Original Article

Differential Expression of CK 19 in follicular adenoma, Well-differentiated tumour of uncertain malignant potential (WDT-UMP) and follicular variant of papillary carcinoma

Uzma Bukhari,[1] Saleem Sadiq,[2] Shanaz Imdad Kehar[3]

Pathology Department, Muhammad Medical College, Mirpurkhas,[1] Pathology Department, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi.[2,3]

Abstract

Objective: To see the expression of cytokeratin 19, a proven helpful marker for the differential diagnosis of neoplastic follicular patterned lesions of thyroid.

Methods: This was a retrospective study carried out in the Department of Pathology, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi from 2000 to 2005. The haematoxylin and eosin (H&E) stained sections were reviewed and the cases were classified according to already published criteria. On the basis of the recent recommendations by Chernobyl Pathologists Group, encapsulated follicular patterned lesions with questionable nuclear changes were categorized as well - differentiated tumours of uncertain malignant potential (WDT-UMP). Formalin fixed paraffin embedded tissues of follicular adenoma, WDT-UMP and follicular variant of papillary carcinoma were obtained for CK 19 immunostaining.

Results: All (16) cases of follicular adenoma were negative for CK19. In a total of 35 cases of WDT- UMP, 10 cases scored 3+ positive for CK19, 15 were 2+ positive and remaining 10 cases were 1+ positive. There were 43 cases of follicular variant of papillary carcinoma with 4+ CK 19 positivity, 14 were 3+ positive and 3 were 2+ positive.

Conclusion: CK19 is a good and useful diagnostic marker for differential diagnosis of follicular adenoma, WDT-UMP and follicular variant of papillary carcinoma. The recommendations by Chernobyl Pathologists Group need to be adopted and the cases of WDT-UMP require strict follow-up (JPMA 59:15; 2009).

Introduction

Follicular adenoma (F.A) is the second common cause of thyroid enlargement after mutinodular goiter.[1] It is a benign, encapsulated tumour of the thyroid showing evidence of follicular cell differentiation.[2]

Pathologic diagnosis and classification of thyroid tumours are based, to a large extent, on the microscopic appearance of the specimens. Despite the general acceptance of the World Health Organization classification of thyroid tumours, the histological diagnosis of neoplastic follicular patterned lesions of thyroid is remains one of the most problematic area in surgical pathology.[3]

A frequent problem posed by encapsulated follicular lesion is that papillary carcinoma like nuclear changes are present focally.[4] Chernobyl Pathologists Group categorized these tumours as well - differentiated tumours of uncertain malignant potential (WDT-UMP).[5,6]

Papillary carcinoma is the most common form of well-differentiated malignant thyroid neoplasm. Follicular variant of papillary thyroid carcinoma (FV-PTC) is the most frequent variant of papillary thyroid carcinoma after the classic type.[7] Usually the diagnosis of this variant is straight forward, however diagnostic problems arise when the characteristic nuclear features are not diffusely distributed throughout the lesion but are present focally or multi focally.[8]

Multiple studies have demonstrated great interobserver variability in the diagnosis of these tumours, even among experts in thyroid pathology, thus underscoring the difficulties in properly defining the criteria for the diagnosis of this particular type of papillary thyroid carcinoma. This fact has immediate practical consequences for these patients, as over diagnosis of this condition may lead to excessive treatment, including total thyroidectomy followed by radioactive iodide therapy.[9]

Obviously the Pathologist's diagnostic decision in dealing with these cases is not simply a matter of tumour classification. The distinction between benign and malignant lesion is clearly important. Further distinction of papillary carcinoma is meaningful at a clinical level because prognosis and metastatic spread differ considerably between the two.[10] Thus new methods that can simply and accurately distinguish benign from malignant tumours are greatly desired.[11]

Cytoskeletal proteins have been utilized in an attempt to resolve this issue. Cytokeratin 19 (CK19) is the most commonly used cytokeratin investigated in thyroid lesions.[12] Papillary carcinoma has been shown to express CK 19 with

strong diffuse cytoplasmic reactivity.[13,14] The expression of this marker was 100% sensitive and 82.5% specific for papillary cancers by Guyetant et al.[15] A study by Schelfhout et al[16] showed that this marker can be helpful in differentiating papillary carcinoma from follicular adenoma.

