Original Article

DOI: 10.21276/APALM.2827

Immunohistochemical Expression of Extracellular Matrix Protein-1 in Neoplastic Thyroid Lesions

Nazoora Khan[1], Hena Ayyub Ansari[1], Naba Hasan[1]* and Mohammad Amanullah Khan[2]

[1]Department of Pathology, Jawaharlal Nehru medical college, AMU, Aligarh (UP), India
[2]Department of Surgery, Jawaharlal Nehru medical college, AMU, Aligarh (UP), India

ABSTRACT

Background: Upregulated Extracellular matrix protein 1 (ECM 1) expression has been observed in several malignancies including breast, thyroid carcinoma, cutaneous melanoma and gastric carcinoma. ECM 1 expression has been evaluated in various epithelial malignancies using real time – reverse transcriptase polymerase chain reaction (RT-PCR). The purpose of current study is to evaluate the immunohistochemical expression of ECM 1 in various neoplastic lesions of thyroid.

Methods: The study included 81 neoplastic thyroid tissue specimens (21 benign and 60 malignant lesions) received over a period of 5 years (2012-2017). Hematoxylin and Eosin stained sections of the tissue specimens were carefully examined under light microscope in order to establish the histopathological diagnosis. The immunohistochemical marker ECM 1 was applied on appropriate sections and the slides were evaluated accordingly.

Result: The expression of ECM 1 was nil (grade 0, 47.62% cases) or weak (1+, 52.38% cases) in benign lesions while majority of malignant lesions (78.33%) showed strong homogenous and crisp staining (3+). The difference in the expression of ECM 1 between benign and malignant lesions was found to be statistically significant ($p < 0.001$). ECM 1 has a diagnostic accuracy of 0.82 and sensitivity of 100% in differentiating benign from malignant lesions.

Conclusion: ECM 1 is uniformly upregulated in all malignant thyroid neoplasms irrespective of the histological type of malignancy. Immunohistochemical expression of ECM 1 shows comparable results to RT-PCR. ECM 1 immunohistochemistry can thus serve as an important standalone marker of malignant thyroid tissue.

Keywords: *Extracellular Matrix Protein-1, Reverse Transcriptase Polymerase Chain Reaction, Neoplastic Lesions Of Thyroid, Immunohistochemistry*

Introduction

The most common presentation of mass lesions of the thyroid is in the form of a palpable nodule. Thyroid nodules are of frequent occurrence both in males and females (single nodules being four times more common in females as compared to males).[1] Although an overwhelming majority of solitary nodules of the thyroid are diagnosed as benign on histopathology but, from a clinical viewpoint the major concern in such patients is to rule out the possibility of malignancy.[1] Fine needle aspiration biopsy (FNAB) is the most widely accepted and cost effective method for initial evaluation of thyroid nodules. The accuracy of FNAB is mainly related to the histological subtype that is being evaluated. The accuracy of FNA is much lower for the follicular patterned lesions like follicular adenoma (FA), follicular thyroid carcinoma (FTC) and follicular variant of papillary thyroid cancer (FVPTC) owing to a considerable overlap of cytomorphological characteristics.[2]

More recently, the use of ultrasound guided core needle biopsy (CNB) has come up as a complementary tool for the evaluation of thyroid nodules, more so in cases where FNAC results are non-diagnostic. CNB also allows for ancillary immunohistochemical and molecular tests to be carried out on the tissue sample. This enables triage of patients with thyroid disease who need to be surgically managed.[3]

ECM 1 is a secretory glycoprotein first isolated from an osteogenic mouse stromal cell line. Human ECM 1 gene has been mapped to chromosome 1q21. Interaction of ECM 1 with other molecules of basement membrane like Perlecan, Matrix Metalloproteinases and Fibulin 1C/1D has been hypothesised as a possible mechanism for its role in a diverse array of biological processes like cell proliferation, angiogenesis, embryonal chondrogenesis, skin differentiation and cancer.[4] Upregulated ECM 1 protein expression has been observed in several malignancies including breast and thyroid carcinoma, cutaneous melanoma, and gastric carcinoma.[5,6] It has been related to poor prognosis in breast, hepatocellular and gastric cancers.[6,7,8]

