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The Diagnostic Utility of CD117 (c-KIT) as Adjunctive Preoperative Marker in Solitary Thyroid Nodule Management

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Abstract **Background**: Unfortunately, about 30% of FNAC findings reveal an intermediate or suspicious follicular proliferation followed by thyroid surgery to establish the diagnosis. Therefore, reliable preoperative molecular markers are immensely needed to distinguish benign from malignant thyroid nodules to avoid unnecessary aggressive surgical interference in benign lesions or reoperation in malignant lesions. **Aim:** To investigate the utility of c-KIT marker in combination with the immunopanel of CD56, Galectin-3 and HBME-1, to distinguish between benign and malignant thyroid lesions on cell blocks in order to reduce unnecessary thyroid surgery. **Patients and methods:** This study was conducted on 113 patients with solitary thyroid nodule, in Pathology Department and Surgical Oncology Unit at General Surgery Department, Tanta University Hospital from June 2015 to May 2017. After histopathological examination of FNAC samples, Cell blocks were prepared for only cases diagnosed as follicular neoplasm/suspicious for follicular neoplasm for further immunohistochemistry of immunopanel including c-KIT, CD56, Galectin -3 and HBME-1, then the selected cases underwent hemithyroidectomy to establish a histological tissue diagnosis. **Results:** Thirty six out of 113 patients (31.8%) were diagnosed as follicular neoplasm/suspicious for follicular neoplasm on FNAC. The combined panel of CD56, Galectin-3 and HBME-1 results on cell blocks showed sensitivity 80.0%, specificity 100%, PPV 100.0%, NPV 92.86% and the accuracy was 94.44%. However after the addition of c-KIT to the immunopanel the diagnostic sensitivity, specificity, PPV, NPV and total accuracy improved to 100% for all. **Conclusion:** The diagnostic preoperative accuracy of the combined CD56, Galectin-3 and HBME-1 panel in solitary thyroid nodules with suspicious cytology could be extremely improved with the addition of c-KIT as a supplementary preoperative immunostain in order to avoid over or under treatment.

Keywords: *FNAC, solitary thyroid nodules, c-KIT, CD56, Galectin-3, HEMB-1, thyroidectomy*

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1. Introduction

Solitary thyroid nodules are common finding, only 5% of nodules are malignant and the vast majority is nonneoplastic lesions or benign neoplasms [1,2]. In order to avoid over treatment by total thyroidectomy (TT) for benign lesions or under treatment by hemithyroidectomy (HT) for a malignant neoplasm, it is essential to distinguish between benign and malignant lesions [2,3,4].

Till now fine needle aspiration cytology (FNAC) is considered the critical initial diagnostic and cost-effective procedure but it has its disadvantages [5,6]. Some FNAC findings fall into the intermediate or suspicious group not obviously showing frank benign or malignant cells diagnosed as follicular neoplasm/suspicious for follicular neoplasm [category 4] according to the Bethesda system, however these nodules could be malignant in 15-30% of cases [7,8,9].

Once a thyroid tumor with this intermediate cytology is identified by FNAC, the current accepted approach is HT which is the minimum surgical procedure should be performed to evaluate the nature of the nodule. This approach is useful to maintain the thyroid functions of the patient without hormone supplementation, lower the risk of the injury of recurrent laryngeal nerve (RLN) and hypoparathyroidism [4,9]. On the other hand, it also has some disadvantages, such as the need to completion of thyroidectomy if the pathology report confirms a malignancy, In addition to the obligation to perform cautious follow up by ultrasound and reoperation if another suspicious nodule appears in this lobe, which could be predicted in 50% of patients in 10 years [2,3,10].

Trying to find a specific diagnostic marker of thyroid malignancy in liquid based cytology with high accuracy, a

growing number of immunomarkers, had been tested as single or in combination as panels showing considerable variability of results and among them [6,11,12,13,14].

