

2001

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Sosa, Carlos; Benetucci, Jorge; Hanna, Colleen; Sieczkowski, Laura; Deluchi, Gabriel; Canizal, Ana Maria; Mantina, Hamakwa; Klaskala, Winslow; Baum, Marianna; and Wood, Charles, "HUMAN HERPESVIRUS 8 CAN BE TRANSMITTED THROUGH BLOOD IN DRUG ADDICTS" (2001). *Virology Papers*. 200.
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HUMAN HERPESVIRUS 8 CAN BE TRANSMITTED THROUGH BLOOD IN DRUG ADDICTS

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Abstract Human Herpes virus type-8 (HHV-8) seroprevalence was studied in a population of HIV positive intravenous drug users (IVDUs) from Argentina. Analysis of this population also indirectly made it possible to study HHV-8 blood transmission, because these individuals frequently engage in needle sharing behavior and are capable of acquiring a broad array of blood borne pathogens, including Hepatitis B/C virus. The seroprevalence of HHV-8 in IVDUs was compared to a group of non-IVDUs and HIV negative individuals. Of the 223 individuals tested, 13.45% were HHV-8 positive, 16.99% in the IVDUs group, and 5.71% in the non-IVDUs. Among HIV positive IVDUs, 25/144 (17.36%) were also HHV-8 seropositive. The seropositivity rate of HHV-8 in HIV negative IVDUs was 11.1%. In contrast, HHV-8 seroprevalence in HIV negative heterosexual individuals without drug usage behavior was even lower (5.71%). The rate of HHV-8 infection in HIV positive IVDUs was three times as high compared to the non IVDU HIV negative individuals, suggesting that IVDU is a risk for HHV-8 infection. Furthermore, it was found that IVDUs showed a very high rate of Hepatitis B/C (52.77%), which also correlate with HHV-8 infection in this population (23.68%). All Hepatitis B/C positive individuals were also HIV positive. Our data confirm other studies showing that individuals who share needles are at risk for acquiring Hepatitis B/C and HIV infections. In addition, our results suggest that they are also at risk to acquiring HHV-8 infection by the same route.

Keywords: HIV, HHV-8, IVDU, hepatitis, seroprevalence

Resumen *El virus Herpes humano 8 puede transmitirse por sangre en adictos a drogas endovenosas.* Se estudió, en la Argentina, la seroprevalencia de HHV-8 en adictos a drogas intravenosas (ADIV) HIV positivos. Este análisis también permitió evaluar, en forma indirecta, la posibilidad de transmisión del HHV-8 por la sangre, debido a que estas personas tienen la costumbre de compartir las agujas, y de esta forma pueden adquirir una amplia cantidad de patógenos que transporta la sangre, inclusive los virus de la Hepatitis B y C. La seroprevalencia del HHV-8 en los ADIV fue comparada con un grupo de individuos no ADIV y HIV negativos. Del total de 223 muestras analizadas, el 13.45% eran HHV-8 positivas; el 16.99% considerando solo los ADIV (153), y el 5.71% de los no ADIV(70). Entre los ADIV HIV positivos, 25/144 (17.36%) eran también HHV-8 positivos. El porcentaje de seropositividad de HHV-8 en los ADIV HIV negativos fue del 11.1% (1/9). A diferencia de esto, el porcentaje de seroprevalencia del HHV-8 en individuos heterosexuales, HIV negativos y sin antecedentes de haber utilizado drogas, fue inclusive más bajo (5.71%). El porcentaje de infección por HHV-8 en personas ADIV HIV positivas fue más del doble comparado con aquellos individuos no ADIV HIV negativos, demostrando que el compartir agujas es un riesgo de infección del HHV-8. Además, se halló que los ADIV presentaban un alto porcentaje de Hepatitis B y C (52.77%), lo cual, en esta población, está también relacionado con la infección por HHV-8 (23.68%). Todos los individuos con Hepatitis B y C, eran a la vez HIV positivos. Nuestra información confirma otros estudios que muestran que aquellas personas que comparten agujas, corren el riesgo de adquirir Hepatitis B, C y HIV. Además, nuestros resultados sugieren que, por la misma vía, pueden adquirir el HHV-8.

