CASE REPORT

Burkitt lymphoma with additional isochromosome 1q in an adult HIV-positive patient

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Abstract

Burkitt's lymphoma (BL) continues to pose challenges in the human immunodeficiency virus (HIV)-infected population and may present at more advance stages with multiple areas of extranodal involvement. Other chromosomal abnormality in addition to the characteristic t (8:14) translocation also associated with poor outcome in BL. Here we report a case of an HIV seropositive adult male with the diagnosis of BL who presented with bone marrow and peripheral blood involvement of the disease. Cytogenetic analysis showed presence of complex chromosomal abnormality, with characteristic translocation of t (8:14) and an additional isochromosome 1q.

Key words

Burkitt lymphoma, HIV positive, Isochromosome 1q

1 Introduction

Burkitt lymphoma (BL) is a high-grade non-Hodgkin lymphoma (NHL) of mature B cell. The tumour has highest mitotic indices and a proliferative index of approximately 100% and is considered as one of the most clinically aggressive form of NHL [1]. It commonly presents in children or young adults and higher risk for those who is in a prolonged state of immunosuppression [2]. In early years of the Acquired Immunodeficiency Syndrome (AIDS) epidemic there was a significant increase in the incidence of B-cell NHLs identified among men who had sex with men [3].

Immunodeficiency-associated BL is primarily seen in association with the human immunodeficiency virus (HIV) infection [4]. In the highly anti-active retroviral therapy (HAART) era, several studies suggest a declining incidence of AIDS-related lymphoma, however, BL remains very frequent, accounting for 25% to 40% of all AIDS-related lymphoma [5]. HIV-associated BL typically presents as an aggressive disseminated disease frequently involving the bone marrow, extra-nodal sites and the central nervous system [6].

Translocation involving MYC gene is highly characteristic but not specific for BL [4]. Translocation of the MYC gene usually to the immunoglobulin (Ig) heavy chain gene on chromosome 14 resulting in t (8:14) (q24:q32) and rarely to

light-chain genes on chromosome 2 or 22 resulting in t (2:8) (p12:q24) or t (8:22) (q24:q11), respectively [7]. Any BL with secondary cytogenetic abnormalities are considered to have adverse prognostic implications [8].

We report a case of a Burkitt lymphoma with characteristic (8:14) translocation and additional chromosome 1 abnormality occurring in an HIV seropositive male.

2 Case presentation

This was a case of a 50-year-old Malay male presented with two weeks history of fever, associated with lethargy, loss of appetite and weight. The patient was also known to have ischaemic heart disease, hypertension and dyslipidaemia.

On physical examination, the patient was febrile. There was no organomegaly noted. Full blood count done showed mild normochromic normocytic anaemia with haemoglobin (Hb) level of 11.5 g/dl and platelet count of 19×10^9/L. His total white blood cell count was increased (21.7×10^9/L). Peripheral blood film revealed leukoerythroblastic picture with 15% suspicious mononuclear cells seen, which were medium in size, having high nuclear-cytoplasmic ratio, inconspicuous nucleoli, minimal amount of basophilic cytoplasm with vacuolation seen in the cytoplasm. The bone marrow aspirate smear showed hypercellular cell trails with presence of more than 90% blast cells which have similar morphology as the peripheral blood film (see Figure 1). Flow cytometric analysis of the bone marrow aspirate sample was performed and showed presence of abnormal cells population gated at CD45 dim to bright and low to intermediate side scatter (SSC). These cells expressed HLA-DR, cyCD79a, CD19, CD20, strong surface IgM with kappa light chain restriction (see Figure 2), and these findings were consistent with B-lymphoproliferative disorder.

