International Journal of Advanced Research in Medicine

E-ISSN: 2706-9575
P-ISSN: 2706-9567
www.medicinepaper.net
IJARM 2024; 6 (1): 53-58
Received: 21-11-2023
Accepted: 28-12-2023

Dr. Adeeb Abdulally Abdulhussien
College of Medicine, Dhi Qar University, Nasiriyah, Iraq

Zainab M Farhan
Ministry of Health, Immam Hussein Teaching Hospital, Thi-Qar, Iraq

Corresponding Author:
Dr. Adeeb Abdulally Abdulhussien
College of Medicine, Dhi Qar University, Nasiriyah, Iraq

A histopathological study to evaluate the role of immunohistochemical technique for evaluating follicular thyroid cancer among cancer patients in Dhi Qar Governorate

Dr. Adeeb Abdulally Abdulhussien and Zainab M Farhan

DOI: https://doi.org/10.22271/27069567.2024.v6.i1a.545

Abstract
Background: Despite rising rates of well-differentiated thyroid cancer, follicular thyroid carcinoma is becoming less common. This paper examines the evolution of morphological and immunohistochemical diagnostic criteria to explain why this phenomenon occurred. Follicular thyroid tumors. This article will cover the differences between follicular carcinoma, follicular adenoma, follicular variant of papillary carcinoma, and poorly differentiated carcinoma. It will also discuss the newly defined categories of follicular tumors: follicular tumor of uncertain malignant potential, well-differentiated tumor of uncertain malignant potential, and well-differentiated carcinoma, not otherwise specified. Finally, interesting molecular biomarkers will be examined in relation to histopathological categorization for their prognostic and therapeutic implications.

Keywords: TPO, Galectin-3, diagnosis, follicular thyroid carcinoma, molecular biomarkers, prognosis

1. Introduction
The most common is thyroid cancer, which is becoming more common each year (Deng, Y., *et al*. 2020) [1]. The third most common malignancy among Saudi adults is thyroid carcinoma (Samargandy, S., Qari, R., *et al*., 2020) [2]. In Saudi Arabia. Thyroid cancer is one of the most common head and neck cancers in Arabia, especially in women, hence more research into potential modifiable risk factors is required (Hussain, F., Iqbal, S., Mehmood *et al*., 2013) [3] (Alkaff, H. H., Besharah, B. O., Bukhari *et al*., 2020) [4]. Thyroid cancer has a complicated etiology that varies greatly by region and is associated with several modifiable risk factors.
Age, gender, and nationality. Compared to males, women are more vulnerable, especially when they are fertile.
The most prevalent histological form is papillary carcinoma, regardless of sex or the existence of risk factors. The most daunting modifiable risk factors include goiter, ionizing radiation exposure, miscarriage or abortion, cigarette smoking, and contraceptive use (Al-Zahrani, A. S., & Ravichandran., 2007) [5]. Immunohistochemistry evaluation of surgically excised thyroid cancer tissues has implications for diagnosis, prognosis, and treatment (Parker, K. G., White *et al*., 2020) [6]. The expression of cytokeratin 19 (CK19) immunohistochemistry is useful in assessing the overall prognosis of thyroid cancer patients (Mohamed, S. Y., Ibrahim *et al*., 2020) [7]. Compared to benign thyroid tumors, high-grade thyroid carcinomas exhibit lower levels of CD56 immunohistochemical expression. As such, it aids in distinguishing benign from malignant thyroid tumors (Pyo, J. S., Kim, D. H., & Yang (2018) [8]. In addition to having diagnostic use in thyroid papillomavirus cases, Galectin-3 immunohistochemistry is often linked to a less severe pathogenesis of papillary thyroid cancer than positivity. Therefore, the current study set out to evaluate thyroid tumor patterns and thyroid cancer marker immunohistochemistry expression in Northern Saudi Arabia (Kim, E. S., Lim, D. J., *et al*., 2012) [9].

