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Detection of Specific Antibodies in Gingival Crevicular Transudate by Enzyme-Linked Immunosorbent Assay for Diagnosis of Human Immunodeficiency Virus Type 1 Infection

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The purpose of this open and multicenter trial was to determine the usefulness of antibody detection by enzyme-linked immunosorbent assay (ELISA) in gingival crevicular transudate (GCT), which was collected with an investigational device (Orasure; Epitope, Beaverton, Oreg.), for the diagnosis of human immunodeficiency virus type 1 (HIV-1) infection and to compare it with antibody detection in serum. A total of 1,880 individuals were tested, as follows: 354 HIV-1-infected individuals (111 asymptomatics individuals and 243 individuals with AIDS), 46 individuals with autoimmune diseases (AD), 296 individuals with dental diseases, 42 individuals with other chronic diseases, and 1,142 healthy individuals. Sera from 356 individuals and GCT from 354 individuals were positive for HIV-1 antibodies. There were two false-negative gingival samples, one from an HIV-1-positive asymptomatic individual and one from a patient with AIDS. HIV-1 antibodies were unexpectedly detected in both serum and GCT of two individuals, one with dental disease and one with pulmonary tuberculosis. None of the sera or GCTs from healthy subjects or patients with AD were positive. Compared with the serum assay, the sensitivity, specificity, and positive and negative predictive values of the GCT assay were 99.5, 100, 100, and 99.9%, respectively. Of 355 paired serum-GCT samples that were HIV-1 positive by ELISA and that were tested by Western blot (immunoblot), all were positive for HIV-1 by using the U.S. Public Health Service interpretation criteria, while among gingival samples, 301 were positive, 52 were indeterminate, and 2 were negative. Of 82 negative paired samples selected at random, 80 were negative by Western blotting of serum and GCT and 2 were indeterminate by Western blotting of serum and negative by Western blotting of GCT (a healthy blood donor and a patient with dermatopolymyositis). Testing for HIV-1 antibodies in GCT is a simple and reliable screening procedure in populations with high and low prevalences of infection because of the high sensitivity and specificity of the method, and it offers improved safety for hospital personnel.

The diagnosis of human immunodeficiency virus type 1 (HIV-1) infection is usually done by such methods as enzyme-linked immunosorbent assay (ELISA), Western blot (immunoblot), and immunofluorescence assay, which detect specific serum antibodies against viral proteins. While there may be slight differences in results depending on the procedure used and the laboratory performing the test, most current methods are highly specific and sensitive (17).

For individual cases and epidemiological purposes, the drawing of blood samples has some disadvantages and poses a potential risk for the personnel collecting them. Testing of gingival crevicular transudate (GCT) could be a solution that would avoid this risk and a patient's unwillingness to provide a blood sample (15).

It is well known that HIV-1 is rarely isolated from saliva (7) and that isolation of the virus from saliva is probably related to contamination of the saliva with blood; thus, the possibility of transmission by this route is highly improbable. On the other hand, the presence of HIV-1 antibodies has been demonstrated in the saliva of infected individuals (2, 4, 6, 10a).

Comparative trials that evaluate the use of whole saliva by various diagnostic methods (radioimmunoassay, ELISA, Western blot, and immunofluorescence assay) show high specificities (98 to 100%) and variable sensitivities (82 to 98.5%) in groups with a prevalence of HIV-1 infection greater than 0.5% (1, 2, 4a, 10, 10b, 11, 15, 16, 19). However, in groups with a low prevalence of infection, the predictive value of HIV-1 detection in serum is lower, and no extensive studies with saliva have been carried out.

Diagnosis of HIV-1 infection is based on the detection of specific immunoglobulin G (IgG) in serum. Since whole saliva consists mainly of secretory IgA and IgG levels are only 1% of those in serum (6a, 8, 16), there is great concern with regard to the use of whole saliva for diagnostic purposes. On the other hand, GCT contains mainly IgG in concentrations similar to those in serum (11), and therefore, it could be used for diagnostic procedures on the basis of detection of specific IgG antibodies. Recently, a device has been developed to collect GCT for diagnostic purposes and has been approved by the U.S. Food and Drug Administration for clinical trials (13).

The purpose of this study was to determine the usefulness of specific antibody detection in GCT for the diagnosis of HIV-1 infection and to correlate these results with those obtained with serum from HIV-1-infected patients, patients

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with other diseases, and healthy individuals in an open, comparative, observational, and multicenter trial.