No study on CK 19 expression in thyroid lesions has been done in Pakistan. Therefore keeping in mind all the difficulties related to these lesions, an attempt was made to see CK 19 expression in these cases.

Material and methods

This was a retrospective study carried out in the Department of Pathology, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi. For this study formalin fixed paraffin embedded tissues were obtained from the department from the period 2000 to 2005. The haematoxylin and eosin (H&E) stained sections were reviewed and the cases were classified according to already published criteria. On the basis of the recent recommendations by Chernobyl Pathologists Group, encapsulated follicular patterned lesions with questionable nuclear changes were categorized as WDT-UMP. The selected (111) cases included F.A (16 cases), WDT-UMP (35 cases) and FVPC (60 cases).

Immunohistochemistry:

Section of 5um thickness were cut and mounted on saline coated slides. Antigen retrieval was performed in citrate by pressure cooker technique. A mouse monoclonal CK19 antibody (Ready to use clone BA-17, Labvision, USA) was used as the primary antibody.The staining was completed with immunoperoxidase technique. Sections where the primary antibody had been omitted served as negative controls and known CK 19 positive colon cancer was used as the positive control (Fig-1).

Quantification of immunohistochemistry results

CK 19 staining profile was classified according to the relative number of positive cells (cytoplasm) of tumour. The scoring system given by Schelfhout et al[16] was used, which is as follows:

a) Negative, no cytoplasmic staining in any tumour cell.

b) 1+, cytoplasmic CK19 staining in 5% or less than 5% tumour cells.

c) 2+, cytoplasmic CK19 staining in 5- 50% tumour cells

d) 3+, cytoplasmic CK19 staining in 50- 95% tumour cells

e) 4+, cytoplasmic CK19 staining in more than 95% tumour cells

Results

All (16) cases of follicular adenoma were negative for CK19 (Fig -1). Out of the 35 cases of well-differentiated tumours of uncertain malignant potential, 10 cases stained 3+ positive for CK 19, 15 cases showed 2+ positivity while remaining 10 cases were 1+ positive (Fig - 2). Out of 60 cases of follicular variant of papillary carcinoma, 43 cases

Table: Expression of CK 19 in Selected Cases.

Type	Score				
	4 +	3+	2 +	1 +	Negative
F.A* (n=16)					16
WDT-UMP ** (n= 35)	-	10	15	10	-
FV-PTC (n=60)	43	14	3	-	-

* encapsulated tumours without nuclear changes.
**encapsulated tumours with questionable nuclear changes.

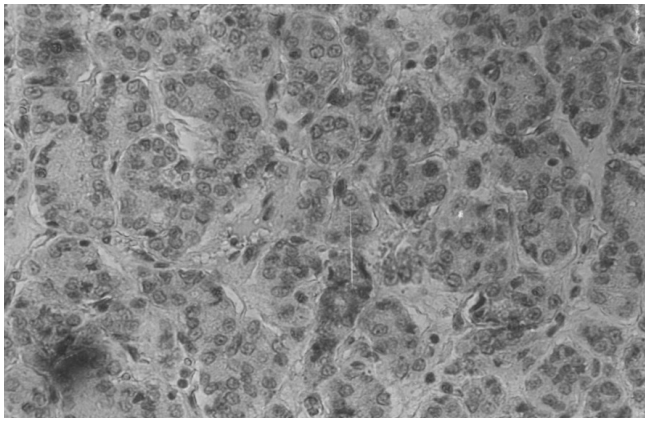

Figure 1: Photomicrograph of follicular adenoma showing negative CK-19 staining IHC X 200.