Kebebew et al. in 2005 and Lal et al. in 2008 have evaluated the expression of ECM 1 in various epithelial

malignancies including that of the thyroid on real time reverse transcriptase polymerase chain reaction (RT-PCR).[9,10] It has been established as an important marker of epithelial malignancies and its possible utilization in differentiating benign and malignant lesions. But since RT-PCR is not a feasible option for routine use in clinical practice, hence immunohistochemical application of this marker can be a breakthrough in management of suspicious thyroid nodules by reducing the number of diagnostic thyroidectomies. However studies are needed to evaluate immunohistochemical application of ECM 1 on histopathology or core needle biopsy specimens. Ours is one such study evaluating usefulness of ECM 1 in differentiating benign from malignant thyroid lesions on paraffin embedded tissue sections.

Materials and Methods

The present study was conducted on 81 thyroid tissue samples received in the department of Pathology, Jawaharlal Nehru Medical College, AMU, Aligarh over a period of 5 years (3 years retrospective and 2 years prospective) from 2012 - 2017. Hematoxylin and Eosin stained sections of the biopsy tissue specimens were carefully examined under light microscope in order to establish the histopathological diagnosis. The immunohistochemical marker ECM 1 was applied on appropriate sections as per the kit staining protocol and the slides were evaluated accordingly.

Tumor sections (2 – 3 μ in thickness) were cut and taken on poly-L-Lysine coated clean glass slides for immunohistochemical assay of ECM 1. One positive and one negative control was set up simultaneously. Infiltrating ductal carcinoma breast (well differentiated) was taken as the positive control while primary antibody omission served as the negative control. The primary antibody used for ECM 1 immunohistochemistry was secretory component antibody; Clone SC-05; Ig class: IgG1 in a dilution of 1:50, supplied by the Thermoscientific.

Immunohistochemistry

All the lesions were subjected to immunohistochemical application ECM 1. Intensity and proportion of staining were combined and a 4-tier grading system was applied (0, 1+, 2+ and 3+) (table 1) which is a modification of the scoring system used by Lal et al. 2008.[10] The results of IHC were analysed under high power (40x). Positive ECM 1 expression was observed as a brown cytoplasmic stain.

The statistical analysis was carried out using STATA 14 software. A p value of <0.05 was considered to be statistically significant. Diagnostic accuracy measures (sensitivity, specificity, positive predictive value and negative predictive value) were calculated.

Results

Out of total 81 neoplastic thyroid tissue samples, 21 were histopathologically diagnosed as benign neoplasms, comprising 18 cases of follicular adenoma (22.2%) and 3 cases of hurthle cell adenoma (3.7%). 60 tissue samples were diagnosed as malignant neoplasms, comprising papillary thyroid carcinoma and its variants (51 cases, 62.9%), follicular thyroid carcinoma (5 cases, 6.1%). Medullary and anaplastic carcinoma were rare, with only 2 cases each (2.4%).

The histopathological variants of PTC included classical and follicular variants (25 cases each, 49%). Only 1 case of oncocytic variant was diagnosed.

