ORIGINAL ARTICLE

Significance of immunohistochemical expression of HBME1 and galectin-3 in differentiation of papillary thyroid carcinoma from benign hyperfunctioning lesions of thyroid with papillary architecture

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ABSTRACT

Papillary thyroid carcinoma is the commonest type (75%-85%) of thyroid malignancy. Females are more affected than males. It can develop at any age group, but the mean age approximately 40 years. the overall 5-year survival rate for papillary thyroid cancer is 96%, with a 10-year survival rate of 93%. Our aim in this study was to evaluate the expression of HBME-1 and galectin-3 proteins in both papillary thyroid carcinoma and hyperfunctioning lesions by Immunohistochemistry and to correlate the expression of both markers in PTC with other clinico-pathological parameters. This study included 60 samples, 30 hyperfunctioning thyroid samples and 30 samples of papillary thyroid carcinoma. Immunohistochemical expressions of HBME-1 and galectin-3 were examined in each case. HBME-1 expression was positive in 100% of PTC samples (30 samples), while it was positive in 13.3% (4 of 30 samples) of hyperfunctioning thyroid lesions. Galectin-3 expression was positive in 83.3% (25 of 30) of PTC samples and 16.7% (5 cases) of hyperfunctioning thyroid samples. HBME-1 and galectin-3 were very sensitive for PTC. Both markers can be used for distinction between benign and malignant lesions. An association of increased HBME-1 expression in primary PTC with the presence of lymph node metastasis, extra thyroid invasion, and tumor size of > 2cm underlines the clinical relevance of its use as independent predictive factor for poor prognosis, but there is no relation between galectin-3 expression and the clinicopathological features of PTC.

Keywords: HBME-1, galectin-3, immunohistochemistry, papillary thyroid carcinoma.

INTRODUCTION

Papillary thyroid carcinoma is the commonest type of thyroid malignancy and comprises about “75%–85%” of thyroid malignancies. In “United States”, PTC encompasses about 1% of all cancers and accounts for 0.2% of cancer deaths. (LiVolsi, 2011)This cancer embraces the furthermost communal malignant growth of thyroid in countries consuming iodine-adequate or iodine-excess nourishments. It is the most cancers that identified in patients in the “third to fifth decades” of life. Men are affected less than women in ratios of 4:1 to 2:1. (Baloch et al., 2010) this cancer type in children represent more than 90% of thyroid malignancies, and the history of exposure to “irradiation” to the neck shown to be found in 5-10% of malignant cases. (Williams et al., 2008) The overall 5-year survival rate for papillary thyroid cancer is 96%, with a 10-year survival rate of 93%. In “one large series, localized disease to the gland was seen in 67% of cases, while within the thyroid gland and “lymph nodes in 13%”, and “lymph nodes only seen in 20% of cases”. (Livolsi et al., 2004)the principal tumor size ranges from very small size (microscopic) to very large (huge), most cases of PTC are solid, firm, whitish, and clearly invasive; fewer than 10% are surrounded by a complete capsule. (NADU, 2018) the typical section of papillary carcinoma contains numerous true papillae with a central fibrovascular core and a lining of “cuboidal cells” which is single or stratiform. (Lin et al., 2011) The diagnosis of papillary carcinoma trusts on the existence of either “papillary architecture or nuclear features” which embrace “optical clearing, elongation, overlapping, micro-nucleoli” and irregular delineations with furrows and pseudo-inclusions. (Lloyd, 2010) Gene rearrangements occur in 20–40% of “papillary thyroid carcinomas”, and the large proportion of them involve RET rearrangement. (Eusebi et al., 2004) **Benign hyperfunctioning lesions** of thyroid gland which have papillary architecture include: multinodular goiter (MNG) and Graves’ disease. MNG: nodular hyperplasia is the most common thyroid disease. It is an enlarged thyroid gland that will have discrete collisions (nodules) on it. The cause is generally unknown. Multinodular goiters are related with an advanced risk of thyroid cancer, and found in two forms: in the form named endemic goiter, the disease is due to low iodine content of the water and soil with frequency at post mortem examination is equal to100% (IJOMONE, 2010), In the form named as sporadic (nodular) goiter which is the greatest communal form seen in the “United States”. The incidence of the disease in adult population is 3–5% clinically and about 50% at autopsy. (Razy et al., 2019) Clinically, furthermost “multinodular goiters” do not cause symptoms and are revealed by routine physical exam or through a test made for another purpose. Patients who have a “toxic multinodular goiter” may expert signs and symptoms of “hyperthyroidism”. (Gullo et al., 2018). The cut surface revealed several brownish or reddish and white nodules that varied in sizes. Histologically, the nodules contained follicles for varied sizes, mostly without capsule. The follicular cells were either normal cuboidal or flattened squamous, with red blood cells occasionally observed within the colloid. (Duangsuwan et al., 2018) Graves’ disease: also known as diffuse toxic goiter, typically presents in young adult

females with goiter, muscle weakness, weight loss, irritability, tachycardia, and mostly a great increase of appetite. Exophthalmos (ophthalmopathy) presents in 25–50% of the patients. Atrial fibrillation may occur. (Goldblum et al., 2017) Graves' disease mostly presents in adults but can also occur in children. Grossly, the gland appears with a mild to moderate symmetric diffuse enlargement. It is succulent and reddish. The cut surface appears uniformly gray or red depending on the degree of vascularity and in longstanding cases, the gland appears friable and dull yellow (Perez-Montiel & Suster, 2008) Microscopically, the "follicular epithelial" cells are tall and packed more than typical lead to the creation of small "papillae", that scheme into the "follicular lumen" and intrude on the colloid, occasionally filling the follicles, and these "papillae" have no fibrovascular hubs, in disparity `to that of "papillary carcinoma". The "colloid" indoors the "follicular lumen" is pale, with crenated boundaries. (Tallini & Giordano, 2017) Graves' disease is currently considered as one of the autoimmune thyroid diseases is thought to be initiated by IgG antibodies directed against specific domains of the TSH receptor. (Kotwal & Stan, 2018)

Galectins are a set of proteins which evolutionarily preserved and found in "vertebrates, invertebrates, and fungi". These proteins have important "carbohydrate-recognition domains (CRD)" which has 130 types of amino acids, that give these proteins the capability to link "β-galactosides". Galectin family can be classified according to their structure into three groups: the first one known as prototype-galectins which include: galectins "1, 2, 5, 7, 10, 11, 13, 14, and 15" that have only one CRD; the second group known as "tandem-galectins include: galectins 4, 6, 8, 9, and 12" with two identical-CRDs; and the third cluster known as chimera-type group, which include only one member (Galectin-3 (Gal-3)), that has a "C-terminal CRD" with a huge "N-terminal (NT) protein-binding domain". (Halimi et al., 2014) Galectin-3 has characteristic structure among all galectins because it is the only member of this family encompasses a "C-terminal CRD linked to an N-terminal protein-binding domain", so it is unique and the chimeric galectin only. Human Gal-3 is prearranged by a single gene, "LGALS3", situated on chromosome 14, and it is stated in the "nucleus, cytoplasm, mitochondrion, cell surface, and extracellular space". (Argüeso & Panjwani, 2011) Galectin-3 is distributed broadly throughout the body; it may be present in a various tissue of the body mainly the gastrointestinal and urogenital tracts, kidneys, lungs, blood & heart. It is discovered with a high percent. in "myeloid cells (monocytes, macrophages, dendritic cells (DCs), neutrophils) and fibroblasts cells". At the level of the cell, gal-3 presents in the "cytoplasm, nucleus, and membranes". Many diverse functions have been exhibited by Gal-3 intracellularly, such as "antiapoptotic" activity and the guideline of "mRNA splicing", direction of mast cell signaling pathway. Gal-3 extracellularly (linked to membrane or free) contributes in a differ array of functions, such as defending the pathogens by the activation of immune system, and in inflammation (both acute and chronic). (Argüeso & Panjwani, 2011) The extensive variety belong to "galectin-3" effects on cancer cells are caused by the exclusive construction and several interaction possessions of the "galectin-3 molecule". Overexpression and fluctuations within "sub- & inter-cellular localization of galectin-3" are mostly shown in cancer circumstances. The numerous interfaces and linking properties of galectin-3 impact many cellular events based on its site. Alteration in the Gal-3 indorses cancer cell growing and differentiation, apoptosis, adhesion, angiogenesis, invasion and tumor spread "metastasis". (Song et al., 2014) Clinical applications of galectin3: 1- Indicator of Cardiovascular Risk (Fortuna-Costa et al. 2014), and 2-Biomarkers especially for thyroid carcinoma. (de Boer et al., 2009)