HHV-8 has been associated with all clinical forms of Kaposi's sarcoma (KS), with pleural effusion lymphomas (PEL) and with Castleman's disease. For a long time, researchers have suspected that an infectious agent could cause KS. Different viruses have been suspected to be associated with the disease, but none was

confirmed until Chang and coworkers discovered HHV-8 in 1994¹.

Despite recent intensive studies, the route of transmission of HHV-8 is still not well understood. Abundant epidemiological and serological studies have indicated a sexual transmission^{2,3}. However, sexual transmission alone does not explain the ample distribution of HHV-8 in some areas of the world; for example in some regions of Africa and Italy, where high HHV-8 seroprevalence has been observed⁴.

In addition to HHV-8 sexual transmission, the possibility of blood transmission may exist. Although the

Received: 18-V-2000

Accepted: 3-IV-2001

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virus can be found in blood during viremic stages of HHV-8 disease⁵, the transmission through blood is still controversial and has not been studied extensively. Lennette and coworkers have reported a low HHV-8 seroprevalence rate among American IVDUs, but the study group was very small and the results could be biased by the geographical distribution and the size of the population⁶. Other groups have also published limited data and found only a 3.17% HHV-8 seroprevalence in a small group of Edinburgh intravenous drug users (IVDUs)⁷. However, the risk of HHV-8 transmission by blood transfusion has been demonstrated by Blackburn et al⁸. They studied a group of blood donors and found one (out of 72 studied) who was consistently positive for HHV-8 after phorbol ester stimulation of his peripheral blood monocyte cells (PBMCs) by RT-PCR. Despite this report, the study of seroprevalence rates for HHV-8 in a large group of IVDUs has not been reported. IVDUs are an ideal population to study parenteral transmission because these individuals usually share contaminated needles, enabling a vast array of blood borne pathogens, such as hepatitis B/C virus and HIV, to be passed from one infected person to another. Therefore, the aim of this work was to study HHV-8 seroprevalence as well as to assess the risk of parenteral transmission by needle sharing in a population of HIV positive IVDUs, from a period of 1994 to 1997 in patients from Buenos Aires, Argentina.

Patients and Methods

This work was a retrospective study on HIV and HHV-8 infection performed by the University of Nebraska school of Biological Sciences and the University of Buenos Aires School of Medicine. A total of 223 men and women were enrolled in the study. A group of 223 patients with a median age of 28.3 years were studied. They were 96.13% men and 3.87% women. The majority of the individuals included in the study had less than ten years of formal education and also were of low socio-economic status. Of all individuals, 56% were from Buenos Aires province and 44% were from the Buenos Aires city. The patients attended clinics at FUNDAI, a non-governmental organization to help AIDS patients, in Buenos Aires city in Argentina. The clinics at FUNDAI provide free HIV testing as well as counseling to the general population. Participants were included in two groups. The first group consisted of 153 male and female HIV positive IVDUs at different stages of AIDS, and the second group consisting of 70 HIV non IVDUs heterosexual negative men and women. Patients attended the clinics between 1994 and 1997, when they were tested for HIV and the leftover sera stored. All eligible participants were included in the study, however, subjects who attended the clinics more than once were not re-enrolled in the study. All participants signed informed consent forms. Information was gathered by an interview at the time of visit and a questionnaire was administered to obtain socio-demographic information on HIV risk factors. Individuals who gave informed consent were tested for HIV at the FUNDAI laboratory by ELISA and Western Blot. Patients with HIV received counseling at the clinics. HHV-8 testing was not available at the clinics at that time, so

information about the HHV-8 serostatus was not given to the patients.

Samples were first screened by an in-house IFA, using tetra decanoyl phorbol acetate (TPA) stimulated cell line. Positive samples were further confirmed by a commercial IFA assay, samples were blindly tested by independent lab technicians.

