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Case Report

Infectious mononucleosis mimicking malignant T-cell lymphoma in the nasopharynx: a case report and review of the literature

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Abstract: Infectious mononucleosis (IM) is Epstein-Barr virus-associated and self-limited lymphoproliferative disorder. The histopathologic features of the nasopharynx in IM are rarely described. In this report, we described a patient of IM with atypical T-cell proliferation in the nasopharynx. In-situ hybridization for EBV-encoded RNA with immunostaining against CD20 was used for evaluation of EBV infection. The histopathologic features of IM could mimic malignant T-cell lymphoma. It should be differentiate reactive T-cell lymphoproliferation from malignant lymphoma in the nasopharynx.

Keywords: Infectious mononucleosis, IM, EBV, lymphoma

Introduction

Epstein-Barr virus (EBV), also called human herpesvirus 4, is a ubiquitous DNA virus belonging to the γ subfamily of herpesviruses. The virus infects more than 90% of individuals during the first two decades of life throughout the world [1]. Primary EBV infection is usually asymptomatic and resolves spontaneously. Occasionally, EBV infection may cause infectious mononucleosis (IM) characterized by fever, pharyngitis and general lymphadenopathy. The histopathologic features of the lymph nodes have been well-documented [2]. However, it is rarely described in the nasopharynx. In this report, we described a patient of IM with atypical T-cell proliferation in the nasopharynx. It mimicked malignant lymphoma microscopically. Previously reported cases were reviewed.

Case report

The patient was a 32-year-old Taiwanese woman who suffered from intermittent sore throat, odynophagia and general malaise for one month. On nasopharyngoscopic examination, bilateral tonsils were enlarged with a bulging mass over the nasopharynx (**Figure 1A**). Head and neck computerized tomography scan showed enlargement of bilateral palatine tonsils and neck lymph nodes with prominent adenoids occupying the nasopharynx (**Figure 1B**). Complete blood count showed normal leukocyte count (9.4×10^9/L) with presence of atypical lymphocytes (3%). Serological tests for EBV revealed high IgM (VCA-IgM: 72.9 U/ml, normal range: 0-36.0 U/ml) and IgG (VCA-IgG: 202.0 U/ml, normal range: 0-18.0 U/ml) antibodies against viral capsid antigen (VCA). Antibody to EBV-induced early antigen (EBEA-Ab) rather than EBV-associated nuclear antigen (EBNA) was positive (1:20). The clinical presentation and serological findings were suggestive of IM. Biopsy for the nasopharyngeal mass was performed.

The patient made a recovery from her illness completely one month later by supportive care and antibiotic treatment. After one-year follow-up, the patient has been well without evidence of malignancy.

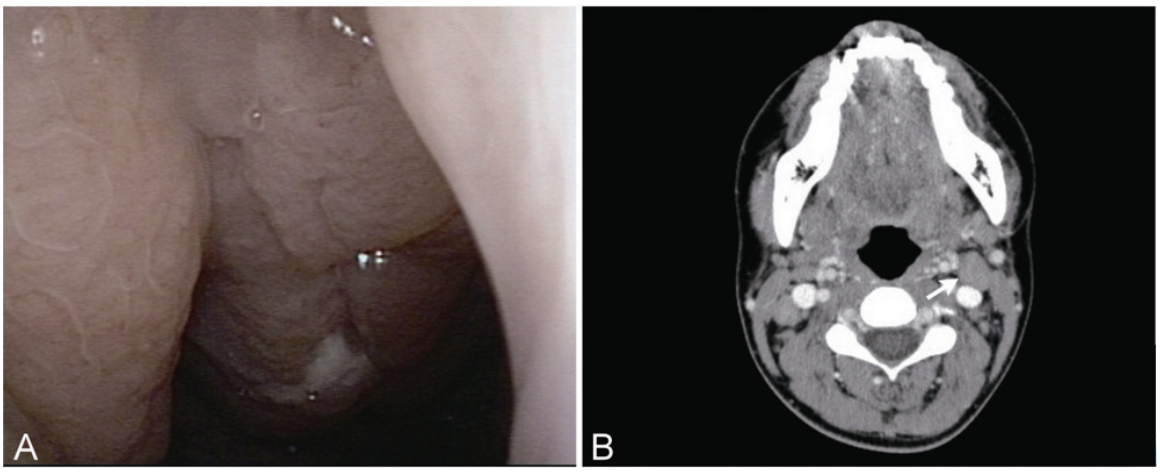

Figure 1. A. The nasopharyngoscopy showed a bulging mass in the nasopharynx coated with pus. B. Head and neck CT study image showed increased number and size of the bilateral neck and submandibular lymph nodes (arrow).

