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THE DIAGNOSTIC ROLE OF BRAF^{V600E} MUTATION AND ULTRASOUND FEATURES IN INDETERMINATE THYROID CYTOLOGY

Alaa Al-Maaitah*[1], Moaath Alsmady[1], Mira Younis[1], Ahmad Al-Alwan[1], Dana Alsmady[1], Shatha Dmour[1], Handan Ankaralı[2], Malek Zihlif[3], Mohammad Al-Shehab[3], Heyam Awad[4], Nader Albsoul[1], Orhan Alimoglu[5,6]

[1]Department of General Surgery, School of Medicine, The University of Jordan, Jordan University Hospital.
[2]Department of Biostatistics, School of Medicine, Istanbul Medeniyet University.
[3]Department of Pharmacology, School of Medicine, The University of Jordan.
[4]Department of Pathology, School of Medicine, The University of Jordan.
[5]Department of General Surgery, School of Medicine, Istanbul Medeniyet University.
[6]Department of General Surgery, School of Medicine, The University of Jordan (Visiting Professor).

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***Corresponding author: Alaa Al-Maaitah**

Department of General Surgery, School of Medicine, The University of Jordan, Jordan University Hospital.

ABSTRACT

Background: Fine needle aspiration biopsies of thyroid nodules are indeterminate in approximately 30% of the cases, so several preoperative molecular markers have been studied to improve the diagnostic utility of such nodules. We aimed to study the role of BRAF ^{V600E} mutation and ultrasonography to predict malignancy preoperatively for indeterminate thyroid nodules. **Methods:** This is a retrospective study that included 78 indeterminate nodules. DNA was extracted from formalin-fixed paraffin-embedded tissues and BRAF^{V600E} mutation was analyzed by direct sequencing. Neck ultrasound records were evaluated for the major sonographic features including size, margin, halo, echogenicity, calcifications, vascularity and cervical lymphadenopathy. A "p" value less than 0.05 was considered statistically significant. **Results:** Seventy-eight indeterminate nodules were distributed in Bethesda categories III, IV and V as 38, 17, 23, respectively. Malignant cases in each category were 6/38, 5/17, 12/23, respectively. All of them were diagnosed with papillary thyroid cancer. Four cases out of 78 harbored BRAF^{V600E} mutation (1 in category III, 3 in category V). Accordingly, malignancy was significantly predicted by cytology (p=0.009) and BRAF^{V600E} mutation (p=0.001). The latter exhibited 100% specificity. However, the overall sensitivity of BRAF^{V600E} mutation was 17.4% and increased only to 25% in category V. Sonographic features that corresponded significantly to malignancy were microcalcifications and cervical lymphadenopathy (p=0.026 and p=0.017, respectively). But only ill-defined margin was associated with BRAF^{V600E} mutation (p= 0.032). Ultrasound features had low sensitivity (15.4%-84.6%), but higher specificity (9.7%-93.9%) of which microcalcifications and ill-defined margin were the most specific signs. **Conclusions:** BRAF^{V600E} mutation was a significant predictor of malignancy for indeterminate thyroid nodules. However, it was most useful in Bethesda category V. Microcalcifications, ill-defined margin and cervical lymphadenopathy were the most suspicious sonographic features of malignancy.

KEYWORDS: Fine Needle Biopsy, Proto-Oncogene Proteins B-raf, Thyroid nodule, Ultrasound.

INTRODUCTION

Thyroid nodules are common. About 50% of the general population have thyroid nodular disease by ultrasonography.[1] This is accompanied by increased incidence of thyroid malignancy, mainly papillary thyroid cancer (PTC).[2] However, only 5-15% of these nodules are malignant,[3] but the risk of tumor necessitates diagnostic workup that includes thyroid stimulating hormone, thyroid ultrasound, and fine-needle aspiration biopsy (FNAB) if indicated followed by diagnostic surgery in suspicious settings.[4]

