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He, Jun; Bhat, Ganapati; Kankasa, Chipepo; Chintu, Chifumbe; Mitchell, Charles; Duan, Wenjing; and Wood, Charles, "Seroprevalence of Human Herpesvirus 8 among Zambian Women of Childbearing Age without Kaposi's Sarcoma (KS) and Mother-Child Pairs with KS" (1998). *Virology Papers*. 155.
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Seroprevalence of Human Herpesvirus 8 among Zambian Women of Childbearing Age without Kaposi's Sarcoma (KS) and Mother-Child Pairs with KS

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The seroprevalence of human herpesvirus 8 (HHV-8) among a group of Zambian women of reproductive age and among mother-child pairs in which either one of them has Kaposi's sarcoma (KS) was determined. A cross-sectional group of 378 pregnant women was randomly recruited into the study, and 183 (48.4%) had HHV-8 antibodies. Among the human immunodeficiency virus (HIV)-1-infected women, 51.1% were HHV-8-seropositive, whereas of HIV-1-negative women, 47.3% were HHV-8-seropositive. In addition, 21 women index patients with KS and 5 young children index patients with KS were studied. All children with KS had mothers who were HHV-8-seropositive, while not all children whose mothers had KS were infected with HHV-8. Our study suggests that there is a high HHV-8 seroprevalence among Zambian women, and the rate is almost the same in HIV-1-positive and -negative women. This high seroprevalence may be a contributing factor toward the increased frequency of KS in this population.

Kaposi's sarcoma (KS) is a soft tissue tumor commonly found in persons with AIDS and iatrogenically induced immune deficiencies [1]. Before the AIDS era, it was known as a rare tumor-like lesion of elderly men from the Mediterranean region and geographic pockets of equatorial Africa. As the human immunodeficiency virus (HIV) epidemic progresses in Africa, the proportion of women and children with AIDS-associated KS is steadily rising, in contrast to the Western world, where AIDS-associated KS is commonly observed in HIV-1-infected homosexual men [2, 3].

Zambia lies in the southern outlying regions of the African tumor belt overlying eastern Zaire, Uganda, Burundi, and Rwanda. Before the HIV epidemic, epidemic KS was uncommon in Zambian adults and was rarely seen in children. Before 1983, the male-to-female ratio was 10–15:1. A significant rise in the incidence of adult KS was observed beginning in 1983 and coincided with the advent of the HIV epidemic. In parallel to the increase in the incidence of KS in women (male-to-female ratio dropping to 1.3:1 by 1996), a 10-fold increase in the incidence of pediatric KS has been observed. It has evolved from

negligible occurrence, of 0%–2%, in the early 1980s to constituting 20%–25% of all pediatric malignancies in 1992 [4].

A novel human herpesvirus 8 (HHV-8), also known as the KS-associated herpesvirus, is being constantly detected in biopsy samples from patients with all types of KS (AIDS-associated, endemic or African, and classical or sporadic) [5]. This finding correlates with a high HHV-8 seroprevalence found in HIV-seropositive homosexual men and fits in with the high incidence of KS in this population. However, it does not rule out the possibility that HHV-8, like other herpesviruses, is widely distributed and may be a mere passenger in these lesions. HHV-8 DNA has also been found in circulating peripheral mononuclear cells (PBMC; mostly of B cell lineage) and hyperplastic lymph nodes of immune-competent persons, strengthening the hypothesis that HHV-8 may establish persistent infection without inducing clinically evident disease [6]. HHV-8 has also been detected in saliva, PBMC, prostate, bone, and nerve tissue of AIDS patients with or without KS [7, 8]. The pathogenic importance of HHV-8 has been further strengthened by studies demonstrating that the presence of HHV-8 in PBMC of HIV-positive persons predicts the subsequent appearance of KS lesions, and seroconversion to positivity for HHV-8 antibodies predates the appearance of KS lesions [9, 10].

In a decade of increasing KS in children in Zambia, no study has shown how children acquired this disease. If HHV-8 is indeed the putative agent, then when and how do children become infected, and have they acquired the infection vertically or horizontally? The rise in pediatric KS could be related to the increase in HIV and HHV-8 infections in women. To determine the seroprevalence of HHV-8 in women and whether

Received 13 April 1998; revised 24 July 1998.

