

A comparison of HIV antibody and HIV viral findings in blood and saliva of HIV antibody-positive juvenile hemophiliacs

Bradford Tucker, DDS L. Daniel Schaeffer, PhD, DDS Robert Berson, DDS
Richard Mungo, DDS, MS Robert Miller, PA Edward Gomperts, MD Donna Warfield, PhD

Abstract

The purpose of this study was to determine the presence or absence of human immunodeficiency virus (HIV) and anti-HIV antibody in the saliva of known anti-HIV positive juvenile hemophiliacs and correlate these findings with blood.

This study utilized a sample population of 13 juvenile hemophiliacs (severe hemophilia A), all of whom were anti-HIV antibody positive. Blood samples, as well as stimulated mixed and parotid saliva, were obtained and subjected to ELISA and Western blot assays as well as electron microscopy.

The results show the presence of HIV in the blood of one subject and anti-HIV antibody in all subjects. Neither HIV nor anti-HIV antibody was detected in whole or parotid saliva of the sample population.

It is now accepted that human immunodeficiency virus (HIV), formerly known as human T-cell lymphotropic virus type III (HTLV-III), is the primary cause of the disease acquired immunodeficiency syndrome (AIDS) and AIDS-related complex (ARC) (Chermann et al. 1984). The virus attacks the T-helper cells, causing the immune system to function at a much reduced level. With this decreased protection, the human body is susceptible to opportunistic infection or neoplasia (Barre-Sinoussi et al. 1983).

HIV virus has been found in a number of body fluids including blood (Groopman et al. 1984), saliva (Groopman et al. 1984), tears (Fujikawa et al. 1985), and semen (Zagury et al. 1984). The finding of HIV virus in human saliva by Groopman et al. (1984) and in saliva and parotid glands of monkeys by Gravell et al. (1984), as well as retrovirus-like particles (putatively associated with AIDS) in the salivary glands of AIDS patients by Lecatsas et al (1985) is of considerable importance to clinical practitioners of dentistry. Indeed, the discovery of HIV in saliva could possibly present a mode of horizontal transmission of the virus from patient to dentist, dentist to patient, and patient to patient. A study by Friedland et al. (1986) examined the possibility of

passing the salivary HIV virus via casual contact with household articles; however, no patient contracted the disease by this route. In 1988, however, Klein et al. reported that a dentist had contracted the AIDS virus by treating an AIDS patient. The Groopman et al. (1984) study indicated that the HIV virus was found in the saliva, but did not indicate how the saliva was collected. Therefore, it is assumed that their study was based on whole saliva. Also, in their studies, saliva may have contained occult blood, exfoliated epithelial cells, and microbial contamination.

In a study reported by Le Baron and Nehrbass (1986), dental treatment of 217 patients with diagnosed AIDS and 35 patients with ARC was described. Following a treatment time of 17 months, none of the auxiliaries or professional staff became virus or antibody positive for HIV, nor did they develop opportunistic infections during the course of treatment. Ho et al. (1986) reported in a study of parotid saliva samples from 83 patients that one tested positive for HIV antibodies. The Centers for Disease Control (CDC) in Atlanta, Georgia, still contend that the virus is not passed by casual contact (personal communication to Donna Warfield, AIDS Epidemiology Branch, June 1, 1987). There are questions, concerning the low incidence of HIV virus in the saliva of infected individuals, and whether or not the virus is in high enough concentrations to bring about a possible transmission threat. Antibodies to HIV have been isolated in whole saliva by Groopman et al. (1984) and parotid saliva by Archibald et al. (1986).

As of June 1, 1987, there have been 504 cases of AIDS in children under the age of 13 reported to the CDC (personal communication to CDC AIDS Epidemiology Branch). Of these, the putative route of HIV transmission was traced to the following: 25 were found in hemophiliacs; 56 through blood transfusions; 397 were children born to parents at risk for AIDS; and 26 unknown. The CDC does not have statistics of the total

number of children who are anti-HIV Ab positive. To date, no studies have been reported utilizing anti-HIV Ab positive children to determine the possibility of HIV transmission via saliva to dental personnel. The purpose of this study was to collect parotid saliva from 13 anti-HIV Ab positive juvenile hemophiliac patients undergoing long-term treatment at the Children's Hospital of Los Angeles, to determine the presence or absence of HIV virus or anti-HIV antibody in saliva, and to correlate these findings to whole blood.

Materials and Methods

The present study utilized a population of 13 patients at Children's Hospital of Los Angeles with severe hemophilia A (patients with less than 2% factor VIII activity) who had received treatment with commercial clotting factor concentrates, and who all became anti-HIV Ab positive. The sample population also was known to be hepatitis non-A, non-B, and hepatitis B positive. Ages ranged from 7 to 19 years. The research procedure and informed consent for the study were approved by the institutional research review committee. After the consent form was signed, blood was obtained via venipuncture to determine the status of the HIV infection. Following collection, the blood samples immediately were placed in RPMI-1640 nutrient broth containing phytohemagglutinin (PHA).

