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Original article

Diagnostic and Prognostic Significance of Immunohistochemistry in Differentiation between NIFTP and other Follicular-Patterned Thyroid Mimics

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Abstract:

Background: It is beneficial to distinguish non- invasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) from other follicular-based lesions like follicular variant of papillary thyroid carcinoma (FVPTC) and follicular adenoma (FA). Galectin-3 plays a role in normal development and tumorigenesis. Cytokeratin 19 (CK19) overexpression is linked to neoplastic transformation. PD-L1 functions as a negative immune regulator. Hypoxia-inducible factor (HIF-1α) plays a role promoting angiogenesis. Aim: Evaluation role of Galectin-3, CK19, PD-L1 and HIF-1α immunoexpressions in distinguishing between benign follicular nodule (BFN), NIFTP and FVPTC with correlation to different clinicopathological variables hoping to find new diagnostic and therapeutic methods. **Material and Methods:** This retrospective study was done on 45 cases of different thyroid follicular-patterned lesions as follows: 25 BFN, 6 NIFTPs, and 14 cases of FVPTCs. Immunohistochemical staining was used to detect Galectin-3, CK19, PD-L1 and HIF-1α expressions in distinguishing those follicular-patterned thyroid lesions. **Results:** PD-L1, Galectin-3 and CK19 showed the highest sensitivity and specificity in distinguishing FVPTCs from BFNs and NIFTPs when used individually. In many configurations, the panel made up of the four markers (positive Galectin-3, positive CK19, positive PD-L1 and high HIF-1α) showed the highest specificity and sensitivity (100% and 88.4% correspondingly). PD- L1 was the most sensitive marker (92.8%), whereas Galectin-3 and CK19 were the most specific (80%) to discriminate FVPTCs from BFNs and NIFTPs. **Conclusion:** The co-expression of Galectin-3, CK19, PD-L1, and HIF-1α increased specificity and sensitivity (100% and 88.4% respectively) while differentiating FVPTCs from BFNs and NIFTPs.

Keywords: NIFTP, Galectin-3, CK19, PD-L1, HIF-1α

Introduction

Thyroid nodules are highly prevalent, affecting approximately 20% of the general population. Their differential diagnosis includes a wide range of conditions, including benign and malignant, as well as neoplastic and non-neoplastic entities [(1)]. The classification of lesions with follicular patterns remains a subject of ongoing discussion.

The World Health Organization's Classification of Tumors of Endocrine Organs recognized the histological type known as noninvasive follicular thyroid tumor with papillary-like nuclear characteristics (NIFTP), which was initially described in 2016 [(2)].

About 9% to 22% of all cases of papillary thyroid cancer are follicular variants (FVPTC), which are classified as either encapsulated or infiltrative. The noninvasive encapsulated follicular variant (NIFTP) exhibits minimal malignant potential and follows an indolent clinical course [(3)]. For research, clinical management, and surgical decision-making purposes, it is essential to accurately differentiate NIFTP from other lesions with a follicular pattern, like follicular adenoma (FA) and follicular variant PTC (FVPTC) [(4)]. However, these lesions showed diagnostic and therapeutic challenges, making the role of the pathologist essential in their evaluation.

In most cases, thyroid nodules associated with NIFTP are identified through physical examination or ultrasound (US). However, as noted by [(5)], NIFTP cannot be diagnosed solely through US. Instead, diagnosis is confirmed postoperatively based on specific pathological criteria observed in the resected specimen. These criteria include complete encapsulation or circumscription, nuclear characteristics of PTC, a follicular growth pattern without psammoma bodies, absence of necrosis and capsular or vascular invasion [(4).]

Since BRAFV600E mutations are rarely found in follicular-patterned thyroid neoplasms like NIFTP, molecular testing for exclusion could be an expensive moreover time-consuming approach. Instead, these tumors more frequently possess RAS mutations or mutations that resemble RAS [(6)].

Several studies have investigated the use of immunohistochemistry (IHC), including TROP-2 (trophoblast cell-surface antigen-2), CITED1 (CBP/p300-interacting trans activator 1), HBME-1 (Hector Battifora mesothelial cell 1), CD56, and the anti-BRAFV600E (VE1) antibody. However, a direct comparison of these markers in differential diagnosis has not been conducted, and research on NIFTP using IHC markers remains limited to only a few markers [(7)].

Galectin-3, a protein that binds beta-galactosides, is mostly found in the cytoplasm. As a physiological target of p53, Galectin-3 plays a role in supporting p53-induced apoptosis. However, its abnormal expression interferes with apoptosis, thereby promoting cancer progression [(8)]. Research suggests that Galectin-3 participates in both normal development and carcinogenesis by controlling important cellular functions, including adhesion, invasion, apoptosis, proliferation, angiogenesis, and metastasis, through interactions with cell surface glycolipids or glycoconjugates containing β-galactose [(9)].

Although Galectin-3 is overexpressed in a considerable number of malignancies, including cancers of the head and neck, stomach, colorectum, endometrium, liver, bladder, and breast [(10)], but its role in distinguishing specific thyroid tumors remains under investigation.

Cytokeratin 19 (CK19) is a low-molecular-weight type I intermediate filament protein found in various simple and glandular epithelial tissues. Unlike other cytokeratins that form heterodimers, CK19 does not pair with other CKs, which is why it is classified as a simple CK. It is significantly expressed in breast tumors and other metastatic malignancies such as liver, lung, pancreas, in addition to

esophagus. CK19 engages in maintaining cell structure, facilitating cellular communication, apoptosis, and regulating protein synthesis and transport [(11)].

