HEAD AND NECK CANCER

Epstein-Barr Virus Latent Membrane Protein-1 Expression in Nasopharyngeal Carcinoma

Valerie E. Salano, MBChB; Amos R. Mwakigonja, MD, MMed, PhD; Ashfaq Abdulshakoor, MD; Aveline A. Kahinga, MD, MMed; and Enica M. Richard, MD, MMed

abstract

PURPOSE Nasopharyngeal carcinoma (NPC), a malignant neoplasm of the epithelium covering the nasopharynx, is a rare disease in most parts of the world. Epstein-Barr virus (EBV), the most potent oncogenic virus, coupled with environmental and genetic factors has been identified to play a role in the development of NPC. An array of methods for detecting the virus do exist, from serologic detection of antibodies to DNA amplification. There is paucity of local data on the status of EBV infection in relation to NPC within the region, and this study attempts to shed more light on the subject.

METHODS This was a retrospective cross-sectional laboratory-based study on histologically confirmed, archived tissues from July 2015 to June 2019. Immunohistochemistry expression of latent membrane protein-1 (LMP-1) was used to detect EBV infection in the tissues.

RESULTS A total of 71 cases were enrolled in this study. The mean age was 47.87 years ± 16.84 years with a male-to-female ratio of 1.5:1. There was a unimodal distribution of EBV detection, with the peak (26.8%) at 36-45 years. About 45.1% of the 71 samples tested positive for LMP-1, all of which were nonkeratinizing carcinoma. Nonkeratinizing carcinoma was the most common histopathologic subtype (n = 67; 94.4%), with the majority (38 of 67; 56.7%) being undifferentiated and 29 of 67 (43.3%) differentiated. Keratinizing and basaloid subtypes had two cases each, representing 2.8%.

CONCLUSION A significant proportion of NPC, particularly nonkeratinizing histologic subtype, seems to show LMP-1 positivity by immunohistochemistry, which may be adopted in resource-constrained settings to detect EBV infection in these tissue biopsies.
expression of its receptor, which is also C3d receptor for the complement system. EBV has been linked to conditions such as infectious mononucleosis, oral hairy leucoplakia, and malignancies such as Burkitt’s lymphoma, B-cell lymphomas, and gastric carcinomas.12 Virtually all human beings become infected with EBV at some point in their lifetime. For those in developing countries and lower socioeconomic status, infection is acquired in early childhood and remains subclinical but for those in the higher socioeconomic status, it is acquired in adolescence, mostly presenting with infectious mononucleosis.11 Transmission is primarily through saliva.11,13

Three types of latency infections have been identified with EBV depending on the viral products expressed. For NPC, it is type 2 latency infection with the expression of EBV nuclear antigen-1, latent membrane protein-1 (LMP-1), latent membrane protein-2, and EBV early RNA.14 These products are what are assayed to detect presence or absence of EBV infection. Detection methods include in situ hybridization (ISH), polymerase chain reaction, and immunohistochemistry (IHC). Serologic assays are becoming a commonplace in testing for EBV but they have their drawbacks such as a wide variability and lack of specificity with false positives seen in autoimmune disorders unrelated to EBV.15

LMP-1 regulates a number of signaling pathways in the pathogenesis of NPC, mainly through the three functional domains of its C terminal activating regions 1, 2, and 3.16,17 Through the NF-κB signaling pathway, LMP-1 regulates cell proliferation, apoptosis, transformation, metastasis, and invasion ultimately causing immortalization through the p53 subunit. LMP-1 plays a crucial role in the chemokine ligand 5–mediated cancer angiogenesis by activating the phosphoinositide 3-kinase protein kinase B (PI3K AKT) and hypoxia-inducible factor 1α signaling pathways. The Janus tyrosine kinase and signal transducer and transcription activator pathway activated by LMP-1 mediates expression of programmed cell death protein ligand 1 aiding the cancer cells escape the body’s immune surveillance.17 LMP-1 makes more cells susceptible to the virus by secretion of matrix metalloproteases, which facilitate the degradation of the extracellular matrix. The stability of p53, a tumor suppressor gene, is interfered with by LMP-1, such that there is a lack of induced cell apoptosis in the cell cycle. LMP-1 also mimics the CD40 causing an overexpression of cancer stem cell markers leading to high metastatic features in NPC.18

METHODS
This was a retrospective, cross-sectional, laboratory-based study conducted at Muhimbili National Hospital, Tanzania. The study population was nasopharyngeal biopsies submitted to the histopathology unit to confirm diagnosis of NPC.