Neck ultrasound is recommended for all patients with thyroid nodules. Both American Thyroid Association (ATA) and Thyroid Imaging Reporting and Data System (TIRAD) are reproducible to stratify the malignant potential of thyroid nodules by ultrasound.[4] Suspicious sonographic features include hypoechogenicity, irregular margins, calcifications, tall thin shape and cervical lymphadenopathy. Nodules with at least one suspicious

feature carries 50-90% risk of malignancy and require further evaluation.[1] In fact, specificity for malignancy increases upon combination of multiple sonographic features.[5] However, these parameters have variable sensitivities and specificities and cannot be relied on solely without taking clinical context into consideration.[6]

FNAB is the first diagnostic modality of choice to evaluate suspicious thyroid nodules since it is safe, inexpensive and has a good sensitivity and specificity ranging between 65- 98%, and 72-100% respectively.[7] However, around 30% of cytology results are inconclusive which includes, according to the Bethesda System for Reporting Thyroid Cytopathology (TBSRTC), atypia of undetermined significance/ follicular lesion of undetermined significance (AUS/FLUS), follicular neoplasm/suspicious follicular neoplasm (FN/SFN) and suspicious for malignancy (SM).[8,9] Since many of these nodules are benign, high number of unnecessary surgeries is performed with its complications and cost.[3,10] On the other hand, the risk of malignancy cannot be overlooked.[11] Therefore, several preoperative molecular markers have been studied to improve the diagnostic utility of indeterminate thyroid nodules. $BRAF^{V600E}$ mutation is highly specific for PTC and anaplastic thyroid cancer originating from PTC with specificity up to 100%.[12,13] However, the limited sensitivity of BRAF mutation restricted its utility, so studies directed their attention to $BRAF^{V600E}$ mutation in indeterminate thyroid nodules rather than a regular test for all Bethesda categories.

The aim of this study was to investigate the diagnostic value of the $BRAF^{V600E}$ mutation in indeterminate FNAB by comparing BRAF status to histopathology. In addition, some ultrasonographic features were evaluated to predict the risk of malignancy.

MATERIALS AND METHODS

This is a retrospective study which was approved by the Institutional Review Board at Jordan University Hospital. It included 78 thyroid nodules from different patients with indeterminate cytology including Bethesda categories III (AUS/FLUS), IV (FN/SFN) and V (SM) between January 2010 and December 2015. FNAB was read according to TBSRTC which includes six diagnostic categories; I = nondiagnostic, II = benign, III = atypia/follicular lesion of undetermined significance (AUS/FLUS), IV = follicular neoplasm/suspicion for a follicular neoplasm (FN/SFN), V = suspicious for malignancy (SM), and VI = malignant. All patients had previously determined histologic diagnoses. Their preoperative ultrasound records were retrospectively analyzed for the size of the dominant nodule (measured to the largest diameter), margin, and the presence of halo, echogenicity, calcification, vascularity and cervical lymphadenopathy.

Five sections of formalin-fixed paraffin-embedded (FFPE) tissues (5 micron/section) were deparaffinized by xylene and absolute ethanol treatment. Genomic DNA was extracted using QIAamp® DNA FFPE Tissue (Qiagen, Germany). DNA was then qualified /quantified with ND-2000 spectrophotometer (Thermo Fisher Scientific, USA). The PCR primer sequences were sense: 5′- TCATGAAGACCTCACAGTAAAAAT -3′; Anti-sense: 5′- TGGATCCA AGACAAC TGTTCAA -3′. PCR was performed in a 20 µl final volume containing 4µl from 5x FIREPol master mix (Solis BioDyne, Eu), 0.5 µM from each primer, 50 ng genomic DNA template and the volume was adjusted to 20 µl by nuclease free water. PCR conditions consisted of the initial denaturation (95°C for 5 min), followed by 36 cycles of denaturation at 94°C for 30 seconds, annealing at 54°C for 60 seconds, and extension at 72°C for 30 seconds and final extension at 72°C for 7 minutes. PCR products were analyzed by 3% agarose gel electrophoresis. Automated dideoxy sequencing and purification were performed by Macrogen® (Korea) after PCR amplification using the sense antisense primer pair.