2. Materials and Methods
2.1 Materials
Archived tissue blocks of follicular thyroid tissue were used in this study.

2.1.2 Method

2.1.1 Study design: This is a hospital based analytical case control aimed to detect gal-3 and TPO expression in follicular thyroid tumor using immunohistochemistry.

Table 1: The design of the study conducted in the laboratory

Age	No.	Gender
21-30 years	18	13 female/ 5 male
31-40 years	28	20 female/ 8 male
41-50 years	28	27 female/ 1 male
51-60 years	18	17 female/ 1male
61-70 years	8	6 female/ 2male

2.1.2 Study samples

Tissue blocks obtained from thirty samples previously diagnosed as follicular thyroid cancer and ten samples which previously as benign thyroid tissue were used. Patient's data (age, histopathology diagnosis) were obtained from the patient's files.

2.1.3 Study area

This study was carried out at the Department Histopathology - College of Health and Medical Technologies, Department of Medical Laboratories during the period from December 2022 through March 2023. All the cases including in this study were collected from the major Al-Hussein Teaching hospital and some private laboratories in Nasiriya Government.

2.1.4 Sample processing

Sections of 3 mm thickness were cut by rotary microtome, mounted in positively charged slides then dewaxed in oven for 30 minutes.

2.1.5 Immunohistochemical staining

The indirect streptoavidin-biotin immune method was employed to perform immunohistochemical staining. Tissue slices with a diameter of 3ºm were subjected to deparaffinization using xylene, followed by rehydration in graded alcohol (100%, 90%, 70%, 50%) and water for a duration of two minutes each section. The process of antigen retrieval was conducted by employing a water bath containing citrate buffer at a pH of 6.8.Subsequently, the slides were immersed in a solution of 0.3% hydrogen peroxide for a duration of ten minutes to inhibit the activity of endogenous peroxidase. The slides underwent a 20-minute treatment with anti Gal-3 and TPO+ primary antibody, followed by a 20-minute washing in phosphate buffer saline (pH 7.4). Subsequently, they were treated with biotinylated secondary antibody for 20 minutes. The slides were then incubated in strepotavidin-horseradish peroxidase for 15 minutes, washed in phosphate buffer saline (pH 7.4), incubated in a subsitute solution of 3-3 diaminobenzidine tetra hydrochloride (DAB) for 7 minutes, and finally washed in running tap water. Subsequently, the sample was subjected to a 1-minute counterstaining with Mayer's haematoxylin stain, followed by dehydration, clearing, and mounting in DPX mounting solution (Erdem, H., Gündogdu., *et al*., 2011) [10].

2.1.6 Data analysis

The statistical analysis included calculating the mean incidence and standard error, as well as determining the differences between the groups using the Two-Way Analysis of Variance (ANOVA). The Least Significant Difference (LSD) test was employed to examine the notable disparities in the mean values of the groups throughout each time. The measured correlation coefficient by Orgien Pro (2021).

3. Result

3.1 The morphology of the lower extremities indicates the association between gender, treatment, and gender.

The correlation result demonstrates a strong positive connection between the treatments (TPO, Gal-3) at the $p<0.01$ level, while the red balls represent a very significant positive correlation between treatments (TPO, Gal-3).

