Section: Pathology

Original Research Article

EXPRESSION OF GALECTIN-3 AND CD117 IMMUNOHISTOCHEMICAL MARKERS IN SURGICALLY EXCISED THYROID LESIONS

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Abstract

Background: Thyroid nodules are common in the general population. While the majority of thyroid neoplasms are benign and can be distinguished by their cellular and architectural features, diagnostic difficulties arise due to overlapping histomorphological characteristics. Galectin-3 plays a role in the processes of apoptosis, malignant transformation, and the spread of tumour cells. CD117 is present in normal thyroid follicular cells, and loss of its expression has been associated with cancer. The aim is to evaluate the expression of CD117 and Galectin-3 IHC markers in surgically excised thyroid specimens. **Materials and Methods:** A Prospective study was conducted in the Department of Pathology of Dr. B.R. Ambedkar Medical College and Hospital, Bangalore over a period of one year. Immunohistochemistry staining of galectin-3 and CD117 was done in 40 thyroid lesions. The data was analysed using SPSS for windows version 22.0, 2013. The level of significance was set at $P<0.05$. **Result:** The mean age was 39.98 ± 9.89 years with predominance of females. Out of 40 cases, 24 were benign, 3 were NIFTP and 13 were malignant lesions. Galectin-3 positivity was noted in 12 malignant neoplasms, 4 benign and 1 NIFTP case. CD117 positivity was seen in 20 benign, 2 NIFTP and 1 malignant case. The sensitivity and specificity for detecting malignancy for Galectin 3 and CD117 were 81%, 84%, 80%, 83%, respectively. The p value for both IHC markers was statistically significant. **Conclusion:** Galectin-3 is found to be a good marker of thyroid malignancy, especially in the diagnosis of papillary thyroid carcinoma. CD117 is found to be a better marker to indicate the benign nature of the tumour. This combined panel of markers were very useful in discriminating malignant from benign thyroid lesions.

INTRODUCTION

Of all endocrine cancers, thyroid carcinoma is the most common. It accounted for 586 000 instances of cancer globally in 2020, making it the ninth most common cause.[1] In India, it is widespread, with a prevalence of 12.2% and an incidence of about 3 per 100,000.[2-4] The vast majority of thyroid nodules are non-neoplastic and only 5-25% of thyroid nodules are true neoplasms.[3] It can be difficult to rule out cancer in certain situations due to aberrant nuclear staining caused by inadequate fixation and tissue processing.[5-7] Thyroid lesions with a follicular pattern can occasionally be challenging to classify. Focused minimum capsular invasion is a common problem that requires examination of multiple deeper areas.[8,9] Therefore, in order to prevent incorrect diagnosis, it is necessary to find markers that will aid in distinguishing between benign and malignant thyroid neoplasms. CD117 (c-KIT) is a type III receptor tyrosine kinase.[12-16] Aberrations in CD117 expression and signaling, including overexpression and reduced/absent expression, have been well characterized in several tumours, including gastrointestinal stromal tumours (GISTs).[13-17] However, few studies have investigated CD117 in the thyroid gland or in thyroid malignancies.[3,12,14-16] These findings indicate that CD117 may be involved in the maintenance of benign follicular cells and that this function may be lost during malignant transformation. Galectin-3 is a member of lectin family. Investigators have found Galectin-3 expression importance in discriminating between benign and malignant thyroid nodules.[18-21] LGALS

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3 gene was found to be up regulated in Papillary Thyroid Carcinoma compared to normal thyroid. It is expressed in nucleus, cytoplasm, cell surface, and extracellular space. It has been shown to be involved in the following biological processes: cell adhesion, cell activation and chemoattraction, cell growth and differentiation, cell cycle, and apoptosis.[22]

Therefore, the aim of the present study was to determine the expression of CD117 and Galectin-3 markers in various surgically excised thyroid lesions.

MATERIALS AND METHODS

This was a prospective study, conducted in the Department of Pathology in Dr. B.R. Ambedkar Medical College and Hospital, Bangalore from July 2023 to June 2024 after getting approval from the Institutional Ethics Committee. All the excised thyroidectomy specimens of all age and gender were included. Autolysed/inadequate specimens or those who were not willing to participate in the study, were excluded from the final sampling. Informed consent was taken from all patients in their own vernacular language. A detailed clinical history and physical examination was carried out on patients.

Ten percent formalin-fixed, paraffin-embedded blocks routinely prepared from representative areas containing tumour and adjacent normal tissues were selected. Two sections of 4 microns' thicknesses were prepared from the corresponding paraffin blocks, one on albumin coated slide for H&E staining and the other on poly-L-lysine coated slide for immunohistochemical staining.

