Original Article

Efficacy of Cytokeratin 19 expression on fine needle aspiration cell blocks in pre-operative diagnosis of malignant thyroid neoplasms

Vimal Bhandari, Yogesh K. Yadav[1], Geetika Khanna[2], Mrinalini Sharma, Mohit Singh, Rajni[2]

Department of Surgery and [2]CIO Laboratory, Safdarjung Hospital and V.M.M.College, New Delhi, [1]Department of Pathology, Integral Institute of Medical Sciences and Research, Lucknow, Uttar Pradesh, India

ABSTRACT

Context: Accurate diagnosis of malignant lesions of thyroid remains a challenge, especially when classical features are lacking on cytological examination. **Aims:** To evaluate the expression of Cytokeratin 19 (CK 19) on cell blocks made from thyroid swellings diagnosed as papillary carcinoma or follicular adenoma/carcinoma on fine needle aspiration cytology (FNAC) smears. **Materials and Methods:** Aspirates from fifty patients diagnosed as papillary or follicular lesions on FNAC were enrolled in the study. Cell blocks were simultaneously made along with aspiration using standard cell block techniques. These cell blocks were subjected to Cytokeratin 19 immunoassay using immunoperoxidase ABC (avidin biotin complex). **Statistical Analysis Used:** Chi-square test for statistical significance and Goldman test for sensitivity and specificity. **Results:** Evaluation of the expression of Cytokeratin 19 on cell blocks revealed that almost all papillary thyroid carcinomas stained strongly positive while all follicular adenomas and follicular carcinomas were found to be negative for CK 19 immunostaining. The difference in frequency of Cytokeratin 19 positive papillary carcinoma and follicular adenomas was statistically significant. The predictive value of a positive CK 19 test was found to be 100% while that of a negative test was 93.9%. **Conclusions:** Cytokeratin 19 immunostaining in conjunction with fine needle aspiration cell blocks can be a valuable marker in pre-operative diagnosis of papillary thyroid carcinoma along with its variants (sclerosing and follicular). This can help the clinician in formulation of treatment as papillary thyroid carcinoma is treated by radical measures as against the more conservative approach for benign follicular lesions.

Keywords: Cell block, cytokeratin 19, fine needle aspiration cytology, thyroid neoplasm

INTRODUCTION

Fine needle aspiration cytology (FNAC) remains the most effective test for distinguishing benign from malignant lesions of the thyroid with accuracy approaching up to 95%.[1] The increasing use of FNAC has reduced the rate of unnecessary thyroid surgery and has also increased the proportion of malignant nodules among all nodules resected.[1] Papillary thyroid carcinoma accounts for approximately 80% of all thyroid cancers and can be diagnosed with remarkable accuracy on cytological examination.[1-4] However, in absence of classical features FNAC of papillary carcinoma may be challenging.[1,3,5] Lesions with follicular patterns are also diagnostic problem on FNA smears and in histological sections as well.[1,5] Immunohistochemical studies using anti-bodies to various cytokeratins have been performed in an attempt to distinguish papillary from follicular tumors of thyroid and to differentiate former from non-neoplastic lesions.[3,6,7] Most of these deal with tissue samples and not with cell block. The present study was conducted to correlate the clinicopathological characteristics and immunohistochemistry (cytokeratin 19 expression) of thyroid neoplasms and to evaluate the possible role of cytokeratin 19 (CK 19) expression in consonance with thyroid fine needle aspiration cell blocks (FNACB) in selecting the treatment modality for such neoplasms.

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Address for correspondence: Dr. Yogesh Kumar Yadav, Department of Pathology, Integral Institute of Medical Sciences and Research, 145, A-Block, South City, Rae Barely Road, Lucknow, U.P–226025, India. E-mail: dr.yogi007@gmail.com

MATERIALS AND METHODS

All patients presenting at our center with thyroid swelling were subjected to FNAC; smears and cell blocks were made simultaneously. Fifty patients diagnosed as papillary carcinoma or follicular neoplasm (adenoma/carcinomas) on FNAC were enrolled in the study.

