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Original Contribution

Epstein - Barr virus infection in de novo diffuse large B-cell lymphoma in Jordan and Turkey

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1. Introduction

Epstein-Barr virus-positive (EBV+) diffuse large B-cell lymphoma (DLBCL), not otherwise specified (NOS) is defined in the 2016 Revised World Health Organization (WHO) classification as an EBV-positive clonal B-cell proliferation, with exclusion of lymphomatoid granulomatosis, cases with a recent EBV infection and other well-defined lymphomas that harbor EBV such as plasmablastic lymphoma, DLBCL associated with chronic inflammation and EBV-positive mucocutaneous ulcer [1]. This neoplasm designated previously as EBV-positive DLBCL of the elderly, with an arbitrary cutoff of an age older than 50 years [2], but the restriction of the elderly has been removed, as these neoplasms can present over a wide age range. The NOS designation was added to emphasize the need to exclude more specific entities. Most patients lack predisposing immunodeficiency diseases [1].

The essential role of EBV in the pathogenesis of DLBCL is supported by numerous findings. EBV is present in a monoclonal form. The viral latent membrane protein-1 (LMP1) is located on the cell membrane of B-cells and can activate CD40 [3], whereas EBV LMP2a enhances MYC-driven proliferation and can stimulate B-cell receptor signaling [4]. Moreover, it has been shown that EBV-positive DLBCL is genetically unique with activation of the NF-κB P13K/AKT and JAK-STAT pathways [5]. Detection of EBV encoded RNA-1 (EBER-1) is considered a standard for diagnosis; however, a clear cutoff for positivity has not been defined [6]. EBV(+) DLBCL is an aggressive disease compared with its EBV(−) counterpart, with a reported median overall survival of 2 years [3]. Factors heralding worse outcome include older age, presence of B-symptoms, and positivity for EBNA2, and CD30 expression [1]. Currently, patients with EBV(+) DLBCL, NOS are staged and managed following guidelines similar to patients with EBV(−) DLBCL [6].

EBV(+)-DLBCL was first described in Asia as a disease of older patients and designated as senile EBV+ DLBCL [7]. Since then, many reports showed a higher disease frequency among Asian populations compared to Western countries [1], similar to other EBV related lymphomas such as extranodal NK/T-cell lymphoma, nasal type, T-cell lymphoproliferative disorders of childhood and EBV+ classical Hodgkin lymphoma. In the Middle East, the incidence of EBV-positive DLBCL is less known due to a shortage of published data. In this report, we assessed a series of 100 DLBCL cases from Jordan and Turkey for EBV by in situ hybridization analysis in two series of DLBCL. The demographic ethnicity in Jordan is predominantly Arab, whereas in Turkey the predominant ethnicity is Turk. We show a similar frequency of EBV in both populations.

2. Materials and methods

2.1. Patient selection

The study was a retrospective analysis of 100 patients with the

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diagnosis of DLBCL, 50 cases from the Department of Hematopathology at Jordan University Hospital and 50 cases from the Department of Pathology at Necmettin Erbakan University in Turkey. Inclusion criteria were histologic diagnosis of DLBCL-NOS. We excluded cases of primary mediastinal large B-cell lymphoma, primary CNS lymphoma, plasmablastic lymphoma and DLBCL associated with chronic inflammation. Clinical correlation was also reviewed to exclude cases with apparent immunodeficiency or autoimmune diseases. All age groups were included in the study. Clinical data including patient gender, age and tumor site were collected. The study was approved by the Institutional Board Review (IRB) and the Committee of Scientific Research.

2.2. Specimen preparation

Histopathologic examination of hematoxylin-eosin (H&E) and immunohistochemical stains for all cases was performed to review diagnosis and ensure tissue adequacy. Unstained, coated slides were cut from formalin-fixed paraffin-embedded blocks at thickness of 4-μm thickness for all cases. In situ hybridization (ISH) for EBV-encoded RNA (EBER) was performed in the Department of Hematopathology at MD Anderson Cancer Center. The analysis used a fluorescein-labeled peptide nucleic acid probe (Dako) in conjunction with the Dako peptide nucleic acid ISH detection kit for formalin-fixed, paraffin-embedded tissue sections and an automated stainer (Ventana Medical Systems, Tucson, AZ, USA). Both positive and negative control probes were used.