Informed consent was obtained from study participants or their parents, and the study was approved by the University Teaching Hospital Research Ethics Committee.

Grant support: NIH (AI-30356, CA-76958, CA-75903 to C.W.; TW-00015 to C.M.); Cancer and Smoking Diseases Research Program, Nebraska (98-49 to C.W.).

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The Journal of Infectious Diseases 1998;178:1787–90

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0022-1899/98/7806-0034\$02.00

HHV-8 infection is correlated with HIV-1 infection, we tested for anti-HHV-8 antibodies in a cohort of Zambian pregnant women (both with and without HIV-1).

Materials and Methods

Patient population. The first group, pregnant women aged 15–45 years, all Lusaka residents, were recruited from the Antenatal Clinic at the University Teaching Hospital in Lusaka, Zambia. The first 25 women attending the Antenatal Clinic were invited to a group discussion led by a nurse-midwife HIV counselor who explained the aims of the study in English and local languages, and patients were allowed to ask questions. Those who were interested in participating in the study received individual pre-HIV test counseling. Blood was drawn from a peripheral vein (antecubital fossa), and serum was separated by centrifugation.

The second group, women aged 15–45 years with a confirmed histopathologic diagnosis of KS, were recruited from the KS section of the Dermatovenereology Clinic. Pre- and post-HIV test counseling and testing were done. The women were encouraged to bring their children aged <10 years for blood sampling. The third group, children aged 0–14 years with a confirmed histopathologic diagnosis of KS, were recruited from the Pediatric Oncology Clinic. Their parents were counseled, and blood was taken from both the mother and child for testing. Remaining sera from all patients was frozen and shipped to the University of Nebraska for detection of HHV-8 antibodies by IFA.

HIV serology test. Two HIV-1 rapid tests (Capillus and RPD; Cambridge Biotechnology, Galway, Ireland) were done the same day, and only discordant results were confirmed by Western blot. Individualized HIV post-test counseling was done regardless of HIV serostatus.

IFA for HHV-8 antibodies. IFA was done with BCBL-1 cells (Epstein-Barr virus-negative, HHV-8 chronically infected B cell lines from body cavity lymphoma expressing HHV-8 latent antigens; obtained from D. Ganem, via NIH AIDS repository) [11]. The assay was done by procedures described by Lennette et al. [12] with minor modifications. Cells were induced into a lytic cycle with 20 $\mu\text{g}/\text{mL}$ tetradecanoyl phorbol ester acetate (TPA) for 3 days before they were harvested. About 10^4 cells were spotted, dried, and fixed onto each well of a 12-well slide. For IFA, induced cells were first incubated with patient serum samples diluted to 1:10 and 1:40 for 30 min at 37°C, followed with mouse anti-human IgG1, IgG2, and IgG3 and then with fluorescein-conjugated anti-mouse antibodies. The samples were then counterstained with 0.004% Evans blue for 5 min at room temperature to suppress nonspecific background and observed under a fluorescence microscope. Only samples with titers of $\geq 1:40$ were considered positive. All positive samples were tested with TPA-induced HHV-8-negative B lymphoma cells (D6) as control. Each sample was tested twice at varying dilutions and was scored by two laboratory workers independently. The samples were also tested by a more traditional direct IFA procedure using fluorescein-conjugated goat anti-human antibody as second antibody. Only samples with titers of $\geq 1:100$ were considered positive.

Results

This was a cross-sectional seroprevalence study carried out over a 6-week period during the first half of 1997. The objectives of this pilot study were to determine the seroprevalence of HHV-8 in a population of pregnant Zambian women and to establish whether there is concordant seropositivity among mother-child pairs, in which either one of them has KS. The cohort consisted of 3 groups: Pregnant women without KS, women with KS, and children with KS. For the first group, 378 pregnant women without KS were recruited. Among them, 275 were HIV-1-seronegative and 103 were HIV-1-seropositive. A total of 183 (48.4%) were found to have an HHV-8 titer of $\geq 1:40$ by IFA (table 1). The frequency of HHV-8 seropositivity in the HIV-negative group was 47.3%, quite similar to that of the HIV-positive group, which had a seroprevalent rate of 51.1% for HHV-8. All positive samples were tested on an HHV-8-negative B lymphoma cell line and were found to be negative. To further confirm the high seroprevalence rate in the studied population, the samples were also tested in parallel with a more traditional direct IFA technique using fluorescein-conjugated goat anti-human antibody as the second antibody. All previously positive samples were also found to be positive ($\geq 1:100$ dilution) by this traditional direct IFA. In addition, $\sim 10\%$ of the negative samples were found to be weakly positive at 1:100. Since this could not be confirmed by the indirect IFA, we considered this to be background.