Method of cell block preparation

Following smear preparations, the needles and syringes used to obtain fine-needle aspirates were rinsed in 10 mL of 50% ethanol in a specimen container. Any residual clot or tissue in the hub of needles was removed carefully in the laboratory with the aid of another needle and rinsed in 50% ethanol. The entire material was centrifuged in a 10-mL disposable centrifuge tube at 4,000 rpm for 6 min to create cell pellets. The supernatant fluid was decanted and the deposit fixed in freshlyprepared Nathan alcohol formalin substitute (NAFS) consisting of 9 parts of 100% ethanol and 1 part of 40% formaldehyde. Fresh working solution is desired, because formalin is capable of oxidizing to formic acid after exposure to air and reacting with blood to form acid hematein pigment artifacts. The fixed cell pellets, at the end of 45 min' fixation, were recentrifuged at 4,000 rpm for 6 min. These pellets should detach themselves or can be removed easily with a disposable Pasteur pipette following centrifugation. The cell pellets were wrapped in crayon paper, placed in a cassette, stored in 80% ethanol and handled as a surgical specimen.

Cytokeratin 19 immunohistochemical staining of the cell blocks

Immunohistochemical staining was performed on 2-3 μ m thick section on silanized slides. The sections were deparaffinized in xylene and rehydrated through graded alcohol. Antigen retrieval was done with prewarmed (95°C) citrate buffer (pH-6) in microwave oven for 20 min. Wash with Tris-buffered saline (pH-7.4) after each step here on. Endogenous peroxidases were inactivated by immersing the sections in hydrogen peroxide for 10 min. The sections were subsequently incubated overnight at 4°C with antibodies to CK 19. The primary antibody employed was prediluted ready to use IgG2a mouse monoclonal anti CK-19 antibody [A53-B/A2] (ab7754) of Dako biotech, reacting with the rod domain of CK peptide 19 (40 kDa) in human tissue at a concentration of 1.000 mg/ml.The following day, the sections were incubated with biotinylated anti-mouse IgG (Maxim biotech Inc.) for 45 min, followed by peroxidise conjugated avidin (Maxim biotech Inc.). The chromogenic reaction was developed with diaminobenzidine for 10 min, and all sections were counterstained with hematoxylin. Controls consisted of omission of the primary antibody.

Post-operatively CK 19 staining was performed on all tissue sections. CK 19 immunoreactivity was scored according to the percentage of stained cells [Table 1]. The statistical analysis was carried out using Chi-square test for statistical significance and Goldman test for sensitivity and specificity.

RESULTS

A total of fifty patients with thyroid swelling were investigated with FNA smears, FNACB, biopsy and immunohistochemistry. Of these, thirty five were diagnosed to have well differentiated thyroid malignancy. Among these thirty five cases, twenty nine patients had papillary carcinoma and its variants (classical 16 patients, Follicular variant 12 patients and Sclerosing variant 1 patient) while 6 patients were found to have follicular carcinoma. Fifteen patients were diagnosed as having follicular adenomas [Table 2]. The age distribution of the patients ranged from 18 years to 50 years. Maximum numbers of patients were found to be in their third or fourth decade with a male to female ratio of 3.4:1. Positive immunoreactivity for CK 19 was identified in 28 cell block preparation out of 29 cases for papillary thyroid carcinoma and this indicated a specificity of 100% and a sensitivity of 96.55% in diagnosis of papillary carcinoma. The strength of cytokeratin positivity however, was found to vary among the various subtypes of papillary thyroid carcinoma with all 16 classical cases staining strongly positive on cell block preparations [Figure 1]. Also, 11 of the 12 follicular variants of papillary carcinomas showed moderate staining for CK 19 on cell block preparations [Figure 2]. Sclerosing variant of papillary thyroid carcinoma also showed strong and diffuse positivity for CK19 on cell block preparations while all follicular carcinomas stained negative for CK 19. Fifteen cases of follicular adenomas included in the study were also negative for CK 19 immunostaining [Table 2].

The differences in frequency of cytokeratin 19 positive papillary carcinoma and follicular adenomas were found to be statistically significant ($P< 0.01$). The predictive value of a positive CK 19 test was found to be 100% while that of a negative test was 93.9%. Further, cytokeratin immunoreactivity had no correlation with the clinicopathological variables of lymph node metastasis, multiplicity and size of the tumor.

