Galactin-3 Protein Expression in Follicular Adenoma, non-invasive Follicular Tumor with Papillary Nuclear Features and Follicular Variant Papillary Thyroid Carcinoma

Fatma-Elzhraa Ahmed Sherkawy[a*], Mai M Elkabsh[b], Mohamed Yousef A[C], Sabah Ahmed Mohamed Fadel[b]

[a]Pathology Department, Faculty of Medicine, South Valley University, Qena, Egypt.
[b]Pathology Department, Faculty of Medicine, Assiut University, Assiut, Egypt.
[c]General Surgery Department, Faculty of Medicine, South Valley University, Qena, Egypt.

Abstract:

Background: Galectin-3 (Gal-3) is a cytoplasmic beta-galactoside-binding protein that is implicated in a many biological processes, including tumor growth, cellular transformation, apoptosis, and cell proliferation. Gal-3 has a diagnostic value in malignant thyroid lesions. Gal-3 is weak or nonexistent in benign or normal thyroid tissue, but overexpressed in some forms of thyroid carcinomas, particularly PTC. Thyroid tumors rank as the tenth most prevalent cancer globally. In Egypt, thyroid cancer makes up around 30% of all endocrine cancers. It ranks the seventeenth malignant tumor among males and the sixth among women. It was crucial to distinguish benign tumors such as follicular adenoma (FA) from follicular variant of papillary thyroid carcinoma (FVPTC) and non-invasive follicular tumor with papillary nuclear characteristics (NIFTP).

Objectives: Evaluation of Galactin-3 expression in FA, NIFTP and FVPTC cases, and correlate its expression with clinicopathological parameters of those cases.

Patients and methods: 48 cases of FA, NIFTP, and FVPTC were histopathologically evaluated using a standard H&E stain and assessed immunohistochemicaly for Galactin-3 protein expression.

Results: There was a significant association in Gal-3 expression between FA and FVPTC (p-value <0.001), where 91.67% of FVPTC cases had high GAL-3 compared to complete absence in FA cases. No significant association in Gal-3 expression between FA and NIFTP was detected (p-value = 0.327).

Conclusion: Gal-3 was significantly higher malignant compared to benign thyroid neoplasms

Keywords: Galactin-3; FVPTC; NIFTP; FA.

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***Correspondence:** fa.ah.sh91@gmail.com

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Introduction

Thyroid tumors represent ninety percent of endocrine tumors, making them the most prevalent neoplasms in the endocrine system. (Al-Ibraheem et al., 2023) Globally, the prevalence of thyroid cancer is rising. It is regarded as the tenth most prevalent cancer globally. **(Almansoori et al., 2022).** In Egypt, thyroid cancer makes up to 30% of all endocrine cancers. It ranks the seventeenth malignant tumor among males and the sixth among women. **(Elbalka et al., 2021)**

The apparent increased prevalence of thyroid cancer has been attributed to several causes, including the use of more advanced diagnostic tools, radiation exposure, increased iodine consumption, chronic lymphocytic thyroiditis, environmental contaminants, and potentially unknown carcinogens. **(Du et al., 2018).**

Follicular derived thyroid carcinomas are papillary thyroid carcinoma, follicular thyroid carcinoma, hurthle cell carcinoma, poorly differentiated thyroid carcinoma, and anaplastic thyroid carcinoma. **(Younis, 2017).**

Approximately 90% of thyroid cancers are PTCs, numerous histopathologic variations exist for it, such as the classic variety, cribriform-morular variant, hobnail variant, follicular variant, tall cell variant, columnar cell variant, and diffuse sclerosing variant. **(Fu et al., 2021).**

FVPTC (follicular variation of PTC) exhibits a diverse morphology, consisting of micro-to macro-follicules with a diffuse development pattern. Its encapsulation or infiltration can sometimes lead to diagnostic confusion with other follicular neoplasms. **(Sadiq et al., 2021)**