Several studies examined CD56, Galectin 3 and mesothelioma antibody (HBME-1: Mesothelioma ab-1) expressions as diagnostic markers in differentiation between thyroid cancer and benign lesions separately or in combination but variable results were obtained [15,16,17]. CD56 is Neural cell adhesion molecule (NCAM) highly expressed in normal thyroid follicles and benign thyroid follicular lesions but lost in papillary thyroid carcinoma (PTC) and other malignant thyroid lesions as reported in many studies [16,18,19].

Galectin-3 is a beta-galactoside binding polypeptide, playing a significant role in many biological processes as neoplastic transformation. Several evidences showed that the expression of Galectin-3 was elevated in thyroid carcinoma of follicular cell origin [14,15]. HBME-1 was firstly known as a specific marker of mesothelial cells. It was observed that HBME-1 up regulated in thyroid malignant follicular lesions [20,21,22].

Although the results of these markers are were generally encouraging and promising but some studies demonstrated inconclusive or conflicting results making difficulty to decide between watchful waiting and diagnostic thyroid surgery in patients with suspicious nodules. Thus the need of other ancillary diagnostic markers is highly recommended [6,7].

CD117 (c-KIT) is a type III receptor tyrosine kinase. Several tumors especially gastrointestinal stromal tumors (GISTs) showed aberrations in CD117 expression and signaling, including overexpression and reduced/absent expression [23]. Recent studies investigated c-KIT expression in thyroid gland and in thyroid malignancies, trying to find its precise role in differentiation and growth control of thyroid epithelium. This role may be lost after malignant transformation [24,25]. Tomei et al, [26] studied the transcription of c-KIT in FNAC of PTC and benign thyroid lesions used quantitative polymerase chain reaction PCR. They suggested the loss of c-KIT expression may be useful as surrogate marker in the diagnosis of PTC in FNAC.

The aim of this study was to investigate the utility of c-KIT marker in combination with the immunopanel of CD56, Galectin-3 and HBME-1, to distinguish between benign and malignant thyroid lesions on cell blocks in order to reduce unnecessary thyroid surgery.

2. Patients and Methods

This a prospective study was conducted on 113 patients with simple solitary thyroid nodule in Pathology Department and Surgical Oncology Unit at General Surgery Department , Tanta University Hospital from June 2015 to May 2017. The patients included in the study aged ≥18 years old, had small nodule less than 5 cm in maximum diameter, unilateral lesion, no enlarged lymph nodes in the neck, no previous neck surgery or irradiation and euthyroid state.

Every patient was subjected to preoperative FNAC, After histopathological evaluation, Cell blocks were prepared for only cases diagnosed as follicular neoplasm/suspicious for follicular neoplasm for further immunohistochemistry of immunopanel including c-KIT, CD56, Galectin -3 and HBME-1. The other well established diagnosed benign and malignant cases were discarded from the study. The selected cases underwent thyroid lobectomy to establish a histological tissue diagnosis.

A written informed consent had been obtained from every patient included in the study after explanation of the nature of the procedure and possible complications. The study was approved by Tanta Faculty of Medicine ethical committee.

3. The Histopathological and Immunohistochemical Procedure

FNAC was performed using 5ml disposable syringe under aseptic condition. Smears were made, immediately fixed in 95% ethyl alcohol. For cell block analysis, the remaining material in the aspirating syringe was pushed in the test tube and centrifuged for 10 minutes. The formed cell button was allowed to fix in 10% formalin overnight. Then the cell button was processed as routine biopsy specimen and stained with hematoxylin and eosin staining.

Immunohistochemical staining was performed on 4-5 µm thick, formalin fixed, paraffin embedded sections. In brief, after dewaxing and hydration, slides were incubated with the following mouse monoclonal antibodies c-KIT (clone C-19; 1: 200; Santa Cruz Biotechnology, Dallas, TX, USA), CD56 (clone 123C3; 1: 100; Dako, Glostrup, Denmark), Galectin-3 (clone 9C4; 1: 600; Novocastra, Newcastle, U.K.) and HBME-1 (clone HBME-1; 1: 100; Dako Cytomation, Carpinteria, CA, U.S.A.). After rinsing with phosphate buffered saline, the sections were incubated with secondary antibody then counterstained with hematoxylin.

