Research Article

No Evidence for Human Papillomavirus in the Etiology of Colorectal Polyps

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Abstract

Background: While some studies have reported detection of oncogenic human papillomavirus (HPV) in colorectal tumors, others have not.

Methods: We examined the association between oncogenic HPV infection and colorectal polyps in a case–control study of individuals with colorectal adenomas ($n = 167$), hyperplastic polyps ($n = 87$), and polyp-free controls ($n = 250$). We carried out real-time PCR for HPV-16 and -18 DNA, and SPF PCR covering 43 HPV types, on lesional and normal colorectal tissue samples. Plasma antibodies for oncogenic HPV types were assessed via a bead-based multiplex Luminex assay.

Results: HPV DNA was not found in any of the 609 successfully assayed colorectal tissue samples from adenomas, hyperplastic polyps, normal biopsies adjacent to polyps, or normal biopsies of the rectum of disease-free controls. Also, there was no association between HPV seropositivity for all oncogenic HPV types combined, for either polyp type, and for men or women. When analyses were restricted to participants without a history of polyps, among men [adenomas ($n = 31$), hyperplastic polyps ($n = 28$), and controls ($n = 68$)], there was an association between seropositivity and hyperplastic polyps when all oncogenic HPV types were combined (OR = 3.0; 95% CI: 1.1–7.9).

Conclusions: Overall, our findings do not support an etiologic relationship between HPV and colorectal adenomas or hyperplastic polyps; however, our finding suggesting an association between HPV seropositivity and hyperplastic polyps in men may warrant further investigations.

Impact: After stringent controls for contamination and three methods to assess HPV infection, we report no evidence for HPV in the etiology of colorectal neoplasia for either men or women. *Cancer Epidemiol Biomarkers Prev; 20(10) 2288–97. ©2011 AACR.*

Introduction

Oncogenic human papillomavirus (HPV) infection is a necessary cause of cervical cancer and is strongly associated with other epithelial malignancies such as oropharyngeal, penile, vaginal, vulvar, and anal cancers (1–3). HPV DNA 16 and other oncogenic types are present in precursor lesions of HPV-related cancers as well as the carcinomas themselves (4). In all of the known HPV-related carcinomas, the most common types detected are types HPV-16 and -18, which cause more than 70% of HPV-related carcinomas (5–8).

Although 100% of cervical cancer is attributable to HPV, the proportion of other anogenital cancers that are etiologically connected to HPV varies (2). In those cancers showing associations with HPV, but with less than 100% attributable to HPV, there are most likely multiple pathways leading to cancer, one (or more) of which involves HPV infection.

Because colorectal cancer originates in epithelial cells and the anal canal, a site known to be associated with HPV-related malignancies (1), leads to the rectum and colon, more than a dozen studies have considered whether colorectal neoplastic tissue contains HPV DNA (9–22). Results from these studies have been inconsistent, with some studies reporting 14% to 84% of colorectal neoplasia positive for HPV DNA (9–17) and others detecting no HPV DNA in colorectal cancers (18–22) or adenomatous polyps (19, 22). Therefore, the association between HPV and colorectal neoplasia is unclear.

Colorectal cancer is a heterogeneous, multifactorial disease with several distinct causal pathways (23). Also, recent evidence supports the hypothesis that specific precursor lesions are associated with different pathways

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(24). Colorectal adenomatous polyps (adenomas) are well-established precursor lesions; however, only a small proportion of these lesions will progress to cancer (25). The adenoma–carcinoma pathway usually involves *APC* mutation as an early event, followed by an accumulation of other mutations and accompanying morphologic changes (26). Hyperplastic polyps, generally considered innocuous lesions, may in some instances progress to cancer along a separate pathway, termed "the serrated pathway" (27, 28). This pathway is hypothesized to involve aberrant methylation and *BRAF* mutation (29, 30). Most reported risk factors for hyperplastic polyps, such as increased risks for male sex and high alcohol consumption and decreased risk for postmenopausal hormone use, calcium intake, and nonsteroidal anti-inflammatory drug use, are similar to risk factors for adenomas (31, 32). However, hyperplastic polyps tend to occur at a younger age than adenomas (31) and have a much stronger association with cigarette smoking (31, 33, 34).

To date, no other studies have examined hyperplastic polyps in relation to oncogenic HPV infection. Also, none of the previous studies used quantitative methods to assess the viral copy number in colorectal case and control tissue samples. Precursor lesions in HPV-related anal and cervical cancer have a higher viral copy number than normal adjacent tissue and normal anal or cervical tissue from disease-free controls (35, 36). If HPV were important in the etiology of colorectal neoplasia, one would expect to observe similarly increased viral copy number specifically in the disease tissue and not in the normal tissue. Finally, most of the previous studies have not compared HPV seropositivity (a marker of past infection) in colorectal neoplasia cases to disease-free controls. This is important because antibody analyses are less susceptible to contamination than DNA analyses. Also, because it takes an average of 8 to 11 months postinfection for HPV-16 antibodies to develop (37, 38), antibody analyses would be better than DNA analyses at differentiating between very recent infection (which would be unlikely to have caused clinically detectable levels of cellular proliferation) and HPV infection occurring further back in time. We conducted a case–control study of the association between oncogenic HPV infection and adenomas and hyperplastic polyps by real-time PCR for HPV-16 and -18, as well as by an assay for serologic HPV antibodies.