NIFTP (non-invasive follicular thyroid neoplasm with papillary-like nuclear features) exhibit the following characteristics: The morphological characteristics that differentiate it from invasive EFVPTC are (1) encapsulation; (2) lack of invasion; and (3) follicular growth pattern, without papillary formation or psammoma bodies; <30% of the tumor has a solid/trabecular/insular growth pattern; low mitotic figures; nuclear score 2-3; and no tumor necrosis. **(Seethala et al., 2018)**

It was crucial to distinguish benign tumors such as follicular adenoma (FA) from follicular variant of papillary thyroid carcinoma (FVPTC) and non-invasive follicular tumors with papillary nuclear characteristics (NIFTP) after NIFTP was identified. **(Alves et al., 2018)**

Galectin-3 (Gal-3) is a cytoplasmic beta-galactoside-binding protein that is implicated in a number of biological processes, including tumor growth, cellular transformation, apoptosis, and cell proliferation. Gal-3 is weak or abscent in benign or normal thyroid tissue, but overexpressed in some forms of thyroid carcinomas, particularly PTC. **(Fu et al., 2021).**

It is clinically difficult to distinguish benign thyroid lesions from cancerous one. According to a study, Gal-3 may be a helpful immunohistochemistry marker for separating PTC patients from non-PTC individuals. Additionally, lymph node metastases were likely in PTC patients with positive Gal-3 expression. **(Tang et al., 2016)**

Our study aimed to determine the immunohistochemical expression of Gal-3 and the relationship between its expression and clinicopathological parameters in FA, NIFTP, and FVPTC patients

Patients and methods

This study was approved by the Ethics Committee of Faculty of Medicine, South Valley University (SVU-MED- PAT005-2-21-12-289). A retrospective study was conducted on forty-eight formalin-fixed paraffin-embedded (FFPE) tissue blocks of FA, NIFTP, and FVPTC which were collected from the archives of the Surgical Pathology Laboratory of South

Valley University Hospital and Surgical Pathology Laboratory of Assuit University Hospital, diagnosed in the period from January 2018 to December 2021.

4 μm thick paraffin-embedded fixed-formalin- tissue sections were placed on slides coated with 3-aminopropyltriethoxysilane (APES). Sections were dewaxed in Xylene and rehydrated using graded alcohols. Tissue slices were incubated with primary mouse monoclonal Gal-3 antibody at an abcam concentration of 1/100 and incubated for 1 hour at room temperature and washed with phosphate buffer saline (PBS) solution. Subsequently, biotinylated Goat Anti-Polyvalent was applied for 10 minutes, then twice washed with PBS. Diammoniobenzidine (DAB) was applied for five to ten minutes.

Counterstaining was done with Mayer's haematoxylin, followed by clearing and mounting.

Immunohistochemical scoring

The average percentage of positive cells was initially assessed for Gal-3 expression using a 4-point index, with 0 representing less than 5%, 1 representing 5-25%, 2 representing 26% -50%, 3 representing 51% - 75%, and 4 representing more than 75%. Second, a four-point scale was used to grade the staining intensity: zero represented a negative stain, one for mild stain, two for moderate, and three for strong. The intensity score and the percentage score were multiplied to get the average weighted immunoreactivity score. **(De Matos et al., 2005)**

Statistical analysis

Data was collected, coded, revised, and inserted to the Statistical Package for Social Science (IBM SPSS) version 27. The data were presented as numbers and percentages for the categorical variables, mean, and standard deviations, for the numerical variables. The data was tested for normality using the Kolmogorov–Smirnov and Shapiro–Wilk tests. The relation between galactin-3 expression and clinicopathological features was determined using a chi-square test for qualitative variables and a student t-test for quantitative variables. The Fisher exact test was used to assess Gal-3 expression in FA, NIFTP and FVPTC. Significance was defined as $p < 0.05$.

Results

Clinicopathological characters of 48 patients with thyroid neoplasm FA, NIFTP, and FVPTC included in the present study were summarized in **(Table 1).** The age of patients ranged from (18-64), the mean age was 36.8 years**.** In this study, 46 cases (95.8%) were females and 2 cases (4.2%) were males.