The immunostains were evaluated by two independent reviewers and the slides were examined with a light microscope at a final magnification of ×400. Galectin-3 displayed cytoplasmic staining, c-KIT, HBME1 and CD56 staining showed membranous positivity. Positive controls were represented by GIST cells for c-KIT, mesothelioma cells for HBME-1, histiocytes and macrophages for Galectin-3 and CD56 positivity. The positivity was assessed, for each case, For all studied markers, Immunoreactivities staining considered positive if >10% of the epithelial follicular cells were stained and graded as 1^+, 2^+ or 3^+ according to the percentage of stained cells (respectively, 10-25%, 26-50% and 51-100%) [16,25].

Cases positive for CD56 expression and negative for both HBME1 and Galectin-3 were considered most probably benign lesions while cases negative for CD56 and positive for Galectin-3 and /or HBME1 were considered most probably malignant lesions according to Nechifor-Boila et al. [15] and Dunđerović et al. [17]. After addition of c-KIT immunostain, the combined results were compared with the previous panels and the postoperative histopathological results.

4. Statistical Analysis of the Data

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. Comparisons

between groups for categorical variables were assessed using Chi-square test (Fisher). Significance of the obtained results was judged at the 5% level.

5. Results

This study was conducted on 113 patients with solitary thyroid nodule, after histopathological examination of FNAC samples as mentioned in Table 1, 36 cases (31.8%) were diagnosed as follicular neoplasm/suspicious for follicular neoplasm. Their age ranged from 18 to 65 years, with a median age of 44.4. Sex distribution of the patients was 23 females (63.9%) and 13 males (36.1%).

Table 1. Distribution of 113 Cases according To FNAC results

Cytological Diagnosis	NO.	%
Colloid goiter / Adenomatoid nodule	55	48.8
Hashimoto thyroiditis	7	6.1
Granulomatous/subacute thyroiditis	1	0.9
Follicular neoplasm/suspicious for follicular neoplasm (6 cases of Hurthle cell type)	36	31.8
Papillary thyroid carcinoma	12	10.6
Anaplastic carcinoma	1	0.9
Large B cell lymphoma	1	0.9
Total	113	100

Cell blocks were prepared from all 36 Follicular neoplasms/suspicious for follicular neoplasms and subjected for immunopanel made up of all the four markers CKIT, CD56, Galectin-3 and HBME-1, as well as for any different

There were 28 cases (77.8%) diagnosed most probably as benign lesion had an immunopanel of CD56+/Galectin3-ve and HBME1 –ve, out of which 2 cases had different diagnosis on postoperative histopathological examination (Table 2, Table 3) (Figure 1 - Figure 3). The combined immunopanel of CD56, Galectin-3 and HBME-1 results showed that sensitivity was 80.0%, specificity was 100%, positive predictive value (PPV) was 100.0%, negative predictive value (NPV) was 92.86% and total accuracy was 94.44% (Table 4).

On the other hand, after the addition of c-KIT marker to the panel (CD56+/– and c-KIT +/Galectin3– and HBME1 –) 26 cases were diagnosed most probably benign which were the same diagnosis postoperatively (16 follicular adenoma, 4 hurthle cell adenoma and 6 colloid nodular goiter) (Table 2, Table 3) (Figure 1 - Figure 3).

This made the sensitivity, specificity, PPV and total accuracy for this immunopanel 100% for all (Table 4).

