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ROLE OF HBME-1 IN DIFFERENTIATING SURGICALLY EXCISED THYROID NODULES

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ABSTRACT

Thyroid nodules represent a wide spectrum of neoplasms with different biological behaviors and thyroid follicular epithelial carcinomas are the most common carcinoma of the endocrine system. Histological evaluation is the gold standard in the diagnosis of thyroid nodules, however, similar morphological features put a burden on pathologist while trying to make diagnosis on H & E slides. The use of immunohistochemical markers offers advantage in cases where histomorphological details are insufficient to establish a definitive diagnosis. The aim of this study to evaluate the role of HBME-1 in Differentiating Surgically Excised Thyroid Nodules. Immunohistochemistry method may plays a complementary role to clarify diagnostic dilemma. **Materials and Methods:** This is a cross sectional study which was conducted in the Department of Pathology, Chittagong Medical College, Chattogram during the period of March, 2019 to February, 2021 with 63 surgically excised thyroid nodule patients attending in Department of ENT, Chittagong Medical College Hospital and other private hospitals in chattogram. All collected samples were processed for routine histopathological study and selected for immunohistochemistry with HBME-1 antibody. Immunohistochemistry was done at Armed Forces Institute of Pathology, Dhaka Cantonment. Immunostaining was done by using primary antibody HBME-1 (Anti–Mesothelioma mouse monoclonal antibody HBME-1 1ab2383.Abcam, UK). Patient's demographic data were collected and recorded in a pre-designed data sheet. Statistical analysis was carried out as required. Ethical practice was ensured in every step of the study. **Results and observations:** In this study the mean age was 39.47 ± 13.67 and male to female ratio was 1: 6.9. Thirty-four patients (76.3%) had multiple nodules and 29 patients had history of consuming iodized salt. Forty-three (68.3%) cases were histomorphologically diagnosed as benign and 20 cases (31.7%) as malignant thyroid nodules according to the 2017 WHO classification of thyroid tumors. In the study, HBME-1 positive expression was showed in 17 cases (85%) out of 20 cases of malignant nodules and expression was not observed in benign nodules. The expression of HBME-1 was not statistically significant ($p>0.250$) means no difference was observed between histopathological examination and HBME-1 expression for diagnosis of benign and malignant nodules. **Conclusion:** Positive HBME-1 staining is a strong indicator of malignancy, although negative staining does not rule it out. The utilization of HBME-1 markers along with histomorphological evaluation is supportive in the differential diagnosis of thyroid nodules.

KEYWORDS: Thyroidnodules, lymphangiogenesis, metastasis.

INTRODUCTION

Thyroid diseases are the most common endocrine disorders worldwide. The prevalence of thyroid nodules based on palpation in the general population is 2–6 %.[1] According to World Health Organization (WHO) GLOBOCAN Statistics, thyroid cancer was responsible for 567,000 new cases and 41,071 deaths in the year 2018 worldwide, was ranking in ninth place for incidence. The global incidence rate in women of 10.2 per 100,000 is 3 times higher than in men. The yearly incidence of thyroid cancer has nearly tripled from 4.9 per 100,000 to 14.3 per 100,000 in approximately 35years.[2] In 2016, thyroid cancer was predicted to be the fifth most common cancer in Canadian women after breast,lung,colon and uterine cancers.[3]

Majority of thyroid nodules are benign, but malignancy is found in approximately 5–15% of cases.[4] The 'gold standard' in diagnosis of thyroid nodules is histopathologic evaluation using routine haematoxylin and eosin staining. Diagnostic dilemma may arise when an encapsulated nodule with a follicular pattern of growth exhibits clear nuclei with grooves and distinguishing follicular adenoma from encapsulated follicular variant papillary thyroid carcinoma becomes difficult. Multinodulargoitre with delicate papillary budding and focal nuclear clearing may be confused with Papillary carcinoma.[5]

