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The use of immunohistochemistry in an oral pathology laboratory

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Abstract

Immunohistochemistry has become part of normal routine diagnostic work in the Stomatology Unit, Institute for Medical Research, Kuala Lumpur. Of 9523 cases received from the year 2000 to 2005, 197 cases (2.1%) required immunohistochemical staining. These cases ranged from benign to malignant lesions. They include lymphomas (n=41), epithelial tumours (n=29), neural lesions (n=21), fibroblastic/myofibroblastic tumours (n=16), small round cell tumour (n=11), vascular tumours (n=4), smooth muscle tumours (n=4), myxomatous tumours (n=4) and skeletal muscle tumours (n=1). In most of the cases (69.5%), immunohistochemical staining was mandatory to reach a definite diagnosis, while 60 cases (30.5%) required immunohistochemistry in confirming the diagnosis. In 32 cases (16.2%), definitive diagnosis could not be made due to the small size of the specimens received or the results of immunohistochemistry were inconclusive. Standardization of techniques, competent medical laboratory technologists and sufficient budget allocation are important in producing a high quality immunohistochemistry service.

Key words: immunohistochemistry, oral pathology

INTRODUCTION

The Stomatology Unit, Institute for Medical Research (IMR), Kuala Lumpur, which was established in 1967, is the main oral pathology diagnostic laboratory in the Ministry of Health, Malaysia. Diagnostic work is an essential part of the unit which receives approximately 1400 specimens each year from the government dental clinics as well as from private dental practitioners. A few cases are also referred from other oral pathologists as well as general pathologists for second opinion. The cases range from benign to malignant neoplasms. In most cases, the diagnosis of the disease is based on the microscopical features of the cells seen using haematoxylin and eosin stained slides. However, in some cases, definitive diagnoses cannot be reached due to the variable and overlapping histological patterns encountered in rare tumours or the pathologists are not familiar and lack exposure to these unusual tumours. In these scenarios, to achieve a more definitive diagnosis, the pathologists have to resort to special techniques like immunohistochemistry.[1,2] The aim of this study is to record the pattern of cases for which imunohistochemical staining were performed from the year 2000 to 2005 in the Stomatology Unit, IMR.

MATERIALS AND METHODS

The records of the Stomatology Unit, IMR were reviewed for cases for which immunohistochemical staining were performed from the year 2000 to 2005. A total of 197 cases were included and the biopsy request forms were reviewed. The clinical features and the interpretation/diagnoses of the cases were obtained from the records.

The interpretation of the diagnosis was then broadly classified according to cellular morphological type i.e. small round cell, spindle cell, or pleomorphic cell tumour. The biological behaviour of the cases were categorised as benign, intermediate or malignant.

We also categorised the reasons for doing the immunohistochemistry staining as: (a) for research purposes or to assist in confirming the diagnosis, or (b) mandatory i.e. without the staining, a definitive diagnosis cannot be reached.

The data was analysed with descriptional statistics.

RESULTS

There were 197 cases for which immunohistochemistry were performed within the 6 year period (2000 to 2005). There were more male patients (n=113, 57.4%) compared to

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female patients (n=84, 42.6%). The majority of the patients were Malay (n=90, 45.7%). There were 38 (19.3%) Chinese, 24 (12.2%) Indians and 45 (22.8%) of other ethnicity. Of these 197 cases, only 5 cases were received for second opinion.

The number of cases received each year and the proportion for which immunohistochemistry were performed are shown in Table 1. Table 2 details the final diagnoses of cases which had immunohistochemistry performed. Of these 197 cases, 60 cases (30.5%) required immunohistochemistry in confirming the diagnosis while in 137 cases (69.5%), immunohistochemistry was mandatory for definitive diagnosis.

DISCUSSION

We reviewed 197 cases for which immunohistochemical staining were performed during a 6-year period. Approximately 35 cases each year had immunostaining and although these cases represented only 2% of the total number of cases received by the Stomatology Unit, it reflects that immunohistochemical staining has become part of the diagnostic work.