Methods

Study population

Participants were 30- to 79-year-old members of Group Health, a large integrated health care system in Washington State, who received a colonoscopy for any indication from September 1998 to March 2003. Prior to colonoscopy, a sequential sample of individuals was invited to take part in a study of colorectal cancer screening markers (39, 40). All study protocols were approved by Institu-

tional Review Boards at Group Health and the Fred Hutchinson Cancer Research Center. Eligibility was limited to participants with at least 1 prevalent adenoma or hyperplastic polyp at the index colonoscopy, and for comparison, participants with disease-free colons and rectums (see "Case–control classification," later); in addition, among these, only those with questionnaire data and a blood sample, a tissue sample, or both available for analyses were included in the present study. Of the 738 participants in the parent study, we excluded those who had a self-reported history of colorectal cancer, ulcerative colitis, Crohn's disease, familial adenomatous polyposis (FAP), or other non-polyp colorectal pathologies ($n = 165$). Thirty-seven additional participants were excluded because of missing or incomplete questionnaire data, which precluded the ascertainment of colorectal disease history, and 32 were excluded because they did not have blood or tissue samples available for analysis. Thus, 504 participants were eligible (Fig. 1).

Case–control classification

For polyp cases, standardized pathology reviews were completed and medical records reviewed to ascertain the size and anatomic site of each polyp. If a participant had at least one of the following polyps, he or she was classified an adenoma case: tubular adenoma, tubulovillous adenoma, or villous adenoma. Cases with microvesicular hyperplastic polyps, goblet cell hyperplastic polyps, serrated polyps, or sessile serrated polyps and no adenomas were classified as hyperplastic polyp cases (41). Controls were participants who had no colorectal pathologies identified during the index colonoscopy. The eligible study population consisted of 167 adenoma cases, 87 hyperplastic polyp cases, and 250 controls (Fig. 1).

Data collection

Study participants completed a self-administered questionnaire, eliciting the following information: demographic characteristics, height, weight, history of colorectal polyps, cancer history, lifetime alcohol consumption, smoking history, aspirin use, and, for women only, reproductive history and hormone use. More than 95% of participants completed the forms prior to colonoscopy, thus minimizing the possibility of recall differences by diagnosis.

Prior to colonoscopy, an EDTA (ethylenediamine tetra-acetic acid) tube of blood was collected, processed within 20 hours, and stored at $-80°C$.

During colonoscopy, tissue samples from polyps and other pathologies (e.g., cancer, ulcerative colitis, and Crohn's disease) were collected for diagnostic review. In addition, up to 8 perilesional biopsies were collected from the normal mucosa adjacent to polyps and up to 8 normal mucosa biopsies were sampled from the rectum of all participants, regardless of clinical findings. Tissues were processed and stored in formalin-fixed, paraffin-embedded (FFPE) blocks.
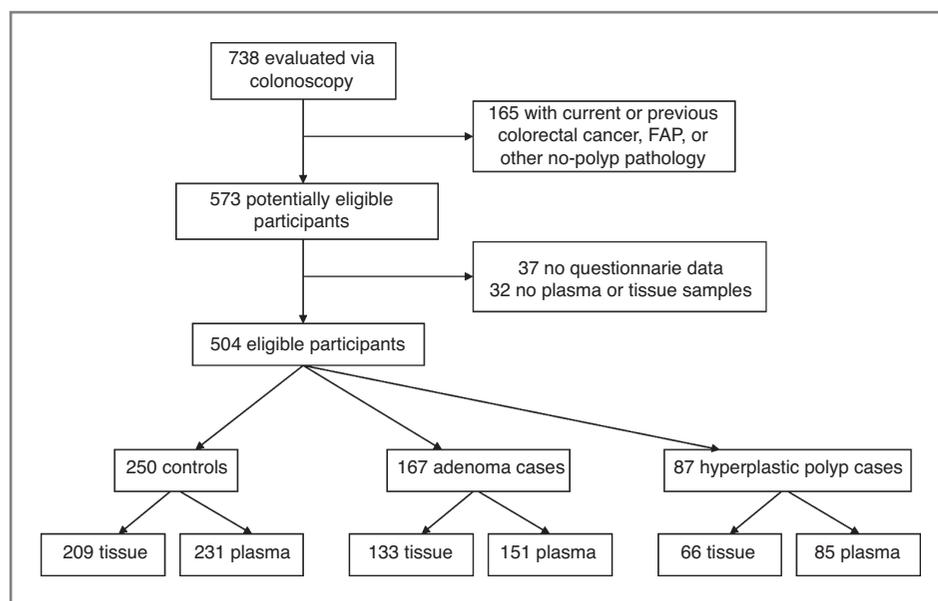
Figure 1. Study eligibility flow chart: Group Health members undergoing colonoscopy 1998–2003.

