SALIVA AS EPIDEMIOLOGICAL TOOL FOR HIV SURVEILLANCE IN DEVELOPING COUNTRIES

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ABSTRACT

Isolation of the human immunodeficiency virus (HIV) as saliva collected from HIV infected individuals and AIDS patients is sporadic, even with highly sensitive methods such as the polmerase chain reaction (PCR), and has therefore a low utility in detecting HIV infection. On the other hand, IgG HIV antibodies persist in saliva after infection with the virus, and studies have shown a complete agreement between saliva and serum antibody testing in diagnosis of HIV infection. The same ELISA and Western blot (WB) kits used for testing sera can be used, with slight modifications, to screen and confirm HIV infection, respectively. Thus confirmatory testing of blood samples should no longer be considered essential when HIV antibody is detected in saliva. The use of saliva for anti-HIV screening appears to be attractive since specimen collection is simple, rapid, safe, cheap and has better compliance compared with blood testing. Thus, saliva is recommended as an effective alternative to serum for HIV surveillance programmes in developing countries.

INTRODUCTION

The emergence of the AIDS epidemic, and with it the recognition of the deadly result of HIV infection coupled with the lack of effective drug treatment or vaccine, have fueled the search for less hazardous, yet reliable specimen types. Body fluids other than blood, such as urine and saliva, have been used for HIV

testing. The non-invasive nature and ease of saliva and urine collection reduces the biohazard and eliminates the pain associated with blood collection, making them extremely attractive for use in HIV surveillance. An increasing number of research groups are working with saliva specimens for HIV detection, as the factors that have limited the feasibility of saliva testing are eliminated by technical advances. This review discusses the utility of saliva testing for HIV surveillance in the developing countries burdened with the HIV pandemic.

HIV in Saliva

Report that HIV could be isolated in saliva of infected persons (1) prompted a number of research workers to determine the utility of this finding in the detection of HIV infection. In subsequent studies, however, it became apparent that the recovery of HIV-1 from oral secretions is sporadic (2), even with highly sensitive methods such as the polymerase chain reaction which amplifies small portions of the HIV-1 proviral genome (3). Goto and co-workers (3) using the PCR technique, probed for three proviral DNA sequences from different portions of the viral genome, and detected HIV-1 proviral sequences in the saliva of only a half of the 20 AIDS patients tested. There was no obvious relationship between a patient's clinical condition and detection of HIV sequences in saliva. The same researchers found, in a second set of investigations, HIV genome in only two out of six AIDS patients tested. Four samples were then collected from the six patients, at intervals os five to sixty days, and HIV-1 proviral DNA could be detected from all the patients in at least two samples. It is important to note that the amount of the virus in saliva, when present, is low (< 1 infectious particle/millilitre) (4). The fact that repeated sampling and a technique as sensitive as PCR are needed to detect HIV indicates that the presence of the virus in saliva cannot be used for the diagnosis of HIV infection.

IgGHIV-specific antibodies in saliva and their utility in detecting HIV infection.

HIV specific IgG and IgM are found in saliva following HIV-1 infection (5). The IgG isotype, unlike the other isotypes, is persistently detectable in saliva after infection and has been used in anti-HIV-1 screening (5,6). Initial studies attempting to detect IgG HIV-1 antibodies in saliva reported wide ranges in sensitivity (50-95%) and specificity (77%-98%) (7-10), and were met with delays up to four weeks in detecting anti-HIV antibodies in saliva after their detection in serum (12,13). The inconsistencies in the utility of saliva specimens in the detection of HIV-1 antibody have been blamed on the specimen inadequacy (14). To solve this problem, several sample devices for collecting saliva for testing purposes have recently been developed. One such device, named the Omni-SAL™ collection device, facilitates the collection of saliva, and is provided with a colour indicator which ensures collection of adequate volume of saliva (14). Recent studies using adequate saliva have all shown 100% specifity and 100% sensitivity in the detection of HIV-1 antibody (6,10,15,17). The problem of delayed detection of anti-HIV in saliva has been overcome with GACELISA (an immunoglobulin antibody capture ELISA) which has shown extremely high sensitivity in detecting IGG anti-HIV-1 antibodies shortly after seroconversion (12,13). A fundamental problem which remained until recently was confirmation of saliva specimens reactive to conventional ELISA methods of detecting HIV antibody (18). Western blot test, which is the most widely accepted confirmatory assay, frequently produced negative or indeterminate reactivities with saliva which have tested positive by conventional ELISA (5,19,21). Thus, confirmatory testing on blood samples remained critical when there is HIV-antibody detection in saliva. However, the conventional serum HIV-1 WB has recently been optimized for use with saliva