Table 2. Distribution of 36 studied cases according to different parameters

	No. (%)
Age (years)	44.6 ± 14.4
<45	15(41.4%)
≥45	21(58.3%)
Sex	
Male	13(36.1%)
Female	23(63.9%)
CD56+/Galectin3- and HBME1 –	28(77.8%)
CD56-/Galectin3 + and or HBME1 +	8(22.2%)
CD56+/- and CKIT +/Galectin3- and HBME1 –	26(72.2%)
Postoperative diagnosis	
Benign	26(72.2%)
Malignant	10(27.8%)

Table 3. Comparison between of Cell blocks immunostaining results of studied 36 cases with Postoperative Histopathological Diagnosis in studied

Cell blocks immunostaining	No.	Postoperative diagnosis	No.	%
Benign		**Benign**	**26**	**72.2**
CD56+/Galectin3- and HBME1 –	*28*	*Follicular adenoma*	*16*	*61.5*
CD56+/- and CKIT +/Galectin3- and HBME1 –	*26*	*Hurthle cell adenoma*	*4*	*15.4*
		Colloid nodular goiter	*6*	*23.1*
Malignant		**Malignant**	**10**	**27.8**
CD56-/Galectin3 + and or HBME1 +	*8*	*Follicular variant papillary thyroid carcinoma*	*5*	*50*
CD56+/- and CKIT -/ Galectin3 + and or HBME1 +	*10*	*Follicular carcinoma*	*3*	*30*
		Hurthle cell carcinoma	*2*	*20*
Total		**36**		**100**

Table 4. Diagnostic value of markers combinations in preoperative discrimination of malignant from benign thyroid lesions:

	Postoperative		p	Sensitivity	Specificity	PPV	NPV	Accuracy
	Benign (n= 26)	Malignant (n= 10)						
Combined CD56,Galectin3andHBME1								
Benign	26(100%)	2(20%)	<0.001	80.0	100.0	100.0	92.86	94.44
Malignant	0(0%)	8(80%)						
Combined CKIT, CD56,Galectin3andHBME1								
Benign	28(100%)	0(0%)	<0.001	100.0	100.0	100.0	100.0	100.0
Malignant	0(0%)	8(100%)						

Qualitative data were described using number and percent and was compared using Fisher Exact test
*: Statistically significant at $p \leq 0.05$.