Tissue sample selection and preparation

We chose FFPE polyp tissue blocks for analysis if a polyp was at least 3 mm in size, based on clinical diagnosis, and contained at least 50% lesional tissue according to the hematoxylin and eosin slide. Of the 167 eligible adenoma cases, 96 cases had 1 block and 37 cases had multiple adenomatous polyp blocks that fit these criteria, resulting in a total of 177 adenomatous tissue samples for analyses. For hyperplastic polyp tissue, 66 of the 87 hyperplastic polyp cases had eligible blocks (50 cases with 1 block and 16 cases with multiple blocks) for a total of 84 hyperplastic polyp tissue samples from cases with only hyperplastic polyps. An additional 25 hyperplastic polyp tissue samples were included from 25 cases that had both adenomatous and hyperplastic polyps.

As noted, 2 types of clinically normal tissue were sampled: (i) normal tissue adjacent to eligible polyps in cases, and (ii) tissue from the rectum of polyp-free controls. Of 177 adenomatous polyps, 60 had normal adjacent tissue available for comparison; 62 of the 109 hyperplastic polyps had normal adjacent tissue available. There were normal rectal tissue samples from 209 polyp-free controls. All lesional and normal tissue samples were labeled with identification numbers that did not reveal the source of the tissue or diagnosis.

In preparation for sectioning, tissue samples were arranged in batches containing lesional-tissue blocks interspersed with clinically normal tissue blocks. Ten 10-μm sections were cut from each block and placed directly into 2 separate 1.5-mL microcentrifuge tubes. All blocks were sectioned by the following protocol to limit contamination: (i) before beginning sectioning and between each block, the microtome, forceps, and work bench were wiped down with RNase, followed by 1% bleach, and then 70% EtOH; (ii) a new microtome blade,

new forceps, and fresh gloves were used for each block; and (iii) a 5-μm section was cut off the front of each block and discarded before cutting any sections for assays. In addition to these procedures, every 11th block sectioned was composed of mouse heart tissue that served as a sentinel for potential contamination.

DNA extraction

Tissue sample tubes were kept at room temperature until DNA extraction. We used QIAamp DNA FFPE tissue kits (Qiagen) for DNA extraction and followed the manufacturer's protocol with modifications to optimize DNA yield. Briefly, 1 mL of xylene was added to samples to remove the paraffin, followed by 1 mL of 100% ethanol; samples were then incubated at 55°C in a proteinase K solution overnight (16–18 hours). After samples were completely lysed, a conditioning buffer was added and samples were incubated for 10 minutes at 70°C; they were then transferred to silica-based QIAamp MinElute columns (Qiagen) that selectively bind nucleic acids. DNA was eluted from the columns with 55 μL of 70°C heated buffer solution, quantified using Picogreen (Invitrogen), and stored at −20°C until HPV DNA testing.

Real-time PCR for HPV-16 and -18 DNA

We carried out a multiplex real-time PCR assay using TaqMan (Applied Biosystems) probes for HPV-16 and -18, based on the methods by Schmitz and colleagues (42), to detect HPV DNA in colorectal lesional and nonlesional tissue from the earlier described FFPE samples. Specific primers and probes for this assay were previously reported (42) and were designed to amplify the HPV *E6* or *E7* genes and to have small product sizes: 128 bp for HPV-16 and 124 bp for HPV-18. The *β-globin* gene, with a product size of 110 bp, was amplified in the same reaction

as HPV-16 and -18 to control for DNA quality and to determine the concentration of HPV-16 and -18 DNA relative to the number of cells in each sample.

Each probe was labeled with a different fluorescent dye: 5′-Fam/MGB-3′ for HPV-16, 5′-NED/MGB-3′ for HPV-18, and 5′-VIC/MGB-3′ for *β-globin*. The reaction used 2 μL DNA, 10 μL TaqMan Gene Expression Master Mix (Applied Biosystems), 10 pmol of each primer, and 1 to 5 pmol of each probe. Forty-five cycles were run at 94°C for 15 seconds, 50°C for 20 seconds, and 60°C for 40 seconds. Heart tissue DNA (HPV-negative), SiHa cell line DNA (HPV-16 positive), and C4-1 cell line DNA (HPV-18 positive) were used for quality control and included in each run. Each sample was run in triplicate, and the mean cycle threshold (C_t) was recorded for *β-globin*, HPV-16, and HPV-18.

SPF PCR with Southern blot hybridization

We used SPF PCR primers and Southern blot hybridization to verify results of the real-time PCR for HPV-16 and -18 and to test for additional HPV types in all rectal adenoma and hyperplastic polyp tissue samples ($n = 99$) and in normal rectal tissue from a subset of randomly selected controls ($n = 99$). These primers, previously described (43), amplify a 65-bp sequence in the *L1* open reading frame and can be used to detect 43 different types of HPV. PCR amplification, HPV DNA detection, and Southern blot hybridization were carried out as described in Kleter and colleagues (43).