Thyroid tumor of uncertain malignant potential (UMP) was first proposed by Williams (2000) for encapsulated follicular pattern thyroid tumors to solve problems due to observer variation. Liu *et al.* proposed well-differentiated tumor with uncertain behavior (WDT-UB) which covered WDT of UMP (WDT-UMP) and non-invasive encapsulated follicular variant of papillary thyroid carcinoma (EFVPTC). These borderline/precursor thyroid tumors were incorporated in the 4th edition WHO classification of thyroid tumors as a new tumor entity. Their behavior codes were decided to be 1 (borderline or uncertain behavior), and 0 (benign), 2 (*in situ* carcinoma) or 3 (malignant). (Kakudo*et al.*,2016) Such morphological features put a burden on pathologist while trying to make diagnosis on H & E slides. Immunohistochemistry method plays a complementary role to clarify these dilemma.[6]

Hector Battifora mesothelial epitope (HBME-1) is a marker of mesothelial cells, named after the laboratory of Dr. Hector Battifora. HBME-1 is a membranous antigen located on microvilli of mesothelial cells and also in follicular thyroid tumour cells.[7] Normal thyroid tissue is negative for HBME-1. In previous study, diagnostic sensitivity was observed highest in differentiating follicular variant papillary thyroid carcinoma from follicular adenoma (100%), follicular variant papillary thyroid carcinoma from follicular carcinoma (100%), Papillary carcinoma from thyroid tumours of uncertain malignant potential (100%) and Papillary carcinoma from other benign non neoplastic lesions (100%).[5]

Patients with differentiated thyroid carcinoma have an excellent prognosis. The treatment includes surgery (near-/total thyroidectomy) usually followed by remnant ablation using radioiodine according to the guidelines of the American Thyroid Association (ATA) and European Association of Nuclear Medicine (EANM).[8]

Borderline/precursor thyroid tumors are indolent tumors biologically and should be treated more conservatively than as previously recommended for thyroid follicular cell carcinomas by western clinical guidelines.[6] If diagnosis can be made correctly, these individuals will be spared of unnecessary, aggressive surgical and radioactive iodine therapy and morbidity and financial costs related to these procedures.

OBJECTIVES

General objectives

To evaluate the contribution of HBME-1 immuno expression in the diagnosis of surgically excised thyroid nodules.

Specific objectives

1. To study the different histological subtypes of thyroid nodules.
2. To evaluate the HBME-1expression in surgically excised thyroid nodules.
3. To assess the association of HBME-1 expression with histological type.

METHODOLOGY

Type of the study: Cross sectional observational study.
Place of study: Department of Pathology, Chittagong Medical College, Chattogram. Immunohistochemistry was done at Armed Forces Institute of Pathology. Dhaka Cantonment.
Study period: March 2019 to February 2021.
Study sample: Total 63 Patients with clinically palpable thyroid nodules excised in the Department of E.N.T, Chittagong Medical College & Hospital, Chattogram, and other private hospitals in Chattogram were the target population during specified time duration.
Sampling technique: Consecutive sampling.

Sample selection criteria

Inclusion criteria

1. Patients with surgically excised palpable thyroid nodules.
2. Those patients who had given written informed consent for the study.

Exclusion criteria

1. Patients with any form of malignancy metastasis to thyroid from other sites.
2. Patients previously treated with chemotherapy or radiotherapy for thyroid tumor.
3. Patients unwilling to give written consent.

Data collection tool

A predesigned Case record form.

Data collection procedure

Data was recorded on variables of interest by interview and using the structured questionnaire after taking properly informed written consent from the patient at the Department of Pathology, Chittagong Medical College and private clinics. Patients were eligible for inclusion if they were undergone surgical resection of thyroid nodular leisons and all the specimens of each case were submitted for histological examination; and finally diagnosed according to the WHO classification.

Histopathologic Examination

All specimens of each case were processed by conventional histopathology method. Hematoxylin and eosin-stained slides of each case was prepared for proper microscopic evaluation. Sections were studied under light microscope to classify benign and malignant lesions and to select one representative paraffin block for immunohistochemical analysis.