specimens by increasing both the specimen/diluent ratio from 20µl/2000µl for serum to 300µl/1200µl and biotin/avidin concentrations by a factor of three (22). Using these modifications were have been able to confirm all the GACELISA reactive saliva samples collected from HIV-1 infected Tanzanian individuals (unpublished observations). Just recently, Emmons et al (23) reported similar modifications pertaining to sample dilution but with unaltered conjugate dilution, and noted a 97.4% Western blot sensitivity based on 195 HIV seropositive subjects. In a nutshell, screening for HIV-specific IGG antibody in saliva has the same sensitivity and specificity as blood testing, and can be used for screening and diagnosis of HIV infection.

Adaption of HIV kits for saliva

Many of the standard kits for testing of HIV in serum can, with minor adjustments, be used for HIV testing in saliva. Sample protocols for four different types are described in SDS technical report (24). These changes include; increasing sample volume, decreasing diluent volume, and lowering the optical density to 70% of the serum value. Specific procedures involved in saliva testing, including interpretation of results are usually provided by the manufacturer.

Recommended testing strategy

The high sensitivy (100%) obtained with the use of a single GACELISA test implies that it is sufficient for the WHO strategy I for surveillance (25), and coupled with the high specificity (100%) means that it can also be used for diagnostic purposes as well. However, we believe that, if positive test results are to be reported back to the patient, confirmatory tests should be mandatory. This can be done by a second Elisa or a conventional serum HIV-1 WB kit optimised for saliva.

Advantages of using saliva in HIV surveillance studies

Saliva has several advantages over serum for HIV surveillance. Saliva

contains antibodies to HIV, but infectious virus is rare (26,27), and is therefore less biohazardous compared to venepunture and blood collection. Specimen collection is very simple, which involves placing of a pad under the tongue until is saturated with saliva, obviating the necessity for highly trained personnel. Collection of saliva is relatively rapid and many samples can be collected at once, a distinct advanctage for surveillance programmes that involve sampling a cohort of individuals on a given day in one or more selected sites. Furthermore, better compliance has been reported with saliva rather than blood collection (18), as it does not entail venepuncture and needle injury, a distinct advantage with children. Also there are no culturally founded or religious objections for saliva as opposed to blood collection, thereby minimising such bias in epidemiological surveys. There is an apparent cost advantage, of not using gloves, associated with saliva testing. However, the cost advantage is reduced if saliva has to be collected with a special device containing a preservative and a buffer. However, recent studies have shown high concentrations of IgG anti-HIV antibodies in dribbled whole saliva, due to local synthesis in oral epithelium (28), and this type of saliva, unlike sub-lingual (crevicular) saliva, can be collected untreated in any convenient device for direct antibody testing implying significant reductions in test costs.

Future consleratlons

The number of early seroconveters involved in saliva studies is low. More research is needed to show that saliva can be used as confidently as serum assays in the early stages of seronconversion. Furthermore, there is a need of developing a test which will require a smaller volume of saliva than the one mL which is currently required. This improvement would reduce the specimen collection time in individuals with xerostomia due to organic illnesses or apprehension, mentally handicapped and infants.

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