A STUDY ON BURKITT' S LYMPHOMA AND EPSTEIN-BARR VIRUS LATENCY IN DUHOK, IRAQ

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ABSTRACT

Background: Burkitt's lymphoma (BL) is the most common lymphoma in children and primarily, but certainly not exclusively, originates in the lymphoid tissue of the distal ileum. This study looks at the Epstein-Barr virus latency and C-MYC gene rearrangement in tumor cells from patients in Iraq's Duhok province.

Methods: Between 2014 and 2020, archived surgical pathology tissue from thirty Kurdish patients diagnosed with BL in the Duhok region of northern Iraq was examined. Formalin-fixed, paraffin-embedded (FFPE) tissue blocks were used to make tissue microarrays (TMA), which were then studied using immunohistochemistry for a panel of lymphoma markers, in-situ hybridization (ISH) for EBV-encoded RNAs (EBER1), and fluorescent in-situ hybridization (FISH) for C-MYC/IGH translocation.

Results: The median age of patients was 7 years (3-22). Twenty-two were male and eight were female. Twenty-five patients had extra-nodal primaries, 21 of them had intrabdominal primaries, the ileocecal region accounted for all of the cases, and five had nodal primaries. All patients had c-MYC rearrangement in their tumors, whereas EBER1 was present in 22 (73%) of the tumors.

Conclusion: This study reveals that Burkitt lymphoma in the Duhok region is a juvenile illness with a preference for the ileocecal region, as opposed to the United States and northern Europe, where it is regarded as an adult disease with a propensity for the lymph nodes.

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Keywords: Burkitt's lymphoma, EBER, EBV, IRAQ, MYC.

Burkitt's lymphoma (BL) is an aggressive germinal center B-cell non-Hodgkin lymphoma[1]. BL is classified into three subtypes: a) endemic BL in central Africa; b) sporadic BL in worldwide distribution with the exception of equatorial Africa; and c) endemic BL in patients with HIV infection[2]. EBV is a human DNA virus that causes infection at various ages and in various populations[3]. In developing nations, more than 95% of the population acquires the virus before the age of five[4,5]. In contrast, EBV exposure in developed nations like northern Europe and the United States is postponed until adolescence and early adulthood[6]. In prior research in the region of Iraqi Kurdistan, BL was described as a pediatric disease[5,7]. The link between BL and latent Epstein-Barr virus (EBV) infection varies geographically and between the subtypes. EBV-encoded small nuclear non-polyadenylated mRNA (EBER) is present in nearly all endemic BL in Africa but in only 10–40% of sporadic BL in western nations[3]. In IRAQ and EYGPT, more than 70% of BL samples tested positive for EBER[7,8]. In the pathogenesis of endemic BL, EBV collaborates with malaria falciparum[9]. Whereas in sporadic BL, including the Middle East, no co-existing infection or

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promoting factor other than EBV has ever been discovered[6].

The expression of the MYC gene, which encodes the C-MYC protein transcription factor and is located on chromosome 8q24, governs cell proliferation, differentiation, and death. BL is characterized by abnormally high levels of C-MYC, which can be caused by several different mechanisms, most commonly by translocation of the long arm of chromosome 8 (containing the MYC gene) and the Ig heavy chain gene on chromosome 14. C-MYC overexpression causes rapid B cell proliferation, which accounts for the rapid doubling time of BL tumor cells (between 24 and 48 hours)[10]. BL has a "starry sky" look histologically, with benign histiocytes possessing copious, transparent cytoplasm distributed among a backdrop of homogenous, basophilic tumor cells. In general, extremely high rates of cell growth and apoptosis are found[10,11].

The goal of this study is to look at the link between EBV latency and Burkitt's lymphoma in Kurdish children from the Duhok region and compare the results with those from nearby countries, Africa, and the West.

MATERIALS AND METHODS

This retrospective review focuses on 30 Burkitt lymphomas detected between 2014 and 2020 in the pathology departments of Duhok Central Public Laboratory and Vajeen Private Hospital Laboratory in the Duhok region. The patient records at the given center were used to get the clinical information, such as age, gender, and the main site of the disease.

Tissue Microarray: Hematoxylin and eosin (HE) stained slides were examined, and relevant areas were selected for inclusion in the construction of a tissue microarray (TMA) block with a TMA kit from 3dhistech in Bulgaria. The 4 mm TM-Block slices were then mounted on charged slides (Dako, Denmark) for immunohistochemistry and in-situ hybridization.

