Potential significance of epstein barr virus-positive mucosa in patients with nasopharyngeal carcinoma

Saleh AlDhahri1*, Raneem Alshareef1, Hanadi Fatani2 and Aziz A. Chentoufi1

1 Otolaryngology Head and Neck Unit; King Fahad Medical City, King Saud University P.O. Box 59046, Riyadh, KSA
2 Pathology and Clinical Laboratory Medicine, Anatomic Pathology Department, King Fahad Medical City, P.O. Box 59046, Riyadh, KSA
3 Pathology and Clinical Laboratory Medicine, Immunology Department, King Fahad Medical City, P.O. Box 59046, Riyadh, KSA

Abstract

Introduction: Nasopharyngeal carcinoma (NPC) is one of the most Virus-associated malignancies. In Saudi Arabia, it represent about one-third of head and neck mucosal malignancies. Early detection and treatment of nasopharyngeal carcinoma are the key factor in the disease outcome. The objective of this study was to determine the possibility of detecting latent Epstein-Barr virus infection in histologically normal mucosa of patients with nasopharyngeal carcinoma and its potential clinical implications in predicting disease’s recurrence or persistence.

Materials and methods: All patients diagnosed with nasopharyngeal carcinoma from 2006 to 2012 at King Fahad Medical City (KFMC) clinics were retrospectively reviewed for EBV-mRNA expression in both healthy and malignant tissues using insitu Hybridization assay. The correlation between Epstein-Barr virus detection and variables of our cohort were evaluated.

Results: Sixty-one patients were enrolled in the study. The majorities of patients were male (80.3%), aged 40-60 years (60.6%) and were classified with WHO type III NPC (85%). While 98.4% of specimens with malignant cells had positive Epstein-Barr virus-in situ hybridization, only 6.6% were also positive for latent EBV infection in normal mucosal cells. All normal epithelium cells that were positive for EBV have positive Epstein-Barr virus-in situ hybridization in tumors. Only one case had Epstein-Barr virus-in situ hybridization negative in both normal and malignant cells.

Conclusion: The detection of EBV in nasopharyngeal mucosa in the absence of a tumor can be strong indication for carcinoma and could be used in the detection of early and recurrent disease.

Introduction

Nasopharyngeal Carcinoma (NPC) is a highly malignant tumor [1] that is considered the most common primary neoplastic tumor of the nasopharynx [2]. Clinically, NPC is characterized by local invasion and early distant metastasis [3]. NPC tumor-genesis is linked to multiple factors including environmental, behavioral, genetic alterations and infectious (Epstein-Barr Virus) factors [4]. NPC is rare in most parts of the world, with an incidence of <1 per 100,000 annually [5]. In Saudi Arabia, it ranks the first (35%) among all head and neck cancers and 17th (2.9%) among all cancers, with male predominance (2:1) with crude incidence for male 2.1/100,000 annually. [6], [7].

Patients with NPC most commonly present with a neck mass, and then to a lesser extent with nasal symptoms such as nasal obstruction, chronic sinusitis, or epistaxis [8]. With the progression of disease otological complaints such as tinnitus, hearing loss or fluid in the middle ear become evident [8]. The presence of neurological symptoms such as headache or cranial nerves palsy usually indicates advanced local disease with skull base or intracranial extension [8]. Although the diagnosis of NPC can readily be made, it can be challenging in most patients due to non-specific symptoms and/or inadequately diagnostic materials [9,10]. Treatment of NPC includes combined radiochemotherapy or radiotherapy alone that usually induces complete remission, especially in the early stages of the disease. Despite this high rate of initial local control [11], it may be difficult to diagnose persistent or recurrent disease postradiotherapy and postchemoradiotherapy, because of necrosis and treatment effect, especially when the recurring tumor is small tissue in the nasopharynx, skull base or a small lymph node in the neck.

The detection of clonal EBV genome in precancerous lesions indicates that EBV latent infection is an early event in the tumorigenesis of NPC [12]. Other studies have shown the value of increasing EBV viral genome plasma load as an indicator of recurrent tumor [13]. Many techniques have been used to detect EBV latent infection, but in situ hybridization for EBV-encoded

RNA (EBER-ISH or EBV-ISH) is considered the gold standard [14] and the most sensitive and practical method for detecting EBV [15]. The presence of EBV-encoded RNA in benign mucosa has not been thoroughly investigated. This study attempted to compare EBV detection by using in-situ hybridization (ISH) in both normal and malignant mucosa of patients with confirmed primary nasopharyngeal carcinoma [16].

Correspondence to: Dr. Saleh AlDhahri, Otolaryngology Head and Neck Unit; King Fahad Medical City Medical City, King Saud University, P.O. Box 59046, Riyadh; KSA, Tel: +966-4786100 ext:1419 & 1420; E-mail: saldhahri@ksu.edu.sa

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Materials and methods

Cohort characteristics

All patients diagnosed with NPC and having archived materials from 2006 to 2012 were included for this study. Patients who received any chemotherapy or radiotherapy to the head and neck regions before nasopharyngeal biopsies and those who did not have available pathological blocks were excluded from the study. The EBV genome expressions were scored using ISH in both normal and malignant cells of the nasopharynx. Pretreatment assessment including complete history and physical examination with endoscopy, and routine blood tests and imaging information were extracted from patients’ medical records. Disease staging and radiological findings and follow-up were collected from patients’ medical records. A senior head and neck pathologist reviewed all pathology slides.