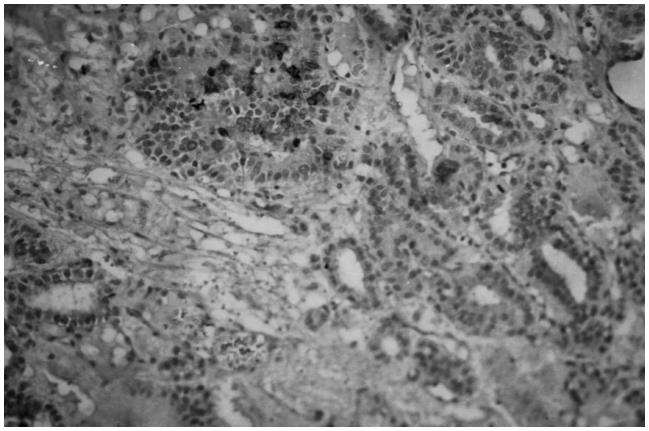

Figure 2: Photomicrograph of WDT-UMP showing 1+ poitivity for CK-19. IHC X 100.

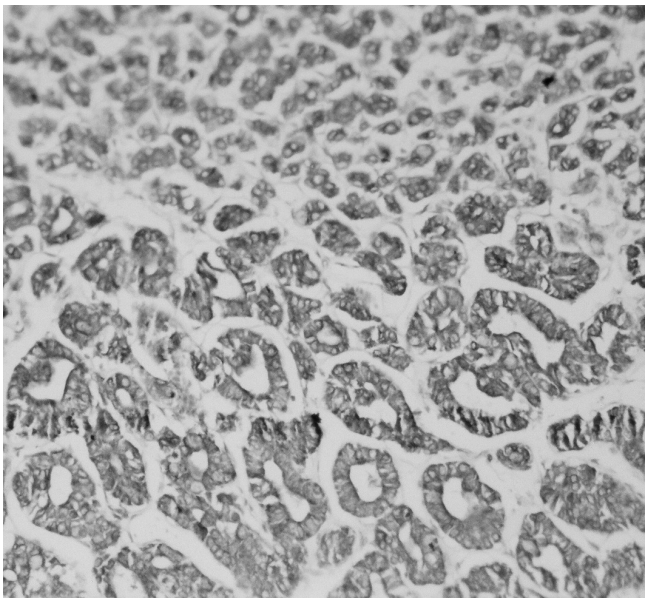

Figure 3: Photomicrograph of follicular variant of papillary carcinoma showing 4+ positivity for CK-19 IHC X 100.

showed 4 + positivity (Fig - 3), 14 were 3 + positive and 3 stained 2+ positive (Table).

Discussion

The histological diagnosis of well circumscribed thyroid nodules without capsular or vascular invasion is not always straightforward. A common diagnostic dilemma arises when an encapsulated nodule with a follicular pattern of growth exhibits some but not all of the features of papillary thyroid carcinoma (PTC). In such instances distinguishing follicular adenoma from encapsulated follicular variant of papillary thyroid carcinoma (FV-PTC) becomes difficult.[17]

Since the recognition of the follicular variant of papillary carcinoma, there has been a gradual extension of the category to include lesions with minor nuclear changes. Pathologists face this problem all the time. Chernobyl Pathologists Group suggested that it is more appropriate to recognize this difficulty than to arbitrarily place well-differentiated encapsulated tumours with a follicular architecture where minor nuclear changes are the only indicator of a papillary carcinoma in a definite malignant or definite benign category. They referred to these tumours simply as well differentiated and used the term WDT-UMP for those lesions having questionable or incomplete nuclear changes and without capsular invasion.[5,6]

The technique of immunohistochemistry has much appeal for the surgical pathologist as it is relatively simple and preserves morphologic detail.[18] Cytokeratin 19 is the low molecular weight cytokeratin.[19] Baloch et al[20] found that CK19 could be helpful in differentiating papillary cancers from follicular adenoma and follicular carcinoma.