PD-L1 functions as a negative immune regulator by binding to the Programmed Cell Death 1 (PD-1/B7-H1) receptor. PD-1 is expressed on different immune cells, including B cells, T cells as well as NKTs (natural killer T cells). T-cell proliferation and the production of cytokines like IL-2 are inhibited when PD-L1 binds to PD-1. This immune suppression allows tumors to evade immune attack by suppressing cytotoxic T-cell activity. Consequently, increased PD-L1 expression in tumors weakens the immune response, promoting tumor progression and metastasis. However, whether PD-L1 overexpression can effectively differentiate between NIFTP and other mimics remains unclear [(12)].

In response to low oxygen levels, the body activates an adaptive hypoxic response mediated by hypoxia-inducible factor (HIF). HIF plays a crucial role in regulating glycolysis-related metabolic pathways and promoting angiogenesis to enhance oxygen supply. HIF primarily consists of two forms: HIF-1 and HIF-2. HIF-1α, the first identified HIF-α subunit, regulates oxygen-dependent stability through its core degradation domain [(13)]. According to research, HIF-1α is expressed in several human malignancies, including glioblastoma, pancreatic and gastric cancers [(14)]. However, research on the ability of HIF-1α overexpression to differentiate EFVPTC from NIFTP remains limited.

This work aims to evaluate the expressions of Galectin-3, CK19, PD-L1, and HIF-1α and help to differentiate between follicular patterned thyroid lesions BFN, NIFTP and FVPTC with correlation to different clinicopathological variables to reach novel approaches to diagnosis and treatment.

Material and Methods:

Study groups:

This retrospective study was done on 45 cases of different thyroid follicular lesions as follow: twenty five cases were diagnosed as Benign follicular nodule (BFN) (ten cases of hyperplastic nodules, fifteen cases as follicular adenoma), six non-invasive follicular thyroid neoplasms with papillary like nuclear features (NIFTPs), and fourteen cases of follicular variant papillary thyroid carcinomas (FVPTCs).

Benha Faculty of Medicine's Pathology Department and Early Cancer Detection Unit provided the cases throughout the 2020–time limit. The study was approved by the Ethical Committee of Faculty of Medicine, Benha University with the code number **(RC 1-9-2024).**

Inclusion criteria: availability of clinicopathological data retrieved from the patients' files which included age, sex, capsular/vascular invasion, lymph node, and distant metastases.

Exclusion criteria: Cases with no available paraffin blocks or clinicopathological data were excluded from the current study.

A-Histopathological Examination:

Formalin fixed /Paraffin embedded blocks were cut at 5 μm thickness and stained using hematoxylin and eosin stain. Three observers reviewed the microscopic sections from all the cases, unaware of their diagnoses and classified them according to the 2022 WHO classification of thyroid tumors **[(15)]**.

B-Immunohistochemical Procedure:

On positive charge slides, and for immunohistochemical analysis, the streptavidin-biotin technique is utilized in compliance with the manufacturer's guidelines. Antibodies are shown in **Table (1).** For the secondary developing reagents, we used a standard labeled streptavidin-biotin system ***(DakoCytomation, Denmark, A/S)*.** The sections were stained with a 0.02% diluted solution of diamethoxybenzidine. After that, hematoxylin was used as a counterstain. The primary antibody stage

was omitted for each marker, and the normal rabbit serum IgG was used as a negative control in its stead.

Galectin-3 interpretation:

Galectin-3 was detected as cytoplasmic brown coloration. Immuno reactivity was evaluated according to distribution and intensity as follows: the intensity was graded from 0 to 3 where 0, 1+, 2+, and 3+ denote no staining, weak/slight staining, moderate staining and intense staining respectively, and the proportion of stained cells were interpreted as 1+ (< 5% of cells), 2+ (5% to 50% of cells) and 3+ (>50% of cells). As per the guidelines, cases that showed specific cytoplasmic staining of more than 5% of the tumour cells, regardless of the intensity, were scored as positive for Galectin-3 according to [16]

CK19 interpretation:

CK19 was detected as cytoplasmic expression. When more than 10% of the cells showed antibody reactivity, the lesion was considered positive as described by [17]

PD-L1 interpretation:

PD-L1 was detected as membranous/cytoplasmic brown coloration. The staining intensity and positivity were used to calculate immunostaining scores. Percentage positive scores were assigned according to the following scale: 0 ≤ 10%; 1 ≥ 11–30%; 2 ≥ 31–50%; 3 ≥ 51–70%; and 4 ≥ 71%. Staining intensity was scored semi-quantitatively as follows: 0 (none); 1 (mild); 2 (moderate) and 3 (intense). A total score was then obtained (ranging from 0 to 7) by adding the percentage positivity scores and intensity scores for each section, samples were considered positive if the score is ≥3 [18].

Table (1): Antibodies used in the study:

Antibody	Source	Working concentration	Incubation period	Positive control
Galectin-3	*(clone9C4; Leica, Bannockburn, IL, USA)*	1:50	Overnight at room temperature	Normal prostatic tissue
CK19	*clone B170; Leica, Newcastle, UK),*	1:400	Overnight at room temperature	Intraductal carcinoma of the breast
PD-L1	*(E1L3N, Cell Signaling Technology, Inc., Danvers, MA)*	1:100	overnight at room temperature	Colonic carcinoma
HIF-1α	*ab51608; Abcam; USA*	1:100	overnight at room temperature	Gastric carcinoma

HIF-1α interpretation:

HIF-1α was detected as nuclear brown coloration. It received the following semiquantitative grades: I denote no staining, II denotes minor staining in less than 10% of cells, III denotes moderate staining in 10% to 50% of cells, and IV denotes severe staining in more than 50% of cells. The low expression group was assigned a final staining score of I or II for statistical analysis, while the high expression group was assigned a final staining score of III or IV [19].