Immunohistochemical Examination

From paraffin-embedded blocks, 4-micrometer thick sections were cut, deparaffinized with xylene and rehydrated through a graded series of alcohol. For antigen retrieval, the samples were carried out with 1 mmol/L of EDTA (pH 6 for HBME-1)with 10 minutes on the hotplate. This was followed by a 10-minute incubation in avidin biotin with tris(hydroxymethyl)aminomethane (Tris) buffer in between. Then the sections were stained successively with Anti –Mesothelioma mouse monoclonal antibody (HBME-1) ab2383. (Abcam UK). DAKO REALTM EnVision TM (HRP RABBIT/MOUSE)(ENV) was used as secondary antibody. For HBME-1 immunostain, positive control was taken from sections of papillary carcinoma of thyroid.

Evaluation of HBME-1 Status

The membranous expression of HBME-1 was scored semi quantitatively by the intensity of staining and percentage of positive cells. Then the 'expression intensity score' was computed as the multiplication of the percentage of positive cells by staining intensity (Expression Intensity score = proportion score x intensity score). immunoreactivity no staining or weak staining was considered as negative (-), staining less than 25% of the cells and buffy staining was considered as weakly positive (+), staining 25%-50% of the cells and buffy staining was considered as midrange positive (++), staining more than 50% of the cells and deep brown staining was considered as strong positive (++ +) The lesion was considered positive for a immunomarker with expression intensity score of at least 2 or more.[9]

Statistical analysis of data

Data were entered into Excel worksheet to generate a master sheet. Then they were fed into software Statistical Package for the Social Sciences, version 27 (SPSS Inc., Chicago, IL) for processing and analysis. Results were shown as table and expressed as frequency & percentage for qualitative data and mean ± SD for quantitative data and McNemartest was applied for compared of the HBME-1 marker with histopathological examination. A 'p' value <0.05 was considered a statistically significant because of all analysis were consider as 95% confidence level.

RESULT

The present study was carried to evaluate the diagnostic role of HBME-1 immuno expression in thyroid nodules. For these purposes total of 63 cases of surgically resected thyroid nodules sample were enrolled in this study. After details gross examinations of the specimens, hematoxylin and eosin-stained sections were examined under the microscope for histological examination, HBME-1immunostaining were done.

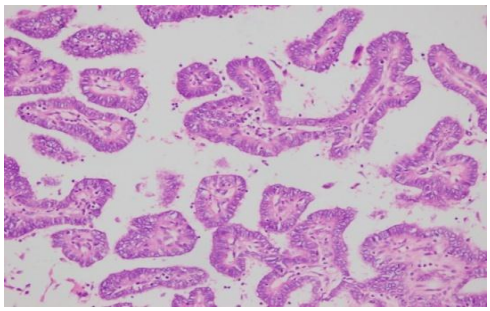

Fig 01: Papillary thyroid carcinoma (x40) H & E

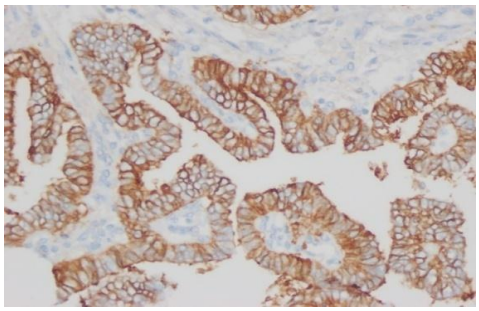

Fig 02: Papillary thyroid carcinoma (x40)
HBME-1 expression: Strong positive (+++).

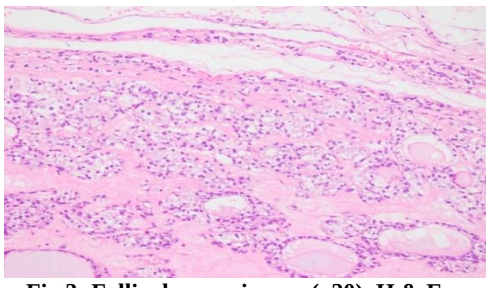

Fig 3: Follicular carcinoma (x20). H & E.

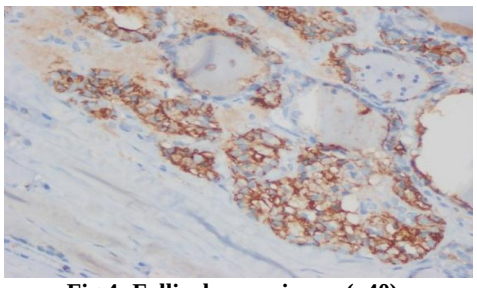

Fig 4: Follicular carcinoma(x40). HBME-1 expression: Capsular invasion, Strong positive (+++).