The distribution of histopathology and $BRAF^{V600E}$ status variables was performed using Pearson's chi-squared or Fisher-Freeman-Halton exact tests followed by the post-hoc Dunn test. The differences between histopathology and $BRAF^{V600E}$ mutation groups regarding age and nodule size were evaluated by independent samples *t* test or Mann-Whitney *U* test according to type of variables. A "p" value derived from two-tailed tests less than 0.05 was considered statistically significant. All calculations were done using the SPSS (IBM Statistics ver. 23) program.

RESULTS

$BRAF^{V600E}$ mutation analysis was performed for 78 specimens which, according to the TBST classification system, included 38 AUS/FUS, 17 FN/SFN and 23 SM. Molecular analysis showed that 4 specimens out of 78 (5.1%) were $BRAF^{V600E}$ mutation positive as all of them were wild/mutant type, while 74 (94.9%) were wild/wild type. On histopathology, 55/78 (70.5%) were benign (14 follicular adenomas, 25 multinodular goiter, 6 Hürthle cell adenoma, 4 Hashimoto's thyroiditis, 1 Graves' disease, and 5 hyperplastic nodules), whereas 23/78 (29.5%) were malignant (20 PTC, 2 follicular thyroid carcinomas (FTC), and 1 anaplastic thyroid cancer) (**Table 1**).

Table 1: Characteristics of thyroid nodules.

Number of nodules	**78**		
Mean age (years)	**47.5 ± 14.7 (19-79)**		
		N	**(%)**
Bethesda category	AUS/FLUS	38	48.7
	FN/SFN	17	21.8
	SM	23	29.5
BRAF^{V600E} mutation	Yes	4	5.1
	No	74	94.9
Histopathology	Malignant	23	29.5
	Benign	55	70.5

AUS/FLUS, atypia of undetermined significance /follicular lesion of undetermined significance; **FN/SFN**, follicular neoplasm/suspicion for a follicular neoplasm; **SM**, suspicious for malignancy.

The status of BRAF^{V600E} mutation and histopathology for each category is described in **Table 2**. In *AUS/FLUS* 6/38 (15.8%) were malignant. In *FN/SFN* 5/17 (29.4%) were malignant while 12/23 (52.2%) of SM category were tumors revealing that malignancy rate was significantly higher in the SM category than in the AUS / FUS and FN / SFN categories (p=0.009).

Only one case of AUS/FLUS and three nodules of SM group were BRAF^{V600E} mutation positive. They were all malignant and represented PTC. No mutation was detected in FN/SFN group. Although the rate of BRAF^{V600E} mutation was higher in SM category (13%) than in AUS/FLUS (2.6%) and FN/SFN (0%), this result was not statistically significant (p=0.134).

Table 2: BRAF ^{V600E} mutation and histopathology distribution for each FNA category.

Bethesda category	**Total**	**BRAF state**		**P**	**histopathology**		**P**
		wild	**mutant**		**benign**	**malignant**	
	N	**n (%)**	**n (%)**		**n (%)**	**n (%)**	
AUS/FUS	38	37 (97.4)	1 (2.6)	0.134	32 (84.2)	6 (15.8)	**0.009***
FN/SFN	17	17 (100)	0 (0.0)		12 (70.6)	5 (29.4)	
SM	23	20 (87.0)	3 (13.0)		11 (47.8)	12 (52.2)	

AUS/FLUS, atypia of undetermined significance /follicular lesion of undetermined significance; **FN/SFN**, follicular neoplasm/suspicion for a follicular neoplasm; **SM**, suspicious for malignancy.
* p value less than 0.05 is statistically significant.

When the distribution of cancer subtypes in each Bethesda category was evaluated, the rate of follicular carcinoma was significantly higher in the FN/SFN category (n=2, 11.8%) than in other categories (0% for AUS/FUS and SM categories, p=0.016). In addition, the rate of Hürthle cell adenoma was significantly higher in the FN/SFN category (n=4, 23.5%) than in AUS/FLUS (n=2, 5.3%, p=0.049) and SM categories (0%, p=0.022). On the other hand, the rate of PTC was significantly higher in SM category (n=11, 47.8%) than in AUS/FLUS (n=6, 15.8%, p=0.007) and FN/SFN (n=3, 17.6%, p=0.030) categories.

The sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) of BRAF^{V600E} mutation to detect malignancy were 17.4%, 100%, 100%, 100% respectively. When calculated for each Bethesda category, they were 16.7%, 100%, 100%, 86.5% for AUS/FLUS, 0%, 100%, 0%, 70.6% for FN/SFN, and 25%, 100%, 100%, 45% for SM category, respectively.

Besides the status of BRAF^{V600E} mutation, we studied pre-operative ultrasound features that included the size of the dominant nodule, margin, and the presence of halo, echogenicity, calcification, vascularity and cervical lymphadenopathy. The relationship between BRAF^{V600E} mutation and different clinicopathologic features is described in **Table 3**. BRAF^{V600E} mutation was significantly associated with malignancy (p=0.001) and irregular nodule margin on ultrasound (p=0.037) while other parameters in relation to BRAF^{V600E} status were not significant (**Table 3**).

Table 3: The relationship between BRAF ^{V600E} mutation and histopathology, FNAB, and sonographic features.

		BRAF status				**p**
		Wild/Wild		**Wild/Mutant**		
		Count	**(%)**	**Count**	**(%)**	
Histopathology	Benign	55	74.3	0	0.0	**0.001***
	Malignant	19	25.7	4	100.0	
Bethesda	AUS/FLUS	37	50.0	1	25.0	0.113

category	FN/SFN	17	23.0	0	0.0	
	SM	20	27.0	3	75.0	
Calcifications	Microcalcifications	12	27.9	2	100.0	0.100
	Macrocalcifications	2	4.7	0	0.0	
	No calcification	29	67.4	0	0.0	
Echogenicity	Hypoechoic	15	36.6	1	50.0	0.281
	Isoechoic	19	46.3	0	0.0	
	Hyperechoic	3	7.3	0	0.0	
	Heterogenous	4	9.3	1	50.0	
Margin	Well-defined	40	93.0	1	50.0	**0.037****
	Ill-defined	3	7.0	1	50.0	
Halo	Present	14	31.8	0	0.0	0.339
	Absent	30	68.2	2	100.0	
Cervical LN	No	37	86.0	2	100.0	0.570
	Yes	6	14.0	0	0.0	
Vascularity†	Perilesional	4	9.5	0	0.0	0.166
	Intralesional	34	81.0	1	50.0	
	No blood flow	4	9.5	1	50.0	
		Mean ± SD		Mean ± SD		
Nodule size on ultrasound (cm)		3.21±2.18 (n=48)		4.8±3.81 (n=2)		0.953
Age (year)		46.8±14.8 (n=74)		57.7±11.6 (n=4)		0.153

AUS/FLUS, atypia of undetermined significance /follicular lesion of undetermined significance; **FN/SFN**, follicular neoplasm/suspicion for a follicular neoplasm; **SM**, suspicious for malignancy; **LN**, lymph node.
†If a nodule demonstrated increase in both perilesional and intralesional blood flow, it was included in intralesional category.
,* p value less than 0.05 is statistically significant.

The association between histopathology and clinicosonographic features is summarized in **Table 4**. Malignancy rate was higher in females than males (p=0.014). Neck ultrasound showing thyroid microcalcifications and cervical lymphadenopathy was significantly associated with malignancy (p=0.026 and p=0.013, respectively). On the other hand, neither age nor nodule size, echogenicity, margin, halo, or vascularity were in significant relationship with malignancy (**Table 4**).

Table 4: The relationship between histopathology and clinicosonographic features.