Statistical analysis: The Statistical Package for the Social Sciences (SPSS) 25 for Windows (SPSS Inc., Chicago, IL, USA) was used to record, present, and statistically analyze the data. Numbers and percentages were used to represent categorical data. The mean ± standard deviation was used to express numerical data. To evaluate the relationships between groups, the Pearson Chi square test (X2) was employed. P-values more than 0.05 were regarded as non-significant (NS) and less than 0.05 as significant (S). The

receiver operating characteristic (ROC) curve was used to calculate sensitivity, specificity, positive predictive and negative predictive value at different cutoff points to detect performance of Galectin-3, CK19, PD-L1 and HIF-1α in differentiation between different thyroid follicular-patterned lesions (BFN, NIFTP and FVPTC).

Results:

Clinicopathological Characteristics:

The clinicopathological features of the studied cases are summarized in **Table (2).** The age of studied cases of BFNs ranged from 21- 56 years with mean age 33.85 years, the age of the studied cases of NIFTPs ranged from 22- 45years with mean age 31.16 years and the age of the studied cases of FVPTCs ranged from 20 - 76 years with mean age 42.35 years.

Immunohistochemical staining results:

IHC expression of Galectin-3 in various histologic types of thyroid tumors indicated that 80% of the BFN were negative, 83.1% of the NIFTP cases were negative and 85.7% of the FVPTC showed cytoplasmic positivity. A statistically significant difference was found between Galectin-3 expression and different thyroid follicular lesions in favor of FVPTCs (P=0.000) as shown in **Table (2)** and **Figure (1): A, B& C.**

The CK19 IHC expression showed that 80% of the BFN cases were negative, but 83.1% of the NIFTP cases were positive. CK19 status in the case of malignant neoplasms (FVPTC), 85.7% of cases showed cytoplasmic positivity. A statistically significant difference was present between CK19 expression and different thyroid follicular lesions in favor of FVPTCs (p =0.000) as illustrated in **Table (2)** and **Figure (2): A, B &C.**

As regards PD-L1 IHC evaluation in the cases studied, it showed that 72% of the BFN cases were negative, 66.7% of NIFTP cases were positive and 92.8% of the FVPTC cases were positive. A statistically significant difference was detected between PD-L1 expression and the different studied cases (BFN, NIFTP and FVPTC) in favor of FVPTCs (p=0.000) as seen in **Table (2)** and **Figure (3): A, B &C.**

Regarding IHC staining of HIF-1α in the studied cases, 64% of the BFN cases showed low expression, half (50%) of the NIFTP cases showed high expression and 64.3% of the FVPTC cases showed high stain. A statistically significant difference was found between different thyroid follicular lesion cases regarding HIF-1α expression in favor of FVPTCs (p=0.03) as shown in **Table (2)** and **Figure (4): A, B &C**

Table (2): Immunohistochemical expression of Galectin-3, CK19, PD-L1 and HIF-1α in the studied cases:

		Galectin-3			CK19			PD-L1			HIF-1α		
Studied cases	**N.**	**-ve**	**+ve**	**P**	**-ve**	**+ve**	**P**	**-ve**	**+ve**	**P**	**Low**	**High**	**P**
BFN	**25**	**20 (80%)**	**5 (20%)**	**0.000***	**20 (80%)**	**5 (20%)**	**0.011***	**18 (72%)**	**7 (28%)**	**0.000***	**16 (64%)**	**9 (36%)**	**0.03***
NIFTP	**6**	**5 (83.3%)**	**1 (16.67%)**		**1 (16.7%)**	**5 (83.3%)**		**2 (33.3%)**	**4 (66.7%)**		**3 (50%)**	**3 (50%)**	
FVPTC	**14**	**2 (14.3%)**	**12 (85.7%)**		**2 (14.3%)**	**12 (85.7%)**		**1 (14.3%)**	**13 (92.8%)**		**5 (35.7%)**	**9 (64.3%)**	
Total	**45**	**27 (60%)**	**18 (40%)**		**23 (51.2%)**	**22 (48.8%)**		**21 (46.7%)**	**24 (53.3%)**		**24 (53.3%)**	**21 (46.7%)**	

*Correlation is significant at 0.05 level BFN: Benign follicular nodule NIFTP: Non-invasive follicular thyroid neoplasm with papillary like nuclear features. FVPTC: Follicular variant papillary thyroid carcinoma.

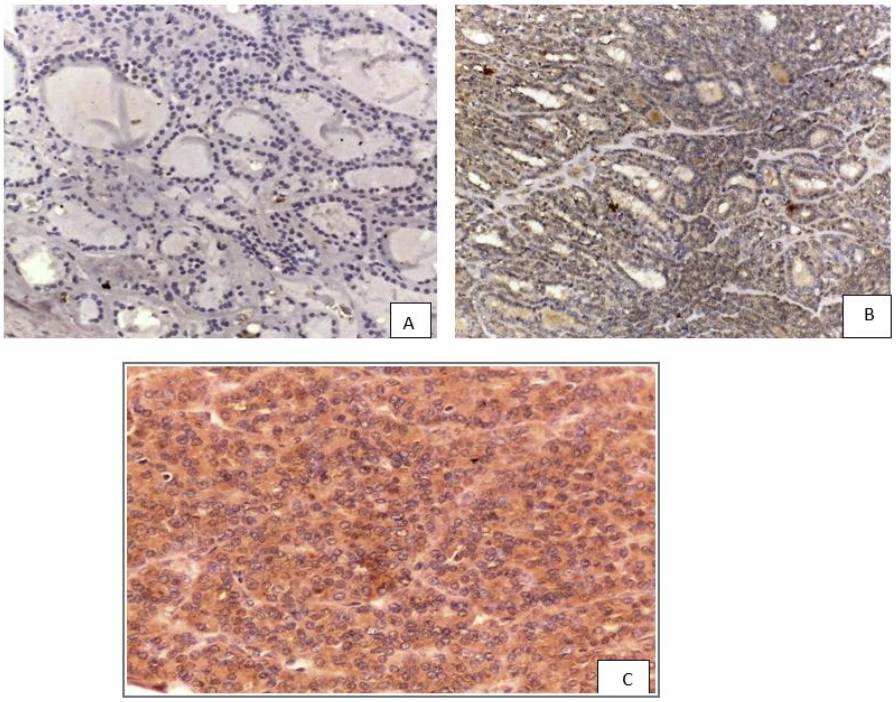

Figure (1): Variable thyroid lesions with Galectin-3 IHC cytoplasmic staining, **A**: BFN (Nodular hyperplasia) showed negative expression (ABCx200), **B**: NIFTP showed positive expression (ABCx200), **C**, FVPTC: showed positive cytoplasmic expression (ABCx200)

