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Detection of anti-HIV antibodies in saliva

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It is sometimes difficult in clinical practice to identify carriers of the AIDS virus. Such identification is of unquestionable value in oral pathology, both for determining the pathogenesis of certain lesions and for assessing their significance to the patient. We evaluated several commercially available diagnostic techniques for the detection of anti-HIV antibodies in serum, and examined the feasibility of adapting such techniques to tests on saliva. The technique chosen for experimental adaptation required only slight modifications for use with this medium. We compared the results obtained in serum from intravenous drug users with a western blot assay designed to detect p24 viral protein, against the findings with a test designed to detect salivary antibodies. The likelihood of cross-reactions in saliva containing high concentrations of other antiviral antibodies was also studied. The specificity and sensitivity of the modified saliva test were 100% and 96% respectively, and no cross-reactions were observed.

Key words: anti-HIV antibodies; HIV-infection; saliva

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In the course of clinical practice and extensive screening programs it is not uncommon to encounter problems in identifying carriers of the AIDS virus (1). Although these patients may refuse to provide blood samples for fear of being denied treatment, it is nonetheless true that the identification of these subjects is of unquestionable value in professional practice, particularly in the area of oral pathology, in order to determine the pathogenesis of certain lesions and to assess the significance of such lesions for the patient.

A given lesion may reflect a banal infection, or may be a warning signal of the development of an established immune deficiency syndrome. For example, oral candidiasis may have some predictive value in identifying AIDS patients (2, 3). Moreover, these patients may also have severe hematological and immunohematologic alterations that lead to the formation of anti-thrombin and anti-platelet autoantibodies, causing severe platelet deficiency (4). This, together with the changes of causing severe infection, occasionally means that extraction may lead to a general worsening of the patient's condition.

However, because of the social stigma associated with HIV-positive status, patients are sometimes unwilling to advise others that they are carriers of the AIDS virus. The fear of being refused treatment, or of being the object of the health professional's discomfiture (5), reinforces the tendency to maintain anonymity.

The current problems with testing for viral antibodies or antigens (6), and the demands of these techniques in terms of specialized skills and time required, make them unfeasible in routine clinical practice. Most practices lack the infrastructure necessary to obtain and interpret the results. Problems in obtaining serum, the patient's refusal to provide a blood sample, and the dangers inherent in working with highly contaminated materials (7), are all factors that stand in the way of a straightforward diagnostic approach that would be informative as to the prognosis for a given lesion and would allow the clinician to choose the most suitable treatment option.

The objectives of the present study were: 1) to assess the usefulness of saliva, a readily available biologic fluid, in diagnosing HIV status, 2) to search for a technique able to detect anti-HIV antibodies in saliva, and 3) to compare the reliability and accuracy of the results in saliva with serologic tests.

Material and methods

Several commercially available diagnostic techniques for the detection of anti-HIV antibodies in serum were considered. An Abbot kit was chosen as the most appropriate for the present study. The study population consisted of 50 HIV-positive and 50 HIV-negative intravenous drug users whose HIV status was determined by serologic analysis. Saliva and serum samples were obtained from all subjects and were frozen until analysis.

Analytical technique

A sandwich indirect immunoassay, with a second alkaline phosphatase-conjugated antibody, was used. The immunoassay device consisted of a small disposable plate with a well set in several layers of white support material and a filter. Viral proteins obtained by recombinant DNA techniques were located along the vertical arm of a cross printed in the bottom of the well. The horizontal arm

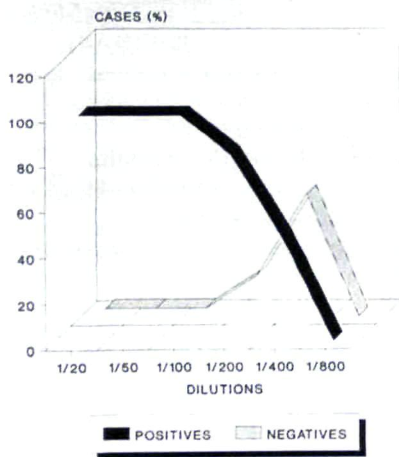

Fig. 1. Percentages of positive (solid curve) and negative (hatched curve) samples at each dilution.

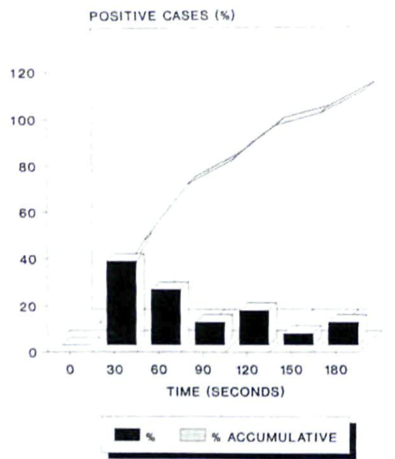

Fig. 2. Progressive incubation time with second antibody and percentage of HIV-positive samples correctly identified with indirect immunoassay performed with saliva after different periods of incubation.