DISCUSSION

Thyroid cancer constitutes the most common endocrine malignancy accounting for 1% of all malignancies and 0.5%

Table 1: Scoring of cytokeratin 19 immunoreactivity based on percentage of stained cells

1+ (negative or weakly positive)	<10% of cells
2+ (moderately positive)	10 – 50% 0f cells
3+ (strongly positive)	>50% of cells

Table 2: Cytokeratin 19 positivity in various thyroid neoplasms

Score on cytology (% of positive cells)	Papillary carcinoma (N=29)			Follicular carcinoma (N=6)	Follicular adenoma (N=15)
	Classical (n=16)	Follicular variant (n=12)	Sclerosing variant (n=1)		
1+	0	1	0	6	15
2+	0	11	0	0	0
3+	16	0	1	0	0

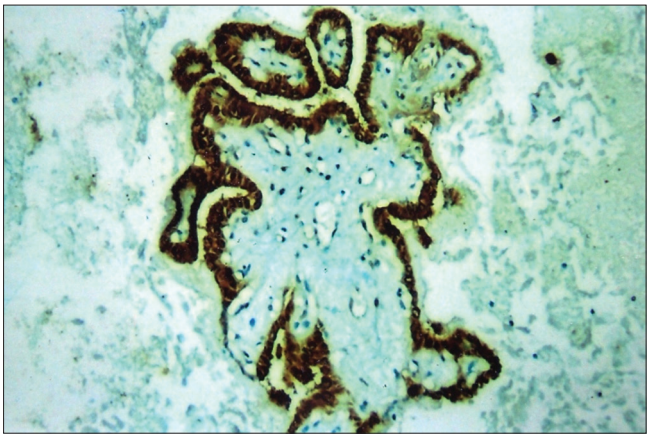

Figure 1: Cell block preparation showing CK-19 positive immunostaining of papillary carcinoma thyroid - classical showing strong positivity (×400)

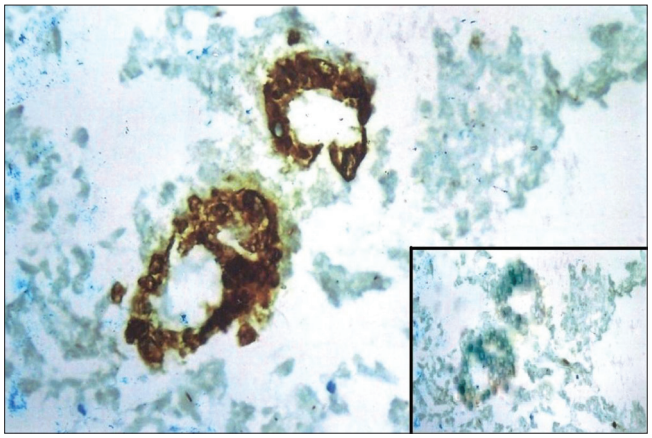

Figure 2: Cell block preparation showing CK-19 positive immunostaining of papillary carcinoma thyroid - follicular variant showing strong positivity (×400). Also seen, negative control in the inset

of all cancer related deaths.[2] Majority of the thyroid cancers are categorized as well differentiated tumors arising from follicular cell origin. Fine needle aspiration cytology (FNAC) of the thyroid is a valuable tool in evaluation of thyroid nodules with accuracy approaching 95% in differentiating benign from malignant lesions.[1] Papillary carcinoma thyroid constitutes 80% of all thyroid malignant neoplasms with its follicular variant being the most common subtype.[3,4] FNAC can reliably diagnose papillary carcinoma but a diagnostic dilemma arises in lesions with follicular patterns. These lesions have a potential for malignant spread but usually have subtle nuclear features of papillary carcinoma making a pre-operative diagnosis difficult due to intraobserver variation in cytological diagnosis.[5]