A large number of cases for which immunohistochemistry were performed were tumours or tumour-like lesions (Table 2). There were 32 cases (16.2%) including 23 malignant tumours and 9 benign lesions in which definitive diagnoses could not be made. Some of the reasons for this are the small size of the specimens received or the immunohistochemistry findings were inconclusive. Despite using antigen retrieval methods,[3] not all specimens could be immunostained successfully. The quality of immunostaining can be affected by the type and period of tissue fixation. Leong and Gilham found that there was a distinct fall-off in staining for some antigens after three days of fixation in 10% buffered formalin.[4] As most of our specimens are sent from other states in Malaysia, transit time for some specimens to reach our laboratory can exceed 3 days.

Most of the cases (n=137, 69.5%) for which the immunohistochemical staining was mandatory for definitive diagnosis were malignant neoplasms such as lymphoma, Ewing's sarcoma/PNET, malignant melanoma and spindle cell carcinoma. This shows that to reach a definitive diagnosis based solely on haematoxylin and eosin staining is not possible in such cases as the histological features of these lesions are composed of pleomorphic cells or cells with overlapping morphological features.

41 cases (20.8%) were lymphoma. The current WHO classification of lymphoma is based on the morphology of the tumour cells as well as the immunohistochemical typing.[5] Since our laboratory performs only a bare minimal antibody panel for lymphoma, it merely differentiates lymphoma from other neoplasms, and subtypes lymphomas on the basis of CD3 antigen (for T cell) and CD20 antigen (or B cell) expressions. Further subclassifications are limited and are based only on the morphological features. In some of the cases, a second opinion was sought. We note that in a study of 134 cases of small B cell lymphomas, Siquera *et al* selected CD10, CD23 and cyclin D1 as the minimal panel for its classification, which gave a final diagnosis in 88.1% of the cases.[6]

Cases which required immunohistochemistry to assist in confirming the diagnosis (n=60, 30.5%) were Langerhan's cell histiocytosis, myofibroma, multiple myeloma and benign neural lesions such as neurofibroma and neurilemomma. This finding shows that these lesions are no stranger to the pathologists but

TABLE 1. Number of cases received by the Stomatology Unit, and proportion for cases which immunohistochemistry was performed over the study period 2000-2005

Year	Total number received	No. (%) of cases with immunostaining
2000	1672	32 (1.9%)
2001	1584	27 (1.7%)
2002	1474	45 (3.1%)
2003	1688	31 (1.8%)
2004	1562	30 (1.9%)
2005	1543	32 (2.1%)
TOTAL	9523	197 (2.1%)

TABLE 2. Summary of cases for which immunohistochemistry was performed between 2000 and 2005

CATEGORY	NO OF CASES
Neural lesion: (n=21)	
Neurilemomma	8
Palisaded encapsulated neuroma	3
Malignant peripheral nerve sheath tumour	3
Neurofibroma	2
Nerve sheath myxoma	1
*Others	4
Lymphoma: (n=41)	
Hodgkin's lymphoma:	1
Non Hodgkin's lymphoma:	
B cell neoplasm	
B cell lymphoma	20
Marginal zone lymphoma	5
Burkitt's lymphoma	3
Small lymphocytic lymphoma	2
Lymphoplasmacytic lymphoma	1
Follicular lymphoma	1
T cell neoplasm	4
T/NK cell lymphoma	1
No typing	3
Smooth muscle tumours: (n=4)	
Smooth muscle hamartoma	2
Leiomyoma	1
Leiomyosarcoma	1
Skeletal muscle tumours: (n=1)	
Rhabdomyosarcoma	1
Fibroblastic/myofibroblastic tumours: (n=16)	
Myofibroma	5
Malignant fibrous histiocytoma	3
Fibromatosis	2
Low grade myofibroblastic sarcoma	2
Benign fibrous histiocytoma	1
Inflammatory myofibroblastic tumour	1
[#]Others	2
Epithelial tumours: (n=29)	
Squamous cell carcinoma	7
Spindle cell carcinoma	2
[£] Malignant epithelial tumour	20
Vascular tumours: (n=4)	
Papillary endothelial hyperplasia	1
Epithelioid haemangioendothelioma	1
Epithelioid angiosarcoma	1
Kaposi's sarcoma	1
Small round cell tumour: (n=11)	
Ewing's sarcoma/PNET	7
Malignant melanoma	3
Melanotic neuroectodermal tumour of infancy	1

