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DETECTION OF HUMAN PAPILLOMAVIRUS IN THE ORAL CAVITIES OF PERSONS WITH FANCONI ANEMIA

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Abstract

Objective—We conducted a cross-sectional study to describe the prevalence and correlates of type-specific human papillomavirus DNA in the oral cavities of persons with Fanconi Anemia.

Materials and Methods—Oral swabs were collected from 67 participants with Fanconi Anemia and tested for 27 human papillomavirus genotypes using polymerase chain reaction-based methods.

Results—Participants were a mean of 18.6 (standard deviation, 10.0) years of age (range 4 to 47 years). The prevalence of oral human papillomavirus infection was 7.5%, and the prevalence of high-risk human papillomavirus infection was 6.0%. Human papillomavirus type 16 was not detected in any samples. Prevalence was higher in adults than in children (13.3% versus 2.7% in those 18 versus <18 years of age). Among adults, prevalence was higher in males than in females (25.0% versus 9.1% respectively).

Conclusions—Prevalence of oral human papillomavirus infection in persons with Fanconi Anemia was comparable to estimates from other studies in the general population. However, in contrast to previous studies, we did not identify human papillomavirus type 16 (the type found in most human papillomavirus-related head and neck cancers) in any participants.

Keywords

Fanconi Anemia; oral human papillomavirus; head and neck squamous cell carcinoma

INTRODUCTION

Fanconi anemia (FA) is a rare, autosomal recessive (and rarely X-linked) genetic disease associated with increased cancer risk (Rosenberg et al., 2008, Rosenberg et al., 2003, Alter et al., 2010). Notably, persons with FA have an ~800-fold increased risk for head and neck squamous cell carcinomas (HNSCC) (Rosenberg et al., 2003, Alter et al., 2013). While most HNSCC are attributable to alcohol and tobacco use (Lubin et al., 2009), HNSCC incidence attributable to high-risk human papillomavirus (HPV) infections, which are oncogenic, is increasing in the US and globally (Joseph & D'Souza, 2012, Chaturvedi et al., 2013), particularly among men (Chaturvedi et al., 2013, Chaturvedi et al., 2011). The majority of HPV-positive HNSCC are oropharyngeal, and >90% are attributable to HPV-16 (Gillison et al., 2000, Joseph & D'Souza, 2012). In the US, HPV prevalence in oropharyngeal cancers increased from 16% during the 1980s to 73% during the early 2000s (Chaturvedi et al., 2011). By 2030, HPV-positive oropharyngeal cancers are projected to comprise the majority of head and neck cancers in the US (Chaturvedi et al., 2011).

For persons with FA, the association between HPV and HNSCC is unclear and published data are inconsistent (Alter et al., 2013, Kutler et al., 2003, van Zeeburg et al., 2008). In 18 FA patients, Kutler et al. (2003) identified HPV in 83% of head and neck tumors (all HPV-16-positive), versus 36% of control samples. However, two other studies failed to detect HPV in HNSCC tumors from FA patients (Alter et al., 2013, van Zeeburg et al., 2008). Furthermore, only one published study has characterized prevalence of HPV infections in the oral cavities of FA patients without oral neoplasias (de Araujo et al., 2011), and no study has explored correlates of infection in persons with FA. We conducted a cross-sectional study to determine prevalence of type-specific HPV DNA in the oral cavities of persons with FA. We also explored correlates of oral HPV infection. Understanding the epidemiology of oral HPV infections in persons with FA may help determine whether their increased risk of HNSCC is partially due to either increased susceptibility to HPV infection (potentially due to impaired immune function [Myers et al., 2011, Comar et al., 2013]), or to differences in the natural history of HPV infection that accelerate carcinogenic progression. In addition, characterizing type-specific HPV prevalence in persons with FA may help determine the potential benefit of prophylactic HPV vaccination to prevent HPV-positive HNSCC. Finally, identifying health and sexual behavior correlates may enhance our understanding of HPV transmission and risk in persons with FA.

MATERIALS AND METHODS

Study Population

Persons with FA were recruited from a longitudinal study conducted by the Cincinnati Children's Hospital FA Comprehensive Care Center (CCFACCC). For this cross-sectional oral HPV study, FA patients visiting the CCFACCC during routine clinic visits from August

2012–March 2013 were invited to participate. Additional recruitment occurred in August 2012 at an annual Fanconi Anemia Research Fund (FARF) Family meeting in Casco, Maine, and in October 2012 at the annual FARF Adult meeting in Austin, Texas. Individuals reporting an FA diagnosis were eligible if they were willing to provide an oral sample for HPV testing. A subset of patients was asked to submit multiple samples at different time points if they attended multiple clinic visits or FARF meetings. Informed consent was obtained from all participants and the study was performed in accordance with the Declaration of Helsinki. The study protocol was approved by the Institutional Review Boards at Seattle Children's Hospital on August 14, 2012 (approval number 13992) and Cincinnati Children's Hospital Medical Center on February 10, 2011 (approval number 2010–3354).