Table 1. Antibodies Used for Immunohistochemical Staining

Antibody	Source	Clone	Dilution
CD2	Neomarkers	AB75	1:60
CD3	Neomarkers	SP7	1:300
CD4	Novocastra	4B12	1:80
CD5	Neomarkers	4C7	1:50
CD7	Novocastra	LP15	1:100
CD8	Neomarkers	SB16	1:100
CD20	DAKO	L26	1:400
CD56	Novocastra	CD564	1:100
Ki-67	DAKO	MIB-1	1:150

Materials and methods

Immunohistochemistry

The specimen was fixed in 10% formalin solution and embedded in the paraffin block. Sections were cut and stained with hematoxylin and eosin for light microscopy. Immunohistochemical (IHC) stains were performed by using standard reagents and techniques on an i6000 Automated Staining System (BioGenex, San Ramon, CA). Briefly, the sections were deparaffinized in xylene and hydrated in a graded series of alcohol. Endogenous peroxidase was blocked with 3% hydrogen peroxide. The sections were then pretreated by a pressure cooker in citric acid buffer (PH 6.0) for seven minutes and incubated with primary antibodies (**Table 1**) followed by PicTure™-Plus kits (ZYMED®: 2nd Generation Polymer Detection System, San Francisco, CA). Positive and negative controls were done according to manufacture's instruction. It was defined as positive when at least 20% of the malignant cells in the slides revealed positive staining.

EBER ISH and CD20 double stain

The paraffin-embedded sections from nasopharyngeal biopsy were used for EBER ISH and CD20 double stain. The sections of EBV-infected nasopharyngeal tissue were deparaffinized and pretreated with proteinase K for 10 min and incubated with fluorescein-conjugated EBER DNA probe (Leica Biosystems, Newcastle, UK) at 37°C for 2 h. The sections were rinsed in water and incubated with horseradish peroxidase-conjugated anti-fluorescein antibody for 15 min before adding fresh DAB color substrate (3,3-Disminobenzidine) to produce an alcohol-insoluble brown intranuclear stain in EBV-positive cells. Immediately following the EBER

