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Detection of Micrometastasis in Lymph Nodes in Various Malignancies Using Immunohistochemical Marker (CK7)

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Abstract

Introduction: Carcinoma from almost all the organs has a tendency for regional metastasis. Nodal status is a key prognostic indicator in such patients, particularly with N0 stage. Occult metastasis in the form of micrometastasis (MM) and isolated tumor cells (ITCs), often goes undetected by routine hematoxylin and eosin (H&E) examination using 1-2 sections for analysis. This limitation could be overcome by combining serial sectioning (SS) with immunohistochemistry (IHC) for the detection of MM. Cytokeratin (CK7) is particularly a useful marker to detect these deposits as their presence has resulted in varied interpretations and different applications of the tumor-node-metastasis system. The objective of the study was to detect micrometastases in lymph nodes reported negative on H & E staining by combining SS and IHC and to compare it with conventional H&E staining. *Methods:* The study was conducted on 181 LNs received from 77 patients which were negative on H &E. The lymph nodes (LNs) positive on H & E were not taken up for the study. IHS with CK7 was performed on these lymph nodes for detection of micrometastsis. *Results:* The application of combination of SS and IHC using CK7 in our study revealed the presence of MM in 14.36% of the LNs diagnosed as negative on routine H&E examination. *Conclusion:* In the view of crucial role of occult LN metastasis in prognosis and survival of patients, diagnostic tools such as IHC staining, particularly with CK7, combined with SS should be preferred over conventional methods as they result in upstaging, thus sparing the low-risk patients the morbidity of unnecessary treatment.

Keywords: Micrometastasis; Lymph Nodes; Immunohistochemistry; Cytokeratin7; Serial Sectioning.

Introduction

Malignant tumors of epithelial tissues are the most common form of cancer and are responsible for the majority of cancer related deaths.

The identification of occult metastases in patients with early stage cancer could have a substantial clinical impact on the prognosis and optimal therapy for patients with cancer. At later stages of the disease, it may be useful to determine the presence of and change in the number of residual malignant cells so that the therapies selected can be monitored and adjusted to the changing needs of the patient [1].

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Micrometastasis has been defined, according to the TNM classification system as metastasis more than 0.2 mm in size, but less than 2 mm. If metastasis is more than 2 mm in size it is referred to as macrometastasis [2]. The accuracy of detecting micrometastases in the pathology laboratory has been a major concern.

A variety of methods including serial sectioning and immunohistochemistry (IHC) on paraffin blocks have been shown to be superior to the original single hematoxylin and eosin (H and E) staining in detecting micrometastases. Sections from cases in which lymph node had been reported free of metastases were stained using monoclonal antibody Cytokeratin 7(CK7).

Our study was conducted to detect lymph node micrometastases in node negative cancer patients using cytokeratin (CK7) and to correlate this with tumor size and lymph node size.

Material and Methods

The present study was conducted to analyse CK7 as a marker of micrometastasis in lymph nodes in various malignancies conducted at Index Medical College, Hospital & Research centre, Indore (M.P.) over a period of 18 months.

A total of 181 LNs received from 77 patients which were negative on H & E were taken up for the study. The specimens were examined for gross details on arrival in the department, routinely processed and 3 to 5 micron thick sections were made from paraffin embedded blocks. These sections were routinely stained with H & E and examined for presence of invasive carcinomas. The lymph nodes reported negative for presence of metastasis are be subjected to immunohistochemical study. Immunohistochemistry was performed using a monoclonal antibody that is reactive with cytokeratin 7.

For this a section of 3-5 μm thickness from the block was made on poly l-lysine coated slides. Antigen retrieval was performed by heat induced epitope retrieval using microwave oven. IHC was performed using avidin biotin technique (using labelled streptavidin biotin (LSAB) + kit) with Dako Monoclonal Mouse anti-Human CK clone AE1/AE3 (dilution 1:50).

CK staining gave brown cytoplasmic reactivity. Cytokeratin positive tumor cells had diffuse cytoplasmic staining with a more intensely stained peripheral band. There were two patterns of micrometastases:

Micrometastases consisting of a single cell were classified as the single-cell type (Fig.1). Micrometastases consisting of clusters of two or more tumor cells were classified as the cluster type (Fig. 2). When both single tumor cells and clusters were observed in a lymph node, they were classified as the cluster type.

Results

Out of 793 lymph nodes dissected from 77 patients of various malignancies, 612 (77.1%) lymph nodes were positive for metastases. 181 (22.8%) lymph nodes were negative for metastases on H and E examination. These 181 cases were taken up for the study.

One hundred fifty nine cases (87.8%) were diagnosed with infiltrating duct carcinoma not otherwise specified, Fourteen cases (7.7%) as squamous cell carcinoma and Eight cases (4.4%) as gastric carcinoma.

The minimum age of the patients was 30 years and the maximum age was 80 years. The mean age of patients was 53.07 years (standard deviation [SD] – 9.933) and the median age was 54 years.