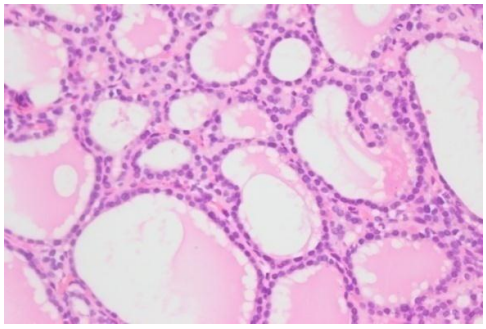

Fig 5: Multinodular goitre (x20). H & E.

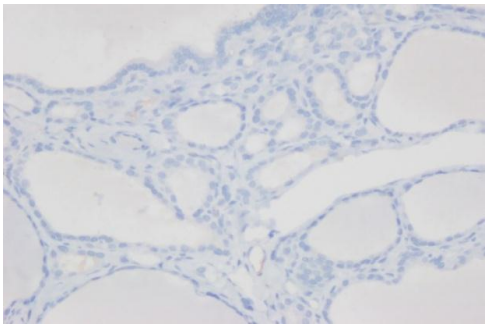

Fig 6: Multinodular goitre (x40).

HBME-1 expression: Negative.

Distribution of the patients according to age (n=63):
Among the 63 cases of present study, 20 patients (31.7%) were in between 26-35 years of age, followed by 17 patients (27%) in between 36-45 years of age. The mean age (±SD) of the patients was 39.47 ± 13.67 years with the youngest and eldest patient in this study were 16 years and 74 years of old respectively (Table 1).

Table 1: Distribution of the patients according to age (n=63).

Age (Years)	Frequency	Percent
• **16-25**	7	11.1
• **26-35**	20	31.7
• **36-45**	17	27.0
• **46-55**	11	17.5
• **56-65**	6	9.5
• **66-74**	2	3.2
Mean ± SD (Min-Max)	39.47 ± 13.67 (16-74)	
Median	38	
Mode	40	

Gender
Female was predominant (55 cases; 87.3 %) and male to female ratio was 1: 6.9 (Table 2). This study showed frank predominance of thyroid nodule in female patients.

Table 2: Distribution of the patients according to gender (n=63).

Gender	Frequency	Percent
Male	8	12.7
Female	55	87.3

Dietary iodine intake
Out of 63 cases, 29 patients (46%) consume iodized salt and 34 patients (54%) consumed non-iodized salt. (Table 3).

Table 3: Distribution of the patients according to dietary iodine intake (n=63)

Dietary iodine intake	Frequency	Percent
Yes	29	46.0
No	34	54.0
Total	**63**	**100.0**

Family history
Maximum patients (59cases; 93.7%) had no significant family history. Four patients (6.3%) had a family history of the thyroid nodule. (Table 5).

Table 5: Distribution of the patients according to family history (n=63).

Family history	Frequency	Percent
Yes	4	**6.3**
No	59	**93.7**
Total	**63**	**100.0**

Histopathological types
In present study, among 63 cases of nodules, 37 cases (58.7%) were histopathologically diagnosed as multinodulargoitre (MNG). Hasimoto thyroiditis and follicular adenoma both were 3cases (4.8%) respectively. Histopathologically diagnosed malignant nodules include – 10 cases of papillary thyroid carcinoma (15.9%), 5 cases (7.9%) of follicular variant PTC and 5 cases (7.9%) of follicular carcinoma. Histological typing were done according to 2017 WHO classification of thyroid tumours.[10]

Table 6: Distribution of the patients according to histological subtypes (n=63).

