Medical Research Archives

ESMED
European Society of Medicine

OPEN ACCESS

Published: June 30, 2023

Citation: Perumal PV, Siddaraju N, et al., 2023. Role of GDF-15 as an Immunohistochemical Marker in Follicular Neoplasms and Lesions of Thyroid, Medical Research Archives, [online] 11(6). https://doi.org/10.18103/mra.v11i6.4036

DOI
https://doi.org/10.18103/mra.v11i6.4036

ISSN: 2375-1924

RESEARCH ARTICLE

Role of GDF-15 as an Immunohistochemical Marker in Follicular Neoplasms and Lesions of Thyroid

Prasanna Venkadesa Perumal, B.Sc. (MLT), M.Sc. (MLT-Cytopathology)
Laboratory Technician, Department of Microbiology
Jawaharlal Institute of Medical Education and Research (JIPMER)
Puducherry-605006, INDIA

Neelaiah Siddaraju, MD
Senior Professor, Department of Pathology
Jawaharlal Institute of Medical Education and Research (JIPMER)
Puducherry-605006, INDIA
Corresponding Email: rajusiddaraju@gmail.com

Sunil Kumar Saxena, MS
Professor, Department of Otorhinolaryngology
Jawaharlal Institute of Medical Education and Research (JIPMER)
Puducherry-605006, INDIA

Soundravally Rajendiran, MD
Professor, Department of Biochemistry
Jawaharlal Institute of Medical Education and Research (JIPMER)
Puducherry-605006, INDIA

Ramachandra V. Bhat, MD
Dean & Professor of Pathology
Indira Gandhi Medical College and Research Institute (IGMC &RI)
Puducherry-605006, INDIA

ABSTRACT

Introduction: A pre-therapeutic distinction between benign and malignant thyroid nodules is critical for deciding the therapeutic strategy, wherein fine-needle aspiration cytology plays a major role. However, there are grey-zone categories in the Bethesda system for reporting thyroid cytopathology, which is currently, the universally accepted pattern for reporting thyroid FNAC. Several immunomarkers studied in these 'indeterminate' lesions have proven to be of limited value. Recent molecular studies have shown the growth differentiation factor-15 (GDF-15) gene to be a promising marker in various malignancies; though, there is sparse literature regarding its use as a surrogate immunohistochemical marker, especially in thyroid neoplasms. The present (pilot) study explores its possible utility in the pre-therapeutic distinction of follicular-patterned lesions and neoplasms of the thyroid by testing it initially on histopathologic sections.

Objective: To assess the utility of GDF-15 as an immunohistochemical marker in distinguishing common follicular-patterned thyroid neoplasms and lesions.

Materials and Methods: GDF-15 immunohistochemistry was performed on 75 surgical specimens of thyroid lesions, comprising 19 adenomatous nodules or follicular hyperplasias, 10 nodular goitres, 17 follicular thyroid adenomas, 8 follicular thyroid carcinomas, 12 follicular variant of papillary thyroid carcinomas, and 9 conventional papillary thyroid carcinomas. All these cases were histologically

proven, and their immunohistochemical interpretation was performed using the immunoreactive scoring system. Pearson's analysis was performed for the immunohistochemical correlation among the study groups.

Results: The findings were inconsistent; 16 follicular-patterned malignancies (5 follicular carcinomas and 11 cases of follicular variant of papillary carcinoma) and seven conventional papillary thyroid carcinomas revealed both cytoplasmic and membranous expression of GDF-15. All benign lesions were negative except one follicular adenoma and three follicular hyperplasias, which exhibited patchy or focal GDF-15 positivity. However, three follicular carcinomas, one follicular variant of papillary carcinoma, and two conventional papillary carcinomas were found to be GDF-15-negative.

Conclusion: Though there is a literature backup supporting the use of GDF-15 gene analysis in differentiating various follicular-patterned neoplasms, the GDF-15 protein, as an immunohistochemical marker failed to distinguish not only between benign and malignant follicular-patterned neoplasms but also between follicular carcinoma and follicular variant of papillary carcinoma.