		Histopathology				**p**
		Benign		**Malignant**		
		Count	**(%)**	**Count**	**(%)**	
Calcifications	Microcalcifications	6	42.9	8	57.1	**0.026***
	Macrocalcifications	1	50.9	1	50.0	
	No calcification	25	86.2	4	13.8	
Echogenicity	Hypoechoic	9	56.3	7	43.8	0.289
	Isoechoic	15	78.9	4	21.1	
	Hyperechoic	3	100.0	0	0.0	
	Heterogenous	4	80.0	1	20.0	
Margin	Well-defined	30	73.2	11	26.8	0.329
	Ill-defined	2	50.0	2	50.0	
Halo	Present	12	85.7	2	14.3	0.164
	Absent	21	65.6	11	34.4	
Cervical LN	No	31	79.5	8	20.5	**0.017****
	Yes	2	33.3	4	66.7	
Vascularity†	Perilesional	4	100.0	0	0.0	0.568
	Intralesional	24	68.5	11	31.5	
	No blood flow	3	60.0	2	40.0	
Gender	Male	7	70.0	3	30.0	0.970
	Female	48	70.6	20	29.4	

		Mean ±SD	Mean ±SD	
Nodule size on ultrasound, mean ±SD (cm)		3.17 ±2.26 (n=34)	3.24 ±2.21 (n=14)	0.918
Age		46.04 ±14.70 (n=55)	51.1 ±14.4 (n=23)	0.164
AUS/FLUS, atypia of undetermined significance /follicular lesion of undetermined significance; **FN/SFN**, follicular neoplasm/suspicion for a follicular neoplasm; **LN**, lymph node. † If a nodule demonstrated increase in both perilesional and intralesional blood flow, it was included in intralesional category. *****, ****** p value less than 0.05 is statistically significant.				

Sensitivity, specificity, NPV, and PPV for each ultrasound feature (calcifications, halo, margin, echogenicity, cervical lymph node and vascularity) to detect malignancy are shown in **Table 5**.

Table 5: The sensitivity, specificity, PPV and NPV of each ultrasound feature.

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	Risk category for diagnostic measures
Margin	15.4 (2/13)	93.8 (30/32)	50.0 (2/4)	73.2 (30/41)	71.1 (32/45)	Ill-defined
Halo	15.4 (2/13)	63.6 (21/32)	14.3 (2/14)	65.6 (21/32)	50.0 (23/46)	Present
Cervical LN	33.3 (4/12)	93.9 (31/33)	66.7 (4//6)	80.5 (31/39)	79.5 (37/47)	Yes
Calcifications	61.5 (8/13)	74.2 (26/32)	57.1 (8/14)	83.9 (26/31)	53.3 (24/45)	Microcalcifications
Echogenicity	58.3 (7/12)	70.9 (22/31)	43.8 (7/16)	81.5 (22/27)	67.4 (29/43)	Hypoechogenicity
Vascularity	84.6 (11/13)	9.7 (3/31)	28.2 (11/39)	60.0 (3/5)	31.8 (14/44)	Intralesional
PPV, positive predictive value; **NPV**, negative predictive value; **LN**, lymph node.						

DISCUSSION

Indeterminate thyroid nodules are usually managed with repeat FNAB, follow-up ultrasound or surgery.[14] Although many of these nodules are benign, the risk of malignancy is significant in the SM cytology reaching 50-75% while this rate is 20-30% in the FN/SFN and 5-10% in the AUS/FLUS categories.[3,15] According to our histopathology results, the rate of malignancy among the nodules with indeterminate cytology was comparable to the literature.

To enhance the sensitivity and accuracy of FNAB, multiple genetic mutations were studied to further triage thyroid nodules preoperatively.[3,9] $BRAF^{V600E}$ mutation is the most common mutation in thyroid cancer[13] with high specificity for PTC reaching 100%.[16,17]

In this study, four cases out of 78 were $BRAF^{V600E}$ mutation positive (5.1%), three of them were in the SM group and one in the AUS/FLUS group. All of them were malignant by histopathology. In fact, the prevalence of $BRAF^{V600E}$ mutation according to a commentary by Pusztaszeri et al was 15-20% in the SM category and 4.6% in AUS/FLUS combined with FN/SFN.