Inclusion Criteria
All histologically confirmed NPC biopsies submitted to the histopathology department from July 2015 to June 2019 were included.

Exclusion Criteria
Crushed tissues, unavailable tissue blocks, and cases lacking patient information were excluded.

Histopathologic Evaluation and Microscopy
This was done as previously described.19 Briefly, review of the diagnoses was done by the first author (V.E.S.) and a qualified, senior anatomical pathologist (A.R.M.) on hematoxylin and eosin sections and classification using the WHO system. EBV status was determined using immunohistochemical expression of LMP-1. Photomicrography was performed by the first author (V.E.S.) and the senior histopathologist (A.R.M.) using an Olympus (CX31RBSF Model) light microscope equipped with a digital camera (Olympus Corporation, Tokyo, Japan) as previously described.19,20

IHC Staining
This was done according to methods previously described.20 The paraffin-embedded tissue blocks were cut into 3 μm–thick sequential sections; the slides were dried, deparaffinized in xylene, and rehydrated through graded

CONTEXT
Key Objective
To determine a difference in expression of Epstein-Barr virus (EBV) infection among the different histologic types of nasopharyngeal carcinoma (NPC).

Knowledge Generated
There is a unimodal peak in age distribution among patients with NPC and EBV, latent membrane protein-1 detection, and only nondifferentiated keratinizing carcinoma, the most prevalent histologic type, tested positive for EBV.

Relevance
With the prospects of immunotherapy for NPC, adoption of routine EBV testing, even with immunohistochemistry in resource-constrained settings, would be an added advantage in identifying those who would benefit from this modality of treatment.
alcohol; tissue sections were circled with a hydrophobic pen (Dako pen); and endogenous peroxidase activity was blocked for 15 minutes using peroxidase blocking solution (Dako ready-to-use reagent) and antigen retrieved by pressure cooking for 10 minutes in citrate buffer (pH = 6). Slides were allowed to cool using tap water for another 10 minutes and then rinsed with wash buffer (phosphate-buffered saline [PBS]) for 5 minutes. Sections were incubated with mouse antibody anti-EBV-LMP (CS1-4) prediluted by Medaysis for 30 minutes, washed with wash buffer for 5 minutes and thereafter incubated with a universal Horseradish peroxidase for 30 minutes and washed with PBS twice each for 3 minutes. Sections were then incubated with 3,3′-diaminobenzidine (Dako DAB) for 10 minutes followed by rinsing in water for 2 minutes then counterstaining with hematoxylin for 17 dips and bluing for 5 minutes. Sections were dehydrated in the ascending grades of alcohol (ethanol 70%, ethanol 80%, ethanol 95%, and ethanol 100%) and then cleared in two changes of xylene for 5 minutes in each and covered using mounting medium by using Sakura Tissue Tek coverslipper. All these procedures were performed in the humidity chamber to make sure slides are not drying in between the steps. Both negative and positive controls were run alongside the tests.

IHC Evaluation

The slides were then reviewed under a light microscope. Brown granular cytoplasmic and membrane staining was interpreted as positive for EBV LMP-1, whereas bluish staining of the cytoplasm and membrane was interpreted as negative for EBV LMP-1. A positive control included a tissue known to have EBV infection, whereas for negative controls the test antibody was omitted and replaced by PBS.19

Furthermore, an internal negative control included parts of the section (stroma) that did not stain to the antibody.

Data Analysis

Data analysis was performed using Statistical Package for Social Sciences SPSS version 26. Results were presented in cross tabulations and figures. Association of EBV with age and sex as well as histologic classification of NPC was analyzed using Fisher’s exact f test and a \( P \) value of < .05 was considered significant.

Ethical Approval

Ethical clearance was sought from the Research and Publication Committee of the School of Medicine and from the Senate Research and Publications Committee of the Muhimbili University of Health and Allied Sciences. Administrative permission to conduct the study and waiver of consent, to be allowed access to the tissue bank, were granted from Muhimbili National Hospital as this was a retrospective study.

