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Use of immunohistochemistry in the differential diagnosis of nasopharyngeal tumours in resource limited-settings: defining a cost-effective approach

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Abstract

Background: Squamous cell carcinomas (SCC) are the most common nasopharyngeal tumors (NP) subtypes, however other tumors particularly Non-Hodgkin's lymphomas (NHL), occur in the nasopharynx and require different treatment approach. Therefore identifying and distinguishing these tumors from carcinomas is crucial for appropriate patient management. This study reviews the diagnostic accuracy of NP with and without the use of immunohistochemistry (IHC) and attempts to define meaningful, cost-effective immunohisto-chemical approach in low-resource setting.

Materials and methods: Nasopharyngeal tumours (52 cases) identified in the database of Department of Pathology, University College Hospital, Ibadan, Nigeria in the period January 2007 to December 2012 were reviewed. The diagnosis based on haematoxylin and eosin stained sections were Nasopharyngeal carcinomas (41 cases), Poor differentiated tumour (2 cases), Non-Hodgkin's lymphomas (7 cases), Adenoid cytic carcinoma (1case) and Small blue cell tumour (1 case). A limited IHC antibody panel consisting of Cytokeratin cocktail (AE1/AE3/ CAM5.2), CD20 and CD3 were performed on all cases and subtyping T-cell NHL using CD30 and Desmin for the small blue cell tumour typing. The previous morphologic diagnosis and post IHC diagnosis were compared to determine accuracy/ error rate.

Results: Reviewed post-IHC classification of available 52 cases includes: NPC (39), NHL (11), adenoid cystic carcinoma (1) and Rhabdomyosarcoma (1), 2 cases of NHL were misclassified as carcinoma based on morphology alone. Therefore, Lymphomas included: B-cell NHL (9 cases); Anaplastic Large Cell Lymphoma (2 cases which were the poorly differentiated tumours on H&E); Error rate post IHC studies was approximately 5% (2/41) for NPC. The diagnosis of Anaplastic Large Cell Lymphoma and Rhadomyosarcoma could only be made definitively with IHC. Overall, error rate for all tumours post IHC was 11.5% (6/52)

Conclusion: Small panel of antibodies (cytokeratin, CD20, CD3) combined with good H&E stained sections is useful and cost effective in distinguishing undifferentiated nasopharyngeal carcinoma from lymphomas and for minimally subtyping NHL in resource-limited regions and crucial for better patient management.

Keywords: *Nasopharyngeal carcinoma, immunohistochemistry, Non Hodgkins, lymphoma. Cytokeratin, CD45*

Abstrait

Contexte : Les carcinomes épidermoïdes (SCC) sont les sous-types de tumeurs nasopharyngées (NP) les plus courants. Cependant, d'autres tumeurs, en particulier les lymphomes non hodgkiniens (LNH), apparaissent dans le nasopharynx et nécessitent une approche thérapeutique différente. Par conséquent, l'identification et la distinction de ces tumeurs des carcinomes sont cruciales pour la gestion appropriée du patient. Cette étude examine l'exactitude du diagnostique des NP avec et sans utilisation de l'immunohistochimie (IHC) et tente de définir une approche immunohistochimique significative et rentable dans un environnement à faibles ressources.

Matériels et méthodes : Les tumeurs nasopharyngées (52 cas) identifiées dans la base de données du département de pathologie du Collège Hospitalier Universitaire d'Ibadan, Nigéria entre janvier 2007 et décembre 2012 ont été passées en revue. Les diagnostics basés sur les coupes colorées à l'hématoxyline et à l'éosine étaient les suivants : carcinomes du nasopharynx (41 cas), tumeur mal différenciée (2 cas), lymphomes non hodgkiniens (7 cas), carcinome adénoïde

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cystique (1 cas) et tumeur à petites cellules bleues (1 cas). Un panel limité d'anticorps IHC consistant en un cocktail de cytokératine (AE1 / AE3 / CAM5.2), CD20 et CD3 ont été réalisés sur tous les cas et sous-typage de LNH des cellules T en utilisant CD30 et Desmin pour le typage de tumeur à petites cellules bleues. Diagnostic morphologique précédent et le diagnostic post-IHC ont été comparés pour déterminer le taux d'exactitude / erreur.