MATERIALS AND METHODS

Study population. Study subjects were recruited from two hospitals, the National Institute of Nutrition and the Hospital of Infectious Diseases from the Centro Médico "La Raza" of the Mexican Social Security Institute and from the dental clinic of the School of Dentistry, National University of Mexico, to constitute the following groups: asymptomatic HIV-1-positive individuals (Centers for Disease Control groups II and III), patients with AIDS (Centers for Disease Control group IV), healthy blood donors and health workers, patients with autoimmune diseases, dental clinic patients, and patients with chronic illnesses.

For the HIV-1-infected groups, patients were required to have two separate ELISAs positive for HIV-1 or one ELISA and another test positive for HIV-1. All individuals participating in the study signed an informed consent and were questioned for demographic data, presence of oral pathology, and for the first two groups, risk factors for HIV-1 infection. Subjects were excluded if they had had any food or drink or had brushed their teeth or had mouthwashed within 1 h prior to sample collection.

Samples. Blood samples were obtained by venipuncture. GCT was collected with an investigational device (Orasure; Epitope, Beaverton, Oreg.), consisting of a chemically treated cotton fiber pad that was placed between the lower gum and cheek, rubbed back and forth, and then kept in place for 2 min. The pad was then transferred to a tube containing a preservative solution; the tube was centrifuged at 454 × *g* for 10 min and stored at 4°C for up to 7 days or at −20°C when it was kept longer. GCT and serum samples were collected from each patient.

ELISA. A commercial kit (Organon Teknika, Durham, N.C.) was used to detect HIV-1 antibodies in both serum and GCT by the manufacturer's recommendations; dilutions of 1:75 and 1:2, respectively, were used. The technician was unaware of the study group to which each sample belonged. Results are expressed as the ratio obtained by dividing the optical density (OD) of a tested sample (serum or GCT) by the cutoff value obtained on the same day. A sample was considered positive when the ratio was ≥1.0.

Western blot. A commercial kit (Organon Teknika, Baxtel, Holland) was used to test all samples that were HIV-1 positive by ELISA, those paired samples (serum-GCT) with discordant ELISA results, and 82 negative paired samples selected at random; 1:50 dilutions were used for serum and 1:40 dilutions were used for GCT. The chromogen was added to the strips and was kept in place for 10 min in serum samples and 30 min in GCT samples. The U.S. Public Health Service criteria were used to interpret Western blot results (3). Briefly, a positive result was the presence of any two of the following bands: p24, gp41, and gp120-gp160; an indeterminate result was the presence of any other band(s) that failed to meet the positive criteria, and a negative result was the absence of all bands.

Statistical analysis. Chi-square, Student's *t*, and Pearson's correlation coefficient tests were used for statistical analysis.

RESULTS

Demographic data. A total of 1,880 individuals were included in the study, as follows: 111 HIV-1-positive asymptomatic individuals, 243 patients with AIDS, 1,142 healthy blood donors; 46 patients with autoimmune diseases (25 patients with systemic lupus erythematosus, 4 patients with rheumatoid arthritis, 4 patients with mixed connective tissue diseases, 3 patients with myasthenia gravis, 3 patients with active chronic hepatitis, 2 patients with scleroderma, 1 patient with Sjögren's syndrome, 1 patient with Graves' disease, 1 patient with polyarteritis, 1 patient with thrombocytopenic purpura, and 1 patient with pure erythrocyte aplasia), 296 dental clinic patients, and 42 patients with other chronic diseases. There were 1,104 men and 776 women, with a mean age of 31.1 years (range, 1 to 80 years). A history of smoking was reported by 628 individuals. Associated oral pathologies were as follows: gingivitis in 95 patients, candidiasis in 90 patients, herpes simplex virus infection in 30 patients, ulcers in 26 patients, dental abscess in 15 patients, and neoplasia in 2 patients. Risk factors for HIV-1 infection in asymptomatic HIV-1-infected individuals and patients with AIDS were as follows: male homosexuality in 236 individuals, history of blood transfusion in 62 individuals, heterosexual promiscuity in 34 individuals, bisexuality in 10 individuals, and unknown in 12 individuals.

TABLE 1. HIV-1 antibodies detected by ELISA in serum and GCT

Study group	No. of subjects	No. of subjects with the following HIV-1 ELISA result:			
		Positive		Negative	
		Serum	GCT	Serum	GCT
Asymptomatic subjects	111	111	110	0	1
Patients with AIDS	243	243	242	0	1
Blood donors and health workers	1,142	0	0	1,142	1,142
Patients with autoimmune disease	46	0	0	46	46
Dental clinic patients	296	1	1	295	295
Patients with other diseases	42	1[a]	1[a]	41	41
Total	1,880	356	354	1,524	1,526

[a] A patient with pulmonary tuberculosis.