Keywords: thyroid, GDF-15, immunohistochemistry, follicular neoplasm, follicular adenoma, follicular carcinoma, follicular hyperplasia, follicular variant of papillary carcinoma

Introduction

The thyroid gland is one of the most vital organs of the human endocrine system, and thyroid disorders are globally prevalent.[1] A 4.2 crore population of India is estimated to suffer from thyroid disorders, with a higher incidence being noted among females (1.8/1,000 females vs. 1/100000 males).[2] The major disorders reported among Indians include goitre due to iodine deficiency, those related to an increase (hyperthyroidism) or decrease in thyroid hormones (hypothyroidism), and thyroid neoplasms.[3] Of all thyroid cancers, papillary, follicular, medullary, and anaplastic thyroid carcinomas (PTC, FTC, MTC, and ATC) represent 80%, 15%, 3%, and 2% of cases, respectively. Though the Bethesda system for reporting thyroid cytopathology (TBSRTC) has significantly improved the cytopathologists' approach to thyroid lesions, the ambiguous TBSRTC categories such as 'follicular lesions of undetermined significance (FLUS)' and 'follicular neoplasm/suspicious for follicular neoplasm (FN and SFN)' frequently pose considerable problems in patient management. TBSRTC-IV (FN/SFN) category does not comment on the benign or malignant nature of a follicular neoplasm, as it is not possible to assess the capsular and vascular invasion on FNAC and thus, has remained an accepted limitation of cytology.[4]

There have been innumerable studies dealing with the role of immunocytochemistry (ICC) and immunohistochemistry (IHC). The important markers include galectin-3, human bone marrow endothelium marker-1 (HBME-1), cytokeratin-19 (CK-19), and thyroid peroxidase (TPO). Most of these markers were studied as IHC panels, either on histologic sections or cell block preparations from FNA material. Galectin-3, which initially emerged as the most 'promising' marker for differentiating benign versus malignant thyroid nodules, was later found to have limited practical value.[5] Several newer molecular markers have been identified in recent years, and these include protein convertase-2 (PCSK2), cyclin D2 (CCND2), human telomerase reverse transcriptase (h-TERT), growth differentiation factor (GDF-15), Ras-related protein-2A (RAP2A), thyroid stimulating hormone receptor (TSHR), and insulin-like growth factor type 1 receptor (IGF-1R).[6-10] Using these markers, some authors also tried to distinguish between benign and malignant follicular-patterned thyroid neoplasms.[8,11]

Among the above genes, growth differentiation factor-15 (GDF-15) belongs to the human transforming growth factor-β (TGF-β) family. It is also known by several other names, such as nonsteroidal anti-inflammatory drug (NSAID)-activated gene-1 (NAG-1), macrophage inhibitory cytokine-1 (MIC-1), prostate differentiation factor (PDF), placental bone morphogenetic protein (PLAB), and placental TGF-β (PTGF-β), and these names reflect its varied functions. Certain GDF-15-related cytokines are shown to have immunomodulatory and cancer progression

activities.[8,F,12] An elevated level of GDF-15, or its increased expression is often noticed during the tumour progression of gastric, ovarian, prostatic, and breast carcinomas.[12-17] Studies have demonstrated its role in the activation of STAT3 signalling as well as stimulation of ERK phosphorylation and SMAD signalling in thyroid cancer cells; GDF15-STAT3 signalling is said to have a crucial role in the progression of mitochondrial stress-induced thyroid cancer.[17]

The present (pilot) study assessed the role of GDF-15 as a surrogate IHC marker in distinguishing between benign and malignant follicular thyroid lesions / neoplasms on histologic sections, presuming that a positive outcome would facilitate its use on cytologic material for a pre-therapeutic distinction of indeterminate thyroid nodules.

Materials and Methods

The study was conducted in the Department of Pathology, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry, India, in collaboration with the Departments of Surgery, Otorhinolaryngology, and Biochemistry, JIPMER, Puducherry, and the Department of Pathology, Indira Gandhi Medical College and Research Institute (IGMC&RI), after obtaining approval from the Research Committees of JIPMER and IGRMC&RI, as well as the JIPMER Ethics Committee. The thyroid samples were selected based on the corresponding fine needle aspiration cytology (FNAC) reports or clinical and ultrasound (US) findings. A total of 75 solitary thyroid nodules with subsequent histopathologic follow-up were studied; these included 19 cases of follicular thyroid hyperplasia (FTH), 10 nodular goitres (NGs), 17 follicular thyroid adenomas (FTAs), 8 follicular thyroid carcinomas (FTCs), 12 follicular variant of papillary thyroid carcinomas (FVPTCs), and 9 conventional papillary thyroid carcinomas (CPTCs). The informed consent of all participant-patients was obtained, both before the fine needle aspiration (FNA) procedure and prior to surgery.