RESULTS

General Findings

A total of 71 cases were enrolled to this study. The patients’ age ranged from 16 to 82 years with a mean age of 47.87 years ± 16.84 years. The most frequent (26.8%) age group was 36-45 years, reflecting a unimodal peak. There were 43 males enrolled, with a male-to-female ratio of 1.5:1. Thirty-two (45.1%) of the 71 samples tested positive for LMP-1. There is a unimodal peak in EBV tissue immunostaining across the age groups, with the highest positivity rate seen among the 36-45 years group (26.8%) and the lowest being among the 26- to 35-year-olds (8.5%) although this was not statistically significant (\( P \) value of .111; Table 1).

Histopathologic and Immunohistologic Results

Nonkeratinizing carcinoma (Fig 1) was the most common (67 of 71; 94.4%) histopathologic subtype, majority (38 of 67; 56.7%) of which were undifferentiated and 29 of 67 (43.3%) differentiated, although this difference was not statistically significant (\( P \) value = .277). Furthermore, keratinizing squamous cell carcinoma and basoloid type each had two cases, representing 2.8%, respectively.

A significant number (32 of 71 [45.1%]) of the tissues tested positive for LMP-1 antibody by IHC and all these were nonkeratinizing carcinoma (Figs 2 and 3). On the contrary, keratinizing and basaloid carcinomas all tested negative for LMP-1, although this difference in EBV immunoreactivity across the three histologic types of NPC was not statistically significant, with a \( P \) value of .248 (Table 2).

Moreover, majority (41 of 71 [58.1%]) of the males tested negative for LMP-1, whereas the females showed equal distribution of the same, although this difference was also not statistically significant, with a \( P \) value of .333 (Table 1).

| Table 1. LMP-1 Immunoexpression by Age and Sex |
|-----------------------------------------------|
| Characteristic | Positive | Negative | Total | \( P \) |
|----------------|----------|----------|-------|-------|
| Age, years     |          |          |       |       |
| 16-25          | 7 (77.8) | 2 (22.2) | 9 (12.7)| .111 |
| 26-35          | 1 (16.7) | 5 (83.3) | 6 (8.5)|     |
| 36-45          | 11 (57.9)| 8 (42.1) | 19 (26.8)|     |
| 46-55          | 4 (30.8) | 9 (69.2) | 13 (18.3)|     |
| 56-65          | 4 (30.8) | 9 (69.2) | 13 (18.3)|     |
| > 65           | 5 (45.5) | 6 (54.5) | 11 (15.5)|     |
| Total          | 32 (45.1)| 39 (54.9)| 71 (100)|     |
| Sex            |          |          |       | .333 |
| Male           | 18 (41.9)| 25 (58.1)| 43 (60.6)|       |
| Female         | 14 (50)  | 14 (50)  | 28 (39.4)|       |
| Total          | 32 (45.1)| 39 (54.9)| 71 (100)|       |

Abbreviation: LMP-1, latent membrane protein-1.
DISCUSSION

Sub-Saharan Africa is considered to be a low endemic group for this particular malignancy with expected bimodal peak in age incidence and a male predominance.\textsuperscript{1,2,7,21} In this study, there was a unimodal peak at the 36-45 years age group similar to other studies within the continent. Edris et al\textsuperscript{22} in Sudan found the same unimodal peak at 41-60 years, so did other studies in Nigeria and Kenya with a peak at 40-49 and 31-40 years, respectively.\textsuperscript{8,23} Mremi et al\textsuperscript{24} in Tanzania found a peak at the 31-50 years age group. This similarity might be suggestive of comparable pathogenetic mechanisms and risk factors. However, a more extensive study on NPC in the region should be carried out to establish whether there is a change in age distribution pattern. In this study, there were more males who tested positive for EBV, a finding that is replicated in a study from Finland with 74\% positive cases being male. Edris et al in Sudan found 60\% of men tested positive for EBV, whereas Abdalazez et al found an equal number of positive and negative cases among men. EBV detection rates were high amongst females with NPC, with positivity rates of 82.7\%, 66.6\%, 77\% in Finland and the last two in Sudan respectively.\textsuperscript{22,25,26}

FIG 1. Hematoxylin and eosin-stained photomicrograph showing nonkeratinizing undifferentiated carcinoma, $\times 40$ magnification.