HBME-1(Hector Battifora Mesothelial -1marker): is a "monoclonal antibody" generated contrary to unidentified antigens prevailing the microvillous surface of mesothelial cells of mesothelioma. (De Micco et al., 2008) HBME-1 has been reported to be a talented biomarker in thyroid pathology and also a worldwide marker of malignant growth because of its high expression in numerous destructive tumors. Anti-HBME-1 tags "thyroid papillary carcinoma and follicular carcinoma" but not normal thyroid making it a appreciated marker for distinctive thyroid malignancies from "benign thyroid lesions". (Cui et al., 2012) It is tangled with cancer cell propagation and exodation. (Banco et al., 2011)

Aims of the study: this study aims to evaluate the Immunohistochemical expression of HBME-1 and galectin-3 proteins in hyperfunctioning lesions of thyroid and papillary thyroid carcinoma, and to differentiate the expression of both markers between the two disease groups. To correlate the results of both markers in PTC samples to some clinico-pathological parameters.

MATERIALS AND METHODS

A retrospective "cross-sectional" study was conducted on human thyroid tissue obtained from patients attending the hospital and labs after surgical removal of thyroid mass during the period from September 2019 to September 2020. The study was involved 60 specimens of patients diagnosed either with hyperfunctioning thyroid lesions (30 specimens) or with papillary thyroid carcinoma (30 specimens). Quality positive control: mesothelioma for HBME1 marker and papillary thyroid carcinoma for galectin-3 marker with each run. Negative control: It was done by deleting the primary antibody and adding antibody diluent (PBS) alone in the same slide and follows the same steps in immunohistostaining. Preparation of tissue section: a serial 4 micrometers thickness sections were obtained for each sample and three sections were taken, the first was stained with Hematoxylin and Eosin to confirm the diagnosis and to determine the histological types of the disease, the other two sections were put on positively charged slides for immunohistochemical staining with anti-HBME-1 and anti-galectin-3 antibodies, respectively. First, we should perform de-waxing of the slides in the oven at a temperature adjusted at 60-65ºC for 60 minutes then transferred to three xylene containers for 5 minutes each then rehydrated by immersing in alcohol concentration baths 100%, 90% and 70% for three minutes each, followed by washing the slides with distal water. Antigen retrieval solution to unmask antigens by heat treatment, with 50 mm Tris-EDTA buffered solution (pH 9.0) in water bath at 95°C for 30 mints. then Peroxidase block was applied to cover the whole specimen, and placed in the humid chamber and incubated at (37o c) for 10 mins. Then

the slides were incubated with protein block for 10 min in a humid chamber to eliminate nonspecific background block staining. Then pre-diluted primary antibody was placed into sections (1: 25 for HBME-1, Abcam code:ab2383; Mouse monoclonal antibody, and galectin-3 ready to use, Pathnsitu; Mouse monoclonal antibody) incubated at room temp. for 60 min in a humid chamber. The slides were washed with fresh PBS for 3 mints, twice. Then slides were "drained and blotted". Apply Goat anti-rabbit HRP-conjugate (horseradish peroxidase –Streptavidin conjugate) and incubate for 10 minutes in humid chamber. Substrate chromogen solution of diaminobenzene (DAB) were added on each section & incubated for (7-10 mints) in humid chamber, then counter stained with Hematoxylin. then the sections were dehydrated by immersing the slides sequentially in ethanol and xylene containing jars. Finally, Mounting and Examination under light microscope. **IHC quantitative scoring method**: The cells were scored as positive or negative staining depending on the presence of distinct brown cytoplasmic and/or membranous staining. The percentage of cells staining were evaluated using 10% as cut-of value. The intensity of staining scored as: 0, none; 1, mild; 2, moderate; 3, strong (Christensen et al, 2019). **Statistical analysis:** Data analysis was performed using IBM(R) SPSS(R) Statistics software for Windows, version 26 (2019) and presented using Microsoft(R) Excel(R) (2016) MSO. Continuous variables were expressed as mean ±standard deviation (SD) or median (interquartile range, 25-75% IQR) when applicable. Categorical variables were expressed as frequency percentage. Numerical data were presented using box. Comparisons were performed using two sample t-test in normally distributed data with equal variance or Mann-Whitney U test when the assumptions of two sample t-test were not met. Chi-square test was used to compare categorical variables. Statistical significance was set at P-value < 0.05 with 95% confidence interval (CI).

RESULTS

4.1 Age and Gender distribution / Discrepancy

There was a statistically significant difference of age among patients with thyroid hyperplasia and those with thyroid carcinoma (table 1, figure 1). Patients with hyperplasia had significantly lower age means than those with carcinoma.

Table 4.1: Difference in age (years) among patients with thyroid hyperplasia & carcinoma. (data presented as Mean±Standard deviation).

Case type	Age (years)	P-value
Hyperplasia	39.5±15.2	0.000
Carcinoma	54.7±15.3	

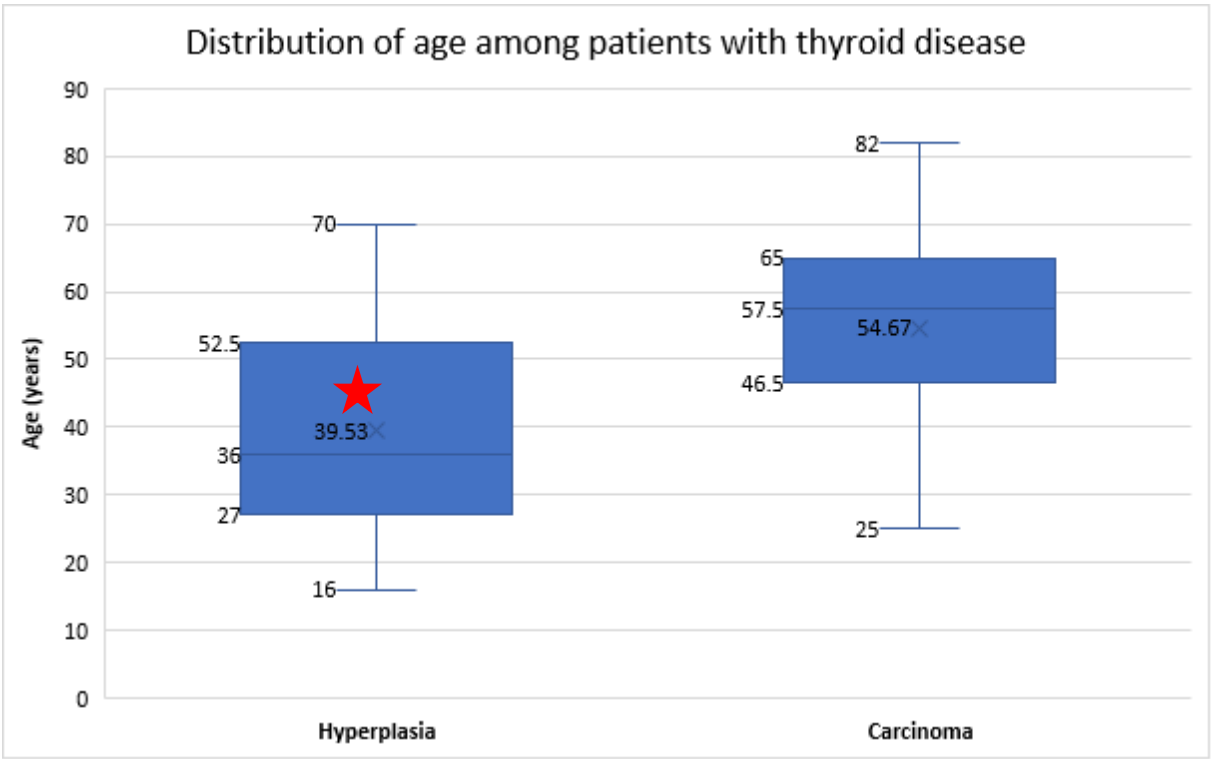


Figure 4.1: Box & whiskers graph showing the age (years) distribution among patients with thyroid hyperplasia & carcinoma. ("Red star" directs statistically significant difference," P<0.05)"

Regarding gender, there was no statistically significant difference in either group of patients (Table 2, figure2). In both groups, the predominant sex was females.