Résultats : La classification révisée postérieure à l'IHC des 52 cas disponibles comprend : NPC (39), LNH (11), carcinome adénoïde cystique (1) et du sarcome Rhabdomyome (1). Deux cas de LNH ont été classés à tort comme des carcinomes basés sur la morphologie seule. Par conséquent, les lymphomes comprenaient : LNH à cellules B (9 cas) ; Lymphome anaplasique à grandes cellules (2 cas, qui étaient les tumeurs mal différenciées sur H&E); Le taux d'erreur après les études IHC est d'environ 5% (2/41) pour les NPC. Le diagnostic de lymphome à grandes cellules anaplasique et de sarcome Rhabdomyome ne peut être posé de manière définitive qu'avec l'IHC. Dans l'ensemble, le taux d'erreur pour toutes les tumeurs après l'IHC était de 11,5% (6/52)

Conclusion : Un petit groupe d'anticorps (cytokératine, CD20, CD3) associé à de bonnes sections colorées H&E est utile et économique pour distinguer le carcinome nasopharyngé indifférencié des lymphomes et pour sous-caractériser de manière minimale l'LNH dans les régions limitées en ressources et crucial pour une meilleure gestion des patients.

Mots-clés : *carcinome* du *nasopharynx, immunohistochimie, non hodgkinien, lymphome, Cytokératine, CD45*

Introduction

Nasopharyngeal Carcinomas (NPC) especially squamous cell carcinomas (SCC) are the most common malignant neoplasm subtype that occur in the nasopharynx in most parts of the world. Other tumors, particularly Non-Hodgkin's lymphomas (NHL) do occur in the nasopharynx and require different treatment approach.[1-5] The differentiation between B-cell and T-cell type NHL, becomes critical and important because of the more aggressive nature of the Tcell tumours compared to B cell type tumours.[2,3,6] Furthermore identifying Natural Killer (NK) cell/Tcell Non- Hodgkins lymphoma as distinct from T cell lymphoma is important because of the markedly different prognosis in the two tumour types [3,6]. Therefore, identifying and distinguishing these tumors from SCC with immunohistochemical staining by the characteristic antibody marker is crucial for appropriate patient management.

In many parts of Nigeria and sub-Saharan Africa, immunohistochemistry is not part of routine histopathology service mainly because of high cost of antibodies and consumables, poor supply chain, lack of steady electric power and lack of technical-know how.

The use of Haematoxylin and Eosin (H and E) stained sections only in diagnosis of Nasopharyngeal Tumours (NPT) is fraught with likelihood of misdiagnosis because diffuse cellular infiltrate of non-cohesive cells admixed with lymphoplasmacytic infiltrate (Schminke pattern) may resemble NHL in many cases. This is in contrast to syncytial pattern of arrangement of cohesive tumour cells with indistinct cell margins (Regaud pattern) which are much easier to diagnose as NPC on H& E stained sections.

The fact that lymphomas are amenable to chemotherapy, radiotherapy and monoclonal antibody treatment makes it imperative that a precise diagnosis is made.

The aim of this study was to review the diagnostic accuracy of nasopharyngeal tumors with and without the use of immunohistochemistry (IHC) and attempts to define meaningful, cost-effective immunohistochemical approach that will assist in distinguishing these tumors.

Material and methods

Nasopharyngeal tumours identified in the database of Department of Pathology University College Hospital, Ibadan, Nigeria in the period January 2007 to December 2012 were reviewed. Only 54 cases fulfilled the inclusion criteria as only cases where nasopharngeal tissue were biopsied directly were included in the study. All lymph nodes biopsied in relation to the primary nasopharngeal tumours were excluded. The 52 cases in this study (based on initial Haematoxylin and Eosin diagnosis) included the following - 1. Nasopharyngeal carcinomas-NPC (41 cases) 2. Poorly differentiated tumour (2 cases) 3. Non-Hodgkin's lymphomas -NHL (7 cases) 4. Small blue cell tumour (1 case) and 5. Adenoid cystic carcinoma (1 case)

Limited IHC using antibody panel that consists of cytokeratin cocktail (AE1/AE3/CAM 5.2), CD20 and CD3 were performed on all cases and to subtype NHL. Further subtyping of NHL using CD 30 and typing the Small blue cell was done using Desmin. The previous morphologic/ histological diagnosis and post IHC diagnosis were compared to determine accuracy rate.