A clinical dental examination was performed on each subject, noting gingival bleeding or inflammation. A simple gingival index as developed by Loe and Silness (1967), was performed on all subjects. Teeth numbers 3, 5, 10, 15, 19, 21, 26, and 31 were evaluated and the gingival tissue surrounding each tooth was divided into 4 scoring units: distal-facial papilla, the entire facial margin, mesial-facial papilla, and the entire lingual margin. Each section was scored by the following criteria.

- 0: Normal gingivae
- 1: Mild inflammation; slight color change, slight edema, no bleeding on probing
- 2: Moderate inflammation; redness, edema, and bleeding on probing
- 3: Severe inflammation; marked redness and edema, ulcerations, tendency to spontaneous bleeding.

Scores were added for each tooth and divided by 4 which gave an index for each tooth. Scores for all teeth were added and divided by the total number of teeth to give a gingival index for the entire mouth, and related to the overall oral gingivitis index as follows:

Gingival score	Condition
0.1 - 1.0	Mild gingivitis
1.1 - 2.0	Moderate gingivitis
2.1 - 3.0	Severe gingivitis

Additionally, dental history was taken to determine the frequency of brushing and the presence and amount of bleeding that occurred at such times.

Stimulated mixed "whole" saliva was collected by having patients chew on approximately 1 g of paraffin wax and expectorating into a sterile test tube. Approximately 15 ml were collected and inoculated into RPMI-1640 nutrient broth containing PHA. Stimulated parotid saliva also was collected via parotid collection cup, utilizing standard collection procedures (Sproles and Schaeffer 1974). Approximately 15 ml of stimulated parotid saliva was collected and inoculated into RPMI-1640 nutrient broth containing PHA. All samples were sent to the CDC where Elisa and Western blot HIV assays were performed on all blood and saliva samples.

The HIV virus isolation procedure utilized co-cultivated lymphocytes with 3-day-old PHA-stimulated normal human lymphocytes in RPMI-1640 medium containing 5% interleukin-2. Additional normal human lymphocytes were added every 4-5 days. The cultures were monitored for virus replication by immunofluorescence, and particulate reverse transcriptase (RT) assays were done at intervals for 28 days. Concentrated culture fluids were assayed for particulate RT activity with synthetic template primer (A)n(dt)12-18 and either 7.5 nmol/L Mg⁺⁺ or 0.1 nmol/L Mn⁺⁺ as cation. The direct immunofluorescence test was performed with a high titer anti-HIV human serum. All cultures were examined by transmission electron microscopy (TEM). Cells were fixed for TEM with 2.5% glutaraldehyde, thin sectioned, post-fixed with osmium tetroxide, embedded in epon-araldite, and stained with lead citrate and uranyl acetate. Cultured cells were considered infected if they produced RT in the supernatant fluid, expressed HIV-specific antigens, and contained HIV-like virus particles when examined under the EM. Primary cultures of 4 different batches of normal human lymphocytes used for co-cultivation were monitored similarly to ensure absence of primary infection or laboratory contamination.

Viral antigens for Western blot were prepared by ultracentrifugation of HIV-infected culture supernates over a 30% w/w sucrose cushion (80,000 xg, 60 min). The pellets were dissolved in 0.01 M Tris, pH 8.0, containing 1% sodium dodecyl sulfate, 25 µg/ml bromophenol blue, 10% glycerol, and 5% 2-mercaptoethanol, and heated at 65° C for 30 min to inactivate complement factor. T-cell phenotypes were determined by indirect immunofluorescence with fluorescence-activated cell sorter using commercial monoclonal antibodies (OKT3,

Results

HIV virus was detected in the blood of only one patient while all patients were anti-HIV Ab positive. No virus was detectable in either the stimulated whole or parotid saliva samples from this population. Anti-HIV Ab status was negative for saliva. No spontaneous or mechanically caused gingival hemorrhage was noted during collection saliva samples from this population. Anti-HIV Ab status was negative for saliva. All patients exhibited normal oral hygiene, no oral lesions or opportunistic infections. The clinical findings, HIV presence, hepatitis status, and HIV antibody presence in blood and saliva are summarized in the Table.

Discussion

Results of this study corroborate those of Ho et al. (1986), indicating that the presence of HIV virus is not as prominent in the saliva as has been previously reported (Groopman et al. 1984). The virus was not found in either the whole or parotid saliva of our cohort of patients. We feel that this may be due to the lack of occult blood (gingival hemorrhage) in the saliva of these children, since all had gingival indices of 1.0 or less. All previous studies which have found the virus in whole saliva were concerned with adult subjects. In adults, gingival hemorrhage is much more prominent and is principally due to advanced gingivitis and periodontal disease occurring at approximately 35 years of age (Karayiannis et al. 1985). The practical question has been

raised as to whether or not the practitioner can contract AIDS while treating a juvenile AIDS patient. Preliminary data from this study indicate that children are less likely to exhibit salivary manifestations of AIDS, presumably due to healthier periodontium and resultant lack of gingival inflammation and bleeding.