HPV antibody testing

Of 167 adenoma cases, 151 had blood samples available for HPV antibody analysis; of 87 hyperplastic polyp cases, 85 similarly donated a blood sample, and of 250 controls, 231 donated a blood sample (Fig. 1). Plasma samples were tested using a multiplex Luminex assay as described by Waterboer and colleagues (44). Briefly, we used 10 distinct bead sets, each carrying a specific GST-HPV L1 antigen to one of the following oncogenic types: type 16, 18, 31, 33, 35, 39, 45, 52, 58, or 68. In addition, we used a bead set with antigens to BK virus, a ubiquitous virus that the vast majority of the general population has antibodies to, and a bead set without antigens as quality control measures. Two microliters of plasma was mixed and incubated with the beads (final dilution of plasma 1:100). The beads were differentiated with a Luminex analyzer, and bound antibodies were detected and quantified using fluorescent reagents. The output for each HPV type is the median fluorescent intensity (MFI), a measure of the amount of antibody bound to the bead.

Statistical analysis

Seropositivity was determined using sex-specific cutoff points from a separate population of healthy men and women in the Puget Sound region with only 1 lifetime sex partner; we used cutoff points equal to the mean MFI value plus 2 SDs for each HPV type. Individuals with MFI values above the cutoff point for a specific HPV type were considered positive for that particular HPV type. Also, we included both a variable for seropositivity to types 16 or 18 and a variable for seropositivity to any of the HPV types tested. We carried out multivariable polytomous regression to estimate the ORs and 95% CIs comparing HPV seropositivity in colorectal adenomas and hyperplastic polyp cases to controls using STATA (version 11.0, 2009; StataCorp LP). Regression models were adjusted for age, race, smoking status (never, former, current), education, body mass index (BMI), and usual alcohol consumption (drinks per week), and stratified by sex. We tested for an interaction between HPV seropositivity and sex using a cross-product term and the likelihood ratio test.

If HPV were associated with polyp formation, the presently polyp-free controls who have had polyps in the past may have a higher prevalence of seropositivity than those without previous polyps, and this could attenuate possible associations. Similarly, because colorectal neoplasia is multifactorial, cases with current polyps that have had previous polyps may be at higher risk for polyp formation irrespective of HPV status (i.e., possible genetic susceptibility to colorectal neoplasia). Thus, *a priori*, our analysis plan included regression analyses restricting the entire study population to participants without a history of colorectal polyps.

We calculated prevalence estimates for HPV-16 or -18 DNA in colorectal adenomatous polyps, colorectal hyperplastic polyps, and in normal tissue samples from the rectum of polyp-free controls.

Results

Table 1 displays selected characteristics of the study population by case–control status. Compared with controls, adenoma cases tended to be older, were more likely to be male, had higher alcohol consumption, and were more likely to have a history of polyps ($P < 0.05$). Hyperplastic polyp cases were more likely to have BMI of 30 or more and to have a history of polyps than controls ($P < 0.05$).

HPV DNA results

We tested a total of 617 colorectal tissue samples from 408 different study participants for the presence of HPV-16 and -18 DNA by real-time PCR. On the basis of standard curves of the positive controls in each plate, if the β-globin C_t was lower than the threshold needed to detect a positive HPV-16 or -18 signal, the sample was considered to have insufficient DNA quantity or quality for HPV-16 and -18 DNA detection with this assay. Among the 617 samples tested, 8 samples failed the assay on the basis of poor β-globin C_t values; thus, 99% of samples would have had sufficient DNA quantity and quality to detect HPV-16 and -18 if either type were present in the sample. Despite successful detection of HPV-16 and -18 in each of the respective positive quality control samples, none of the 609 successfully

Table 1. Characteristics of controls, adenoma cases, and hyperplastic polyp cases: Group Health members undergoing colonoscopy, 1998–2003

Characteristic	Controls (N = 250), n (%)	Adenomas (N = 167), n (%)	P^{a}	Hyperplastic polyps (N = 87), n (%)	P^{b}
Age, y					
30–49	54 (22)	19 (11)		11 (13)	
50–59	88 (35)	45 (27)		28 (32)	
60–69	57 (23)	58 (35)		29 (33)	
70–79	51 (20)	45 (27)	<0.01	19 (22)	0.13
Men	99 (40)	84 (50)	0.03	41 (47)	0.22
Race/ethnicity					
Caucasian	221 (88)	151 (90)		76 (87)	
African American	8 (3)	3 (2)		4 (5)	
Asian American	10 (4)	5 (3)		1 (1)	
Hispanic	2 (1)	5 (3)		4 (5)	
Other	9 (4)	3 (2)	0.41	2 (2)	0.18
Cigarette smoking status					
Never	108 (43)	68 (41)		26 (30)	
Former	119 (48)	86 (51)		51 (59)	
Current	22 (9)	13 (8)	0.75	10 (11)	0.09
Education					
High school graduate or less	31 (12)	24 (14)		13 (15)	
Some college/vocational school	70 (28)	57 (34)		29 (33)	
College graduate	61 (24)	26 (16)		19 (22)	
Post–college graduate school	88 (35)	60 (36)	0.15	26 (30)	0.66
BMI, kg/m^2					
<25	90 (36)	53 (32)		23 (26)	
25–29.9	100 (40)	70 (42)		31 (36)	
≥30	58 (23)	43 (26)	0.77	33 (38)	0.05
Alcohol consumption, drinks/wk					
0	51 (20)	42 (25)		21 (24)	
1–7	174 (70)	96 (57)		56 (64)	
8–14	19 (8)	16 (10)		5 (6)	
>14	6 (2)	13 (8)	0.02	5 (6)	0.36
History of colorectal polyps	51 (20)	90 (54)	<0.01	35 (40)	<0.01