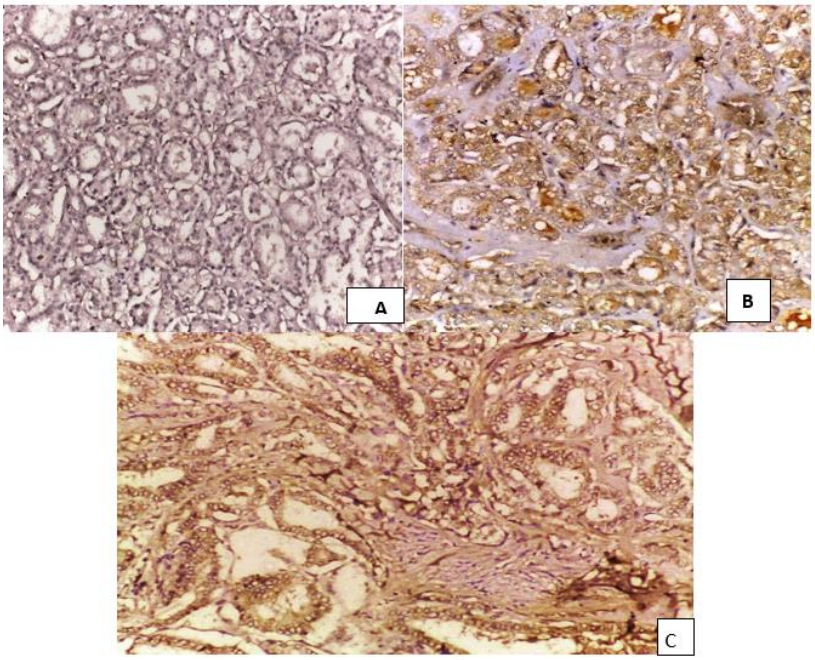

Figure (2): Variable thyroid lesions with CK19 IHC cytoplasmic staining, **A**: BFN (Follicular adenoma) showed negative expression, **B**: NIFTP showed positive cytoplasmic expression, **C**, FVPTC: showed positive cytoplasmic expression (ABCx200).

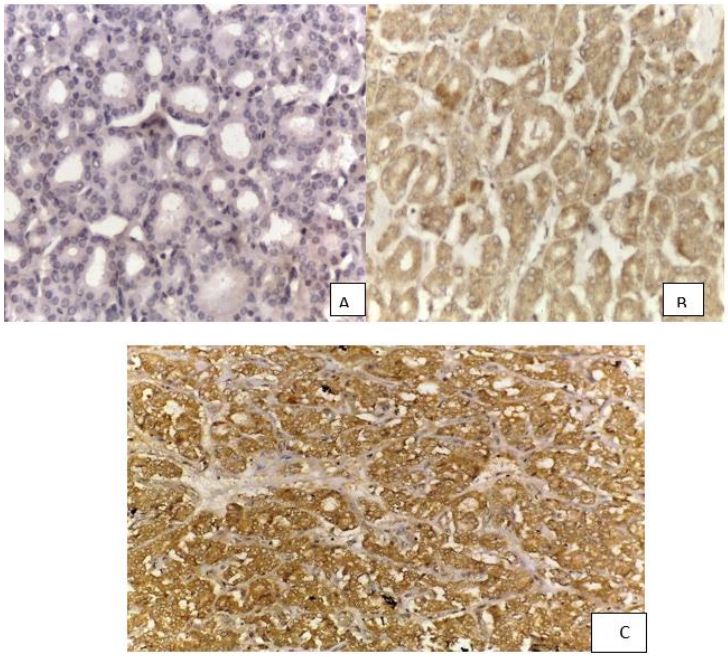

Figure (3): Variable thyroid lesions with PD-L1 IHC cytoplasmic staining, **A**: BFN (Follicular adenoma) showed negative expression, **B**: NIFTP showed positive cytoplasmic expression, **C**, FVPTC: showed positive cytoplasmic expression (ABCx200).

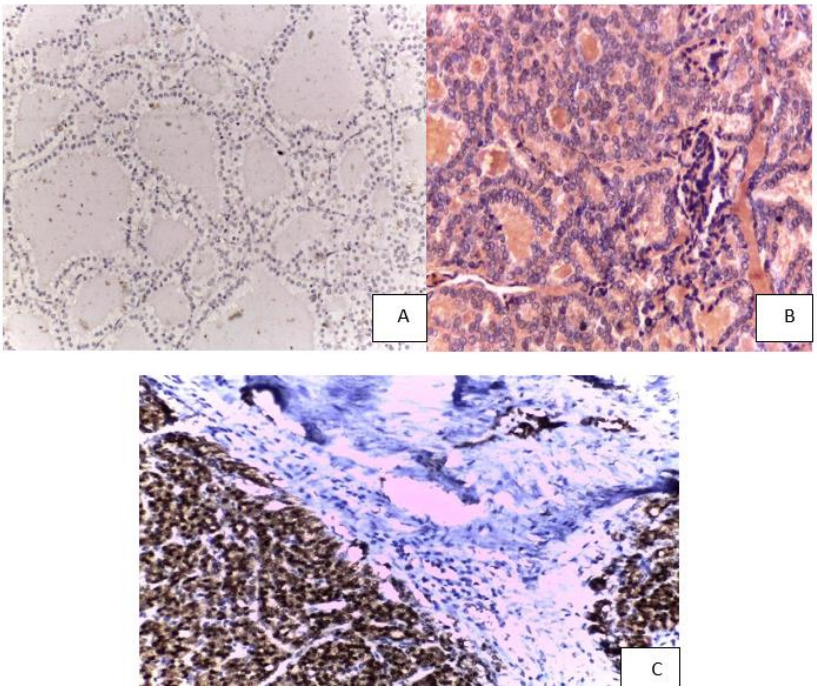

Figure (4): Variable thyroid lesions with HIF-1α IHC nuclear staining, **A**: BFN (Nodular hyperplasia) showed negative expression, **B**: NIFTP showed negative expression, **C**, FVPTC: showed high nuclear expression (ABCx200).