Survey Instrument

Participants were asked to complete a self-administered questionnaire on demographics, health history, oral hygiene habits, and sexual behaviors. The questionnaire was administered by paper or by electronic survey using the online program Research Electronic Data Capture tools (REDCap) (Harris et al., 2009). For participants <15 years of age, a parent was asked to complete the questionnaire on his or her child's behalf. For participants aged 12–14 years, the sexual history subsection of the questionnaire was completed by confidential participant interview with a trained study coordinator. Questionnaires were administered at the time of the oral swab collection when possible. If not administered, the study coordinator contacted the participant up to three times by email or mail to request that it be completed.

Oral Swab Collection

A trained research coordinator obtained samples for HPV testing by swabbing two locations of the patient's oral cavity with Dacron swabs. The first swab sampled the tonsils or palatine arches if tonsils were not present, and the second sampled the tongue and pharynx. Both swabs were placed into one collection tube with 1.5 mL Specimen Transport Media (STM) (Qiagen, Inc., Valencia, CA). Samples were stored at 4°C and shipped via overnight FedEx to the University of Washington Pathology laboratory for HPV testing.

DNA isolation, amplification and detection

DNA was isolated via the QIAamp DNA Mini Kit vacuum protocol (Qiagen Inc., Valencia, CA) using 400 microliter (µL) of the sample collected in STM. Final elution volume was 50 µL. As previously described (Cherne et al., 2012, Feng et al., 2009), HPV and HPRT1 PCR product was generated by amplification with HPV primers MY09 and MY11 (500nM), HMB01 (100nM) and HPRT1 forward and reverse primers (100nM). Excess primers were removed using the QIAquick Multiwell PCR Purification Kit (Qiagen, Inc., Valencia, CA) per protocol with a final elution of 60 µL followed by a second amplification (20 cycles) with only biotin labeled reverse primers (MY11 and HPRT1 reverse) resulting in single stranded biotin labeled product. Detection of the 27 HPV types (16/18/26/31/33/35/39/45/51/52/53/55/56/58/59/62/64/66/67/68/69/71/73/82/83/84/IS39) plus HPRT1 was performed following the Liquid Bead Microarray assay (LBMA) protocol using 20 µL of the product (Cherne et al., 2012, Feng et al., 2009). Four negative controls

(K562) were processed in parallel with every 42 samples and used to determine the cut-off median fluorescent intensity (MFI) for each HPV type. Signals greater than seven times the average control signal were considered positive. HPRT1 was monitored to ensure sufficient DNA was present in the sample.

Statistical analyses

One oral sample and one survey were included from each participant for analysis. Since participants were recruited from a larger longitudinal study, flexibility was afforded in the timing of oral sample and questionnaire collection. For participants who submitted >1 oral sample or >1 survey, we chose the sample and survey that were chronologically closest to one another. To minimize misclassification of correlates of oral HPV prevalence, we created reliability criteria for inclusion or exclusion of observations when the oral swab and survey collection dates occurred > 30 days apart. These criteria were applied to the following variables: cancer history, bone marrow transplant, open mouth kissing, sexual experience, HPV vaccination, and genital or anal warts (see Table 1).

We described oral HPV positivity by demographics, health history, oral hygiene habits, and sexual history characteristics. We then stratified the descriptive analyses by age (<18 versus 18 years). Due to sample size constraints, statistical significance testing was not performed.

RESULTS

Study Population

Sixty-seven participants submitted oral swabs for HPV testing, and 64 (95.5%) also completed a questionnaire. Eight participants submitted two samples and/or two surveys each; for each participant, the sample and questionnaire that were chronologically closest to one another were selected for analysis.

At the time of oral swab collection, participants' mean age was 18.6 (SD,10.0) (range 4–47) years. Forty-two percent of all participants reported ever having a sexual experience with a partner (82.8% [24/29] of participants 18 years of age, and 3.2% [1/31] of participants <18 years of age). Of those reporting a sexual experience, all but one responded to additional questions about types of sexual activity; all reported ever having vaginal or anal sex, and 83.3% reported ever having given oral sex. The majority of participants reported daily tooth brushing (90.6%) and yearly dental visits (85.9%), whereas less than half reported flossing at least weekly (39.1%). Two participants reported a history of HNSCC. Other participant characteristics are described in Table 1.

