

Original Research Article

Human Herpes Virus 8 (HHV-8) – associated lymphoproliferative disorders in Kampala, Uganda

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Abstract

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Both Human Immunodeficiency Virus (HIV-1) and Human herpesvirus-8 (HHV-8) are endemic in sub Saharan Africa. While the prevalence of HIV-1 ranges from 6-10% that of HHV-8 ranges from 30-60%. Apart from one case of primary effusion lymphoma and another of diffuse large B cell lymphoma, HHV-8 related lymphoproliferative disorders such as Multicentric Castleman's Disease and plasmablastic lymphoma have, hitherto, not been described in Uganda. To describe cases of HHV8 associated lymphoproliferative disorders seen at the department of Pathology, Makerere University, Kampala, Uganda. We retrospectively analyzed pathologic tissue obtained from 456 formalin fixed paraffin embedded tissue blocks with a diagnosis of malignant lymphoma or adenopathy from the Department of Pathology, Makerere University, from 2009-2011. They were examined using Haematoxylin and eosin and Giemsa stains for morphology. They were further evaluated using immunohistochemistry and in situ hybridization. Of the 456 biopsies studied, 5 tested positive for HHV 8, and five had Castleman's' disease (mainly multicentric). The remainder had a spectrum of lymphoproliferative disorders. HHV8-associated lymphoproliferative disorders occur in Ugandan patients infected with HIV that is endemic in the country. Their diagnosis depends on a high index of suspicion and use of immunohistochemistry. Clinicians and pathologists should keep them in mind as they ponder the diagnosis of HIV infected patients with lymphadenopathy and B-symptoms.

Key words: HHV8, lymphoproliferative, Castleman's disease, HIV, Uganda

INTRODUCTION

In 1994, Chang and Cesarman identified a herpes-like DNA virus in AIDS associated Kaposi sarcoma (KS). This was named Kaposi's sarcoma herpesvirus (KSHV) or Human herpesvirus 8 (HHV8) (Chang et al., 1994). Since then this virus has been detected in several disorders including multi-centric Castleman's disease and primary effusion lymphoma (PEL) (Cesarman et al., 1995).

Although both KS and HHV8 are endemic in sub Saharan Africa, the association between HHV8 and other lymphoproliferative disorders is scanty and has only been described in Uganda in a few cases of lymphoma (Tumwine et al., 2011) and three cases of lymphoproliferative disease (Engels et al., 2007). Indeed this is in line with what others (Carbone et al., 2005) have

observed elsewhere: the number of solid lymphoma cases associated with HHV8 has remained low.

Both the Human Immunodeficiency Virus (HIV-1) and Human herpesvirus-8 (HHV-8) are endemic in sub Saharan Africa (Shebl et al., 2011). While the prevalence of HIV-1 is around 6-10% (Green et al., 2006), Human herpesvirus-8 (HHV-8) infection is endemic with an estimated 36% - 60% sero-prevalence (de- The et al., 1999; Mayama et al., 1998).

Other HHV-8 related lymphoproliferative disorders such as Multicentric Castleman's Disease and plasmablastic lymphoma have not been described in Uganda.

Table 1. Clinical and laboratory findings of patients with HHV8 and lymphoproliferative disorders

Case	Age/ sex	Clinical findings	Diagnosis	Subtype	HIV result	HHV8 result	Histology
1	- /M	Single lymph node	Castleman's Disease	Unicentric	positive	Positive	Hyaline vascular mixed plasma cell variant
2	28/M	Adenopathy	Castleman's Disease	Multicentric (MCD)	positive	Positive	Atypical plasma cell proliferation
3	13/F	Adenopathy; On HAART	Castleman's Disease	Multicentric (MCD)	positive	Positive	Lymphocyte depleted
4	34/M	Fever, rigors, adenopathy	Plasmacytic lymphoproliferative disorder	-	positive	Positive	Sheets of atypical plasma cells.
5	-	-	Large cell lymphoma	-	NA	Positive	Large cell lymphoma
6	35/M	-	Plasmablastic lymphoma	-	NA	negative	Plasmablastic lymphoma
7	- /M	-	Castleman's Disease	Multicentric	NA	-	-
8	-	-	Castleman's Disease	Unicentric	NA	-	-

In this paper, we report several cases of HHV8 associated lymphoproliferative disorders, which we identified during lymphoma surveys in Uganda.

