



Cutaneous HPV and alpha-mucosal 9-valent HPV sero-status associations

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ABSTRACT

Seroepidemiology of human papillomaviruses (HPV) among men is poorly understood. We examined the association between seropositivity to cutaneous HPV and 9-valent HPV (9vHPV) types. Six hundred men were randomly selected from the *HPV Infection in Men (HIM) Study*. Archived serum specimens were tested for antibodies against 9vHPV types [low-risk (6/11) and high-risk (16/18/31/33/45/52/58)], and 14 cutaneous types, including β -types 5/8/12/14/17/22/23/24/38/47, α -type-27, γ -type-4, μ -type-1, and ν -type-41, using a GST L1-based multiplex serology assay. Risk factor data were collected through questionnaires. Logistic regression was used to evaluate associations between mucosal and cutaneous HPV types. Approximately 21% of men were positive for ≥ 1 cutaneous HPV type, and ≥ 1 nine-valent HPV vaccine type at the same time. Men who were seropositive for any-cutaneous HPV were nearly twice as likely to be seropositive for 9vHPV (adjusted odds ratio (AOR) = 1.97, 95% confidence interval (CI): 1.30–2.99), high-risk (AOR = 1.83; 95% CI: 1.04–3.20), low-risk (AOR = 1.92; 95% CI: 1.16–3.18), and four-valent, 4vHPV, (AOR = 2.01; 95% CI: 1.25–3.21). Type-specific cutaneous HPV seropositivity (types: 8/14/17/23/38/27/4/1) was also positively associated with seropositivity to 9vHPV, high-risk, and low-risk categories. These data indicate that exposure to cutaneous HPV and 9vHPV types is common. Future longitudinal studies are needed to assess the temporality of these associations.

1. Introduction

Over 200 types of Human Papillomaviruses (HPV) have been identified. Based on their site of infection, HPV are classified into mucosal and cutaneous HPV. Several mucosal HPV types are considered causal for multiple cancers, and a role for cutaneous HPV in cutaneous cancers is also suspected. Exposure to cutaneous HPV occurs early in life [1], whereas exposure to mucosal HPV usually occurs at sexual debut [2]. Immune response to HPV infection is complex and poorly understood, particularly among men. Generally, the immune response against HPV infection consists of cell mediated cytotoxicity followed by antibody production [3]. Type specific antibodies against L1 HPV capsid protein provide evidence of past or present infection. Although alpha mucosal HPV types 6, 11, 16 and 18 have been widely studied, less is known about the seroepidemiology of other HPV types.

Previously, we reported the seroprevalence and factors associated with seropositivity for 9-valent HPV vaccine types (9vHPV) and 14 cutaneous HPV types [4,5]. In this short report, we examined the

association between seropositivity to type-specific and grouped cutaneous HPV and 9vHPV types. The main route of transmission for cutaneous HPV and 9vHPV types is skin-to-skin contact, and previously we reported [4,5] that certain shared sexual behaviors, such as anal sex, could possibly influence the infection status of both cutaneous HPV and 9vHPV types.

2. Methods

2.1. Study population

This analysis included 600 men randomly selected from the *HIM Study*, a prospective cohort of the natural history of HPV infection in men. Both the subset of these 600 men and the *HIM Study* population overall, have been described previously [6,7]. Briefly, between July 2005 and September 2009, the *HIM Study* enrolled over 4000 men aged 18–70 years at baseline from Tampa, Florida, Sao Paulo, Brazil, and Cuernavaca, Mexico. Every six months, participants underwent

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interview, physical examination, and laboratory analysis for up to seven years of follow-up. All participants provided written informed consent, and study protocols were approved by the institutional review boards at the University of South Florida (Tampa, FL, USA), the Ludwig Institute for Cancer Research (Sao Paulo, Brazil), and the Instituto Nacional de Salud Publica (Cuernavaca, Mexico).

2.2. Data collection

Data on demographic, lifestyle, and sexual behavior factors were collected through a questionnaire. Archived baseline serum samples from 600 participants were tested for seroreactivity to the L1 protein of 14 cutaneous HPV types, including β -types (5, 8, 12, 14, 17, 22, 23, 24, 38 and 47), α -type 27, γ -type 4, μ -type 1, and ν -type 41, and 9vHPV types [low-risk (6, 11) and high-risk (16, 18, 31, 33, 45, 52 and 58)]. We restricted the analysis to 14 HPV types due to limited funds; these HPV types were carefully selected based on previous reports of their association skin lesions [8,9]. Antibodies were detected using glutathione S-transferase (GST) capture ELISA in combination with fluorescent bead technology previously described [10,11]. Median fluorescence intensity (MFI) values were used to define type-specific seropositivity. After excluding inadequate serologic results of two subjects, the final sample included 598 men.