The differential expression of the four immunohistochemical markers Galectin-3, CK19, PD-L1 and HIF-1α among different thyroid lesions:

- As shown in **Table (3),** the expression of the four markers between BFNs and FVPTC was all statistically significant ($p < 0.05$**)**.

- As regards the comparison between BFN and NIFTPs, Galectin-3 and CK19 expressions showed a positive statistically significant relation (p=0.033 & p=0.017 respectively**),** while PD-L1 and HIF-1α expression showed no statistically significant relation.
- The expressions of Galectin-3, PD-L1 and HIF-1α showed a positive statistically significant relation ($p < 0.05$) in comparison between NIFTPs and FVPTCs while CK19 expression showed no statistically significant relation.

Relation between IHC expressions of the four markers and different clinicopathological parameters in FVPTC cases (Table 4):

A statistically significant direct relation was identified between positive Galectin-3 IHC expression and lymph node metastasis (P=0.02) among the examined cases. A statistically significant direct relationship was found between positive CK19 IHC expression and capsular invasion & lymph node metastasis (P=0.011& P=0.029) respectively. A statistically significant direct relation between positive PD-L1 IHC expression and both capsular and vascular invasions was found (P=0.005 and P=0.003). A statistically significant relation was found between high HIF-1α IHC expression and capsular invasion (P=0.002) and vascular invasion (P=0.038) as illustrated in **Table (4).**

The diagnostic validity of Galectin-3, CK19, PD-L1 and HIF-1α in diagnosis of the studied thyroid follicular-patterned lesions when each marker is used individually (ROC curve):

Using ROC curve, when Galectin-3 is applied alone, its diagnostic efficacy in distinguishing FVPTCs from other cases (BFNs & NIFTPs) demonstrated 85.7% sensitivity and 80.0% specificity. CK19 showed 85.7% sensitivity and 80.00% specificity. Regarding the expression of PD-L1, it revealed 92.8% sensitivity and 72.0% specificity. While HIF-1α revealed 64.3% sensitivity and 64.0% specificity as shown in **Table (5)** and **Figure (5): A, B, C & D.**

In terms of discrimination against FVPTCs from other cases (BFNs and NIFTPs), PD-L1 was the single most sensitive marker (92.8%), while Galectin-3 and CK19 were the most specific (80%).

Table (3): The comparison of expression of the four immunohistochemical markers Galectin-3, CK19, PD-L1 and HIF-1α among the studied thyroid follicular-patterned lesions:

IHC markers	**BFN/NIFTP**	**BFN/**FVPTC	**NIFTP / FVPTC**
Galectin-3	P=0.033*	P=0.00*	P=0.001*
CK19	P= 0.017*	P=0.00*	P=0.08
PD-L1	P=0.8	P=0.00*	P=0.003*
HIF-1α	P=0.2	P=0.047*	P=0.014*

*Correlation is significant at 0.05 level
BFN: Benign follicular nodule
NIFTP: Non-invasive follicular thyroid neoplasm with papillary like nuclear features.
FVPTC: Follicular variant papillary thyroid carcinoma

Table (4): Association of markers' expression with different clinicopathological parameters in FVPTCs cases:

			Galectin-3 expression			CK19 expression			PD-L1 Expression			HIF-1α expression		
Variables		**No. (%)**	**-ve**	**+ve**	**P**	**-ve**	**+ve**	**P**	**-ve**	**+ve**	**P**	**Low**	**High**	**P**
Age	**< 45**	**11 (78.5%)**	**2 (18.2%)**	**9 (81.8%)**	**0.6**	**1 (9%)**	**10 (91%)**	**0.3**	**1 (9%)**	**10 (91%)**	**0.7**	**5 (45.5%)**	**6 (54.5%)**	**0.9**
	≥ 45	**3 (21.5%)**	**0 (0%)**	**3 (100%)**		**1 (33.3%)**	**2 (66.7%)**		**0 (0%)**	**3 (100%)**		**0 (0%)**	**3 (100%)**	
Sex	**Male**	**3 (21.5%)**	**0 (0%)**	**3 (100%)**	**0.3**	**1 (33.3%)**	**2 (66.7%)**	**0.3**	**۰ (0%)**	**۳ (0%)**	**0.45**	**1 (33.3%)**	**2 (66.7%)**	**0.7**
	Female	**11 (78.5%)**	**2 (18.2%)**	**9 (81.8%)**		**1 (9%)**	**10 (91%)**		**1 (9%)**	**10 (91%)**		**4 (36.4%)**	**7 (63.6%)**	
Capsular invasion	**Absent**	**7 (50%)**	**2 (28.6%)**	**5 (71.4%)**	**0.07**	**2 (28.6%)**	**5 (71.4%)**	**0.011***	**6 (85.7%)**	**1 (14.3%)**	**0.005***	**5 (71.4%)**	**2 (28.6%)**	**0.002****
	Present	**7 (50%)**	**0 (0%)**	**7 (100%)**		**0 (0%)**	**7 (100%)**		**0 (0%)**	**7 (100%)**		**0 (0%)**	**7 (100%)**	
Vascular invasion	**Absent**	**6 (42.8%)**	**2 (33.3%)**	**4 (66.7%)**	**0.08**	**2 (33.3%)**	**4 (66.7%)**	**0.08**	**6 (100%)**	**0 (0%)**	**0.003***	**4 (33.3%)**	**2 (66.7%)**	**0.038***
	Present	**8 (57.2%)**	**0 (0%)**	**8 (100%)**		**0 (0%)**	**8 (100%)**		**1 (12.5%)**	**7 (87.5%)**		**1 (12.5%)**	**7 (87.5%)**	
LN metastasis	**Absent**	**9 (64.3%)**	**2 (22.2%)**	**7 (77.8%)**	**0.02***	**2 (22.2%)**	**7 (77.8%)**	**0.029***	**1 (11.1%)**	**8 (88.9%)**	**0.02**	**5 (55.5%)**	**4 (44.5%)**	**0.063**
	Present	**5 (35.7%)**	**0 (0%)**	**5 (100%)**		**0 (0%)**	**5 (100%)**		**0 (0%)**	**5 (100%)**		**0 (0%)**	**5 (100%)**	
Distant metastasis	**Absent**	**10 (71.4%)**	**1 (10%)**	**9 (90%)**	**0.4**	**1 (10%)**	**9 (90%)**	**0.50**	**0 (0%)**	**10 (100%)**	**0.2**	**4 (40%)**	**6 (60%)**	**0.54**
	Present	**4 (28.6%)**	**1 (25%)**	**3 (75%)**		**1 (25%)**	**3 (75%)**		**1 (25%)**	**3 (75%)**		**1 (25%)**	**3 (75%)**	