Table 1: Age Calculation

Age group (yrs)	No. of Patients
<50	74
≥50	107

Table 2: Calculation of tumor size

Tumor size (cms)	No. of Patients	% of Patients
< 2	5	2.8
2-5	137	75.7
>5	37	20.4

Table 3: Calculation of Lymph node size

Lymph Node Size	No. of Patients	% of Patients
< 1	47	25.7
1-2	125	68.3
>2 cm	9	4.9

Twenty six out of 181 cases (14.36%) were positive for lymph node metastases by CK IHC. The sensitivity of detection of occult metastases by CK was 65.4% and specificity was 34.6%.

Most of the cases i.e 137(75.7%) had tumor size 2-5 cm. Thirty seven cases (20.4%) had tumor size > 5cm and five cases (2.8%) had tumor size < 2cm.The mean tumor size was 4.13 cm (SD – 2.36) and median 11.2 cm.

One hundred twenty five (68.3%) cases had lymph node size measuring between 1 and 2 cm, forty seven (25.7%) cases < 1 cm and nine (4.9%) cases with > 2 cm. The mean lymph node size was 1.27 cm (SD – 0.55) and median 5.7 cm.

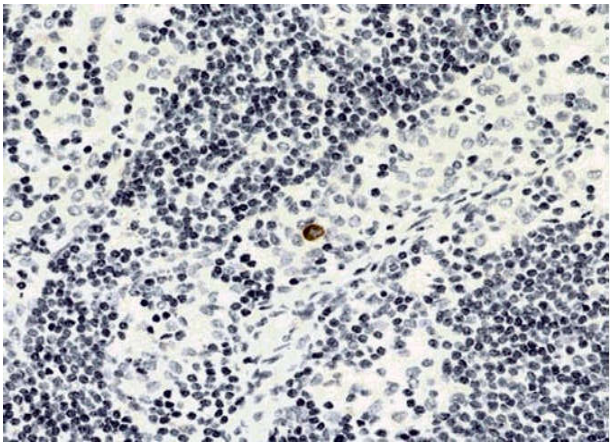

Fig. 1: Micrometastases of the single-cell type. Cytokeratin positive cells are involved in the lymph nodes (original magnification, 3200)

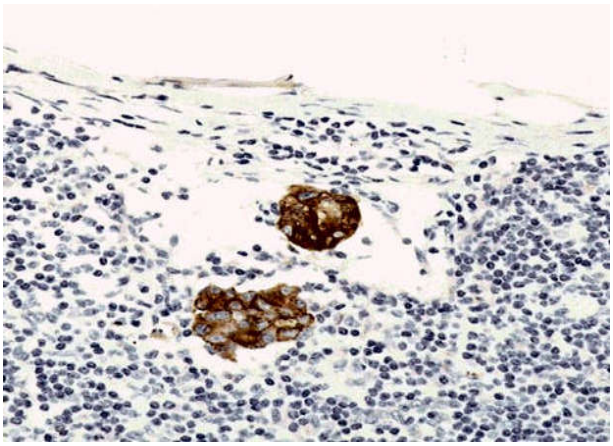

Fig. 2: Micrometastases of cluster type. Small clusters consisted of cytokeratin positive cells (original magnification, 3200)

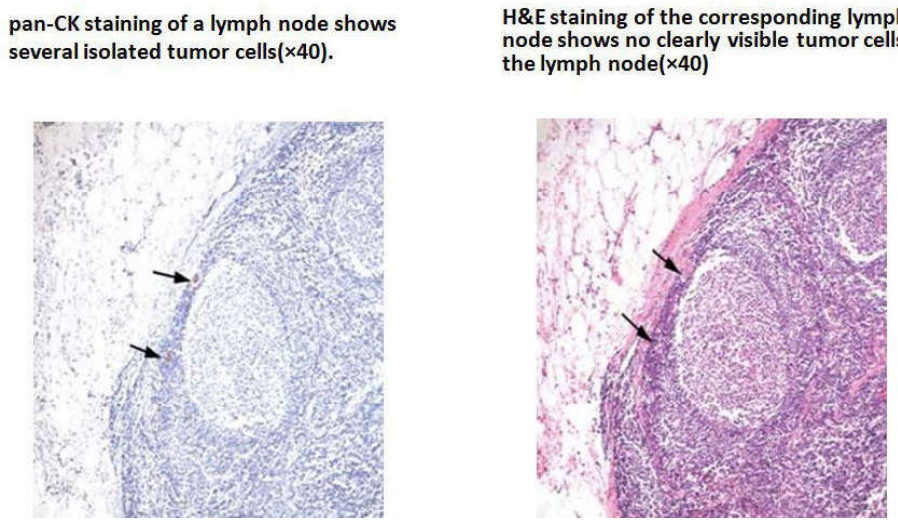

Fig. 3: H & E misdetection

Table 4: Correlation of metastasis detected by CK with age, tumor size and lymph node size.