The low prevalence of $BRAF^{V600E}$ mutation in AUS/FLUS and FN/SFN can be explained by the fact that wild-type BRAF nodules are usually follicular cell-derived cancers such as follicular variant PTC and FTC or benign follicular growth.[18-20] So $BRAF^{V600E}$ negative indeterminate nodules cannot exclude malignancy. In our study, 5/37 BRAF negative nodules among AUS/FLUS that were malignant all turned to be PTC, and none of FN/SFN group harbored $BRAF^{V600E}$ mutation instead it included FTC and Hürthle cell adenoma significantly.

On the other hand, some reports studied wild type $BRAF^{V600E}$ nodules for RAS mutation among indeterminate thyroid cytology[3,18,21-23] as RAS mutation is the most common mutation in indeterminate thyroid nodules.[24]

Yoon et al reported low prevalence of RAS mutation in $BRAF^{V600E}$ negative AUS/FLUS thyroid nodules with low specificity as 31 out of 198 FNABs were RAS mutation positive of which 22.6% were malignant by histopathology.[18] On the other hand, the probability of cancer in indeterminate nodules which harbored exclusively RAS mutation was higher in other studies taking into consideration that some of them included FN with or without SM cytology besides AUS/FLUS.[3,21] In addition, according to risk assessment and study methodology, only a portion of the patients underwent surgery which may mask benign conditions as RAS mutations can be found in a wide range of thyroid growth including follicular cancer and anaplastic thyroid cancer, follicular adenoma and hyperplastic nodules. On the contrary, some argue that follicular adenoma that are RAS mutated are more likely to transform into follicular carcinoma justifying its removal.[25]

The low specificity of RAS mutation decreases its utility and cannot exclude surgery. However, combined BRAF and RAS mutations increase the diagnostic value for 60-70% of indeterminate thyroid nodules.[21]

Although testing for $BRAF^{V600E}$ mutation in indeterminate thyroid nodules allows the decision for initial total thyroidectomy because of its high specificity, its low sensitivity is the major drawback. A meta-analysis by Su et al reported $BRAF^{V600E}$ sensitivity in

indeterminate thyroid nodules to be 44.2% which is relatively low.[23]

In our study, the overall sensitivity of $BRAF^{V600E}$ mutation was 17.4%. Other studies that reported higher sensitivities did not include histopathology results for all of their cases, their FNAs were all ultrasound-guided and they adopted different mutation analysis methods besides their different sample size which may explain the difference in $BRAF^{V600E}$ mutation rate between the studies.[24,26]

When we studied the role of $BRAF^{V600E}$ mutation in Bethesda categories, we found that $BRAF^{V600E}$ profile was more useful in the SM group as 13% of them were $BRAF^{V600E}$ mutation positive with improved sensitivity in comparison to other indeterminate results (25% for SM versus 16.7% for AUS/FLUS) as this result is concordant with other studies.[9,12,23,26-29] However, ATA recommends management of SM cytology similar to malignant one, finding the routine preoperative testing for $BRAF^{V600E}$ mutation in SM category of limited value and not cost-effective.[14]

Neck ultrasound is essential in evaluating thyroid nodules.[14] We found that female gender and nodules with microcalcifications or cervical lymphadenopathy were significantly associated with malignancy (p=0.014, p=0.026 and p=0.017, respectively).

Many studies reported that neither age nor gender was correlated with thyroid cancer in indeterminate nodules.[28-30] On the other hand, Rago et al found male gender riskier to develop thyroid cancer.[31] Additionally, males remain to have worse prognosis at presentation.[32]

Microcalcifications can be found in both benign and malignant thyroid nodules.[33,34] However, it is one of the common sonographic features of PTC and it is highly specific for thyroid cancer.[33,35] In the present study, microcalcifications were significantly predictive of malignancy with a sensitivity and specificity of 61.5% and 74.2% respectively, a result concordant with other publications (p=0.026).[33,36-38]

Approximately half PTC patients have lymph node metastasis at diagnosis and about 20% of patients present only with cervical lymphadenopathy.[39] We found that cervical lymphadenopathy was significantly associated with malignancy (p= 0.017) with high specificity 93.9%, but poor sensitivity 33.3%. There are several sonographic signs of suspicious lymph nodes such as microcalcifications, cystic aspect, peripheral vascularity, and hyperechogenicity. Their specificity (34-100%) is much higher than their sensitivity (5-87%).[40] Obviously, normal cervical lymph node cannot rule out thyroid cancer especially with the early detection of thyroid tumors.