Table 1. Clinicopathological parameters of the studied group

Parameters		Frequency	Percentage %
	≤ 40	32	66.67%
	>40	16	33.33%
	Mean ± SD	36.79 ± 9.525	
	Median (range)	35.50 (18-64)	
Gender	**Male**	2	4.20 %
	Female	46	95.83%
Histopathology	**Follicular adenoma**	4	8.33 %
	Papillary thyroid carcinoma	24	45.83%
	Non-invasive follicular thyroid neoplasm with papillary nuclear features	20	41.667%
T staging of	**T1**	8	33.33%

FVPTC	**T2**	6	25%
	T3	6	25%
	T4	4	16.67%
Extra-thyroid extension of FVPTC	**Positive**	12	50%
	Negative	12	50%

Immunohistochemical expression of galactin-3

Galactine-3 was expressed in thyroid tumor cells as a cytoplasmic staining pattern. Diffuse strong staining pattern was noticed in malignant thyroid neoplasms compared to benign one. Galactin-3 low IRS expression was identified in 22 cases (45.8%) and high expression in 26 cases (54.2%). (**Table.2**, **Fig.1 A, B, C, D**)

Table 2. Difference of Gal-3 IRS between FA, NIFTP, and FVPTC

Histopathology	**GAL-3 IRS**				**P value**
	Low expression (n=22)		**High expression (n=26)**		
	No.	**%**	**No.**	**%**	
Follicular adenoma	4	18.2%	0	0%	**<0.001***
Follicular variant of papillary thyroid carcinoma	2	9.1%	22	84.6%	
NIFTP	16	72.2%	4	15.4%	