Majority of malignancies (78.33%) showed strong homogenous and crisp staining (3+) with ECM 1, whereas benign neoplasms showed a variable staining pattern. Importantly, all histopathological subtypes of thyroid carcinoma showed moderate to strong positivity for ECM 1 (figure 1). As shown in table 2, amongst the benign neoplasms, 47.62% (10 cases) were negative for ECM 1, while 52.38% lesions (11 cases) stained positively. Out of the 11 positive cases of adenomas, the majority (i.e. 10 cases, including 8 cases of follicular adenoma and 2 cases of hurthle cell adenoma) showed weak patchy and granular staining (1+) (figure 2). Only 1 case of hurthle cell adenoma showed moderate degree of staining (2+) (figure 3). The difference in expression of ECM 1 between these two categories (benign vs malignant) was found to be markedly significant ($p<0.001$)

The expression of ECM 1 in various follicular patterned lesions has been shown in Table 3. It was noted that the difference in ECM 1 expression between follicular adenoma and follicular patterned malignant neoplasms (FTC and FVPTC) was statistically significant ($p<0.05$). While 55.56% cases of follicular adenoma were negative for ECM 1, 44.4% showed weak patchy (1+) staining in 10-30% cells. However on comparing ECM 1 expression between the two follicular patterned malignant neoplasms (FTC and FVPTC), the results were found to be statistically insignificant ($p> 0.05$) since all the histological types of malignant lesions were consistently positive for ECM 1 with moderate (2+) to strong (3+) staining pattern (figure 4 and 5).

Diagnostic accuracy measures of ECM 1 for differentiating benign from malignant thyroid lesions were calculated. Sensitivity of 100% was recorded for the differentiation of benign from malignant thyroid neoplasms. However a lower specificity of 45.5% was obtained. The diagnostic accuracy of ECM 1 for the differentiation of benign from malignant lesions was 0.82.

Table 1: Interpretation of immunohistochemistry.

Percentage of cells stained	Intensity	Score
<10%	No staining	0
>10 - 30%	Weak, patchy staining	1+
>31 - 60%	Moderate, uniform staining	2+
>60%	Strong uniform staining	3+

Table 2: Expression of ECM 1 in neoplastic lesions of thyroid.

S. No.	Type of lesion	ECM1 staining grade				TOTAL
		0	1+	2+	3+	
a.	*Benign	10	10	1	0	21
b.	**Malignant	0	0	13	47	60
TOTAL						**81**

**Benign: Follicular adenoma-18; Hürthle cell adenoma-3*

***Malignant: PTC and variants-51; FTC-5, Medullary thyroid carcinoma-2, Anaplastic thyroid carcinoma-2*

Statistical analysis carried out using STATA 14 software. P value (a:b) <0.05

Table 3: Expression of ECM 1 in follicular patterned thyroid neoplasms.

S. No.	Diagnosis		ECM1 staining grade			TOTAL
		0	1+	2+	3+	
1.	FA	10	8	0	0	18
2.	FTC	0	0	2	3	5
3.	FVPTC	0	0	12	13	25
	TOTAL					**48**

Statistical analysis carried out using STATA 14 software. 1:2 ($p<0.05$); 1:3 ($p<0.05$)

FA=follicular adenoma, FTC=follicular thyroid carcinoma, FVPTC=follicular variant of PTC

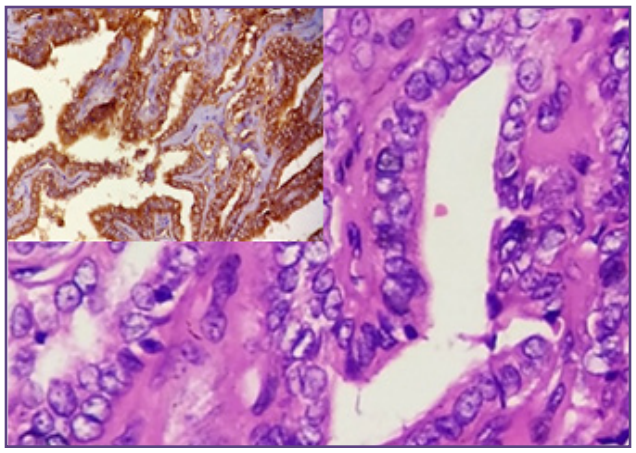

Fig. 1: Papillary thyroid carcinoma (H&E 40x) atypical optically clear nuclei (orphan annie eye nuclei),