The prevalence of any oral HPV was 7.5% (5 of 67 participants), and the prevalence of high-risk HPV was 6.0% (4 of 67 participants). HPV-16 was not detected in any samples. One patient sample tested positive for multiple HPV types (Table 2). Although this was a cross-sectional analysis, paired longitudinal samples were available from six subjects. For five of these subjects, both samples tested HPV-negative. The sixth subject tested positive for HPV-84 on two consecutive samples submitted three months apart. Both participants who reported a history of HNSCC tested negative for HPV.

HPV prevalence was higher among participants who reported infrequent dental visits (<1 per year) than among participants who reported 1 dental visit per year (22.2% [2/9] versus 5.5% [3/55]). HPV prevalence was also higher among participants who reported flossing their teeth infrequently (<1 time per week) than among those who reported flossing 1 time per week (10.3% [4/39] versus 4.0% [1/25]). HPV prevalence was similar among participants who did and did not report ever having had a bone marrow transplant (6.3% [2/32] versus 7.1% [2/28]).

Oral HPV prevalence was 13.3% (4/30) among those 18 years of age versus 2.7% (1/37) among those <18 years of age (in the latter group, the one HPV-positive patient was <12 years of age and reported no history of sexual experience or open mouth kissing). Among those 18 years of age, oral HPV prevalence was 25.0% in males (2/8) versus 9.1% in females (2/22). HPV was not detected in any of the six adult participants who reported current tobacco use, whereas prevalence was 16.7% (4/24) among those who reported no current tobacco use. Among adult participants who reported ever having had vaginal or anal sex, oral HPV prevalence was 13.0% (3/23) compared to 20.0% (1/5) in those reporting no history of vaginal or anal sex (the one HPV-positive participant who reported no history of vaginal or anal sex reported no history of oral kissing, oral-to-genital contact, finger-to-genital contact, or genital-to-genital contact). All three participants who tested HPV positive and reported a history of vaginal or anal intercourse also reported having given oral sex to a partner.

DISCUSSION

In this study of FA patients, the prevalence of oral HPV infection was low (7.5% for any type, and 6.0% for high-risk types). Our study did not include a comparison group of persons without FA. However, while differences in sample size, age distributions, oral sampling methods (collection instruments and sites sampled), and HPV detection assays complicate comparisons across studies, our estimates are comparable to those observed in other large studies of the general population. A systematic review of 18 studies comprising over 4,000 healthy adults worldwide reported an oral HPV prevalence of 4.5% (3.5% for high-risk types) (Kreimer et al., 2010). Furthermore, a US population-based study using 2009–2010 National Health and Nutrition Examination Survey (NHANES) data reported that the prevalence of oral HPV infection in oral rinse samples from 14–69 year old participants was 6.9% (3.7% for high-risk types) (Gillison et al., 2012). Notably, none of the HPV-positive swab samples in our study were positive for HPV-16, the type most commonly associated with HPV-positive HNSCC (Gillison et al., 2000, Joseph & D'Souza, 2012) (in the systematic review and US study described above, HPV-16 prevalence was 1.3% and 1.0%, respectively). To our knowledge, only one other study has characterized the prevalence of HPV infection in oral exfoliated cell samples from FA patients (de Araujo et al., 2011). In that study, 42 FA patients without oral malignant lesions were recruited from a bone marrow transplantation clinic in Brazil, and samples of the hard and soft palate, gums, cheek, floor of mouth and tongue were collected by cytobrush. In contrast to our study, oral HPV prevalence was high (35.0% and 27.3% in patients with and without a history of bone marrow transplant, respectively), and HPV-16 was the most frequently detected type (detected in 6 of 13 positive samples). Reasons for the discrepant results are unclear, but

could be attributable to sample size, or to differences in participant characteristics, oral collection methods and oral sampling sites (contributing to differential collection of cell types), or HPV detection assays and protocols (whereby the nested PCR and two-step genotyping procedure described by de Araujo et al. [2011] may have enhanced sensitivity for detecting HPV DNA).