Table 4.2: Difference in sex among patients with thyroid hyperplasia & carcinoma. (Data presented as frequency percentage)

Gender	Hyperplasia	Carcinoma	P-value
Male	5(16.7%)	8(26.7%)	0.347
Female	25(83.3%)	22(73.3%)	

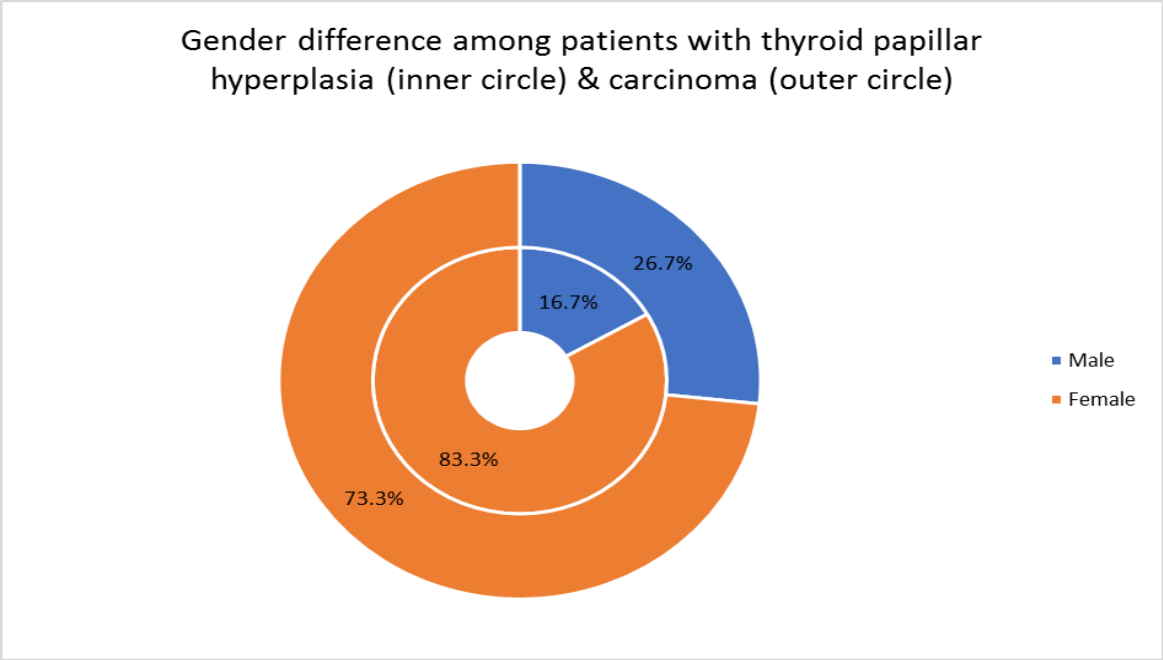


Figure 4.2: D onut chart showing gender distribution in patients with thyroid hyperplasia (inner circle) and carcinoma (outer circle).

4.2 Immunohistochemical marker staining discrepancies

Regarding HBME1, there was “statistically significant” difference in staining intensity between the groups “two”. Most samples of hyperplasia (86.7%) tested negative for staining (fig.4.14) and while most samples of carcinoma had a staining intensity of “+2” (66.7%) followed by “+3” (26.7%) fig 4.14. None of the cases for carcinoma tested negative for HBME1. (Table 3, figure 3)

Table 4.3: Difference in staining intensity of HBME1 marker in samples of thyroid hyperplasia & carcinoma. (Data presented as frequency percentage).

HBME1 staining intensity	Hyperplasia	Carcinoma	P-value
Negative	26(86.7%)	0%	0.000
+1	4(13.3%)	2(6.7%)	
+2	0%	20(66.7%)	
+3	0%	8(26.7%)	

Regarding galectin, slightly similar results were found. Most samples of hyperplasia (83.3%) tested negative and the rest had

Figure 4.3: Stacked column graph showing the difference in staining intensity of HBME1 in samples of thyroid hyperplasia and carcinoma. (“Red star” directs statistically significant difference”, “P<0.05) a staining intensity of “+1” as seen in fig.4.15. In carcinoma samples, most samples stained “+2” (66.7%), (fig 4.15) and only 16.7% tested negative (table4, figure 4).

Table 4.4: Difference in staining intensity of galectin marker in samples of thyroid hyperplasia & carcinoma. (Data presented as frequency percentage).

Galectin staining intensity	Hyperplasia	Carcinoma	P-value
Negative	25(83.3%)	5(16.7%)	0.000
+1	5(16.7%)	2(6.7%)	
+2	0%	20(66.7%)	
+3	0%	3(10%)	

Using 2X2 tables for sensitivity, specificity & accuracy, HBME1 showed significantly higher values for all three parameters as shown in table 5 /figure 5.

Table 4.5: Difference in sensitivity, specificity & accuracy of HBME-1 and galectin-3 staining in papillary thyroid carcinoma.
Figure 4.4: Stacked column graph showing the difference in staining intensity of Galectin in samples of thyroid hyperplasia and carcinoma. (Red star indicates statistically significant difference, P<0.05)

Validity Parameter	HBME1	Galectin
Sensitivity	100%	80%
Specificity	86.7%	83.3%
Accuracy	93.3%	81.7%

4.3 Factors affecting immunohistochemical staining in papillary thyroid carcinoma. **1.Age & gender:** Neither age (table6, figure 6) nor gender (table 7, figure 7) type had statistically significant effect on staining intensity of either HBME1 or Galectin in thyroid carcinoma samples. However, it is worth mentioning that "+3" staining intensity was associated with younger females for both makers.

Table 4.6: Difference in age (years) in relation to staining intensity of HBME1 & Galectin in patients with thyroid carcinoma. (data presented as Mean±Standard deviation).

HBME1 staining intensity	Age (years)	P-value	Galectin staining intensity	Age (years)	P-value
Negative	-	0.254	Negative	51.2±16.9	0.063
+1	53.5±4.95		+1	76±8.5	
+2	57.8±15.1		+2	55.6±12.5	
+3	47.1±15.97		+3	40±21.8	

Table 4.7: Effect of gender on staining intensity of HBME1 & Galectin in patients with thyroid carcinoma. (data presented as frequency percentage).