MATERIALS AND METHODS

Cases of lymphoproliferative disorders associated with HHV8 described in this paper were identified during lymphoma surveys in Uganda conducted by the sub Saharan Africa Lymphoma Consortium (SSALC) in Uganda.

Patient Samples

We retrospectively analyzed pathologic tissue obtained from 456 formalin fixed paraffin embedded tissue blocks with a diagnosis of malignant lymphoma or adenopathy from the Department of Pathology, School of Biomedical Sciences, Makerere University College of Health Sciences from 2009-2011. Haematoxylin and eosin (H&E) and Giemsa stains for were used for morphology.

Immunohistochemistry

We constructed tissue micro arrays paraffin blocks and subjected them to monoclonal antibodies (CD3, CD5, CD10, CD20, CD30, CD38, CD79a, BCL-2, BCL-6, Ki-67, CD138, IRTA-1, MUM-1/IRF4, LANA-1) as previously described (Tumwine et al., 2008; Sabbatini et al., 1998).

In situ hybridization

EBV was detected by looking for the presence of EBV encoded RNA (EBER). It was assayed using in situ hybridization with a FITC labeled probe to EBER-1 and 2 (Dako Y0017) and mouse anti-FITC (Dako M0878), rabbit anti-mouse serum and APAAP complexes as described

previously (Tumwine et al., 2008). Positive and negative controls were run concurrently.

Microscopy

Slides were mounted with a glass cover slip and analyzed with an Olympus BX61 microscope (Olympus, Tokyo, Japan).

Ethical considerations

Permission to conduct the study and ethical clearance were obtained from the Research and Ethics committee of the Faculty of Medicine, Makerere University and the Uganda National Council of Science and Technology. A waiver of consent to use the patients' delinked HIV serology data was also obtained.

To summarize the methodology: samples of 456 malignant lymphoma and adenopathy cases in formalin-fixed paraffin-embedded (FFPE) blocks from the Uganda SSALC and the Uganda AIDS and Cancer Specimen Resource (ACSR) were examined for morphology and LANA-1 (immunohistochemical, IHC) for diagnosis of HHV-8 lymphoproliferative disorders. Samples were also tested (IHC and in situ hybridization, ISH) using 20 monoclonal antibodies for common NHL antigens, ISH for EBV-encoded RNA, and kappa/lambda light chains (ISH, Ventana, Tucson).

RESULTS

Of the 456 biopsies studied, eight were lymphoproliferative disorders. Of the 8, five tested positive for HHV-8, five had Castleman's disease while of the remaining three, one had plasmacytic lymphoproliferative disease, another was a large cell lymphoma and the other was plasmablastic lymphoma.

