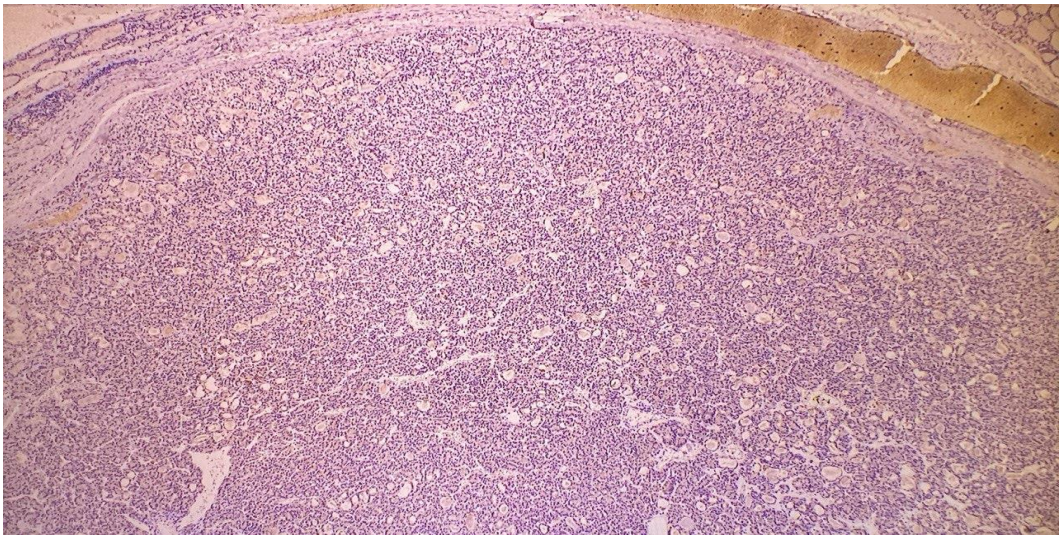

Figure (5): IEFVPTC negative for BRAF staining 100x.

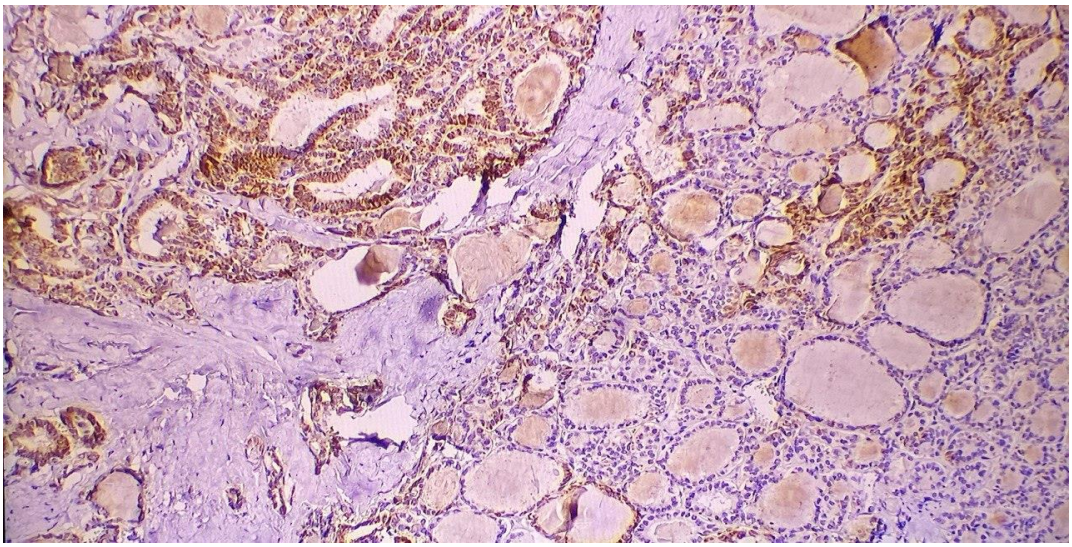

Figure (6): IFVPTC positive cytoplasmic staining for BRAF 400x.

DISCUSSION

In histopathologic practice it is necessary to differentiate between follicular patterned thyroid neoplasms, especially in the last years with the introduction of NIFTP as a diagnostic entity, and in this study we employed BRAFV600E IHC for that purpose. The current study revealed a significant difference in the rate of BRAFV600E IHC positivity in FVPTC (71%) compared to NIFTP (40%), and this agrees with a study conducted by Daniel and Peter in Boston[4], but there is disagreement in that the current study revealed 40% (12/30) BRAFV600E-positive NIFTP compared to 0% (0/62) in Daniel and Peter's study[4] and 0% (0/10) in a study conducted in Al-Najaf city by Maha and Rihab.[7] This rate of BRAFV600E positivity in NIFTP is considered high in the background that BRAF mutation excludes the diagnosis of NIFTP according to WHO[8], and this may be interpreted in the context that the tumour was not entirely submitted and morphologic NIFTP exclusion criteria, such as papillae, psammoma bodies or solid growth, may be present in the non-submitted part; thus, BRAF may act as a suitable surrogate for NIFTP exclusion criteria in such conditions, and positivity favours a diagnosis other than NIFTP. However, even sub-cm tumours (2 cases) that were entirely submitted showed BRAFV600E positivity. This may agree with a Korean study that showed 28.6% NIFTP BRAFV600E positive.[9] So still, complete submission of the entire nodule is an important practice. Some suggest that BRAF-positive NIFTP may indicate carcinoma in situ. However, many authors feel that since no non-malignant thyroid neoplasm with a BRAFV600E mutation has been documented, referring to a positive BRAFV600E-associated tumour as a tumour of uncertain malignant potential.[4] The current study also revealed no significant association between BRAFV600E positivity and tumour size in both NIFTP and FVPTC, and this agrees with Maha and Rihab's study[7] and with Daniel and Peter's study in regard to NIFTP. Only while the rate of BRAFV600E positivity in the subcentimeter IEFVPTC was significantly greater than those greater than 1.0 cm in Daniel and Peter's study[4], this disagreement may be contributed to the limited cases of the current study. The current study also revealed that female is the predominant gender in all types of studied cases (NIFTP and FVPTC), which is consistent with Daniel and Peter's study[4] and Maha and Rihab's study.[7] The current study also revealed no significant association between BRAF positivity and age group, which agrees with Maha's study.[7] The current study also revealed no significant association between BRAF positivity and tumour focality that also agrees with Maha and Rihab's study.[7]

CONCLUSION

BRAFV600E can be a helpful diagnostic adjuvant for NIFTP and act as a surrogate for NIFTP exclusion criteria such as papillae especially in larger nodules that are incompletely submitted in case positivity of BRAF favors diagnosis other than NIFTP such as IEFVPTC.

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