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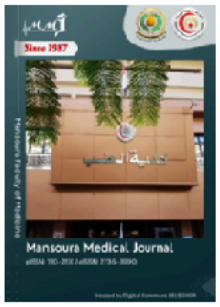

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IMMUNOHISTOCHEMISTRY IN THE DIFFERENTIAL DIAGNOSIS OF SOLITARY THYROID NODULE.

Maha Amin

Pathology Department, Faculty of Medicine, Mansoura University, Egypt.

Dina El-Tantawy

Pathology Department, Faculty of Medicine, Mansoura University, Egypt.

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By

Maha M Amin, MD and Dina A El-Tantawy, MD

From

Pathology Department, Faculty of Medicine,

Mansoura University, Egypt.

ABSTRACT

Evaluation of solitary thyroid nodule is the most common thyroid problem; the prevalence varies according to the method of screening used. Solitary thyroid nodule can be caused by any of the thyroid diseases; the majority is benign. Lesions characterized by a follicular growth pattern constitute the most common type encountered by pathologists. The vast majority of such lesions do not pose difficulties for histopathological interpretation. A subset of these tumors, however, can represent a serious challenge for diagnosis, management & fall under the "indeterminate" category. Thyroid tumors with follicular growth pattern include a broad range of lesions that range from benign, hyperplastic nodules to follicular adenoma and follicular carcinoma. In addition, other types of tumors with follicular growth pattern belonging to separate diagnostic categories, including the follicular variant of papillary carcinoma, atypical adenomas, Hürthle cell tumor & medullary carcinoma can be also encountered. The histologic features used for distinguishing these conditions can be sometimes subtle & subjective.

The present study was performed on 77 excision specimens of solitary thyroid nodules with predominate follicular architecture. Immunohistochemical stains were done for selected cases for high molecular weight cytokeratin, chromogranin A, S-100 & factor VIII.

CONCLUSION : Most thyroid tumors with follicular architecture are diagnosed by morphology alone; however, immunohistochemistry can be used to differentiate between problematic cases exhibiting follicular pattern & to detect true vascular invasion in follicular carcinoma.

INTRODUCTION

The most common thyroid problem is the evaluation of the patient with a single thyroid nodule; which are rare in children, their frequency increases in each decade & more common in females than males [1]. True solitary nodules occur in 0.22-1.35% of the pediatric population & need close attention because of increased frequency of malignancy & more aggressive behavior than adults (4%)[2].The majorities of these nodules are benign and only 8% to 17% of nodules removed are proved to be malignant[3].

Factors that increase the risk of malignancy includes; extremes of age, the incidence of carcinoma is higher in men [1], head and neck radiation especially in young age, family history of thyroid cancer, familial polyposis & Autoimmune thyroiditis [4].

Solitary thyroid nodules may be neoplastic or non-neoplastic; the non-neoplastic nodules include cyst, multinodular goiter, prominent area of thyroiditis and glandular hyperplasia. The neoplastic nodules include adenoma and thyroid cancer [5]. The majority of thyroid neoplasms are primary and epithelial including both adenoma and carcinoma [6]. However, tumors with follicular pattern often show diagnostic dilemma. Differential diagnosis includes; dominant nodule of nodular hyperplasia, follicular adenoma, follicular carcinoma, follicular variant of papillary carcinoma, insular carcinoma & Medullary carcinoma [6,7].

Follicular adenoma is the most common thyroid neoplasm. Follicular carcinoma, on the other hand, is rare and its identification depends mainly on the presence of capsular or vascular invasion which needs thorough pathological examination [7, 8 & 9].

Papillary carcinoma is the most common thyroid cancer, and is common incidental finding in thyroid glands removed for other reasons.

Multicentricity is a common feature of papillary carcinoma; however, it may present as solitary nodule. The diagnosis of papillary carcinoma depends on the presence of nuclear features rather than a papillary formation [10]. The immunohistochemical profile of papillary carcinoma is different from that of normal follicles, especially positivity to HMW CK [11].

