Original Article

An Immunohistochemical Expression of CK5/6, CK7, and CK20 on Cell Blocks in Metastatic Cervical Lymphadenopathy

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Abstract

Background: The neck is a common site of both primary and secondary malignancies. Many tumors from the head and neck (oral cavity, larynx, and pharynx), lung, and gastrointestinal tract metastasize to cervical lymph nodes. At most times, tumors are diagnosed by morphology, sometimes it is difficult to diagnose an unknown primary presenting as metastatic lymphadenopathy solely on the basis of morphology. Specific histological cell types can be confirmed by the use of immunohistochemistry. **Aim:** The present study evaluated the utility of cell block over fine-needle aspiration cytology (FNAC) and immunohistochemical expression of CK5/6, CK7, and CK20 in metastatic cervical lymphadenopathy. **Methods:** This prospective study design was used on a total of 50 cases. FNAC smears and cell blocks were made in all the cases. All the cell blocks were compared with FNAC findings and further subjected to immunohistochemical (IHC) analysis. The necessary statistical analysis was done. **Results and Conclusion:** Our study showed that the combined use of the cell block technique and FNAC was more useful and sensitive in diagnosing the metastatic cervical lymph nodes and the accuracy can be further improved by the use of IHC on the cell blocks. The combined use of CK5/6, CK7, and CK20 in metastatic cervical lymphadenopathy is helpful in diagnosing squamous cell carcinoma and adenocarcinoma with known/unknown primary sites.

Keywords: *Cellblock, CK20, CK5/6, CK7, fine-needle aspiration cytology, immunohistochemical, metastatic cervical lymphadenopathy*

Introduction

Lymphadenopathy is one of the most common clinical presentations of patients, who attend outdoor clinics in most hospitals. The etiology varies from a mild inflammatory process to malignant conditions. Many tumors from the head and neck (oral cavity, larynx, and pharynx), lung, gastrointestinal tract, and other areas metastasize to cervical lymph nodes. There are different histological types of tumors such as squamous cell carcinoma (SCC), adenocarcinoma, malignant melanoma, and sarcoma that can metastasize to lymph nodes. Many tumors are diagnosed by morphology, at times, it is difficult to diagnose an unknown primary presenting as metastatic lymphadenopathy on the basis of morphology alone.

For an initial diagnosis and management of patients with lymphadenopathy, fine-needle aspiration cytology (FNAC) of lymph node is the most common and widely used investigation due to the early availability of results, simplicity, and minimal trauma. However, it has the disadvantage of false-negative or intermediate/incomplete diagnoses. To overcome these problems, the cell block technique has been resorted to as a corollary investigation. The cell block technique allows the recovery and processing of minute amounts of cytological material and facilitates in better classification of tumors when reviewed along with cytological smears. It is also used as a reliable preparation for immunohistochemical (IHC) studies. Immunohistochemistry involves the detection and localization of antigens or proteins in tissue sections by the use of antibodies that bind specifically to the antigen of interest.

Monoclonal antibodies to specific CK subtypes have been used to classify tumors according to their site of origin. CK5/6 is a high-molecular-weight cytokeratin and expressed in neoplasms of epithelial origin including SCC, mesothelial carcinoma,

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and urothelial carcinoma.[1] Similarly, CK20 and CK7 have been used successfully in studies determining the primary location of adenocarcinomas. CK20 is distributed predominantly in carcinomas of the gastrointestinal and pancreatobiliary tracts, urothelium, and mucinous ovarian tumors.[2] CK7 is expressed in a wide variety of epithelia including the lung, breast, endometrium, urothelium, stomach, pancreatobiliary tract, and skin adnexal glands.[3]

The coordinated expression of CK7 and CK20 has been used to determine the site of origin of carcinomas in known and unknown primaries.[4,5]

Methods

This study was done on a total of 50 cases of metastatic cervical lymphadenopathy from carcinoma of the known and unknown primaries received in the cytology section of the Department of Pathology and Department of Radiotherapy and Oncology, Government Medical College, Amritsar. All the patients were subjected to FNAC and cytological smears were prepared and the cell blocks were also prepared in all the cases. Cell blocks, thus prepared were further subjected to IHC staining using antibodies for CK7, CK20, and CK5/6. All the procedures and methods used were in accordance with the ethical standards of the Institutional Research Committee.