The surgical specimens were routinely processed in all 75 cases, and the histopathologic diagnosis was made on hematoxylin and eosin (H&E) sections. The antibody kit for GDF-15 was procured from Origene Technologies (US). Antibody dilution was done according to the manufacturer's protocol in the range of 1:1000. The working condition of the antibody was tested using the control tissue recommended by the manufacturers. The 4 µm thick microsections were prepared from the paraffin-embedded tissue blocks and mounted on the poly-L-lysine coated slides for immunostaining. Each slide was treated with 3% hydrogen peroxide in methanol for 20 minutes for endogenous peroxidase blocking. Antigen retrieval was done by the pressure cooker method with citrate buffer (pH 6.4). The primary antibody was incubated overnight at 4 °C, and the polymer horseradish peroxidase was incubated for 40 minutes. Diaminobenzidine (DAB) was used as a chromogen. The slides were examined under a microscope, and an IHC assessment was done using the immunoreactive scoring (IRS) system.[18] The scoring pattern is shown in Table 1. Pearson's correlation analysis was performed to assess the correlation of IHC expression between the study groups. All statistical tests were done using GraphPad Instant Version 3.06. To determine the sensitivity and specificity of IHC markers for diagnosis, the area under the receiver operator characteristic curve (ROC) was calculated using the R statistical software R-3.6.1.

Table 1: Immunoreactive Scoring System (IRS)

A (Percentage of Positive Cells)	B (Intensity of expression)	Final score (AxB)
0= no positive cells	0= no color reaction	0-1 = negative
1=<10% of positive cells	1 = mild reaction	2-3 = mild
2=10-50% positive cells	2 = moderate reaction	4-8 = moderate
3=51-80% positive cells	3 = intense reaction	9-12 = strongly positive
4=>80% positive cells	Final IRS score (A×B): 0-12	

Results

As for IHC expression of GDF-15, most of the malignant thyroid tumours expressed moderate to strong positivity (Fig. 1A, 1B, 1C), and the cases included four FTCs and seven each of FVPTCs and CPTCs. In contrast, all benign lesions, including FTAs, FTHs, and NGs, were mostly negative, though one FTA and three FTHs showed a mild and focal expression. Notably, malignant thyroid neoplasms like FTC (1 case) and FVPTC (2 cases) also revealed mild positivity, while three cases of FTC and one case of FVPTC, as well as two conventional PTCs, had a negative expression, raising doubts about its practical utility. Strikingly, all our NGs were GDF-15 negative (Table 2). The details of GDF-15 IHC expression in our study groups are highlighted in

Table 2. The percentage of malignant lesions expressing GDF-15 ranged from 62.5 to 77.8% (CPTCs: 77.8%; FVPTCs: 91.6%; and FTCs: 62.5%), while 0.0–8.69% of benign lesions expressed the marker, though it was unexpected. Further, among the benign lesions, the FTH (non-neoplastic) group had a greater number of GDF-15 positive cases than the FTA (neoplastic) group (Table 3).