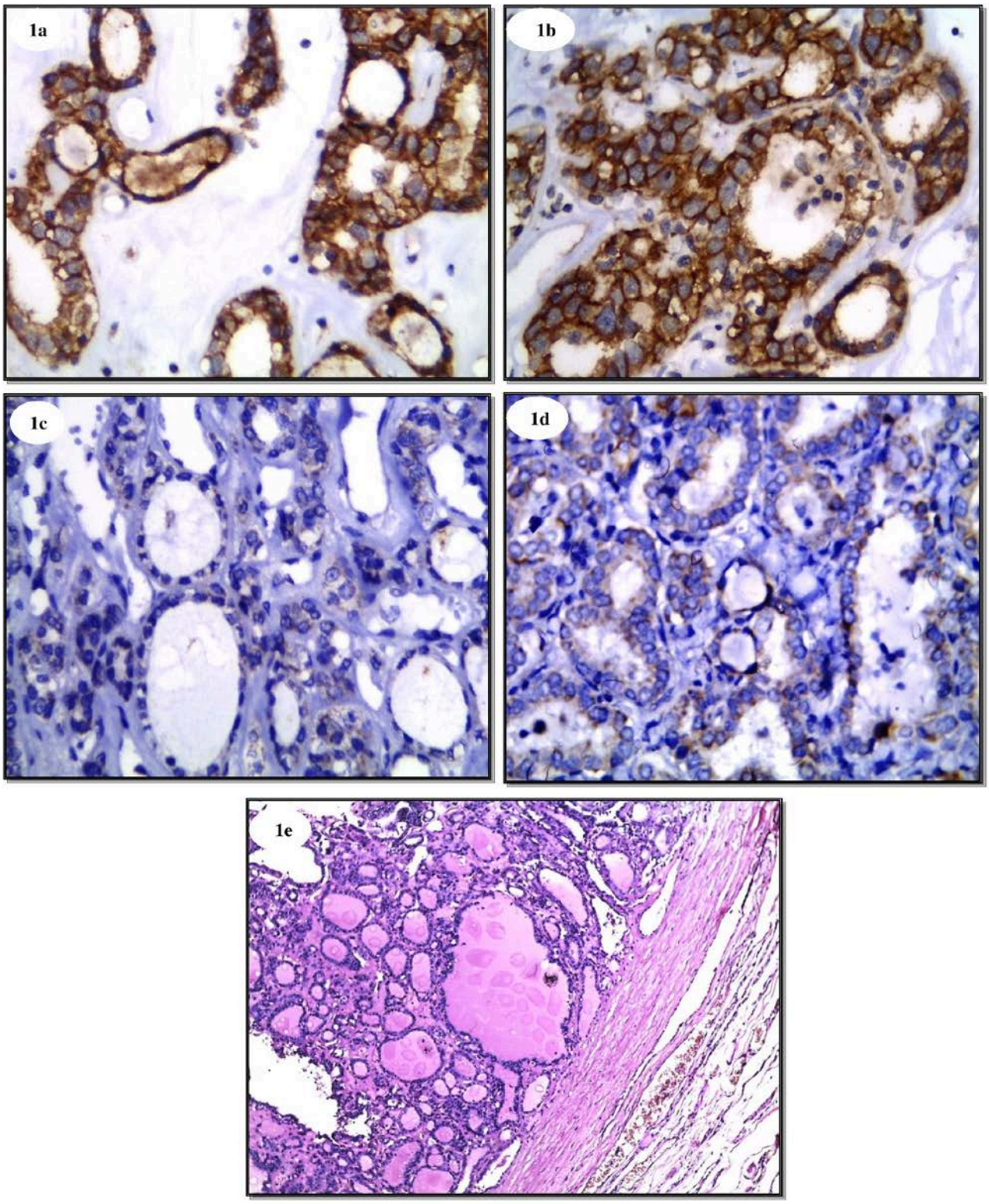

Figure 1. Immunopanel on a case cell block **a**, Positive c-KIT membranous expression (immunohistochemistry, x400), **b,** Positive CD56 membranous expression (CD56 immunohistochemistry, x400), **c,** Negative Galectin-3 expression (Galectin-3 immunohistochemistry x400), **d,** Negative HBME-1 expression (HBME-1 immunohistochemistry x400), **e,** Postoperative histopathological examination was diagnosed as follicular adenoma (H&E x100)