Results

The present study consisted of a total of 50 cases of metastatic cervical lymphadenopathy. The major age group in our study was 60–70 years and the majority of the patients were male (84%). Most (85%) of the malignancies in males were reported as SCC and the rest as adenocarcinoma. In comparison, the female patients reported half the cases as SCC and the other half as adenocarcinomas. Of the total, 30 cases were of various malignancies of known origin and 20 cases were unknown primaries [Table 1]. Malignancies of the head and neck were responsible for the most cervical metastasis in known cases.

On noting the level of the cervical lymph nodes, the maximum number of cases (52%) showed level II lymphadenopathy, followed by level III lymphadenopathy (22%). The malignancies of the head and neck were most commonly associated with level II lymph node involvement, followed by level III lymph node involvement. In cases of carcinoma breast, metastasis was seen in level V lymph node.

As depicted in Table 2, we were able to diagnose SCC on FNAC [Figure 1] alone in 31 out of 50 cases (62%), the percentage of which increased with the use of cell blocks [Figure 2] (70%) and IHC (80%). More importantly, 15 (30%) cases were reported as metastatic carcinomatous deposits (MCD) on FNAC, and eventually by the use of IHC, we were able to categorize all the cases of MCD.

Table 3 illustrates that 10 out of the total cases of unknown primaries were diagnosed on FNAC, followed by three cases on cell block and further seven cases by the use of IHC.

All the cases were analyzed by the use of IHC and all 40 cases of SCC were positive for CK5/6 [Figure 3] and negative for both CK7 [Figure 4] and CK20 [Figure 5]. In 10 cases of adenocarcinoma, CK7 was positive in all cases but CK20 came out positive in seven cases.

The intensity and quantity scores of all three markers were noted in all cases. As understandable from Table 4, CK5/6 intensity was moderate to strong in almost all cases, and in most cases (29 out of 40), more than 50% of the cells were positive. In adenocarcinoma cases, CK7 was moderately positive in all cases (10) and CK20 staining was weak to moderate.

Quantity score: 0 – no staining; 1 – 1%–10% of cells; 2 – 11%–50% of cells; 3 – 51%–80% of cells; and 4 – 81%–100% of cells.

Table 1: Suspected primary malignancy

Site	*n* (%)
Ca larynx	8 (16)
Ca tongue	7 (14)
Ca oral mucosa	4 (8)
Ca supraglottis	4 (8)
Ca parotid	2 (4)
Ca breast	2 (4)
Ca hypopharynx	1 (2)
Ca lower lip	1 (2)
Ca pharynx	1 (2)
Unknown	20 (40)
Total	50 (100)

Ca: Carcinoma

Table 2: Diagnosis on the basis of investigation modality

Diagnosis	FNAC	Cell block	IHC
SCC, *n* (%)	31 (62)	35 (70)	40 (80)
Adenocarcinoma	4	4	10
MCD	15	11	0
Total	50	50	50

SCC: Squamous cell carcinoma; MCD: Metastatic carcinomatous deposits; FNAC: Fine-needle aspiration cytology; IHC: Immunohistochemistry

Table 3: Diagnosis of cases with unknown primary

	SCC	Adenocarcinoma	Total
Diagnosed on FNAC	7	3	10
Diagnosed on cell block	3	0	3
Diagnosed on IHC	3	4	7
Total	13	7	20

SCC: Squamous cell carcinoma; FNAC: Fine-needle aspiration cytology; IHC: Immunohistochemistry

Table 4: Immunohistochemistry intensity score and quantity score

Score	CK5/6		CK7		CK20	
	Quantity	Intensity	Quantity	Intensity	Quantity	Intensity
0	10	10	40	40	43	43
1	0	1	0	0	2	5
2	11	27	7	10	5	2
3	19	12	3	0	0	0
4	10	-	0	-	0	-
Total	50	50	50	50	50	50

Intensity score: 0 – No staining, 1 – Weak staining, 2 – Moderate staining, 3 – Intense staining, Quantity score: 0 – No staining, 1 – 1%-10% of cells, 2 – 11%-50% of cells, 3 – 51%-80% of cells, 4 – 81%-100% of cells. CK: Cytokeratin

Discussion

Lymph nodes are the most common sites of metastatic tumor and sometimes constitute the first clinical manifestation of the disease.[6] The asymptomatic enlargement of one or more cervical lymph nodes in an adult has at least an 85% chance of being malignant and of all the malignancies about 70% of metastases are from primary head-and-neck cancers.[7]

The present study was aimed to access the utility of cell blocks in increasing the cytodiagnosis of fine-needle aspirates and to compare the data with the cytological smears. Further, this study applied IHC markers (CK7, CK20, and CK5/6) on the cell blocks to see the expression and their diagnostic utility in known/unknown primary in metastatic cervical lymphadenopathy.