$^{a}\chi^2$ P value comparing adenoma cases to controls.
$^{b}\chi^2$ P value comparing hyperplastic polyp cases to controls.

assayed study samples were positive for HPV-16 or -18 DNA.

In addition to real time-PCR for HPV-16 and -18, we conducted a PCR assay using SPF primers on 198 rectal tissue samples, of which 99 were either adenomatous or hyperplastic polyp tissue samples and 99 were normal tissue samples from disease-free controls. For this assay, the failure rate was 4% (failures were defined by blank wells, indicating insufficient quality or quantity of DNA). For the remaining 190 samples that were successfully assayed, no HPV DNA was detected.

HPV antibody results

The distribution of MFI values for each HPV type differed between men and women, with women having higher mean values. For example, for HPV-16, the mean MFI among women was 1,461 (SD = 4,576), and among men it was 942 (SD = 4,827; data not shown). The finding of higher HPV antibody levels in women is consistent with other HPV antibody analyses (45, 46) and is the rationale for setting sex-specific cutoff points for HPV seropositivity. Also, as previously mentioned, all analyses were stratified by sex, because previous studies have reported stronger associations between HPV seropositivity and anal cancer in men than in women (47, 48).

There was no association between HPV seropositivity for all types combined, and either type of polyp, for either men or women in the full study population (Table 2). Among women, the adjusted OR for the association between HPV seropositivity to any of the HPV types and adenomas was 0.78 (95% CI: 0.43–1.41),

Table 2. ORs and 95% CIs comparing HPV seropositivity in adenoma and hyperplastic polyp cases to controls, stratified by sex: Group Health members, 1998–2003

	Women (N = 261)						
	Controls (n = 138)		Adenomas (n = 78)			Hyperplastic polyps (n = 45)	
	Pos, n (%)	Pos, n (%)	Crude OR	Adjusted[a] OR	Pos, n (%)	Crude OR	Adjusted[a] OR
Any type	61 (46)	29 (37)	0.68 (0.39–1.21)	0.78 (0.43–1.41)	18 (40)	0.77 (0.39–1.53)	0.88 (0.43–1.80)
HPV-16	35 (25)	11 (14)	0.48 (0.23–1.02)	0.58 (0.27–1.24)	12 (27)	1.07 (0.50–2.30)	1.32 (0.59–2.96)
HPV-18	18 (13)	5 (6)	0.46 (0.16–1.28)	0.60 (0.21–1.75)	0 (0)	0 (0–0.65)	N/A[b]
HPV-16 or -18	43 (31)	15 (19)	0.53 (0.27–1.03)	0.63 (0.31–1.26)	12 (27)	0.80 (0.37–1.71)	0.97 (0.44–2.13)
HPV-31	18 (13)	8 (10)	0.76 (0.31–1.84)	0.90 (0.36–2.25)	3 (7)	0.48 (0.13–1.70)	0.65 (0.18–2.40)
HPV-33	15 (11)	3 (4)	0.33 (0.09–1.17)	0.31 (0.09–1.14)	2 (4)	0.38 (0.08–1.74)	0.31 (0.06–1.42)
HPV-35	9 (7)	5 (6)	0.98 (0.32–3.04)	1.14 (0.35–3.74)	3 (7)	1.02 (0.26–3.96)	1.31 (0.31–5.47)
HPV-39	6 (4)	1 (1)	0.29 (0.03–2.42)	0.41 (0.05–3.71)	1 (2)	0.50 (0.06–4.27)	0.86 (0.09–8.01)
HPV-45	3 (2)	1 (1)	0.59 (0.06–5.72)	0.57 (0.06–5.72)	0 (0)	0 (0–4.38)	N/A[b]
HPV-52	8 (6)	2 (3)	0.43 (0.09–2.07)	0.42 (0.08–2.06)	1 (2)	0.37 (0.04–3.04)	0.41 (0.05–3.50)
HPV-58	9 (7)	5 (6)	0.98 (0.32–3.04)	1.05 (0.32–3.45)	0 (0)	0 (0–1.42)	N/A[b]
HPV-68	1 (1)	0 (0)	0 (0–3.56)	N/A[b]	0 (0)	0 (0–6.71)	N/A[b]
	Men (N = 206)						
	Controls (n = 93)		Adenomas (n = 73)			Hyperplastic polyps (n = 40)	
	Pos, n (%)	Pos, n (%)	Crude OR	Adjusted[a] OR	Pos, n (%)	Crude OR	Adjusted[a] OR
Any type	26 (28)	20 (28)	0.97 (0.49–1.93)	1.02 (0.48–2.16)	14 (35)	1.39 (0.63–3.06)	1.44 (0.61–3.41)
HPV-16	12 (13)	10 (14)	1.07 (0.43–2.64)	1.01 (0.39–2.59)	6 (15)	1.19 (0.41–3.43)	1.22 (0.40–3.66)
HPV-18	3 (3)	2 (3)	0.84 (0.13–5.20)	0.87 (0.13–5.75)	4 (10)	3.33 (0.71–15.64)	3.19 (0.63–16.28)
HPV-16 or -18	13 (14)	12 (16)	1.21 (0.52–2.84)	1.20 (0.49–2.95)	7 (18)	1.31 (0.48–3.56)	1.36 (0.48–3.89)
HPV-31	6 (6)	6 (8)	1.30 (0.40–4.21)	1.55 (0.45–5.30)	5 (13)	2.07 (0.59–7.23)	2.20 (0.59–8.20)
HPV-33	3 (3)	6 (8)	2.69 (0.65–11.13)	2.56 (0.59–11.03)	6 (15)	5.29 (1.25–22.37)	5.23 (1.17–23.24)
HPV-35	2 (2)	2 (3)	1.28 (0.18–9.32)	1.04 (0.14–7.93)	6 (15)	8.03 (1.55–41.73)	6.74 (1.24–36.57)
HPV-39	1 (1)	1 (1)	1.28 (0.08–20.78)	0.98 (0.06–16.41)	1 (3)	2.36 (0.14–38.68)	1.76 (0.10–30.52)
HPV-45	0 (0)	2 (3)	∞ (0.61–∞)	N/A[c]	1 (3)	∞ (0.03–∞)	N/A[c]
HPV-52	4 (4)	4 (5)	1.29 (0.31–5.34)	1.43 (0.33–6.23)	3 (8)	1.80 (0.38–8.46)	1.78 (0.35–9.12)
HPV-58	2 (2)	2 (3)	1.28 (0.18–9.32)	1.24 (0.14–10.81)	0 (0)	0 (0–4.75)	N/A[b]
HPV-68	0 (0)	0 (0)	N/A[d]	N/A[d]	0 (0)	N/A[d]	N/A[d]