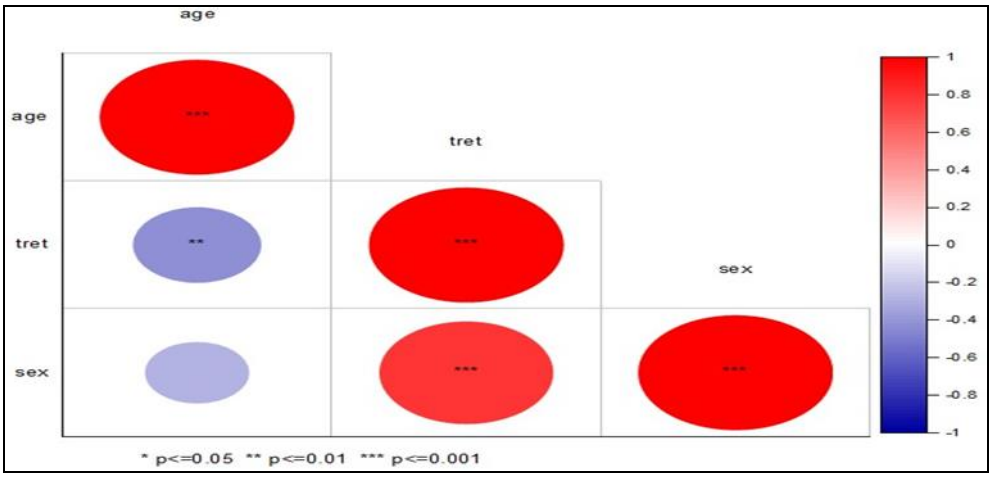

Chart 1: The correlation between the age, treatment addition to gender

3.2: The correlation between TPO and Gal-3, gender in follicular adenoma cases

Chart 2 illustrates the investigation of connection between gender and two marker types utilized in follicular adenoma. It indicates that there was a substantial positive association between the positive protein of Gal-3$^+$ and TPO$^+$ in this kind of thyroid cancer, with a p-value of less than 0.01.According to table (1) in pictures 1, 2, and the microscopic analysis, galectin-3 expression was present as a dark hue in the cytoplasm and/or nucleus on the nuclear membrane. Additionally, a microscope analysis revealed that Gal-3$^+$, the positive expression of TPO$^+$, was present in

the cytoplasm of cells as seen in fig 1 and 2. Chart No. 2 shows a highly significant positive expression was found between positive Gal-3 protein expression and samples taken from female sex at the $p<0.01$ probability level. Red balls in the association show a highly significant correlation between positive Gal-3 protein expression and TPO protein expression. Although there is no positive link between TPO positive expression and male gender, there is a positive correlation with female gender. At the probability level $p<0.05$, a significant positive expression was found between TPO positive protein expression and samples from the female sex, but a highly significant negative expression was recorded between TPO positive protein expression and samples from the male gender that did not reach the level of significance as show in chart 2 respectively.

Table 2: The relation between gender period and gender immune - expression in patients with follicular adenoma

Gender Age period	Female (M ±S.D)	Male (M ±S.D)	Mean gender (M ±S.D)
21-30	1.25± .957	.75±.500	1.00±.756 b
31-40	2.25± .957	1.75±.957	2.00±.926 b
41-50	2.00± .816	1.00±1.155	1.50±1.069 ab
51-60	1.0±1.155	.75±.500	.88±835b
61-70	.75 ± .500	.50±0.577	.63±0.46291 b
Mean Gender	1.45 ± .999	.95±.826	1.20±.939