Cytokeratins are intermediate filament proteins responsible for the structural integrity of epithelial cells. Cytokeratin 19 is a type I keratin encoded by the KRT19 gene. CK-19 was proposed as a possible marker for epithelial tumors by Bjorklund in 1957.[6] Miettinen *et al*.[7] in 1984 observed the utility of intermediate filament proteins reactivity in cases of thyroid cancers. Among other tumors, trichoblastoma show consistent expression of CK-19.[8] Malignant ameloblastomas exhibited constant CK19 expression in neoplastic cells.[9] The CK19 expression in suprabasal cell layers of oral mucosa can be used as a marker of diagnosis of oral precancerous lesions and CK19 expression is the initial events during oral carcinogenesis.[10] CK19 has been shown to be an independent prognostic factor for pancreatic neuroendocrine tumors, especially the insulin-negative tumors.[11] CK19 is positive in the most of neuroendocrine tumors occurring in the rest of the GIT, except rectal tumors, which are negative.[12] In the liver, CK19 is of prognostic value in hepatocellular carcinomas and is of use in distinguishing cholangiocarcinoma from hepatocellular carcinomas.[12] The vast majority of adenocarcinomas in the GIT and pancreas are CK19 positive.[12] CK19 immunohistochemistry was useful in showing the extent of myometrial invasion and subtle foci of lympho-vascular space invasion in cases of low-grade endometrioid adenocarcinoma of the endometrium.[13] CK-19 expression can also be used to detect tumour cells in peripheral blood by RT-PCR in cases of prostate cancer, early breast Cancer, gastric cancer, colorectal cancer and lung cancer patients.[14-18]

The use of Cytokeratin 19 expression in cases of thyroid tumors is well established. Cytokeratin 19 has been proposed as an immunohistochemical marker to distinguish papillary thyroid carcinoma from other tumors with follicular pattern and with benign lesions. Several studies have reported the diffuse positive reactivity for Cytokeratin 19 in papillary thyroid carcinomas. Miettinen *et al*.[7] observed positive CK 19 reactivity for all papillary thyroid cancers and 50% of all follicular carcinomas. Liberman *et al*.[19] reported uniform reactivity for CK 19 in 100% of papillary tumors. In their study 9 out of 10 follicular adenomas also stained weakly positive. In another study, 16 out of 18 cases of papillary thyroid

carcinomas showed moderate to strong staining with CK 19 and almost all non-neoplastic lesions stained negative.[20] Sahoo *et al*.[21] concluded that immunoreactivity for CK19 is not specific for papillary thyroid carcinoma, although, they acknowledge that the extent and intensity of staining are considerably greater in this tumor than in follicular adenoma. Cheung *et al*.[22] also reported diffuse cytokeratin staining in 80% of usual variants of papillary carcinoma. With majority of the studies affirming the role of CK19 immunoreactivity for diagnosis of papillary carcinoma thyroid, the practical usefulness of CK19 for differential diagnosis of various benign and malignant thyroid lesions remains ill defined. Zhu *et al*.[23] also concluded that CK19 was very useful not only for the differentiation of benign and malignant papillary structure, but also for the differential diagnosis of follicular PTC and FTC. They observed that CK19 expression was diffuse and strong in the papillary structure of PTC, but weak and focal in the papilla of tissue with benign disease. And also, the expression of CK19 in follicular PTC was significantly higher than in follicular thyroid carcinoma.[24]

Certain benign lesions of thyroid like multinodulargoiter may mimic papillary carcinoma if it contains papillary areas. Although, it is not usually very difficult to distinguish between these benign and malignant lesions, some cases may be problematic in differential diagnosis. Erikilc *et al*.[24] used CK19 for differentiation of these goitres with papillary areas from those with papillary carcinomas and found diffuse and intense CK19 positivity in all papillary carcinomas. A focal reactivity was also observed in 5 of 25 cases of multinodulargoiter and some follicular adenomas as well, a finding which they attributed as false positive owing to hemorrhage. They, thus concluded that staining pattern with CK19 together with histopathological finding may be helpful in differential diagnosis between foci mimicking papillary carcinoma and true papillary carcinoma in Grave's disease.

Immunohistochemistry using CK19 has also been showed to be useful in detecting Papillary carcinoma in lymph nodes of the neck.[25] Boutross-Tadross *et al*.[26] examined 10 cases of follicular variant papillary thyroid carcinoma in strumaovarii and 3 cases of benign strumaovarii and found all of the carcinomas were diffusely positive for CK19 (cytokeratin 19) whileall 3 benign strumaovarii were negative for CK19.

