Original Article

The role of immunohistochemistry for diagnosing undifferentiated malignancy in the head and neck region

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Abstract

Background: *Undifferentiated tumours in the head and neck region are not uncommon. They can arise from different sites like in mucosa as well as in salivary glands, soft tissues or lymph nodes. Histopathological examination plays a central role in the diagnosis but difficulties arise with some tumours which are poorly-differentiated due to their high inter- and intra-observer variability. In those cases, immunohistochemistry has greatly assisted to diagnose the tumours that cannot be accurately identified using routine histopathological procedures. The correct histopathological diagnosis is essential especially in case of malignant tumourwhere subsequent specific therapy is required. The aim of this study was to determine the role of immunohistochemistry for diagnosing undifferentiated malignancy in the head and neck region.*

Methods: *This cross-sectional study was conducted during July 2014 to June 2015. A total of 35 Bangladeshi patient's specimens of head and neck swelling were collected from two renowned laboratories in Dhaka city.These cases were diagnosed as undifferentiated tumour in histopathological examination.Standard protocol was followed for immunohistochemistry.Then primary immunohistochemical panel which included the markers for Epithelial CK (AE1/AE3),mesenchymal marker (Vimentin) and lymphoid marker (LCA) were used.The cases which were not resolved by primary immunohistochemistry panel, the second panel was applied for further sub classification (Desmin, Chromogranin, CK20, CEA, CD20, CD30, HMB45, NSE). Based on interpretation of immunohistochemical findings final diagnoses were made.Data analysis was performed using the Statistical Package for the Social Sciences for Windows version 22.0 (SPSS, Chicago, Illinois, USA).*

Results: *A total of 35 undifferentiated tumorsof head-neck region were studied.The mean age was 46.3±17.6 years and male to female ratio was 4.8:1. The majority 13 (37.1%) patient had cervical lymphadenopathy, 11 (31.4%) had neck mass and 4(11.4%) had in nasal/sinonasal mass.Regarding histopathologicalcell types, round cell was 21 (60.0%), spindle cell 6(17.1%), pleomorphic cells 6(17.1%) and epithelioid cells 2(5.7%). In initial histopathological examination, majority 25(71.4%) were undifferentiated malignant neoplasm, 8(22.8%) were metastatic undifferentiated carcinoma, 1(2.9%) was pleomorphic sarcoma and the remaining 1(2.9%) had malignant adnexal tumour. By application of immunohistochemistry, most (33, 94.3%) of the cases were resolved and the two cases remained unresolved. Among the resolved cases majority (15, 45.5%) were lymphoma, 4(12.1%) were metastatic carcinoma, 3(9.1%) were Ewing's sarcoma and malignant melanoma were found in 3(9.1%) cases.*

Conclusion: *This study supports that the immunohistochemical technique has a fundamental role in the investigation of undifferentiated tumour origin, to determine the correct guidance for treatment and improving the prognosis for head and neck tumour patients.*

Keywords: *undifferentiated malignant tumour, head and neck, lymph nodes, histopathological diagnosis, immunohistochemistry, round cell, lymphoma.*

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Introduction

Malignancy in the head and neck region are a common form of cancer in South East Asian countries accounting for 23% of all cancer in males and 6% in females.[1] Among these 1.1% of all tumours diagnosed are undifferentiated. The undifferentiated tumour refers to a heterogeneous group of neoplasms with little or no evidence of differentiation on routine histopathological examination.[2]They can arise from different sites in the head and neck region like in mucosa as well as in salivary glands, soft tissues or lymph nodes.[3,4]The diverse tissues in this anatomic region give rise to a broad spectrum of tumours and tumour-like conditions. Routine histopathological examination plays a central role in diagnosis, classification, grading and staging of malignancy but sometimes it creates great challenge for some tumour groups because of the absence of definite morphology that characterizes their lymphoid, epithelial or mesenchymal origin.[5] Beside these, routine histological analysis sometimes influenced by the practitioner's experience as well as skill.With poorly-differentiated neoplasm, inter- and intra-observer variability appear to be high.[6]

The introduction of the immunohistochemical method by Coons et al.[7] has become a powerful adjunct in tumour analysis. It has increased the possibilities for histogenetic diagnosis of undifferentiated tumours. However, there are only a few references in the literature to the immunohistochemical technique applied to the identification of undifferentiated head and neck tumours.[8,9] By application of specific panel of monoclonal or polyclonal antibodies depending on the cellular components of the undifferentiated tumour, 90% of tumours posing diagnostic difficulties by morphology could be accurately classified by immuno-histochemistry.[6,9]

The present study was aimed to immunohistochemical evaluation of undifferentiated tumours in the head and neck region. The key objective was a broad lineage determination of the malignancy by applying a primary or screening panel. Moreover, based on the results of the screening panel, a more detailed or specific panel was applied to further sub classify the tumour and to confirm a particular diagnosis.

