Research Article

Cancer Epidemiology, Biomarkers & Prevention

Case–control Study of Merkel Cell Polyomavirus Infection and Cutaneous Squamous Cell Carcinoma

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Abstract

Background: Merkel cell polyomavirus (MCV) DNA has been reported in 0% to 25% of squamous cell carcinomas (SCC) occurring in immunocompetent individuals. We conducted the first serologic case–control study of MCV and SCC.

Methods: Patients with histologically confirmed cutaneous SCC ($n = 173$) were recruited from a university dermatology clinic. Controls were individuals who screened negative for and had no history of skin or other cancers ($n = 300$). Levels of antibodies against capsid antigens for MCV and another polyomavirus, JC virus (JCV), were determined by fluorescent bead-based multiplex serology. Fresh-frozen tumor tissues were obtained from 145 SCC cases and tested for MCV DNA by multiplexed PCR. Associations between MCV seroreactivity and SCC were estimated by ORs and 95% CIs calculated using logistic regression with adjustment for age and sex.

Results: MCV DNA was detected in SCC tumor tissues from 55 (38%) of 145 cases. A statistically significant association was observed between MCV seropositivity and MCV DNA-positive SCC (OR = 2.49, 95% CI = 1.03–6.04), with an almost four-fold association observed when comparing those with MCV antibodies in the fourth versus first quartiles (OR = 3.93, 95% CI = 1.43–10.76, $P_{trend} = 0.01$). No significant associations were observed between MCV seropositivity and MCV DNA-negative SCC (OR = 1.38, 95% CI = 0.76–2.48) or between JCV seropositivity and MCV DNA-positive or DNA-negative SCC.

Conclusion: Past exposure to MCV may be a risk factor for SCC.

Impact: Understanding the role of viral infections in the development of nonmelanoma skin cancer could lead to novel prevention strategies. *Cancer Epidemiol Biomarkers Prev; 21(1); 74–81. ©2011 AACR.*

Introduction

Merkel cell polyomavirus (MCV) was first discovered in Merkel cell carcinoma (MCC) tumor tissues, with 80% of MCC tissues testing positive for MCV DNA (1). Subsequent studies have confirmed that MCV DNA is present in 40% to 100% of MCC tissues, as recently reviewed (2). A subset of these MCC studies investigated the presence of MCV in other types of nonmelanoma skin cancers for comparison with MCC (3–6), such as cutaneous squamous cell carcinoma (SCC). Taken together with studies that specifically assessed MCV in SCC (7–10), MCV DNA prevalence estimates range from 0% to 25% for SCC among immunocompetent individuals.

Similar to MCC, SCC occurs more often among immunocompromised patients, with organ transplant recipients experiencing a significant 56-fold increased risk of developing cutaneous SCC compared with the general population (11). One study reported that MCV DNA was more common among SCC tumors arising in immunocompromised patients (52%) than immunocompetent patients (25%; ref. 8). However, a separate study of 85 organ transplant recipients reported MCV DNA in 0% of 85 SCC tumor tissues (10). Thus, the proportion of SCC patients with MCV DNA-positive tumors remains unclear. Furthermore, there have been no epidemiologic studies investigating the association between MCV seroreactivity and SCC.

To investigate the association between exposure to MCV and SCC, we conducted a case–control study, comparing MCV seroreactivity between SCC cases and controls. Comparisons in antibody levels were conducted

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separately for cases with and without MCV DNA present in the SCC tumor tissues. To address the possibility that MCV seroreactivity could be a marker of immunosuppression, antibodies to another human polyomavirus known to reactivate among immunosuppressed individuals (12), JC virus (JCV), were also measured.

Materials and Methods

Study design and population

A clinic-based case–control study was conducted at the Moffitt Cancer Center (Moffitt) and the affiliated University of South Florida (USF) Dermatology and Family Medicine clinics located in Tampa, FL. Cases were defined as histologically confirmed cutaneous SCC patients who were approached for recruitment consecutively through the USF Dermatology clinic in 2007 to 2009. Pathology reports were obtained for all cases to verify SCC diagnoses. Controls comprised of patients undergoing skin cancer screening exams at Moffitt's Lifetime Cancer Screening Clinic (LCS) or the USF Family Medicine clinic that were determined not to have any type of skin cancer and reported no history of skin or other cancers. All study participants had to be between the ages of 18 and 80 years. Individuals were eligible to participate regardless of immune status, and 9 SCC cases (5%) reported a history of organ transplantation. All participants provided written informed consent, and all study procedures were approved by the Institutional Review Board at the USF.