2.3. Interpretation of results

EBER1-ISH slides were reviewed primarily by two pathologists (TA and AM). Only nuclear staining of large cells was considered positive. Scoring of positive cells was performed using a semiquantitative technique. A cutoff of 10% of large neoplastic cells with nuclear reaction was required to label a neoplasm as positive. We reviewed the morphology of positive cases according to WHO classification criteria as monomorphic or polymorphic variants [1].

3. Results

EBER1 positivity was detected by ISH in 5 of 50 (10%) Jordanian cases. The group consisted of 2 men and 3 women with a median age of 57 years (range 47–84). Four patients had disease based in lymph nodes and 1 patient had disease in the spleen. All patients underwent excisional biopsy. The histology of DLBCL was monomorphic variant in 3 cases and polymorphic variant in 2 cases. Positivity for EBER1 ranged from 10 to 90% of neoplastic cells, with a median of 30% cells. Only 1 case showed a diffuse positivity in > 90% of neoplastic cells (Fig. 1), whereas the other 4 cases showed focal reactivity in neoplastic cells with a range of 10–30% positive neoplastic cells. In cases with focal positivity two patterns were seen; either a focal confluence of positive neoplastic cells in a rather negative tumor (Fig. 2), or the positive tumor cells were evenly distributed within the neoplasm (Fig. 3). According to Hans algorithm, 4 cases were immunohistochemically of non-germinal center (non-GC) origin and 1 of GC origin.

Similarly, 6 of 50 (12%) Turkish patients were positive for EBER1. Five patients were women and 1 was a man. The age range was 25–68 years (median 54 years). Four patients underwent excisional biopsy and 2 patients needle core biopsy. Three patients had lymph node-based disease, 2 patients had spinal masses, and 1 patient had disease involving tonsil. Morphologically, 4 cases were monomorphic variant and 2 polymorphic variant. One case showed diffuse positivity in > 90% of neoplastic cells, whereas the other cases had EBER1 positive cells ranging between 10 and 30% (median 10%). One case was excluded from the group as the positive cells were small reactive lymphocytes in an adjacent lymphoid aggregate (Fig. 4). Half of cases were of non-GC and the second half of GC origins. Table 1 summarizes the clinical and pathologic results of the Jordanian and Turkish patients.

4. Discussion

In this study, we analyzed two groups of patients with DLBCL, from Jordan and Turkey, for the presence of EBV infection by in situ hybridization analysis. The prevalence among Jordanian (10%) and Turkish (12%) patients was similar to that reported in Asian populations in which EBV positivity has ranged from 8 to 16% [7-11]. The positivity rate in Jordan and Turkey was also close to what was reported in Mexico (7%), Poland (12%) and Peru (14%) [12-14]. In contrast, studies from Western countries showed a lower incidence, ranging from 0 to 4% in the USA [15,16] and 2% in Germany [12]. Studies from Middle East on this topic are few and the frequency of EBV positivity in the Arab population is less known. Our study was the first from Jordan and the second from Arabic states. A previous study from Kuwait showed a frequency of 18% in DLBCL assessed by EBER1-ISH [17]. In Turkey, one previous study showed a lower incidence of EBV in DLBCL of 5.3% [18], which could be attributed to different sample size (340 vs 50 patients) and due to no-age restriction in our study.

