Deciphering early childhood infection by the Kapsosi's sarcoma associated human herpesvirus in Zambia

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INTRODUCTION

Human herpesviruses are large double stranded DNA viruses that are ubiquitous in nature. There are currently eight known human herpesviruses belonging to three subfamilies: alpha, beta and gamma herpesvirinae. They were sub-divided based on morphology, biological properties, genome structure and sequence homology . Human herpesvirus-8 (HHV-8), also known as Kaposi's sarcoma-associated herpesvirus (KSHV), is the most recently described human herpesvirus. HHV-8 was co-discovered by Chang and Moore in 1994, from lesions of a Kaposi's sarcoma (KS) patient by representational differential analysis . HHV-8 DNA sequences in the KS tissue, which were not found in normal skin tissue, could then be amplified. Human herpesvirus-8 (HHV-8) is the infectious etiologic agent of all forms of Kaposi's sarcoma (KS), primary effusion lymphoma and multicentric Castleman's disease . KS is an AIDS-defining illness and is the most common malignancy present in HIV infected patients . During the early stages of the AIDS epidemic, KS was the most common AIDS defining illness. In fact, it was the sudden appearance of Kaposi's sarcoma (KS) and shortly thereafter, the appearance of high-grade non-Hodgkin's lymphoma (NHL) in a handful of young homosexual men who otherwise were in good health signaled the start of the AIDS epidemic . Due to the ongoing HIV epidemic in sub-Saharan Africa, KS has become one of the most frequently diagnosed cancers in this region .

Global seroprevalence of HHV-8 varies greatly and is generally high in areas where non-HIV associated forms of KS (classic or endemic forms) have been common . HHV-8 seroprevalence in the United States and Northern Europe is generally low, but ranges from 20 to 80 percent in adult populations in African and Mediterranean regions . Zambia is a part of the “KS belt” where endemic KS was prevalent and where significant increase in KS incidence in adults and children has coincided with the emergence of the HIV-1 epidemic . By 1992, KS accounted for approximately 25% of all childhood cancers diagnosed in Lusaka, the capital of Zambia .

The modes of transmission of HHV-8 may be different in different parts of the world depending on the endemicity of that region, and are still being investigated but both horizontal and vertical transmission has been reported . Horizontal transmission via heterosexual and homosexual contact has been reported in adults . Vertical transmission to children seems to occur at a very low rate; a likely source of non-sexual transmission is via saliva, and rare transmission may occur also through breast milk . A report from Uganda has provided evidence for HHV-8 transmission through blood transfusion . HHV-8 can be found in the PBMCs, saliva, oropharyngeal mucosa, semen and prostate glands which represent the source of both vertical and horizontal transmission .

KSHV TRANSMISSION STUDIES: 1998 TO 2004

Our cohort studies first started in 1998 with the aim to study the epidemiology of HHV-8 in Zambia and also examine the major risk factors in the acquisition of this infection. We were also interested in determining the major routes of transmission of this virus in adults and to young children.

Study Cohort

Between October 1998 and April 2004, 3161 pregnant women visiting the labor ward at the University Teaching Hospital (UTH) in Lusaka, Zambia were screened for HHV-8 and HIV-1 infections and enrolled into the study . Women in early stages of labor were enrolled in this prospective cohort study after being counseled, educated about the study, and giving written informed consent. After delivery, mothers were encouraged to come back with their children for follow-up visits. Demographic, medical and exposure data was collected via structured interviews at each visit. A total of 1424 mother-infant pairs (MIPs) who returned for at least one postpartum visit constituted the longitudinal cohort.

Results

We initially determined HHV-8 prevalence estimates and the risk factors that were associated with HHV-8 infection in the enrolled women. We found that 40.2% of the women were seropositive for HHV-8 infection . HHV-8 seropositive women were more likely to be co-infected with HIV-1 than those who were HHV-8 negative. We also evaluated 154 variables to understand the independent predictors of HHV-8 seropositivity. We found that diagnosis of genital warts, HIV-1 coinfection and primary education were independent predictors of HHV-8 infection.

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We also wanted to determine if HHV-8 seropositive women could vertically transmit the infection to the child before or during delivery. We randomly chose a sub-group of 89 women who were found to be HHV-8 seropositive. We checked for the presence of HHV-8 DNA in mothers and infants in PBMCs. We found that 2 out of 89 samples drawn at birth from infants has HHV-8 DNA detectable in PBMCs . These findings suggested that HHV-8 can be transmitted perinatally, but infrequently. Other routes of transmission such as horizontal transmission could be the most likely route of transmission.

Therefore, we examined whether transmission of HHV-8 to infants by mother could occur through breast milk feeding or through salivary contact. Our findings showed that there was a lack of HHV-8 DNA in the breast milk of seropositive mothers . This suggested that breast milk was not a likely source of horizontal transmission of virus to infants. But we have frequently found HHV-8 DNA in saliva and buccal cells. Therefore, as is the case with EBV, our results indicate that salivary contact may be the primary source of horizontal transmission.

In this cohort, the children were followed for 48 months after delivery. Based on 1,532 total child-years of follow-up, we determined that in this cohort HHV-8 seroconversion occurs early in life and hazard rate of HHV-8 seroconversion is 13.8 infections per 100 child-years . HIV-1 infected children were at substantially higher risk for HHV-8 seroconversion (adjusted Hazard ratio = 4.60). Maternal HIV-1 and HHV-8 infection status were not independently associated with risk of HHV-8 seroconversion in the child. We also observed that HHV-8 antibody titers in children followed at all consecutive time-points seroreverted for HHV-8 antibodies with undetectable titers in some children at one or more time points after seroconversion. These results demonstrated that cross-sectional serological screening likely underestimates true HHV-8 seroprevalence in young Zambian children due to fluctuations in detectable antibody titers.