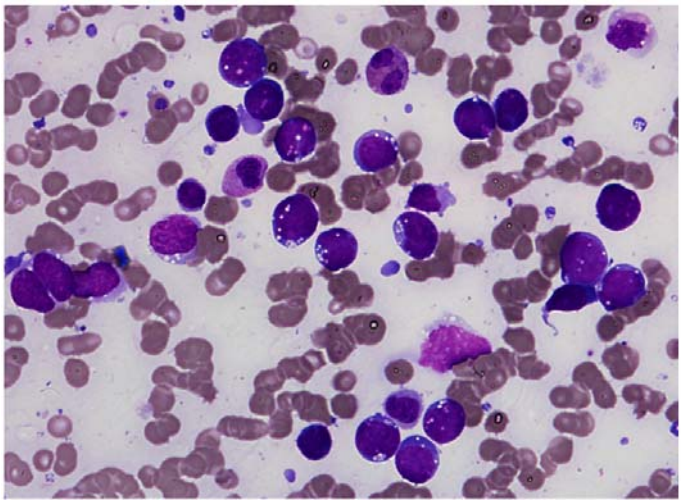

Figure 1. Bone marrow aspirate smear showed presence of atypical mononuclear or blast cells which were heterogenous in size, having high nuclear-cytoplasmic ratio with strongly basophilic vacuolated cytoplasm (MGG staining, ×40).

The trephine biopsy revealed cohesive sheets of malignant lymphoid cells proliferation which exhibit large to intermediate size, rounded nuclei, multiple nucleoli and scanty cytoplasm. Numerous mitosis, tangible-body macrophages and apoptotic bodies were seen (see Figure 3). Immunohistochemical staining was positive for CD20, CD79a and CD10 and the cells were negative for CD3, Tdt, CD34, CD5, BCL-2 and cyclin D1. The Ki67 proliferative index was very high (100%).

Karyotype analysis was performed on cultured bone marrow cells and revealed two cell lines, with the major cell line showed an additional isochromosome for the long arm of the chromosome 1 and a derivative chromosome 14 with additional material of unknown origin attached at band 14q32 (see Figure 4). In addition, a normal male karyotype was identified in three metaphase cells. Fluorescence in situ hybridization (FISH) was performed using the Vysis IGH/MYC/CEP8 tri-color, dual fusion translocation probe to confirm the reciprocal t (8:14) (q24:q32) involving the IGH and MYC gene regions. The probes consist of three colours - the SpectrumOrange probe spans approximately 821 kb and

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covers the MYC gene region, the approximately 1.6 Mb SpectrumGreen probe spans the IGH region and the aqua CEP 8 probe serves as a control for the copy number of chromosome 8. The Vysis ToTelVysion 1p spectrum green and Vysis ToTelVysion 1q spectrum orange were applied to confirm isochromosome (1q). The FISH analysis demonstrated fused (green/orange) signals, indicating the presence of the reciprocal t (8:14) (q24:q32) in 52% of the cells (metaphases and nuclei) analysed (see Figure 5A). Analysis using the telomeric 1p (spectrum green) and 1q (spectrum orange) probes confirmed the presence of the isochromosome 1q (see Figure 5B). Therefore, based on the hematological and cytogenetics findings, the patient was diagnosed to have Burkitts lymphoma. He was also found to have HIV infection with serological evidence of past EBV and CMV infection. At presentation, his CD4+ count was 115 cells/μl with a viral load of 23,600 copies/ml.

