Research

Pathological features of nasopharyngeal carcinoma: A single-center study in Vietnam

Nguyen Cuong Pham¹, Thanh Xuan Nguyen², Nguyen Tuong Pham³, Thanh Chinh Phan¹, Hai Thanh Phan²

¹Department of Pathology, Hue Central Hospital, Vietnam
²Department of Abdominal Emergency and Pediatric Surgery, Hue Central Hospital, Vietnam
³Oncology Center, Hue Central Hospital, Vietnam

Abstract

Aims: We carried out this research to investigate the pathological characteristics of NPC to broaden the knowledge in diagnosing and treating this type of cancer.

Materials and Methods: This is a retrospective analysis of 106 patients diagnosed with nasopharyngeal carcinoma (NPC) at the Department of Pathology of Hue Central Hospital from January 2018 to June 2020. This study demonstrates the clinical data (age and sex) and histopathological findings of all patients. The histopathological diagnosis is based on the WHO classification in 1991.

Results: The data of 73 male patients and 33 female patients were analyzed and showed that the proportion of undifferentiated carcinoma was the most common type (70.8%), followed by well-differentiated keratinizing squamous cell carcinoma (19.8%) and other types (9.4%). In terms of immunohistochemistry, 21/21 of well-differentiated keratinizing squamous cell carcinoma cases expressed positive reactivity to CKAE1/3; meanwhile, only 5/75 of undifferentiated cases had the same result.

Discussion: Biopsy is essential for the identification of cancerous tissues. Therefore, the specimen should be obtained precisely and sufficiently in the area of injury. It is notable for avoiding collecting the necrosis areas and taking the edge of the lesion. We recommended a clinical consultation between otolaryngologists and oncologists to determine the area and the general characteristics of the lesion for making a more accurate diagnosis and prognosis. Immunohistochemistry can be helpful in confirming the type of nasopharyngeal carcinoma cases that are already stained with hematoxylin and eosin but are still challenging to diagnose.

Keywords: Nasopharyngeal Carcinoma; Hematoxylin-Eosin; Immunohistochemistry

(Rceived September 23, 2020; Accepted October 23, 2020)

Introduction

Nasopharyngeal carcinoma is a kind of tumor arising from the epithelium of the nasopharynx¹ ². Nasopharyngeal carcinoma is the most prevalent tumor among head and neck tumors and one of the ten malignant diseases that are diagnosed with the highest frequency in Viet Nam³. The proportion of nasopharyngeal carcinoma is growing, and the mean age of this disease has been falling³ ⁴. There has not been much detailed research about pathological and immunohistological characteristics of nasopharyngeal carcinoma (NPC) carried out in our hospital. We carried out this research to investigate the pathological characteristics of NPC to broaden the knowledge in diagnosing and treating this type of cancer.

Materials and Methods

Study population

This study was approved by the Board of Ethics in Biomedical Research at Hue Central Hospital, Vietnam under the approval number of 12-2019/NCKH-BVH. Informed consent was waived by the Board due to the retrospective study.

The clinical data (age and sex), histopathological and immunohistochemistry (IHC) results of 106 patients diagnosed with NPC were obtained at the Department of Pathology of Hue Central Hospital from January 2018 to June 2020. The histopathological diagnosis was made according to the World Health Organization (WHO) classification in 1991⁵ ⁶.
Immunohistochemistry procedure

Plenty of significant steps are performed in IHC procedure including proper handling of the specimen, appropriate fixation, paraffin block preparation, antigen retrieval, selection and preparation of antibody and reagents, incubation, washing, and counterstaining. Since the invention of automated IHC machines, they have played a key role in improving the reliability and reproducibility of IHC, notably in the clinical setting. However, the manual staining method still brings more flexibility, allowing scientists to optimize an antigen-antibody reaction and therefore achieve better results, especially in terms of the research setting. Both techniques have their own advantages and disadvantages. However, they all have the same basic principles and procedures. Table 1 demonstrates the standard procedure of IHC.

TBS-T, Tris-buffered saline and Tween 20; DAB, diaminobenzidine; PBS, phosphate buffered saline; DW, dextrose 5% in distilled water.

Table 1 Basic protocols of immunohistochemistry.

| Step                  | Protocol                                                                                       |
|-----------------------|------------------------------------------------------------------------------------------------|
| Fixation              | 10% Neutral buffered formalin for 24 hours at room temperature                                 |
|                       | Frozen section: cold acetone for 1 min                                                         |
| Embedding and sectioning | Paraffin embedding                                                                           |
|                       | Mostly 4 μm                                                                                    |
|                       | Frozen sections: between 4 μm and 6 μm in thickness                                              |
| Deparaffinization and hydration | 60°C hot plate                                                                |
| Antigen (or epitope) retrieval | Heat induced epitope retrieval is most widely used                                            |
| Blocking               | Normal serum of same species of a secondary antibody or premixed                               |
|                       | Varies from 30 min to overnight, from 4°C to room temperature                                  |
| Adding primary antibody | Dilution of antibodies with protein blocking solution or premixed Ab diluents                |
| Incubate              | Appropriate antibody selection and titration                                                     |
| Incubate              | 30–60 min, at room temperature                                                                |
| Wash (TBS-T)          | 3 × 5 min                                                                                     |
| Incubate              | 30–60 min, room temperature                                                                   |
| Wash                  | 3 × 5 min, TBS-T                                                                              |
| Add substrate         | 250 μL of 1% DAB, and 250 μL of 0.3% hydrogen peroxide to 5 mL of PBS, 1–3 minutes, room temperature |
| Wash                  | 3 × 5 min, DW                                                                                 |
| Counterstain          | Hematoxylin, 1 min                                                                            |