ELISA. Among the 1,880 subjects tested, the sera of 356 subjects were positive for HIV-1 antibodies and the GCTs of 354 subjects were positive for HIV-1 antibodies (Table 1). There were two false-negative GCT samples, one from an HIV-1-positive asymptomatic individual and one from a patient with AIDS. HIV-1 antibodies were unexpectedly detected in both the sera and GCTs of two individuals, one from the dental clinic and one with pulmonary tuberculosis, who were not previously known to be HIV-1 seropositive; in both cases the Western blotting of serum and GCT was positive for HIV-1. None of the sera or GCTs from blood donors, health workers, or patients with autoimmune diseases were positive for HIV-1 antibodies. Compared with the serum assay, the sensitivity, specificity, and positive and negative predictive values of the GCT assay were 99.5, 100, 100, and 99.9%, respectively. The means of the OD ratios that were >1 were 3.48 ± 1.39 for GCT and 4.65 ± 0.84 for serum; the means of the OD ratios that were <1 were 0.32 ± 0.12 and 0.37 ± 0.11, respectively. A high correlation was observed between HIV-1 antibody levels in serum and GCT ($r = 0.9091$; $P < 0.0001$) (Fig. 1).

Western blot. All but one pair of samples that were HIV-1 positive by ELISA were tested by Western blotting. Of 355

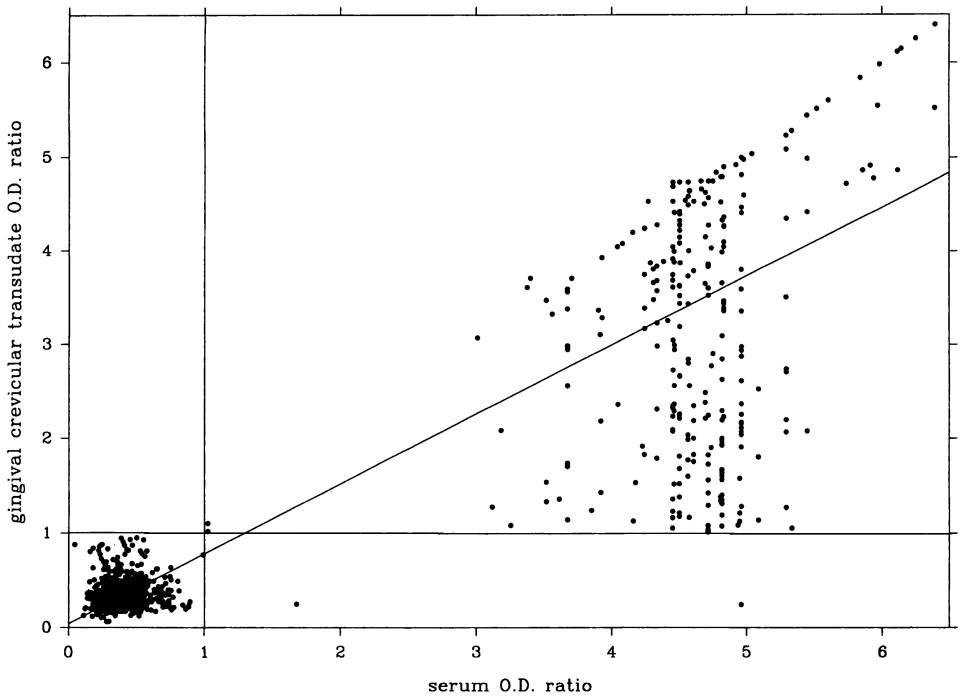

FIG. 1. Correlation between antibodies against HIV-1 in GCT and serum. Values are expressed as the OD ratio obtained by dividing the actual OD value of GCT or serum by the cutoff value obtained on the same day (Pearson's correlation coefficient, $r = 0.09091$; $P < 0.0001$).

paired serum-GCT samples, all sera were positive and 301 GCTs were positive, 52 GCTs were indeterminate, and 2 GCTs were negative. However, gp160 and gp120 envelope glycoproteins were present in the GCTs of at least 95% of those seropositive individuals (Table 2), while *gag* and *pol* gene products, especially p18 and p55, were significantly less common in GCT. Of 82 negative paired samples selected at random, 80 were negative for HIV-1 antibodies in serum and GCT and 2 were indeterminate for HIV-1 antibodies in serum and negative for HIV-1 antibodies GCT. A p24 band was present in the serum of a healthy blood donor and a patient with dermatopolymyositis. The presence of the p24 band in both serum and GCT was significantly less frequent in patients with AIDS than in HIV-1-infected asymptomatic individuals (Table 3).

