Case Report

Epstein–Barr Virus-Positive Diffuse Large B-cell Lymphoma, Not Otherwise Specified: A Diagnostic Challenge

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Abstract

Epstein–Barr virus-positive diffuse large B-cell lymphoma, not otherwise specified (EBV + DLBCL) is a new separate entity included in 2016 WHO classification with dismal prognosis. Hence, awareness is needed for prompt diagnosis and swift treatment. Here, we present a case of a 48-year-old male with multiple cervical lymphadenopathy for 5 months. On examination, the patient had severe pallor and multiple firm to hard nontender cervical lymph nodes. Bone marrow aspiration was a dry tap. Bone marrow biopsy revealed nodular collection of atypical monomorphic lymphoid cells admixed with lymphocytes, eosinophils, and plasma cells in the background. Lymph node biopsy demonstrated total effacement of normal lymph node architecture with atypical lymphoid cells. These cells were positive for CD20, CD30, and CD38, LMP1, myc, IRF4/MUM1, and FOXP1 and were negative for anaplastic lymphoma kinase, CD3, CD10, bcl2, bcl6, and CD15. Diagnosis of EBV-positive DLBCL was rendered.

Keywords: Epstein–Barr virus, immunohistochemistry, lymphoma

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Introduction

Epstein–Barr virus-positive diffuse large B-cell lymphoma, not otherwise specified (EBV + DLBCL, NOS) is a separate entity included in 2016 WHO classification. The median age of patients with EBV + DLBCL is 71 years (50–91 years) with male predominance (M: F ratio-1:4), though younger patients can be affected.[1] EBV + DLBCL has a higher prevalence in Asia and Latin America (5%–15%) as compared to the Western population (<5%).[2]

Case Report

A 48-year-male presented with multiple cervical lymphadenopathy for 5 months. The patient had severe pallor and multiple firm to hard nontender cervical lymphadenopathy. Laboratory investigations revealed pancytopenia with no atypical cells on peripheral smear.

Bone marrow aspiration was a dry tap. Bone marrow imprint smears showed occasional particles with marked crushing in trails. Few small collections of atypical monomorphic cells were seen. Bone marrow biopsy revealed cellular marrow for age with nodular collection of atypical monomorphic cells. These cells vary from cleaved lymphoid to lymphoplasmacytic. Occasional large mononuclear cells with prominent nucleoli were seen. Lymphocytes, plasma cells, and eosinophils were present in the background [Figure 1a and b]. Grade 2 fibrosis was detected on reticulin staining. The differential diagnosis was diffuse large B-cell lymphoma, T cell-rich B-cell lymphoma, and Hodgkin's lymphoma. On immunohistochemistry, these cells were positive for CD20, CD30 [Figure 2a], and CD38 and were negative for CD3 and CD15. Diagnosis of CD30-positive DLBCL was considered. Due to the presence of reactive background and CD30 positivity, LMP1 was performed which was positive [Figure 2b]. Hence, the final diagnosis of EBV-positive DLBCL, NOS was proffered.

The lymph node biopsy demonstrated total effacement of normal lymph node architecture and showed perinodal and pericapsular involvement. Monomorphic atypical lymphoid cells were arranged in sheets. These cells had a high nucleo-cytoplasmic

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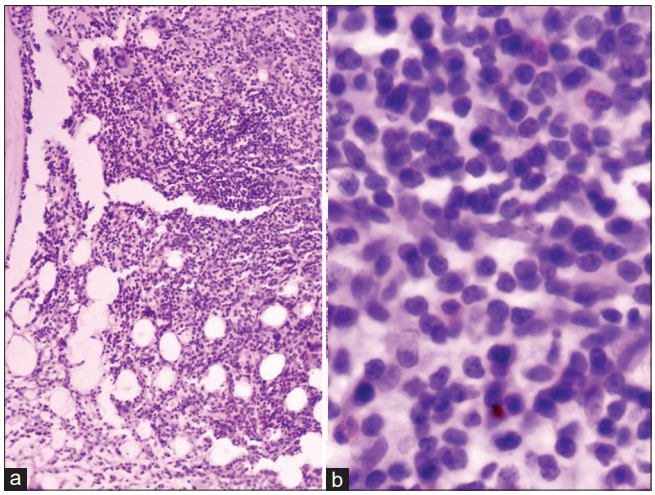

Figure 1: (a) Section of bone marrow biopsy showing nodular collection of atypical lymphoid cells (H and E, ×40). (b) High power showing atypical lymphoid cells admixed with lymphocytes and eosinophils (H and E, ×600)