The presence of anti-HHV-8 was also determined in the second and third groups of patients, women with KS and children with KS, respectively. Twenty-one women with KS and 5 young children with KS were studied. The results are summarized in table 2. We found HHV-8 antibodies in all except 2 KS patients (patients 13 and 14, table 2). These 2 patients may have antibody titers below the detection limit by our IFA. Among children from the 7 women with KS who had children (table 2), those from 3 mothers were found to be positive; however, the 1-month-old infant (daughter of patient 6) could have acquired maternal antibodies. These data suggest that not all HHV-8-seropositive women will have children who are also infected. In addition, we cannot exclude the possibility that some children have acquired infection from their mothers horizontally, and even if HHV-8 can be transmitted vertically, not all pregnancies will lead to transmission. This may be similar to HIV-1, which has a 30% vertical transmission rate. In contrast, among the 5 children with KS who were tested for HHV-8 antibodies (children 1–5), all 3 surviving mothers were infected

Table 1. HHV-8 seroprevalence in Zambian women of childbearing age without KS.

	HHV-8-positive	HHV-8-negative	Total
HIV-positive ^a	53 (51.1%)	50	103
HIV-negative	130 (47.3%)	145	275
Total	183 (48.4%)	195	378

^a HIV-1 seroprevalence is $\sim 27\%$ in general female population in Zambia.

Table 2. HHV-8 seroprevalence in Zambian women and children with clinical KS.

Age group, no.	Sex/age (years)	Clinical data	Seropositivity		Relatives	Seropositivity	
			HHV-8	HIV		HHV-8	HIV
Adult							
1	F/25	KS skin lesions, LAD	+	ND			
2	F/26	KS skin lesions	+	+			
3	F/33	KS skin & oral lesions, LAD	+	+	Daughter (5 yr)	-	±
4	F/31	KS skin lesions	+	ND			
5	F/32	KS skin & oral lesions, LAD	+	+			
6	F/28	KS skin & oral lesions	+	+	Daughter (1 m)	+	+
7	F/46	KS oral lesions	+	+	Son (6 yr)	+	-
8	F/31	KS skin lesions, LAD	+	+	Daughter (1 yr 8 m)	-	-
9	F/35	KS skin & oral lesions, LAD	+	ND	Husband	+	ND
					Son (5 yr)	+	-
10	F/32	KS skin lesions, LAD	+	+	Son (3 yr)	-	-
					Daughter (5 yr)	-	+
11	F/33	KS skin lesions, LAD	+	+			
12	F/18	Skin lesions, LAD	+	+			
13	F/36	KS oral lesions	-	ND			
14	F/20	KS, LAD	-	ND			
15	F/27	KS, LAD	+	ND			
16	F/27	KS skin & oral lesions, LAD	+	+			
17	F/26	KS skin & oral lesions, LAD	+	+	Daughter (5 yr)	-	+
18	F/19	KS skin & oral lesions, LAD	+	+			
19	F/17	KS skin & oral lesions, LAD	+	+			
20	F/34	KS skin lesions, LAD	+	+			
21	F/36	KS skin lesions	+	+			
Children							
1	M/5	KS skin lesions, LAD	+	ND	Mother	+	ND
					Sister (7 yr)	-	-
2	M/12	Oral KS lesions, emaciated, LAD	+	+	Mother (deceased)		
3	M/4.5	LAD	+	+	Mother	+	+
4	M/8	KS skin lesions, LAD, carditis	+	+	Mother	+	+
5	F/8	KS	+	+	Mother (deceased)		
					Father	+	+
Control (clinically normal mother/infant pairs)							
1	M/1	Normal	-	ND	Mother	-	ND
2	F/1	Normal	+	ND	Mother	+	ND
3	F/1	Normal	-	ND	Mother	-	ND
4	F/1	Normal	-	ND	Mother	-	ND
5	F/1	Normal	-	ND	Mother	-	ND
6	M/8	Normal	+	ND	Mother	+	ND
					Sister (6 yr)	+	ND

NOTE. ND, not determined; LAD, lymphadenopathy; yr, years; m, months; ±, weak positive.

with HHV-8. Interestingly, patient 1, the 5-year-old, has a 7-year-old sister who is HHV-8-negative. Our preliminary results thus showed that not all HHV-8-positive mothers will have children with HHV-8, but all HHV-8-infected young children or infants have HHV-8-positive mothers.