We did not find a correlation between age, nodule size, echogenicity, margin, halo, and vascularity with malignancy. In this study, sonographic nodule size measured to the largest diameter was not correlated with malignancy. Rossi et al did not find nodule size helpful to discriminate the malignant potential of thyroid nodules. However, not all of their patients underwent surgery and more than half of them had subcentimetric nodules.[41] On the other hand, He et al reported malignant nodules to be significantly smaller than benign ones among indeterminate cytology.[42] Wharry et al, who studied thyroid nodules larger than 4 cm, found nodule size and other sonographic features not predictive of malignancy.[43] It is notable that our patients had larger nodules. It may be explained in part by seeking medical attention late after nodules become visible or palpable.

Hypoechogenicity is a suspicious sign for thyroid cancer in indeterminate nodules.[33, 38] But about half benign nodules are hypoechoic compared to the surrounding tissues which decreases its specificity that was 70.9% in our study.[14]

In the present study ill-defined margin was not correlated with malignancy as supported by other reports.[33,38] Nevertheless, it was the only sonographic feature significantly associated with $BRAF^{V600E}$ mutation and showed a specificity of 93.8% (p=0.037). Since $BRAF^{V600E}$ mutation strongly predicted malignancy in our study (p=0.001), it is reasonable to consider ill-defined margin a red sign.[36,37] We consider that margin regularity is disputable, operator dependent, and differs according to ultrasound resolution.[39] Thus, it is recommended to use speculated/microlobulated category rather than ill-defined margin which is a non-specific term.[39]

A study on the AUS/FLUS nodules found peripheral vascularity a significant indicator of malignancy, and another study on impalpable thyroid nodules indicated central vascularization predictive of malignancy.[36] In the study by Su et al, doppler flow was sensitive to detect thyroid cancer while a systematic review reported intranodular vascularity the most specific sign in indeterminate nodules.[44,45,46]

In our study, if a lesion demonstrated increase in both intralesional and perilesional blood flow, it was included in central vascularity category. Accordingly, we did not find vascularity to be correlated with histopathology or $BRAF^{V600E}$ mutation. In addition, vascularity was a sensitive rather than a specific marker (84.6% vs 9.7%). In the study by Moon et al, malignant nodules had significantly no blood flow which was explained by the frequent fibrosis found in PTC while central vascularization was more detected in benign nodules ($p<0.0001$) attributed possibly to hyperplastic nodules or granulation tissue of cystic lesions.[47] But this study is limited by the dependance on cytology results alone in the vast majority of cases to define malignancy. On the

other hand, internal blood flow in malignant nodules is distinct from benign ones being chaotic and irregular.[45]

There are some limitations to our study. Firstly, it is retrospective and reflects a small sample size. Secondly, neck ultrasound was performed by different operators which may increase inter-observer variability. In addition, not all FNABs were ultrasound-guided. On the other hand, all our specimens had confirmed histopathology diagnoses.

CONCLUSION

$BRAF^{V600E}$ mutation is a specific marker for PTC. However, its application in indeterminate thyroid nodules was restricted by its low rate and sensitivity. If to be used, we recommend its role for triaging the SM category only. On the other hand, FNAB was significantly predictive of malignancy among indeterminate cytology.

Neck ultrasound was of low sensitivity. But the combination of multiple suspicious sonographic features is predictive of thyroid cancer of which microcalcifications and cervical lymphadenopathy were significantly associated with thyroid cancer.

The most specific ultrasound features for thyroid cancer were cervical lymphadenopathy and ill-defined margin. The latter was more likely to harbor $BRAF^{V600E}$ mutation.

In conclusion, the clinical context, ultrasound features and FNAB are the corner stones to evaluate indeterminate thyroid nodules. $BRAF^{V600E}$ mutation analysis could be an adjuvant for the SM category only. Alternatively, we recommend fresh frozen sections to determine the extent of initial surgery when indicated.

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