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Short communication

Comparison of oral fluid and serum ELISAs in the determination of IgG response to natural human papillomavirus infection in university women

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Abstract

Background: Venipuncture (phlebotomy) is an obstacle to subject recruitment and ongoing participation in cohort studies investigating human papillomavirus (HPV) infection. Anti-HPV antibodies are not only detected in serum but also in oral fluid.
Objectives: To evaluate if oral fluid specimens can be used in lieu of blood specimens for determining HPV antibody status.
Study design: One hundred and seven paired oral fluid and blood specimens from female university students were tested in a HPV16 ELISA and compared to sexual history and serial genital HPV16 DNA status.
Results: ELISA results were in agreement in 97% (104/107) of paired sera and oral fluid. Of six women with positive anti-HPV16 serum samples, only three had positive oral fluid specimens. However, the specificity of the oral fluid test was 100% compared to the blood test.
Conclusions: Detection of antibodies in oral fluid correlated with antibodies but was less sensitive than sera. A larger validation study is required to fully characterize the oral fluid assay.

Keywords: Female; Genital; Venipuncture; HPV

1. Introduction

Serial venipuncture has been a standard procedure in studies investigating the natural history of human papillomavirus (HPV) infections. Over a decade ago, Parry described the use of saliva samples in antibody assays (Parry, 1989). The translocation of IgG from blood to extracellular fluid occurs most notably in the dental–capillary bed and the transudate can be obtained from fluid lying in the dental–gingival crevice. The concentration of IgG in human plasma is approximately 1.5×10^4 mg/l and in crevicular fluid it is 3.5×10^3 mg/l (Cordeiro et al., 1993). In oral fluid the concentration is 4.7×10^1 mg/l which is 320 times more dilute then serum.

As part of a large ongoing cohort study investigating the natural history of HPV infections in university women, we initiated an investigation as to whether or not oral fluid specimens could be used in lieu of blood specimens for antibody testing. The impetus for this was to see if we could eliminate

Abbreviations: ELISA, enzyme-linked immunosorbent assay; HPV, human papillomavirus; VLP, virus-like particles

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venipuncture, a procedure that was an obstacle to subject recruitment and ongoing participation.

2. Methods

2.1. Study subjects and procedures

Recruitment methods have been reported elsewhere (Carter et al., 1996). Briefly, between September 1990 and September 1998, 18–20-year-old female students at the University of Washington were invited to participate in a longitudinal cohort study of genital HPV infection. After informed-consent, subjects underwent standardized interviews including current and past sexual behavior and standardized pelvic examinations. Dacron-tipped swab specimens were collected from multiple sites for HPV DNA analysis by polymerase chain reaction (PCR) based methods as described elsewhere (Carter et al., 2000). Twenty milliliters of blood was collected for serologic testing. The study was approved by the Institutional Review Board and informed consent was obtained.

2.2. Collection of oral fluid

From October 2000 to April 2001, oral fluid specimens were obtained from participants. An OraSure device (OraSure Technologies, Bethlehem, PA) was gently rubbed back and forth along a dental–gingival crevice and then left to lie in the buccal gingival sulcus. The volume of fluid collected by this method was approximately 0.3 ml. The swab was then placed in the accompanying vial that contained 0.8 ml transport buffer. Specimens were stored at room temperature and transported to the laboratory weekly. The buffer was collected by centrifugation according to the manufacturer's instructions, and then stored at −70 °C until subjected to antibody testing. The mean volume of liquid available for testing was 1.0 ml (range: 0.5–1.4 ml), with a small amount remaining on the pad.

2.3. HPV antibody test

To test for oral fluid IgG antibodies to HPV16, a capture antibody test similar to the assay described elsewhere (Carter et al., 1996) was used. Mouse anti-H16L1 monoclonal antibodies (H16.V5) were diluted in 0.1 M sodium bicarbonate pH 9.5 (1:5000) and used to coat microtiter plates (Immulon II; Dynex Technologies, Chantilly, VA). Monoclonal antibody was generously provided by Dr. N. Christensen (Pennsylvania State University, Hershey Medical Center, Hershey, PA). Non-specific binding was blocked by the addition of PBS with 5% goat serum (Sigma Chemical Co.) and 0.05% Tween-20 (Fisher Scientific, Fair Lawn, NJ) for 2 h at room temperature. HPV16 L1 virus like particles (VLPs) were produced by infecting SF-9 embryologic insect cells with recombinant baculovirus and partially purified on CsCl gradients (Hagensee et al., 1993). VLPs were added to half the wells at an optimum dilution, determined with a positive control serum. Incubation at 37 °C was performed for 1 h. Undiluted fluid from the OraSure device was aliquoted into wells and left overnight at 4 °C. Alkaline phosphatase conjugate goat antihuman IgG (Roche Diagnostics Corporation, Indianapolis, IN) diluted 1:3000 in PBS with 5% goat serum was incubated at 37 °C for 1 h. Final development of the ELISA was performed with Sigma 104® Phosphatase Substrate (*p*-nitrophenyl phosphate disodium salt) (Sigma Chemical Co.) for 3 h at room temperature.

All volumes were 50 μl and wells were washed with PBS between steps. All specimens were measured in sextuplicate (triplicate wells with VLPs and triplicate wells without VLP). The ELISA values are the difference between the natural logarithm of the median triplicate well containing capsid and the natural logarithm of the median triplicate well containing no VLP.

Human serum samples that had been frozen at −70 °C were thawed, diluted 1:100 in blocking buffer, and tested in triplicate in wells containing HPV16 VLPs and in triplicate wells containing no VLPs according to protocols already described (Carter et al., 1996; Xi et al., 2002).

2.4. Data analysis

Statistical analyses were performed using Stata (Stata Corporation, College Station, TX). Absorbance of each well was recorded and transformed to the natural logarithm.

Of 121 oral fluid specimens for which there was a paired serum sample, 14 were withdrawn from further analysis either because there was insufficient specimen ($n = 4$) or because the variance in the triplicate wells was larger than a prespecified limit (Nelson, 1982) ($n = 10$).

Agreement between serum testing and the oral fluid testing was evaluated with the kappa statistic. Reproducibility of oral fluid-based ELISA was assessed using factorial ANOVA.

3. Results

Amongst the 107 paired specimens provided by 93 unique subjects, 10 pairs were from subjects who were virgins and had never been HPV PCR positive during their enrolment in the study. When their oral fluid was tested, the mean ELISA value was −0.03 (S.D. = 0.06). A positive oral fluid specimen was deemed to be one that generated an ELISA value that was more than two standard deviations above the virgin mean (i.e. ELISA > 0.09). From the same virgins, their serum ELISAs generated a mean signal of −0.02 (S.D. = 0.14). For all paired specimens obtained from study subjects that were not virgins the mean oral fluid ELISA was −0.02 (S.D. = 0.08) and the mean serum ELISA was 0.04 (S.D. = 0.22).

Of the 107 paired specimens, 6 sera were positive for HPV16. Three of the corresponding oral fluid specimens were positive and three were not. None of the remaining 101 oral