In the current study, CK19 expression was seen in 60 cases of follicular variant of papillary carcinoma. Most of our cases were 4+ (>95% cells) positive. These findings are in agreement with those of Schelfhout et al.[16] Beesley et al[19] and Sahoo et al[22] who also found diffuse CK19 positivity in their cases of papillary carcinoma.

All our cases of follicular adenomas were negative for CK19. These results are somewhat in accordance to several authors[13,16,21,23] who reported nil or focal positivity for CK19 in follicular adenomas, which were diagnosed on the basis of existing criteria.

We reviewed and categorized encapsulated lesions with questionable or incomplete nuclear changes as WDT-UMP according to the recent recommendations by Chernobyl Pathologists Group. In Turkey, Koseoglu et al[22] also followed these recommendations and categorized 2 cases of follicular patterned lesions with questionable nuclear changes as WDT-UMP. Total thyroidectomy was performed in those cases.

In our study significant positivity for CK19 was seen in WDT-UMP. However no immunohistochemical study on WDT-UMP was found in literature. This might be due to the fact that the use of this nomenclature has not as yet been universally accepted.

It is possible that lesions in which the nuclear changes are "questionable" represent an early development of papillary carcinoma in a preexisting benign lesion as suggested by the fact that in microdissection experiments the RET/PTC rearrangements are restricted to these foci.[25]

CK19 can be a good and useful ancillary diagnostic tool for diagnosing follicular lesuons with questionable nuclear changes and there is a need to separate them from common adenomas (encapsulated follicular neplasms without nuclear changes) in order to analyze predictive factors.

Conclusion

In conclusion, our study documents the advantage of CK 19 immunostaining in routine surgical pathology for the differential diagnosis of follicular patterned lesions, particularly the cases of encapsulated follicular patterned lesions with questionable nuclear changes (WDT-UMP) and the cases of follicular variant of papillary carcinoma.

The recommendations by Chernobyl Pathologists Group need to be adopted and the cases of WDT-UMP required strict follow-up in order to manage them in the most appropriate way.

References

1. Hussain N, Anwar M, Nadian N, Ali Z. Pattern of surgically treated thyroid diseases in Karachi. Biomedica 2005; 21:18-20.