2.3. Statistical analysis

Outcome categories of grouped 9vHPV, high-risk, low-risk, four-valent HPV vaccine types (4-vHPV), any-cutaneous, and any-beta were created. The 9vHPV category was defined as the proportion of men who were seropositive to ≥ 1 of the 9 types included in the 9-valent HPV vaccine; high-risk category, seropositive to ≥ 1 of the 7 types (16, 18, 31, 33, 45, 52 and 58); low-risk category, seropositive to ≥ 1 of the 2 types (6, 11); 4vHPV category, seropositive to ≥ 1 of the 4 types (6, 11, 16, 18). Any-cutaneous and any-beta categories were defined as the proportion of men who were seropositive to ≥ 1 of the 14 cutaneous types or to ≥ 1 of the 10 beta types tested for in this study, respectively.

The proportion of men who were seropositive for at least one cutaneous HPV type and at least one 9vHPV type was also calculated. Multivariable logistic regression was used to estimate the independent associations between individual HPV types and grouped HPV categories. Seronegative for both exposure and outcome were selected as the reference groups in the models. Final models were adjusted for country of residence, age, education, circumcision, and male anal sex lifetime partners. These factors were selected based on their associations with seropositivity to cutaneous HPV and 9vHPV types in our previous analyses [4,5]. All analyses were performed using SAS 9.3.

3. Results

Distribution of the socio-demographic and behavioral characteristics, and the seroprevalence estimates for these 600 men have been published previously [Rahman, 2016 #528; Rahman, 2016 #527]. Table 1 shows the proportion of men who were seropositive for both cutaneous HPV and 9vHPV types. Approximately 21% of men were seropositive to both any-cutaneous and 9vHPV categories, and nearly 15% of men were positive for both any-beta and 9vHPV types. Similarly, 10.7% of men were seropositive for any cutaneous HPV and high-risk HPV, and 13.4% were seropositive for both any-cutaneous and low-risk HPV. Proportions of men who were positive for a given cutaneous type and any 9vHPV type was highest for γ -HPV 4 (10.9%) and μ -HPV 1 (10.7%).

Table 2 shows results from multivariable analysis of associations. Men who were seropositive for any-cutaneous HPV were nearly two times more likely to be seropositive for 9vHPV, high-risk, low risk, and 4vHPV types compared to men who were seronegative for all cutaneous HPV types. Similarly, men who were seropositive to any-beta HPV were

two times more likely to be seropositive to 9vHPV, high-risk, low-risk, and 4vHPV types compared to men who were seronegative for all beta HPV types. Seropositivity to individual types of cutaneous HPV (beta types: 8, 14, 17, 23, 38; alpha 27; gamma 4; and mu 1) was also significantly associated with seropositivity to any 9vHPV, with men who were seropositive for these cutaneous HPV types being 2–2.5 times more likely to be seropositive for grouped 9vHPV, depending on the cutaneous HPV type. Similar associations were also observed for other categories (i.e. high-risk, low-risk), with this association being more pronounced for α -HPV 27 and high-risk (AOR 3.87; 95%CI: 1.98–7.57), and β -HPV 14 and low-risk (AOR 3.65; 95%CI: 1.66–8.03).

4. Discussion

Previously, we reported the seroprevalence of 14 cutaneous HPV and 9vHPV types and investigated factors associated with the seropositivity of each [4,5]. In this short report, we examined the association between seropositivity to cutaneous HPV and 9vHPV types. Men who were seropositive for type-specific and grouped cutaneous HPV were nearly two times more likely to be seropositive for 9vHPV types. Although some of the type-specific associations did not reach statistical significance, our findings consistently showed a positive association between seropositivity to cutaneous HPV and seropositivity to 9vHPV types. To our knowledge, this is the first study evaluating these associations; no published literature exists that we can use to contextualize our findings.

One possible explanation for the observed association is shared exposure and with similar immunogenicity of the viruses. The primary method of transmission for cutaneous HPV and mucosal HPV is contact with infected skin or mucosal surfaces. Therefore, individuals who were exposed to cutaneous HPV were also exposed to mucosal HPV, which resulted in immune response, and antibody production or vice versa. This might indicate shared exposure for cutaneous and mucosal HPV types. However, usually exposure to cutaneous HPV occurs at a young age which is evident by high level of anti-cutaneous HPV antibodies in the serum in children [12], whereas exposure to mucosal HPV usually occurs at sexual debut [2]. It is more likely that exposure to cutaneous HPV in our sample preceded exposure to mucosal HPV. Another possible explanation for the observed association is that individual who were able to successfully mount an immune response against cutaneous HPV were also able to successfully mount an immune response against mucosal HPV or vice versa. This might indicate a common higher or lower immune response to HPV regardless of genera or type showing individuals with hyperactive immune system.