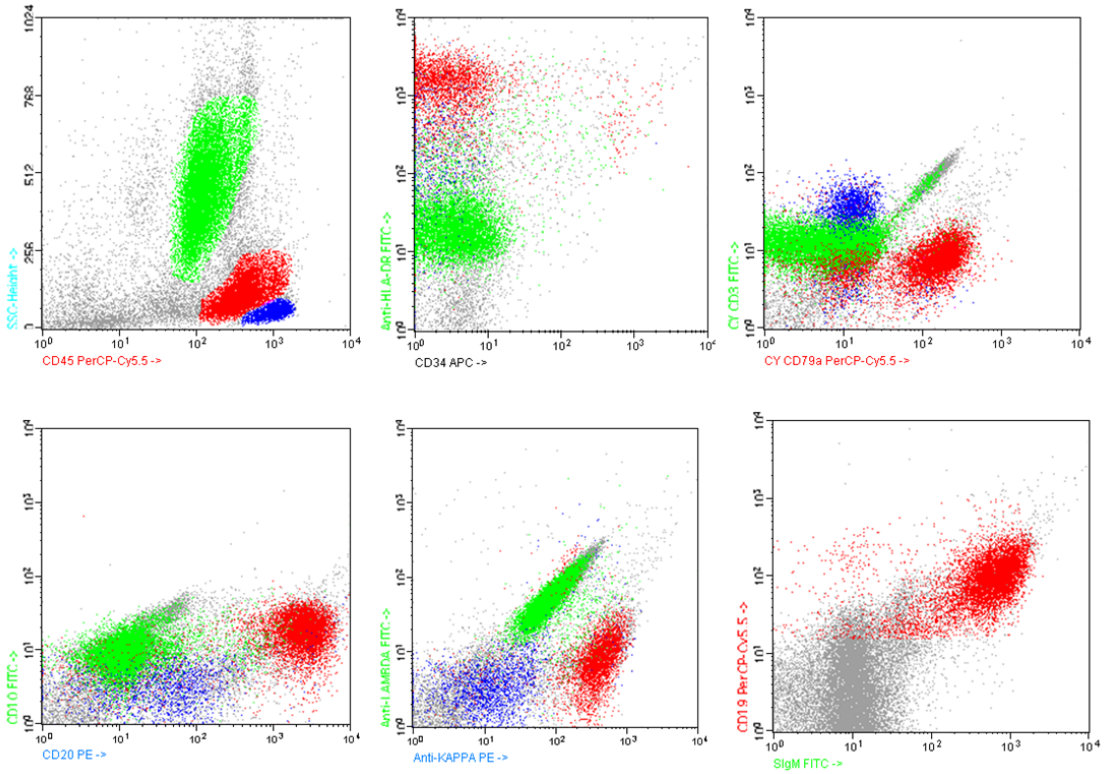

Figure 2. Flow cytometric analysis of the bone marrow aspirate sample showed presence of abnormal cells population (in red) gated at CD45 dim to bright and low to intermediate side scatter (SSC). These cells expressed HLA-DR, cyCD79a, CD19, CD20, strong surface IgM with kappa light chain restriction.

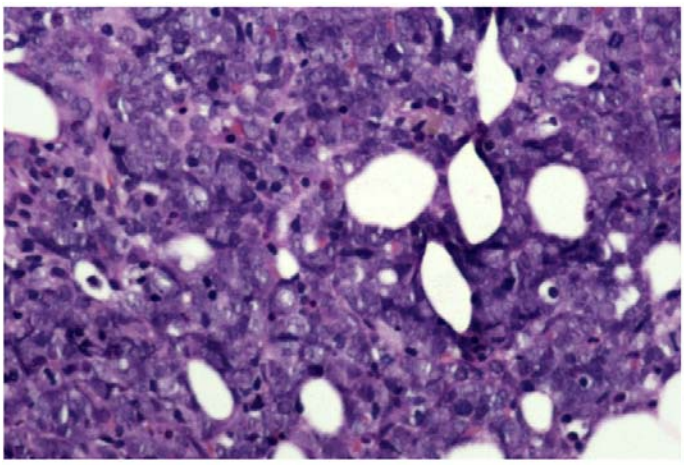

Figure 3. Trephine biopsy showed diffuse infiltrate of malignant lymphoid cells with numerous mitosis and multiple tingible-body macrophages, giving a starry-sky pattern. The malignant lymphoid cells exhibit large to intermediate size, rounded nuclei, multiple nucleoli and scanty cytoplasm (H&E, ×20).