Table 1: The classification of the 52 cases with only Haematoxylin and stained sections and post immunohistochemical studies.

	Nasopharyngeal tumour	Number of cases with Haematoxylin and eosin diagnosis only	Diagnosis/Number of cases after application of IHC makers	IHC Makers used
1	Nasopharyngeal Carcinoma(NPC)	41	NPC- 39 NHL(B-cell)-2	AE1/AE 3, CD3, CD20
2	Poorly differentiated tumour	2	ALCL-2	CD3,CD30, AE1/AE3
3	NHL	7	NHL (B-cell)-7	CD3,CD20,
4	Small blue cell tumour	1	Rhadomyosarcoma	AE1/AE3, DESMIN
5	Adenoid cytic carcinoma	1	Adenoid cystic carcinoma	AE1/AE3

ALCL - Anaplastic large cell lymphoma, NHL - Non Hodgkin' lymphoma

The FFPE tumour tissue for each case was sectioned into 4μm thick sections. The slides were deparaffinized, rehydrated and the protocol specified for each antibody by the manufacturer was used for antigen retrieval, primary antibody dilution and staining. The slides were then washed with a buffer solution twice for five minutes. The bound antibody was visualized using a DAB-chromogen substrate. The sections were then stained with hematoxylin and mounted with a cover-slip. Negative control cases were obtained by omission of the primary antibody in the staining protocol. A suitable tissue was used as the positive control and stained along with all the sections.

This study was conducted in compliance with the guidelines of the Helsinki declaration on biomedical research in human subjects. Confidentiality of the identity of the patients and personal health information was maintained.

Results

Table 1 shows the reviewed classification of the 52 cases after immunohistochemical studies. These included: NPC (39 cases), NHL [11], adenoid cystic carcinoma [1] and Rhabdomyosarcoma [1]. Two cases of NHL were originally misclassified as carcinoma based on H&E stained sections alone. These were thereafter reclassified. Table 1 also shows details of the cases pre and post-IHC staining. Overall, Lymphomas included: B-cell NHL (9 cases); T-Cell NHL (2 cases), both cases were anaplastic large cell lymphoma-(ALCL)- (These were the 2 cases with H&E diagnosis of poorly differentiated tumour probably carcinoma). The error rate post-IHC studieswais approximately 5% (2/41) for NPC. The diagnosis of ALCL and rhadomyosarcoma could only be made definitively with IHC. Therefore, the error rate for all tumours post- IHC, which will affect