The kits for Galectin-3 mouse monoclonal antibody and CD117 primary polyclonal rabbit anti-human immunohistochemical staining were obtained. Staining was done and positive control was obtained for each markers. Cytoplasmic/nuclear staining of Galectin-3 in cells was regarded as positive. The staining intensity was noted on a scale ranging from 0 to 3 where 0, 1+, 2+, and 3+ indicate no staining, weak 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).[19,20]

For CD117, Cytoplasmic staining in cells was regarded as positive. Staining percentages of >10% was accepted as positive. Staining extent was scored as (1) when 10–50% of the cells were stained, and (2) when >50% were stained. Staining intensity was assessed solely in positive cases and rated as (1) for weak staining (faint, light yellow), (2) for moderate staining (brown), and (3) for strong staining (dark brown). For negative cases, the score was accepted as zero.[18]

Statistical analysis: The statistical analysis was performed with Statistical package for social sciences (SPSS) windows version 22. Chi square test was used to compare the Galectin-3 and CD117 expression based on the type of thyroid lesions. The level of significance was set at P value <0.05.

RESULTS

The Histopathological examination was carried out in 40 cases of thyroid lesions. Each case was evaluated for the immunohistochemical staining proportion and intensity of Galectin-3 and CD117. The study patients ranged in age from 20 to 60 years. The majority fell within the 31–40 years of age group, accounting for 47.5% (n=19) of the population. The mean age of the participants was 39.98 ± 9.89 years. [Table 1] A significant majority of the study population was female, comprising 87.5% (n=35) of the participants, while males represented only 12.5% (n=5). This indicates a predominance of female subjects within the study. [Table 1]

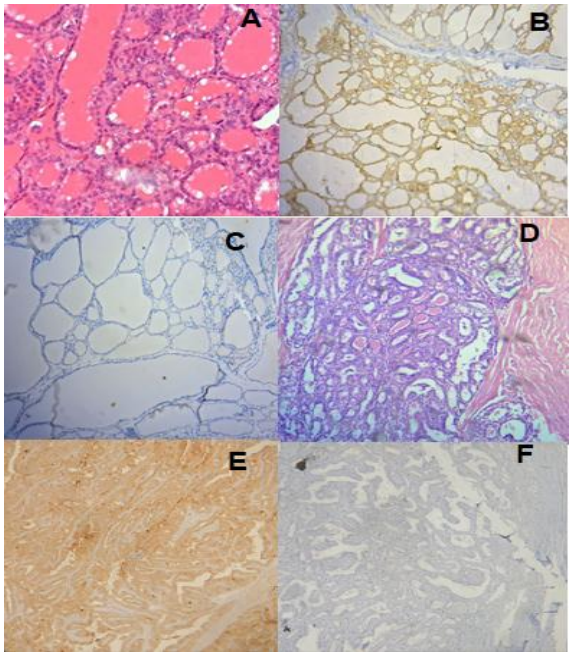

Figure 1: a) Multinodular goitre. H&E 100X; b) Galectin-3 negativity. 100X; c) CD117 strong positivity. 100X; d) Papillary thyroid carcinoma. H&E 100X; e) Galectin-3 strong positivity. 100X; f) CD117 loss of expression. 100X.

Table 1: Distribution of Age & Gender among study patients.

Variable	Category	n	%
Age	20-30 yrs.	5	12.5%
	31-40 yrs.	19	47.5%
	41-50 yrs.,	9	22.5%
	51-60 yrs.	7	17.5%
		Mean	SD
	Mean	39.98	9.89
	Range	20 - 58 yrs.	
Gender	Males	5	12.5%

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	Females	35	87.5%

Among the study population, the majority of lesions were categorized as benign, accounting for 60.0% (n=24) of the total cases. This suggests that non-malignant conditions are the most commonly observed type of thyroid lesion in this cohort. NIFTP were identified in 7.5% (n=3) of the patients. Malignant lesions comprised 32.5% (n=13) of the cases, highlighting a substantial prevalence of cancerous conditions in the study group. [Table 2]

Table 2: Distribution of Type of Thyroid Lesion among study patients

Variable	Category	n	%
Type of Lesion	Benign	24	60.0%
	NIFTP	3	7.5%
	Malignant	13	32.5%