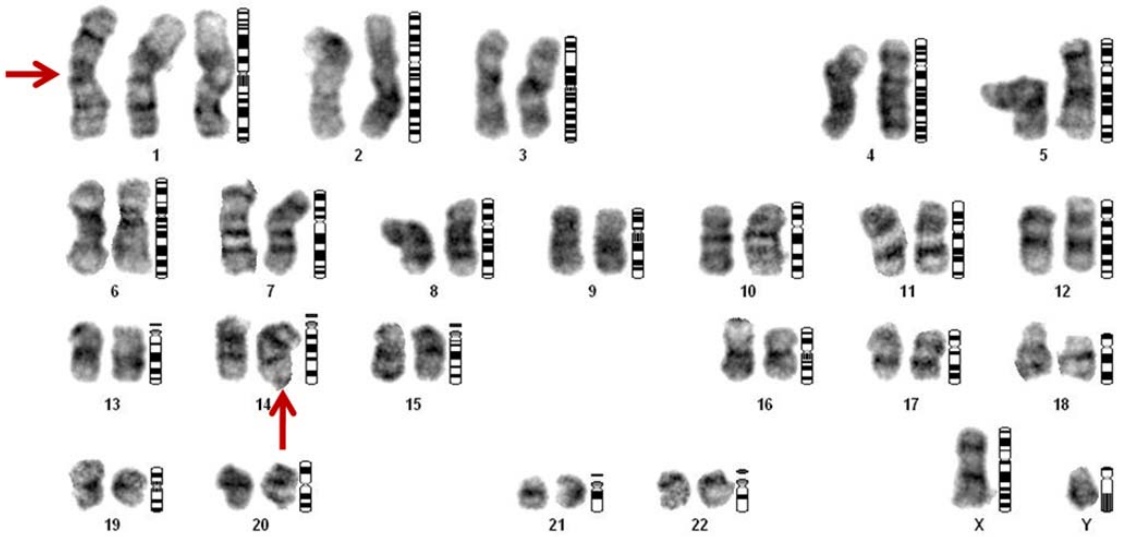

Figure 4. Karyotype analysis revealed additional isochromosome for the long arm of the chromosome 1 and a derivative chromosome 14 with additional material attached at band 14q32 (red arrows)

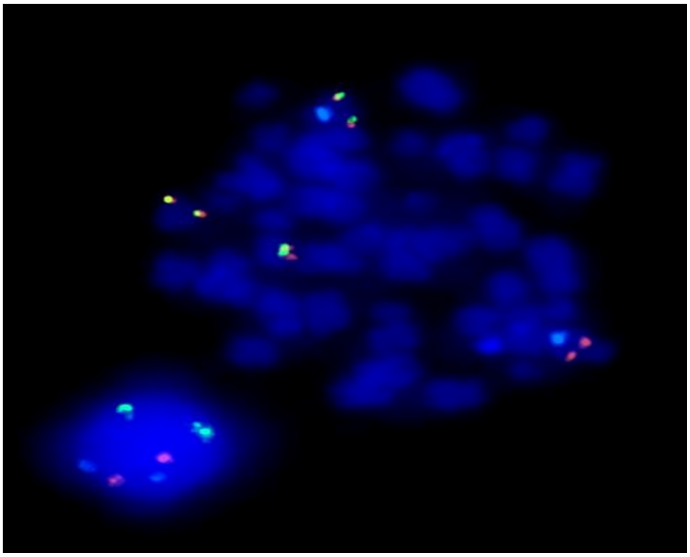

Figure 5A. FISH analysis using the Vysis IGH/MYC/CEP8 tri-color, dual fusion translocation probe demonstrated three fused (green/orange), two aqua and one orange signal pattern on the metaphase cell indicating the presence of t (8:14) (q24:q32). Hybridization of the probes on a normal nucleus showed two aqua, two orange and two green signal patterns.