As shown in the Table, all the subjects exhibited 2 types of hepatitis (hepatitis B and non-A, non-B hepatitis). This is of concern to all health professionals in treating juvenile hemophiliacs, especially since the titers of hepatitis-associated virus in blood is infinitely higher in comparison to HIV (Ho et al. 1986).

The hepatitis B surface antigen (HBsAg), indicating chronic virus infection, has been found in the saliva as has been the viral DNA, but this again was from mixed whole saliva (Karayiannis et al. 1985). This study did not rule out presence of occult blood in the saliva samples. A study by Shikata et al. (1985) was unable to find the hepatitis B core antigen (HbcAg) in the parotid which would have indicated the replication of hepatitis virus in the gland. They concluded that the finding of HBsAg in the saliva was probably due to capillary leakage of serum HBsAg into the saliva. The Shikata study, as well as the present one, seems to indicate that saliva may not be an important mode of HIV virus transmission in juvenile hemophiliacs with no or mild gingivitis.

The transmission of hepatitis B rather than HIV should be of greater concern to health professionals. Interestingly, as of June, 1987, the CDC had reported 36,058 AIDS cases; whereas it reported 23,457 hepatitis A cases; 26,453 type B cases, and 4,192 non-A, non-B cases.

TABLE. Hepatitis and HIV Antibody and HIV Viral Status in Blood and Saliva of Juvenile Hemophiliacs

Patient	Clinical Findings			Hepatitis Status		HIV Virus and HIV Antibody Status						
	AIDS ¹	ARC ¹	Gingival Index ²	A	B ³	Non A Non B	Blood HIV ⁴	Ab ⁵	Saliva Parotid		Saliva Whole	
									HIV	Ab	HIV	Ab
1	-	-	0.15	-	+	+	+	+	-	-	-	-
2	-	-	0.0	-	+	+	-	+	-	-	-	-
3	-	-	1.0	-	+	+	-	+	-	-	-	-
4	-	-	1.0	-	+	+	-	+	-	-	-	-
5	-	-	1.0	-	+	+	-	+	-	-	-	-
6	-	-	0.25	-	+	+	-	+	-	-	-	-
7	-	-	1.0	-	+	+	-	+	-	-	-	-
8	-	-	0.25	-	+	+	-	+	-	-	-	-
9	-	-	1.0	-	+	+	-	+	-	-	-	-
10	-	-	0.25	-	+	+	-	+	-	-	-	-
11	-	-	0.0	-	+	+	-	+	-	-	-	-
12	-	-	0.75	-	+	+	-	+	-	-	-	-
13	-	-	0.0	-	+	+	-	+	-	-	-	-

¹ AIDS and ARC clinical findings determined according to AMA and ADA Guidelines.¹⁰

² Gingival index as described by Loe and Silness.

³ Anti HBsAg.

⁴ HIV virus.

⁵ Anti-HIV antibodies.

These statistics lead to the current phobias of health professionals in treating AIDS patients. Also, the high mortality rate of AIDS, approaching as high as 100%, lends credence to the concern. A health professional utilizing barrier protection methods, as recommended by ADA guidelines (Le Baron and Nehrbass 1986) should be adequately protected from AIDS and hepatitis infection.

The question remains as to whether there is a correlation between the presence of hepatitis B or non-A, non-B hepatitis virus to the HIV virus. This is of particular interest as none of the juveniles in this study exhibited any clinical signs or symptoms of AIDS or ARC. Additional studies currently are underway to assess the relationship of HIV virus and antibody status in saliva derived from ARC and AIDS juvenile patients.

Conclusions

1. From the present study, neither HIV viruses, anti-HIV antibodies, nor antihepatitis antibodies were detectable in whole and parotid saliva of juvenile hemophilia A subjects. All patients were anti-HBsAg and hepatitis non-A, non-B positive.
2. Lack of HIV virus in saliva of this study indicates that saliva is not an important mode of transmission.
3. Patients with normal gingival indices in this study indicate that occult blood in saliva may be an important factor in HIV virus transmission. Future investigations will examine this possibility.
4. Further research is needed to attempt isolation of the HIV virus from the saliva of clinically diagnosed ARC and AIDS juvenile and adult patients and to determine if salivary enzymes deactivate the HIV virus or HIV antibody.

Dr. Tucker is a resident in pediatric dentistry, Dr. Berson is an attending dental service staff member, Dr. Mungo is dental service chief, Mr. Miller is a physician's assistant in hematology and oncology, and Dr. Gomperts is chief of hematology and oncology, all at Children's Hospital of Los Angeles; Dr. Schaeffer is an associate professor, basic sciences, University of Southern California; and Dr. Warfield is a staff virologist, Centers for Disease Control, Atlanta, Georgia. Reprint requests should be sent to: Dr. L. Daniel Schaeffer, Dept. of Basic Sciences, School of Dentistry, University of Southern California, Los Angeles, CA 90089-0641.

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