In our study, 40% (20 out of 50) of cases had an unknown primary location and just presented with neck lymph node metastasis. The maximum cases (16%) belonged to carcinoma larynx as their primary malignancy in all of the patients in which the primary location was known followed by carcinoma tongue in 14% of the cases. Various studies found the oropharynx or oral cavity as the primary site of malignancy in case of metastatic cervical lymphadenopathy.[7,8] Naeimi M *et al*. found in their study that the larynx and hypopharynx were the most common source of metastatic cervical lymphadenopathy which is in concordance with the results in our study.[9]

Analyzing the results of our study, a final diagnosis of SCC, whether on FNAC, cell block H and E examination, or IHC, was made in the majority (80%) of the cases, and diagnosis of adenocarcinoma was made in the rest (20%). Mangal *et al*. found in their study that 86% of metastatic lymphadenopathy was of squamous cell type arising chiefly from the oropharyngeal region[8] which was corroborated by another study done by Alam *et al*. which found that 67.87% of the total cases were of SCC and 9.04% were adenocarcinomas.[10]

On evaluating the sensitivity and diagnostic accuracy of both the investigation modalities, it was observed that the use of cell block was a superior technique to FNAC in the diagnosis of SCC [Table 5]. The results of our study in comparing the investigation techniques are comparable to the other studies in the literature on the diagnosis of SCC.[11,12] In cases of adenocarcinoma, both the techniques are comparable as both sensitivity (40%) and diagnostic accuracy (88%) are the same in both. This might be attributed to the fact that the number of cases of adenocarcinoma in our study was very less in number.

As discussed and concluded above, cell block microscopy yields a higher degree of morphological diagnosis in comparison to FNAC, besides its diagnostic accuracy can be enhanced further by the use of IHC to reach a definitive diagnosis of the histological type of metastatic carcinoma. In the present study, all the cases were subjected to IHC analysis for expression of CK5/6, CK7, and CK20.

On observing the quantity score [Table 4], the maximum number (73%) of cases of SCC had quantity scores of 3 and 4 (moderate-to-strong intensity). The results of our study are comparable to the study done by Kaufmann *et al*. which demonstrated a high expression of CK5/6 in 81% of SCCs that were included in their study, with an intense immunoexpression, which was diffuse in the majority of the tumoral cells.[13] Similarly, Rahman *et al*. found in their study that CK5/6 was expressed in 90.9% of the SCC cases.[14]

CK5/6 expression was observed in all the cases of SCC included in our study (sensitivity 100%). This finding is in line with the results of a study which reported that CK5/6 is detected in 100% of all the SCC.[15] Another study conducted on metastatic SCCs also reported that CK5/6 has 100% sensitivity.[16]

On the basis of the heterogeneity of CK7 and CK20 expression in malignant epithelial tumors, the CK7/CK20 expression has served as a useful diagnostic tool for the discrimination of primary and metastatic carcinomas of unknown origin.

On studying the patterns of CK7 expression in the present study, it was found to be positive in all the 10 cases (100% sensitivity) diagnosed with adenocarcinoma with more than 11% of cells being positive (quantity score of 2) and staining with moderate intensity (intensity score of 2). A study done by Chu and Weiss found in their study that

CK7 shows diffuse cytoplasmic positivity in the majority of adenocarcinoma cases.[17] According to Alwahaibi *et al*., CK7 has a high sensitivity (91.7%) and it was very good in predicting the positive cases as the positive predictive value (PPV) showed 95.6%.[15] Another study done by Kim *et al*. reported that CK7 was expressed in 96.6% of all the total gastric carcinoma specimens.[18]

The present study also noted the expression pattern of CK20 and it came out positive in only seven cases of adenocarcinoma (sensitivity of 70% with PPV of 100%). Majority of the cases were weak positive with a quantity score of 2 (11%–50% of cells). In the literature, CK20 sensitivity and specificity is variable and is dependent on the site of malignancy. According to Bayrak *et al*., CK20 reactivity was found in 84% of colorectal carcinomas, 53% of gastric carcinomas, and only 22% of pancreatic adenocarcinomas.[19]