IHC markers staining		Male	Female	P-value
HBME1 staining intensity	+1	0%	9.1%	0.323
	+2	87.5%	59.1%	
	+3	12.5%	31.8%	
Galectin staining intensity	Negative	25%	13.6%	0.496
	+1	0%	9.1%	
	+2	75%	63.6%	
	+3	0%	13.6%	

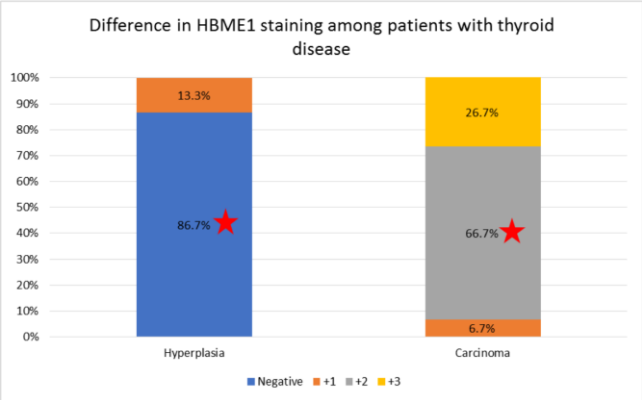


Figure 4.5: Clustered column graph showing the difference in sensitivity, specificity & accuracy of HBME1 and Galectin in thyroid carcinoma detection.

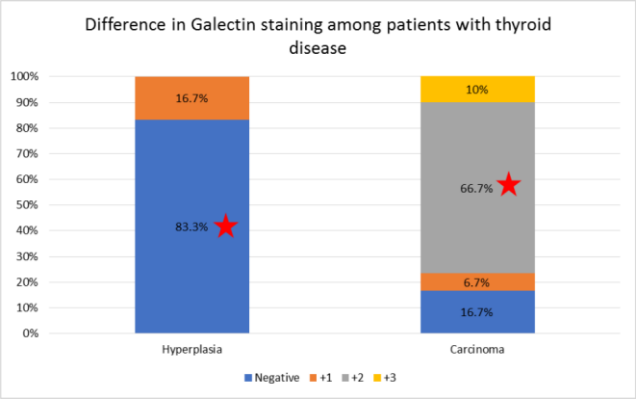


Figure 4.6: Clustered column graph showing the difference in age (years) in relation to staining intensity of HBME1 & Galectin in patients with thyroid carcinoma. (columns represent Mean, error bars represent Standard deviation).

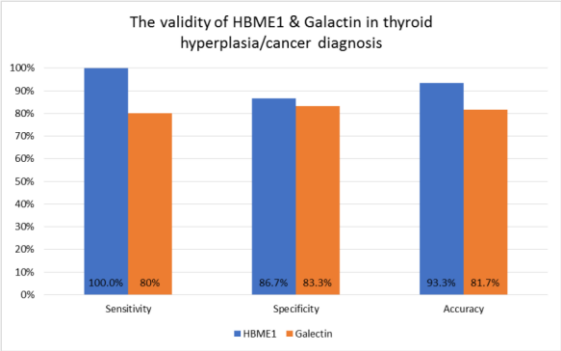
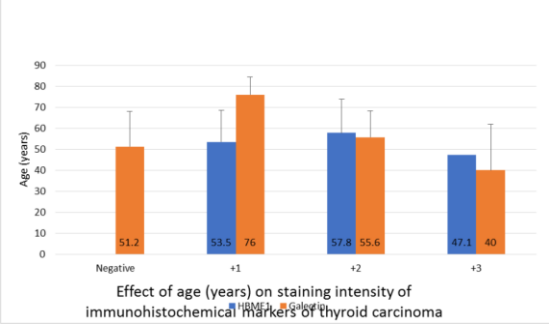


Figure 4.7: Clustered column graph showing effect of gender on staining intensity of HBME1 & Galectin in patients with thyroid carcinoma. (Columns represent frequency percentage).



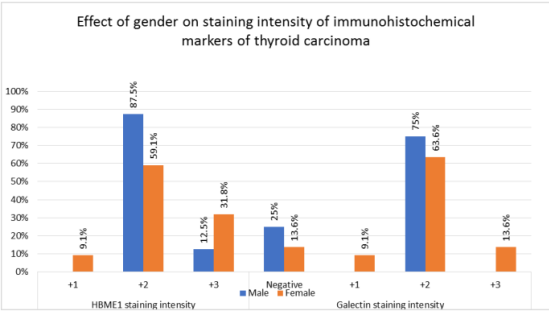


Figure 4.8: Column graph showing distribution of tumor size, lymph node involvement & extrathyroid metastasis in patients with thyroid carcinoma.

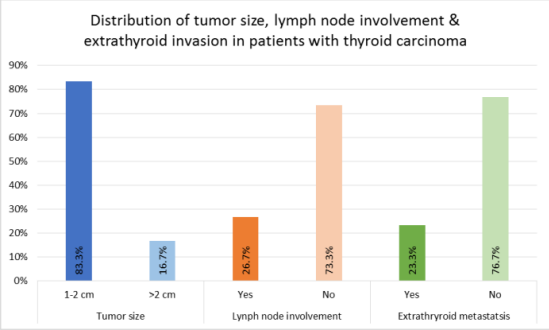


Figure 4.9: Clustered column graph showing effect of tumor size on staining intensity of HBME1 & Galectin in patients with thyroid carcinoma. (Columns represent frequency percentage. "Red star" directs statistically significant "difference", "P<0.05).

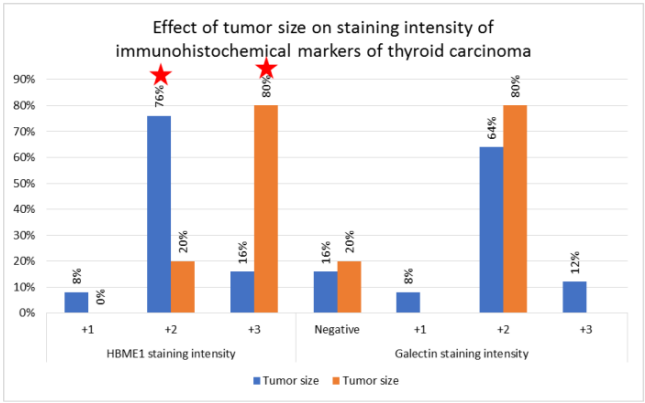
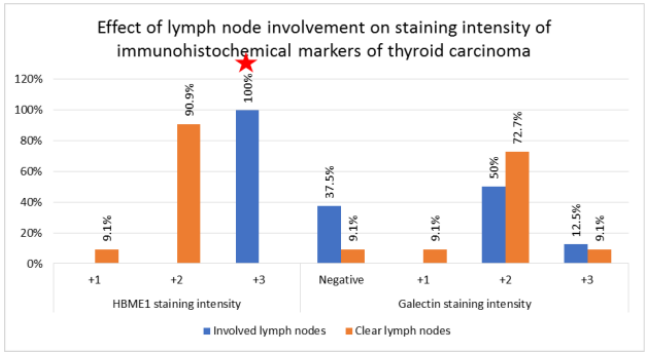


Figure 4.10: Clustered column graph showing effect of lymph node involvement on staining intensity of HBME1 & Galectin in patients with thyroid carcinoma. (Columns represent frequency percentage. "Red star" directs statistically significant "difference", "P<0.05).



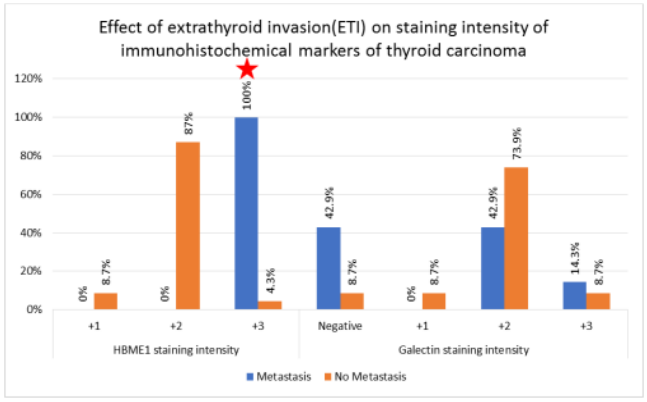


Figure 4.11: Clustered column graph showing effect of extra thyroid invasion on staining intensity of HBME1 & Galectin in patients with thyroid carcinoma. (Columns represent frequency percentage. "Red star" directs statistically significant "difference", "P<0.05).