Histological diagnosis	Frequency	Percent
Benign MultinodularGoitre (MNG)	37	58.7
Hasimoto Thyroiditis	3	4.8
Follicular Adenoma (FA) Malignant	3	4.8
Papillary Thyroid Carcinoma(PTC)	10	15.9
Follicular variant PTC	5	7.9
Follicular Carcinoma (FC)	5	7.9
Total	63	100

Histopathological diagnosis (n=63)

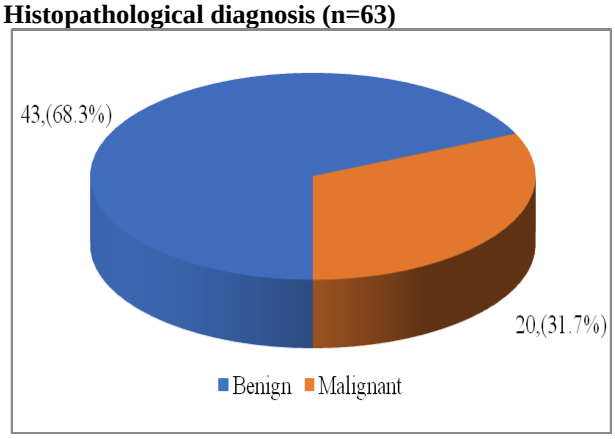

Figure 7: Pie chart of the patients according to histopathological diagnosis (n=63).

Figure 7 shows among the 63 patients, 43cases (68.3%) were histologically reported as benign and 20 cases (31.7%) as malignant according 2017 WHO classification of thyroid tumours.[10]

Histopathological diagnosis and number of the nodule (n=63)

Among 29cases of single nodule,17cases (58.6%) were malignant nodules,and the rest 12 cases (41.4%) were benign. On the other hand, among 34 cases of multiple nodules, mostly represented nodular goiter and other benign lesions -31 (91.2%) and only 3cases (8.8%) were malignant nodules. So, single nodules have a high possibility of being malignant. This association reached statistical significance ($p<0.05$) and was calculated by chi-square test (Table7).

Table 7: Histopathological diagnosis and number of the nodule (n=63).

No. of nodule	Histopathological diagnosis	
	Malignant	Benign
Single (29)	17(58.6)	12(41.4)
Multiple (34)	3(8.8)	31(91.2)
Total	**20 (31.7)**	**43 (68.3)**

HBME-1 expression

HBME-1 was expressed positive in 17 out of 20 malignant cases. Benign cases were negative for HBME-1. The expression of HBME-1 was not statistically significant ($p>0.05$) means no difference was observed between histopathological examination and HBME-1 expression for diagnosis of benign and malignant nodules. It was calculated by McNemar test.

Table 8: HBME-1 expression with histopathological diagnosis(n=63).

HBME-1 expression	Histopathological diagnosis		P value*
	Malignant	Benign	
Positive	17 (85.0)	0 (0.0)	**0.250**
Negative	3 (15.0)	43(100.0)	
Total	**20 (100.0)**	**43(100.0)**	

DISCUSSION

In the diagnosis of thyroid nodules, gold standard is histopathological evaluation. In the cases of morphological overlap, immunohistochemistry is needed for differential diagnosis.[11] The purpose of this study is to observe the expressions of HBME-1 antibodies in surgically excised thyroid nodular lesions for diagnosis of benign and malignant nodules. A total 63 histopathologically diagnosed cases of surgically excised thyroid nodules were enrolled in this study.

In present study, the mean age (±SD) of the patients was 39.47 ± 13.67 years with female predominance (55 cases; 87.3%). Male to female ratio was found 1: 6.9. These findings are nearly similar to the study of Durmus *et al*.(2016),who found a mean age of 48.2 for malignant and 50.8 for benign lesions (range: 21-77 years) with 83.6% female patients. The male to female ratio was 1:5.2, which is much similar to the present result.[11]

Female sex was an independent risk factor for the development of Thyroid nodules. Clinical studies have shown that gender differences might be due to the combined effect of estrogen and progesterone. Estrogen stimulated thyrotropin (TSH) generation in both normal and neoplastic thyroid tissues that have estrogen receptor expression. So, it can indicate that estrogen plays a possible role in the growth of thyroid cells and nodule formation. It was demonstrated in vitro studies that 17-estradiol may stimulate the growth of normal thyroid cells and that thyroid follicular cells contained functional estrogen receptors.[12]

This study showed that 46% (29) patients consume household iodized salt and 54% (34) patients consume non-iodized /inadequate iodized salt (commonly known as open salt). Although, presence of iodine amount in household salts was not assessed in our study. Benign disease mainly multinodular goitre was seen in 25 (73.5%) patients under low coverage of adequately iodized household salt and 18 (62.1%) patients with consuming adequately iodized salt. Yusuf *et al*., 2008 was conducted a Similar survey in Bangladesh[13]. They observed inverse relationship between the coverage of adequately iodized household salt and the prevalence of iodine deficiency in children and women, which is similar to this study.