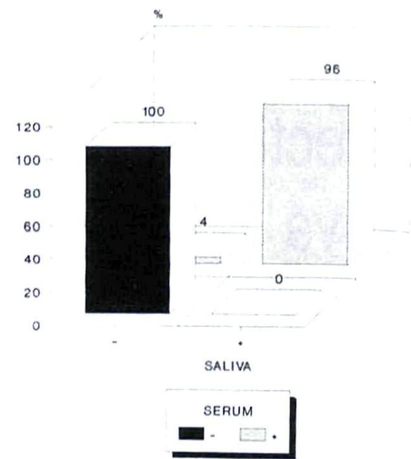

Fig. 3. Correlation between the results produced by the indirect immunoassay technique with saliva and serum.

contained unspecific human antigens as a control; the horizontal arm only was stained in negative tests, while both arms were stained in positive tests. The addition of the saliva sample diluted in buffer solution (0.1% sodium azide and Tween-20 as preservatives), together with a wetting agent to eliminate surface tension of the fluid, allowed binding of the anti-HIV antibodies to viral proteins.

After washing (Immunotech), the second alkaline phosphatase-conjugated goat antihuman Fc antibody was added. If the test was positive, the second antibody became bound to immunocomplexes formed in the solid phase along the vertical arm of the cross. The reaction was developed by adding a substrate that reacted with an enzyme to produce a violet color in the vertical arm of the cross, appearing against the white background as a positive result. The entire test took 6–8 min to perform.

The results were compared with the findings obtained with serum assays for the determination of p24 viral protein using western blot and with the results obtained using serum instead of saliva in the indirect immunoassay described above.

Results

The technique required minor adaptations for use with saliva. A series of dilutions of saliva from HIV-positive subjects with circulating antibodies was tested to determine the lowest concentration that yielded a positive result in the solid phase.

Fig. 1. illustrates the percentages of positive samples (solid curve) at each dilution. HIV proteins were correctly detected at dilutions of 1/20, 1/50 and 1/100; the percentage of positive results decreased at dilutions greater than 1/100. Negative results were obtained with dilutions of up to 1/400; at greater dilutions the results became difficult to read.

The duration of the substrate-enzyme reaction also influenced the results. We determined optimal incubation time with the second antibody, and also the percentage of HIV-positive samples that were correctly identified with indirect immunoassay performed with saliva after different periods of incubation. Repeated readings for up to 180 s led to the identification of all positive results, which correlated well with the number of positives detected by serological analysis (Fig. 2).

The correlation between the results produced by the indirect immunoassay technique with saliva and serum is shown in Fig. 3. The bar chart illustrates the number of seronegative cases that were also identified as negative by the saliva test (solid bar). The saliva test produced no false positives. The hatched bar shows the number of cases in which anti-HIV antibodies were detected in both serum and saliva with the indirect immunoassay test. The test performed with saliva gave a false negative reading in two cases.

We also compared the results obtained with western blot analysis for the detection of p24 viral protein in serum and saliva (Fig. 4). The sensitivity of the assay with saliva approached that of serum; only two cases in which viral protein was detected in serum yielded negative results with saliva. Specificity was 100% when saliva was used (i.e., the chances that saliva from a normal subject would produce a false positive result were 0%).

We also studied the possibility that the saliva test might show cross-reactions with samples from subjects who were not carriers of HIV, but who did have high salivary concentrations of antibodies against other viruses. Fifteen subjects with high salivary concentrations of anti-type 1 herpes simplex virus (HSV) IgG and anti-CMV (cytomegalovirus) IgG were further tested. Fig. 5 shows that no false positives for HIV were detected by the saliva test.

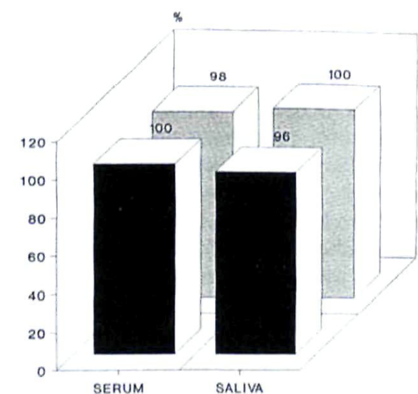

Fig. 4. Specificity and sensitivity of the saliva test and of western blot analysis for the detection of p24 viral protein in serum and saliva.