2.Tumor related factors Figure 8 demonstrates the distribution of tumor size categories, lymph node involvement & extra thyroid invasion in the patients with thyroid carcinoma.

Tumor size, lymph node involvement and the presence of metastasis all had statistically significant effect on HBME1 staining but not on Galectin staining properties.
Most of tumor sizes greater than 2cm had a "+3" staining intensity for HBME1, those with sizes of 1-2cm mostly had a "+2" intensity. For Galectin, various staining intensities were fairly distributed in both tumor sizes (table 8, figure 9).

Table 4.8: Effect of tumor size (cm) on staining intensity of HBME1 & Galectin in patients with thyroid carcinoma. (data presented as frequency percentage).

IHC marker staining		Tumor size		P-value
		1-2 cm	>2 cm	
HBME1 staining intensity	+1	8%	0%	0.013
	+2	76%	20%	
	+3	16%	80%	
Galectin staining intensity	Negative	16%	20%	0.753
	+1	8%	0%	
	+2	64%	80%	
	+3	12%	0%	

Regarding lymph node involvement, all samples with positive node involvement tested with "+3" intensity for HBME1 while most of those with clear lymph nodes had "+2" intensity. For Galectin, the most frequent intensity was "+2" in both involved and clear lymph nodes (table 9, figure 10).

Table4.9: Effect of lymph node involvement on staining intensity of HBME1 & Galectin in patients with thyroid carcinoma. (data presented as frequency percentage).

IHC marker staining		Involved lymph nodes	Clear lymph nodes	P-value
HBME1 staining intensity	+1	0%	9.1%	0.000
	+2	0%	90.9%	
	+3	100%	0.0%	
Galectin staining intensity	Negative	37.5%	9.1%	0.252
	+1	0%	9.1%	
	+2	50%	72.7%	
	+3	12.5%	9.1%	

Also, in the case of extra thyroid invasion, all samples with ETI tested with "+3" intensity for HBME1 while most of those with no invasion had "+2" intensity. For Galectin, cases with ETI had equal frequencies of negative & "+2" results while those with no invasion were most frequently at "+2" intensity (table 10, figure 11).

Table 4.10: Effect of extra thyroid invasion on staining intensity of HBME1 & Galectin in patients with thyroid carcinoma. (data presented as frequency percentage).

IHC marker staining		Extra thyroid invasion	No extra thyroid invasion	P-value
HBME1 staining intensity	+1	0%	8.7%	0.000
	+2	0%	87%	
	+3	100%	4.3%	
Galectin staining intensity	Negative	42.9%	8.7%	0.15
	+1	0%	8.7%	
	+2	42.9%	73.9%	
	+3	14.3%	8.7%	

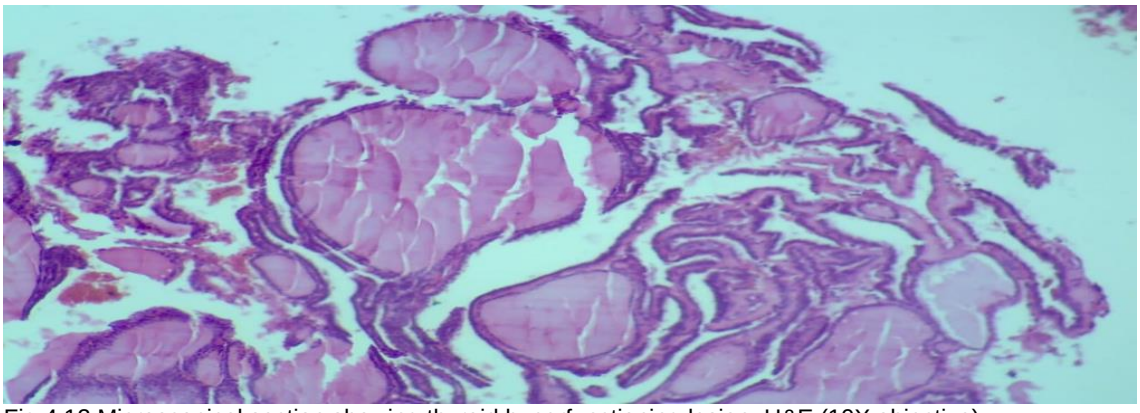


Fig 4.12 Microscopical section showing thyroid hyperfunctioning lesion, H&E (10X objective)

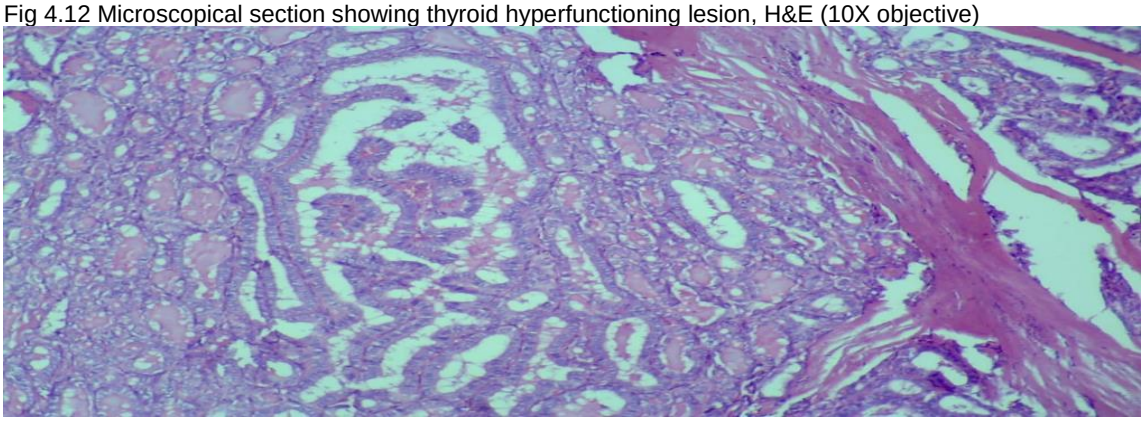


Fig 4.13 Microscopical section showing PTC H&E (10X objective)

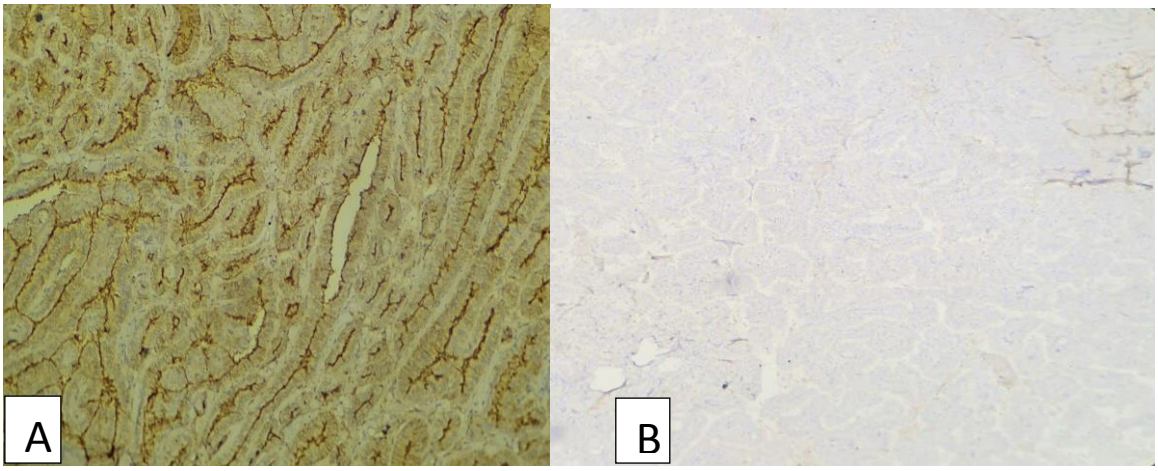


Fig 4.14 A- Microphotograph showing +ve IHC expression of HBME-1 in PTC, brownish discoloration of the membraneous stain, high intensity, 40X objective. B- Microphotograph showing -ve IHC expression of HBME-1 in hyperfunctioning lesion of thyroid, bluish discoloration of the membraneous stain, 40X objective