*Fisher's exact test

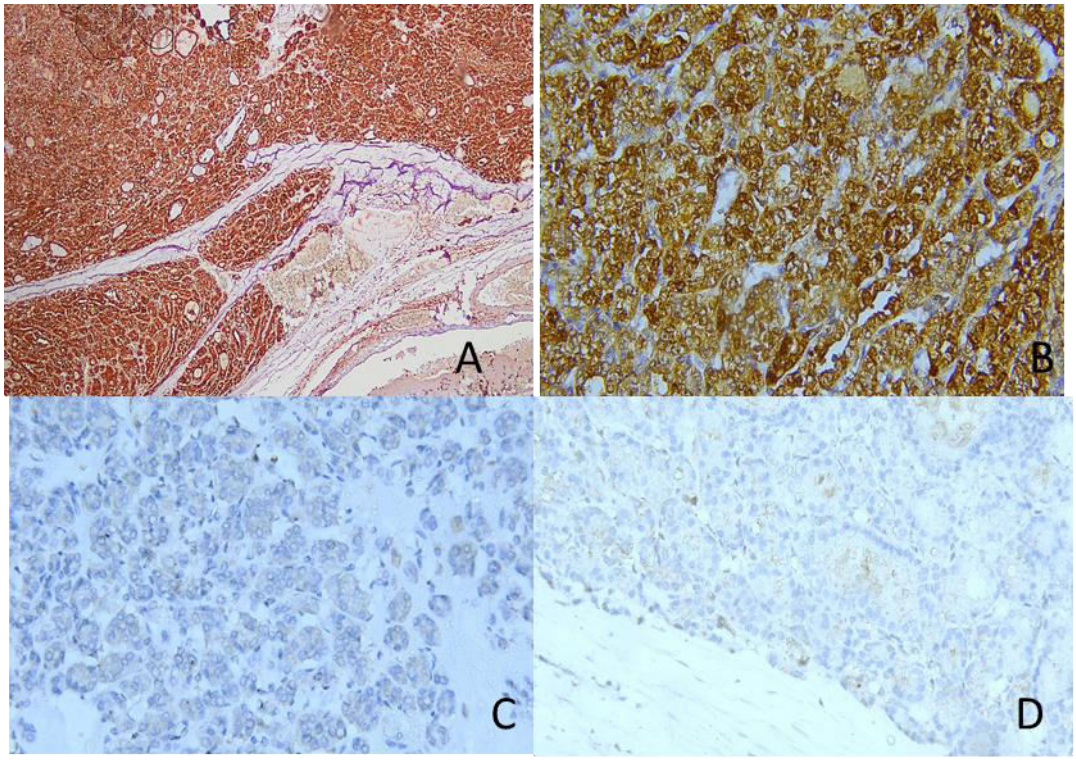

Fig.1: A. FVPTC with strong cytoplasmic Gal-3 expression (x40). **B.** FVPTC with strong cytoplasmic Gal-3 expression (x400). **C.** NIFTP with weak cytoplasmic Gal-3 expression (x400). **D.** FA with negative cytoplasmic Gal-3 expression (x400)

Regarding FVPTC, there was a statistically significant difference in the expression of Gal-3 between cases of FA and FVPTC (p-value <0.001), where 91.67% of FVPTC cases had high GAL-3 compared to none of FA cases expressed GAL3, (**Table.3**). No statistically significant difference in the expression of Gal-3 was detected between cases of FA and NIFTP (p-value = 0.327), **(Table.4**)

Table 3. Difference of Gal-3 IRS between FVPTC and FA

Variables		Histopathology		P value
		FA (n=4)	FVPTC (n=24)	
		Number (%)	**Number (%)**	
GAL-3 expression	Low	4 (100%)	2 (8.33%)	<0.001*
	High	0 (0%)	22 (91.67%)	

*Chi-square test

Table 4. Difference of Gal-3 IRS between NIFTP and FA

Variables		Histopathology		P value
		FA(n=4)	NIFTP (n=20)	
		Number (%)	**Number (%)**	
GAL-3 expression	Low	4 (100%)	16 (80%)	0.327
	High	0 (0%)	4 (20%)	

*Chi-square test

Association between Gal-3 expression and clinic-pathological features

Statistical analysis using Student t-test, and One-way ANOVA demonstrated Gal-3 expression in relation to different pathological variables such as age, gender, T staging of FVPTC, and extra-thyroid extension of FVPTC, (**Table.5**)

Table 5. Clinicopathological characteristics based on Gal-3 expression

Histopathology		GAL-3 IRS				P value
		Low expression (n=22)		**High expression (n=26)**		
		No.	**%**	**No.**	**%**	
Age (years)	**≤40**	**18**	**81.8%**	**14**	**53.8%**	**0.041***
	>40	**4**	**18.2%**	**12**	**46.2%**	
Gender	**Male**	**0**	**0%**	**2**	**7.7%**	**0.184**
	Female	**22**	**100%**	**24**	**92.3%**	
Tumor Stage (PTC)	**T1**	**2**	**100%**	**6**	**27.3%**	**0.232**
	T2	**0**	**0%**	**6**	**27.3%**	
	T3	**0**	**0%**	**6**	**27.3%**	
	T4	**0**	**0%**	**4**	**18.2%**	
Extra-thyroid extension (PTC)	**Positive**	**0**	**0%**	**12**	**54.5%**	**0.140**
	Negative	**2**	**100%**	**10**	**45.5%**	

*Chi-square test

Discussion

In this study, we collected 48 cases of FA, NIFTP, and FVPTC with a mean age of 36.79 ± 9.525 years, This finding was in agreement with a study done by **Priyadarshini et al. (2023)** that recorded that the age varied from the third to the eighth decade, with the most prevalent age for benign and malignant thyroid lesions being the fourth decade.