Methods

This was a cross-sectional study,carried out in the Department of Histopathology, Armed Forces Institute of Pathology, Dhaka Cantonment between July 2014 to Jun 2015. A total of 35 patients of all age group of either sex who had been suffering from head and neck swelling and diagnosed as undifferentiated tumour in routine histopathology were included in this study. The samples were collected from two renowned histopathology laboratory of Dhaka city, AFIP and Delta Medical Services. The inclusion criterion was the patients with neck swelling diagnosed as undifferentiated malignant tumour in histopathological examination. The exclusion criteria were patients with head and neck masses who did not have undifferentiated malignant lesion and who have been suffering from swelling in deep seated organ or organs and thyroid gland. After receiving the biopsy specimens of head and neck swelling, tissue were processed for histopathological examination by routine H&E method. Interpretation, confirmation of malignant lesion and identification of the cases diagnosed as undifferentiated tumour were performed. For immunohistochemistry, standard protocol was followed. Then a primary immunohistochemical panel was applied for Epithelial (AE1/AE3), mesenchymal marker (Vimentin) and lymphoid marker (LCA). Based on the result of primary panel, the secondary panel of immunohistochemical markers ware applied for further sub classification (CD99, CEA, CD20, CD30, HMB45, NSE, Desmin, chromogranin). The final diagnosis was achieved by analyzing the slides under microscope. The main outcome variables were age, sex,location of the head and neck swelling, cellular morphology and pattern of undifferentiated tumour in head and neck region in histopathology andimmunohistochemical findings in case of those undifferentiated tumour.Statisticaldata analysis was carried out by using the Statistical Package for Social Sciences (SPSS) version 22.0 for windows.

Results

A total of 35 patients of head and neck swelling diagnosed on histopathology as undifferentiated tumour were studied.Among them male were 29 (82.9%) and female were 6 (17.1%). Male to female ratio was 4.8:1.Age distribution of the patients were that, out of 35,18 (51.4%) patients belonged to age 41-60 years and their mean age was found 46.3±17.6 years. According to the site of the lesion, that majority (13, 37.1%) had cervical lymphadenopathy, 11(31.4%) had neck mass, 4(11.4%) had mass in nasal/sinonasal region (Table I).Considering the cellular morphology of studied cases, it was found that round cell was 21(60.0%), spindle cell 6(17.1%), pleomorphic cells 6(17.1%) and epithelioid cells were 2(5.7%).In initial histopathological examination, majority (25, 71.4%) were undifferentiated

malignant neoplasm, 8(22.8%) metastatic undifferentiated carcinoma, 1(2.9%) was pleomorphic sarcoma and the remaining 1(2.9%) was malignant adnexal tumour.Out of 35 cases diagnosed as undifferentiated tumour in histopathology, 33(94.3%) were resolved and 2(5.7%) remained unresolved by using immunohistochemistry. Among the resolved cases, 15(45.5%) were lymphoma, 4(12.1%) were metastatic carcinoma, 3(9.1%) were Ewing's sarcoma and 3 (9.1%) were malignant melanoma (TableII).

Table I Distribution of the study patients by site of the lesion (n=35)

Site of the lesion	Number of patients	Percentage
Cervical lymph node	13	37.1
Face	1	2.9
Neck	11	31.4
Parotid gland	1	2.9
Nasal /sinonasal	4	11.4
Nasopharynx	2	5.7
Oral cavity	1	2.9
Scalp	1	2.9
Infratemporal lesion	1	2.9

Table II Distribution of the resolved cases with immunohistochemistry (n=33)

Immunohistochemistry	No. of patients	Percentage
Lymphoma	15	45.5
Metastatic carcinoma	4	12.1
Ewing's sarcoma	3	9.1
Small cell carcinoma	2	6.1
Olfactory neuroblastoma	2	6.1
Malignant melanoma	3	9.1
MPNST	1	3.0
Myoepithelial carcinoma	1	3.0
Rhabdomyosarcoma	1	3.0
Epithelioid sarcoma	1	3.0

Considering the cell pattern of the resolved cases with immunohistochemistry,21 patients had round cell type and the two unresolved cases,one was rounds cell and the another one case was spindle celltype.The use of immunohistochemistry panels in resolved cases, it was observed that by using primary panel which included three markers 8 cases were resolved. Out of them 6(75.0%) had lymphoma. Secondary panel was used in 27 patients, among them 25 (92.6%) were resolved and 9(36.0%) had lymphoma (Table III).