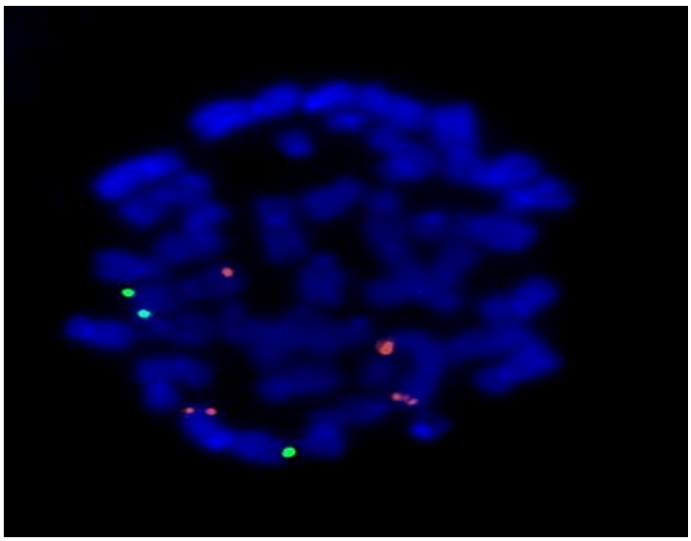

Figure 5B. Hybridization of the telomeric probes, Vysis ToTelVysion 1p spectrum green and Vysis ToTelVysion 1q spectrum orange hybridized to a metaphase cell identified two copies of normal chromosome 1 and an isochromosome 1q.

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Patient's basal tuberculosis (TB) status were also assessed at diagnosis and findings for sputum acid fast bacilli (AFB), mycobacterium TB culture and mycobacterium TB polymerase chain reaction (PCR) were negative. Screening for fungal infection such as *Aspergillus Fumigatus* and *Candida Albicans* were done and negative. Blood culture for fungus also showed no growth. His computed tomography (CT) scan pulmonary showed no significance finding.

In view of the highly aggressive disease, he was started on BFM-90 A and B protocol which consist of vincristine, methotrexate, ifosfamide, etoposide, cytarabine and dexamethasone for cycle A and vincristine, methotrexate, cyclophosphamide, doxorubicin, dexamethasone for cycle B. At the same time, HAART consist of tenofovir disoproxil fumarate 300 mg/emtricitabine 200 mg (Tenvir-EM) 1 tablet daily and efavirenz 600 mg daily was also started for the HIV infection. He went into complete remission and was planned for HLA-matched sibling donor allogeneic peripheral blood stem cell transplantation.

Unfortunately, nine months later he had relapsed of the disease when he presented with fever, haemoptysis, leukocytosis and thrombocytopaenia. Bone marrow aspiration at this stage showed presence of more than 90% Burkitt lymphoma cells. He was started on modified FLAG chemotherapy protocol which consists of fludarabine, cytarabine and granulocyte stimulating colony factor (G-CSF), however he developed tumour lysis syndrome, severe sepsis and pulmonary haemorrhage requiring intubation. He was also treated empirically for pulmonary tuberculosis and covered with anti-fungal for suspected fungal infection. The patient however succumbed to the disease despite aggressive treatment and management.

3 Discussion

Currently there are three variants of BL and our patient fall the under category of immunodeficiency-related BL [4]. Previous reports showed that BL with HIV commonly presented with advanced disease [9]. The usual B symptoms which consists of fever, night sweats, and weight loss which occur in 60% to 70% of BL patients [9], were also seen in our patient. Extranodal involvement is very common in all types of variant BL with bone marrow and CNS involvement being the highest in immunodeficiency-related BL [10].

A leukaemic phase also can be observed in patients with bulky disease, but only rare cases (mainly males) present purely as acute leukaemia with peripheral blood and bone marrow involvement [4, 10]. If present, the circulating neoplastic cells have the characteristics of acute lymphocytic leukaemia-L3 (according to the former FAB classification) with oval nuclei, small but distinct nucleoli, and a deep basophilic cytoplasm with prominent vacuoles. As in this case, the patient presented with peripheral blood and marrow involvement of BL with no lymphoid tissue or other organs infiltration.