In this study, 34 (54%) patients had multiple thyroid nodules and 29 (46%) patients had solitary nodules. Most of the multiple thyroid nodules 31 (91.2%) were histologically benign and only 3 (8.8%) were malignant. In the case of a solitary nodule, predominant nodules 17 (58.6%) were malignant followed by 12 (41.4%) were benign. Jena *et al*., (2015) found that Solitary thyroid nodules have a high likelihood of harboring a malignancy. In their study

106 patients of STN confirmed by USG, 49 (46.2%) turned to be malignant on histopathology, while only 9 (22.5%) of the 40 patients with MNG were malignant (P = 0.009) [14]. Similar findings were observed byTai D J., *et al* (2012).[15]

Among 63 cases of thyroid nodules in the current study 59 (93.7%) patients had no family history of thyroid disease. Only 4 (6.3%) patients had a family history of goiter and thyroid carcinoma. Three patients with multinodular goiter had a family history of MNG in their first-degree relatives and one patient had a malignant thyroid disease(PTC) who was the son of a father having thyroid carcinoma.

Among the patients according to histopathological classification, 43 (68.3%) patients had identified benign disease, 20 (31.7%) patients had identified malignant disease.

In the present scenario, the HBME-1 marker is most promising antibody for identifying thyroid malignancy. HBME-1 stains mostly follicular derived malignant tumours, including both well-differentiated and poorly differentiated carcinomas. HBME-1 proved to be the most specific marker in distinguishing benign from malignant thyroid pathology. In study, observed positive expression of HBME-1 in 17 out of 63 cases (27%). It was negative in histomorphological diagnosed benign cases. Histomorphologically diagnosed 17 out of 20 malignant cases (85%) showed positive expression. In a study, PALO *et* al. found positive expression of HBME-1 in 87.5% of malignant cases [16]. Nasr *et al.* have worked a lot of immunohistochemical markers in 51 PTC and 57 benign thyroid lesions. They have found HBME-1 staining in 96% of the malignant group and staining was not observed in 93% of benign lesions. This was quite similar to our study[17]. Saleh *et al.* worked with galectin‑3, HBME‑1, CK19, and Ret oncoprotein immunohistochemically in 98 benign thyroid nodules (52 hyperplastic nodules, 46 follicular or HHA) and 54 malignant tumours (22 FC, 20 classic PTC, and 12 FVPTC). In the same study, staining percentage of HBME‑1 in classic PTC and FVPTC was 90% and 91.7%, respectively. Similar results were obtained in our study for staining percentage of HBME‑1[7]. Cheung et al. reported HBME1 positivity in 70% classic PTC and 45% FVPC with no expression 7.5% of malignant cases. This study was compatible with present study.[18]

HBME-1 was showed positive expression for 17(85%) out of 20 cases of malignant nodules and negative expression was observed in benign nodules. No statistically significant (p>0.250) difference was found between HBME-1 expression and histopathological diagnosis.

CONCLUSIONS

In this study, Positive HBME-1 staining is a strong indicator of malignancy, although negative staining does not rule it out. So this study has improved the better understanding of thyroid nodules by expression of this immunomarker and thus may help the patients for selecting appropriate management protocol.