Table III Association between immune panel with final diagnosis (n=35)

Final diagnosis with immunohistochemistry		Panel			
		Primary panel(n=8)		Secondary panel (n=27)	
		n	%	n	%
Final diagnosis					
	Unresolved	0	0.0	2	7.4
	Resolved	8	100.0	25	92.6
Immunohistochemistry					
	Lymphoma	6	75.0	9	36.0
	Metastatic carcinoma	1	12.5	3	12.0
	Ewing's sarcoma	0	0.0	3	12.0
	Small cell carcinoma	0	0.0	2	8.0
	Olfactory neuroblastoma	0	0.0	2	8.0
	Malignant melanoma	0	0.0	3	12.0
	MPNST	0	0.0	1	4.0
	Myoepithelial carcinoma	0	0.0	1	4.0
	Rhabdomyosarcoma	0	0.0	1	4.0
	Epithelioid sarcoma	1	12.5	0	0.0

Resolved case: Male 70, soft tissue mass neck. Histopathological Diagnosis: Undifferentiated malignant neoplasm
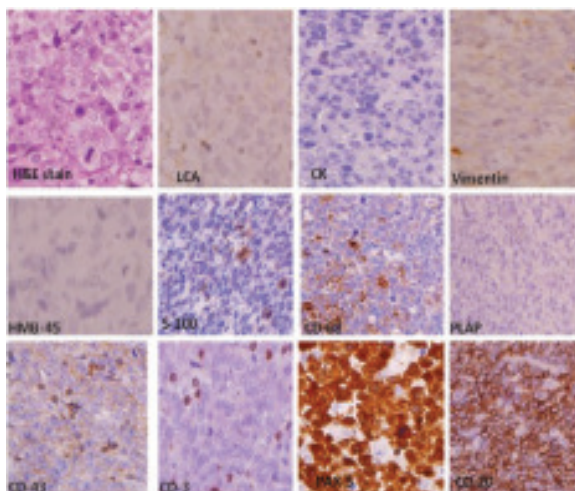**Final diagnosis:** Diffuse large B-cell lymphoma

Figure 1 Photomicrographs showing the immunohistochemical profile of an undifferentiated malignant neoplasm. The haematoxyl and eosin section shows pleomorphic cells with nuclear vessiculation and prominent nucleoli disposed in islands. The intermediate to large sized round cells are showing strong immunopositivity to PAX-5 and moderate reactivity CD-20. These cells are negative for primary immuno-histochemistry panel. [Unresolved case], M 41 years, core biopsy from neck mass].
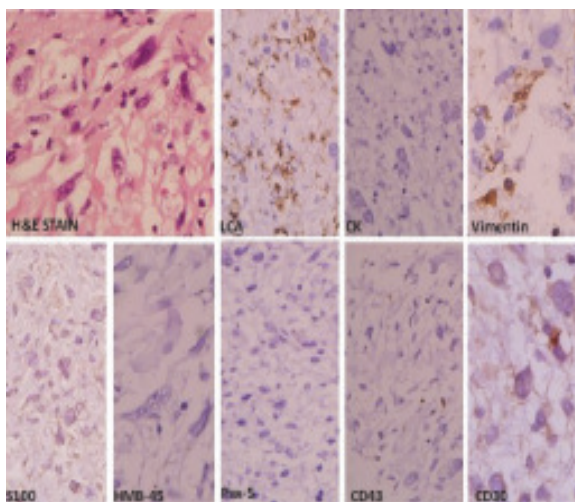

Figure 2 Photomicrographs showing the immunohistochemical profile of an undifferentiated malignant neoplasm. The haematoxylin and eosin section reveals highly pleomorphic spindle cells show no immunoreactivity to primary and secondary panel except focal positivity to vimentin.

Discussion

The surgical pathology knowledge has revolutionized by applying immunohistochemical technique, because it is essential for the correct diagnosis of the neoplasia and for successful therapy, prognosis and outcome.[10]

This cross-sectional study was carried out with an aim to know the role of immunohistochemistry to diagnose undifferentiated malignancy in head and neck region.