FIG 2. LMP-1 immunohistochemistry photomicrograph showing absence of staining of the nuclei and cytoplasm, which is defined as negative for LMP-1 immunoexpression, $\times 40$ magnification. LMP-1, latent membrane protein-1.
In our current study, the prevalence of EBV infection in NPC tissue sections was found to be 45% using LMP-1 IHC, although this was not statistically significant, which could partly be because of the small sample size. This compares well with a previous study from Sudan that showed almost similar findings at 41%. However, among studies that used a similar EBV detection method, a previous report from Finland showed a prevalence of 62%, whereas two in Nigeria showed 77.3% and 86%, respectively. These studies were in low endemic regions for NPC but the type of antibody used and the detection system applied might partly account for the differences seen in the rates. On the contrary, a previous report from Ghana showed a low 25% detection rate despite using EBV DNA polymerase chain reaction, a method considered to be superior to IHC. LMP-1 is expressed in all NPCs but the cells express variable levels of the protein within the tumor foci and this might explain the low detection rate of EBV observed in this study. Despite EBV early RNA ISH being considered the gold standard for EBV detection, other methods such as IHC are now being adapted in low-resource settings. This is especially true considering that a number of studies have noted that there is no significant difference among the various tests in detection rates. However, Fanaian et al compared the ISH and IHC methods of EBV detection and found that automated ISH had a sensitivity and specificity of 94% and 69%, respectively, whereas IHC had a sensitivity of 44% and specificity of 93%.

With advancements in genomic research, there are biomarkers such as p53R2, fibronectin, Mac-2 binding protein, plasminogen activator inhibitor-1, ceruloplasmin, and serum amyloid A that are being identified to be key in early diagnosis, response to treatment, and prognosis of NPC. It has been shown that expression of LMP-1 and Cripto-1, a member of the epidermal growth factor and a modulator in embryogenesis and oncogenesis, is positively related. LMP-1 can therefore be used as biomarker in tumor progression and metastasis. There is also promising use of immunotherapy in NPC with LMP-1–specific autologous cytotoxic lymphocytes–targeted therapy for recurrent disease. A vaccine based on LMP-1 has also shown tumor growth and metastasis suppression in mouse models; however, human trials are yet to be done.

The histopathologic classification of NPC has undergone several changes, with the current classification by WHO identifying three distinct types: nonkeratinizing, keratinizing, and basaloid carcinoma. Nonkeratinizing carcinoma was the most prevalent type of NPC in this study, at 94.4%, which seems to be similar to the global picture across all risk strata. This histologic type is further classified into undifferentiated and differentiated with the former accounting for majority of the cases at 36%-95%, but this classification has no clinical significance and there might be cases wherein the two coexist in one tumor. In our current study of the various histologic types, EBV was apparently only detected in nonkeratinizing

**TABLE 2.** Histologic Type of Nasopharyngeal Carcinoma by EBV Status

| Histology     | EBV Status, No. (%) | Total, No. (%) | P     |
|---------------|---------------------|----------------|-------|
|               | Positive | Negative |               |       |
| Keratinizing  | 0 (0)     | 2 (100)  | 2 (2.8)       | .248  |
| Nonkeratinizing | 32 (47.8) | 35 (52.2) | 67 (94.4)     |       |
| Basaloid      | 0 (0)     | 2 (100)  | 2 (2.8)       |       |
| Total         | 32 (45.1) | 39 (54.9) | 71 (100)      |       |
carcinoma tissues, a finding that seems comparable with reports from elsewhere.

In conclusion, this current study determined the association of EBV infection in NPCs at Muhimbili National Hospital, Tanzania, using the LMP-1 IHC and found that apparently about half (45%) of the cases tested positive, reflecting almost one in every two patients, although this appears to be lower than in majority of previous reports from elsewhere. The age and sex distribution of NPC appeared similar to other studies from Africa showing a unimodal peak, but this is contrary to previous studies that indicate a bimodal peak with a majority of those tested positive for EBV being males and younger than 45 years. The most common histologic type of NPC appeared to be nonkeratinizing carcinoma, and only this type seemed to be associated with LMP-1 tissue positivity.

With the prospects of immunotherapy for NPC, adoption of routine EBV testing would be an added advantage in identifying those who would benefit from this modality of treatment.

AFFILIATIONS
1Department of Otorhinolaryngology, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania
2Department of Pathology, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania

CORRESPONDING AUTHOR
Valerie E. Salano, MBChB, Muhimbili University of Health and Allied Sciences, P.O. Box 65001 Dar es Salaam, Tanzania; e-mail: vasalano@gmail.com.