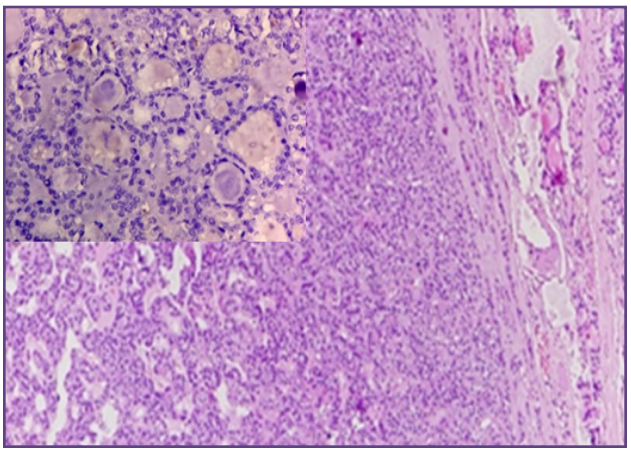

Fig. 2: follicular adenoma (H&E 10x). IHC for ECM 1 (inset, 40x) showing weak and granular staining in 10-30% cells (grade 1+)

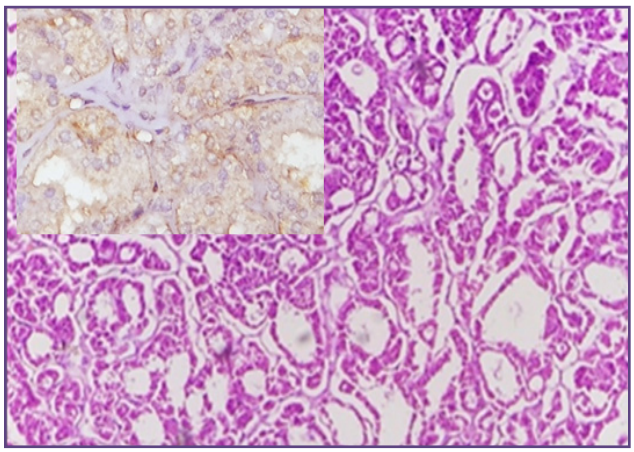

Fig. 3: Hurthle cell adenoma (H&E 10x). IHC for ECM 1 (inset, 10x) showing weak patchy staining in >10-30% cells (1+).

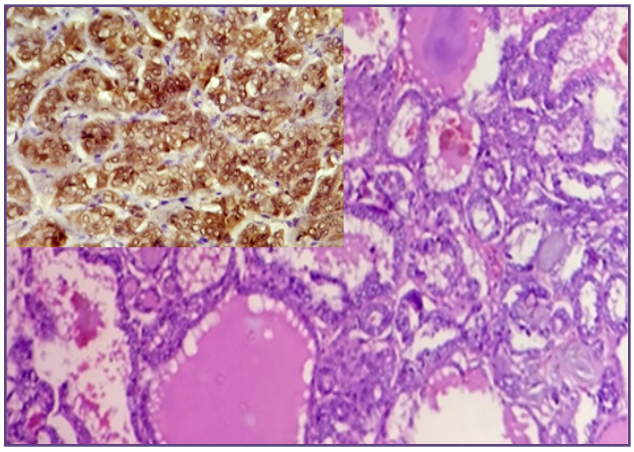

Fig. 4: Follicular variant of papillary thyroid carcinoma (H&E 10x). IHC for ECM 1 (inset, 40x)showing 3+ staining in >60% tumour cells.