Of the nonmelanoma skin cancer (NMSC) patients approached at the USF Dermatology Clinic, 79% agreed to participate, including 185 SCC cases. There were no statistically significant differences in age or gender between those who agreed to participate and those who refused. Of the 756 patients recruited through the LCS and USF Family Medicine Clinic, 432 (57%) agreed to participate, of whom 281 (65%) screened negative for skin cancer. Of the 151 (35%) who were referred for a follow-up exam with a dermatologist, 95 (63%) were successfully recontacted and had completed the follow-up exam, 77 of whom were determined not to have skin cancer and were included in the study as controls. The 18 patients who were determined to have skin cancer based on the follow-up exam included 6 patients diagnosed with SCC, 9 patients diagnosed with BCC, and 3 patients diagnosed with melanoma or NMSC not otherwise specified. The 6 SCC patients were included as cases in this study and the other NMSC patients were excluded. The 56 screening patients who were referred to a dermatologist and never completed the follow-up exam or were unsuccessfully recontacted by study staff on 3 separate occasions were considered lost to follow-up and excluded from the study. There were no statistically significant differences in age or gender between those that completed screening follow-up and those that did not.

Blood samples were available for 91% of SCC cases and 95% of controls. The study population for the current serologic analysis was restricted to White individuals, with the exception of 2 non-White controls, resulting in a final sample size of 173 SCC cases and 300 controls. Of the 300 controls included in the current analysis, 220 (73%) were recruited from LCS and 80 were recruited from the USF Family Medicine Clinic. There was no significant difference in MCV seroprevalence between controls recruited from the 2 different clinics. In addition to blood sample collection, a 3 mm punch of tumor was obtained at the time of surgical excision and flash frozen in liquid nitrogen. Of the 191 SCC cases who agreed to participate in the study, tumor tissues were obtained from 162 (85%) individuals who contributed a total of 185 tumor samples.

All participants were asked to complete a questionnaire that contained questions on demographics and skin cancer risk factors including smoking status, alcohol consumption, hair and eye color, and measures of sun susceptibility and/or exposure, including one's skin reaction to repeated sun exposure (unable to tan, tan if you work at it, tans easily), history of a blistering sunburn, and ever having a job in the sun for at least 3 months.

MCV antibody measurement

Seroreactivity against MCV (isolate 344) capsid (VP1) protein was measured by fluorescent bead-based multiplex serology and expressed as the median fluorescence intensity (MFI) of 100 or more beads per set per serum sample, as previously described for human papillomaviruses (13) and the polyomaviruses BK virus (BKV), JCV, and simian virus 40 (SV40; ref. 14). To assess specificity of the SCC association with MCV and to address the possibility that MCV seroreactivity may be a marker of increased immunosuppression among the SCC cases compared with controls, antibodies to JCV, which is known to reactivate upon immunosuppression, were also measured. To determine the cut points for defining polyomavirus seropositivity, percentile plots of the strength of the antibody reactions were created, the inflection points were determined by visual inspection, and for each virus, the binary cut point was calculated as the median of the data points immediately below and above the inflection point (15, 16).

MCV DNA detection

DNA extraction from fresh-frozen SCC tumor tissues was carried out with the QIAGEN BioRobot EZ1 with the EZ1 DNA Tissue Kit according to the manufacturer's instructions (QIAGEN). Briefly, frozen tissues were incubated in proteinase K and a buffer G2 (QIAGEN) at 56°C until the tissue was completely lysed. To monitor the possible occurrence of cross-contamination between the different specimens during DNA extraction, tubes containing buffer only were also included.