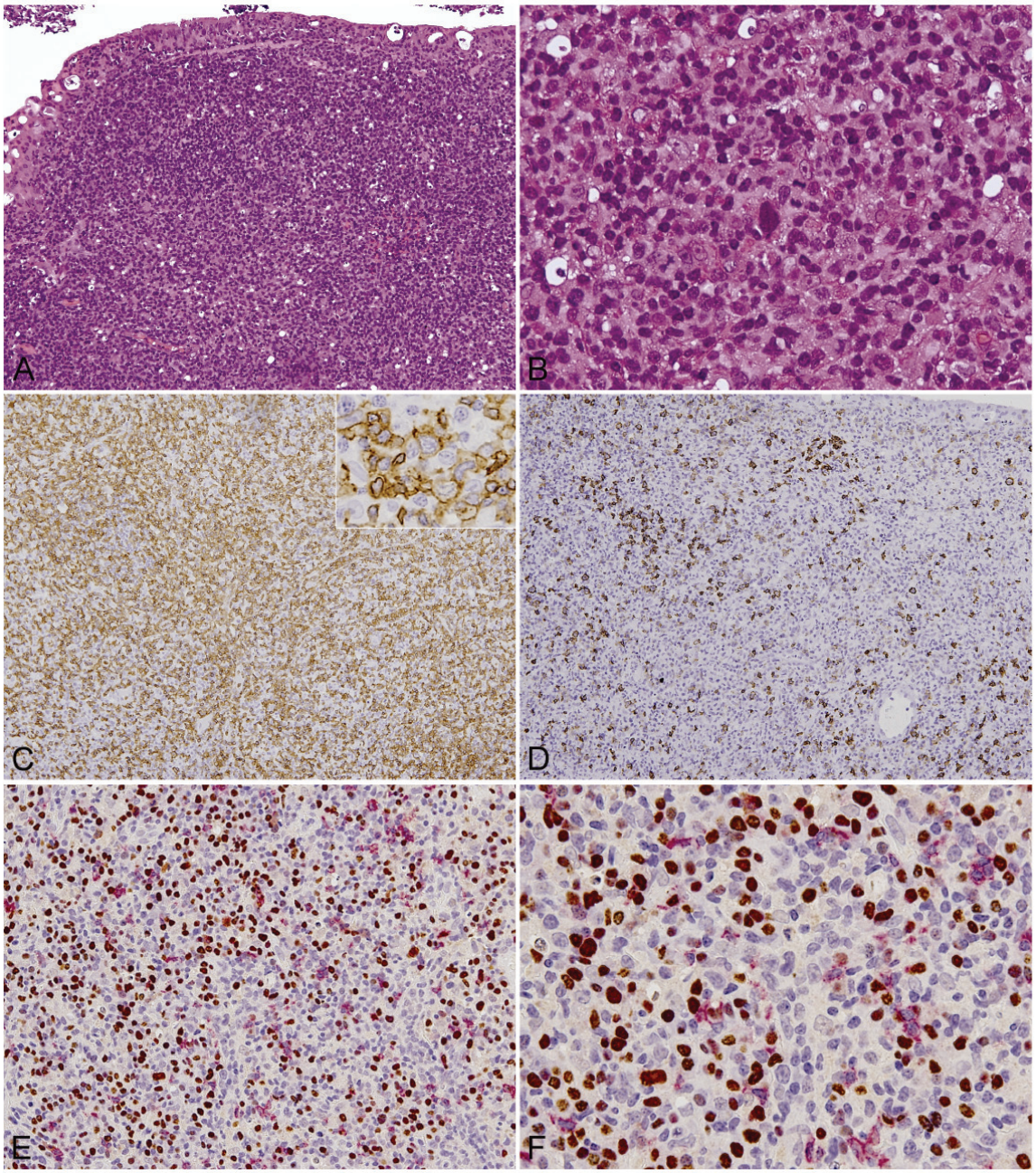

Figure 2. A. On hematoxylin and eosin-stained sections, the nasopharyngeal tissue was effaced by atypical lymphocytes (×100). B. Most of them had small to medium-sized nuclei, and there were also some immunoblast-like cells with cytologic atypia and brisk mitotic activity (×400). C and D. Atypical lymphocytes were immunostained positive for CD3 (C, ×100; inset, ×400), rather than for CD20 (D, ×100). E. By using double staining of combined in-situ hybridization for EBV-encoded RNA (EBER) with immunostaining against CD20, about one half of the infiltrating lymphoid cells were positive for EBER (×200). F. Most of the EBER-positive cells (brown) were actually atypical T lymphocytes without expression of CD20(red) (×400).

ISH, immunohistochemical stain with CD20 (Zytomed, Berlin, Germany) was performed on an Leica Bondmax autostainer (Leica Microsystems, Bannockburn, IL) using standard reagents and techniques to produce fast red membranous stain in CD20-positive cells.

Results

Histopathologic examination of the nasopharynx revealed effaced architecture without lymphoid follicle formation (**Figure 2A**). There was diffuse infiltration of atypical polymorphous

lymphocytes with small to medium-sized nuclei. Some immunoblast-like cells with cytologic atypia and brisk mitosis were focally found (**Figure 2B**). In the IHC study, the infiltrating atypical lymphocytes were predominantly composed of CD3-positive T cells without expression of CD56 and CD20 (**Figure 2C** and **2D**). Most of the lymphoid cells were also positive for CD2, CD5 and CD7 with either CD4 or CD8 expression. The MIB-1/Ki-67 labeling index was about 75%. By using double staining with in-situ hybridization for EBV-encoded RNA (EBER) and immunostaining against CD20, about one half (45%) of the infiltrating lymphoid cells were positive for EBER (**Figure 2E**). Only a few (20%) of the EBER-positive cells co-expressed CD20 (**Figure 2F**). When compared with CD3 immunostaining, most of the EBER-positive cells were atypical T lymphocytes.