There is no consensus cutoff for the number of EBV-positive neoplastic cells to establish the diagnosis of EBV(+)-DLBCL, but a substantial number of positive cells is required along with exclusion of EBV-positive reactive cells in the background [3]. We set a threshold of 10%, as most of published series used a threshold between 5 and 50% [13,16,19-21]. The higher the cutoff applied, the lower the frequency of positive cases found. The argument in the literature for excluding cases with low percentage of positive cells is that these EBV+ cells might represent a secondary infection of the neoplastic cells, or that they are clonally unrelated to the original tumor. Yet, one study showed an inferior outcome even when the percentage of EBV-positive cells was as low as 5%, justifying including these cases [13]. In our study, the median percentage of EBV-positive cells was 30% in Jordanian and 10% in Turkish patients. Thus, we recommend including cases with positivity below 50% within the category of EBV(+)-DLBCL, as they

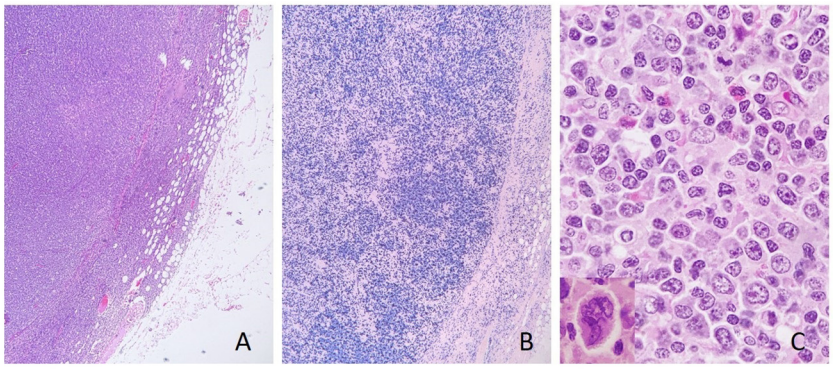

Fig. 1. (A) Diffuse large B-cell lymphoma effacing lymph node architecture and infiltrates through adjacent fat (H&E, 200×). (B) EBER1-ISH shows extensive positivity in almost all neoplastic cells. (C) Upon high power view (H&E, 600×), the lymphoma cells are composed of a mixture of centroblasts and immunoblasts in a background eosinophils and small lymphocytes. In some foci, large pleomorphic cells are present (inset), qualifying for polymorphic variant.

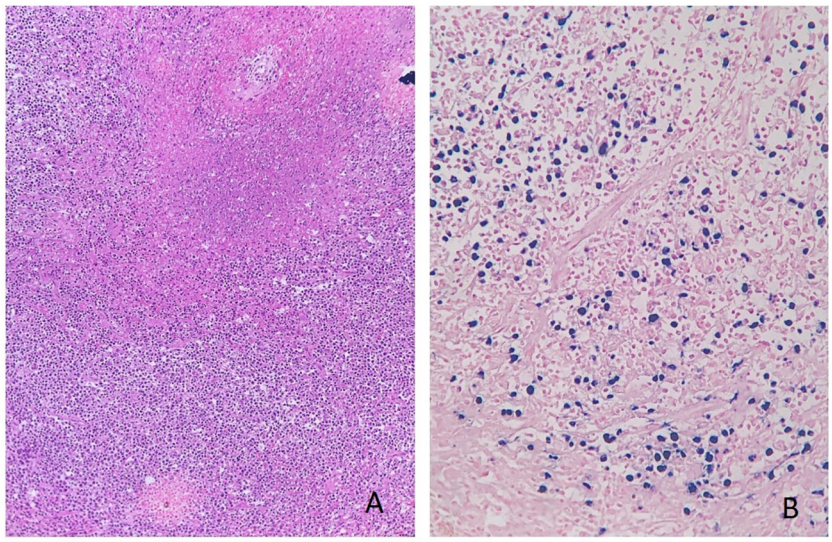

Fig. 2. (A) A case from spleen shows diffuse large B-cell lymphoma with prominent geographic necrosis (H&E, 200×). (B) Upon staining with EBER1-ISH, one focus shows an aggregate of positive large cells, while most of other areas are negative (not shown).