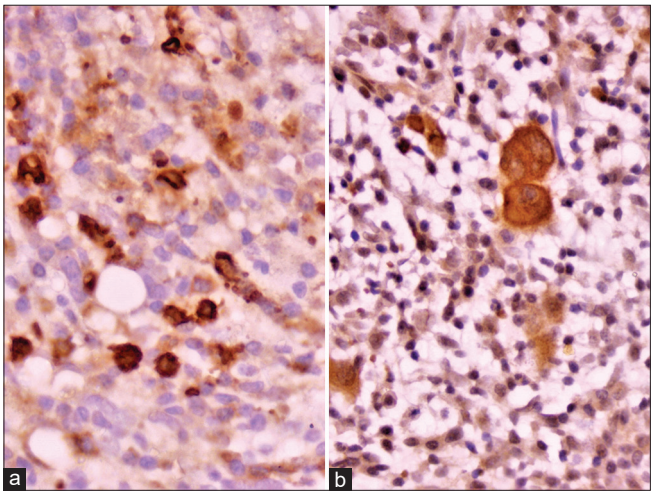

Figure 2: (a) Tumor cells showing cytoplasmic and membranous immunopositivity for CD30 (immunohistochemistry, DAB chromogen, ×200). (b) Tumor cells showing cytoplasmic and membranous immunopositivity for LMP1 (immunohistochemistry, DAB chromogen, ×400)

ratio with scant to moderate amount of cytoplasm, large irregular nuclei, and few showing prominent nucleoli. Mitosis was 4–5 per high-power field [Figure 3a and b]. These cells were immunopositive for CD20, CD19, IRF4/MUM1, myc, FOXP1 and showed kappa light chain restriction. Ki67 was <20%. These cells were immunonegative for CD3, CD10, anaplastic lymphoma kinase, and bcl6. Bcl2 was positive in <50% of cells. These features were suggestive of activated B-cell (ABC) subtype of DLBCL. The patient was started on rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone (R-CHOP), but after two cycles of chemotherapy, the patient succumbed to the disease.

Discussion

The magnitude of EBV infection is pervasive, with almost every person being exposed to the virus in their lifetime. The virus may cause minor discomfort to the infected body. After the acute phase wears off in an immunocompetent patient, the virus enters into the latent phase. Cytotoxic T cells are responsible for clearing the EBV-infected B cells. In the elderly, there is senescence of the immune system, with a decrease in B-cell and T-cell diversity. Cytotoxic T cells are responsible for clearing the EBV-infected B cells. Due to the decrease in B-cell diversity and cytotoxic T cells, there is a clonal expansion of EBV-positive B cells.[3] This immune senescence is hypothesized as one of the causes of EBV-positive DLBCL, NOS. Other hypothesis states that inflammation induces reactive oxygen species that activates p53, retinoblastoma, and nuclear factor kappa pathway.[4]

The disease occurs in a higher age group with male preponderance (M: F = 2.5:1) and has an aggressive clinical course.[5-7] Lymph node involvement was seen in 65% cases; however, this tumor frequently involves extranodal sites such as skin, soft tissue, bones, nasal cavity, pharynx, tonsils, lung, pleura, stomach, liver, spleen, peritoneum, caecum, and bone marrow. Prevalence of EBV-positive DLBCL in the East Asian population is around 8%–15%.[2,5-7]

Morphologically, EBV-positive DLBCL, NOS are of two types: monomorphic and polymorphic. Monomorphic subtype was more common than polymorphic subtype with the ratio being 1.75: 1. Microscopically, the tumor comprises a uniform population of large cells with extensive necrosis, mitoses, and apoptosis. The disease is classified as polymorphic if reactive cells such as lymphocytes, plasma cells, histiocytes, centroblast, immunoblast, and plasmablast are present in the background, and monomorphic if reactive cells are absent or minimal. Reed–Sternberg-like cells and Hodgkin cells may be present in both subtypes. Mixed pattern with both monomorphic and polymorphic areas can also be found.[8] In the present case, we found monomorphic subtype in the lymph node biopsy while polymorphic type in the bone marrow biopsy. This creates a diagnostic dilemma, as the presence of Reed–Sternberg-like cells in polymorphic background poses a diagnostic challenge that too on bone marrow biopsy. The differential diagnosis of EBV-positive DLBCL, NOS has been discussed in Table 1.