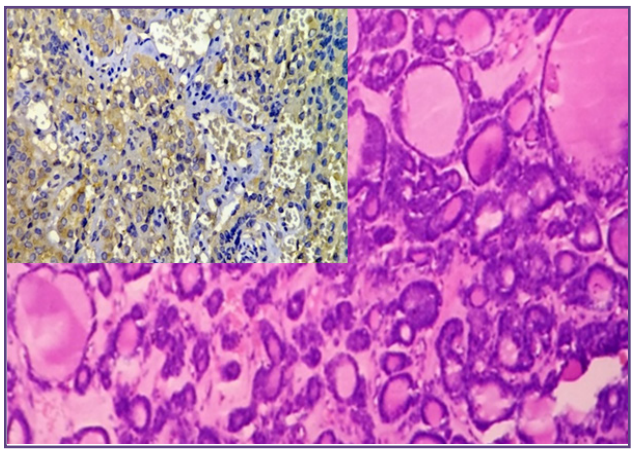

Fig. 5: Follicular thyroid carcinoma (a) H&E 40x, IHC for ECM 1 showing 3+ staining in >60% tumour cells (b) 40x.

Discussion

Fine needle aspiration cytology (FNAC) is the most widely accepted and cost effective method for initial evaluation of thyroid nodules. Follicular patterned lesions like follicular adenoma, follicular carcinoma and follicular variant of papillary thyroid cancer cannot be precisely subtyped in all cases on FNAC, owing to overlapping cytomorphological characteristics, hence they are usually assigned a diagnostic category of 'Follicular neoplasm/ Suspicious for follicular neoplasm'.[2] Ancillary tests involving various immunomarkers particularly target the cases falling under the above mentioned diagnostic category. The use of core needle biopsy (CNB) in thyroid lesions is gradually becoming commonplace.[3] A biopsy sample is superior to a FNAC sample in terms of providing architectural preservability and an adequately representative sample both in terms of quality and quantity, thus allowing appropriate application of immunomarkers.[11] An immunomarker with high sensitivity could thus be utilized to identify neoplastic tissue on a CNB specimen which would help triage patients in need of excisional procedures. This would greatly reduce unnecessary surgical procedures and patient morbidity by acting as an important bridge between a non diagnostic/ suspicious FNAC and histopathology.

Several immunomarkers for thyroid neoplasms have been studied till date both singly and in combination. Most studies have been in favour of use of a panel of markers, but the results are variable and cost effectiveness of this method is debatable. Moreover, keeping in view the future trend of use of biopsy specimens in evaluation of suspicious thyroid nodules a single marker with high sensitivity would nonetheless be preferred over a panel of markers.[12] Izkhakov et al. (2016) showed mRNA expression of ECM 1 to be an independent predictor of thyroid malignancy.[13] ECM 1 is amongst one of the seven hub-genes whose expression levels are validated by RT-qPCR in thyroid malignancies.[14] Kebebew et al. in 2005 and Lal et al. in 2008 studied ECM 1 m-RNA expression on real time Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) and reported ECM 1 to be an independent marker of malignancy in thyroid.[9,10] Both however recommended further studies to evaluate the immunohistochemical utility of the marker. Pauws et al. (2004) observed that ECM 1 is over expressed in papillary and follicular thyroid carcinoma but not in benign thyroid nodules.[15] Gene expression analysis of ECM 1 has been studied by using Real Time Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). However this technology is more complex than IHC based assessment of protein expression and is not easily available at all the centers hence study of IHC expression

of ECM 1 would make it a feasible option in diagnostically challenging cases.

Benign tumours evaluated for ECM 1 expression in this study included 18 cases of follicular adenoma and 3 cases of hurthle cell adenoma. 47.6 % of these were negative for ECM 1 and 52.3% lesions stained positively with majority showing weak patchy granular staining. Wang et al. (2003) reported ECM 1 positivity of 11.7% for benign tumours of thyroid while Lal et al. (2008) noted 25% benign tumours to be positive for ECM 1. [5,10] Poma et al. (2018) also showed a differential expression of ECM 1 in benign and malignant follicular lesions. [16]