Currently, two vaccines are approved by the US Food and Drug Administration to protect against infection with high-risk HPV-16 and HPV-18. Vaccine efficacy has been demonstrated against HPV-16/HPV-18-related genital and anal neoplasias (reviewed in Schiller et al., 2012), and limited data suggest that vaccines may be similarly efficacious for preventing oral HPV-16/HPV-18 infections (Herrero et al., 2013). Given that we did not detect HPV-16 or HPV-18 in any oral samples, our results do not support the hypothesis that immunization of FA participants would prevent a significant portion of HNSCC. Notably, however, half of study participants reported a history of receiving 1 dose of HPV vaccine; conceivably, vaccination may have prevented HPV-16/HPV-18 infections in some participants.

While we did not have sufficient power to formally evaluate correlates of oral HPV infection, we observed several notable trends. First, in accordance with other studies (Gillison et al., 2012, Pickard et al., 2012, Kreimer et al., 2004), HPV prevalence was higher in males than females. The higher prevalence of oral HPV infection in males may be due to different patterns of sexual behavior, or increased rates of HPV transmission during male-female oral-genital contact (Gillison et al., 2012). Second, we observed that oral HPV was more common in participants 18 years of age (13%) than in those <18 (3%). This is consistent with results from other studies in the general population enrolling children (Smith et al., 2007), adolescents (Smith et al., 2007), and adults (Gillison et al., 2012, Edelstein et al., 2012, D'Souza et al., 2009). History of bone marrow transplant (a risk factor for HNSCC in FA patients [Rosenberg et al., 2005]) was not associated with oral HPV prevalence.

In the general population there is mounting evidence that oral HPV infection is associated with sexual behavior, including lifetime numbers of oral and vaginal sex partners (Gillison et al., 2012, D'Souza et al., 2009, Pickard et al., 2012), recent frequency of performing oral sex on female partners (Edelstein et al., 2012), and lifetime number of open-mouth kissing partners (D'Souza et al., 2009, Pickard et al., 2012). In our study, the majority of adults reported a history of vaginal or anal sex and a history of performing oral sex; therefore, we were limited in our ability to assess correlations between oral HPV prevalence and sexual behavior. Nonetheless, the lower prevalence of HPV infection in children versus adults may be attributable to the rarity of sexual experience in participants <18 years of age. It is also worth noting that the frequencies of sexual behaviors reported by our adult participants were comparable to those reported in oral HPV studies in the general population. For example, among respondents who reported ever having a sexual experience with another person, 83% reported ever having given oral sex, compared to previous studies in the general population that have reported 70–76% (Gillison et al., 2012, Pickard et al., 2012, Smith et al., 2007).

Two previous studies reported on associations between oral hygiene and oral HPV infection. Four measures of oral health (poor-to-fair self-reported overall oral health, self-perceived

diagnosis of gum disease, self-reported use of mouthwash to treat dental problems in the past week, and number of teeth lost calculated from oral examination) were associated with increased oral HPV infection in 30–69 year old 2009–2010 NHANES participants (Bui et al., 2013). A study of 18–24 year old male university students reported that more frequent tooth brushing was associated with a lower likelihood of incident oral HPV infection, although the difference was not statistically significant (Edelstein et al., 2012). While infrequent tooth brushing was rarely reported in our study population, we did observe that HPV prevalence was higher in participants reporting less frequent flossing or dental visits than in participants reporting higher frequencies of these behaviors.

Our study may be limited by survival bias, since only those FA participants who were healthy enough to attend clinic visits and meetings were recruited. Despite this limitation and the small sample size, our study has several important strengths. Studies of persons with FA are limited, and the majority of previous studies have only assessed oral HPV in patients with squamous cell carcinomas (Alter et al., 2013, Kutler et al., 2003, van Zeeburg et al., 2008). Our study is the second (and largest) to provide information on the prevalence of HPV in the oral cavity of FA participants without HNSCC (only 2 of 67 participants reported a history of HNSCC), and the first to describe risk and protective correlates of oral HPV infection in persons with FA. Additional longitudinal studies are needed to further establish the epidemiology of oral HPV infections in persons with FA, and to determine whether the natural history of oral HPV infections differ between individuals with and without FA. These studies are ongoing, and expected to yield data to further our understanding of mechanisms contributing to the high incidence of HNSCC in persons with FA.