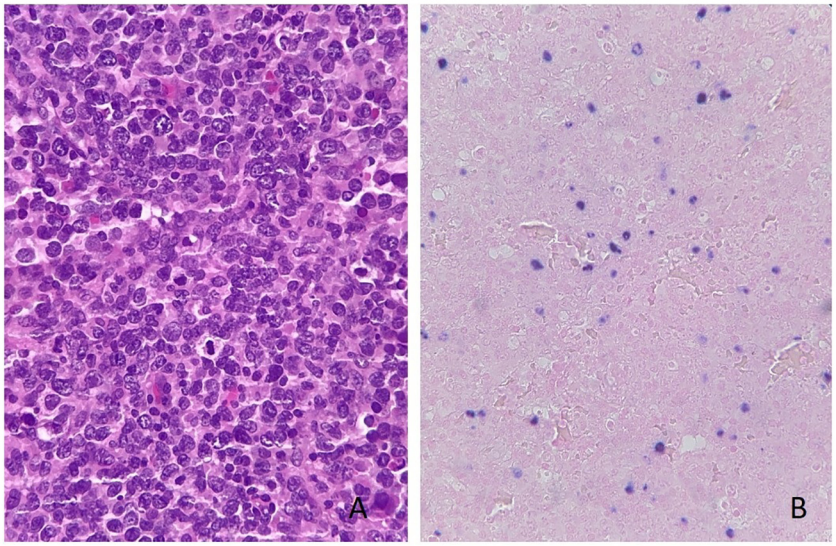

Fig. 3. (A) A case of monomorphic diffuse large B-cell lymphoma. The neoplasm is composed of sheets of centroblasts with prominent mitosis (H&E, 400×). (B) Only few large cells (10%) were positive for EBER1-ISH, scattered throughout the tumor. Most positive cases showed a similar pattern.

represented the majority of cases in this study.

EBV(+)-DLBCL was first described as a disease of elderly, with an arbitrary age cutoff of > 50 years [2]. Later studies demonstrated a younger age of presentation and even during childhood [22]. Thus, the term "of elderly" was omitted in the revised 2016 WHO Classification [1]. It is noteworthy to mention that most of the published studies from different countries – such as Turkey, Peru, Mexico, Germany, USA, Japan – were before 2016 and included only patients above the age of 50 years. Therefore, the exact frequency might differ from that reported if the age criterion was not applied. In our study, the median age of presentation was 57 and 54 years in Jordanian and Turkish patients, respectively, which was younger than patients in series from Japan and Mexico [7,8,12]. With the exception of three patients, all patients in this study were older than 50 years of age.

Although the pathogenesis of EBV(+)-DLBCL is not fully known, a close interaction between chronic EBV infection and immunosenescence seems likely [14]. It has been reported that 30% of EBV(+)-DLBCL cases express the type-III latency protein EBNA2, thus reflecting immunodeficiency – associated lymphoma [12]. In our series, none of the patients had clinical manifestations of immunodeficiency or autoimmune diseases, supporting the view that the suggested immunodeficiency status is subclinical. In the original description of EBV(+)-DLBCL, an extranodal presentation was the hallmark of disease [7]. Nevertheless, a larger study of the same group found that EBV(−)-DLBCL had a similar frequency of extranodal disease [8], and more recent studies showed that EBV(+)-DLBCL is predominantly a nodal disease [12,15,22-24]. Our study confirms this finding as lymph node presentation appeared in 80% of Jordanian and in 50% of Turkish patients.

Histologically, EBV(+)-DLBCL demonstrates a wide spectrum of findings that can be divided into polymorphic and monomorphic subtypes [1]. Polymorphic lesions show a broad range of B-cells, including centroblasts and immunoblasts, admixed in a background reactive small lymphocytes, histiocytes and plasma cells. Sometimes, large Reed-Sternberg-like cells are seen. On the other hand, the monomorphic variant is composed of sheets of large cells resembling DLBCL-not otherwise specified. Areas of geographic necrosis are commonly seen. The malignant cells express pan B-cell markers and commonly