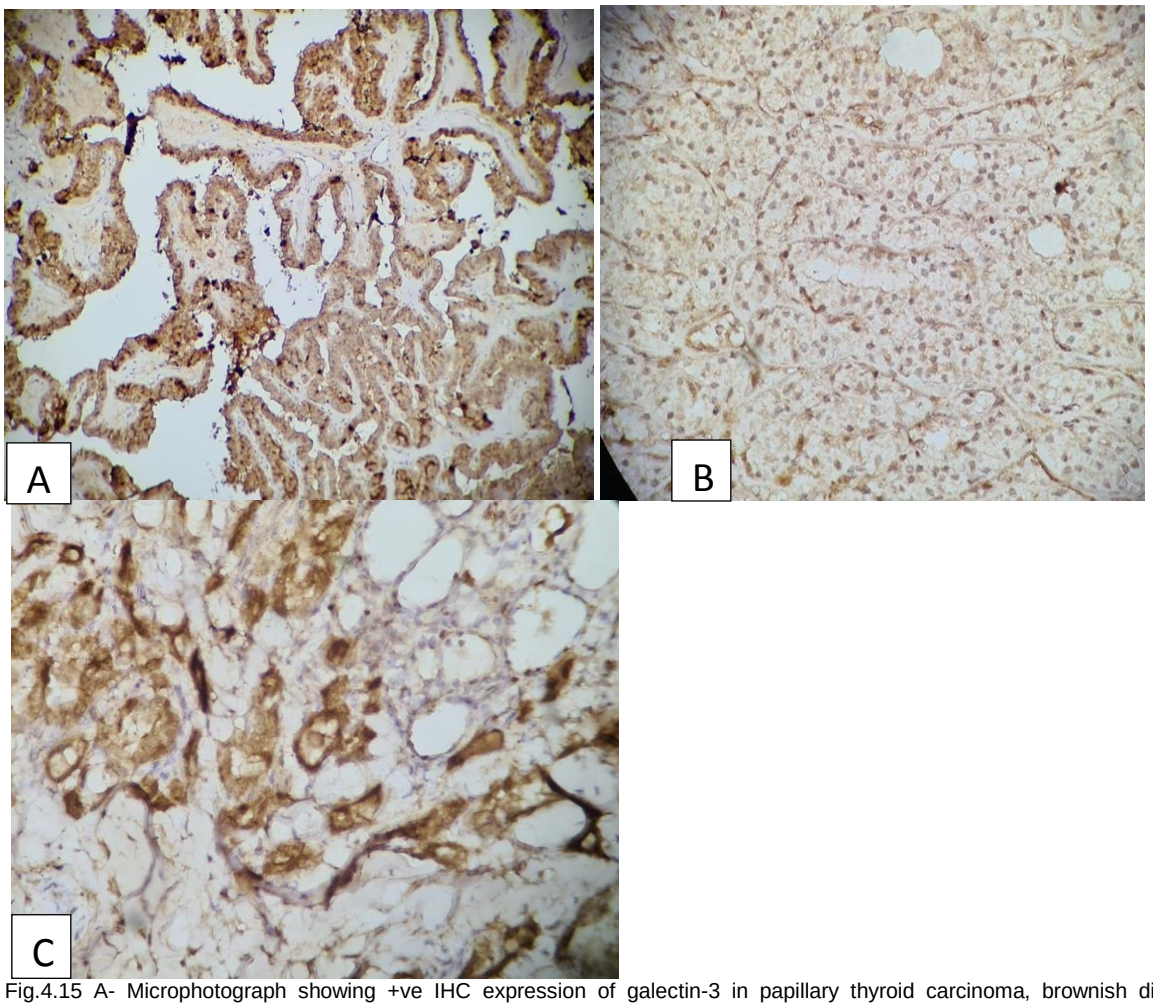


Fig.4.15 A- Microphotograph showing +ve IHC expression of galectin-3 in papillary thyroid carcinoma, brownish discoloration of the cytoplasmic stain, hihg intensity, 40X objective. B- Microphotograph showing -ve IHC expression of galectin-3 in hyperfunctioning thyroid lesion, bluish discoloration of the cytoplasmic stain, 40X objective. C- Microphotograph showing +ve IHC expression of galectin-3 in papillary thyroid ca, moderate staining of the cytoplasm, 40X objective.

DISCUSSION

"Papillary thyroid" carcinoma is the commonest thyroid malignancy, constitute "75% to 85%" of all cases of thyroid carcinoma. It documented more often in females and detected in the "20–55" years group of age. It is considered as the principal cancer type in "children" with this type of cancer. The "histopathology" of papillary carcinoma and papillary hyperplasia is parallel enough to produce a diagnostic "dilemma" in some cases. Both disease lesions may have "papillary" fronds with "fibrovascular" cores, nuclear flocking, and nuclear "anisocytosis". One of the most recurrent problems in thyroid "pathology" is the differentiation of hyperfunctioning thyroid lesions from "carcinomas", particularly those with papillary construction and nuclear "irregularity".

"In fact", the histological finding of an "encapsulated" thyroid nodule which have no "capsular" or "vascular" assault is not always easy, particularly when thyroid cells "thyrocytes" exhibit nuclear clearing and "pleomorphism" or papillary constructions as may be realized in "hyperfunctioning" lesions. In these conditions, the main discrepancy diagnosis is papillary carcinoma. The diagnosis of papillary carcinoma trusts on the existence of either "papillary architecture or nuclear features" which embrace "optical clearing, elongation, overlapping, micronucleoli" and irregular delineations with furrows and "pseudoinclusions", these features occasionally communal by benign hyperplastic lesions. In this study two immunohistochemical symbols ("HBME-1" and "galectin-3") were used to simplify the differential diagnosis amid "papillary" thyroid carcinoma and benign "hyperfunctioning" lesions.

Age and sex: in our study the mean age with SD for thyroid hyperplasia is 39-15.2 years while mean age for papillary carcinoma is 54 years, so there was significant difference "statistically" between them with a "p-value" less than 0.001 which mean that hyperfunctioning lesions with papillary changes were seen in younger age group than carcinoma cases.

This result was in agreement with study done by (Casey et al., 2003) who found the mean age for benign cases is 36 years while those for carcinoma cases is 51 years old, also agree with study done by (Liu et al., 2014) About sex we found that the female is affected more than

male in both groups of thyroid hyperplasia and papillary thyroid carcinoma which was in agreement with study done at (Hsieh et al., 2012) who also reported that the females were more distributed in both benign and malignant thyroid disorders.

The difference in gender distribution can be explained as it is exist due to sex hormones and reproductive factors that may impact the taxes of cancer cell "proliferation", exodus, or "apoptotic change" (thyroid "carcinogenesis"). Numerous epidemiologic studies propose that premature or late "menarche" increases the jeopardy of thyroid cancer by "50%" (Sakoda & Horn-Ross, 2002).

Immunomarkers and their role in differentiation between carcinoma and hyperplastic cases: immunohistochemical expression of HBME1 in our study demonstrates that all cases of papillary thyroid carcinoma are positive and most of them (66.7%) were +2 intensity staining while only 13.3% of hyperplastic cases are positive which with weakly staining (+1 intensity), so there was statistically momentous variance in staining among the two groups with p-value less than 0.001. (86.7%) for papillary carcinoma and can be regarded as a valuable indicator in discrepancy between "benign" and "malignant" lesions because Previous studies such as study done by (Roti et al., 2006) agree with our study that show 98.3% of hyperfunctioning lesions were negative while 92.8% of papillary carcinoma cases were positive. Another agreement with (Casey et al., 2003) that show 100% of papillary carcinoma cases express positive staining for HBME1 and 70% of hyperplastic cases were negative, so this immunohistochemical marker is sensitive (100%) and specific it act as antibody against abnormal antigens that found within the thyrocytes and exists during the process of tumorogenesis (thyroid carcinogenesis).