2. Chan JKC, LiVolsi, Bondeson L, Albores-Saavedra J, Geisinger K, Sobrinho-Simoes M. Sclerosing Mucoepidermoid Carcinoma with Eosinophilia. In: WHO Classification of Tumours, Pathology & Genetics, Tumours of Endocrine Organs. Ed: DeLellis RA, Lioyd RV, Heitz PU, Eng C. Edn: IARC Press, 2004; pp 84.
3. Hirokawa M, Carney J, Goellner J. Observer variation of encapsulated follicular lesions of the thyroid gland. Am J. Surg Pathol. 2002; 26:1508-14.
4. Suster S. Thyroid tumours with a follicular growth pattern: Problems in differential diagnosis. Arch Pathol and Lab Med. 2006; 130:984-8.
5. Williams ED. Guest Editorial. Two Proposals Regarding the Terminology of Thyroid Tumours. Int J Surg Pathol 2000; 8:181-3.
6. Rosai J. Handling of thyroid follicular patterned lesions. Annual meeting, endocrine Pathology society. 2005; pp 1-3.
7. Kesmodel SB, Terhune KP, Canter RJ, Mandel SJ, LiVolsi VA, Baloch ZW et al. The diagnostic dilemma of follicular variant of papillary thyroid carcinoma. Surgery 2003; 134:1005-12.
8. Rosai J, Carcangiu ML, DeLellis RA.Tumors of Thyroid Gland. Atlas of Tumour. Pathology. AFIP, 3rd series, fascicle 5, Washington D.C 1992; pp 42.
9. Chan JKC. Strict criteria should be applied in the diagnosis of papillary thyroid carcinoma. Am J Clin Pathol. 2002; 117:16-18.
10. Caruso D, Mazzaferri EL. Fine needle aspiration biopsy in the management of thyroid nodules. Endocrinologist 1991; 1:194-202.
11. Matos PS de, Ferreire AP, Facuri FDO, Assumpcao LVM, Metze K, Ward LS. Usefulness of HBME-1, cytokeratin 19 and galectin-3 immunostaining in the diagnosis of thyroid malignancy. Histopathol 2005; 47:391-401.
12. Lam KY, Lui MC, Lo CY. Cytokeratin expression profiles in thryoid carcinoma. Eur J Surg Oncol 2001; 27: 631-5.
13. Baloch ZW, Abraham S, Robert S, LiVolsi VA. Differential expression of cytokeratin in follicular variant of papillary carcinoma, an immunohistochemical study and its diagnostic utility. Hum Pathol 1999; 30:1166-71.
14. Kragsterman B, Grimelius L, Wallin G, Werga P, Johansson H. Cytokeratin 19 expression in papillary thyroid carcinoma. App Immunohistochem Mole Morph 1999; 7:181-5.
15. Guyetant S, Michalak S, Valvo I, Saint-Andre JP. Diagnosis of the follicular variant of papillary thyroid carcinoma. Significance of immunohistochemistry. Ann Pathol 2003; 23:11-20
16. Schelfhout LJDM, Muijen GNPV, & Fleuren GJ. Expression of keratin 19 distinguishes papillary thyroid carcinoma from follicular carcinoma & follicular thyroid adenoma. Am J Clin Pahol 1989; 92:654-8.
17. Sahoo S, Hoda SA, Rosai J, DeLellis RA. Cytokeratine 19 Immunoreactivity in the diagnosis of papillary thyroid carcinoma. Am J Clin Pathol 2001; 116:696-702.
18. Permanetter W, Narthrath WBL, Lohrs U. Immunohistochemical analysis of thyroglobulin and keratin in benign and malignant thyroid tumours. Virchows Arch 1982; 398:221-8.
19. Raphael SJ, McKeown- Eyssen G, Asa SL. High molecular weight cytokeratin & cytokeratin 19 in the diagnosis of thyroid tumours. Mod Pathol 1994; 7:295-300.
20. Baloch ZW, Abraham S, Robert S, LiVolsi VA. Differential expression of cytokeratin in follicular variant of papillary carcinoma, an immunohistochemical study and its diagnostic utility. Hum Pathol 1999; 30:1166-71.
21. Beesley MF, Mclaren KM. Cytokeratin 19 and galectin-3 immunohistochemistry in the differential diagnosis of solitary thyroid nodules. Histopathol 2002; 41:236-43.
22. Sahoo S, Hoda SA, Rosai J, DeLellis RA. Cytokeratine 19 Immunoreactivity in the diagnosis of papillary thyroid carcinoma. Am J Clin Pathol 2001; 116:696-702.
23. Choi YL, Kim MK, Suh JW, Han J, Kim JH, Yang JH et al. Immunoexpression of HBME-1, High molecular weight cytokeratin, cytokeratin 19, thyroid transcription factor-1 and E-cadherin in thyroid carcinoma. J Korean Med Sci 2005; 20:893-9.
24. Koseoglu RD, Filiz No, Aladag I, Eyibilen A, Goven M. Problems encountered in the diagnosis of encapsulated follicular variant of papillary thyroid carcinoma and the morphological diagnostic criteria. Turk J Med Sci 2006; 36:17-22.
25. Fusco A, Chiappetta G, Hui P, Garcia-Rostan G, Golden L, Kinder BK et al. Assessment of RET/PTC oncogene activation and clonality in thyroid nodules with incomplete morphological evidence of papillary carcinoma. A search for the early precursors of papillary cancer. Am J Pathol 2002; 160:1944-2157.