[a]Adjusted for age, race, smoking status, BMI, alcohol consumption, and education.
[b]Zero cases seropositive, only crude OR (95% CI) can be calculated.
[c]Zero controls seropositive, only crude OR (95% CI) can be calculated.
[d]Zero cases and 0 controls seropositive.

and among men, it was 1.02 (95% CI: 0.48–2.16). For hyperplastic polyps, the adjusted OR in women was 0.88 (95% CI: 0.43–1.80), and in men, it was 1.44 (95% CI: 0.61–3.41).

When analyses were restricted to participants without previous polyps, among men, there was a 3.1-fold increase (95% CI: 1.04–9.29) in the odds of hyperplastic polyps associated with HPV seropositivity for all HPV types combined (Table 3). The association between HPV seropositivity and hyperplastic polyps in men without

previous polyps seemed to be largely explained by seropositivity to HPV-33 (OR = 13.71; 95% CI: 2.28–82.29). No associations were seen for adenomas in men, nor for either polyp type in women when we restricted analyses to participants without previous polyps (Table 3).

The likelihood ratio test for an interaction between sex and HPV seropositivity suggested that the association between HPV seropositivity for all types combined and hyperplastic polyps among participants without a history

Table 3. ORs and 95% CIs comparing HPV seropositivity in adenoma and hyperplastic cases to controls, stratified by sex, participants without previous polyps: Group Health members, 1998–2003