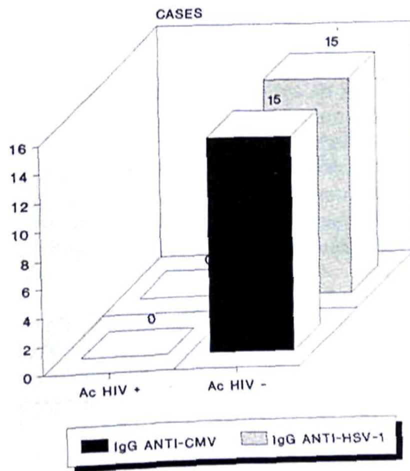

Fig. 5. Absence of cross-reaction in saliva samples from subjects who were not carriers of HIV, but who did have high titers of anti-HSV-I IgG and anti-CMV IgG antibodies.

Discussion

The number of carriers of the human immunodeficiency virus is rising, and many carriers who belong to none of the easily identifiable risk groups will develop AIDS in the coming years. This makes it necessary to develop alternative screening techniques for use in large populations (6), and underscores the importance of finding a readily obtainable but non-contaminating medium to be tested in such screening programs. ARCHIBALD & COLE (8) described the presence, in all buccal salivary glands except the parotid, of factors that curtail viral replication. O'SHEA *et al.* (9) found a lower concentration of virus in the saliva. Although other authors (10, 11) have claimed that contamination can occur via saliva, the evidence noted above (8, 9) suggests that the infectious capacity of saliva is so low as to be considered negligible, as is borne out by the exceptionally low percentages of confirmed cases.

The need for a readily obtainable medium of low contaminating potential is obvious; in addition, such a medium should reliably reproduce the prevalence of antibodies detected in serum (9). The results of the present study show that in 50 subjects in whom the presence of HIV had been confirmed in serum, 46 again tested HIV-positive in analyses of saliva. Most commercially available techniques state their usefulness, specificity and sensitivity with reference to western blot analysis. We also compared our findings with those obtained by western blotting; our saliva test achieved 96% sensitivity (the ability to detect HIV in subjects for whom serological analysis had revealed viral proteins p24, GP 120 and GP 160). This figure seems high enough to make our method useful for routine screening in individual practice, hospitals and clinics.

Several earlier studies recommended the use of saliva for population-related research. ARCHIBALD & HEBERT (12) showed that 20 of 21 samples of saliva from seropositive patients contained measurable concentrations of antibodies against one of the six viral antigens used.

These authors also checked their findings against data obtained with western blotting, and reported similar results with the two methods. MAJOR *et al.* (13) recently recorded 98.35% sensitivity and 100% specificity with saliva tests for HIV prevalence.

The pioneering work of PARRY *et al.* (14) and JOHNSON *et al.* (15) in the use of saliva as an alternative to serum deserves particular mention. Using a technique similar to that described in the present study, they obtained 100% sensitivity and 99.8% specificity. These findings contrast somewhat with our results, in which sensitivity was lower than specificity. An earlier study in our laboratory with the Genelavia MIXT technique (Pasteur) demonstrated a highly significant association ($p<0.001$) between the findings in serum and saliva. This study involved a total of 150 subjects, 75 of whom were seropositive, and 75 healthy controls (16). However, because of its ease of implementation, the Abbott technique assayed in the present study is more suitable for daily clinical practice.

CAO *et al.* (17) compared antibody titers in urine and serum with western blot and enzyme-linked immunosorbent assay (ELISA) techniques, using urine that was concentrated 200-fold. They observed no significant differences between concentrated and unconcentrated urine, and reported sensitivity as 90% or lower with urine ELISA. The sensitivity of western blotting was similar regardless of whether urine or serum was tested, but only for viral proteins GP 120 and GP 160. These authors speculated that urine rather than serum samples may be of use for population studies in parts of the world such as Africa, where working with serum is problematic.

Of the studies that have examined the feasibility of using saliva to diagnose HIV, none published to date has dealt with the potential problem of cross-reactions in subjects with high salivary titers of antibodies against other viruses. To rule out this possibility, we examined saliva from 15 subjects (not part of the original study population) in whom high concentrations of anti-type 1 SHV IgG and anti-CMV IgG had previously been documented (18). The presence of HIV in serum was ruled out, and in no subject were HIV antigens detected in saliva.

Studies published to date have been limited in scope, reporting sensitivity and specificity rates for given techniques with reference to serum antibody titers (19). Sensitivity and specificity rates might be expected to differ between tests on saliva from subjects with low serum concentrations of anti-HIV antibodies and seropositive subjects with high serum concentrations; this hypothesis is currently under investigation in our laboratory.

The sensitivity reported in the present study (96%) is slightly higher than that obtained by CONSTANTINE *et al.* (1) in their comparison of serum samples analyzed with five different techniques (81.3–93.8%). Our findings nonetheless concur with their observations regarding the excellent specificity (100%) of ELISA techniques.

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