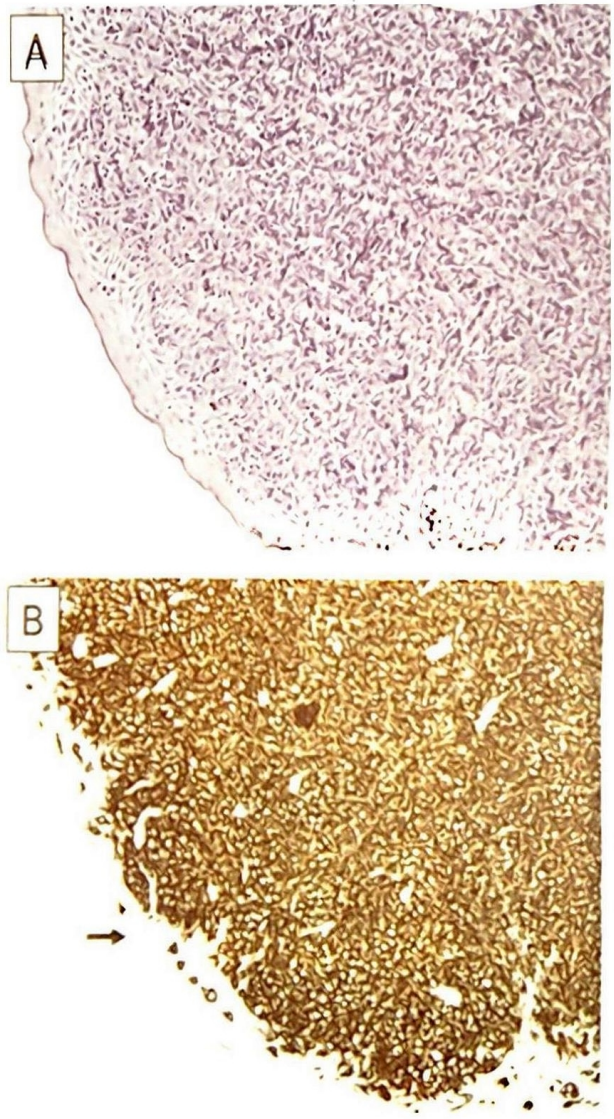

Fig. 1: (A) H&E stained section of submucosal nasopharyngeal tumor with sheets of tumor cells.
(B) The same tumor stained with CD20 with strong diffuse membrane staining of the tumor cells and the unstained negative superficial epithelial mucosa (arrow).

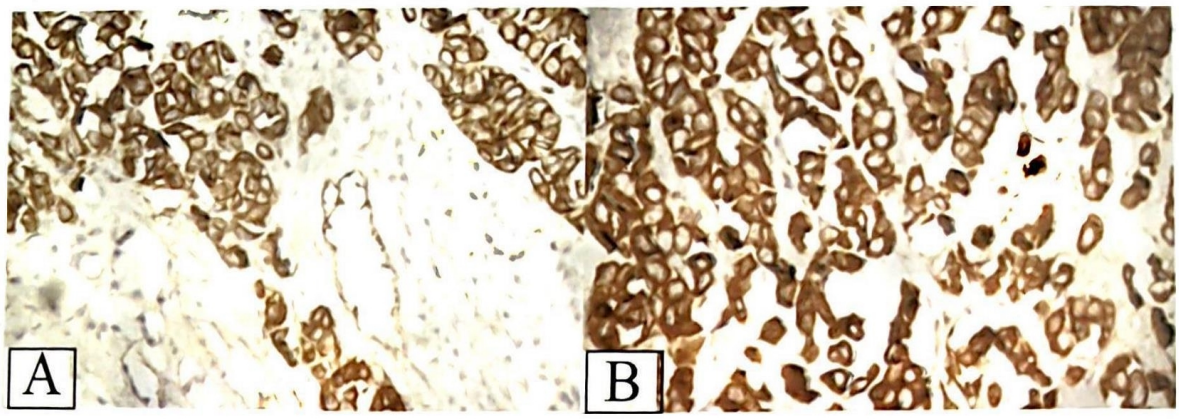

Fig. 2 (A) Cytokeratin stained section with the unstained lymphoid cells in the background in a Nasopharyngeal carcinoma. (B) Higher magnification of the stained in Fig. 2 (A)