LSD: 0.087

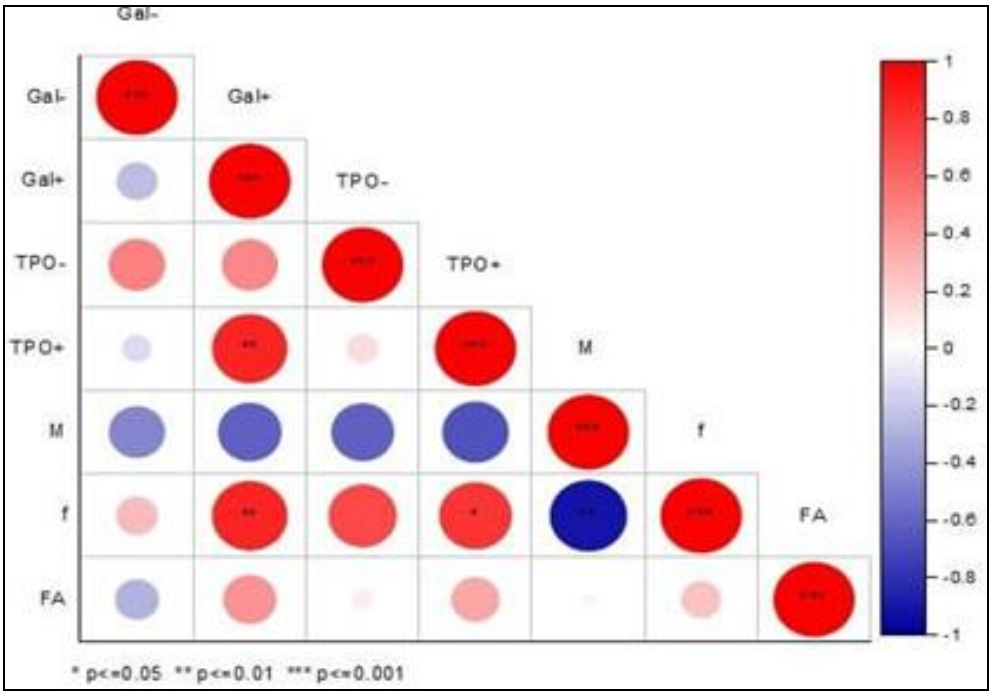

Chart 2: The correlation between TPO and Gal-3, gender in follicular adenoma

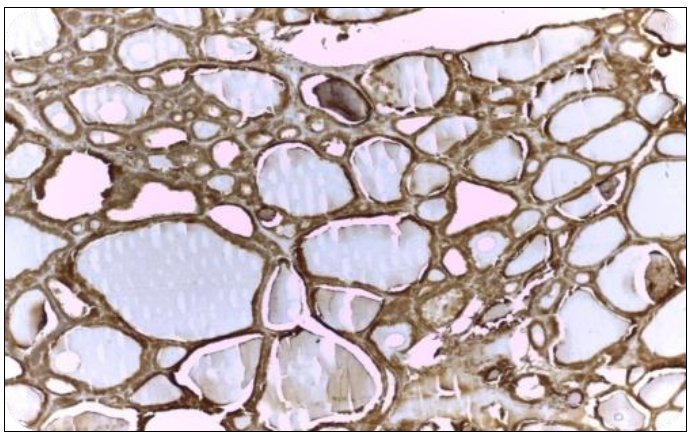

Fig 1: Immune-expression of TPO protein

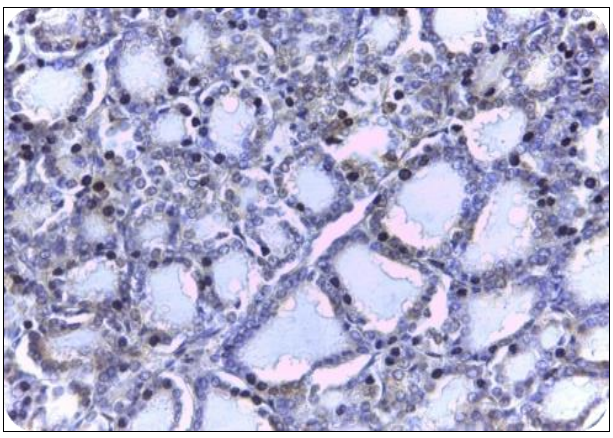

Fig 2: Immune-expression of Gal-3 protein

3.3 The relation between gender period and gender in patients with Follicular carcinoma cases

In the follicular carcinoma patients, we also noted that there was a significant difference between the mean of expression of two groups, gender in immune-expression of both markers, which had means of there were (1.20±1.105 and 0.65±.671) respectively as show in table (3) and figure (2 and 3) respectively.

Table 3: The relation between gender period and gender in patients with Follicular carcinoma cases

Gender & Age period	Female (M ±S.D)	Male (M ±S.D)	Mean gender (M ±S.D)
21-30	.00± .000	0.0±.000	0.0±.000
31-40	1.00± .816	0.5±.577	0.75±..707
41-50	2.25± .957	0.75±.500	1.50±1.069
51-60	2.25±.500	1.25±.957	1.75±.886
61-70	.50 ± .577	0.75±.500	0.63±.518
Mean Gender	1.20 ± 1.105	0.65±..671	0.93±.944