However, the study of Fu et al., 2021 revealed that the examined cases with FA, NIFTP, and FVPTC had a mean age of 50.1 years. This could be due to

different population pyramids in developing countries. **(Fu et al., 2021)**

In the present work there was female predominance, as female to male ratio was 95.83% to 4.20%, this finding was in agreement with a study done by **Sara et al., 2019** that found the male to female ratio in the FVPTC and NIFTP cases ranged from 3.33 to 67.77, and the study of **Dina et al, 2019** revealed that 45 (90%) out of the 50 PTC patients were female, and 5 (10%) were male. This also agrees with the results of study done by **Sung-IM et al, 2017** who found that of the 110 PTC patients, 75 (68.2%) were female. There is significant uncertainty regarding the cause of the gender gap. The gender gap in thyroid cancer cases has been attributed to reproductive factors and the presence or absence of estrogen receptors, which may be hormonal in nature. **(Sung-IM et al., 2017)**

However, other study revealed that the number of men and women of PTC was equal. as noticed by ***Fang*** **et al., 2021** who found a female to male ratio is 1.3:1. This might be due to different sample sizes between different studies. **(Fang et al., 2021)**

In our study, we detected that, there was a statistically significant difference in the expression of Gal-3 between cases of FA and FVPTC (p-value <0.001), where 91.67% of FVPTC cases had high Gal-3 compared to 0% in FA cases and no association of Gal-3 expression between cases of FA and NIFTP (p-value = 0.327). This finding was in agreement with the results of study done by **Fu et al.(2021)** which found that there was no significant correlation found between NIFTPs and benign lesions (p = 0.064), but invasive FVPTCs had considerably higher levels of Gal-3 expression than NIFTPs and benign nodules (p-value < 0.001, < 0.001), respectively. This also agrees with the results of study done **by Haeyon et al.(2018**)which revealed that infiltrative FVPTC exhibited considerably higher levels of Gal-3 expression compared to encapsulated FVPTC.

However, Our results were in disagreement with the study done by **Feilchenfeldt et al.(2003)** as they found that the expression of Gal-3 has been observed in every instance of PTC, as well as in certain benign thyroid lesions, 1/8 of hyperplastic nodule cases, and 1/5 of FA cases. Gal-3 RNA levels in benign and normal thyroid tissue range from 0.03 to 2.75 amol/µg, which could explain this observation. Additionally, positive results in benign thyroid lesions could indicate problems if cytological control is not carried out. **(Feilchenfeldt et al., 2003)**

The current results detected that Gal-3 expression is significantly associated with the age of the studied cases (p value= 0.041), where 81.8% of cases with low GAL-3 expression were younger than 40 years, compared to 53.8% among cases with high GAL-3 expression.

Our study detected that no significant difference in Gal-3 expression and gender or extra thyroid extension (p value = 0.184 and 0.14) respectively. This was in agreement with Tokmak et al., 2021 who found that there was no statistically significant correlation between Gal-3 expression and the clinicopathological parameters. Israa et al., 2021 detected that in thyroid cancer samples, there was no statistically significant correlation seen between the staining intensity of either HBME1 or Gal-3 and gender, or tumor size. They reported that Gal-3 is not a reliable marker of limited metastatic spread or extra thyroid extension of PTC since only a small percentage of positive

instances of Gal-3 expression predict extra thyroid invasion. (Israa et al., 2021) The contradictory findings in the literature show that more research is necessary to definitively identify the association between Gal-3 and clinicopathologic characteristics like (age, gender, and extra thyroid extension).

No statistically significant association between Gal-3 expression and T staging of FVPTC (p value = 0.232), this finding was in agreement with the results of two studies, one done by Kusuhara et al., 2021 who found that no significant association between Gal-3 expression and age, sex, tumor size of NSCLC and smoking habits, and the other done by Shuster et al., 2024 that revealed no statistically significant association between Gal-3 expression and NSCLC stage (p = 0.806). Our results were in disagreement with a study done by Saraswati et al., 2022 that found a correlation between Gal-3 expression and pathological stage (p=0.012) and nodal metastasis (p= 0.013). This discrepancy may be owing to the small number of cases in our study.

Conclusion

Gal-3 was highly expressed in FVPTC compared to FA and NIFTP so it can be helpful in the diagnosis of FVPTC.

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