ECM 1 staining was done in 51 cases of papillary thyroid carcinoma (including follicular and oncocytic variants), 5 cases of follicular thyroid carcinoma and 2 case each of medullary and anaplastic thyroid carcinoma. 100% of the malignancies were positive for ECM 1 with 78.3% showing strong and uniform staining (3+) in >60% tumour cells. This is in concordance with the findings of Lal et al. (2008) and Poma et al. (2018) who also noted a significantly higher uptake in malignant tumours.[10,16]

A markedly significant difference in the expression of ECM 1 levels was observed between benign versus malignant neoplasms ($p < 0.001$). This demonstrates that ECM 1 is indeed overexpressed in malignant thyroid neoplasms, while the expression is nil or weak in benign lesions. Our observations are similar to those of Kebebew et al. (2005) and Lal et al. (2008) who studied ECM 1 m - RNA expression using RT-PCR and reported significantly higher expression levels in malignant neoplasms as compared to benign lesions. [9,10] The present study also establishes that the results of ECM 1 expression on immunohistochemistry are comparable to the results on RT-PCR.

The above analysis also shows that ECM 1 is highly sensitive in identifying malignant thyroid neoplasms and can be utilized as a standalone screening test, while lower sensitivities obtained with other markers and variable expression with reference to PTC and FTC requires them to be used in panels. The reported sensitivity of Gal-3, HBME-1, CK-19 used in combination was 85%, and the specificity was 97%.[17] Keeping in view that a biopsy specimen is limited and clinicians often anticipate early response from a pathologist, application of a single marker with a higher sensitivity would nonetheless be preferred over application of a panel of markers.

Conclusion

The major conclusion of this study is that ECM 1 is uniformly upregulated in all malignant thyroid neoplasms. ECM 1 can be easily applied to biopsy samples and can be used as an independent screening test to identify malignancies pre- operatively. This will help in clinical decision making and reduce patient morbidity and expenses. Other markers, while having higher specificity, show lower sensitivity and their expression is often restricted to PTC, hence usually applied in a panel. In this regard again, ECM 1 has an advantage over other markers for thyroid malignancies.

The sensitivity of the marker for identifying malignancies approaches 100%, although false positivity was observed in a small percentage of benign neoplasms. As previous studies evaluated ECM 1 m RNA expression on RT-PCR, the present study was undertaken with the purpose of evaluating immunohistochemical utility of the same. [10,13,14] The findings of our study reveal that immunohistochemical expression of ECM 1 shows comparable results to RT-PCR method adopted by and can prove to be a feasible option in resource poor clinical setting.

Acknowledgements

Mr. Raju Medhi, Technical assistant, Histopathology Laboratory, Department of Pathology, AMU.

Funding

Nil

Competing Interests

There are no conflicts of interest

Reference

1. Maitra A. The endocrine system. In: Kumar V, Abbas AK, Aster JL, editors. Robbins & cotran pathologic basis of disease.9th ed. New Delhi: Reed Elsevier India Private Limited; 2014. 1082-1100.
2. Freitas BCG and Cerutti JM. Genetic markers differentiating follicular thyroid carcinoma from benign lesions. Molecular and Cellular Endocrinology. 2009; 321(1): 77-85.
3. Jung CK, Min HS, Park HJ, Song DE, Kim JH, Park SY et al. Pathology reporting of thyroid core needle biopsy: a proposal of the Korean Endocrine Pathology Thyroid Core Needle Biopsy Study Group. Journal of pathology and translational medicine. 2015; 49(4): 288-299.
4. Sercu S, Zhang M, Oyama N, Hansen U, Ghalbzouri AE, Jun G et al. Interaction of extracellular matrix protein 1 with extracellular matrix components: ECM1 is a basement membrane protein of the skin. Journal of Investigative Dermatology. 2008; 128(6):1397-1408.
5. Wang L, Yu J, Ni J, Xu XM, Wang J, Ning H et al. Extracellular matrix protein 1 (ECM1) is over-expressed in malignant epithelial tumors. Cancer letters. 2003; 200(1): 57-67.
6. Lal G, Hashimi S, Smith BJ, Lynch CF, Zhang L, Robinson RA et al. Extracellular matrix 1 (ECM1) expression is a novel prognostic marker for poor long-term survival in

breast cancer: a Hospital-based Cohort Study in Iowa. Annals of surgical oncology. 2009; 16(8): 2280-2287.