The coordinated expression of CK7 and CK20 has been used to determine the site of origin of carcinomas.[4,5] Each immunophenotype is associated with a group of epithelial neoplasms. A review article by Selves *et al*. while focusing on carcinomas of uncertain origin states that these malignancies can be separated into four main diagnostic groups: (1) $CK7^{+}$/CK20−, (2) $CK7^{+}$/CK20+, (3) CK7−/CK20+, and (4) $CK7^{-}$/CK20.[20] As shown in Table 6, the malignancies included in our study showed two types of IHC patterns, CK7+/CK20+ and CK7+/CK20−.

Evidently, CK7+/CK20+ pattern was seen in 7 out of 10 cases (70%) of adenocarcinoma included in the study and out of which five cases were of unknown primary. Majority of the adenocarcinoma cases were weekly positive with a quantity score of 2. Roh and Hong found in their study that CK 20 was positive in adenocarcinoma cases presenting with metastatic carcinomas of the unknown primary site and it should be the first choice as a component of antibody panel to prove or exclude the Gastrointestinal Tract (GIT) origin.[21]

Of the known primaries of adenocarcinoma, we reported only one case of parotid adenocarcinoma which had moderate positivity for CK7 and CK20. Many studies in the literature including a study done by Chu and Weiss reported that malignant salivary gland tumors have a typical CK7+/CK20− profile.[17] However, a study done by Nikitakis *et al*. reported on their observation that although overall 92.9% of malignant salivary gland tumors were characterized by a CK7+/CK20− immunoprofile but the remaining 7.1% of cases were positive for both the cytokeratins.[22]

Table 5: Comparison of fine-needle aspiration cytology and cell block microscopy

	SCC		Adenocarcinoma	
	FNAC	Cell block	FNAC	Cell block
Sensitivity (%)	77.5	87.50	40	40
Specificity (%)	100	100	100	100
PPV (%)	100	100	100	100
NPV (%)	52.63	66.67	86.96	86.96
Accuracy (%)	82	90	88	88

SCC: Squamous cell carcinoma; FNAC: Fine-needle aspiration cytology; PPV: Positive predictive value; NPV: Negative predictive value

Table 6: Immunohistochemistry expression pattern in adenocarcinoma

	CK7+/CK20+	CK7+/CK20−	Total
Known primary cases	2	1	3
Unknown primary cases	5	2	7
Total cases	7	3	10

CK: Cytokeratin

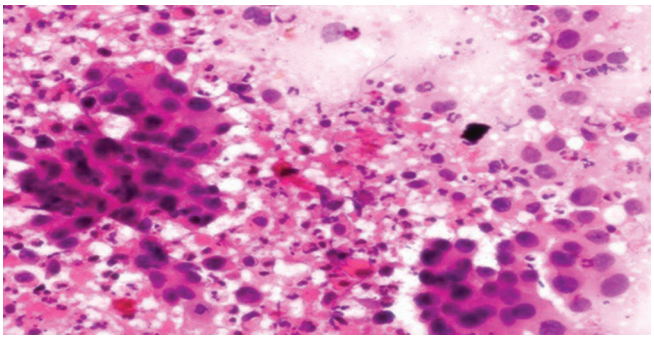

Figure 1: Microphotograph of squamous cell carcinoma on FNAC smear (H and E × 400). FNAC: Fine-needle aspiration cytology

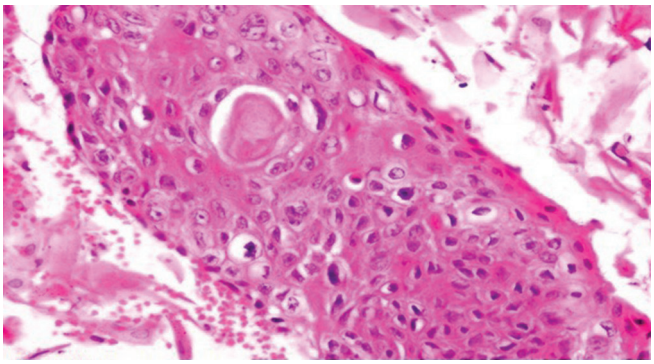

Figure 2: Microphotograph showing squamous cell carcinoma with keratin pearl formation on cell block microscopy (H and E, ×400)