In our case CK19 was found to be strongly positive in 28 of 29 cases of papillary carcinoma. Further, all cases of classical papillary carcinoma showed strong positivity while 11 out of 12 follicular variant showed moderate positivity. None of the fifteen follicular adenomas was found to be positive for CK19 immunostaining. All follicular carcinomas were also negative for CK19. The findings are thus in accordance with other studies.[25] The above figures indicate a specificity of 100% and sensitivity of 96.55% of cytokeratin 19 in diagnosing papillary carcinomas. This is in contrast to some previous studies where few cases of follicular adenomas and follicular carcinomas stained weakly or moderately positive with CK19.[7] Various immunohistochemical studies using anti-bodies to different cytokeratins have been performed in an attempt to distinguish papillary carcinoma from follicular neoplasms of thyroid.[3,7] Most of these deal with tissue samples and smears but not with cell block. In the present study, we reveal the importance of CK19 as the single most important marker in diagnosis of papillary carcinoma thyroid with high degree specificity of (100%) and sensitivity of (96.55%) In the present study, we have also highlighted the importance of cell block which can be used as important tool for immunohistochemistry. The advantage of cell block is that, we can cut several sections from a single specimen and hence a marker can be repeated, if required or several markers can be applied simultaneously. Furthermore, no correlation was found between the clinicopathological variants of age, sex, lymph node metastasis, tumor size, multiplicity and CK19 expression. Similar observations have also been made in other series.[27] Though, we did not encountered any case of occult metastasis from thyroid malignancy in our series, evidence from previous studiesindicate that immunohistochemical staining using CK19 may be contributory in that scenario as well.[25,26] However, further research may be warranted in this context. In our study, CK19 reactivity done pre-operatively on FNA smears correlated with that done on post-operative tissue specimens and an accurate pre-operative diagnosis was thus achieved in all the cases. This fact can have important implications. Taking into consideration that obvious difference in treatment and expected outcome exists between various malignant lesions of thyroid a positive immunostaining for CK19 performed on pre-operative smears can be of immense use in distinguishing these lesions accurately, pre-operatively and can also direct in selecting the appropriate treatment modality for such neoplasms. It can thus, be safely concluded that CK19 immunostaining in conjunction with FNAB can be a valuable marker in pre-operative diagnosis of papillary thyroid carcinoma. It can be especially useful in differentiation of follicular variant of papillary carcinoma from follicular carcinoma and adenoma, the latter being negative for CK19 staining. This can help the clinician in formulation of treatment options of atypical papillary thyroid carcinomas, the follicular variant of papillary thyroid carcinomas being treated by radical measures as against the more conservative approach for benign follicular lesions.

REFERENCES

1. Gharib H. Fine-needle aspiration biopsy of thyroid nodules: Advantages, limitations, and effect. Mayo Clin Proc 1994;69:44-9.
2. Samuel AM, Sharma SM. Differentiated thyroid carcinomas in children and adolescents. Cancer 1991;67:2186-90.
3. Nasr MR, Mukhopadhyay S, Zhang S, Katzenstein AL. Immunohistochemical markers in diagnosis of papillary