KSHV TRANSMISSION STUDIES: 2004 TO 2009

The results from our previous cohort study suggested that the major mode of HHV-8 transmission to children is by person-to-person contact, likely via saliva. Several studies have reported salivary transmission leading to the emergence of saliva as the major route of HHV-8 transmission . We also observed that HHV-8 infection in young children could occur independently of the HHV-8 serostatus of the mother, suggesting the possible role of other household members or others in the community in transmitting infection . Therefore we were interested in evaluating the role of household contacts in transmitting infection to young children. Thus, the Zambia Children's KS-HHV8 study was designed in which longitudinal 4 year follow-up of recruited households was undertaken with the specific objective of determining the rate and source of horizontal transmission of HHV-8 to young children. We aimed to investigate whether transmission of HHV-8 to young children could occur through casual, person-to-person contact within a household.

Study Cohort

In this study we enrolled complete households that had at least one HHV-8 negative child (referred to as index child) . The HHV-8 negative children along with their primary caregiver were requested to return for follow-up visits every 4 months until age 48 months; the other household members were followed annually. During each visit, all the study participants received a physical exam and free medications for common ailments such as worm infestation, fever and minor aches and pains, and multivitamin supplements in case of malnourishment. Structured interview questionnaires were developed to collect information on factors that may be associated with increased risk of horizontal transmission of HHV-8 to the index child. The content of these questionnaires were based on discussions with focus groups conducted in March 2004 . Discussions with men and women from diverse ethnic and socioeconomic backgrounds were conducted to determine various behavioral and socio-cultural practices that could impact the risk of the index child acquiring HHV-8. Questions pertained to household living conditions (electricity, water source, toilet facilities, number of rooms/sleeping areas, household density), behaviors involving food and drinks (premastication, sharing sweets and/or drinks), health and personal care practices (example, bathing habits, oral hygiene, use of traditional medicine and the use of saliva to soothe injuries), demographic variables (sex, age, education of the primary caregiver, household size, playmates), medical history (ailments and hospitalizations) and health assessments of the primary caregiver and the child. Developmental milestones of the child were also recorded.

Preliminary results

In this study we enrolled 464 index children and 1,335 household members (from 368 households). Of the 464 index children initially enrolled, 395 children (85%) returned for one or more visits. We have analysed the baseline characteristics of the 368 enrolled complete households. Homes were generally small in size (mean - 2 rooms), with mean of 4.7 household members per household. A majority of the households were of lower socioeconomic status having no electricity, running water or in-home toilet facilities. Of the 464 enrolled index children from 368 households, 97% of caregivers were mothers to these children, while others included grandmothers, aunts, father and even cousins. A majority of primary caregivers were between 20-29 years of age (54.3%, age range - 14 to 78). Households were classified as 'two parent' if both a mother and a father to at least one index child in the household were present, 'single parent' if only one parent was available in the household (all the single parents in our enrolled cohort were mothers), and if there were no parents, or if the household included another adult such as an aunt or uncle, these households were classified as 'extended family'. Nearly half (48%) of the households were classified as either 'single parent', or 'extended family', which reflects a community continuing to suffer a high adult mortality rate from HIV/AIDS and related conditions. Most of the primary caregivers in the cohort (54%) had completed primary

education; no primary caregiver reported education beyond high school. Data analysis of factors associated with HHV-8 transmission is still ongoing.

KSHV TRANSMISSION STUDIES: 2009 TO PRESENT

Our previous cohort studies have shown that HIV-1 is a major risk factor for HHV-8 infection. We found HIV-1 infected children to have a five-fold higher risk for infection by HHV-8 as compared to uninfected children, most likely due to immune suppression as a result of HIV-1 infection. These studies were conducted when there was limited or no availability of ART in Zambia. Zambia, which is ground zero in the HIV/AIDS epidemic, has recently increased ART availability among its HIV/AIDS infected patient population. Unfortunately, the impact of ART on HHV-8 transmission and on HHV-8 disease pathogenesis is unknown. Restoration of the immune response via ART will likely reduce HHV-8 infection of HIV-1 positive children and enhance the immune response against HHV-8 in infected individuals. There is no study reported in the current literature that has focused on the impact of anti-retroviral therapy on HHV-8 transmission in children or adults. The overall goal of the current cohort study is to determine the impact of ART on HHV-8 transmission, on anti-HHV-8 immune response, and on viral reactivation. This study is of significance because it will be useful in assessing the impact of ART in HHV-8 transmission and in the development of future intervention strategies to prevent HHV-8 transmission in HIV-1 infected children (which is at largest population at risk for HHV-8 infection).

CONCLUSIONS

Our studies over the past decade on HHV-8 transmission have shown that this infection is endemic in Zambia. HHV-8 infection seems to be acquired in early childhood through household contacts and contacts from outside the household. In our cohort we observed very low rate of vertical transmission pointing to the role of horizontal transmission as the major source of transmission. Saliva and not breast milk is the likely source of horizontal transmission to young children. Taken together, these results have the potential to contribute towards the design of effective health behavior interventions for prevention of HHV-8 infection and overall community health promotion.

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