The distribution of histopathological diagnoses among the patients reveal a diverse range of thyroid conditions. Nodular goitre was the most prevalent diagnosis, accounting for 42.5% (n=17) of the cases. This highlights the prominence of non-cancerous nodular conditions. Papillary thyroid carcinoma was the next most common diagnosis, observed in 30.0% (n=12) of the patients. This finding underscores the significance of this malignancy as a frequent thyroid cancer type. Follicular adenoma was identified in 17.5% (n=7) of the cases. Non-invasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) was seen in 7.5% (n=3) of cases, suggesting its relatively rare presentation. Medullary carcinoma, a more uncommon thyroid malignancy, was observed in 2.5% (n=1) of the patients, reflecting its lower prevalence within the population. [Table 3]

Table 3: Distribution of Histopathological Diagnosis among study patients

Variable	Category	n	%
Histopathological Diagnosis	Follicular adenoma	7	17.5%
	Nodular goitre	17	42.5%
	NIFTP	3	7.5%
	Medullary Carcinoma	1	2.5%
	Papillary thyroid carcinoma	12	30.5%

Expression of Galectin-3: In our study, Malignant group involved 12 Papillary Thyroid Carcinomas (PTC), one Medullary Thyroid Carcinoma (MTC). Positive staining was noted in all cases of Papillary thyroid carcinoma. Among 24 benign cases, 3 nodular goitre, 1 follicular adenoma and 1 case of NIFTP were positive. Galectin-3 was negative in one case of medullary thyroid carcinoma, 14 nodular goitre, 6 follicular adenomas and 2 NIFTP cases. [Figure 1]

Expression of CD-117: Out of 24 benign lesions, 17 were nodular goitre and 7 were follicular adenoma. Out of these, 14 cases of nodular goitre and 6 cases of follicular adenomas expressed CD117. 2 cases of NIFTP showed CD117 positivity. All malignant cases showed loss of CD117 expression except for 1 case of papillary thyroid carcinoma, which showed faint positivity. [Figure 1]

The frequency distribution of Galectin-3 and CD-117 expressions among various thyroid lesions revealed key diagnostic patterns. Galectin-3 showed a distinct expression profile, displaying positive expression, making it a highly specific marker for papillary thyroid carcinoma. In contrast, Galectin-3 negativity is predominantly observed in Nodular Goitre and Follicular Adenoma, with minimal negative expression in Papillary Thyroid Carcinoma. Meanwhile, CD-117 showed positivity more commonly with benign conditions. CD-117 negativity, on the other hand, is predominant in Papillary Thyroid Carcinoma.

The distribution of these markers emphasize the diagnostic relevance of Galectin-3 for Papillary Thyroid Carcinoma and highlights the role of CD-117 in identifying benign thyroid lesions such as Nodular Goitre and Follicular Adenoma. This information underlines the utility of these markers in differentiating between benign and malignant thyroid conditions. The p value of galectin 3 and CD117 were statistically significant in present study. [Table 4]

Table 4: Frequency distribution of Galectin-3 & CD-117 positivity among different thyroid lesions

Markers (p value)	Follicular adenoma		Nodular goitre		NIFTP		Medullary Carcinoma		Papillary thyroid carcinoma	
	n	%	n	%	n	%	n	%	n	%
Galectin-3 (p<0.001)	1	14.0%	3	17%	1	33%	0	0.0%	12	100%
CD117 (p<0.001)	6	85%	14	82%	2	66%	0	0.0%	1	8%

Table 5: The Overall Analysis of Galectin 3 and CD-117 among different thyroid lesions

Marker	AUC	p-value	Sn	Sp	PPV	NPV	Accuracy

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Galectin-3	0.82	<0.001	81%	84%	77.0%	87.0%	83.0%
CD-117	0.80	<0.001	80%	83%	86.9%	76.5%	82.0%