Galectin-3 expression in our study was 83.3% of hyperplastic samples were negatively stained and the remaining 16.7% were stained positive to but with +1 intensity (weak staining), while most samples of papillary carcinoma stained positively (83.3%), and only 16.7% of them were negative for this marker, and there was "statistically" important variance in the expression "of" this marker among hyperplasia & carcinoma (p-value <0.001), therefore galectin-3 can be regarded also as sensitive (88%) and specific (83%) immunohistochemical marker for distinction between "benign" and "malignant" lesions and aid in diagnosis "of" papillary thyroid carcinoma. In prior revisions on "gal-3" expression in "thyroid" disorders, there is agreement of our work with study of (Weber et al., 2004) which reported 100% sensitivity of galectin-3 for typical papillary thyroid carcinoma, another study of (Arcolia et al., 2017) demonstrates 97% sensitivity and 83% specificity of galectin-3 for papillary carcinoma of thyroid. Therefore galectin-3 marker considered as a specific marker for papillary thyroid carcinoma because it is associated with the pathogenesis of carcinoma by interference with cell differentiation, proliferation, and apoptosis. (Mehrotra et al., 2004) showed different result from our study that galectin-3 was expressed in a large proportion of benign thyroid lesions like adenomas, multinodular goiters, and Hashimotos thyroiditis. Probable clarifications for this discrepancy are "differences" in sample physical characters, varied "methodological"/"technical" measures and practical "cut-off "values (from 1 to 25%).

Correlation between immunomarkers and "clinicopathological" topographies of "papillary" thyroid cancer: in this study, although most samples of "papillary" thyroid cancer (83.3%) that discolored positive to HBME-1 have a tumor size of 1-2 cm but the resting cases (16.7%) which have a tumor size of > 2 cm were intensely and diffusely stained (+3 staining intensity),this result was statistically significant. All cases of papillary carcinoma that have lymph node metastasis (26.7%) and those with extra-thyroid invasion (23.3%) also stained with +3 intensity (highly staining) and there was a statistically significant relation between these cases and the high expression of HBME-1 marker as demonstrated by the "p-value" of > 0.001 & > 0.001 correspondingly. Therefore, HBME-1 marker can be used as "independent" extrapolative issue for a possibly "poor" prognosis and describe the aggressive behavior in thyroid carcinoma.

Our outcomes are in contract with the result of (Milosevic et al., 2014)". and those result of (Cheng et al., 2011) both relating to an increased hazard of "lymph node" metastasis in thyroid cancer "patients" with p-value equal to 0.001 and associated with extra thyroid invasion with p-value of 0.03. on the other hand, the study of Cui et. al. that done at 2012 institute that there was no connotation between "HBME-1" expression and papillary thyroid cancer destructive behavior. This variety in results may clarified by using of unfit sample sizes, dissimilar "cut-off" value for "HBME-1" or technical deliberations in "immunohistochemistry".

About galectin-3 relation to the clinicopathological features of carcinoma cases in our study, we found that most of samples (21 of 30 cases,70%) were with tumor size of 1-2 cm and positive to galectin-3 while only four cases stained positively to galectin-3 with weak to moderate intensity and have tumor size of > 2cm. The lymph node metastasis found only in 5 cases (16.7%) of positive staining to galectin-3 with only one case appear highly stained (with +3 intensity of staining), and few cases of positive staining predict extra thyroid invasion, while the rest cases were positive to galectin-3 but have no lymph node metastasis or extra thyroid invasion (66.7% and 70%, respectively). Therefore, in our work, we settled that "galectin-3" immunohistochemical countenance is not a pointer of limited metastatic feast or extra thyroid invasion of papillary carcinoma of thyroid, because this marker "immunopositivity" was institute in a large number of cases which have no lymph node metastasis /or extra thyroid invasion immersion. This result agree with result of (Cvejic et al., 2005) that reported no relation between galectin-3 expression and clinical data (including tumor size, "lymph node metastasis", & extra thyroid invasion) of "papillary" thyroid cancer. This result can be explained by that it is due to "galectin-3" localization in thyroid cancerous cells is permanently "cytoplasmic", and only seldom "membranous" or "nuclear". In our study, we will not approve the verdict of (Htwe et al., 2010). study done at 2010 who reported that primary papillary thyroid carcinoma involving lymph node "metastasis" confined "significantly" advanced concentrations of "galectin-3" than tumor growth that have no metastasis, and explanation of this result is galectins

were stated in numerous “neoplastic” lesions and have a relation with tumor development, assault, and metastasis creation. In the “human” thyroid, downregulation of “galectin-3” can enable the announcement of “tumor” cells from the principal lesions, resultant in metastasis of “papillary” carcinoma.

CONCLUSIONS

we reported that both HBME-1 and galectin-3 were very sensitive for papillary thyroid carcinoma. Both markers can be used for distinction between benign and malignant lesions. Among the two markers, the HBME-1 was more specific for diagnosis of papillary thyroid carcinoma. We demonstrated that patients with papillary thyroid carcinoma were have older age than patients with hyperfunctioning lesions. Also reported that females were affected more than males in both groups, There is an association between increased HBME-1 expression in principal “papillary thyroid” cancer “with” the existence of “lymph node” metastasis, extra thyroid invasion, and tumor size of > 2cm emphasizes the clinical importance of its use as “independent” predictive factor for possibly “poor” prognosis. There is no relation between galectin-3 expression and the clinicopathological features of papillary thyroid carcinoma.