Five microliters of purified DNA was analyzed for the human PyVs BKV, JCV, KIV, WUV, and MCV, as well as the monkey PyVs SV40 by multiplex PCR using the Multiplex PCR Kit (QIAGEN) as previously described (17, 18). Type-specific PCR primers target a conserved region of the large T-antigen (LT) gene at the N-terminus

and amplify a DNA fragment of approximately 200 nucleotides. PCR products were obtained even when only 10 copies of the viral genome were used as template (data not shown). Two primers for the amplification of β-globin were also added in the assay to provide a positive control for the quality of the template DNA (19). Typing of the specific polyomaviruses were carried out by hybridization of the PCR products to type-specific Luminex-bead coupled PyV probes as described by Steinbeis Transfer Centre Multiplexion and Luminex corporation. The sequence of the PCR primers and Luminex probes could be provided upon request. PCR products were denatured and hybridized to the beads coupled with specific probes for the 6 polyomaviruses. Results were expressed as the MFI of at least 100 beads per bead set. For each probe, MFI values with no respective PCR product added to the hybridization mixture were considered background values. The cutoff was computed by adding 5 MFI to 1.1× the median background value. For specificity evaluation, cloned HPV genomes and human specimen were used. No crossreactivity has been found (data not shown).

Statistical analysis

Demographic and skin cancer risk factors were compared between SCC cases and controls and between MCV-seropositive and MCV-seronegative controls by the χ^2 test, with the exception of age, for which the Wilcoxon rank-sum test was used. Boxplots of MCV antibody levels expressed in MFI were created for SCC cases (overall and stratified by MCV DNA status of the tumor) and controls. Mean MCV antibody levels were compared between these groups using the Wilcoxon rank-sum test. Using binary cut points (792 and 885 MFI for MCV and JCV respectively), polyomavirus seropositivity was defined separately for each virus, and associations with SCC were estimated by calculating ORs and 95% CIs using logistic regression. Quartiles of MCV and JCV seroreactivity were defined based on the control distributions of these antibody levels, and associations with SCC were estimated by calculating the OR and 95% CI for the second, third, and fourth quartiles, compared with the first (i.e., lowest) quartile. Tests for trend were conducted by including an ordinal variable in the logistic regression model indicating the quartile of polyomavirus seroreactivity. Demographic and skin cancer risk factors that were associated with case–control status (education, smoking, alcohol consumption, eye and hair color, skin reaction to the sun, sunburn history, and having had a job in the sun) were evaluated as potential confounders of the association between MCV and SCC by individual placement in a model including age and sex as covariates. None of the factors resulted in more than a 10% change in the risk estimate for MCV and SCC, and therefore, only age and sex were included in the final models. Analyses were conducted excluding the 9 SCC cases with a history of organ transplantation, and results were similar, thus, these 9 cases were included in the final analysis.

SCC cases who contributed tumor tissues were classified according to the presence or absence of MCV DNA in their tumor tissue. For the 19 SCC cases who contributed tissue from multiple, distinct, concurrent tumors, cases were considered MCV DNA positive if at least 1 tumor tested positive for MCV DNA. Associations between MCV seroreactivity and SCC stratified by MCV DNA status were calculated using separate logistic regression models comparing MCV DNA-positive SCC cases to controls and MCV DNA-negative SCC cases to controls, adjusting for age and sex. A case–case analysis was also conducted, directly comparing MCV seroreactivity between MCV DNA-positive and MCV DNA-negative SCC. All analyses were conducted using SAS. All tests were considered statistically significant at $P < 0.05$.

Results

Demographic and skin cancer risk factors are presented for cases and controls in Table 1. Compared with controls, SCC cases were older, less educated, and more likely to be male. Cases were also statistically significantly more likely to be ever smokers and less likely to have had at least 1 alcoholic drink in the past year. All but 2 study participants were White. Markers of sun sensitivity and exposure were all associated with SCC, including eye color, hair color, skin's reaction to repeated sun exposure, history of a blistering sunburn, and ever having had a job in the sun for at least 3 months (Table 1). Among the controls, there were no significant differences in MCV seropositivity by age, education, or sex with MCV seroprevalences of 72% and 74% observed for males and females, respectively (Table 2). Smoking status was not associated with MCV seropositivity, whereas those who had at least 1 alcoholic drink in the past year were less likely to be MCV seropositive than those who had no drinks. None of the measures of sun sensitivity and exposure were associated with MCV seropositivity (Table 2).