Table 2 Gender distribution

| Gender | Frequency (n) | Percentage (%) |
|--------|---------------|----------------|
| Male   | 73            | 68.9           |
| Female | 33            | 31.1           |
| Total  | 106           | 100            |

Table 3 Age groups

| Age groups | Frequency (n) | Percentage (%) |
|------------|---------------|----------------|
| < 30       | 13            | 12.3           |
| 30–<55     | 58            | 54.7           |
| ≥55        | 35            | 33.0           |
| Total      | 106           | 100            |

Table 4 The histopathological diagnosis, according to the WHO 1991 classification.

| WHO 1991 classification | Frequency (n) | Percentage (%) |
|-------------------------|---------------|----------------|
| Undifferentiated carcinoma | 75          | 70.8           |
| Well-differentiated carcinoma | 21          | 19.8           |
| Other carcinoma          | 10            | 9.4            |
| Total                   | 106           | 100            |

Table 5 Immunohistological characteristics

| WHO 1991 classification | CKAE1/3 positive | Percentage (%) |
|-------------------------|------------------|----------------|
| Undifferentiated carcinoma (n = 75) | 5                | 6.7            |
| Well-differentiated carcinoma (n = 21) | 21              | 100            |
| Other carcinoma (n = 10) | 0                | 0              |

Statistical analysis

Data were analyzed using SPSS 16.0 (IBM, Chicago, IL, USA). All categorical variables were expressed as frequency and percentage.

Results

The data of 106 patients diagnosed with NPC were analyzed. The rate of male patients was two-fold higher than that of female patients. The mean age of males was 43 years, and the median age of females was 41 years (range: 27–89 years). The largest proportion was observed in the group of patients aged 30–55 years (accounted for 54.7%). The characteristics of the patients are shown in Table 2 and Table 3.

Sixty-four (60.4%) tumors were raised on the lateral wall of the nasopharynx (particularly in the fossa of Rosenmüller). Most of the tumors were exophytic (accounted for 81.1%), with 10 (9.4%) cases described as ulcerated. Cervical lymph node metastases were also commonly seen.

The most common histological type was undifferentiated carcinoma (accounted for 70.8%, as seen in Fig. 1), followed by well-differentiated carcinoma (accounted for 19.8%, as seen in Fig. 2) and other carcinomas (9.4%) (Table 4). The immunohistological characteristics are shown in Table 5. The percentage of histological type positive for CKAE1/3 was 6.7% and 100% for the type of undifferentiated carcinoma and well-differentiated carcinoma, respectively.

Discussion

NPCs are primary tumors in adult patients with a peak prevalence between the ages of 40 and 60, although this type of cancer can also occur in children. The predominant male-to-female ratio is about 3/1, irrespec-
tive of geographic location\(^5\). In our study, male patients had a higher rate of NPC than females. The ages of 106 patients enrolled in this study ranged from 28 to 92 years. Those aged 30–55 years accounted for the highest percentage (54.7%).

In 1978, the WHO first classified NPCs into 3 histologic types: squamous cell carcinoma, nonkeratinizing carcinoma, and undifferentiated carcinoma\(^6\). In 1991, the WHO classification was modified into two major types, including squamous cell carcinoma and nonkeratinizing carcinoma. The latter consists of differentiated carcinoma and undifferentiated carcinoma. The current NPC classification includes nonkeratinizing carcinoma (undifferentiated carcinoma and differentiated carcinoma), keratinizing carcinoma, and basaloid squamous cell carcinoma. We prefer using the histological classification of nasopharyngeal carcinoma developed by WHO in 1991, because it is simple and has been proven to be suitable in the context of our hospital. According to these criteria, our study demonstrated that the undifferentiated NPC constituted the highest proportion (70.8%), while the percentage of the well-differentiated carcinomas and other types were 19.8%, 9.4%, respectively. In general, the portion of undifferentiated carcinoma was the highest\(^13,14\).

With regard to immunohistochemistry, CKAE1/3 marker was used in order to identify each type of carcinoma based on immunohistochemistry markers. Our study showed that all well-differentiated nasopharyngeal carcinoma cases were positive for CKAE1/3 marker, while the number of cases in nasopharyngeal carcinoma and other types were relatively low. This was consistent with theory, since the CKAE1/3 marker is a specific marker of epithelial cells. The negativity of undifferentiated and other types of cancer for CKAE1/3 marker can be explained in this case. As a result, staining immunohistochemistry with CKAE1/3 marker is the more favorable choice over the conventional hematoxylin-eosin staining method in order to distinguish well-differentiated squamous carcinoma from the remaining types of nasopharyngeal carcinoma.