Inclusion of one laboratory protocol for detection of antibodies in serum specimens and the use of computer assisted questionnaires are some of the strengths of this study. Some limitations should be acknowledged while interpreting these results. Although the observed associations were adjusted for a number of known and suspected confounders that we have reported for the same study population previously [4,5], it is possible that the observed association is due to confounding by unmeasured factors. Immune response against HPV infections among men, particularly against cutaneous HPV, is not well understood. Limited studies of mucosal HPV have shown that not all men have measurable antibody responses to anogenital HPV after infection [13,14]. Therefore, the role of seroconversion and antibody decay, among those who develop antibodies, on the observed association cannot be evaluated.

In conclusion, these data indicate that concomitant exposure to cutaneous HPV and 9vHPV types is common, immune response against different types of HPV is present among men, and that there exists a positive association between seropositivity to cutaneous HPV and 9vHPV types. Since exposure to cutaneous HPV usually occurs early in life, antibodies against cutaneous HPV in serum of children has the potential to serve as marker for exposure to mucosal HPV later in life. However, future longitudinal studies are needed to assess the

Table 1
Proportions of men who were seropositive for both cutaneous HPV and 9-valent HPV vaccine types.

Cutaneous HPV types	9vHPV types (%)	High-risk (%)	Low-risk (%)	HPV 6 (%)	HPV 11 (%)	HPV 16 (%)	HPV 18 (%)	HPV 31 (%)	HPV 33 (%)	HPV 45 (%)	HPV 52 (%)	HPV 58 (%)
Any-cutaneous	21.4	10.7	13.4	12.5	3.0	2.5	3.0	3.0	1.7	3.2	2.2	3.8
Any-beta	14.7	7.5	9.4	8.5	2.7	2.0	2.3	1.8	1.5	2.5	1.3	2.7
β-HPV 5	2.8	1.3	2.0	1.7	0.8	0.7	0.8	0.3	0.2	0.7	0.2	0.2
β-HPV 8	8.4	4.7	5.4	4.8	1.7	1.3	1.5	1.3	1.2	1.7	1.2	1.3
β-HPV 12	2.7	1.5	1.8	1.7	0.7	0.5	0.8	0.3	0.3	0.8	0.5	0.8
β-HPV 14	3.0	1.5	2.5	2.3	0.8	0.5	1.2	0.5	0.5	1.0	0.7	0.8
β-HPV 17	5.4	3.0	3.8	3.2	1.5	1.0	1.5	1.0	0.5	1.5	0.8	1.2
β-HPV 22	2.8	2.0	2.0	1.5	1.0	0.8	1.3	0.7	0.5	1.2	0.7	0.8
β-HPV 23	5.2	2.7	3.8	3.3	1.2	0.7	1.2	0.8	0.5	1.7	0.7	1.0
β-HPV 24	3.5	2.2	2.3	1.8	0.8	0.3	1.3	0.8	0.8	1.3	0.8	1.0
β-HPV 38	5.5	3.2	3.8	3.3	1.0	1.0	1.3	1.2	0.5	1.7	0.7	1.0
β-HPV 47	4.5	2.7	2.8	2.5	1.2	0.5	1.7	1.0	0.8	1.3	0.8	1.0
α-HPV 27	4.2	3.0	2.3	2.0	1.2	1.2	1.2	0.7	0.5	1.8	0.7	1.0
γ-HPV 4	10.9	6.2	6.0	5.7	1.5	1.3	1.5	2.3	1.2	1.8	1.5	2.0
μ-HPV 1	10.7	5.0	7.7	7.0	2.0	1.3	1.8	1.7	0.7	1.5	0.8	1.8
ν-HPV 41	3.5	2.0	2.3	2.2	0.3	0.2	1.2	0.7	0.5	1.2	0.7	1.0

Any-cutaneous HPV category included seropositivity to at least one of the 14 types.

Any-beta HPV category included seropositivity to at least one of the 10 beta types.

9vHPV category included seropositivity to at least one of the 9 vaccine types.

High-risk category included seropositivity to at least one of these 7 types: 16, 18, 31, 33, 45, 52, 58.

Low-risk category included seropositivity to at least one of these 2 types: 6 and 11.

If a person was seropositive for any-cutaneous HPV and at the same time seropositive for 9vHPV then s/he was considered seropositive for both (i.e. any-HPV+ and 9vHPV+).

Percentage is out of 100. % positive = Positive for any-HPV and 9vHPV divided by 598.