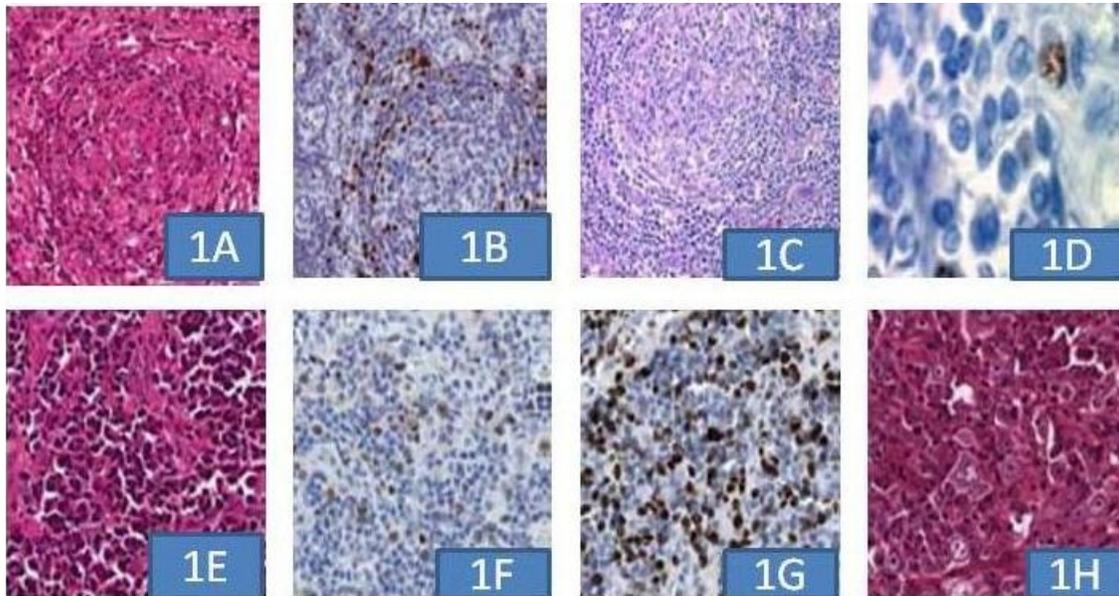


Figure 1. 1A Multicentric Castleman's disease (H&E staining X 100), 1B Multicentric Castleman's disease (HHV-8 in situ hybridization with LANA-1 staining X 100), 1C Unicentric Castleman's disease (H&E Staining X 100), 1D Unicentric Castleman's disease (HHV-8 in situ hybridization with LANA-1 staining X 400), 1E Plasmacytic lymphoproliferative disorder (H&E staining X 200), 1F Plasmacytic lymphoproliferative disorder (HHV-8 in situ hybridization with LANA-1 staining X 200), 1G Plasmacytic lymphoproliferative disorder Ki-67 immunostaining X 200), 1H Plasmablastic lymphoma (H&E staining X 200).

DISCUSSION

In this paper, we describe five cases of HHV-8-associated lymphoproliferative disorders identified during a survey of lymphomas over a two year period at the Department of Pathology, Makerere University in Kampala, Uganda.

All were male except one thirteen year old girl. This is in keeping with previous findings where most of the patients were male. The reason for this is not clear. In the USA, men who have sex with men have the highest prevalence of HHV-8-associated disorders including Kaposi's sarcoma and multicentric Castleman's disease (Lanchant et al., 1985). The sexual orientation of our patients was not known.

Three of our patients were middle aged men (age range 28-35 years): much younger than the cases reported from Europe and North America where most of the affected patients were in their sixth decade (Frizzera, 1988).

The most common site of involvement was the lymph node; one patient had only one lymph node involved while the others had generalized lymphadenopathy: very much in keeping with findings of studies elsewhere (Frizzera, 1988). Most authors have reported that the lymph node is the most common site of involvement, although other organs such as the spleen may also be involved (Keller et al., 1972).

In the current study, the most common lymphoproliferative disorder was Castleman's disease occurring in four out of the seven patients studied. Of the remaining patients, one had a plasmacytic lymphoproliferative disorder, while the other two had lymphoma. Of the two lymphomas, one was large cell lymphoma while the other was an HHV8 negative plasmablastic lymphoma.

Only four patients had known HIV serology results, and all were HIV-infected. They had the following diagnoses: multicentric Castleman's disease with morphology of hyaline vascular and mixed plasma cell variant, two multicentric Castleman's disease, one with atypical plasma cell proliferation and the other typically lymphocyte depleted.

The last one had plasmacytic lymphoproliferative disorder with sheets of atypical plasma cells. These atypical plasma cells that are present in all the HIV-infected patients in this study were infected with human herpesvirus 8 (HHV-8) and had strong LANA-1 staining.

This is a very interesting but disturbing finding. We now know that HIV-infected patients tend to present with multicentric Castleman's disease especially the plasma cell variant (Loi et al., 2004; Oskenhendler et al., 1996). These patients rarely survive more than two years: they have rapid disease progression and poor outcome, with mortality nearing 70% in most case series (Oskenhendler et al., 1996).