	Women (N = 184)						
	Controls (n = 118)		Adenomas (n = 39)			Hyperplastic polyps (n = 27)	
	Pos, n (%)	Pos, n (%)	Crude OR	Adjusted[a] OR	Pos, n (%)	Crude OR	Adjusted[a] OR
Any type	53 (45)	14 (36)	0.69 (0.33–1.45)	0.79 (0.35–1.75)	10 (37)	0.72 (0.30–1.71)	0.87 (0.34–1.75)
HPV-16	31 (26)	6 (15)	0.51 (0.19–1.33)	0.60 (0.22–1.64)	5 (19)	0.64 (0.22–1.83)	0.81 (0.25–2.54)
HPV-18	13 (11)	4 (10)	0.92 (0.28–3.01)	1.22 (0.34–4.28)	0 (0)	0 (0–1.17)	N/A[b]
HPV-16 or -18	35 (30)	9 (23)	0.71 (0.31–1.65)	0.86 (0.35–2.11)	5 (19)	0.54 (0.19–1.54)	0.65 (0.21–2.06)
HPV-31	14 (12)	3 (8)	0.74 (0.19–2.84)	0.70 (0.18–2.69)	3 (11)	0.93 (0.25–3.49)	1.84 (0.44–7.72)
HPV-33	13 (11)	2 (5)	0.44 (0.09–2.03)	0.38 (0.08–1.82)	2 (7)	0.65 (0.14–3.05)	0.41 (0.07–2.20)
HPV-35	6 (5)	2 (5)	1.00 (0.20–5.21)	1.16 (0.21–6.37)	2 (7)	1.49 (0.28–7.84)	2.28 (0.37–14.02)
HPV-39	5 (4)	1 (3)	0.59 (0.07–5.25)	0.70 (0.08–6.60)	1 (4)	0.87 (0.10–7.76)	1.84 (0.18–18.77)
HPV-45	3 (3)	1 (3)	1.01 (0.10–9.99)	0.92 (0.09–9.60)	0 (0)	0 (0–5.87)	N/A[b]
HPV-52	7 (6)	1 (3)	0.42 (0.05–3.50)	0.41 (0.05–3.45)	1 (4)	0.61 (0.07–5.18)	0.75 (0.08–6.80)
HPV-58	8 (7)	1 (3)	0.36 (0.04–2.98)	0.38 (0.04–3.34)	0 (0)	0 (0–2.13)	N/A[b]
HPV-68	1 (1)	0 (0)	0 (0–5.72)	N/A[b]	0 (0)	0 (0–9.29)	N/A[b]
	Men (N = 124)						
	Controls (n = 68)		Adenomas (n = 32)			Hyperplastic polyps (n = 24)	
	Pos, n (%)	Pos, n (%)	Crude OR	Adjusted[a] OR	Pos, n (%)	Crude OR	Adjusted[a] OR
Any type	17 (25)	12 (38)	1.80 (0.73–4.44)	1.74 (0.65–4.64)	12 (50)	3.00 (1.14–7.91)	3.10 (1.04–9.29)
HPV-16	8 (12)	5 (16)	1.39 (0.42–4.64)	1.33 (0.37–4.76)	5 (21)	1.97 (0.58–6.76)	2.42 (0.62–9.44)
HPV-18	2 (3)	2 (6)	2.20 (0.30–16.37)	1.84 (0.23–14.87)	4 (17)	6.60 (1.12–38.73)	5.72 (0.84–38.71)
HPV-16 or -18	9 (13)	7 (22)	1.84 (0.62–5.48)	1.88 (0.59–5.97)	6 (25)	2.19 (0.69–6.97)	2.70 (0.75–9.65)
HPV-31	5 (7)	4 (13)	1.80 (0.45–7.21)	1.71 (0.39–7.42)	4 (17)	2.52 (0.62–10.30)	2.12 (0.46–9.70)
HPV-33	2 (3)	4 (13)	4.71 (0.82–27.24)	4.54 (0.75–27.66)	6 (25)	11.00 (2.04–59.20)	13.71 (2.28–82.29)
HPV-35	1 (1)	0 (0)	0 (0–4.18)	N/A[b]	4 (17)	13.39 (1.42–126.68)	10.21 (0.76–137.82)
HPV-39	0 (0)	1 (3)	∞ (0.03–∞)	N/A[c]	1 (4)	∞ (0.04–∞)	N/A[c]
HPV-45	0 (0)	2 (6)	∞ (1.13–∞)	N/A[c]	1 (4)	∞ (0.04–∞)	N/A[c]
HPV-52	4 (6)	2 (6)	1.07 (0.19–6.15)	1.02 (0.16–6.41)	3 (13)	2.29 (0.47–11.05)	2.08 (0.35–12.31)
HPV-58	0 (0)	1 (3)	∞ (0.03–∞)	N/A[c]	0 (0)	N/A[d]	N/A[d]
HPV-68	0 (0)	0 (0)	N/A[d]	N/A[d]	0 (0)	N/A[d]	N/A[d]

[a]Adjusted for age, race, smoking status, BMI, alcohol consumption, and education.
[b]Zero cases seropositive, only crude OR (95% CI) can be calculated.
[c]Zero controls seropositive, only crude OR (95% CI) can be calculated.
[d]Zero cases and 0 controls seropositive.

of polyps differed between men and women ($P = 0.05$). However, the likelihood test for an interaction between sex and seropositivity for adenomas was not statistically significant ($P = 0.08$).

Among men, there was also a statistically significant interaction between HPV seropositivity and history of polyps for adenomas ($P = 0.04$) and for hyperplastic polyps ($P = 0.01$). No interactions were observed between history of polyps and HPV seropositivity for either polyp type among women.

Discussion

Despite some previous studies detecting oncogenic HPV DNA in as much as 38% of colorectal adenomas (12), our HPV DNA and antibody analyses showed that there is no association between oncogenic HPV and colorectal adenomas. Previous studies of colorectal neoplasia and HPV have primarily focused on HPV DNA detection in colorectal polyps and tumors by PCR-based methods (9–15, 17–22) and, in some cases, even nested PCR assays

(9, 13, 15, 17); only one previous study included HPV antibody analyses of colorectal cancer cases (49).