Medullary thyroid cancer and anaplastic or undifferentiated carcinomas are much more rare [3]. The frequency of carcinoma in nodular goiter is about 25- 60 % of that in solitary nodules. The exception to this may be cases of multinodular goiter with a dominant nodule. They are reported to have 5% carcinomas. Thus, it must be evaluated as if it were a single nodule [12].

MATERIAL & METHOD

The present study is performed on 77 excision specimens from solitary thyroid nodule cases with follicular architecture. All were received from Mansoura University Hospital and Mansoura Oncology Center.

Routine hematoxylin and eosin (H&E) were used for morphologic diagnosis. Classification into benign and malignant tumors was done according to that mentioned by [13].

Cases were classified into benign (which included both neoplastic and non neoplastic) and malignant; and all the neoplastic cases were classified based on the histogenetic origin of the tumor. Immunohistochemistry was applied for selected problematic cases, and for the delineation of some details as vascular invasion.

Antibodies required :

Pre-diluted, Monoclonal mouse high molecular weight (DAKO code No. M0630); diluted 1:50, Polyclonal rabbit anti-cow S-100 antibody (DAKO code No. Z0311), Monoclonal mouse anti-human chromogranin A (DAKO code No. U 7030) & Polyclonal rabbit anti-human factor VIII (DAKO code No. N1505).

Statistical analysis of data was done by using Excel program and SPSS program (statistical package of social science). Chi- square test and ANOVA test were used. P is significant if <0.05.

RESULTS

In our study, benign lesions were about 71.43 % of total & malignant lesions were 28.57% of the total cases. The commonest cause of solitary nodule was the goitrous nodule (35.06%) of total. The commonest neoplasm was follicular adenoma (32.47%) of total. The commonest malignant tumor was papillary carcinoma (15.58%) of total. *Table (1)*

As regard age distribution, the age was between 15-80 years. The percent of malignancy was higher in the older age group, reaching more than 50% above the age of 60 year. *Table (2)*

Table (3) demonstrates female predilection for both benign and malignant cases. Total female to male ratio was 2.5: 1.

Table (4) shows that both benign and malignant cases were more common on the right side (49.4% of the total); the left side (44.2%); the isthmus contained 6.4% of the total.

Results of the immunostains used for selected cases (Table (5):

Nineteen cases required immunostaining for definitive diagnosis. S-100 and HMWK were helpful in the diagnosis of nodules with borderline nuclear features of papillary carcinoma; Chromogranin was used to help diagnosis of medullary carcinoma and finally factor VIII to differentiate between the endothelial hyperplasia and the true vascular invasion.

The number of papillary carcinoma in this study was 12 cases (about 15.58% of all cases) and they represented the highest incidence in the malignant cases (Table 1) . There were eight cases with questionable focal clearing and grooving and all had follicular pattern. Three of them were diagnosed by S100 and HMWK as papillary carcinoma *(fig.1 &2)* and five cases showed no reactivity to S-100 or HMWK and confirmed to be of hyperplastic nature.

The number of medullary carcinoma cases was two cases (3.9%) of the malignant tumors. They showed positive immunostaining for chromogranin A *(fig. 4).*

There were four cases of follicular carcinoma (5.19 % of the cases) & two cases of Hurthle cell carcinoma (2.6 % of cases). They showed mitoses and definite vascular

and capsular invasion.

Immunostaining for factor VIII was done in one case of follicular carcinoma in which vascular invasion was suspected to differentiate between the endothelial hyperplasia and the true vascular invasion *(figure 6)*.