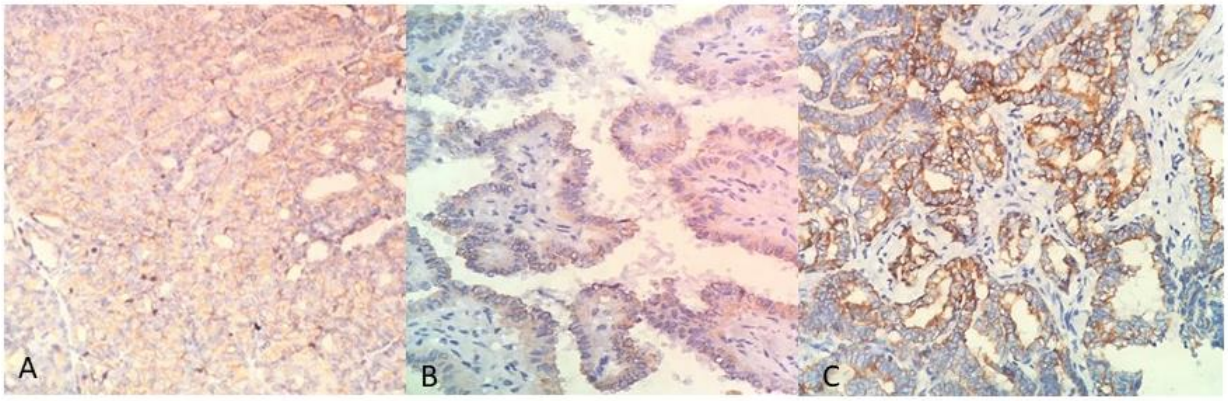


Figure 1: (A) A diffuse, strong expression of GDF-15 in FTC; (B) a case of CPTC with moderate GDF-15 expression; (C) A diffuse, strong expression of GDF-15 in FVPTC (A, B&C: IHCx40, DAB chromogen).

Table 2: Assessment of GDF-15 IHC expression by using IRS scoring system

Histopathology Diagnosis	Number of Cases	GDF-15 Expression			
		Negative (0-1)	Mild (2-3)	Moderate (4-8)	Strong (9-12)
FTC	8	3	1	2	2
FTA	17	16	1	0	0
FVPTC	12	1	2	6	3
PTC	9	2	0	6	1
FTH	19	16	3	0	0
NG	10	10	0	0	0

Table 3: Percentage distribution of GDF-15 IHC expression in different study groups

Immuno Marker	FTC	FTA	FVPTC	PTC	FTH	NG	Total
GDF-15 + n%	5 62.50%	1 5.80%	11 91.60%	7 77.80%	3 15.78%	0 0%	27
GDF-15 – n%	3 37.5	16 94.20%	1 8.40%	2 22.20%	16 84.22%	10 100%	48
Total No. of Cases	8	17	12	9	19	10	75

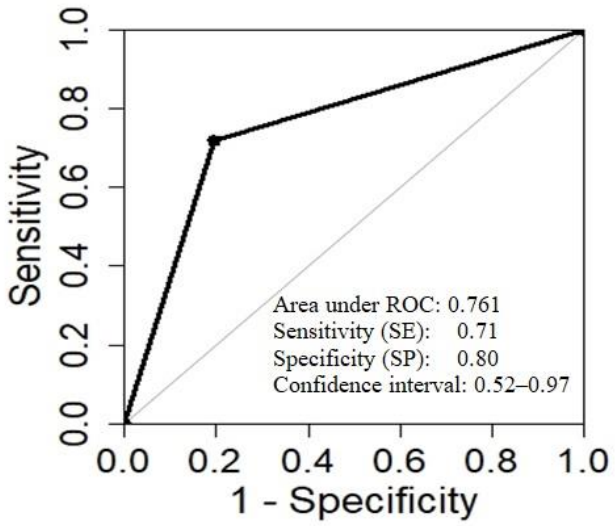
The Pearson correlation analysis of GDF-15 expression among the study groups was carried out, and the values ranging from 0.7 to 0.89 were considered a 'strong correlation'. The values ranging from 0.4 to 0.69 were taken as 'moderate correlation', while the values ranging from 0.1 to 0.39 were considered 'mild correlation'. The R-value of the GDF-15 IHC correlation among all study groups is shown in Table 3. Cases of NG were not considered for correlation analysis as none of them were positive for GDF-15. No significant correlation was observed between FTA and FTC with regard to the IHC expression of GDF-15. As per the correlation analysis, GDF-15 expression in FTA and FTC showed a weak correlation with an R-value of 0.117 and an insignificant '*p-value* of 0.634 (Table 4). A strong correlation was observed between FTC and CPTC with an R-value of 0.758 and a highly significant '*p-value* of 0.0002, indicating a similar IHC expression pattern in both lesions. FVPTC and PTC had a similar percentage of positive GDF-15 expression in 75% of our cases; however, as per the correlation analysis, both had a weak correlation with an R-value of 0.152 and a '*p-value* of 0.535. A moderate correlation was observed between the FTH (a non-neoplastic benign lesion) and PTC (a malignant neoplasm) groups with an R-value of 0.403 and a '*p-value* of 0.086, which was statistically not significant; this moderate correlation decreases the utility of GDF-15 in routine diagnostic practice. A weak correlation was observed between an FTA and an FTH with an R-value of 0.206, which indicated a difference in their IHC expression. This was an example of dissimilarity in IHC expression between two non-malignant categories. Such results decrease the sensitivity of

GDF-15 as an immunomarker for differentiating follicular-patterned tumours and discriminating between benign and malignant thyroid neoplasms (Table 4). The GDF-15 expression showed 71% sensitivity and 80% specificity for differentiating the follicular-patterned thyroid neoplasms under the area of 0.761 (Graph 1).