LSD: 0.75

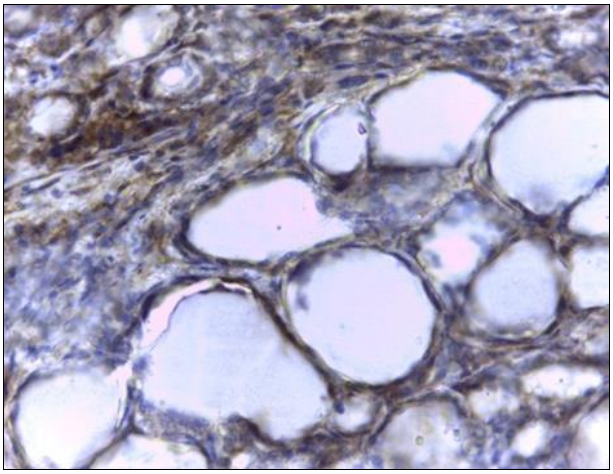

Fig 3: Immune-expression TPO protein

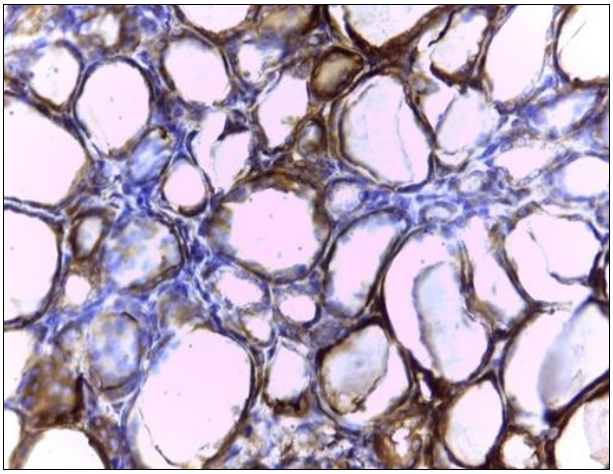

Fig 4: Immune-expression Gal-3 protein

Chart 3 displays the results of correlation studies between the expression of the Gal-3^{+} and TPO^{+} proteins and gender in instances of follicular carcinoma. It explains why there is a substantial positive association between the two, with a p<0.01 value.

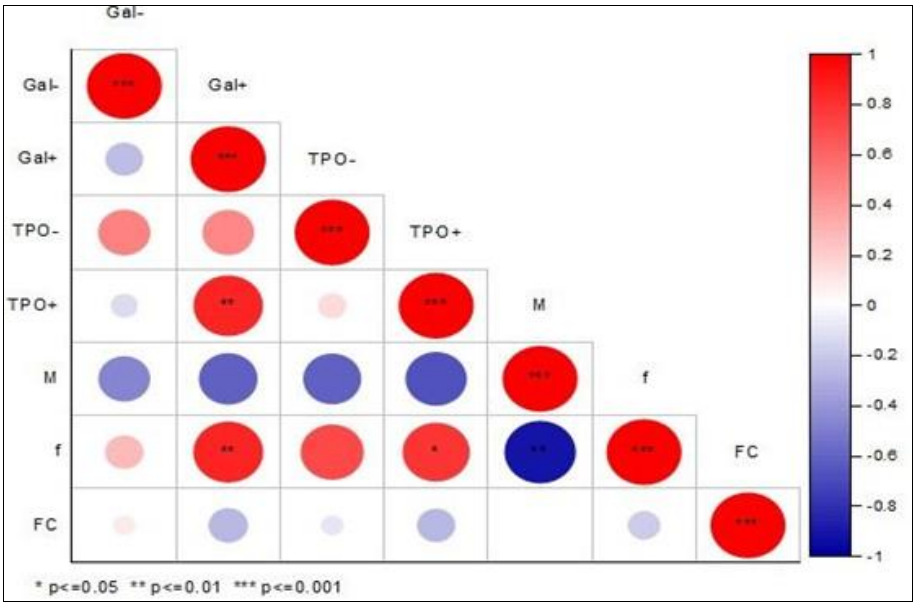

Chart 3: The relation between gender period and gender in patients with Follicular carcinoma cases

3.4 The relation between gender period and gender in patients with Nodular thyroid hyperplasia cases

Table 4: The relation between gender period and gender in patients with Follicular carcinoma cases