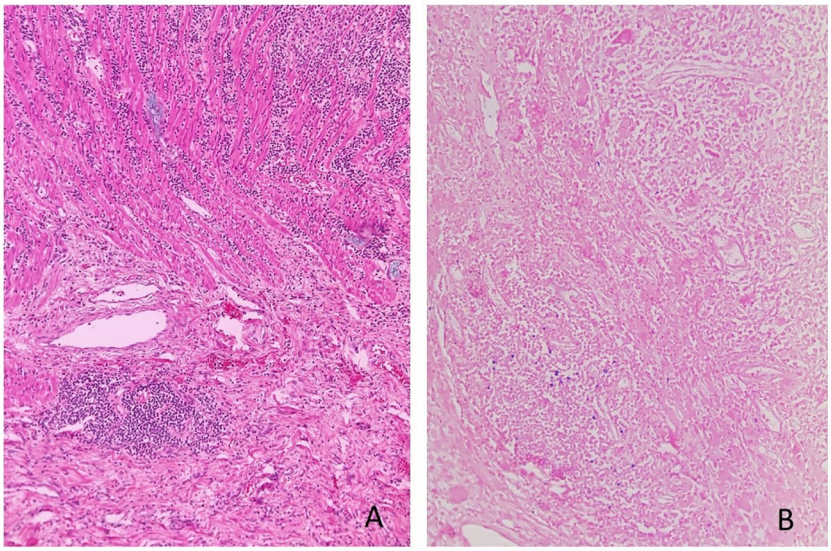

Fig. 4. (A) Diffuse large B-cell lymphoma arising in colon. The tumor cells dissect through the muscularis propria in the right upper border (H&E, 200×). Note the small reactive lymphoid aggregate in the left lower border, which shows (B) few scattered small cells positive for EBER1-ISH, while the tumor cells are completely negative. This case was regarded as negative for EBER1.

Table 1
Patients characteristics for EBV(+)-DLBCL. COO: cell of origin, F: female, GC: germinal center, M: male, LN: lymph node.

Case	Origin	Age	Sex	Site	Biopsy	Morphology	Percentage of EBER (+) cells	COO
1	Jordan	54	M	LN	Excision	Polymorphic	> 90%	Non-GC
2	Jordan	84	F	LN	Excision	Monomorphic	30%	GC
3	Jordan	47	F	LN	Excision	Monomorphic	30%	Non-GC
4	Jordan	57	F	spleen	Excision	Polymorphic	20%	Non-GC
5	Jordan	63	M	LN	Excision	Monomorphic	10%	Non-GC
6	Turkey	60	F	Spine	Excision	Polymorphic	> 90%	Non-GC
7	Turkey	58	F	Tonsil	Excision	Monomorphic	30%	Non-GC
8	Turkey	46	F	LN	Excision	Polymorphic	10%	GC
9	Turkey	68	F	LN	Excision	Monomorphic	10%	GC
10	Turkey	50	M	Spine	Core	Polymorphic	10%	Non-GC
11	Turkey	25	F	LN	Core	Polymorphic	10%	GC

CD30. In 70% of cases, they are of non-germinal center type [3]. In our entire series, both polymorphic and monomorphic variants were almost equal (55% vs 45%, respectively). The frequency of polymorphic variant in previous studies was variable as 32% [14], 42.9% [13] and 77% [12]. To date, these variants do not correlate with clinical presentation or prognosis and its importance relies in awareness of the spectrum of the disease for establishing the diagnosis [3]. Most of our cases (73%) were of non-GC type. We did not explore the detailed immunophenotypic profile of positive cases due to financial limitations and clinical outcome due to missing data of many positive cases.

In short, our study showed that EBV(+)-DLBCL is a relatively common disease among Arabic and Turkish patients, with a frequency similar to Asian populations as compared with the West. Most patients are above the age of 50 years, but the disease can present earlier. Patients do not suffer immunodeficiency and commonly have a nodal disease. EBV positive cells are < 50% in the majority of positive cases. The presence of EBV did not correlate with the morphology of DLBCL, and thus we recommend testing all newly diagnosed cases of DLBCL for EBER1 by in situ hybridization.

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Authors' contribution

TNA: concept of research, interpretation of results, writing of manuscript.
AM: interpretation of slides, writing manuscript.
RM: collection and archiving of slides, blocks and data.
PO: providing cases from Turkey.
JK: performance of staining, reviewing manuscript.
LJM: performance of staining, reviewing manuscript.

Declaration of competing interest

The authors have no conflict of interest of financial disclosure.

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