In-house Immunofluorescence Assay (IFA) to detect human HHV-8 antibodies. This technique was based on a report by Lennette *et al*⁶, with minor modifications and was carried out using an HHV-8 positive cell line (BCBL-1) (from Dr. Ganem via the AIDS Repository Program), as a source of antigen. An HHV-8 negative cell line (BJAB), and an EBV producer cell line (P3HR-1) were used as controls. Cells were induced with 20 ng/ml of TPA for three days and spotted (20,000 cells) onto specially designed IFA slides (Erie Scientific). Cells were air dried inside the laminar hood and then fixed with ice cold acetone for ten minutes. Slides were then washed once in PBS and dried for IFA. Serum samples diluted in PBS were applied to individual wells on the slide and incubated for 30 min at 37 °C in a humid chamber. Two washes in PBS were performed to eliminate nonspecific binding, and an anti-human IgG_{1,2,3} monoclonal antibody (ATCC HF6508) was added to the wells and incubated at 37 °C in the humid chamber for another 30 min. After that, two more washes of PBS were performed and an anti-mouse FITC conjugate was added (Lampire Laboratories). Incubation in a humid chamber at 37 °C was carried out for 30 min, followed by two more washes in PBS. Finally, the slides were counter-stained with 0.05% Evans Blue, and mounted in a solution of PBS/glycerol (Sigma). Samples were examined using an epifluorescent Olympus BX60 microscope. All samples were coded and an inverse antibody titer of 10 or more against HHV-8 infected BCBL-1 but not against BJAB cells was considered positive.

All positive samples were confirmed using a commercial HHV-8 IFA kit (kindly provided by Dr. D. Ablashi, ABI). The assay was carried out according to manufacture's protocol.

Results

In order to study the risk of parenteral transmission, two groups of individuals were tested. The first group contained 153 IVDUs, the great majority (144/153) were HIV positive; and the second group consisted of 70 non IVDUS HIV negative heterosexual individuals. The HHV-8 seroprevalence rates between these two groups were compared.

Of the 223 individual tested, 30 (13.45%) were found to be HHV-8 positive, 26/153 (16.99%) in the IVDU population, and 4/70 (5.71%) in the non IVDU group (Table1). Among the HIV positive IVDUs, 25/144 (17.36%) were also HHV-8 positive. There were very few HIV negative individuals in our IVDU population, only 9/153 (5.88%). Among these nine only one patient was seropositive for HHV-8 (11.1%). In contrast, the HHV-8 seroprevalence in HIV negative heterosexual individuals without drug usage behavior was even lower, 4/70 (5.71%). Interestingly, the rate of HHV-8 infection in HIV positive IVDUs was almost three times as high when compared to the HIV negative individuals (17.36% vs. 5.71%), suggesting that intravenous drug usage is a risk for HHV-8 infection (O.R.=3.37; $X^2 = 4.32$, $p = 0.05$).

TABLE 1.— *Human Herpesvirus 8 positive samples in selected populations*

	n	HIV positive	HHV-8 Positive	HIV+/ HHV-8 Positive
IVDUs	153	144/153 (94.11)+	26/153 (16.99)	25/153 (16.33)
Non-IVDUs		0/70	4/70 (5.71)	0/70
Total Study	70	144/223 (64.58)	30/223 (13.45)	25/223 (11.21)
	223			

+Percent of seropositivity

Table 2.— *HHV-8 seroprevalence among Hepatitis B/C positive, HIV positive IVDUs*

	HIV Positive	HIV/HHV-8 Positive
Hepatitis B/C Positive	76/144 (52.78)+	18/76 (23.68)
Hepatitis B/C Negative	68/144 (47.22)	7/68 (10.29)

+ Percent of seropositivity

We next analyzed the relationship of other blood borne diseases (Hepatitis B or Hepatitis C) associated with intravenous drug usage in our seroprevalence groups. HIV positive IVDUs showed a very high rate of Hepatitis B or C infection, 76/144 (52.78%). In this group of 76 hepatitis and HIV dually infected individuals, 18/76 (23.68%) patients were HHV-8 infected. In contrast, in the HIV positive Hepatitis B/C negative individuals (68/144), only 7/68 (10.29%) were HHV-8 positive (Table 2). Interestingly, all Hepatitis/HHV-8 positive individuals were also HIV positive; there were no hepatitis B/C positive in our group of 70 non IVDU HIV negative men (0%).