Immunohistochemistry:

TMA slides were baked and antigens were retrieved in an automatic pressure cooker (PT link—Dako, Denamrk). This study used a polymer-based detection kit and automatic-stainer (Dako, Denmark) to identify comparable antigens using a panel of primary monoclonal antibodies that include: CD20, CD3, TdT, CD10, Bcl2, Ki67, and BCl6[11].

In-situ hybridization detection of EBER and C-MYC: TMA slides were baked, deparaffinized by xylene, rehydrated by a series of alcoholic solutions, washed in de-ionized water, and emersed in a buffered solution (TBS). In accordance with the manufacturer's instructions, a 20-minute incubation in a pre-hybridization 98°C heated solution in a water bath is followed by cooling in a washing buffer, drying, and digestion with the enzyme proteinase K. After that, the slides were incubated in hybridization solutions containing fluorescent (Zytovision-Germany) and chromogen-congregated MYC gene break-apart probes and EBV-encoded RNA (EBER1) probes (Leica biosystem, UK) for 16–18 hours, respectively. Following washing in buffer solution, post-hybridization incubation in 75°C heated buffer solution for 2–3 minutes, dehydration, drying, and mounting with DAPI (4,6-Diamidno-2-phenylindole) for scoring by fluorescent microscope (Olympus BX43) with DP74 camera (Olympus, Japan) and triple filter (Aqua/FITC/Texas Red Triple Filter Set, Chroma Technology Corporation, USA),

all cases exhibit a break-apart signal that represented by one yellow fusion, one green and one red signals, while ISH for EBER seen as a brown nuclear staining[12]. For each sample, a positive and negative case study was conducted.

RESULTS:

The ages of the 30 patients diagnosed with BL ranged from 3 to 17, with a median age of 7. The 30 BL instances included 22 males and 8 females, representing a male-to-female ratio of 2.75:1. All but three of the participants in this study were children under the age of 15.

Among the 25 patients with BL who had extra-nodal malignancies, the majority (84%) had primary tumors in the ileocecal region of the abdomen. Only two of the patients in this series had jaw disease. Twenty-seven of them (or 90%) were under the age of 15, while only three (or 10%) were over the age of 15. Five people, or 17%, had primary nodal lesions; all five were Weldayer's rings. Four children under the age of 15 and one patient over the age of 15 were affected (Table 1). The HE-stained sections (FFPE) of tissue exhibit starry-sky growth of small lymphoid cells with round nuclei and scant cytoplasm. These cells expressed CD20, CD10, and BCL6, with a 100% proliferation index (Ki67), but no CD3, TdT, or BCL-2 expression was detected (Figure 1). By FISH, all 30 BL cases were determined to be positive for C-MYC gene rearrangement, whereas 73% of the cases, which include 17 males and 4 females, exhibit nuclear expression for EBER1 (Figure 2). Eight patients (27%), four males and four females, tested negative for EBER in contrast to the C-MYC score, with four (50%) of the cases being extra-nodal and two (25% of the cases) being adult.

Table-1 Duhok Burkitt's lymphoma distributed according to the primary site of disease in relation to age, sex, and EBER

	Nodal	Extra nodal	Total (%)
Age >=15	1	2	3 (10%)
Age<15	4	23	27(90%)
Male	4	18	22(73%)
Female	1	7	8(27%)
EBER Positive	1	21	22(73%)
EBER Negative	4	4	8(27%)