Table 4: The correlation of GDF-15 IHC expression between the study groups

Study Groups	Mean	Standard Deviation	R-Value	P-Value	Correlation
FTC Vs FTA	2.053 0.211	4.0342 0.917	0.117	0.634	Weak
FTC Vs FVPTC	2.053 3.526	4.034 4.623	0.216	0.375	Weak
FTC Vs PTC	2.053 2.421	4.034 3.625	0.758	0.0002	Strong
FTC Vs FTH	2.053 1.737	4.034 2.663	0.317	0.1864	Weak
FTA Vs FVPTC	0.211 3.526	0.917 4.623	0.13	0.597	Weak
FTA Vs PTC	0.211 2.421	0.917 3.625	0.239	0.324	Weak
FTA Vs FTH	0.211 1.737	0.917 2.663	0.206	0.398	Weak
PTC Vs FVPTC	2.421 3.526	3.625 4.623	0.152	0.535	Weak
FVPTC Vs FTH	3.526 1.737	4.623 2.663	0.098	0.691	Weak
PTC Vs FTH	2.421 1.737	3.625 2.663	0.403	0.086	Moderate

Graph 1: The ROC curve analysis of GDF-15 IHC expression



Discussion

The FNAC procedure plays a major role in the pretherapeutic distinction between benign and malignant thyroid nodules, which is critical for deciding the therapeutic strategy. However, the lesions encompassing follicular neoplasms (FTA and FTC), FTH, and FVPTC often cause diagnostic dilemmas by presenting as 'indeterminate lesions' representing the grey-zone TBSRTC categories-III, IV, and V. The IHC markers used on cell blocks prepared from FNA material have yielded varied results, which are of limited utility.[5] Currently, molecular assays like Afirma or ThyroSeq v2 are being effectively utilised on thyroid FNA material, but they are too expensive for routine application in developing nations like India.[19] In recent years, certain newer molecular markers have emerged but have not been validated to an extent that would allow their routine application. The use of corresponding IHC markers would be of some benefit, but the related literature is too sparse.[13] It is noteworthy that there are occasional studies discussing the use of GDF-15 as an IHC marker.[11,20] The present study tested the efficacy and practical utility of GDF-15 as a surrogate IHC marker on histologic sections of follicular-patterned lesions/neoplasms. A few cases of conventional PTC were also included in the study as a sort of control.

Normally, a significant IHC expression of GDF-15 is seen in placental tissue and macrophages. Elevated serum GDF-15 levels are often associated with injury as well as inflammation; but more significant is its overexpression in malignant tissues.[11,14-17,21] A recent study by Kang et al.[11] demonstrated elevated GDF-15 levels in patients with aggressive and recurrent PTCs. In cancer biology, GDF15 is reported to have various roles, such as cellular proliferation and migration, inflammation, metabolism, and DNA damage response. The pro-angiogenic property is one of its tumour-promoting activities.[17,22] Even cancer cachexia is linked to GDF-15 overexpression.[11]

A positive IHC expression of GDF-15 is described in several malignant tumours. Wallin et al.[14] and Peake et al.[15] reported cytoplasmic or membranous expression of GDF-15 in colonic and breast adenocarcinomas, respectively, while Liu et al. found membranous expression, chiefly in high-grade hepatocellular carcinoma (grade-III HCC).[16]

A recent study by Kang et al.[11] evaluated the role of GDF-15 IHC and its quantification by enzyme-linked immunosorbent assay (ELISA) in a series of 266 papillary thyroid carcinomas (PTCs). The authors analysed GDF-15 protein expression and the GDF-15 gene knockdown effects. The cell viability, cell migration, and cell invasion assays were also carried out by the authors. It was found that overexpression of GDF-15 was associated with decreased mitochondrial activity in PTC. The circulating GDF-15 level in serum was also measured by ELISA, and the level was found to be significantly elevated in patients with PTC as compared to normal individuals. Reporting an increased IHC expression of GDF-15 in PTCs with extrathyroidal invasion and/or lymph node metastasis, the authors suggested its association with aggressive behaviour.[11]

As for the role of GDF-15 in other thyroid neoplasms, there are only two rare studies by Weber et al.[8] and Prabakaran et al.[23] that investigated the utility of GDF-15 mRNA expression in follicular-patterned neoplasms of the thyroid. However, there has been extremely limited data on its role as a surrogate IHC marker in follicular-patterned neoplasms like FTA, FTC, and FVPTC.