Discussion

To our knowledge this is the first study in an attempt to determine the relationship between HHV-8 infection and IVDUs in a Latin American country. In fact, there have been no reports on the HHV-8 seroprevalence in a large IVDU population. Lennette et al have studied a relatively small group of American IVDUs and found the HHV-8 seroprevalence rate of about 23%⁶. In contrast, Simpson and coworkers studying 63 IVDUs from Edinburgh, found an HHV-8 seroprevalence of only 3.17%⁷. Our study from a large group of IVDUs from Argentina shows higher HHV-8 seroprevalence rate than those reported by Simpson, and similar to the one published by Lennette.

We have studied a group of high risk individuals who have provided us with the unique opportunity to assess

the HHV-8 blood transmission through needle sharing practices. This route of transmission has not been evaluated and it is still controversial whether this route of transmission can occur. A case of HHV-8 transmission by blood transfusion was published recently⁸, but its significance is uncertain in view of the fact that in some areas of the world such as Italy and some African countries, where there is a high rate of HHV-8 among normal blood donors^{1, 9, 10}.

We have found that the rate of HHV-8 infection in HIV positive IVDUs was about three times as high as compared to the non IVDU HIV negative individuals (17.36% vs. 5.71%), suggesting that intravenous drug usage is a risk for HHV-8 infection, and that blood transmission through needle sharing is possible. The 5.71% may reflect the seroprevalence of HHV-8 in the general heterosexual population in Buenos Aires, similar to those found in the US². Our results indicate that blood exposure is an alternative route of HHV-8 transmission and is in agreement with the report published by Mendez and coworkers, who showed that most patients with post-transplant KS were already infected with HHV-8 prior to transplantation¹¹. Those patients were exposed to blood borne diseases throughout years of chronic dialysis. In contrast, the studies reported by Beral and coworkers challenge this notion, contending that blood transfusion does not appear to represent a risk for AIDS-associated KS¹².

When we analyzed the relationship of blood borne diseases (Hepatitis B or C) associated with intravenous drug usage in our groups, we found that HIV positive IVDUs showed a very high rate of hepatitis B or C infection, 52.77%. Infection by Hepatitis B/C in this group correlated with an increased risk for HHV-8 infection. A 23.68% of the hepatitis infected HIV positive IVDUs were infected by HHV-8 as compared to 10.29% of the hepatitis negative HIV positive individuals. These data strongly support the notion that individuals who shared contaminated needles and acquired hepatitis B or C, can also acquire HHV-8 infection by the same route. These findings are provocative and suggest an alternative route of transmission, which may not be as efficient as sexual

transmission, possibly due to the low viremia rate in HHV-8 infected individuals, but may explain some of the distinct geographical distributions of HHV-8 infection.

Acknowledgments: This study was supported in part by PHS grant CA-75903 and CA-76958 to C.W.

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(Mujeres de ciencia en la Italia del siglo XVIII)*

En el siglo XVIII la ciencia experimental constituía un ámbito de investigación con continuas interacciones, que en el Instituto, propiciaban la continuidad del laboratorio. Los estudios sobre la influencia del fluido eléctrico sobre el organismo animal y el uso terapéutico de la electricidad conocieron un enorme desarrollo en la segunda mitad del siglo XVIII. En ese período, durante el papado de Benedetto XIV, el Instituto de la Ciencia pudo adquirir numerosos instrumentos y aparatos para la observación y la experimentación científica. Una serie de medidas legales del Papa permitieron enriquecer el patrimonio instrumental del Instituto. Entre los aparatos se encuentra un generador eléctrico con un globo de vidrio. La Universidad y el Instituto de la Ciencia no eran la única sede de investigación científica. El gabinete privado de Laura Bassi, única mujer profesora del Instituto, tenía importantes visitantes y representó un punto de referencia para los investigadores jóvenes. Entre estos estaban Felipe Fontana y Leopoldo Caldani los que, en el salón de Bassi y su marido Veratti, desarrollaron muchos de los experimentos sobre la irritabilidad halleriana y la electricidad.

Extractado y traducido de *Guida ai Musei di Palazzo Poggi, Scienza e Arte*. Bologna: Editrice Compositori, 2001, pp 96-7