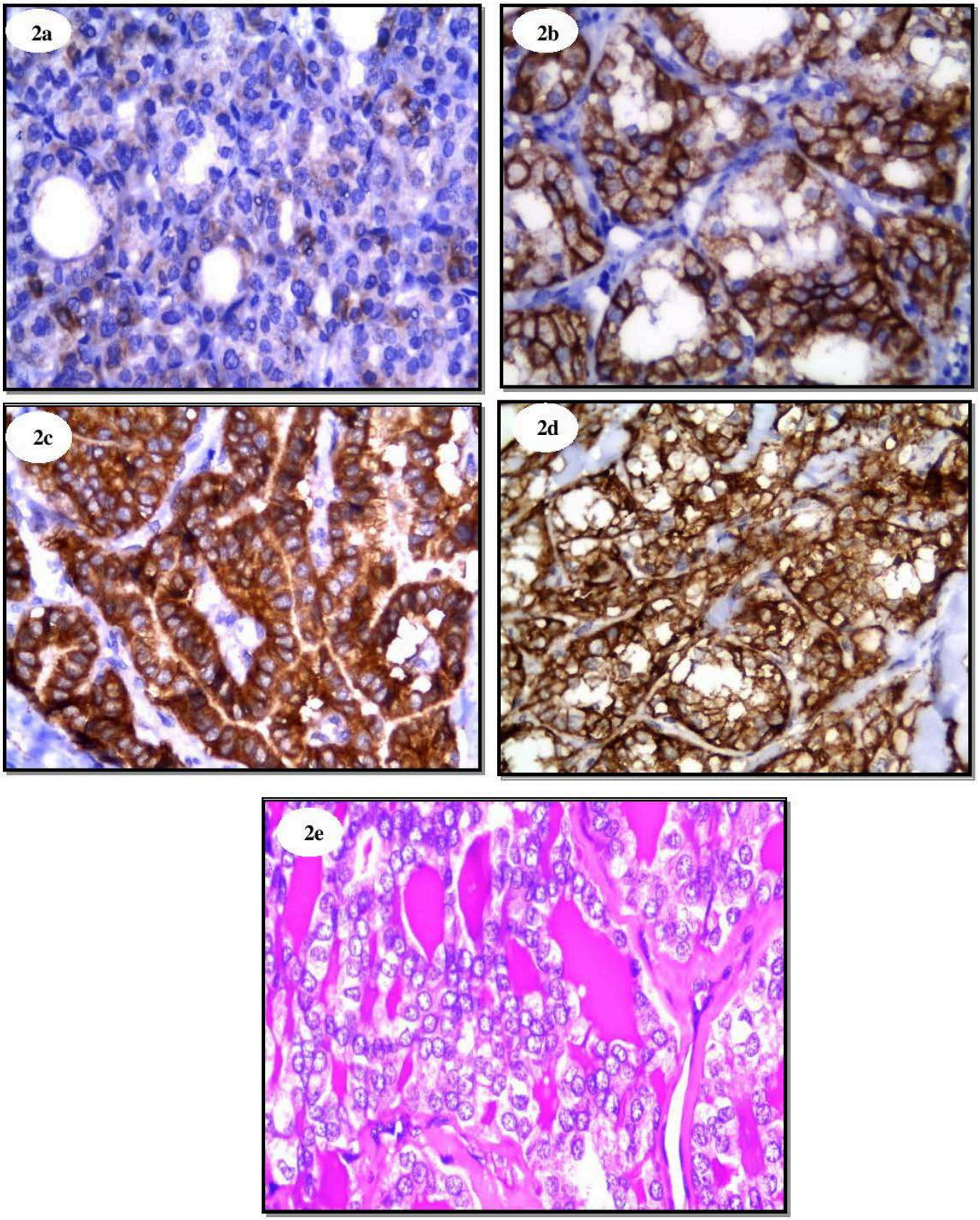

Figure 2. Immunopanel on a case cell block **a**, Negative c-KIT expression (immunohistochemistry, x400), **b,** Positive CD56 membranous expression (CD56 immunohistochemistry, x400), **c,** positive Galectin-3 cytoplasmic expression (Galectin-3 immunohistochemistry x400), **d,** positive HBME-1 membranous expression (HBME-1 immunohistochemistry x400), **e,** Postoperative histopathological examination was diagnosed as follicular variant papillary thyroid carcinoma (H&E x400)