On immunophenotyping, tumor cells are positive for CD19, CD20, CD22, CD79a, CD30, PAX5 and are negative for CD15, CD10, and T-cell markers such as CD3, CD7, and CD2.[8] These cells express EBER, LMP1, and EBNA2. EBV-positive DLBCL, NOS commonly displays ABC immunophenotype, that is , MUM1/IRF4 and bcl2 positive and CD10 negative. Germinal center B-cell (GCB) immunophenotype has also been reported in the literature with ABC: GCB ratio being 2.6:1. CD30 positivity has been seen in 50.6% of cases. This tumor has a poor prognosis when compared to EBV-negative DLBCL, and CD30 positivity has a worse prognosis.[1]

Treatment of EBV-positive DLBCL, NOS entails the addition of R-CHOP. Overall, response rates with R-CHOP (50%–90%) is higher than the CHOP regimen (30%–80%).[9] Role of antiviral therapies in EBV-positive DLBCL along with inducers of lytic phase such as methylase transferase inhibitors, histone deacetylase inhibitors, and proteasome inhibitors are under investigation.[10]

Table 1: Differential diagnosis of Epstein-Barr virus-positive diffuse large B-cell lymphoma, not otherwise specified

	Age of presentation (years)	Site	Histological finding	IHC	Stage at diagnosis
EBV-positive DLBCL, NOS	Elderly (median=71)	Nodal, extranodal-skin, soft tissue, bone, lung, bone marrow	Polymorphic > monomorphic Geographic necrosis	CD20+, CD30+, CD79a+, PAX5+, LMP1+, Mum1+, CD10−, bcl2−, bcl6−	III or IV
EBV-positive classical Hodgkin's lymphoma	15-34	Nodal, mediastinal	Reed-Sternberg cells in reactive background	CD20−, CD79a−, CD15+, CD30+	II
T-cell histiocyte rich B cell lymphoma	Middle aged men	Nodal, extranodal-bone marrow, liver, spleen	Large B cells in the background of abundant T cells and histiocytes	CD19+, CD20+, CD79a+, bcl6+, CD15−, CD30−, CD138−	III or 1V
ALK-negative anaplastic large cell lymphoma	40-65	Nodal, extranodal-bone, soft tissue, skin	Large pleomorphic cells, multinucleated cells, wreath like cells, hallmark cells	CD30+, CD3+, CD4+, CD43+	III or IV
Grey zone lymphoma (mediastinal/nonmediastinal)	20-40	Mediastinal Mass, supraclavicular LN±	Pleomorphic tumor cells in fibrotic stroma with sparse inflammatory infiltrate	CD20+, CD79a+, CD30+, CD15±, Bcl6+, CD23+, CD10−, IRF4/MUM1+, LMP1−	III or IV

EBV: Epstein–Barr Virus, DLBCL: Diffuse large B-cell lymphoma, NOS: Not otherwise specified, ALK: Anaplastic lymphoma kinase, IHC: Immunohistochemistry, LN: Lymph node

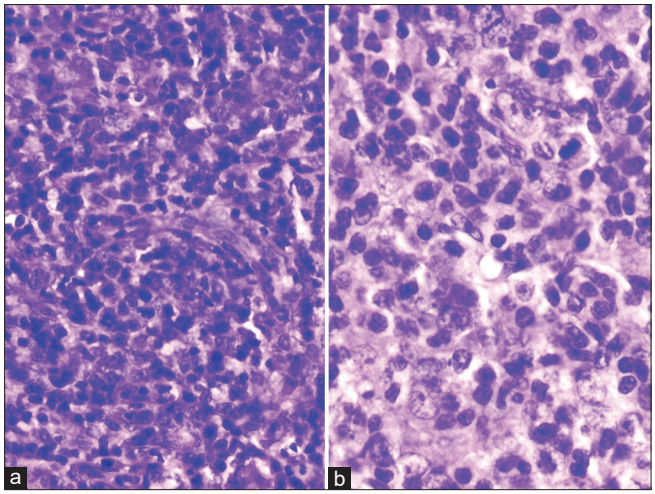

Figure 3: (a) Sections of lymph node biopsy showing effacement of lymph node architecture by atypical lymphoid cells (H and E, ×400). (b) Sections of lymph node biopsy showing atypical lymphoid cells with high N: C ratio, large irregular nuclei, and few showing prominent nucleoli (H and E, ×600)

Conclusion

EBV-positive DLBCL is an uncommon lymphoma associated with poor prognosis. The incidence of DLBCL, NOS is underestimated as EBV tests are not available in many institutes. EBV tests must be done in all cases of DLBCL, especially when encountered to have CD30 positivity, as this lymphoma has a poor prognosis and requires prompt detection.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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