Gender & Age period	Female (M ±S.D)	Male (M ±S.D)	Mean gender (M ±S.D)
21-30	3.00± 2.944	2.00±2.160	2.50±.2.449 a
31-40	4.00± 2.944	1.50±1.291	2.75±2.493 a
41-50	2.50± 1.291	1.50±1.732	2.00±1.512 a
51-60	1.50±.1.915	1.00±.816	1.25±1.389 ab
61-70	0.25 ± .500	0.50±.577	0.38±.518 b
Mean Gender	2.25 ± 2.314	13.30±1.380	1.78±1.941

LSD: 1.78

Chart 4 illustrates the gender-related association between the two markers utilized in the current investigation, and it indicates a high positive correlation (p<0.01) between the positive protein expression of Gal-3^{+} and TPO^{+} expression as show in chart 4 and fig (5and 6) respectively.

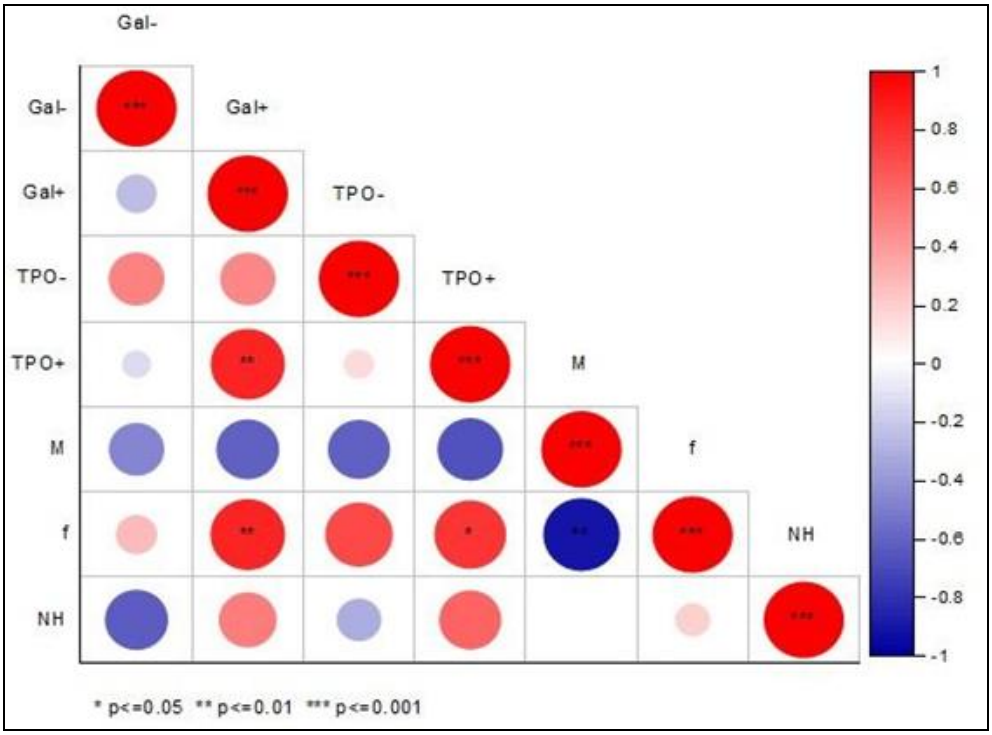

Chart 4: The correlation between TPO and Gal-3, gender in nodular hyperplasia

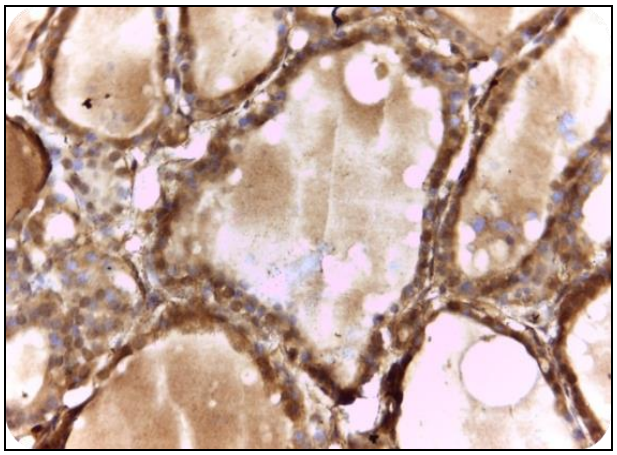

Fig 5: Immune-expression of TPO protein

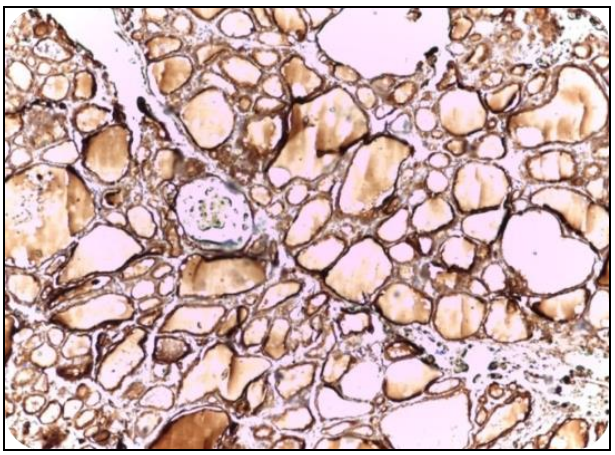

Fig 6: Immune-expression of Gal-3 protein

4. Discussion

Moreover, pathological features like papillary structure with characteristic complex branches and nuclear features have been widely used in the diagnosis of thyroid disease. Immunohistochemistry is crucial in the diagnosis of thyroid follicular carcinoma cases. Papillary formation is frequently observed in benign and malignant thyroid diseases.

However, because of the variety of tumors, it is difficult to distinguish FTC from thyroid papillary hyperplasia and solitary nodules with papillary alterations. The findings of this study show that there is a relationship between the immune expression of both positive Gal -3 and TPO immune markers in thyroid follicular carcinoma tissue. Hypoxia is a major microenvironmental determinant for tumor growth and treatment resistance in solid tumors. Gal-3 has been shown to protect cells from hypoxic death (Rabinovich, G. A. (2005) [11], and it also plays