Discussion

IM is a benign and self-limited lymphoproliferative disorder usually caused by EBV infection. It typically occurs in adolescents and young adults with presence of fever, pharyngitis and general lymphadenopathy. Microscopically, the architecture of lymph nodes is focally destroyed with hyperplastic and irregular-shaped follicles, expansion of paracortex and dilatation of sinuses. The infiltration cells are heterogeneous including small and large lymphocytes, immunoblasts, histiocytes, and variable numbers of plasma cells and eosinophils. There is extensive proliferation of immunoblasts in the paracortex with a mottled appearance [2]. The histologic features of IM are so varied that sometimes misdiagnosed as malignant lymphoma, especially when numerous large immunoblasts and Reed-Sternberg-like cells are present [3, 4]. The differential diagnosis includes large B-cell lymphoma, anaplastic large cell lymphoma, Hodgkin lymphoma and other viral infection. The histopathologic features of nasopharynx in IM are rarely described. In 1988, Takimoto et al. [5] reported an 8-year-old Japanese girl with mixed small and large atypical lymphoid cells in the nasopharynx. The histology mimicked malignant lymphoma. About 50% of the lymphoid cells were EBNA-positive and 2% EA/VCA-positive. Mitotic activity and immunophenotype were not described in detail. In the presenting case, most of the infiltrating lymphoid cells were T lymphocytes with cytologic atypia. Malignant lymphoma was highly suspected. If the tumor occurred in the nasal cavity, extranodal nasal NK/T-cell lymphoma (NKTCL) would be first considered. However, it should be cautious to make the diagnosis of NKTCL in the nasopharynx. IM featuring florid reactive T-cell proliferation may be misdiagnosed as NK/T-cell lymphoma only on the basis of morphologic and immunophenotypic findings. Further genetic study and careful clinical correlation are required to prevent erroneous diagnosis.

EBV infects B-lymphocytes via the C3d complement receptor (CD21) [6]. In the immunocompetent patient, EBV infection induces CD8-positive cytotoxic T cell proliferation against EBV-infected B cells. These activated T cells are the characteristic atypical lymphocytes seen in the peripheral blood of IM patients [7, 8]. The infection is controlled and enters into a latent phase where only a few infected B cells are present. Although mature T cells express low levels of CD21, EBV infection of T cells seems to be rare [9]. In the study of EBER ISH conducted by Hudnall et al, a randomly chosen subset of 20 from 50 EBNA1 PCR-positive tonsils was used. The male/female ratio was 1.2 with a mean age of 24 years old (range: 5 to 57). Six tonsils with 50–150 positive cells per section were selected to localize EBV within various phenotypically defined cell types by combined EBER ISH and IHC study [10]. They analyzed 1191 EBER-positive human tonsil cells from these six tonsils. Most (82.2%) of EBER-positive cells were CD20-positive B cells, while 6.7% were CD3-positive T cells, 1.2 % were epithelial cells and 10.9% were null cells with dim CD20 expression and having plasmacytoid features, likely to be plasmacytoid B cells. Nearly 60% of the EBER-positive cells were found within the T-cell-rich interfollicular regions, while 15% were present within the B-cell-rich germinal centers of secondary follicles. Most of EBER-positive CD3-positive tonsillar T cells are located within the interfollicular areas. Dual EBER/CD4 and EBER/CD8 immunostains reveal that most of the infected T cells are CD4-positive helper/inducer T cells rather than CD8-positive cytotoxic T cells. In our case, most (80%) of the EBER-positive cells were CD3-positive T cells. The reason for why EBV-infected T cells were much more than B cells was unknown. Both CD4- and CD8-positive T cells are potentially susceptible to EBV infection that may cause T-cell lymphoproliferative disorder, including

NK/T-cell lymphoma, chronic active EBV infection, and hemophagocytic lymphohistiocytosis [11-13]. The presenting case demonstrated that EBV-associated atypical T-cell proliferation could occur in the nasopharynx of IM patient.

The pattern of EBER staining is correlated with the clonality of lymphocytes. Lee et al reported 15 cases of EBV-associated primary gastrointestinal lymphoma in non-immunocompromised patients [14]. The patterns of EBER staining in 8 cases were diffuse (>50% of the tumor cells showed staining all over the hybridized sections) or localized (>30% of the tumor cells showed staining but in a localized field). The other 7 cases showed scattered EBER staining pattern (more than 20 scattered tumor cells showed staining). The diffuse and localized patterns of EBER expression might be associated with the pathogenesis of lymphoma. In contrast, the scattered EBER staining pattern might represent secondary infection due to depressed immunosurveillance. The EBER staining of the presenting case was between diffuse and localized, similar to lymphoma. Further study is necessary to explore EBER staining pattern between lymphoma and IM.

In summary, we reported a case of IM with atypical T-cell proliferation in the nasophaynx. The EBV-infected cells were relatively diffuse, mimicking malignant lymphoma. This case indicates that it is important to differentiate florid lymphoid proliferation from malignant lymphoma in IM.

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