*Correlation is significant at 0.05 level

Table (5): The diagnostic validity of Galectin-3, CK19, PD-L1 and HIF-1 α in diagnosis of the studied thyroid follicular-patterned lesions when each marker is used individually:

Test Result Variable(s)	**AUC**	**P value**	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Accuracy**
Galectin-3	0.88	0.000	85.7%	80.0%	66.7%	74.1%	71.1%
CK19	0. 88	0.000	85.7%	80.0%	54.5%	87.0%	73.3%
PD-L1	0.96	0.005	92.8%	72.0%	59.1%	78.3%	68.9%
HIF-1α	0.69	0.03	64.3%	64.0%	42.9%	66.7%	55.6%

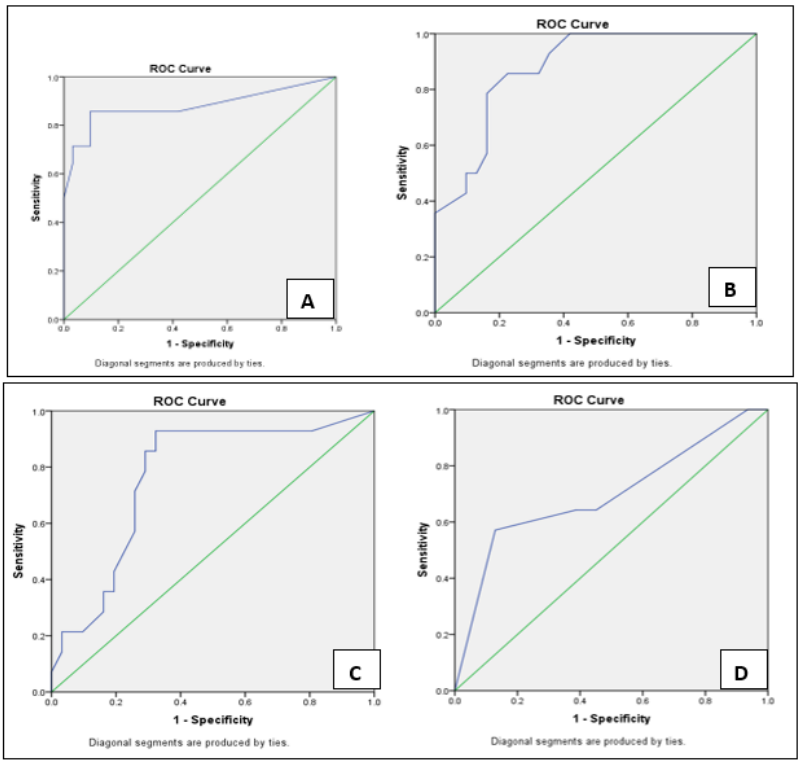

Figure (5): ROC curves for performance of each marker individually in the diagnosis of different thyroid lesions: **A**, Galectin-3, **B**, CK19, **C**, PD-L1 and **D**, HIF-1α.

The diagnostic validity of combined Galectin-3, CK19, PD-L1 and HIF-1α in diagnosis of the studied thyroid follicular-patterned lesions:

In various combinations, the panel exhibiting increased HIF-1α and positive CK19 demonstrated the least sensitivity and specificity (46% and 84%, respectively). The addition of Galectin-3 to the immuno-panel improved both sensitivity and specificity (50% and 100%, respectively). The association of the four markers (positive Galectin-3, positive CK19, positive PD-L1, and high HIF-1α) showed the greatest sensitivity and specificity (100% and 88.4%, respectively) in differentiating FVPTCs from other instances (BFNs and NIFTPs) as illustrated in **Table (6)** and **Figure (6).**

The relation between markers' expressions in different studied thyroid follicular-patterned lesions:

The Spearman correlation analysis revealed a positive statistically significant relation between the expression of Galectin-3 and CK19 (r=.472), Galectin-3 and PD-L1 (r=.355), & Galectin-3 and HIF-1α (r=.346). Furthermore, PD-L1 and HIF-1α had a positive statistically significant correlation in the cases studied (r=-0.442) and CK19 and HIF-1α (r=.384). However, there was insignificant statistical correlation between CK19 and PD-L1 (r=0.289).