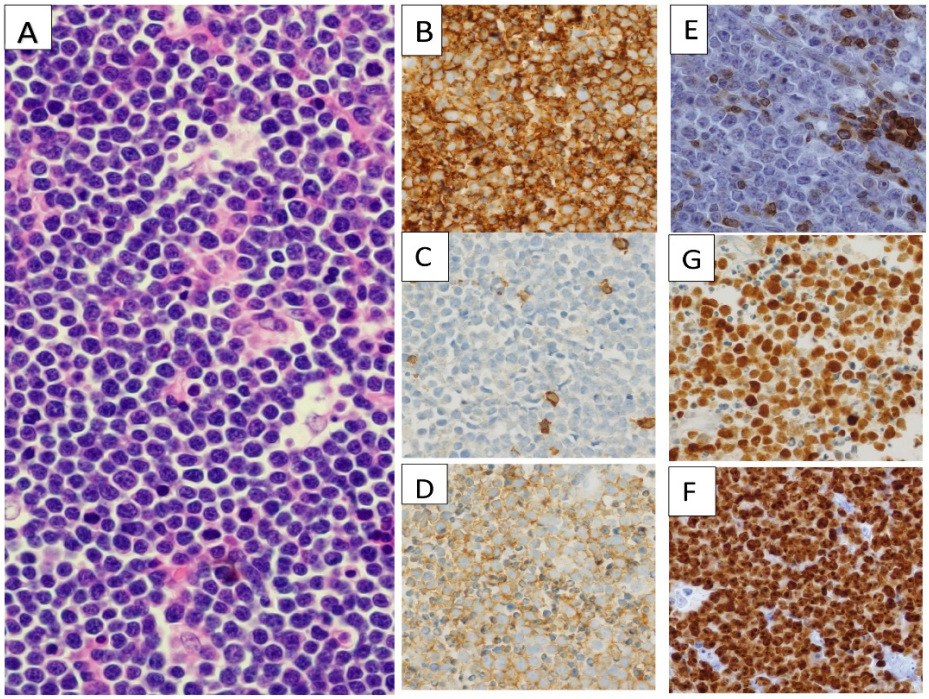

Figure 1: Histology and immunophenotyping features of a 4 years old male presented with ileocecal Burkitt's Lymphoma.

A- H&E, Histological morphology of BL of 4-year-old child with Ileocecal region mass,
B- CD20, diffuse membranous staining, **C- CD3; Not expressed by tumor cells**
D- CD10; Membranous staining, **E- BCL2; Not expressed by tumor cells,**
F- BCL6; Diffuse nuclear expression **G- Ki67; proliferation index ~100%**

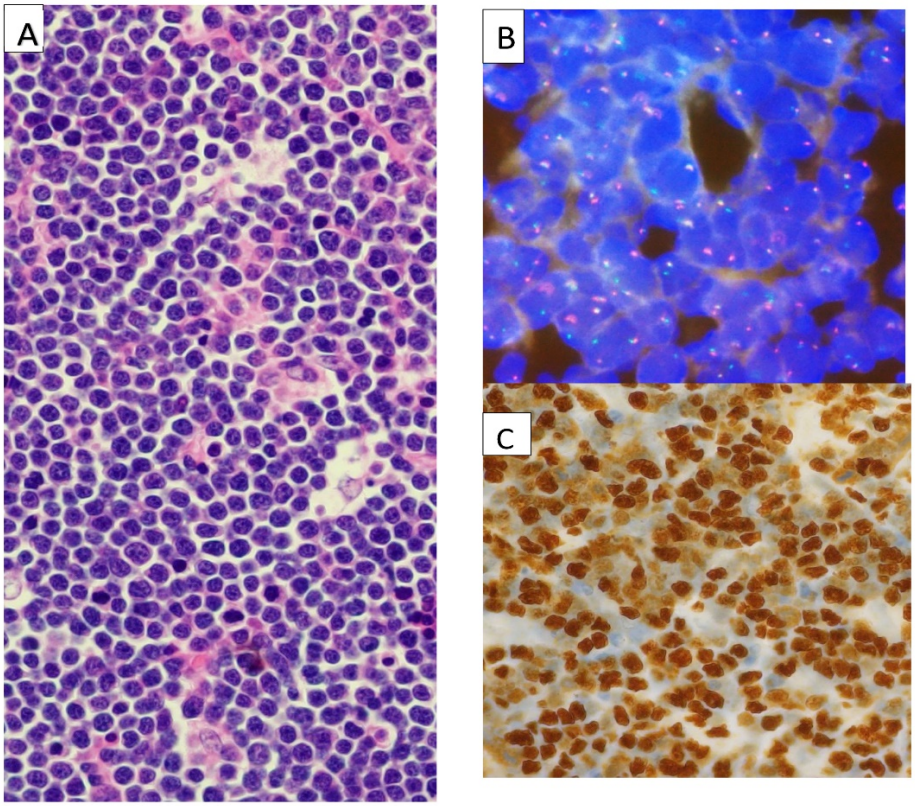

Figure 2: Histology, C-MYC-FISH and EBER-ISH of a 4 years old male presented with ileocecal Burkitts Lymphoma.

A-Histology of BL: small to medium size cells, round to ovoid nucleus, 1-2 nucleoli, scanty cytoplasm, stary-sky.
B-FISH C-MYC Dual Break Apart probe, 1 fusion (yellow) with two separate red and green signals,
C-ISH for EBER1: Nuclear expression (Brown)

DISCUSSION

Burkitt's lymphoma is the most prevalent lymphoma subtype among Kurdish children[6] with an age incidence rate of 6.2 per million children, which is 2 times the rate reported in the United States at 3.2 per million but significantly less than the reports of an endemic variant in Africa (30.0 per million)[4,5]. indicating that Burkitt's lymphoma in Iraq is distinct from endemic and sporadic variants[5].