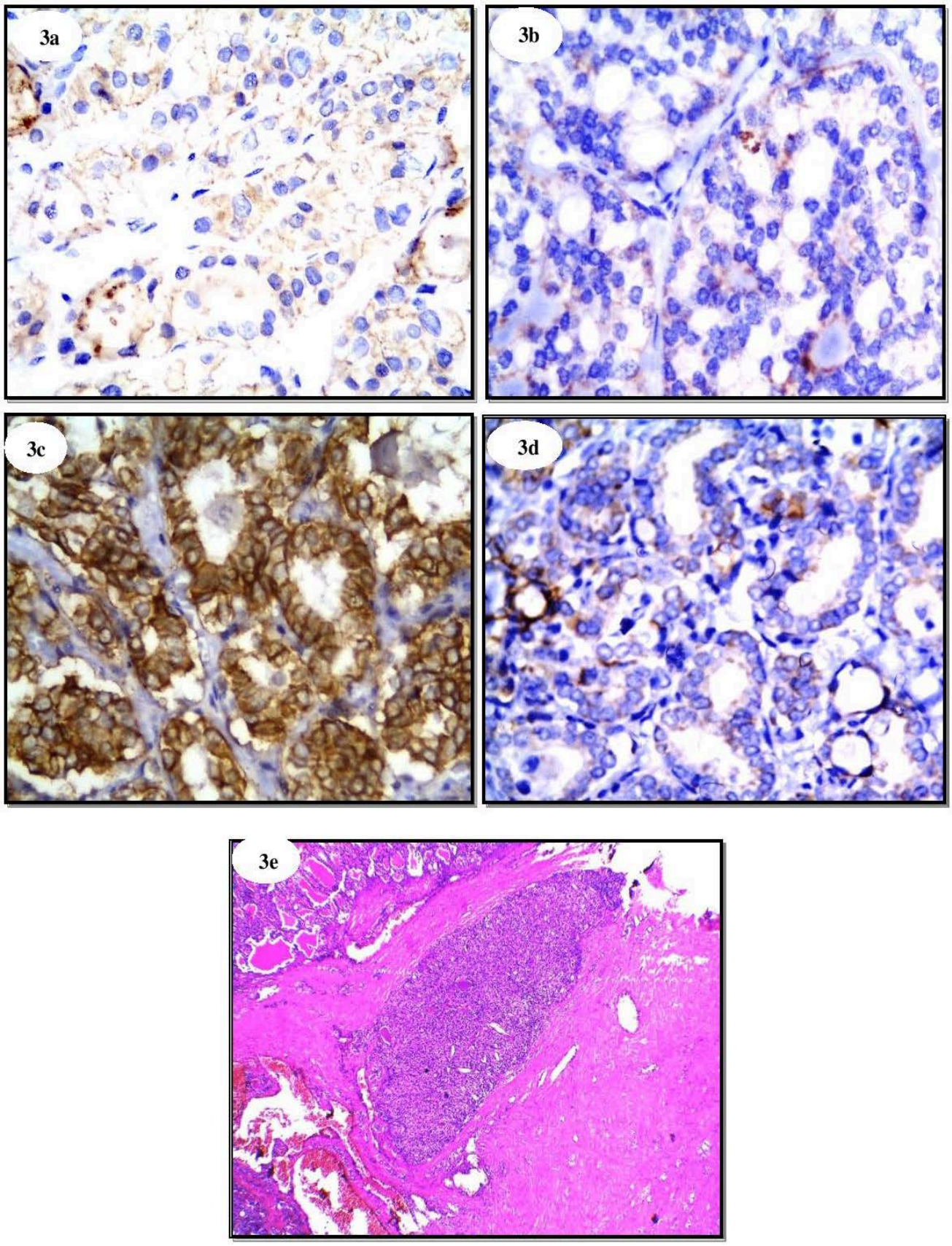

Figure 3. Immunopanel on a case cell block a, negative c-KIT expression (immunohistochemistry, x400), b, negative CD56 membranous expression (CD56 immunohistochemistry, x400), c, positive Galectin-3 cytoplasmic expression (Galectin-3 immunohistochemistry x400), d, negative HBME-1 membranous expression (HBNE-1 immunohistochemistry x400), e, Postoperative histopathological examination was diagnosed as follicular carcinoma showing capsular invasion (H&E x100)

6. Discussion

Unfortunately, about 30% of FNAC findings reveal an intermediate or suspicious follicular proliferation followed by thyroid surgery to establish the diagnosis [6,7]. Therefore, reliable preoperative molecular markers are immensely needed to distinguish benign from malignant thyroid nodules to avoid unnecessary aggressive surgical interference in benign lesions or reoperation in malignant lesions [2].

In the current study, four markers (c-KIT, CD56, Galectin-3 and HBME-1), in various combinations were evaluated in a series of benign and malignant thyroid lesions, aiming to determine their preoperative diagnostic value according to sensitivity, specificity, PPV, NPV and accuracy.

According to this study, the combined immunopanel of CD56, Galectin-3 and HBME-1 results showed sensitivity 80.0%, specificity 100%, PPV 100.0%, NPV 92.86% and the accuracy was 94.44%.