thyroid carcinoma: Utility of HBME1 combined with CK19 immunostaining. Mod Pathol 2006;19:1631-7.
4. Rosai J, Zampi G, Carcangiu ML. Papillary carcinoma of the thyroid. A discussion of its several morphologic expressions, with particular emphasis on the follicular variant. Am J Surg Pathol 1983;7:809-17.
5. Lloyd RV, Erickson LA, Casey MB, Lam KY, Lohse CM, Asa SL, *et al*. Observer variation in the diagnosis of follicular variant of papillary thyroid carcinoma. Am J Surg Pathol 2004;28:1336-40.
6. Bjorklund B, Bjorklund V. Antigenicity of pooled human malignant and normal tissues by cyto-immunological technique; presence of an insoluble, heat-labile tumor antigen. Int Arch Allergy Appl Immunol 1957;10:153-84.
7. Miettinen M, Franssila K, Lehto VP, Paasivuo R, Virtanen I. Expression of intermediate filament proteins in the thyroid gland and thyroid tumors. Lab invest 1984;50:262-70.
8. Kurzen H, Esposito L, Langbein L, Hartschuh W. Cytokeratins as markers of follicular differentiation: An immunohistochemical study of trichoblastoma and basal cell carcinoma. Am J Dermatopathol 2001;23:501-9.
9. Kumamoto H, Yoshida M, Ooya K. Immunohistochemical detection of amelogenin and cytokeratin 19 in epithelial odontogenictumors. Oral Dis 2001;7:171-6.
10. Nie M, Zhong L, Zeng G, LiB. The changes of cytokeratin 19 during oral carcinogenesis. Zhonghua Kou Qiang Yi Xue Za Zhi 2002;37:187-90.
11. Schmitt AM, Anlauf M, Rousson V, Schmid S, Kofler A, Riniker F, *et al*. WHO 2004 criteria and CK19 are reliable prognostic markers in pancreatic endocrine tumors. Am J Surg Pathol 2007;31:1677-82.
12. Jain R, Fischer S, Serra S, Chetty R. The use of Cytokeratin 19 (CK19) immunohis to chemistry in lesions of the pancreas, gastro intestinal tract, and liver. Appl Immunohistochem Mol Morphol 2010;18:9-15.
13. Stewart CJ, Crook ML, Lacey J, Louwen K. Cytokeratin 19 expression in normal endometrium and in low-grade endometrioid adenocarcinoma of the endometrium. Int J Gynecol Pathol 2011;30:484-91.
14. Tobias-Machado M, Fonseca F, Fantinato AP, Bendit I, Wroclawski ML, Wroclawski E, *et al*. Cytokeratin 19 expression by reverse transcriptase-polymerase chain reaction in the peripheral blood of prostate cancer patients. Tumori 2005;91:248-52.
15. Xenidis N, Ignatiadis M, Apostolaki S, Perraki M, Kalbakis K, Agelaki S, *et al*. Cytokeratin-19m RNA-positive circulating tumor cells after adjuvant chemotherapy in patients with earlybreast cancer. J Clin Oncol 2009;27:2177-84.
16. Koga T, Tokunaga E, Sumiyoshi Y, Oki E, Oda S, Takahashi I, Detection of circulating gastric cancer cells in peripheral blood using real time quantitative RT-PCR. Hepatogastroenterology 2008;55:1131-5.
17. Xu D, Li XF, Zheng S, Jiang WZ. Quantitative real-time RT-PCR detection for CEA, CK20 and CK19 mRNA in peripheral blood of colorectal cancer patients. J Zhejiang Univ Sci B 2006; 7:445-51.
18. QuYM, Liao GQ, Liu PH, Wang HM, LiuL, LiLL, *et al.* Clinical Significance of CK20, CK19, CEA mRNAs in Peripheral Blood from Lung Cancer Patients. Zhongguo Fei Ai ZaZhi 2009;12:1013-7.
19. Liberman E, Weidner N. Papillary and follicular neoplasms of the thyroid gland. differential immunohistochemical staining with high molecular weight keratin and involucrin. Appl Immunohistochem Mol Morphol 2000;8:42-8.
20. Raphael SJ, McKeown-Eyssen G, Asa SL. High molecular weight cytokeratin and cytokeratin 19 in the diagnosis of thyroid tumors. Mod Pathol 1994;7:295-300.
21. Sahoo S, Hoda SA, Rosai J, DeLellis RA. Cytokeratin 19 immunoreactivity in the diagnosis of papillary thyroid carcinoma: A note of caution. Am J Clin Pathol 2001;116:696-702.
22. Cheung CC, Ezzat S, Freeman JL, Rosen IB, Asa SL. Immunohistochemical diagnosis of papillary thyroid carcinoma. Mod Pathol 2001;14:338-42.
23. Zhu X, Sun T, Lu H, Zhou X, Lu Y, Cai X, *et al*. Diagnostic significance of CK19, RET, galectin-3 and HBME-1 expression for papillary thyroid carcinoma. J Clin Pathol 2010;63:786-9.
24. Erkilic S, Kocer NE. The role of cytokeratin 19 in the differential diagnosis of true papillary carcinoma of thyroid and papillary carcinoma like changes in Grave's disease. Endocr Pathol 2005;16:63-6.
25. Wang Z, Qiu S, Eltorky MA, Tang WW. Histopathologic and immunohistochemical characterization of a primary papillary thyroid carcinoma in the lateral cervical lymph node. Exp Mol Pathol 2007;82:91-4.
26. Boutross-Tadross O, Saleh R, Asa SL. Follicular variant papillary thyroid carcinoma arising in strumaovarii. Endocr Pathol 2007;18:182-6.
27. Shin E, Chung WY, Yang WI, Park CS, Hong SW. RET/PTC and CK 19 expression in papillary thyroid carcinoma and its clinicopathological correlation. J Korean Med Sci 2005;20:98-104.

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