AUTHOR CONTRIBUTIONS
Conception and design: All authors
Provision of study materials or patients: Valerie E. Salano, Amos R. Mwakigonja
Collection and assembly of data: Valerie E. Salano, Amos R. Mwakigonja
Data analysis and interpretation: Valerie E. Salano, Amos R. Mwakigonja, Ashfaq Abdulshakoor, Aveline A. Kahinga

REFERENCES
1. Leon B, John E, Peter R, et al (eds): World Health Organization Classification of Tumors Pathology & Genetics Head and Neck Tumors. Lyon, France, IARC Press, 2005, pp 81-97
2. Stalow EB, Wening BM: Update from the 4th edition of the World Health Organization classification of head and neck tumours: Nasopharynx. Head Neck Pathol 11:16-22, 2017
3. Yua F, Lu Y, K Tay J, et al: Establishment of EBV latency in nasopharyngeal tumor epithelial cells by in vivo cell-mediated transfer infection. Otorhinolaryngol Neck Surg. 3:1-7, 2018
4. Lao TD, Anh NTH, Nguyen DH: Pattern of EBNA-1, EBNA-2, LMP-1 and LMP-2 in nasopharyngeal carcinoma in Vietnamese patients. International Conference on the Development of Biomedical Engineering in Vietnam, Ho Chi Minh City, Vietnam, June 2016 (pp 243-247)
5. Mwansasu C, Liyombo E, Moshi N: Pattern of head and neck cancers among patients attending Muhimbili National Hospital Tanzania. Tanzan J Health Res 17, 2015. https://doi.org/10.4314/thrb.v17i1
6. Umar B, Ahmed R: Nasopharyngeal carcinoma, an analysis of histological subtypes and their association with EBV, a study of 100 cases of Pakistani population. Asian J Med Sci 5:16-20, 2014
7. Salehiniya H, Mohammadian M, Mohammadian-Hafshejani A, et al: Nasopharyngeal cancer in the world: Incidence, mortality and risk factors. World Cancer Res J 5:e1046, 2018
8. Muchiri M: Demographic study of nasopharyngeal carcinoma in a hospital setting. East Afr Med J 2008;85:181-186
9. Flint PW, Haughey BH, Lund VJ, et al: Head and Neck Surgery, in: Cummings Otolaryngology (ed 6). Saunders, Philadelphia, PA, 2015, pp 1420-1431
10. Chamberlin M, McGrath J, Washek F: EBV DNA in biopsies of Burkitt tumours and anaplastic carcinomas of the nasopharynx. Nature. 228:227-231, 1970
11. Raab-Traub N: Epstein–Barr virus in the pathogenesis of NPC. Semin Cancer Biol 12:431-441, 2002
12. Asante DB, Asnain RH, Adjei AA, et al: Detection of human papillomavirus genotypes and Epstein-Barr virus in nasopharyngeal carcinomas at the Korle Bu Teaching Hospital, Ghana. Sci World J 2017:2721367, 2017
13. Jha HC, Pei Y, Robertson ES, et al: Epstein–Barr virus: Diseases linked to infection and transformation. Front Microbiol 2016;7:1-16
14. Borthakur P, Kataki K, Keppen C, et al: Expression of Epstein Barr virus encoded EBNA1 and LMP1 oncoproteins in nasopharyngeal carcinomas from northeast India. Asian Pac J Cancer Prev 17:3411-3416, 2016
15. Smatti MK, Al-Sadeq DW, Ali NH, et al: Epstein–Barr virus epidemiology, serology, and genetic variability of LMP-1 oncogene among healthy population: An update. Front Oncol 8:211, 2018
16. Fernandez Q, Merhi M, Raza A, et al: Role of Epstein–Barr virus in the pathogenesis of head and neck cancers and its potential as an immunotherapeutic target. Front Oncol 8:257, 2018
17. Luo Y, Liu Y, Wang C, et al: Signaling pathways of EBV-induced oncogenesis. Cancer Cell Int 21:93, 2021
18. Cheerathodi MR, Meckes DG: The Epstein–Barr virus LMP1 interactome: Biological implications and therapeutic targets. Future Virol 13:863-887, 2018

AUTHORS’ DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST
The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated unless otherwise noted. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO’s conflict of interest policy, please refer to www.asco.org/rcw or ascopubs.org/go/authors/author-center.

Open Payments is a public database containing information reported by companies about payments made to US-licensed physicians (Open Payments).

No potential conflicts of interest were reported.

Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

No potential conflicts of interest were reported.
19. Mremi A, Yahaya J, Abraham Z, et al: The role of a minimum immunohistochemical antibody panel in confirming undifferentiated nasopharyngeal carcinoma: A cross-sectional study at the Muhimbili National Hospital, Dar-es-Salaam, Tanzania. Niger Med J 60:279, 2019
20. Kaaya EE, Castaños-Velez E, Ekman M, et al: AIDS and non AIDS-related malignant lymphoma in Tanzania. Afr Health Sci 6:69-75, 2006
21. Nasopharyngeal Carcinoma, 2014 Review of Cancer Medicines on the WHO List of Essential Medicines. Geneva, Switzerland, Union for International Cancer Control, 2014, pp 1-9
22. Edris A, Mohamed MA, Mohamed NS, et al: Molecular detection of Epstein-Barr virus in nasopharyngeal carcinoma among Sudanese population. Infect Agent Cancer 11:60, 2016
23. Yates S, Illyasu Y, Ahmed S, et al: Nasopharyngeal carcinoma at the Ahmadu Bello University Teaching Hospital, Zaria: A 22-year histopathological review (1992–2013). Arch Med Surg 3:24, 2018
24. Mremi A: The Histopathological Diagnosis of Nasopharyngeal Carcinoma at Muhimbili National Hospital. Muhimbili University of Health and Allied Sciences, Tanzania, 2015
25. Adam A, Abdullah N, El Hassan L, et al: Detection of Epstein-Barr Virus in Nasopharyngeal Carcinoma in Sudanese by in Situ Hybridization. J Cancer Ther 5:517-522, 2014
26. Ruuskanen M, Irjala H, Minn H, et al: Epstein-Barr virus and human papillomaviruses as favorable prognostic factors in nasopharyngeal carcinoma: A nationwide study in Finland. Head Neck 41:349-357, 2019
27. Yates SM, Illyasu Y, Ahmed SA, et al: Immunohistochemical expression of Epstein-Barr virus latent membrane protein-1 in nasopharyngeal carcinoma. Ann Trop Pathol 9:99-103, 2017
28. Omoseebi O, Akinde OR, Obadofin OO, et al: Association of Epstein–Barr virus (EBV) with malignancy of the nasopharynx in Lagos, Nigeria. Ann Trop Pathol 8:29-33, 2018
29. Shair KHY, Reddy A, Cooper VS: New insights from elucidating the role of LMP1 in nasopharyngeal carcinoma. Cancers (Basel) 10:1-22, 2018
30. Qi ZL, Hon XQ, Hu J, et al: Comparison of three methods for the detection of Epstein-Barr virus in Hodgkin’s lymphoma in paraffin-embedded tissues. Mol Med Rep 7:89-92, 2013
31. Adam AAM, Abdullah NE, El Hassan LAM, et al: Detection of Epstein-Barr virus in nasopharyngeal carcinoma in Sudanese by in situ hybridization. J Cancer Ther 5:517, 2014
32. Kurniawan AN, Kodariah R, Elisabeth -M, et al: Evaluation of EBV-LMP1 as prognostic indicator of nasopharyngeal carcinoma in Indonesian patients. Med J Indonesia 11:81, 2002
33. Fanaian NK, Cohen C, Waldrop S, et al: Epstein-Barr virus (EBV)-encoded RNA: Automated in-situ hybridization (ISH) compared with manual ISH and immunohistochemistry for detection of EBV in pediatric lymphoproliferative disorders. Pediatr Dev Pathol 12:195-199, 2009
34. Chen J, Li S, Xiao Y, et al: p53R2 as a novel prognostic biomarker in nasopharyngeal carcinoma. BMC Cancer 17:846, 2017
35. Zhang S-Q, Pan S-M, Liang S-X, et al: Research status and prospects of biomarkers for nasopharyngeal carcinoma in the era of high-throughput omics (Review). Int J Oncol 58:9, 2021
36. Ye Q, Li J, Wang X, et al: In vivo and in vitro study of co-expression of LMP1 and Cripto-1 in nasopharyngeal carcinoma. Braz J Otorhinolaryngol 86:617-625, 2020
37. Teow S-Y, Yap H-Y, Peh S-C: Epstein-Barr virus as a promising immunotherapeutic target for nasopharyngeal carcinoma treatment. J Pathog 2017:1-10, 2017
38. Chang ET, Adami HO: The enigmatic epidemiology of nasopharyngeal carcinoma. Cancer Epidemiol Biomarkers Prev 15:1765-1777, 2006