As control, we have also tested randomly 6 child-mother pairs that were all clinically normal. Two of the children were found to be positive, and both of them have seropositive mothers (table 2). One of them, a 1-year-old baby (control 2), could have acquired maternal antibodies passively.

Discussion

To our knowledge, this is the first report of a survey for HHV-8 infection in a well-characterized cohort from an African country with a high incidence of KS in both women and children. We have chosen pregnant women as our study group, as

they are usually used as a sentinel population for monitoring the trends in sexually transmitted diseases, including AIDS. The study of this group of women for HHV-8 seroprevalence will also enable us to determine whether HHV-8 infection in the heterosexual female population is also correlated with HIV-1 seropositivity, as seen in the male homosexual population. Our results suggest that there is a high HHV-8 seroprevalence in this population. Almost 50% of pregnant women without KS in our study had HHV-8 antibodies. This high seropositive rate is in agreement with previous studies that used sera with no accompanying clinical information from other African countries where HHV-8 seroprevalences of 30%–100% have been reported [13, 14]. This high seroprevalence may also reflect epidemiologic characteristics of KS in different geographic regions of the world, such as those seen in an Italian cohort, which has high incidence of classical KS [13, 15]. The correlation between HHV-8 seroprevalence and KS supports the notion

that HHV-8 plays an important role in KS development. The HIV seroprevalence of 27% found in our study correlates with urban high HIV seroprevalence of 20%–30% among Zambian women of childbearing age. The fact that the HHV-8 seroprevalence is similar in both HIV-positive and HIV-negative populations suggests that infection by HHV-8 is independent of HIV, and HIV infection did not predispose infection by HHV-8 and vice versa. However, given the high HHV-8 seroprevalence and the much lower incidence of KS in normal women, it is likely that HHV-8 infection alone may not be enough for KS development. The fact that all KS patients are HIV-positive confirms that KS in this population is epidemic (AIDS-related), and infection with HIV or immune suppression may be a necessary prerequisite for clinical expression of KS in this latently infected population.

Our HHV-8 seroprevalence data among Zambian women of childbearing age has important implications. This may explain why in Zambia, in contrast to the Western world, HIV-positive women often develop KS. The high HHV-8 seroprevalence among women of childbearing age may be linked to the rising incidence of KS in Zambian infants and young children. While epidemiologic features suggest the involvement of a sexually transmissible agent, KS in children points to a nonsexual mode of transmission, and if HHV-8 is indeed the putative agent, then like other herpesviruses, it can be horizontally transmitted from mother to child. KS has been reported in a 2-week-old infant [16], preliminary data from Kasolo et al. [17] have identified HHV-8 in a small number of infants and children, and our preliminary data have demonstrated that young children with KS were also infected with HHV-8.

Our seroprevalence studies on a limited number of mother-infant pairs also demonstrated that all HHV-8–positive children had HHV-8–seropositive mothers, but we cannot make definite conclusions at this stage because the cohort under study was small, and it is possible that the mothers could have become infected by HHV-8 subsequent to the children's birth. We propose to expand on these initial observations to a much larger cohort. With an estimated HIV seroprevalence of ~25% and HHV-8 seroprevalence of almost 50% among the Lusaka urban obstetrical population, many of these women and their offspring may develop KS. Therefore, it is of utmost importance to continue follow-up on these women and determine whether HHV-8 can be transmitted from mother to child.

Acknowledgments

The following reagent was obtained through the AIDS Research and Reference Reagent Program (Division of AIDS, NIAID, NIH):

BCBL-1 from Michael McGrath and Don Ganem. We thank P. Maitondo and the University Teaching Hospital Board of Management for their support and cooperation. We also thank Kara Alexander-Nielsen for her help in manuscript preparation.

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