7. Chen H, Jia WD, Li JS, Wang W, Xu GL, Ma JL et al. Extracellular matrix protein 1, a novel prognostic factor, is associated with metastatic potential of hepatocellular carcinoma. Medical Oncology. 2011; 28(1): 318-325.
8. Wu Q, Li X, Yang H, Lu C, You J, Zhang Z. Extracellular matrix protein 1 is correlated to carcinogenesis and lymphatic metastasis of human gastric cancer. World journal of surgical oncology. 2014; 12(1): 132-137.
9. Kebebew E, Peng M, Reiff E, Duh QY, Clark OH, McMillan A. ECM1 and TMPRSS4 are diagnostic markers of malignant thyroid neoplasms and improve the accuracy of fine needle aspiration biopsy. Annals of surgery. 2005; 242(3): 353-359.
10. Lal G, Padmanabha L, Nicholson R, Smith BJ, Zhang L, Howe JR et al. ECM1 expression in thyroid tumors—a comparison of real-time RT-PCR and IHC. Journal of Surgical Research. 2008; 149(1): 62-68.
11. Na DG, Kim JH, Sung JY, Baek JH, Jung KC, Lee H, et al. Core-needle biopsy is more useful than repeat fine-needle aspiration in thyroid nodules read as nondiagnostic or atypia of undetermined significance by the Bethesda system for reporting thyroid cytopathology. Thyroid. 2012; 22:468-475.
12. Sung JY, Na DG, Kim KS, Yoo H, Lee H, Kim JH et al. Diagnostic accuracy of fine-needle aspiration versus core-needle biopsy for the diagnosis of thyroid malignancy in a clinical cohort. European radiology. 2012; 22(7):1564-1572.
13. Izkhakov E, Somjen D, Sharon O, Knoll E, Aizic A, Fliss DM, Limor R, Stern N. Vitamin D receptor expression is linked to potential markers of human thyroid papillary carcinoma. The Journal of steroid biochemistry and molecular biology. 2016; 159:26-30.
14. Zhang K, Liu J, Li C, Peng X, Li H, Li Z. Identification and validation of potential target genes in papillary thyroid cancer. European journal of pharmacology. 2019; 843:217-25.
15. Pauws E, Veenboer GJ, Smit JW, de Vijlder JJ, Morreau H, Ris-Stalpers C. Genes differentially expressed in thyroid carcinoma identified by comparison of SAGE expression profiles. The FASEB journal. 2004; 18(3):560-571.
16. Poma AM, Giannini R, Piaggi P, Ugolini C, Materazzi G, Miccoli P, Vitti P, Basolo F. A six-gene panel to label follicular adenoma, low-and high-risk follicular thyroid carcinoma. Endocrine connections. 2018; 7(1):124-32.
17. De Matos LL, Del Giglio AB, Matsubayashi CO, De Lima FM, Del Giglio A, Da Silva PMA. Expression of ck-19, galectin-3 and hbme-1 in the differentiation of thyroid lesions: systematic review and diagnostic meta-analysis. Diagnostic Pathology. 2012; 7:95-97.

***Corresponding author:**
Naba Hasan, Flat no. 607, the castles, anoopshaher road, Aligarh
Phone: +91 7500068648
Email: naba2k8@gmail.com

Financial or other Competing Interests: None.

Date of Submission : 04-03-2020
Date of Acceptance : 14-08-2020
Date of Publication : 30-09-2020