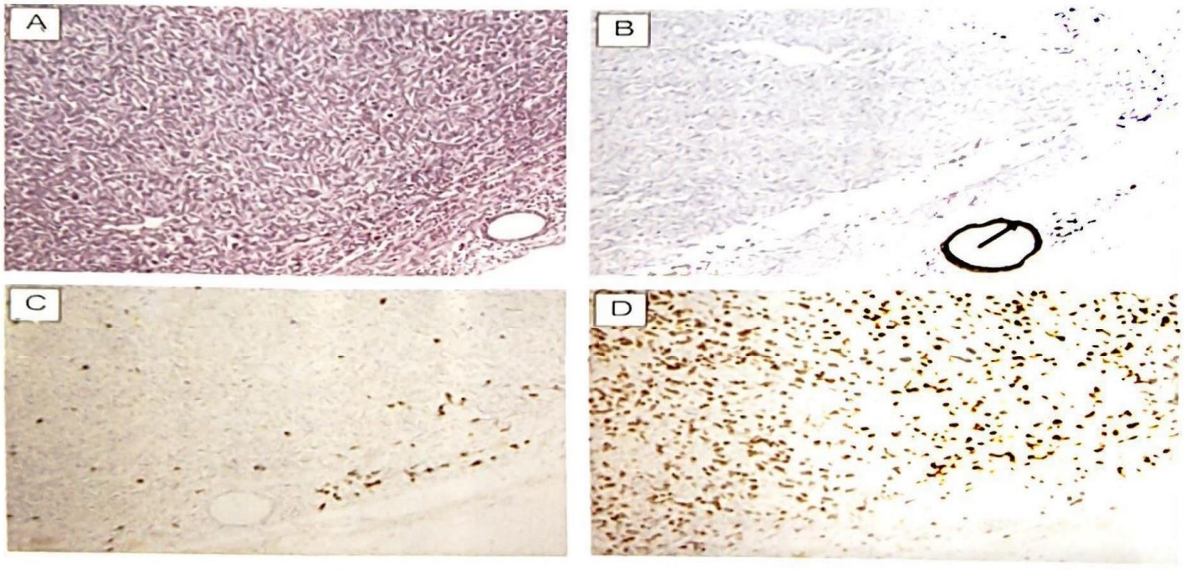

Fig.3: (A) H&E section of pleomorphic submucosal nasopharyngeal tumour with scattered large cells. (B) Cytokeratin immunostained section with negative staining tumour cells; small mucosa gland staining as internal positive control (arrow). (C) Scattered B-lymphocytes stained with CD20 but tumour cells are negative. (D) CD3immunostain shows predominance of CD3-positive atypical small and intermediate sized T lymphoid cells.

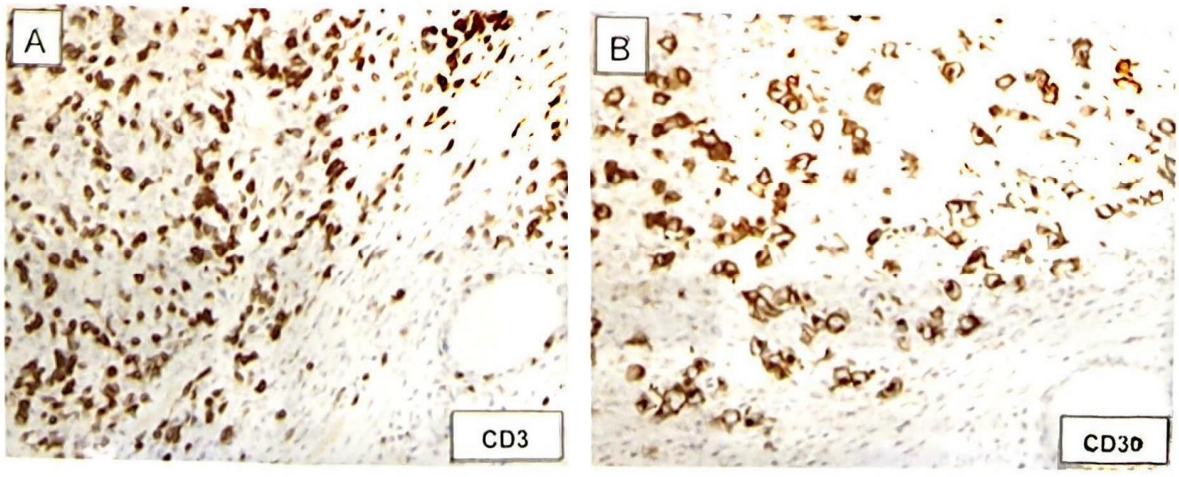

Fig. 4: (A) Higher magnification of CD3 immunostained section in Fig 3(D) with scattered unstained large cells amongst the atypical small and intermediate-sized T-lymphoid cells. (B) Additional CD30 immunostain was performed which shows scattered CD30 positive large cells, supporting the diagnosis of ALCL

treatment plan of the patients was 11.5% (6/52). Figure 1 illustrates the usefulness of CD20 in confirmation and subtyping NHL. Figure 2 illustrates a poorly differentiated nasopharyngeal carcinoma.

Figures 3 and 4 illustrate a case of anaplastic large cell lymphoma (ALCL) misdiagnosed as poorly differentiated tumour.