Of the SCC lesions diagnosed among the cases in this study, 52% occurred on the head or neck, 30% on the arms, 12% on the legs, and 6% on the torso. Of the 185 SCC tumor tissues obtained for DNA analysis, 179 (97%) were β-globin positive, including 66 (37%) that were MCV DNA positive. All tissues were negative for the other 5 polyomaviruses (JCV, BKV, KIV, WUV, and SV40). Among 16 patients for whom tissue samples were obtained from 2 distinct, concurrent tumors, 4 had MCV DNA in both of their tumors, 9 had MCV DNA in neither tumor, and 3 had MCV DNA in 1 tumor but not the other, for an overall percent agreement across tissues of 81%. Among 3 patients for whom tissue samples were obtained from 3 distinct, concurrent tumors, 1 had MCV DNA in all 3 tumors, and the other 2 were MCV DNA negative across all 3 tissues.

MCV antibody levels were significantly higher among SCC cases [mean (SD) = 8,228 (6,617) MFI] compared with controls [mean (SD) = 6,495 (5,891); $P = 0.004$]. Associations between MCV seroreactivity and SCC overall are presented in Table 3. MCV seropositivity was associated

Table 1. Characteristics of SCC cases and controls, Tampa, FL, 2007 to 2009

	SCC cases ($n = 173$)		Controls ($n = 300$)		
Characteristic	***n***	**%**	***n***	**%**	***P*[a]**
Age, y (mean, SD)	64.4	(9.9)	55.4	(11.7)	<0.0001
Sex					
Male	114	65.9	114	38.0	
Female	59	34.1	186	62.0	<0.0001
Education					
≤12 y	33	21.4	29	9.8	
>12 y	121	78.6	267	90.2	0.001
Smoking status[b]					
Never smoker	47	30.7	150	49.8	
Ever smoker	106	69.3	149	50.2	<0.0001
Alcohol consumption[b]					
1+ drinks in past year	122	79.7	260	87.3	
No drinks in past year	31	20.3	38	12.8	0.04
Eye color[b]					
Blue	62	40.5	85	28.6	
Green	24	15.7	48	16.2	
Hazel	30	19.6	48	16.2	
Light brown	17	11.1	35	11.8	
Dark brown	20	13.1	81	27.3	0.01
Hair color[b]					
Black/brown	102	66.2	234	78.3	
Blond/red	52	33.8	65	21.7	0.01
Skin's reaction to repeated sun exposure[b]					
Unable to tan	25	16.3	22	7.5	
Can tan if you work at it	72	47.1	99	33.6	
Tans easily	56	36.6	174	59.0	<0.0001
History of a blistering sunburn[b]					
Yes	118	77.1	202	68.0	
No	35	22.9	95	32.0	0.04
Ever had a job in the sun for 3+ months[b]					
Yes	76	49.4	81	27.3	
No	78	50.7	216	72.7	<0.0001

[a]*P* values obtained from χ^2 tests comparing characteristics of SCC with controls, exceptfor age, which was compared between SCC cases and controls by the Wilcoxon rank-sum test.
[b]Numbers do not sum to the total because of missing data on these factors.

with an increased risk of SCC, although the association was not statistically significant (OR = 1.58, 95% CI = 0.96–2.60), and there was no clear trend in SCC risk with increasing levels of MCV seroreactivity ($P_{trend} = 0.15$). Similarly, SCC was not associated with seropositivity for the other polyomavirus measured, JCV (OR = 1.40, 95% CI = 0.89–2.20).

Both serology and DNA data were available from 145 SCC cases, of whom 55 (38%) were MCV DNA positive. MCV seroreactivity, expressed in microfluorescence intensity units (MFI), is plotted for SCC cases and controls in Fig. 1. Compared with controls, MCV seroreactivity was significantly greater among the 55 SCC cases that had MCV DNA in their SCC tumor tissues ($P < 0.0001$). No differences were observed when the 90 SCC patients who were MCV DNA negative were compared with controls ($P = 0.85$). MCV seropositivity was significantly associated with MCV DNA-positive SCC (OR = 2.49, 95% CI = 1.03–6.04; Table 4). In addition, risk of MCV DNA-positive SCC increased with increasing antibody levels (OR for quartile 4 versus quartile 1 = 3.93, 95% CI = 1.43–10.76, $P_{trend} = 0.01$). Similar associations were observed when 5 organ transplant patients were excluded from the MCV DNA-positive SCC case group (OR = 3.45, 95% CI = 1.25–9.52, $P_{trend} = 0.01$) and when MCV DNA-positive cases were compared directly with MCV DNA-negative cases (OR = 5.76, 95% CI = 1.82–18.28, $P_{trend} = 0.0004$). In contrast, there was no association between MCV seropositivity and SCC