Table (1) : Total number of solitary thyroid nodule cases with follicular pattern and their histological diagnosis:

Histological diagnosis	No.	% of total
non neoplastic follicular lesions:		
Goiterous nodule (GN)	27	35.06%
Benign neoplastic follicular lesions:		
1-Follicular adenoma (FA)	25	32.47%
2-Hürthle cell adenoma (HA)	2	2.60%
3-Atypical adenoma (AA)	1	1.30%
Total benign neoplastic cases	28	36.37%
Total benign cases	55	71.43%
Malignant lesions with follicular pattern:		
1-Papillary carcinoma (PC)	12	15.58%
2-Follicular carcinoma (FC)	4	5.19%
3- Insular carcinoma (IC)	1	1.30%
4- Hürthle cell carcinoma (HC)	2	2.60%
5- Medullary carcinoma (MC)	2	3.90%
Total No. of malignant cases	22	28.57%
Total cases	77	100%

Table (2): Age distribution in the three categories of diagnosis:

Histological diagnosis	No.	Range	Mean	Median	S.D.
Non-neoplastic	27	15 - 60	36.74	37.00	11.04
Benign neoplastic	28	19 - 67	40.70	40.00	12.20
Malignant	22	18 - 80	46.04	45.00	16.51
Total	77	15 - 80	40.69	40.00	13.70

Table (3) : Gender distribution in the three categories of diagnosis:

Histological diagnosis	No.	Female		Male		female: Male
		No.	% of group	No.	% of group	
Non-neoplastic	27	20	74.1%	7	25.9%	2.9:1
Benign neoplastic	28	21	75.0%	7	25.0%	3:1
Malignant	22	14	63.6%	8	36.4%	1.8: 1
Total	77	55	71.4%	22	28.6%	2.5: 1

Table (4) : Site distribution of both benign and malignant categories:

Type	No.	Right side		Left side		Isthmus	
		No	%	No	%	No	%
Non-neoplastic	27	13	16.9%	12	15.6%	2	2.6%
Benign neoplastic	28	14	18.2%	12	15.6%	2	2.6%
Malignant	22	11	14.3%	10	13.0%	1	1.3%
Total	77	38	49.4%	34	44.2%	5	6.4%

Table (5) : Immunostaining results and final diagnosis of the problematic cases:

Initial diagnosis	No	Immuno-staining	Result	Final diagnosis
?Papillary carcinoma ?? hyperplastic nodules	8	High Molecular weight keratin & S-100	3 cases both (+ve)	Papillary carcinoma
			5 cases both (-ve)	Hyperplastic Nodules
?Follicular carcinoma	8	Factor VIII	3 (+ve)	Follicular carcinoma
			5 (-ve)	Follicular adenoma
?Medullary carcinoma	2	Chromogranin A	(+ve)	Medullary carcinoma
?Medullary carcinoma ?insular carcinoma	1	Chromogranin A	(-ve)	Insular carcinoma

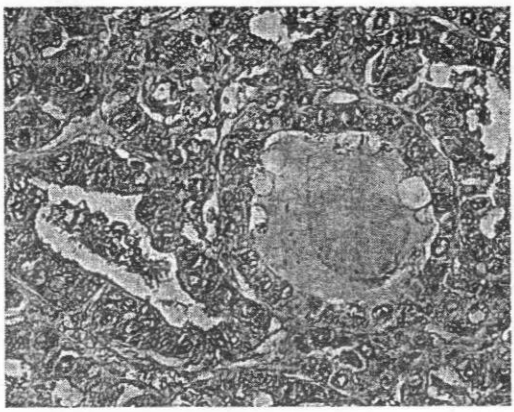

Fig. (1): Positive S100 in papillary carcinoma (immunostain x400)

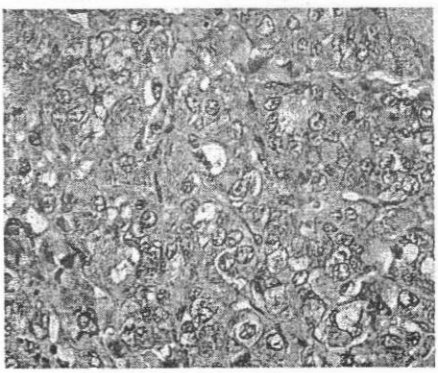

Fig. (2): Positive HMWK in papillary carcinoma (immunostain x400).