These results were quite in accordance with the results of other studies tested the previous markers individually and in combination with other markers as preoperative diagnostic markers. Barroeta et al,[20] , Scognamiglio et al. [21] and Nakamura et al.[22] and reported nearly similar results of HBME1, Gal-3, CK19 markers co-expression sensitivities (83 %, 54 %, and 87 %) as well as specificities of 100 %, 89 % and 100 %, respectively. According to Dunđerović et al. [17], the application of a forth marker CD56 to the previous immunopanel not only did not improve the sensitivity, but it lowered the specificity.

In addition, Dunđerović et al. [17] reported that combined two, three or four co-expressed markers did not reach specificity of 100 % for malignancy, however values increased compared to single marker expression. Nechifor-Boilă et al. [15] found that panels consisting of CD56 and/or CK19/Gal-3, and CD56 and/or HBME-1 had the highest sensitivities (90.9 %) and NPV (87.5 and 83.3, respectively). They stated that CD56 and/or HMBE-1 immunopanel was of the highest sensitivity and specificity (100 %, 90 % respectively) used in distinguishing benign thyroid lesions from malignant thyroid lesions especially follicular variant papillary thyroid carcinoma. On the other hand the reported sensitivities of the previous immunopanel in other studies of Park et al. [18] and Mi et al. [19] were 93,8 % and 88.1% respectively.

This marked diversity of diagnostic value of investigated markers could be due to variable reasons. Firstly, cut off values varied from > 0 to 25 % of positive cells for different marker expression. Secondly, some study designs included only papillary thyroid carcinomas in the malignant group. Thirdly, there is still controversial interpretation of marker staining for example some authors considered HMBE-1 positivity for membranous and/or cytoplasmic staining; on contrary to this study the only membranous HMBE-1 staining considered positive. Lastly, the negativity does not mean that the cytopathology report should be considered 100% benign because some of the malignant neoplasms showed a false negative Galectin-3 reactivity [14,15,16,17].

As a result of this entire dilemma, it is an obligation to continue the quest of searching more reliable preoperative markers. We tried to evaluate c-KIT expression as a forth marker in the panel, we found that c-KIT analysis improves the diagnostic sensitivity, specificity, PPV and total accuracy for this immunopanel to 100% for all.

The results of our study were consistent with results of Pusztaszeri el al. [25] who suggested that c-KIT may be useful as an ancillary marker for thyroid carcinoma in FNAC. They found that c-KIT expression was mostly absent in tumor cells of thyroid carcinoma especially papillary thyroid carcinoma in contrast to benign lesions ($P < .0001$) with highest sensitivity and specificity (100%) in thyroid carcinoma. This is higher than the sensitivity and specificity of other commonly immunochemical markers like HBME-1, galectin-3, CD56 and CK-19 used for thyroid carcinomas. Besides other previous histologic studies reported decreased CD117 expression in papillary thyroid carcinoma compared with benign thyroid lesions by immunohistochemistry, Northern blot analysis, Western blot analysis, and qPCR [23,24,26].

This could be explained by Franceschi et al. [24] who showed that c-KIT overexpression leads to a significant inhibition of cellular proliferation through Western blot analysis. Furthermore, c-KIT- cells showed a greater number of cells with malignant morphological criteria like high nuclear-cytoplasmic ratio, powdery chromatin, multiple prominent nucleoli, multinucleated cells than c-KIT+ cells. They suggested that c-KIT overexpression may be linked to regression of thyroid cancer cells malignant features and tumor proliferation.

Moreover, these findings enhance the importance of c-KIT expression as a marker of thyroid malignancy. In this line, this study suggests the application of c-KIT expression as an adjunctive diagnostic marker in the preoperative management of solitary thyroid nodules.

7. Conclusion

The diagnostic preoperative accuracy of the combined CD56, Galectin-3 and HBME-1 panel in solitary thyroid nodules with suspicious cytology could be extremely improved with the addition of c-KIT as a supplementary preoperative immunostain in order to avoid over or under treatment. Moreover further studies on larger scale are required to test these immunomarkers in combination, especially in cases with suspicious thyroid cytology.

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