Castleman's disease

Castleman's disease (CD) is a rare atypical lymphoproliferative disorder whose etio-pathogenesis is not yet clearly defined. It is sub classified according to clinical features and histopathological type. It may be unicentric or multicentric, hyaline vascular, plasma cell or mixed cell types (Castleman et al., 1956; Gaba et al., 1978).

The hyaline vascular type is characterized by numerous small to medium sized lymph node germinal follicles, hyalinised vessels and a concentrically arranged mantle zone producing a characteristic "onion- skinning" appearance (Peterson and Frizzera, 1993).

In the plasma cell type, there are prominent plasmacytic infiltrates in the interfollicular areas of the nodes. The mature plasma cells in the interfollicular areas are polytypic. Mixed forms of CD exist with both features of hyaline vascular and plasma cell elements present (Peterson and Frizzera, 1993).

Human herpesvirus 8 (HHV8) has been found to be an important etiological factor of multicentric Castleman's disease (MCD) in HIV infected and HIV negative patients (Soulier et al., 1995). It has also been seen in most cases of MCD found in the setting of acquired immunodeficiency syndrome (AIDS) (Soulier et al., 1995). The disease most commonly presents in the lymph nodes or spleen. In Western Europe and America, MCD presents mainly in older men and is associated with lymphadenopathy and constitutional symptoms (Loi et al., 2004).

MCD pathogenesis is thought to be related to the production of viral interleukin-6 (vIL-6). HHV 8 associated large B cell lymphoma may also develop in patients with MCD. HIV infected patients with MCD present with aggressive disease (Oskenhendler et al., 1996). It has also been observed to develop in people who have recently been started on highly active antiretroviral therapy (HAART) as part of the immune reconstitution inflammatory syndrome (IRIS) (Zietz et al., 1999). In fact one of our patients had recently been initiated on HAART.

The treatment of MCD patients is still a challenge since all current protocols are based on case reports rather than clinical trials (Bowne et al., 1999; Chronowski et al., 2001). This is because of the rarity of the disease, however it has been noted that HIV infected patients who develop MCD while on HAART do better with chemotherapy regimens than those that develop MCD while they are HAART naïve (Aaron et al., 2002; Lanzafame et al., 2000; Kenyon et al., 2007). The treatment of MCD is evolving with use of antiviral therapy, immunotherapy with anti-CD20 antibodies and anti-IL-6 antibodies showing positive results (Cobellino et al., 2001).

HHV8 associated lymphomas include primary effusion lymphomas (PEL), solid PEL, HHV8 positive

plasmablastic lymphomas and plasmablastic lymphoma associated with MCD (Ferry et al., 2009).

These conditions are generally a rare group of disorders that are often missed in low resource settings (Tumwine et al., 2011). This is mainly because diagnosis of HHV8 associated lymphoproliferative disorders can only be made after doing immunohistochemistry with antibodies against the viral antigen, latent antinuclear antigen-1 (LANA-1) for detection of HHV8 in tissue sections or by in situ hybridization (Soulier et al., 1995).

The HHV-8-associated lymphoproliferative disorders have derived a lot of interest because they mimic malignant lymphoma both clinically and morphologically, however the difference is that they are not monoclonal (Anderson and Talal, 1972; Weaver, 1981). Traditionally, HHV-8 has been linked to three main disease categories: Kaposi's sarcoma, primary effusion lymphoma and Multicentric Castleman's disease and the plasmablastic proliferations that arise from Multicentric Castleman's disease (Ferry et al., 2009).

CONCLUSION

HHV8 associated lymphoproliferative disorders occur in Ugandan patients infected with HIV that is endemic in the country. Their diagnosis depends on a high index of suspicion and use of immunohistochemistry. Clinicians and pathologists should keep them in mind as they ponder the diagnosis of HIV infected patients with lymphadenopathy and B-symptoms.

Competing Interests

The authors declare that they have no competing interests

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