TABLE 2. Western blot bands in 355 paired serum-GCT samples from HIV-1-infected asymptomatic individuals and patients with AIDS

Band	Percent in:			
	Serum		GCT	
	Asymptomatic individuals	Patients with AIDS	Asymptomatic individuals	Patients with AIDS
p18	74	52	10	2
p24	96	90	65	56
p31	97	48	37	12
gp41	99	100	85	88
p51	96	93	41	17
p55	47	23	3	2
p65	97	94	50	29
gp120	100	100	95	95
gp160	100	100	95	99

DISCUSSION

Our data confirm and extend previous observations of detection of HIV-1 antibodies in oral fluid (whole or parotid saliva) (1, 2, 8–10) and suggest that detection of HIV-1 antibodies in GCT obtained with an investigational device (Orasure) is a promising screening procedure for the diagnosis of HIV-1 infection in populations with either a high or a low prevalence of infection. It is also an easy and inexpensive method for epidemiological studies and is valuable as a noninvasive procedure. This is the first large open study done to analyze the usefulness of GCT for the diagnosis of HIV-1 infection by means of ELISA and Western blotting. Previous studies have been done with a limited number of selected samples of whole saliva. The sensitivity of the ELISA in those studies, when comparing simultaneously paired serum and whole-saliva samples, ranged from 82 to 98.5% (1, 2, 8, 10, 10b, 11), which is lower than that observed in our study of GCT. Although comparative stud-

TABLE 3. Characteristics of p24 band on Western blotting of serum and GCT samples in HIV-1-infected asymptomatic individuals and patients with AIDS

p24 band	No. (%) of samples in[a]:			
	Asymptomatic individuals		Patients with AIDS	
	Serum	GCT	Serum	GCT
Positive	102 (91.9)	57 (51.4)	163 (67.1)	85 (35.0)
Weakly positive	4 (3.6)	14 (12.6)	53 (21.8)	41 (16.9)
Negative	5 (4.5)	40 (36.0)	27 (11.1)	117 (48.1)
Total	111	111	243	243

[a] For serum, $\chi^2 = 25.663$ and $P = 0.0000027$; for GCT, $\chi^2 = 8.502$ and $P = 0.014$.

ies with whole saliva and GCT will be necessary to define the true sensitivity of the ELISA in these fluids, it is possible to explain this difference by the fact that the IgG content of GCT is several times greater than that of saliva (12). Furthermore, a recent study that analyzed saliva and GCT obtained with the Orasure device demonstrated that concentrations of IgG in transudate were four times greater than those in saliva (5). However, the two false-negative results found in this study may limit use of GCT testing for accurate diagnosis; nevertheless, it may be useful for screening children or the general population and may be especially useful for screening by insurance companies, for admission to the armed services, and of health care employees.

Results of the Western blot with GCT in this study differed from those of a previous study with whole saliva (10). We observed a substantial number of indeterminate results, which could be explained by a dilution factor, since in our study a 1:40 dilution of GCT was used; a lower dilution may therefore be needed. Another explanation could be the very low titers of IgG antibodies to the *gag* and *pol* antigenic determinants present in GCT (Table 2).

The predominance of gp120 and gp160 bands in GCT may be explained by the local production of specific IgG (6a, 18); and it is also possible that the gp120-gp160 bands are more immunogenic than the other HIV-1 proteins, and thus, the elicited response is more intense (11a). To overcome this lower sensitivity obtained by using GCT, it may be necessary to restandardize the dilutions suggested by the manufacturer. Preliminary studies in our laboratory suggest that dilutions can be lowered while still maintaining good specificity and further increasing sensitivity.

Another interesting finding in the Western blot (Table 3) was that the p24 band was seen significantly less frequently in both the sera and the GCTs of patients with AIDS than in the sera and GCTs of asymptomatic individuals. The loss of anti-p24 antibodies may be an early sign of progression of HIV-1 infection, as has been suggested by others (14, 16).

The accuracy of testing of GCT was not modified by the presence of oral lesions or a history of smoking. The collection device that we used greatly reduces the possibility of contamination with sputum and modifies dilutional problems, thus enhancing immunoglobulin collection (5). Also, it was possible to use the GCT sample directly in the assay without previous manipulation, thus decreasing the time and labor requirements of the test.

In conclusion, because of the ease of collection and handling and high sensitivity and specificity, testing for HIV-1 antibodies in GCT represents an inexpensive and reliable screening procedure, regardless of the rate of prevalence of HIV-1 infection in the population under study. It also has the advantage of offering increased safety for hospital personnel who perform sample collection and processing.

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