In this present study it was observed, out of 35 patients, more than half patients belonged to the age 41-60 years and their mean age was found 46.3±17.6 years. Shinde and Hashmi[1] reported the commonest age group of head and neck malignancy is the 6th decade comprising of 39.58% cases. In a study done in Pakistan, Bhurgri et al.[11] reported that majority of the cases were in age group 51- 60 years which are comparable with the current study. Shinde and Hashmi[1] showed that male cases were more common than female comprising of male to female ratio was 2:1. In our study it was observed that male to female ratio was 4.8:1. Similar observations regarding the head and neck region malignancy is predominant in male subjects also observed by Mandalet al.[12]

In our study it was observed that over one-third patients had cervical lymphadenopathy, one-third had neck mass and one-tenth had mass in nasal/sinonasal region. Almost similar findings was reported Bianchini et al.[13] who obtained the lymph nodes (20.9%), pharynx and neck mass (16.3%), paranasal sinus (14.0%) and nose (11.6%) constituted the majority of sites.

In this study, it was observed that round cell was predominant cell pattern. Round cell tumours, the most (51.16%) prevalent cell pattern also reported by Bianchini et al.[13]

In our study it was observed that lymphoma was found in majority cases, followed by metastatic carcinoma, Ewing's sarcoma and malignant melanoma. Shinde and Hashmi[1] reported that about 86.95% of cases of cervical lymph node cancers were found to be SCC and rest 15% cases were Hodgkins lymphoma and non-Hodgkins lymphoma.

In this series, 21 patients had round cell among them 90.5% cases were resolved and 66.7% patients had lymphoma. Adisa et al.[2] mentioned in their study that undifferentiated carcinomas were the most prevalent group constituting 64% of the undifferentiated malignancies and 7.6% of all head and neck malignancies.

In this current study, 25 patients had undifferentiated malignant neoplasm (UMN) among them 23(92.0%) cases resolved of which 11 (44.0%) patients had lymphoma. In Adisaet al.[2] study showed the original histological diagnosis of 62 lesions was carcinoma, but only 34(54.8%) were confirmed by immuno-histochemistry. This proportion is higher than the 27.9% confirmation rate of carcinomas in a study by Bianchini et al.[13] in Italy. This demonstrates the value of immunohistochemistry in distinguishing between anaplastic carcinomas and malignant lymphomas of the head and neck. Histopathological differentiation of undifferentiated carcinoma from neuroendocrine carcinoma is challenging and is significantly aided by immunohistochemistry.[14,15]

Our results showed diagnostic guidance in over 90% patients. It was not possible in 2 patients (5.7%) of cases. Bianchini et al.[13] reported 18.6% and Gatter et al.[5] reported 6.7% inconclusive results. This could be due to technique differences, different antigen retrieval methods or the unavailability of the antigen suspected. In addition, some poorly-differentiated tumours might require other techniques such as electron microscopy and molecular studies before an accurate diagnosis can be achieved.

Conclusive diagnosis was not possible in the single case of pleomorphic cell tumour and one round cell tumour. The least number(three) of antibodies were used to solve the cases to clarify the confusion between carcinoma and lymphoma. In our study 8 cases were confused between carcinoma and lymphoma. After using immunohistochemistry, 7(87.5%) cases were proved as lymphoma and 1 (12.5%) casewas diagnosed as carcinoma. Gatteretal.[5] also reported 29 cases (67.4%) out of 43 cases initially thought to be anaplastic carcinomas, were revised as lymphomas by immunohistochemistry. This demonstrates the value of immunohistochemistry in distinguishing between anaplastic carcinomas and malignant lymphomas of the head and neck by Stricker and Kumar.[16]Therefore, the present study thusconsistent with other studies which suggest that immunohistochemical technique has a great role in the definition of undifferentiated tumours.

Limitations

Further studies can be undertaken by including large number of patients. Moreover, molecular or cytogenetic study can be included with this study to evaluate exact nature of the undifferentiated tumours.

Conclusion

This study was undertaken to determine the role of immunohistochemistry for diagnosing undifferentiated malignancy in the head and neck region. By applying panels of immunohistochemistry most of the cases were resolved. This study reveals that the immunohisto-chemical technique has a fundamental role in the investigation of undifferentiated tumour. It determines correct guidance for treatment and improving the prognosis for head and neck oncological patients.The diagnostic results are appropriately transmitted to concern specialist dealing with the patients the oncologists, radiation oncologists and surgeons to create a coordinated plan of care for patients with these disorders affecting the head and neck.

Conflits of interest: Nothing to declare.

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