Discussion

Immunohistochemistry (IHC) is indispensable in modern practice of histopathology. In resource-limited setting, low income countries (LIC) and low middle income countries (LMIC) like Nigeria, IHC is not routinely carried out in practice. The reasons commonly adduced for this include cost consideration in setting up immunohistochemistry service, poor supply chain and issues of infrastructural challenges like electrical power to preserve the reagents used in the IHC procedure including the antibodies which are temperature sensitive.

This study illustrates that a good section of haemtoxylin and eosin can still be used to arrive at a good diagnosis in 88 % of nasopharyngeal tumours. However, there were 11.5% of cases that could be misdiagnosed based only on H&E sections alone. This will definitely have changed treatment plans for these patients. Therefore, IHC as addition helps in making precise diagnosis and offering a better prognosis.

The panel of IHC markers that is useful in arriving at a specific diagnosis of a NPT can essentially be applied only after a detailed examination of a good Haemtoxylin and eosin stained sections. Broad linage makers for epithelia (AE1/AE3) and lymphoid (CD3 and CD 20) can be applied as first line IHC markers. As illustrated from this study only 3 cases needed further makers to diagnose the ALCL (Figures 3 and 4) and the Desmin needed to diagnose the case of Rhabdomyosarcoma. Therefore 49 out of the 52 cases (94% of the cases) were diagnosed easily with an H&E stained sections and the 3 first line markers (AE1/AE3, CD3 and CD 20) used.

In a study by Sugimoto *et al*, 74 cases of nasopharyngeal malignant neoplasms were analyzed immunohistochemically and classified into 16 lymphomas and 58 carcinomas. Eight lymphomas were of T-cell origin and eight were of B-cell origin. [6] With immunophenotyping, the ratio of carcinomas to other malignant tumours in the nasopharynx from the study by Sugimoto et al was 78% which is similar to 75% we got in the current study. Similarly, the ratio of carcinomas to lymphomas was similar to the study by Sugimoto *et al.* [6]. However the ratio of B-Cell to T-cell was equal in that study but was about 4:1 in this study. This observation may be related to the high incidence of endemic type NPC which is mainly driven by Epstein Barr Virus (EBV) in the part of the world where that study was carried out. A high percentage T-cell type lymphomas in the head and neck region are Natural Killer (NK) cell/Tcell Non- Hodgkins lymphoma which are EBV driven as distinct from T cell lymphomas which are not EBV driven [3,6]. It is therefore important for this discrimination because of the markedly different prognosis in these two distinct tumour types [3,6].

Our current study was able to identify ALCL which are usually EBV driven based on a systematic and stepwise of use of IHC as illustrated in figures 3 and 4.

Futhermore, the importance of the use of IHC in discriminating between different types of lymphomas and NPC is critical when the only tissue submitted from a patient is a lymph node that is biopsied for definitive diagnosis. This is more critical as IHC can help discriminate from the possible differential diagnosis on H&E stained sections as illustrated in the case reported by Jabbour *et al* where metastatic NPC, a NHL and HL were considered [8]

The challenge of cost of treatment of a nasopharyngeal tumour which is daunting in resource limited setting therefore makes precise diagnosis of a specific tumour more important. This is because a misdiagnosis as illustrated from this study when no IHC was applied towards arriving at a diagnosis could lead to wrong treatment making the prognosis in patients worse [5].

It must be emphasized that a good clinical history and detailed physical examination of the patient - the traditional pathway of arriving at diagnosis are still very crucial in resource limited settings where cost of health care is still borne with out of pocket payment. Therefore, the Pathologist has to take note of this challenge when ordering IHC panel. This study reinforces and illustrates the need for judicious use of IHC markers in the practice of histopathology and this can be used to arrive at very specific diagnosis to enable precise treatment in about 94 % of cases of NPT.

In conclusion, a small panel of antibodies (cytokeratin, CD20, CD3) combined with good H&E section is useful and cost effective in distinguishing

undifferentiated nasopharyngeal carcinoma from lymphomas and for minimally subtyping NHL in limited resource areas and crucial for better patient management.

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