REFERENCES

1. Arcolia, V., Journe, F., Renaud, F., Leteurtre, E., Gabius, H., Remmelink, M., & Saussez, S. (2017). Combination of galectin-3, CK19 and HBME-1 immunostaining improves the diagnosis of thyroid cancer. *Oncology Letters*, *14*(4), 4183–4189.
2. Argüeso, P., & Panjwani, N. (2011). Focus on molecules: galectin-3. *Experimental Eye Research*, *92*(1), 2.
3. Baloch, Z. W., Shafique, K., Flanagan, M., & LiVolsi, V. A. (2010). Encapsulated classic and follicular variants of papillary thyroid carcinoma: comparative clinicopathologic study. *Endocrine Practice*, *16*(6), 952–959.
4. Banco, B., Antuofermo, E., Borzacchiello, G., Cossu-Rocca, P., & Grieco, V. (2011). Canine ovarian tumors: an immunohistochemical study with HBME-1 antibody. *Journal of Veterinary Diagnostic Investigation*, *23*(5), 977–981.
5. Casey, M. B., Lohse, C. M., & Lloyd, R. V. (2003). Distinction between papillary thyroid hyperplasia and papillary thyroid carcinoma by immunohistochemical staining for cytokeratin 19, galectin-3, and HBME-1. *Endocrine Pathology*, *14*(1), 55–60.
6. Cheng, S.-P., Yin, P.-H., Hsu, Y.-C., Chang, Y.-C., Huang, S.-Y., Lee, J.-J., & Chi, C.-W. (2011). Leptin enhances migration of human papillary thyroid cancer cells through the PI3K/AKT and MEK/ERK signaling pathways. *Oncology Reports*, *26*(5), 1265–1271.
7. Cui, W., Sang, W., Zheng, S., Ma, Y., Liu, X., & Zhang, W. (2012). Usefulness of cytokeratin-19, galectin-3, and Hector Battifora mesothelial-1 in the diagnosis of benign and malignant thyroid nodules. *Clinical Laboratory*, *58*(7–8), 673–680.
8. Cvejic, D. S., Savin, S. B., Petrovic, I. M., Paunovic, I. R., Tatic, S. B., & Havelka, M. J. (2005). Galectin-3 expression in papillary thyroid carcinoma: Relation to histomorphologic growth pattern, lymph node metastasis, extrathyroid invasion, and tumor size. *Head & Neck: Journal for the Sciences and Specialties of the Head and Neck*, *27*(12), 1049–1055.
9. de Boer, R. A., Voors, A. A., Muntendam, P., van Gilst, W. H., & van Veldhuisen, D. J. (2009). Galectin-3: a novel mediator of heart failure development and progression. *European Journal of Heart Failure*, *11*(9), 811–817.
10. De Micco, C., Savchenko, V., Giorgi, R., Sebag, F., & Henry, J. F. (2008). Utility of malignancy markers in fine-needle aspiration cytology of thyroid nodules: comparison of Hector Battifora mesothelial antigen-1, thyroid peroxidase and dipeptidyl aminopeptidase IV. *British Journal of Cancer*, *98*(4), 818–823.
11. Duangsuwan, P., Plirat, W., Nitiruangjaras, A., & Sinkijcharoenchai, W. (2018). Gross and histopathologies of thyroid gland in cadavers from the South of Thailand. *Chulalongkorn Medical Journal*, *62*(3), 541–553.
12. Eusebi, V., Tallini, G., & Rosai, J. (2004). Nuclear alterations and RET/PTC activation. *The American Journal of Surgical Pathology*, *28*(7), 974–975.
13. Goldblum, J. R., Lamps, L. W., McKenney, J. K., & Myers, J. L. (2017). *Rosai and Ackerman's Surgical Pathology E-Book*. Elsevier Health Sciences.
14. Gullo, I., Batista, R., Rodrigues-Pereira, P., Soares, P., Barroca, H., do Bom-Sucesso, M., & Sobrinho-Simões, M. (2018). Multinodular goiter progression toward malignancy in a case of DICER1 syndrome: histologic and molecular alterations. *American Journal of Clinical Pathology*, *149*(5), 379–386.
15. Halimi, H., Rigato, A., Byrne, D., Ferracci, G., Sebban-Kreuzer, C., ElAntak, L., & Guerlesquin, F. (2014). Glycan dependence of galectin-3 self-association properties. *PLoS One*, *9*(11), e111836.
16. Hsieh, S.-H., Chen, S.-T., Hsueh, C., Chao, T.-C., & Lin, J.-D. (2012). Gender-specific variation in the prognosis of papillary thyroid cancer TNM stages II to IV. *International Journal of Endocrinology*, *2012*.
17. Htwe, T. T., Karim, N., Wong, J., Jahanfar, S., & Mansur, M. A. (2010). Differential expression of galectin-3 in advancing thyroid cancer cells: a clue toward understanding tumour progression and metastasis. *Singapore Med J*, *51*(11), 856–859.
18. IJOMONE, E. A. (2010). HISTOPATHOLOGICAL REVIEW OF THYROID DISEASE AT THE UNIVERSITY OF PORT HARCOURT TEACHING HOSPITAL-A TEN YEAR RETROSPECTIVE STUDY (1999-2008). *Faculty of Pathology*.
19. Kotwal, A., & Stan, M. (2018). Current and future treatments for Graves' disease and Graves' ophthalmopathy. *Hormone and Metabolic Research*, *50*(12), 871–886.
20. Lin, J.-D., Hsueh, C., & Huang, B.-Y. (2011). Papillary thyroid carcinoma with different histological patterns. *Chang Gung Med J*, *34*(1), 23–34.
21. Liu, Z., Li, X., Shi, L., Maimaiti, Y., Chen, T., Li, Z., Wang, S., Xiong, Y., Guo, H., & He, W. (2014). Cytokeratin 19, thyroperoxidase, HBME-1 and galectin-3 in evaluation of aggressive behavior of papillary thyroid carcinoma. *International Journal of Clinical and Experimental Medicine*, *7*(8), 2304.
22. LiVolsi, V. A. (2011). Papillary thyroid carcinoma: an update. *Modern Pathology*, *24*(2), S1–S9.
23. Livolsi, V. A., Albores-Saavedra, J., Asa Sl, B. Z. W., Baloch, Z. W., & Baloch, Z. W. (2004). Pathology and genetics of tumours of endocrine organs. *DeLellis RA, LLoyd RV, Heitz PU, Eng Ch (Eds). Oxford University Press: Oxford*, 57–66.
24. Lloyd, R. V. (2010). *Endocrine Pathology:: Differential Diagnosis and Molecular Advances*. Springer Science & Business Media.
25. Mehrotra, P., Okpokam, A., Bouhaidar, R., Johnson, S. J., Wilson, J. A., Davies, B. R., & Lennard, T. W. J. (2004). Galectin-3 does not reliably distinguish benign from malignant thyroid neoplasms. *Histopathology*, *45*(5), 493–500.
26. Milosevic, Z., Pesic, M., Stankovic, T., Dinic, J., Milovanovic, Z., Stojsic, J., Dzodic, R., Tanic, N., & Bankovic, J. (2014).

Targeting RAS-MAPK-ERK and PI3K-AKT-mTOR signal transduction pathways to chemosensitize anaplastic thyroid carcinoma. *Translational Research*, *164*(5), 411–423.
27. NADU, T. (2018). A STUDY OF CK 19 EXPRESSION IN PAPILLARY LESIONS OF THYROID. *PATHOLOGY*.
28. Perez-Montiel, M. D., & Suster, S. (2008). The spectrum of histologic changes in thyroid hyperplasia: a clinicopathologic study of 300 cases. *Human Pathology*, *39*(7), 1080–1087.
29. Razy, N. H. M. P., Rahman, W. F. W. A., & Win, T. T. (2019). Expression of vascular endothelial growth factor and its receptors in thyroid nodular hyperplasia and papillary thyroid carcinoma: a tertiary health care centre based study. *Asian Pacific Journal of Cancer Prevention: APJCP*, *20*(1), 277.
30. Roti, E., Rossi, R., Trasforini, G., Bertelli, F., Ambrosio, M. R., Busutti, L., Pearce, E. N., Braverman, L. E., & Degli Uberti, E. C. (2006). Clinical and histological characteristics of papillary thyroid microcarcinoma: results of a retrospective study in 243 patients. *The Journal of Clinical Endocrinology and Metabolism*, *91*(6), 2171–2178. https://doi.org/10.1210/jc.2005-2372
31. Sakoda, L. C., & Horn-Ross, P. L. (2002). Reproductive and menstrual history and papillary thyroid cancer risk: the San Francisco Bay Area thyroid cancer study. *Cancer Epidemiology and Prevention Biomarkers*, *11*(1), 51–57.
32. Song, L., Tang, J., Owusu, L., Sun, M.-Z., Wu, J., & Zhang, J. (2014). Galectin-3 in cancer. *Clinica Chimica Acta*, *431*, 185–191.
33. Tallini, G., & Giordano, T. J. (2017). Normal Anatomy and Thyroid Gland Development. *Rosai and Ackerman's Surgical Pathology E-Book*, 277.
34. Weber, K. B., Shroyer, K. R., Heinz, D. E., Nawaz, S., Said, M. S., & Haugen, B. R. (2004). The use of a combination of galectin-3 and thyroid peroxidase for the diagnosis and prognosis of thyroid cancer. *American Journal of Clinical Pathology*, *122*(4), 524–531.
35. Williams, E. D., Abrosimov, A., Bogdanova, T., Demidchik, E. P., Ito, M., LiVolsi, V., Lushnikov, E., Rosai, J., Tronko, M. D., & Tsyb, A. F. (2008). Morphologic characteristics of Chernobyl-related childhood papillary thyroid carcinomas are independent of radiation exposure but vary with iodine intake. *Thyroid*, *18*(8), 847–852.