Total sample was N = 598 men.

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Conflict of interest

None to declare.

Table 2
Association between seropositivity to grouped and individual cutaneous HPV types and seropositivity to 9vHPV, high-risk, low-risk and 4-valent HPV categories.

Cutaneous HPV Type	Seronegative Reference	9vHPV (6,11,16,18,31,33, 42,52,58)	High-risk HPV (16,18,31,33,42,52,58)	Low-risk (6 and 11)	4-valent HPV vaccine (6, 11, 16, 18)
		Seropositive AOR (95%CI) ^a	Seropositive AOR (95%CI) ^a	Seropositive AOR (95%CI) ^a	Seropositive AOR (95%CI) ^a
Any-cutaneous	1.00	1.97 (1.30–2.99)	1.83 (1.04–3.20)	1.92 (1.16–3.18)	2.01 (1.25–3.21)
Any-beta	1.00	2.11 (1.43–3.10)	2.05 (1.24–3.38)	1.92 (1.22–3.02)	2.18 (1.42–3.32)
β-HPV 5	1.00	1.27 (0.63–2.54)	1.26 (0.52–3.03)	1.29 (0.60–2.76)	1.63 (0.80–3.34)
β-HPV 8	1.00	1.89 (1.20–2.98)	2.07 (1.18–3.62)	1.72 (1.02–2.90)	1.83 (1.12–2.98)
β-HPV 12	1.00	1.81 (0.85–3.85)	2.11 (0.86–5.13)	1.64 (0.72–3.71)	1.91 (0.87–4.17)
β-HPV 14	1.00	2.76 (1.29–5.93)	2.18 (0.90–5.30)	3.65 (1.66–8.03)	4.18 (1.91–9.14)
β-HPV 17	1.00	1.70 (1.01–2.86)	1.96 (1.05–3.67)	1.92 (1.08–3.41)	1.96 (1.13–3.4)
β-HPV 22	1.00	1.58 (0.78–3.20)	2.48 (1.14–5.39)	1.79 (0.83–3.87)	2.08 (1.01–4.31)
β-HPV 23	1.00	2.01 (1.16–3.48)	2.00 (1.01–3.93)	2.35 (1.30–4.25)	2.18 (1.23–3.88)
β-HPV 24	1.00	1.90 (0.98–3.70)	2.52 (1.17–5.41)	1.77 (0.85–3.68)	1.82 (0.90–3.69)
β-HPV 38	1.00	1.74 (1.03–2.94)	2.01 (1.07–3.78)	1.80 (1.01–3.21)	1.71 (0.98–2.99)
β-HPV 47	1.00	1.53 (0.88–2.64)	1.88 (0.98–3.62)	1.45 (0.77–2.72)	1.79 (1.00–3.20)
α-HPV 27	1.00	2.46 (1.33–4.53)	3.87 (1.98–7.57)	1.70 (0.85–3.40)	2.35 (1.23–4.47)
γ-HPV 4	1.00	1.52 (1.02–2.27)	1.83 (1.10–3.05)	1.15 (0.71–1.85)	1.22 (0.78–1.91)
μ-HPV 1	1.00	2.06 (1.36–3.11)	1.66 (0.98–2.81)	2.45 (1.53–3.92)	2.32 (1.48–3.62)
ν-HPV 41	1.00	1.61 (0.89–2.92)	1.95 (0.95–3.99)	1.58 (0.81–3.09)	1.62 (0.86–3.07)

AOR = adjusted odds ratio.

Any-cutaneous HPV category included seropositivity to at least one of the 14 types.

Any-beta HPV category included seropositivity to at least one of the 10 beta types.

9vHPV category included seropositivity to at least one of the 9 vaccine types.

High-risk category included seropositivity to at least one of these 7 types: 16, 18, 31, 33, 45, 52, 58.

Low-risk category included seropositivity to at least one of these 2 types: 6,11.

How to read table: For both row and column variables, the reference group is seronegative. For example, when evaluating association between any-cutaneous HPV and 9vHPV categories, in logistic regression model any-cutaneous HPV was considered as an outcome, and 9vHPV as a predictor while controlling for other co-variables (i.e. country, age, education, circumcision, and male anal sex lifetime partners). Probabilities were modeled for (outcome = 1) which represented seropositivity to at least one of the 14 cutaneous types tested for in this study. The reference was then (outcome = 0) which represented seronegative for any-cutaneous HPV category. Similarly, for the exposure (9vHPV) the reference group was seronegative (exposure = 0) and (exposure = 1) represented seropositivity to at least of the nine types included in the 9vHPV vaccine.

^a Adjusted for country of residence, age, education, circumcision, male anal sex lifetime partners.

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