The median age of patients included in this study is 7 years old (3–17 years), similar to a nearby Mid-East country and Africa that contrast records from US at 43 years old[11, 13]. And Bahrain[8]. where a modest increase in adult BL, which may be indicative of new regional tendencies overall[14].

The male predominance of BL lymphoma in the Duhok region of Iraq had similar rates to previous reports from Iraq[4,7], Egypt[8], and Jordan[15], this is steady with overall pattern, where sex-connected hypothesis was proposed in some previous review, at this point this should be affirmed by germline investigation of this speculation[3].

This study reveals predominant extra-nodular disease at a rate of 80% and mostly 76% intra-abdominal lesions in the ileocecal region, followed by jaw primaries at 6%. Similar results were found in Turkish children[16] and previous samples from Iraq (5-7) and Egypt[8]. This is in contrast to the reports from western countries such as the United States and Northern Europe, where nodal primaries were predominant[10,11]. Also, the problem is different from what happens to children in Africa, where the jaw is more affected than the GI tract[3,11].

All the BL cases included in this study reveal “starry sky” morphology on hematoxylin and eosin-stained slides, which is another characteristic of BL due to foamy macrophage infiltration as a response to rapid proliferation of tumor cells, even though it is found in other highly proliferative non-Hodgkin lymphomas like diffuse large B-cell lymphoma (DLBCL)[11]. Likewise, the immunophenotype of all cases reveals the germinal center type that is indicated by the co-expression of CD10 and BCL6, although it is not unique to Burkitt’s as it is co-expressed in germinal center DLBCL. All tumor cells have c-MYC gene rearrangement, which is thought to be a common genetic problem linked to BL[10], even though some rare cases of BL are negative for c-MYC gene rearrangement and c-MYC gene rearrangement is found in 5–18% of DLBCL[11,17].

According to the results of this investigation, latent EBV infection (EBER 1) was present in 73 percent of Burkitt's lymphomas. The same sort of random disease pattern that was documented in southern Italy and Spain[18] was also reported in Egypt[8]. This points to EBV infection in early childhood, which may be linked to the low socioeconomic position of the population in the Duhok region (a result of the many hostilities that have happened there)[4]. Although comparable patterns of poor socioeconomic position and EBV exposure are observed in India, there are fewer reports of BL[3]. Variations in EBV exposure between locations and the prevalence of latent EBV infection in particular cancers further complicate efforts to determine the nature of the relationship between EBV and BL[2,3], and[10]. Molecular analysis of EBV variants in areas with varying BL incidence rates may lead to the identification of EBV variants associated with a higher risk of BL[3].

Unlike the African (endemic) type of BL, there is no strong evidence that a

microbiological cofactor, such as malaria falciparum[9], led to the growth of EBV-infected memory B-cells and contributed to the development of Burkitt's lymphoma in different parts of Iraq[4, 10].

CONCLUSIONS

This research proves that Burkitt lymphoma in the Duhok area is a juvenile disease that tends to affect the ileo-cecal region, in contrast to the United States and Northern Europe, where it is typically diagnosed in adults and has a nodal preference. However, a high sporadic pattern of EBV latency has been documented. In contrast to Africa's ubiquitous Malaria falciparum, where there is evidence of a microbial cofactor that increases the multiplication of EBV-infected B cells, no such evidence exists for EBV.

Disclosure: The author discloses no interest

Ethical Statement: This article received approval from the ethical committee in the General Directorate of Health in Duhok.

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پوختە

لێکۆڵینەوە لە لیمفۆمای بورکیت و شاراوەیی ڤایرۆسی ئێپشتاین-بار لە شاری دۆهوک لە عێراق

پێشینە: لیمفۆمای بورکیت (BL) باوترین لیمفۆمایە لە منداڵاندا، کە بە شێوەیەکی سەرەکی، بەڵام بە دڵنیاییەوە بە تەنیا لە شانە لیمفاوییەکانی ئیلیۆمی دووردا سەرهەڵدەدات. ئەم توێژینەوەیە لێکۆڵینەوە لە شاراوەیی ڤایرۆسی ئێپشتاین-بار و ڕێکخستنەوەی جینی C-MYC دەکات لە خانە شێرپەنجەییەکان لە نەخۆشانی پارێزگای دۆهوک لە عێراق.