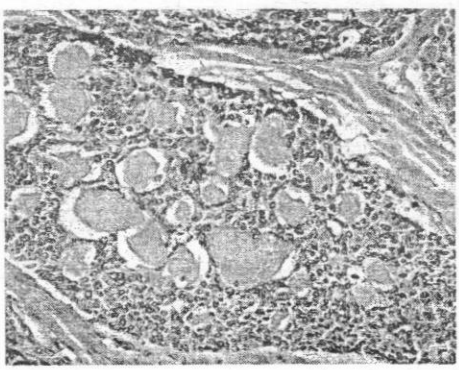

Fig. (3): Medullary carcinoma, follicular variant (x100).

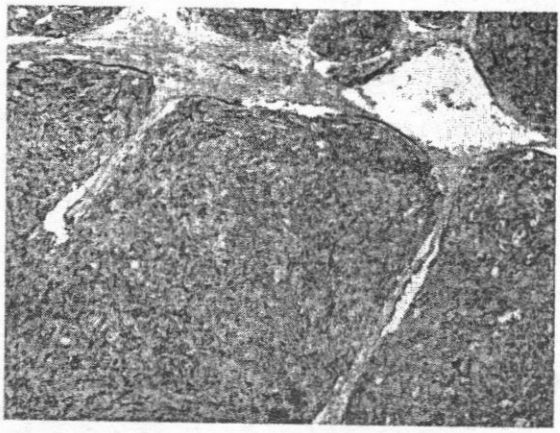

Fig. (4): Positive chromogranin in medullary carcinoma (immunostain x100).

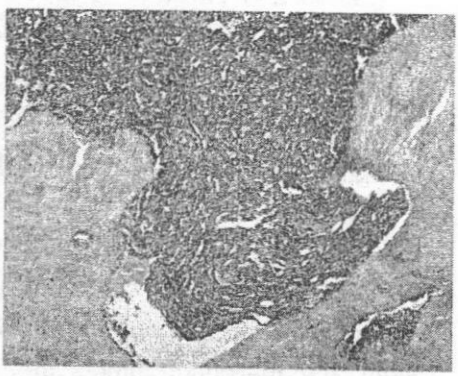

Fig. (5): Follicular carcinoma with mushroom shaped capsular invasion (x100).

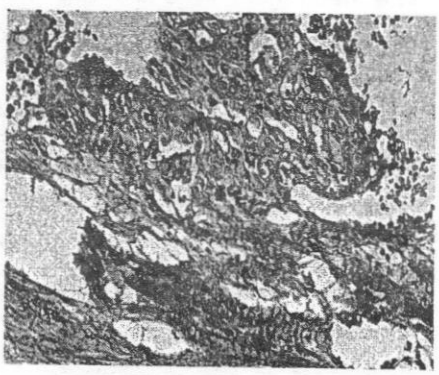

Fig. (6): Positive VIII in equivocal vascular invasion (immunostain x200)

DISCUSSION

The problems involved in the histopathological interpretation of thyroid tumors with follicular pattern has been repeatedly alluded. The main problems seem to involve the distinctions between hyperplastic nodules, follicular adenoma, follicular carcinoma & follicular variants of papillary and medullary carcinoma. Immunohistochemistry has been utilized in an attempt to resolve these issues.

The evaluation of solitary thyroid nodule is a common thyroid problem. The main goal in evaluating these nodules remains whether the detected nodule is malignant, especially in presence of any of the risk factors. They are found in 4%ʌȝ% of adults by palpation and in 13%٦Vȝ% when ultrasound detection is used. In autopsy studies, they have a prevalence of approximately 50%.(2).

In our study, the solitary thyroid nodule represented 32% of all thyroidectomy specimens during the period of the study, since the specimen sample included only surgically removed glands, and because many of the cases that were diagnosed as solitary nodule on clinical and radiology level proved on pathologic examination to be a dominant nodule of multinodular gland. On the other hand, Namou and Lavertu(14), reported nodule prevalence as 49.5%. They reported that the prevalence varies according to the method of screening used in different studies, but the true prevalence of thyroid nodules can be detected from pathologic examinations of thyroid glands at autopsy rather than from surgically excised specimens.