The prototype morphology of BL is observed in endemic BL and in a high percentage of sporadic BL cases, particularly in children [4]. The tumour cells of BL are medium-sized cells and show a diffuse monotonous pattern of growth. The cells appear to be cohesive but sometimes exhibit squared-off borders of retracted cytoplasm. The nuclei are round with finely clumped and dispersed chromatin, with multiple basophilic medium-sized, paracentrally located nucleoli. The cytoplasm is deeply basophilic and usually contains lipid vacuoles. These cellular details are better perceived in imprints. The tumour has an extremely high proliferation index (many mitotic figures) as well as a high fraction of apoptosis. A "starry sky' pattern is usually present, which is imparted by numerous tangible-body macrophages. In some cases, tumour cells exhibit eccentric basophilic cytoplasm often with a single central nucleolus. Such cases, defined as BL with plasmacytoid differentiation, can occasionally be observed in children but are more common in immunodeficiency states. Regardless of subtype or variant, BL typically expresses monotypic surface IgM, pan-B-cell antigens, including CD19, CD20, CD22, and CD79a, and some cases may co-expresses with CD10, Bcl-6, CD43, and p53, but not CD5, CD23, Bcl-2, CD138, or TdT. The proliferation fraction is very nearly 100% as evidenced by the Ki67. The immunophenotype suggests follicle center origin for this lymphoma [11].

Prolong immunosuppression, such in our patient, is a risk factor for BL. The risk is increase with duration of HIV infection. Those who have been HIV seropositive for eight years or more have triple risk compare to those who have been seropositive for less than four years [12]. Most of the cases of HIV seropositive BL have higher CD4+ counts (> 150 cells/μl) compared to diffuse large B cell lymphoma (~ 60 cells/μl) [12]. However, our patient has a quite low CD4+ counts which was 115 cells/μl.

EBV plays a significant role in the pathogenesis of lymphoma and was identified in 40% – 50% cases of HIV-associated. A study done by Thorley-Lawson et al, 2004 have showed that EBV infects, immortalizes, and transforms B cells in vitro and establishes a persistent latent infection [13]. Failure to control EBV-infected B cells may lead to the development of post-transplantation lymphoproliferative disorder. Additional oncogenic mutations lead to clonal selection and evolution toward monoclonal tumors such as BL, Hodgkin disease and diffuse large B-cell lymphomas in immunocompromised patients [13]. All cases of BL have abnormality in the MYC gene and are known to be essential for the initial tumorigenic process. The translocation t (8:14) (q24:q32) has been found in 60% to 70% of the cases while variant translocations which are t (8:22) (q24:q11) and t (2:8) (p12:q24) occur in approximately 10% to 15% and 2% to 5% of the cases, respectively [14]. However, other chromosomal abnormalities are also frequently observed in BL. Lai *et al*. (1989) described additional chromosomal abnormalities in chromosomes 1, 7 and 6 in 23 patients with BL, usually in translocations, duplications, deletions (chromosome 6), or isochromosome of the long arm (chromosome 1 or 7) [15]. The present case showed characteristic translocation of t (8:14) with additional abnormality of isochromosome of the long arm of chromosome 1 (iso[1q]). Although initially the patient responded well with the chemotherapy regime given, he passed away due to the disease relapse and associated complications. Lones *et al*. (2004) and Garcia *et al*. (2003) suggested that abnormalities of the long arm of chromosome 1 are associated with a poor outcome in BL [16]. In addition to the complex chromosomal abnormalities, the underlying HIV infection also impart a poor prognostic factor for the patient. Kersten *et al*. (2001) described that in patients with HIV-related lymphoma, the prognosis is poor and the survival rates are variable [10].

In view of the highly aggressive disease, our patient was given BFM-90 A and B protocol which were also a standard regimens for the treatment of AIDS-related Burkitt lymphoma. However, HIV disease is myelosuppressive, thus some patient with poor haematopoietic reserve, low-dose chemotherapy might helpful in reducing the exacerbations of the disease [17].

In conclusion, BL with HIV continues to pose challenges in the management of the patients. As demonstrated in our study, complex chromosomal abnormality in addition to the characteristic t (8:14) indicates poor prognosis and increase the risks of treatment outcome.

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