شێوازەکان: لە ساڵی ٢٠١٤ تا ٢٠٢٠، پێداچوونەوە بە شانەکانی پاتۆلۆژی نەشتەرگەری ئەرشیڤکراوی سی نەخۆشی کوردی تووشبوو بە بی ئێڵ لە دەڤەری دۆهوک لە کوردستانی عێراق کرا. بلۆکی شانە جێگیرکراوەکانی فۆرمالین، پارافین-چەسپاو (FFPE) بەکارهێنران بۆ دروستکردنی مایکرۆئەرای شانە(TMAs) ، کە دواتر شیکرانەوە بە بەکارهێنانی بەرگری بافتە کیمیاییەکان بۆ کۆمەڵێک نیشاندەری لیمفۆما، و تێکەڵکردن لە شوێنی خۆیدا (ISH) بۆ RNA کۆدکراوی EBV (EBER1)لێکۆڵینەوەکە کراوە. . ، و تێکەڵکردنی فلۆرۆسەنت لە شوێنی خۆیدا (FISH) بۆ گواستنەوەی.C-MYC/IGH

دەرەنجامەکان: مامناوەندی تەمەنی نەخۆشەکان ٧ ساڵ بوو (٣-٢٢). بیست و دوو پیاو و هەشت ژن. بیست و پێنج نەخۆش وەرەمی سەرەتایی لە دەرەوەی گرێ لیمفاوییەکان هەبووە، ٢١ نەخۆش وەرەمیان لە ناو سکدا هەبووە، ناوچەی ئیلیۆسیکاڵ بەرپرسیار بووە لە هەموو حاڵەتەکان، پێنج نەخۆش وەرەمی سەرەتایی لە گرێ لیمفاوییەکاندا هەبووە. هەموو نەخۆشەکان ڕێکخستنەوەی c-MYC یان لە وەرەمەکانیاندا هەبوو، لە کاتێکدا EBER1 لە 22 (73%)ی وەرەمەکاندا دۆزرایەوە.

دەرەنجام: ئەم توێژینەوەیە دەریدەخات کە لیمفۆمای بورکیت لە دەڤەری دهۆک نەخۆشییەکی گەنجە و پێی باشترە لە ئیلیم دەربکەوێت، بە پێچەوانەی ئەمریکا و باکووری ئەوروپا کە ئەم نەخۆشییە لە گەورەکاندا دەبینرێت و لە لیمفدا دەردەکەوێت. گرێبەست.

الخلاصة

دراسة عن سرطان الغدد الليمفاوية بوركيت وكمون فيروس إبستين بار في دهوك، العراق

الخلفية: سرطان الغدد الليمفاوية بوركيت (BL) هو سرطان الغدد الليمفاوية الأكثر شيوعا لدى الأطفال، وينشأ في المقام الأول، ولكن بالتأكيد ليس حصرا، في الأنسجة اللمفاوية من اللفائفي البعيد. تبحث هذه الدراسة في كمون فيروس Epstein-Barr وإعادة ترتيب الجينات C-MYC في الخلايا السرطانية في المرضى في محافظة دهوك في العراق.

الطرق: من عام 2014 لغاية 2020، تم فحص الأنسجة الباثولوجية الجراحية المؤرشفة لثلاثين مريضًا كورديا تم تشخيص إصابتهم بـ BL في منطقة دهوك في كوردستان العراق. تم استخدام كتل الأنسجة المضمنة بالفورمالين والمضمنة بالبرافين (FFPE) لصنع مصفوفات الأنسجة الدقيقة (TMA) ، والتي تمت دراستها بعد ذلك باستخدام الكيمياء المناعية لمجموعة من علامات سرطان الغدد الليمفاوية، والتهجين في الموقع (ISH) للـ RNA المشفر بـ EBV (EBER1). ، والتهجين الفلوري في الموقع (FISH) لإزفاء C-MYC / IGH.

النتائج: كان متوسط عمر المرضى هو 7 سنوات (3-22). اثنان وعشرون من الذكور وثمانية من الإناث. كان لدى 25 مريضًا أورامًا أساسية خارج الغدد اللمفاوية، حيث كان لدى 21 منهم أورامًا داخل البطن، وكانت المنطقة اللفائفية الأعورية مسؤولة عن جميع الحالات، وكان لدى خمسة حالات أورامًا أساسية في الغدد اللمفاوية. كان لدى جميع المرضى إعادة ترتيب c-MYC في أورامهم، بينما وجد EBER1 في 22 (73%) من الأورام .

الاستنتاج: توضح هذه الدراسة أن لمفوما بوركيت في منطقة دهوك هي مرض يصيب الصغار ويفضل الظهور في منطقة اللفائفية،على النقيض من الولايات المتحدة وشمال أوروبا، حيث يُعتبر هذا المرض عند البالغين ويميل إلى الظهور في الغدد اللمفاوية.