In Situ hybridization technique

ISH were performed for EBV in formalin fixed paraffin-embedded tissue (FFPET); ISH for mRNA negative and positive probes was also performed. Samples were fixed in 10% neutral formalin and two sections of 5-μm thickness were made. One was stained with haematoxylin and eosin for histopathological examination. The tumors were classified based on World Health Organization nasopharyngeal classification.

The other section was used for ISH. ISH was performed with EBV peptide nucleic acid (PNA) probe/fluorescein (Dako, Denmark) and detected using a PNA ISH detection kit. Hybridization lasted 1.5 h at 55°C and was visualized by alkaline phosphatase (AP)-conjugated antifluorescein antibodies. Nitro blue tetrazolium (NBT)/bromochloro indoyl phosphate (Dako, Denmark) was used as a substrate for AP. To test the sensitivity and specificity of ISH, both negative and positive controls supplied by the manufacturer were used. A case was considered positive if the nucleus of a tumor cell stained dark blue or black.

Results

Of the 119 patients treated at the Otorhinolaryngology Head and Neck Surgery Clinics in KFMC between 2006 and 2012, only 61 patients were eligible for this study. The remaining cases were excluded because of the lack of pathology archived materials and/or prior treatment. Table 1 shows the study cohort characteristics. The majority of patients (60.6%) were in the age range of 40–60 year with a median age of 50 years. The male: female ratio of patients was 4:1 (80.3%:19.7%). Fifty-six patients (60.6%) were in the age range of 40–60 year with a median age of 50 years. The male: female ratio of patients was 4:1 (80.3%:19.7%).

Table 1. Characteristics of patients at presentation with nasopharyngeal carcinoma.

| Characteristics | No. | % |
|-----------------|-----|---|
| Age (median=50 years) |     |   |
| ≤20              | 2   | 3.3|
| 20-40            | 10  | 16.4|
| 40-60            | 37  | 60.6|
| ≥60              | 12  | 19.7|
| Gender           |     |   |
| Male             | 49  | 80.3|
| Female           | 12  | 19.7|
| WHO type         |     |   |
| I                | 2   | 3.3|
| II               | 7   | 11.5|
| III              | 52  | 85.2|

WHO—World Health Organization

Table 2. EBV-ISH Results.

| EBV-ISH Status | Normal epithelium | Cancerous epithelium |
|----------------|-------------------|----------------------|
| Positive (%)   | 4 (6.6%)          | 60 (98.4%)           |
| Negative (%)   | 57 (93.4%)        | 1 (1.6%)             |

Discussion

NPC is a common and serious disease in Saudi Arabia, as it accounts for about 30-40 % of all head and neck cancers. With variation and development of different modalities for the detection of EBV in NPC, ISH is a unique method because it can confirm the existence of the EBV genome in the nuclei of malignant cells, regardless of infected lymphocytes in the peripheral blood.

The presence of a viral genome in the normal nasopharyngeal epithelium in a subset of the cohort of this study is a novel finding, and positive EBV results in the absence of tumor cells may suggest recurrent disease in a previously treated patient and thus require the performance of additional biopsies. A histologically normal looking mucosa may require EBV testing and if it is positive, then the possibility of a malignant tumor may be high and a repeat biopsy is
results may be limited because we excluded patients without paraffin block specimens; despite this, the majority of the WHO type carcinoma in our patients was WHO type III. However, there was no significant difference in EBV-ISH positivity in relation to histological types.

Conclusion

EBV is found at a high frequency among Saudi patients with nasopharyngeal carcinoma, which supports its role in the pathogenesis of the tumor. EBV-ISH in benign nasopharyngeal epithelium may be found in small number of patients and this finding may have a potential to be useful as a histologic tumor marker for diagnosis and screening for post-treatment recurrence. However more studies with larger number of patients (including treatment failure) are needed to come up with a firm conclusion in this regards.

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Table 3. Follow-up and treatment of patients with positive EBV in the normal mucosa.

| Patient | TNM staging | Last F/U | Status | Treatment modality |
|---------|-------------|----------|--------|-------------------|
| 35 y old male | T4N2M1 | December/2013 | Died with the disease at 26 months post diagnosis | Cisplatin based neoadjuvant followed by Concurrent chemoradiation |
| 24 y old male | T3N1M0 | July/2012 (lost F/U) | Free of disease at last F/U at 27 months post treatment | Cisplatin based neoadjuvant followed by Concurrent chemoradiation |
| 39 y old male | T2N2M0 | December/2014 | Free of disease 48 month post treatment | Cisplatin based neoadjuvant followed by concurrent chemoradiation |
| 52 y old male | T3N1M0 | February/2015 | Free of disease 26 months post treatment | Cisplatin-based concurrent chemoradiation |