Myxomatous tumours: (n=4)	
Fibromyxoma	2
Superficial angiomyxoma	1
Myxomatous lesions	1
Other tumours: (n=34)	
Langerhans' cell histiocytosis	7
Osteosarcoma	3
Fibrous epulis/Fibroma	3
Multiple myeloma	2
Pyogenic granuloma	2
Inflammatory phenomenon	2
Desmoplastic fibroma of bone, ectomesenchymal chondromyxoid tumour, leukaemic infiltrate, angiolymphoid hyperplasia with stromal eosinophilia, intramucosal naevus, alveolar soft part sarcoma, chondroid lipoma, salivary adenoma, chronic gingivitis, irritative hyperplasia, non specific ulcer, eosinophilic granuloma, benign lymphoid hyperplasia, reactive lymph node, atypical lymphoid proliferation	1 each
Others (unclassifiable): (n=32)	
Malignant neoplasm	23
Benign lesions	9

* suggestive of neural lesions/differential diagnosis of neural lesion
\# suggestive of fibroblastic/myofibroblastic tumours
£ include cases of nasopharyngeal carcinoma, adenocarcinoma (not otherwise specified), metastatic carcinoma

immunohistochemical staining is helpful in confirming the diagnosis. We have also found immunohistochemical staining to be useful in highlighting the patterns that might be required in the diagnosis of these lesions.

There were 21 cases (10.7%) of neural lesion. Typically, neurilemomma and palisaded encapsulated neuroma are lesions that exhibit histological features which are usually recognized even without the aid of immunohistochemistry, although in some cases with atypical histological features, immunohistochemistry is useful to identify the neural elements. S100 protein is the antibody which is normally used for neural lesions.[7] S100 protein is a ubiquitous antigen as it is also positive in other tumours such as melanoma,[8] chondrosarcoma[9] and granular cell tumour.[10] S100 protein and a panel of antibodies

TABLE 3. Panel of antibodies commonly in use in the laboratory

Panel	List of antibodies
Spindle cell	SMA or Muscle actin, vimentin, S-100 protein, desmin, possibly CK, HMB45, CD34, CD31
Small round cell/lymphoid	LCA, CD3 (T cell), CD20 (B cell), possibly kappa, lambda, EMA
Round blue cell/pleomorphic	CK, LCA, S-100 protein, HMB45, CD99, desmin

SMA= smooth muscle actin (clone 1A4), Muscle actin (clone HHF35), CK= cytokeratin (clone MNF116), HMB45= anti Human Melanosome, LCA= leucocyte common antigen (CD45), EMA= epithelial membrane antigen (clone E29)

comprising of smooth muscle actin, desmin and vimentin is usually requested to rule out other benign spindle cell lesions.

For a laboratory to produce uniform immunohistochemical results, standardization of the immunohistochemical technique is critical. Inconsistencies can often be directly related to improper tissue fixation and processing, inadequate unmasking of antigenic epitopes and low sensitivity of the detection system.[11] Although immunohistochemistry cases represented only 2% of the cases reported by our laboratory, the cost of the reagents approached almost 90% of the operating budget. This means only a fairly limited panel of antibodies is offered in our laboratory (Table 3). The antibodies chosen are based on common antibodies previously validated by studies in other laboratories. Recent studies have shown that each marker should preferably be validated by at least 2 antibodies, e.g. MNF116 and CAM 5.2 as cytokeratin,[12] or CD20 and CD 79a as B cell markers.[13,14] Other markers such as Ki-67, a proliferative marker, has also been shown to be useful for distinguishing carcinoma-in-situ from non-neoplastic epithelium.[15]

With the advent of immunohistochemistry, it has become a standard tool for diagnosis and prognosis of tumours. Standardization of the techniques, competent medical laboratory technologists, sufficient budget allocation are important in producing a good quality immunohistochemistry service. However, advances and new technological developments in molecular biology also pose other ventures for the diagnostic oral pathology laboratory.

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