a major role in interactions between cells and between cells and matrixes by binding to endogenous glycans (Chiariotti, L., Salvatore., *et al*., 2002) [12]. As a result, Gal-3 is highly expressed in tumors, where it increases tumor survival and metastatic dissemination. Moreover, increased Gal-3 expression at some point during the development of most cancers also promotes tumor growth (Dumic, J., Dabelic, *et al*., 2006) [13].

The findings of our investigation corroborated those of [Cvejic, D., Savin, *et al*., 2005] [14], who found that Gal-3 immune activity was present in the cytoplasm of thyroid papillary carcinoma cells as well as in the nucleus, surface, and outside of the cell. Additionally, [Kawachi K, Matsushita Y, *et al*., 2000] [15] reported that, in stomach malignant tissues, the staining was diffuse or granular at a high power field magnification, and that the expression was

stained in bright yellow, dark yellow, or brown yellow at a low power field.

With the exception of low-risk malignancies like minimally invasive follicular carcinoma (MIFC), TPO+ can be utilized as a prognostic factor for thyroid differentiation cancer as well as patient follow-up and other indicators. It can also be used to confirm or rule out benign illnesses of differentiated thyroid cancer [Ab, T., and Ab, T. (2020)] [16].

5. Conclusion

Along with other markers, Gal-3 and TPO^{+} may be utilized as a prognostic factor for follicular thyroid cancer. In southern Iraqi, follicular thyroid carcinoma is the most common kind of thyroid cancer. The majority of patients are female. And more youthful. In terms of specificity and sensitivity, the combined use of TPO and Galectin-3 tumor markers aids in the precise differential diagnosis of thyroid neoplasms.

Financial support and sponsorship: Nil.

Conflict of Interest: Nil.