Interestingly, GDF-15 expression was observed in all our thyroid lesions, though its expression varied among the different neoplastic and nonneoplastic groups. As in its non-thyroid malignant counterparts, its expression was both cytoplasmic and membranous, with the stronger expression being noted in FTCs. Paradoxically, one of our FTAs was also GDF-15 positive, with its intensity being similar to that of the FTC. In addition, three of our FTCs did not express the marker, indicating that not all GDF-15 negative tumours with a follicular pattern represented FTA. Apparently, such observations limit the practical utility of GDF-15 in differentiating FTA from FTC.

The positive aspect of our (pilot) study is that it dealt with the less-explored GDF-15 protein as a surrogate IHC marker in distinguishing follicular-patterned lesions, though we had a mixed outcome. There are a few limitations, of which the major one is its low sample size with a fairly smaller number of FTAs and FTCs, with which we could not determine a cut-off range of GDF-15 expression for differentiating benign and malignant follicular-patterned neoplasms. A panel of relevant newer markers was not used, and an IHC correlation with clinical parameters was not done. There is a need for further studies with an IHC panel comprising some newer markers in addition to GDF-15 on a larger series of cases to validate its use for routine diagnostic applications. Also, an IHC correlation with the clinical parameters could shed some light on the prognostic significance of GDF-15 IHC in the follow-up of patients with thyroid cancer.

Summary &conclusions

To summarise, GDF-15 as an IHC marker displayed a higher frequency of expression in malignant than benign thyroid tumours and was negative in all NGs. Nevertheless, the fact that patchy and focal GDF-15 expression was noted in a few of our FTAs and FTHs and that some of our malignant thyroid neoplasms lacked GDF-15 expression reduces its significance as an IHC marker for differentiating thyroid follicular tumours. Hence, we conclude that GDF-15 alone cannot be a magic marker for distinguishing various follicular-patterned thyroid lesions or neoplasms.

Ethical statement: This was approved by the Institute Ethics Committee (IEC), Human Studies of the Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry, India.

Acknowledgements: We acknowledge Professor Prathiba Devuru, the Dean of Sri Ramachandra Medical College and Research Institute, Porur, Chennai, and Professor Abdul Hamid, Professor (senior scale), Department of Medicine, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry, for their valuable inputs in our study.

Conflict of interest: The authors declare none.

References:

1. Vanderpump M. The epidemiology of thyroid disease. *Br Med Bull*. 2011;99:39-51. doi:10.1093/bmb/ldr030.
2. Unnikrishnan A, Menon U. Thyroid disorders in India: an epidemiological perspective. *Indian J Endocrinol Metab*. 2011;15:78. doi:10.4103/2230-8210.83329.
3. Subasree S. Prevalence of thyroid disorders in India: an overview. *Research J of Pharm and Tech*. 2014;7:1165–8. doi: 10.5958/0974- 360X.
4. DeMay R. Follicular lesions of the thyroid. *Am J Clin Pathol*. 2000;114:681-3. doi:10.1309/bul2-7v05-dgxu-hu6e.
5. Chan J. Strict criteria should be applied in the diagnosis of encapsulated follicular variant of papillary thyroid carcinoma. *Am J Clin Pathol*. 2002;117:16-8. doi:10.1309/p7ql-16kq-qlf4-xw0m.
6. Cerilli L, Mills S, Rumpel C, Dudley T, Moskaluk C. Interpretation of RET immunostaining in follicular lesions of the thyroid. *Am J Clin Pathol*. 2002;118:186-93. doi:10.1309/53uc- 4u88-rrtn-h33g.
7. Bose D, Das R, Chatterjee U, Banerjee U. Cytokeratin 19 immunoreactivity in the diagnosis of papillary thyroid carcinoma. *Indian J. Med. Paediatr. Oncol*. 2012;33:107- 11. doi:10.4103/0971-5851.99746.
8. Weber F, Shen L, Aldred M, Morrison C, Frilling A, *et al*. Genetic classification of benign and malignant thyroid follicular neoplasia based on a three-gene combination.*J. Clin. Endocrinol. Metab*. 2005;90:2512-21. doi:10.1210/jc.2004-2028.
9. Saleh H, Jin B, Barnwell J, Alzohaili O. Utility of immunohistochemical markers in differentiating benign from malignant follicular-derived thyroid nodules. *Diagn Pathol*. 2010;5. doi:10.1186/1746-1596-5-9.
10. Liu H, Lin F. Application of immunohistochemistry in thyroid pathology. *Arch. Pathol. Lab. Med*. 2015;139:67-82. doi:10.5858/arpa.2014-0056-ra.
11. Kang Y, Kim J, Lim M, Lee S, Yi S, *et* al. Growth differentiation factor 15 is a cancer cell-induced mitokine that primes thyroid cancer cells for invasiveness. *Thyroid*. 2021;31: 772-786. doi:10.1089/thy.2020.0034.
12. Vaňhara P, Hampl A, Kozubík A, Souček K. Growth/differentiation factor-15: prostate cancers suppressor or promoter? *Prostate Cancer Prostatic Dis*. 2012;15:320-8. doi:10.1038/pcan.2012.6.
13. Tamhane S, Gharib H. Thyroid nodule update on diagnosis and management. *Clin Diabetes Endocrinol*. 2016;2:17.doi:10.1186/s40842- 016-0035-7.
14. Wallin U, Glimelius B, Jirström K, Darmanis S,Nong R, *et al*. Growth differentiation factor 15: a prognostic marker for recurrence in colorectal cancer. *Br J Cancer*. 2011;104:1619-27. doi:10.1038/bjc.2011.112.
15. Peake B, Eze S, Yang L, Castellino R, Nahta R. Growth differentiation factor 15 mediates epithelial-mesenchymal transition and invasion of breast cancers through IGF-1R-FoxM1 signaling. *Oncotarget*. 2017; 8:94393-406. doi:10.18632/oncotarget.21765.
16. Liu X, Chi X, Gong Q, Gao L,Niu Y, et al. Association of serum level of growth differentiation factor 15 with liver cirrhosis and hepatocellular carcinoma. *PLoS One*. 2015;10:e0127518. doi: 10.1371/journal.pone.012751.
17. Zhang, H, Zhang W, Tu X, Niu Y, Li X, *et al*. Elevated serum growth differentiation factor 15 levels are associated with thyroid nodules in type 2 diabetes aged over 60 years. *Oncotarget*, 8, pp. 41379– 41386. https://doi.org/10.18632/oncotarget.17328.
18. Fedchenko N, Reifenrath J. Different approaches for interpretation and reporting of immunohistochemistry analysis result in the bone tissue-a review. Diagn Pathol. 2014:9.doi:10.1186/s13000-014-0221-9.
19. Poller DN, Glaysher S. Molecular pathology and thyroid FNA. *Cytopathology*. 2017;28:475-523. doi: 10.1111/cyt.12492.
20. Ni Z, Wang Y, Peng S, Yuan Z. Expression of growth differentiation factor 15 in papillary thyroid carcinoma and its clinical significance. *Cancer Research and Clinic*. 2021;6:282–6.ID: wpr-886049.
21. Joo M, Kim D, Lee M, Lee H, Kim J. GDF15 promotes cell growth, migration, and invasion in gastric cancer by inducing STAT3 activation. *Int J Mol Sci*. 2023;24. doi:10.3390/ijms24032925.
22. Rochette L, Méloux A, Zeller M, Cottin Y, Vergely C. Functional roles of GDF-15 in modulating microenvironment to promote carcinogenesis. *Biochim Biophys Acta Mol Basis Dis*. 2020;1866:165798. doi:10.1016/j.bbadis.2020.165798.
23. Prabakaran I, Grau J, Lewis R, Fraker D, Guvakova M. Rap2AIs upregulated in invasive cells dissected from follicular thyroid cancer. *J Thyroid Res*. 2011;2011:1-6. doi: 10.4061/2011/979840.