In previous studies of colorectal adenomas, HPV DNA detection varied, ranging from 0% to 38% (11, 12, 22), and one of these studies reported a statistically significant difference in the proportion of adenomas positive for HPV DNA (38%) compared with normal colonic tissue from disease-free controls (8%; ref. 12). However, PCR, and particularly nested PCR, is prone to contamination. Also, lesional tissue from cases is often from diagnostic specimens and, as such, is processed with other pathology specimens in a clinical setting; thus, lesional tissue from cases has more opportunity to come into contact with sources of HPV DNA contamination, such as diagnostic cervical biopsies. Evaluation of the association between HPV and colorectal neoplasia by PCR methods alone may be inadequate, and strict contamination control procedures need to be followed at all stages of sample preparation and testing.

Several previous studies examining the association between HPV and colorectal neoplasia included laboratory-based methods to assess potential contamination, such as the use of HPV-negative quality control samples in PCR assays (9, 11), 12, 17, 21), and contamination control procedures, such as changing gloves and pipettes between samples while conducting PCR assays (9, 11, 17). However, none of the previous studies detailed their contamination control procedures for sectioning tissue, including methods to control for contamination that could have occurred during clinical processing. This is an important step at which significant contamination could occur, and it needs to be addressed in study protocols.

In our evaluation of the association between oncogenic HPV and colorectal polyps, we controlled for contamination not just during sample testing but also during sample preparation. We also used 2 separate PCR assays to evaluate our samples and included both positive and negative controls in each run. Using 2 separate HPV DNA assays improved the overall sensitivity for HPV detection. In addition to our HPV DNA assays, we evaluated the association between oncogenic HPV infection and colorectal polyps by an antibody assay. Not only are antibody assays less susceptible to contamination than PCR but also plasma samples from polyp cases and controls have equal opportunity for other measurement errors. In total, 3 assays were used to evaluate the association between HPV and colorectal adenomas; all 3 assays failed to provide evidence for an association between HPV and colorectal adenomas.

For hyperplastic polyps, we found slightly different results. As with colorectal adenomas, no HPV DNA was detected in colorectal hyperplastic polyp tissue and there was no association between HPV antibodies and hyperplastic polyps among women. However, we did observe a marginally statistically significant association between seropositivity to HPV and hyperplastic polyps among men without previous polyps (OR = 3.1; 95% CI: 1.04–9.29). Because this is the first study to examine

hyperplastic polyps in relation to HPV infection, and because our sample size for hyperplastic polyps among men without previous polyps was small ($n = 24$), this result should be interpreted with caution. Given that none of the hyperplastic polyp tissue samples were positive for HPV DNA and that the association with HPV antibodies was largely driven by HPV-33, a type rarely found outside the cervix, our antibody findings among men with hyperplastic polyps do not provide evidence for a biological relationship between HPV and hyperplastic polyps. HPV antibody analyses need to be conducted in a separate population of hyperplastic polyp cases and controls to determine whether these results can be reproduced or whether this is a chance finding.

Persistent infection with HPV is necessary for the virus to cause cancer (50). Therefore, if HPV were important to the etiology of hyperplastic polyps, one would expect to find HPV DNA in the lesions. In the absence of HPV DNA in colorectal tissue, a reproducible positive correlation between HPV seropositivity and colorectal hyperplastic polyps in men could, nonetheless, be a clue to other risk factors for hyperplastic polyps among men. In this case, HPV seropositivity would be serving as a surrogate for the true, and yet unidentified, risk factor. Further investigation of hyperplastic polyps is warranted to test this hypothesis.

We conducted this study among colonoscopy patients from a single medical care delivery system to ensure that cases and controls were comparable with one another and to ensure that controls were in fact polyp free. This is particularly important when evaluating risk factors for colorectal polyps because most polyps, regardless of type, do not cause symptoms, and misclassification of disease status could attenuate associations.

Our study results should be interpreted in light of the several limitations. Even though this is the largest evaluation of the association between colorectal polyps and HPV infection to date, small sample sizes, especially for stratified and restricted analyses, resulted in some imprecise estimates. Also, by conducting both the stratified and restricted analyses, we increased the chances of a type I error. However, our analysis plan was informed by previous studies reporting differences between men and women for the association between HPV antibodies and anal cancer. Finally, we did not collect sexual behavior information, such as number of previous sex partners and history of anal intercourse. Because HPV is a sexually transmitted disease, these data would have allowed us to evaluate the association between colorectal polyps and sexual behavior; in particular, we could have determined whether the HPV seropositivity results among men with hyperplastic polyps are consistent with the sexually transmitted nature of these viruses.

In summary, we conducted a rigorous evaluation of the association between HPV and 2 types of colorectal polyps and found no evidence to support an etiologic role for HPV in either adenomas or hyperplastic polyps. However, our results suggest that further investigation

of HPV seropositivity and colorectal hyperplastic polyps among men may be warranted.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interests were disclosed.

Acknowledgments

The authors thank Dr. Elena Kuo for her contributions.

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