Namou and Lavertu(14), reported that excision biopsies give a higher yield of malignancy than needle aspirate since many of the cases diagnosed by aspirate as non neoplastic will be treated medically not surgically. They reported that the frequency of cancer in surgical specimens increased to 32.9%. In agreement with them, the malignant tumors in this study showed a high incidence up to 28.57 %. This can be explained by the collection of cases of this study from the oncology center, which is considered as a referral center for neoplastic cases especially the malignant ones.

As regard gender predilection, this study showed an overall female predominance; the female to male ratio was 2.5: 1, which is in agreement with Hebra et al. and Mazzaferri (3 &15), who reported that solitary thyroid nodules are 3-4 times more common in women than men.

Regarding the age of the patients, there was a wide range of age (15-80 years). This came in agreement to Mazzaferri (15).

Yeung and Serpell (2), reported that the commonest cause of nodule is the goiterous nodule, the commonest neoplastic lesion is follicular adenoma and the commonest malignancy is papillary carcinoma. In agreement with that, in this study the goiterous nodule was (35.1%), follicular adenoma (32.5%) and the commonest malignant neoplasm was papillary carcinoma (16.5%).

Regarding atypical adenoma, Livolsi, et al. (16), stated that it is benign and regarded it as a variant of follicular adenoma. Welker and Orlov (17), also considered atypical adenoma as benign but they mentioned it as a separate entity in their classification. Vasko et al. (18), on the other hand, suggested that atypical adenomas are a heterogeneous group, some are truly benign tumors and some are premalignant. In this study, atypical adenoma was considered as a benign neoplasm, but separated from follicular adenoma in terms of prognosis, post-operative therapy and value of follow up, as mentioned in the classification of (17).

Regarding Hürthle cell tumors, Livolsi et al.(16), suggested that oncocytic tumors are treated like follicular tumors. DeLellis and Williams (19), also stated that completely oncocytic tumors are still regarded as a variant of follicular neoplasms, but it is important to identify them separately since they show different molecular features. In this study, Hürthle cell tumors managed as follicular tumors in terms of capsular and vascular invasion and staging. They represented 2.6% of the malignant nodules, this was the same figure mentioned by Rosai et al. (20), they reported that Hürthle cell carcinomas account for 2 to 3% of all thyroid carcinoma.

DeLellis and Williams [19], stated that the incidence of papillary carcinoma has increased world wide but the figures show great variability (5-35%) depending on the area, sampling technique and the source of data; autopsy specimens show much higher rates than specimens from clinically apparent patients (caused by detection of many subclinical microcarcinomas). In our study, papillary carcinoma was also the commonest malignant tumor, and it represented 15.58 % of the total.

Yamamoto et al.[10], reported that both clear and grooved nuclei are also reported in many benign thyroid lesions; however they were focal and do not stain for HMWK or S-100. In this study also, staining for HMWK or S-100 was useful; it was done to three cases with questionable nuclear clearing and inclusions & were positive in the three cases, so they were considered papillary carcinomas.

DeLellis and Williams [19], reported that medullary carcinoma showed a variety of patterns, including follicular variant but with no clinical significance. In this study, there were two cases of medullary carcinoma with follicular pattern and were strongly and diffusely positive for chromogranin.

Regarding follicular carcinoma, Livolsi et al.[16], stated that it accounts for 10-15% of thyroid malignancies. In our study, it was (5.19%) & it was explained by the low number of cases.

Conclusion :

Most thyroid tumors are diagnosed by morphology alone. However, thyroid tumors with a follicular growth pattern could be sometimes a diagnostic challenge. Immunohistochemistry can be used for definitive diagnosis in case of unusual looking tumor. In this study, immunohistochemistry was used to confirm the diagnosis for all cases of medullary carcinoma with follicular pattern. Also it was used in detecting true vascular invasion in follicular carcinoma by using factor VIII. S-100 and HMWK were also very useful in nodules with questionable nuclear features of papillary carcinoma.

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