Table (6): The diagnostic validity of combined Galectin-3, CK19, PD-L1 and HIF-1α in the diagnosis of different studied thyroid follicular-patterned lesions:

	AUC	95% Confidence Interval		Sensitivity	Specificity
		Lower Bound	Upper Bound		
Positive Galectin-3 & high HIF-1α & positive PD-L1	.750	.572	.928	50%	100%
Positive Galectin-3 & high HIF-1α	.721	.546	.896	57.1%	87.1%
Positive Galectin-3&positive PD-L1	.877	.742	1.000	78.6%	97%
High HIF-1α &positive PD-L1	.753	.581	.926	57.1%	94%
Positive Galectin-3 &HIF-1α &PD-L1 &CK19	.750	.572	.928	88.4%	100%
Positive Galectin-3 & HIF-1α &CK19	.750	.572	.928	50%	100%
Positive Galectin-3&PD-L1&CK19	.750	.572	.928	50%	100%
Positive HIF-1α & PD-L1& CK19	.753	.581	.926	57.1%	87.1%
Positive HIF-1α &CK19	.741	.572	.909	46%	84%
Positive PD-L1 &CK19	.844	.704	.985	78.6%	91%

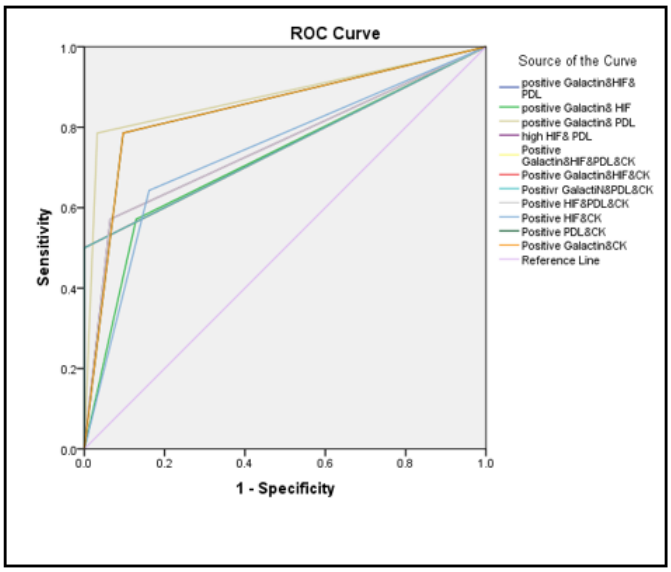

Figure (6): ROC curve for the validity of combined Galectin-3, CK19, PD-L1 and HIF-1 α in the diagnosis of different thyroid follicular lesions.

Discussion:

Thyroid follicular-patterned lesions frequently present diagnostic challenges because of the structural and morphologic similarities between benign and malignant tumors. The range of thyroid follicular-patterned neoplasms includes malignancies, low-risk neoplasms with borderline behavior, and benign follicular adenoma (FA) [(1)]. Patients undergoing surgery as well as follow-up and research are benefited by the precise distinction of NIFTP from other follicular-based diseases, such as FVPTC and follicular adenoma (FA) [(4)].

The non-invasive encapsulated follicular variant, or NIFTP, is distinguished by its slow progression and limited propensity for malignancy. FVPTC, or follicular variation of papillary thyroid cancer, is categorized as either infiltrative or encapsulated and makes for 9–22% of all PTC [(20)].

After fulfilling a number of requirements, including complete capsule of the tumor, nuclear features of PTC, a follicular pattern of growth devoid of psammoma bodies, no capsular or vascular invasion, and no necrosis, NIFTPs are diagnosed post-operatively [(4)]. However, it remains subjective. As a result, this needs scientific and objective markers that can describe these lesions more accurately than traditional morphology.

Galectin-3 (Gal-3) is a member of lectins family (carbohydrate-binding proteins) that bind betagalactoside. Numerous physiological and pathological processes are mediated by this protein, including cell proliferation, apoptosis, inflammation, adhesion, cellular transformation, tumor growth, and cancer cell metastasis [(9)].

The FVPTC in the current study exhibited cytoplasmic expression of Galectin-3 at significantly higher levels than the benign lesions (BFN and NIFTP) (p=0.00 and p=0.001, respectively). Galactin-3 expression demonstrated a positive statistically significant relationship (p = 0.033) when comparing BFN and NIFTPs. In line with research by Fu et al. [(8)], which demonstrated that invasive FVPTCs had more cytoplasmic Gal-3 expression than either benign thyroid nodules or NIFTPs.

Furthermore, the study by Chiu et al. [(21)] found that when differentiation progressed from widely invasive follicular carcinoma to locally invasive follicular carcinoma to follicular adenoma, the expression of Galactin-3 gradually declined. Similar patterns of Galectin-3 expression have also been observed in colon cancers by Tao et al. [(22)] and brain gliomas by Binh et al. [(23)] All of these findings suggest that Galectin-3 contributes to thyroid cancer development & pathogenesis and can be used as a diagnostic tool.

Many simple and glandular epithelia contain the low molecular weight type I intermediate filament protein known as cytokeratin-19 (CK19). Neoplastic transformation has been associated with CK19 upregulation [(11)].

When CK19 was evaluated immunohistochemically in this study, we found that NIFTP cases had significantly different CK19 expression compared to other benign follicular lesions, such as nodular hyperplasia and follicular adenoma (BFN) (p=0.017). This is comparable to the findings of Sadiq et al study. [(24)]

The expression of CK19 was also higher in FVPTC (85.7%) than in BFN (20%), with a statistically significant difference (P=0.000). Comparable results were reported by Liu et al. [(25)] and Tastekin et al. [(26)], who found that malignant thyroid follicular tumors had a statistically significant higher level of positive CK19 expression than benign thyroid lesions. There was no statistically significant difference in the expression of CK19 between the NIFTP and FVPTC cases in our study (P=0.08). This is consistent with Sadiq et al. study [(24)] who reported that CK19 cannot distinguish FVPTC from NIFTP, but it can be used to differentiate BFNs from NIFTP and FVPTC.