References

1. Deng Y, Li H, Wang M, Li N, Tian T, Wu Y, *et al*. Global burden of thyroid cancer from 1990 to 2017. JAMA network open. 2020 Jun;3(6):e208759-e208759.
2. Samargandy S, Qari R, Aljadani A, Assaqaf Sr D, Etaiwi A, Alghamdi D, *et al*. Clinicopathological characteristics of thyroid cancer in a Saudi academic hospital. Cureus, 2020 May, 12(5).
3. Hussain F, Iqbal S, Mehmood A, Bazarbashi S, ElHassan T, Chaudhri N. Incidence of thyroid cancer in the Kingdom of Saudi Arabia, 2000–2010. Hematology/oncology and stem cell therapy. 2013;6(2):58-64.
4. Alkaff HH, Besharah BO, Bukhari DH, Sayed SI, Alessa MA, Abdelmonim SK, *et al*. Thyroid neoplasm in Makkah region, Saudi Arabia: A retrospective epidemiological study. Saudi Medical Journal. 2020 Dec;41(12):1330.
5. Al-Zahrani AS, Ravichandran K. Epidemiology of thyroid cancer: A review with special reference to Gulf Cooperation Council (GCC) states. Gulf J Oncolog. 2007;2:17-28.
6. Parker KG, White MG, Cipriani NA. Comparison of molecular methods and BRAF immunohistochemistry (VE1 clone) for the detection of BRAF V600E mutation in papillary thyroid carcinoma: A meta-analysis. Head and neck pathology. 2020;14:1067-1079.
7. Mohamed SY, Ibrahim TR, Elbasateeny SS, Abdelaziz LA, Farouk S, Yassin MA, *et al*. Clinicopathological characterization and prognostic implication of FOXP3 and CK19 expression in papillary thyroid carcinoma and concomitant Hashimoto's thyroiditis. Scientific reports. 2020 Jul 6;10(1):10651.
8. Pyo JS, Kim DH, Yang J. Diagnostic value of CD56 immunohistochemistry in thyroid lesions. The International journal of biological markers. 2018;33(2):161-167.
9. Kim ES, Lim DJ, Lee K, Jung CK, Bae JS, Jung SL, *et al*. Absence of galectin-3 immunostaining in fine-needle aspiration cytology specimens from papillary thyroid carcinoma is associated with favorable pathological indices. Thyroid. 2012 Dec;22(12):1244-1250.
10. Erdem H, Gündogdu C, Şipal S. Correlation of E-cadherin, VEGF, COX-2 expression to prognostic parameters in papillary thyroid carcinoma. Experimental and molecular pathology. 2011;90(3):312-317.
11. Rabinovich GA. Galectin-1 as a potential cancer target. British Journal of Cancer. 2005 Apr;92(7):1188-1192.
12. Chiariotti L, Salvatore P, Frunzio R, Bruni CB. Galectin genes: regulation of expression. Glycoconjugate journal. 2002;19(7):441-449.
13. Dumic J, Dabelic S, Flögel M. Galectin-3: an open-ended story. Biochimica et Biophysica Acta (BBA)-General Subjects. 2006 Apr 1;1760(4):616-635.
14. Cvejic D, Savin S, Petrovic I, Paunovic I, Tatic S, Krgovic K, *et al*. Galectin-3 expression in papillary microcarcinoma of the thyroid. Histopathology. 2005 Aug;47(2):209-214.
15. Kawachi K, Matsushita Y, Yonezawa S, *et al*. Galectin-3 expression in various thyroid neoplasms and its possible role in metastasis formation. Hum Pathol. 2000;31(4):428–433.
16. Ab T, Ab T. Test Definition: TPO test Definition: TPO. 2020;1-4.
17. Neil R, Borley. Grasy's anatomy the anatomical basis of clinical practice. 40th edition. London: Livingstone; c2008. p. 462-4.

How to Cite This Article
Abdulhussien AA, Farhan ZM. A histopathological study to evaluate the role of immunohistochemical technique for evaluating follicular thyroid cancer among cancer patients in Dhi Qar Governorate. International Journal of Advanced Research in Medicine. 2024;6(1): 53-58.