Classification	Number and percentage of total cases	Number and percentage of cases with metastases detected by CK (%)	P value for CK
Age (In years)			
<50	74(40.88)	11(14.86)	-0.039(NS)
≥ 50	107(59.12)	15(14.01)	
Tumor Size (in cm)			
≤ 2	4(2.21)	1(25)	0.323** (S)
2-5	140(77.34)	15(10.71)	
≥ 5	37(20.44)	10(27.02)	
Lymp node Size (in cm)			
≤ 1	37(20.44)	0(0)	
1-2	133(73.48)	18(13.53)	.255**(S)
≥ 2	11(6.07)	8(72.72)	

Classification Number and percentage of total cases Number and percentage of cases with metastases detected by CK (%) P value for CK

Discussion

Lymph node metastasis is an important prognostic factor for patients with malignancy. The clinical

significance of nodal metastasis has long been well appreciated. The cure rate drops to nearly half with involvement of regional lymph nodes. Histopathological examination is highly sensitive and specific test for detection of metastasis but earliest stage of metastasis can be difficult to identify by light microscopy. If a single 5um section is required, the 1cm lymph node has to be sectioned 2000 times.

Lindeman et al. demonstrated the absence of cytokeratins in any types of cells of a normal lymph nodes except the metastatic cells from epithelial primary [3]. He observed the CK7 expression on metastatic cells only in a lymph node which was secondary to epithelial primary tumors.

The results of this study confirmed the predicted sources of error in standard pathological assessment of lymph nodes with both immunohistochemical detection and step sectioning resulting in increased detection of metastasis. Serial sectioning of the lymph nodes as a routine procedure is far too time consuming to be practical. This method is labour intensive for both laboratory technician and pathologist.

About 14.36% of the cases were detected to have metastasis by staining with CK.

In addition, however occult metastasis were seen more frequently with age of patient more than or equal to 50 years but we did not found a statistically significant correlation between age and lymph node metastases detected by CK IHC, when patients were grouped as <50 years or >50 years. McGuckin et al. found that immunohistochemically detected micrometastases were associated with a shorter survival by univariate analysis in the under 50 years old group of patients corresponding to 41% (26/64) of the patients in the series [4]. McGuckin et al., de Mascarel et al. and Grabau et al. also found astatistically significant correlation between age and lymph node metastases detected by IHC, when the patients were grouped as <50 years and ≥50 years [5].

In our study, tumor size correlated significantly with lymph node metastases detected by CK (P = 0.323). Nasser et al. found that occult metastases were more frequent with larger tumor size. McGuckin et al. found lymph node metastases detection rate of 35% in cases with tumor size >2 cm as compared with 20% in cases with tumor size ≤ 2 cm and obtained a statistically significant correlation between tumor size and lymph node metastases detected by IHC. In a study by Cote et al., 26% of the cases with tumor size >2 cm were positive for lymph node metastases detected by IHC as compared with 16% of the cases with tumor size ≤ 2 cm positive for lymph node metastases by IHC [6]. Umekita et al. also found a higher incidence of micrometastases detection in cases with tumor size >2 cm [7].

There was a statistically significant correlation between lymph node size and lymph node metastases detected by CK 7. However, Hainsworth et al. and Grabau et al. found a statistically significant correlation between lymph node number and lymph node metastases detected by IHC [8].

Zhi-Wei Zhou et al in 2005 found no correlation with all these parameters (all p >0.05) [9]. While Noura et al in 2002 found that tumor size alone was significantly larger in the CK-positive group than in the CK-negative group (p = .014) [10].

The detection of micrometastases correlates significantly with the recurrence and survival rate of patients. Immunohistochemical staining is a sensitive and specific method of detecting nodal metastases. It highlights the fallibility of conventional microscopic assessment of lymph nodes. Although experienced histopathologists, given adequate time and a large number of sections might pick up many of these cases, the important point to emphasize is the ease and confidence with which these micrometastases can be identified by immunostaining. This is of potential clinical significance in the context of chemotherapy trials, in which the treatment is dependent upon the number of involved lymph nodes. Furthermore, metastasis by IHC can be detected by adequately trained scientific staff, which minimizes the workload of specialist pathologists and therefore reduces the cost of implementing such procedures. IHC can be performed in the same time frame as conventional histology and is applicable to most methods of tissue processing including frozen sections.

Conclusion

The present study was conducted to analyse CK7 as a marker of micrometastasis in lymph nodes in various malignancies conducted at Index Medical College , Hospital & Research centre, Indore (M.P).

We studied 181 lymph nodes in patients of various malignancies which were confirmed negative on histopathological examination.

Immunohistochemical staining for CK7 was done according to CAP protocol.

CK7 level significantly correlated with increasing tumor size and lymph node size. Age of patient was not found significant with lymph node metastasis.

The sensitivity of CK was 65.4% whereas the specificity was 34.6%.

It is therefore postulated that the higher tumor size and lymph node size show statistically significant CK7 positivity and therefore pronounce a poor prognosis in these cases.

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