By preventing T-cell activation and cytokine production, including IL-2, programmed cell death 1 (PD-1) and its ligand (PD-L1) play a crucial role in the antitumor immune response. Overexpression of PD-L1 in a malignant neoplasm prevents the immune system from attacking malignant cells by suppressing cytotoxic T cells. So, higher PD-L1 expression in tumors will prevent antitumor immunological attack and promote tumor growth and invasion [12].

In the current study, immunohistochemical evaluation revealed high significant variations in cytoplasmic PD-L1 staining expression between BFN & FVPTC (p=0.00) and between NIFTP & FVPTC (p=0.003). However, the difference between BFN and NIFTP cases was insignificant (p=0.8). This is comparable to a study by Fu et al. [8] that found no significant difference between benign nodules and NIFTP (p = 0.554). Cytoplasmic PD-L1 expression was significantly elevated in FVPTC as compared to NIFTP (p<0.001) and benign nodules (p<0.001).

HIF-1α nuclear expression in the current study revealed significant differences between NIFTP and FVPTC (p=0.014) and significant differences between BFN and FVPTC (p=0.047). However, the difference between BFN and NIFTP cases was insignificant (p=0.2). This supports the findings of Zhou et al study. [27]

For the FVPTC cases, the association between cytoplasmic PD-L1 expression and risk of invasion is consistent with our study of PD-L1 as a prognostic and aggressive indicator of thyroid cancer, as PD-L1 expression demonstrated a strong statistical relationship with both capsular and vascular invasion (p=0.005 and p=0.003, respectively), which is consistent with the Ulisse et al study. [28]

A statistically significant direct association between positive HIF-1α IHC expression and capsular and vascular invasion was found (P = 0.002 and 0.038, respectively). This is in line with a study by Klaus et al. [29] that found a strong association between HIF-1α with invasion and metastasis (p < 0.001).

By using ROC curve, the diagnostic performance of Galectin-3 alone in distinguishing FVPTCs from other lesions (BFN and NIFTPs) was found to have 85.71% sensitivity and 80.0% specificity. This was in line with the study performed by Fu et al. [8] The validity of CK19 showed a sensitivity of 85.7% and a specificity of 80%. This is consistent with research by Priyadarshini et al. [30]

In terms of PD-L1's diagnostic ability, the current study found 92.85% Sensitivity and 72.00% Specificity. Therefore, that PD-L1 expression can be used as a predictor to differentiate invasive FVPTC from NIFTP. The cytoplasmic PD-L1 expression of the benign and NIFTP subgroups does not significantly differ, suggesting that NIFTPs are more like benign nodules and that they are non-malignant neoplasms. This goes parallel to the studies done by Ulisse et al. [28] and Nikiforov et al. [31]

In this study, in terms of discrimination of FVPTCs from other cases (BFNs and NIFTPs) and when we used each marker individually, we found that PD-L1 was the single most sensitive marker (92.8%), while Galectin-3 and CK19 were the most specific (80%).

To improve the diagnostic value for differentiating FVPTC from BFNs, the use of combined markers was investigated. The panel exhibiting increased HIF-1α and positive

CK19 demonstrated the least sensitivity and specificity (46% and 84%, respectively). The addition of Galectin-3 to the immuno-panel improved both sensitivity and specificity by (50% and 100%, respectively). The association of the four markers (positive Galectin-3, positive CK19, positive PD-L1, and high HIF-1α) showed the greatest sensitivity and specificity by (100% and 88.4%, respectively) in differentiating FVPTCs from other instances (BFNs and NIFTPs).

Therefore, when a patient has questionable features that meet NIFTP criteria, immunopanels are superior to single markers in distinguishing NIFTP from other follicular patterned lesions.

The Spearman correlation analysis in this study, revealed a positive statistically significant relation between the expression of Galectin-3 and CK19 (r=.472) as reported by Dunđerović et al. [32] study showing that the upregulation of CK19 and galectin-3 was related to neoplastic thyroid transformation.

A positive statistically significant relation was also found between the expression of Galectin-3 and PD-L1 (r=.355) & HIF-1α (r=.346) in line with the Zhang et al. [33] study that reported that Galectin-3 increased PD-L1 expression via the upregulation of STAT3 phosphorylation and Zheng et al. study [34] that detected that Galectin-3 could act as a modulator of thyroid cancer migration and progression, especially in hypoxic microenvironments. Furthermore, PD-L1 and HIF-1α had a positive statistically significant correlation in the studied cases (r=-0.442) going parallel to Zhang et al. study. [33]

Although the sample size is small and the correlation for genetic testing is absent, our study is still novel, and it broadens the use of IHC markers to differentiate between BFNs and FVPTCs.

Conclusion:

Galectin-3 IHC expression could be a helpful diagnostic marker for determining the invasiveness of FVPTC and differentiating between NIFTP and infiltrative FVPTC. The co-expression of positive Galectin-3, positive CK19, positive PD-L1, and high HIF-1α increased the immuno-panel's specificity and sensitivity (100% and 88.4%, respectively) in differentiating FVPTCs from other instances (BFNs and NIFTPs). The study of PD-L1 immunoexpression supported the concept that NIFTP is a non-malignant tumor and offered an additional method for differentiating it from invasive FVPTC hoping for the early management of patients at-risk of thyroid cancer progression who may benefit from anti-PD-1/PD-L1 medications. In terms of discrimination against FVPTCs from other cases (BFNs and NIFTPs), PD-L1 was the single most sensitive marker (92.8%), while Galectin-3 and CK19 were the most specific (80%).

No conflicts of interest

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