Biopsy is essential for the identification of cancerous tissues. Therefore, the specimen should be obtained precisely and sufficiently in the area of injury. It is notable for avoiding collecting the necrosis areas and taking the edge of the lesion. We recommended a clinical consultation between otolaryngologists and oncologists to determine the area and the general characteristics of the lesion for making a more accurate diagnosis and prognosis.

Nasopharyngeal carcinoma is a fatal disease, but quite challenging to diagnose\(^15\). So far, screening for lesions is the only method to detect signs of cancer in general and nasopharyngeal carcinoma in particular. Patients are highly recommended to get a check-up by specialists every six months or whenever having symptoms of sore throat, to get the diagnosis and treatment early.

**Conclusion**

The percentage of males suffering from NPC was nearly double than that of females. The most common type of this disease was undifferentiated carcinoma. All cases of well-differentiated squamous carcinoma were positive for CKAE1/3 marker, while most of the undifferentiated cases were negative. Immunohistochemistry characteristics can be helpful in confirming the subtype of the nasopharyngeal carcinoma cases which are already stained with hematoxylin-eosin but still are challenging to diagnose.

**Acknowledgment:**

We would like to thank the Hue Central Hospital Publication Support Team that assisted the facilities to do this research.
Funding: None

Conflict of interest:
The authors declare no conflict of interest.

References
1) Brennan B. Nasopharyngeal carcinoma. Orphanet J Rare Dis. 2006. 1: 23.
2) Carle LN, Ko CC, Castle JT. Nasopharyngeal carcinoma. Head Neck Pathol. 2012. 6: 364–8.
3) Mahdavifar N, Ghoncheh M, Mohammadian-Hafshejani A, Khoresavi B, Salehiniya H. Epidemiology and Inequality in the Incidence and Mortality of Nasopharynx Cancer in Asia. Osong Public Health Res Perspect. 2016. 7: 360–372.
4) Adham M, Kurniawan AN, Muhtadi AI, Roezin A, Hermani B, Gondhowiardjo S, Tan IB, Middeldorp JM. Nasopharyngeal carcinoma in Indonesia: epidemiology, incidence, signs, and symptoms at presentation. Chin J Cancer. 2012. 31: 185–96.
5) Stelow EB, Wenig BM. Update From The 4th Edition of the World Health Organization Classification of Head and Neck Tumours: Nasopharynx. Head Neck Pathol. 2017. 11: 16–22.
6) Wei KR, Xu Y, Liu J, Zhang WJ, Liang ZH. Histopathological classification of nasopharyngeal carcinoma. Asian Pac J Cancer Prev. 2011. 12: 1141–7.
7) O’Hurley G, Sjostedt E, Rahman A, Li B, Kampf C, Ponten F, Gallagher WM, Lindskog C. Garbage in, garbage out: a critical evaluation of strategies used for validation of immunohistochemical biomarkers. Mol Oncol. 2014. 8: 783–98.
8) Gustavson MD, Bourke-Martin B, Reilly D, Cregger M, Williams C, Mayotte J, Zerkowski M, Tedeschi G, Pinard R, Christiansen J. Standardization of HER2 immunohistochemistry in breast cancer by automated quantitative analysis. Arch Pathol Lab Med. 2009. 133: 1413–9.
9) Kim SW, Roh J, Park CS. Immunohistochemistry for Pathologists: Protocols, Pitfalls, and Tips. J Pathol Transl Med. 2016. 50: 411–418.
10) Bray F, Haugen M, Moger TA, Tretli S, Aalen OO, Grotmol T. Age-incidence curves of nasopharyngeal carcinoma worldwide: bimodality in low-risk populations and aetiologic implications. Cancer Epidemiol Biomarkers Prev. 2008. 17: 2356–65.
11) Zhu Y, Song X, Li R, Quan H, Yan L. Assessment of Nasopharyngeal Cancer in Young Patients Aged <\= 30 Years. Front Oncol. 2019. 9: 1179.
12) Thompson LD. Update on nasopharyngeal carcinoma. Head Neck Pathol. 2007. 1: 81–6.
13) OuYang PY, Zhang LN, Lan XW, Xie C, Zhang WW, Wang QX, Su Z, Tang J, Xie FY. The significant survival advantage of female sex in nasopharyngeal carcinoma: a propensity-matched analysis. Br J Cancer. 2015. 112: 1554–61.
14) Xie SH, Yu IT, Tse LA, Mang OW, Yue L. Sex difference in the incidence of nasopharyngeal carcinoma in Hong Kong 1983-2008: suggestion of a potential protective role of oestrogen. Eur J Cancer. 2013. 49: 150–5.
15) Xu T, Tang J, Gu M, Liu L, Wei W, Yang H. Recurrent nasopharyngeal carcinoma: a clinical dilemma and challenge. Curr Oncol. 2013. 20: e406–19.