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Table 1

Demographic, health, oral hygiene, and sexual behavior characteristics of 67¹ participants with Fanconi Anemia

	n	(%)
Age at time of oral swab collection (years) (n=67)		
<18	37	(55.2)
18+	30	(44.8)
Gender (n=67)		
Female	37	(55.2)
Male	30	(44.8)
Race (n=63)²		
Black/African American	3	(4.8)
White	55	(87.3)
Other ³	5	(7.9)
Ethnicity (n=56)⁴		
Hispanic/Latino	9	(16.1)
Not Hispanic/Latino	47	(83.9)
Ever had Cancer (n=53)⁵		
No	46	(86.8)
Yes ⁶	7	(13.2)
Ever had genital or anal warts (n=55)⁷		
No	51	(92.7)
Yes	3	(5.5)
Don't know	1	(1.8)
Ever received HPV vaccine⁸ (n=58)⁹		
No	29	(50.0)
Yes	29	(50.0)
FA complimentary group (n=63)¹⁰		
FancA	27	(42.9)
FancB	—	—
FancC	8	(12.7)
Other	8	(12.7)
Don't know	20	(31.7)
Ever had a bone marrow transplant (n=60)¹¹		
No	28	(46.7)
Yes	32	(53.3)
Brush 1 time per day (n=64)		
No	6	(9.4)
Yes	58	(90.6)
Floss 1 time per week (n=64)		
No	39	(60.9)

	n	(%)
Yes	25	(39.1)
Visit dentist 1 time per year (n=64)		
No	9	(14.1)
Yes	55	(85.9)
Regularly drink alcoholic beverages (n=64)		
No	52	(81.3)
Yes	12	(18.7)
Currently use tobacco (n=64)		
No	58	(90.6)
Yes	6	(9.4)
Ever engaged in open mouth kissing (n=53)¹²		
No	27	(50.9)
Yes	26	(49.1)
Ever had a sexual experience with another person¹³ (n=60)¹⁴		
No	35	(58.3)
Yes	25	(41.7)
Ever had vaginal or anal sex (n=24)¹⁵		
No	–	–
Yes	24	(100.0)
Ever given oral sex (n=24)¹⁶		
No	4	(16.7)
Yes	20	(83.3)

¹ 3 participants did not complete a survey. For these participants, only data on age and gender were available. Therefore, the maximum n for all other variables is 64.

² 1 observation was excluded because of missing data.

³ “Other” includes participants reporting more than 1 race.

⁴ 8 observations were excluded because of missing data.

⁵ 9 observations were excluded because the oral swab was collected more than 30 days after the survey completion and the participant reported no history of cancer, and 2 observations were excluded because the oral swab was collected more than 30 days prior to survey completion and the participant reported a history of cancer.

⁶ 2 participants reported a history of HNSCC.

⁷ 9 observations were excluded because the oral swab was collected more than 30 days after survey completion and the participant reported no history of genital or anal warts.

⁸ Participants reporting having received 1 dose of the HPV vaccine were coded as “yes.”

⁹ 2 observations were excluded because the oral swab was collected more than 30 days prior to survey completion and the participant reported HPV vaccination and the year of the last shot administered was not prior to the year of the oral swab; 4 additional observations were excluded because the oral swab was collected more than 30 days after survey completion and the participant reported not having received the HPV vaccine.

¹⁰ 1 observation was excluded because of missing data.

¹¹ 3 observations were excluded because the oral swab was collected more than 30 days after survey completion and the participant reported no history of a bone marrow transplant; 1 additional observation was excluded because the oral swab was collected more than 30 days prior to survey

completion and the participant reported a history of a bone marrow transplant, but the age at time of bone marrow transplant was not prior to the age at time of oral swab collection.

¹² 8 observations were excluded because the oral swab was collected more than 30 days after survey completion and the participant reported no history of open mouth kissing. 3 additional observations were excluded because the oral swab was collected more than 30 days prior to survey completion and the participant reported a history of open mouth kissing.

¹³ Defined as mouth to genital, finger to genital, or genital to genital contact with another person.

¹⁴ 3 observations were excluded because the oral swab was collected more than 30 days after survey completion and the participant reported having no sexual experience. 1 observation was excluded because the oral swab was collected more than 30 days prior to survey completion and the participant reported a history of sexual experience, but did not provide age at first sexual experience.

¹⁵ 1 observation was excluded because of missing data.

¹⁶ 1 observation was excluded because of missing data.

Table 2

HPV types detected in FA participants with HPV-positive oral swab samples (n=5)

Participant	HPV Type ^{<i>I</i>}
1	33
2	84 ²
3 ³	66
4 ⁴	51, 52, 62, 82
5	51

¹ High-risk HPV types are in italics.

² Participant submitted 2 samples at 0 and 3 months that were both positive for HPV-84.

³ This participant was <12 years of age. (The other 4 participants were 18 years of age.)

⁴ This participant reported having received 3 doses of HPV vaccine.