REFERENCE

1. Tamhane, S. and Gharib, H. Thyroid nodule update on diagnosis and management. Clinical Diabetes and Endocrinology, 2016; 2(1).
2. Dirikoc, A., Faki, S., Baser, H., Özdemir, D., Aydin, C., Ersoy, R., Kilic, M., Kilicarslan, A. and Çakir, B. Thyroid malignancy risk in different clinical thyroid diseases. TURKISH JOURNAL OF MEDICAL SCIENCES, 2017; 47: 1509–1519.
3. Topstad, D., Dickinson, J, A., (2017) Thyroid cancer incidence in Canada: a national cancer registry analysis.10.9778/cmajo.20160162
4. De, D., Dutta,S., Tarafdar, S., Kar, S.S., Das, U., Basu, K., et al Comparison Between Sonographic Features and Fine Needle Aspiration Cytology with Histopathology in the Diagnosis of Solitary Thyroid Nodule. Indian J EndocrMetab, 2020; 24: 349-54.
5. Alshenawy, H., Utility of immunohistochemical markers in differential diagnosis of follicular cell-derived thyroid lesions. Journal of Microscopy and Ultrastructure, 2014; 2(3): 127.
6. Kakudo, K., K, Adel., Naggar, El., P, Steven., Hodak., Khanafshar, E., E,Yuri., Nikiforov., *et* al, Noninvasive follicular thyroid neoplasm with papillary- like nuclear features(NIFTP)in thyroid tumor classification, 2018; 1–7. Available online atdoi:10.1111/pin.12673
7. Saleh, H.A., Jin, B., Barnwell, J. and Alzohaili, O., Utility of immunohistochemical markers in differentiating benign from malignant follicular derived thyroid nodules. Diagnostic Pathology, 2010; 5(1).
8. Schmidbauer, B., Menhart, K., Hellwig, D., Grosse, J., Differentiated ThyroidCancer—Treatment: StateoftheArt. Int. J. Mol.Sci., 2017; 18: 1292.
9. Zhou Y., Chen Z Lu., Jiang H., Diagnostic significance of Ki67, CK19, Galectin-3 and HBME-1 expression for papillary thyroid microcarcinoma. Biomedical Research, 2017; 28: 4002-4006.
10. Lloyd R. V., Osamura R. Y., Kloppel G.,Rosai J. (Eds): WHO Classification of Tumours of Endocrine Organs (4th edition). IARC: Lyon 2017.
11. Durmus, E.S., Ozcan, D., Yarikkaya, E., Kurt, A. and Arslan, A. CD56, HBME-1 and cytokeratin 19 expressions in papillary thyroid carcinoma and nodular thyroid lesions. Journal of Research in Medical Sciences, 2016; 21(1): 49.
12. Jiang, H., Tian, Y., Yan, W., Kong, Y., Wang, H., Wang, A., et al. The Prevalence of Thyroid Nodules and an Analysis of Related Lifestyle Factors in

Beijing Communities. International Journal of Environmental Research and Public Health, 2016.
13. Yusuf, H., Rahman, A. K. M, Chowdhury F P., Mohiduzzaman, M., Banu, C.P., Sattar, M.A., et al(2008) Iodine deficiency disorders in Bangladesh, 2004-05: ten years of iodized salt intervention brings remarkable achievement in lowering goitre and iodine deficiency among children and women. Asia Pac J ClinNutr., 2008; 17(4): 620-628.
14. Jena, A., Patnayak, R., Prakash, J., Sachan, A., Suresh, V., and Lakshmi. A., Malignancy in solitary thyroid nodule: A clinicoradiopathological evaluation, 2015; 19(4): 498–503 Available online at doi: 10.4103/2230-8210.1
15. Tai, J.D., Yang, J.L., Wu, S.C., Wang, B.W., Chang, C.J. Risk factors for malignancy in patients with solitary thyroid nodules and their impact on the management. J Can Res Ther., 2012; 8: 379-383.
16. Palo, S., and Biligi, D.S., Differential diagnostic significance of HBME-1, CK19 and S100 in various thyroid lesions. *Malaysian J Pathol, 2017; 39(1): 55–67.*
17. Nasr, M.R,, Mukhopadhyay, S., Zhang, S., Katzenstein, A.L., Immunohistochemical markers in diagnosis of papillary Thyroid carcinoma: Utility of HBME1 combined with CK19 immunostaining. Mod Pathol, 2006; 19(12): 1631–7.
18. Cheung, C.C, Ezza,t S., Freeman, JL, Rosen, I.B, Asa, SL. Immunohistochemical diagnosis of papillary thyroid carcinoma. Mod Pathol, 2001; 14: 338–42. Available online at doi: 10.1038/modpathol. 3880312