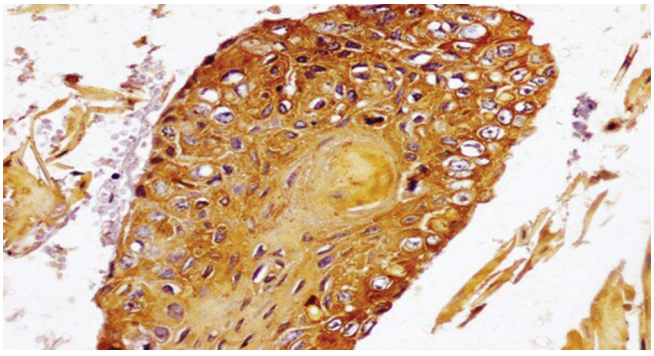

Figure 3: Microphotograph showing immunoreactivity of CK5/6 in SCC (IHC, ×400)

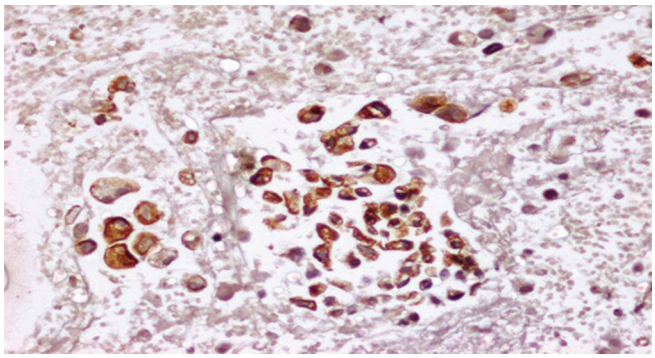

Figure 4: Microphotograph showing immunoreactivity for CK7 in case of adenocarcinoma (IHC, ×400)

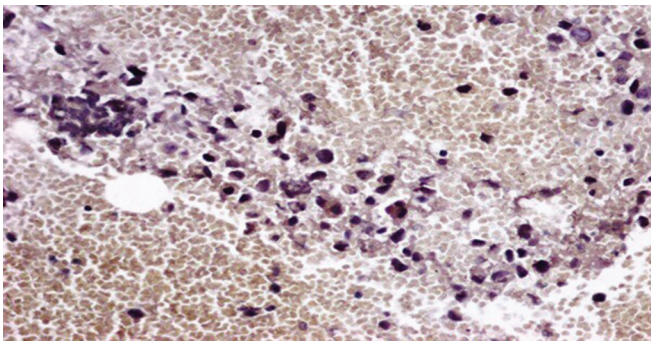

Figure 5: Microphotograph showing immunoreactivity for CK20 in case of adenocarcinoma (IHC, ×400)

Wang *et al*. showed that the application of both CK7 andCK20 to the epithelial tumors aids in the differential diagnosis by narrowing the consideration of the organ of origin to a few primary sites.[4]

Although in the present study, we could not do the follow-up of the patients diagnosed with metastasis with an unknown primary; these two immunoprofiles should be further investigated to narrow down the primary site of malignancy in cases of metastatic lymphadenopathy.

As a coordinate expression of CK7 and CK20 defines the subsets of carcinomas, the next step will be to determine the likely primary site based on their expression profiles.

A study done by Lin and Liu proposed that once the CK7/CK20 expression profile is established, complementary organ-specific antibodies allow refinement or more precise guidance toward the origin of carcinoma of unknown primary.[23]

The staining pattern for CK7 and CK20 gives an overall indication but has some limitations as many cancers can have variable CK7 and CK20 profiles.[24]

Conclusion

The current study showed that the combined use of the cell block technique and FNAC was more useful and sensitive in diagnosing the metastatic cervical lymph nodes and the accuracy can be further improved by the use of IHC on the cell blocks. For the diagnosis of SCC, a single IHC marker CK5/6 can be used which has high sensitivity and specificity. The combined use of CK7 and CK20 can be very helpful in diagnosing adenocarcinomas on cell blocks. The present study also recommends a systematic approach with the further use of organ-specific immunomarkers to aid in the diagnosis of metastatic malignancies of unknown primaries. Further, despite advances in imaging and IHC, unknown primaries still cannot be categorized and newer molecular biology techniques should be used to complement IHC.

Ethical clearance

Study was approved by the Institutional Ethics Committee vide letter no. 5757-58/D-26 dated 27th February 2019.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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