DISCUSSION

Histopathological examination is the cornerstone to make an absolute diagnosis in thyroid lesions. The spectrum of thyroid neoplasms ranges from benign follicular adenoma (FA) to low-risk neoplasms with borderline or uncertain behaviour to malignancies. Low-risk neoplasms consist of non-invasive follicular thyroid neoplasms with papillary-like nuclear features (NIFTP), thyroid tumours of uncertain malignant potential, and hyalinizing trabecular tumours. The malignant thyroid tumours originating from follicular cells include papillary thyroid carcinomas (PTC), follicular thyroid carcinomas (FTCs) and poorly differentiated thyroid carcinomas (PDTCs). Anaplastic thyroid carcinoma (ATC) is an undifferentiated malignancy of follicular thyroid cell origin, showing one of the most aggressive clinical behaviour among human cancers. Medullary thyroid carcinoma (MTC) arises from parafollicular C cells that produce calcitonin. Since thyroid cancers comprise a wide variety of tumours that differ in their molecular and histological characteristics as well as in clinical behaviour, it is essential to identify robust biomarkers for precise diagnosis and treatment. IHC markers are also helpful in improving the categorization of thyroid nodules.[23] Most of the studies in literature have evaluated a panel of positive immune markers for diagnosing this thyroid malignancy. We evaluated the expression of Galectin-3 and CD117 in thyroid lesions to assess their validity for categorising thyroid lesions into benign and malignant category. The present study was done on 40 Thyroid lesions. Mean age of patients in the present study was 39.98 ± 9.89 years.

The most common staining pattern of Galectin-3 observed in our study was both cytoplasmic and nuclear positivity which agrees with the other studies.[24-26] In our study, Galectin -3 showed a strong cytoplasmic positivity for 12 out of 13 malignant thyroid lesions. The overall sensitivity and specificity of Galectin-3 reported in the studies done by Dixit et al. and Soremekun et al. were similar with our observations. Galectin-3 is a sensitive marker for thyroid malignancy, but its specificity has been debated by some authors.[27-36] [Table 6] highlights the study done by other authors:

Table 6: Comparative analysis of Galectin-3 positivity thyroid lesions in other studies.

Studies	Nodular Goitre	Follicular Adenoma	NIFTP	Medullary thyroid carcinoma	Papillary thyroid carcinoma
Prasad ML et al.[28]	18%	10%	-	-	94%
Tastekin E et al.[33]	0%	0%	15%	-	85%
Dunderovic et al,[34]	13%	40%	-	-	92%
Soremekun et al,[36]	-	24%	-	33%	95%
Borkar et al,[37]	-	10%	-	0%	100%
Abouelfadl et al,[39]	-	16%	21%	-	86%
Present study	17%	14%	33%	0%	100%

CD117 is a well-established marker for various tumours like GIST. Although few studies have evaluated the role of CD117 in thyroid gland lesions, but there is deficiency of literature documenting the utility of CD117 in thyroid lesions. Pusztaszeri et al evaluated CD117 immune expression in thyroid cases and reported that CD117 gene expression is lost when the normal thyrocytes transform into PTC (Papillary thyroid carcinoma) and showed 100% positivity of CD117 in thyroid benign lesions.[30,38]

In present study, overall sensitivity and specificity of CD117 for diagnosing benign thyroid lesions was found to be 80% and 83%, respectively. These findings suggest that CD117 can be used as a potential negative marker for thyroid malignancies. Dixit et al found both sensitivity and specificity of CD117 to be 100% for diagnosing PTC of thyroid which is similar to our study.3 Our results confirm the concept presented by Pusztaszeri et al that the combination of CD117 with additional positive markers like Galectin-3 may enhance the positive and negative predictive value for diagnosis.[30]

Table 7: Comparative analysis of CD117 positivity thyroid lesions in other studies.

Studies	Nodular Goiter	Follicular Adenoma	NIFTP	Medullary thyroid carcinoma	Papillary thyroid carcinoma
Pusztaszeri et al,[30]	100%	100%	-	-	0%
Natali et al,[14]	56%	64%	-	-	5%
Dixit et al,[3]	100%	100%	-	-	0%
Present study	82%	85%	66%	0%	8%

In summary, both Galectin-3 and CD-117 show good diagnostic accuracy. For Galectin-3, negative expression was consistently observed in most of the benign and NIFTP cases, while malignant cases demonstrated a notable increase in positive expression levels. This trend suggests that higher Galectin-3 expression is strongly associated with malignant thyroid lesions, making it a valuable

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indicator for distinguishing them from non-malignant cases. In the case of CD-117, benign lesions primarily exhibited moderate or strong expression. In contrast, malignant lesions consistently displayed negative expression of CD-117. This pattern indicates that the loss of CD-117 expression is a characteristic feature of malignant cases, while its presence in varying intensities may be indicative of benign condition.[40]

CONCLUSION

Overall, the distinct expression patterns of these Galectin-3 and CD117 across the cases provide valuable insights into their diagnostic relevance. Galectin-3 appears to be more sensitive in detecting malignant lesions, whereas CD-117 offers high specificity towards benign lesions, making them complementary markers for identifying and classifying thyroid lesions. These findings underscore the importance of incorporating such markers into diagnostic protocols for improved accuracy and precision.

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