Table 2. Demographic and skin cancer risk factors in association with MCV seropositivity among 300 controls

		MCV seropositive		
Variable	**Total**	***n***	**%[a]**	***P*[b]**
Age, y				
18–29	8	7	87.5	
30–39	20	15	75.0	
40–49	54	42	77.8	
50–59	104	78	75.0	
60–69	85	57	67.1	
70–80	29	21	72.4	0.66
Education, y				
≤12	29	21	72.4	
>12	267	198	74.2	0.84
Sex				
Male	114	82	71.9	
Female	186	138	74.2	0.67
Smoking status				
Never smoker	150	113	75.3	
Ever smoker	149	107	71.8	0.49
Alcohol consumption				
1+ drinks in past year	260	185	71.2	
No drinks in past year	38	33	86.8	0.04
Eye color				
Blue	85	59	69.4	
Green	48	40	83.3	
Hazel	48	38	79.2	
Light brown	35	25	71.4	
Dark brown	81	55	67.9	0.27
Hair color				
Black/brown	234	171	73.1	
Blond/red	65	48	73.8	0.90
Skin's reaction to repeated sun exposure				
Unable to tan	22	19	86.4	
Can tan if you work at it	99	69	69.7	
Tans easily	174	127	73.0	0.28
History of blistering sunburn				
Yes	202	147	72.8	
No	95	72	75.8	0.58
Ever had a job in the sun for 3+ months				
Yes	81	58	71.6	
No	216	159	73.6	0.73

[a]Percentages indicate the proportion of controls that are MCV seropositive versus MCV seronegative in a given row.
[b]χ^2 *P* value.

among DNA-negative cases (OR = 1.38, 95% CI = 0.76–2.48). Seropositivity for JCV was not associated with SCC, regardless of tumor MCV DNA status (Table 4).

Discussion

In this first serologic case–control study of MCV infection and SCC, MCV seroreactivity was statistically significantly associated with MCV DNA-positive SCC. There are several possible explanations for the observed serologic associations. MCV seroreactivity could simply be a marker of a general systemic immunosuppression, an established risk factor for SCC. If this was the case, then associations with SCC would be expected to be observed also for JCV seroreactivity, given that JCV reactivates with immunosuppression (20). Although a

Table 3. Associations between polyomavirus seroreactivity and cutaneous SCC, Tampa, FL, 2007 to 2009

	Controls ($n = 300$)		SCC cases ($n = 173$)			
Polyomavirus seroreactivity	***n***	**%**	***n***	**%**	**OR[a]**	**95% CI[a]**
MCV						
Seronegative	80	26.7	33	19.1	1.00	Reference
Seropositive	220	73.3	140	80.9	1.58	0.96–2.60
Quartile 1	75	25.0	31	17.9	1.00	Reference
Quartile 2	75	25.0	39	22.5	1.44	0.78–2.69
Quartile 3	75	25.0	53	30.6	1.71	0.94–3.11
Quartile 4	75	25.0	50	28.9	1.53	0.84–2.78
P_{trend}					0.15	
JCV						
Seronegative	108	36.0	45	26.0	1.00	Reference
Seropositive	192	64.0	128	74.0	1.40	0.89–2.20
Quartile 1	75	25.0	29	16.8	1.00	Reference
Quartile 2	75	25.0	42	24.3	1.74	0.92–3.27
Quartile 3	75	25.0	38	22.0	1.22	0.65–2.29
Quartile 4	75	25.0	64	37.0	1.47	0.81–2.69
P_{trend}					0.44	

[a]ORs and 95% CIs adjusted for age and sex.

greater proportion of SCC cases were JCV-seropositive than controls, the difference was not statistically significant, and no trend was observed between increasing quartiles of JCV antibody levels and SCC risk. Uncontrolled MCV replication resulting from localized cutaneous immunosuppression is theoretically possible, given the previously described effects of UV radiation on antigen presentation and cytokine production in the skin (21–24). However, no associations between any of the markers of sun exposure or skin sensitivity to sun were associated with MCV seropositivity among the controls, and adjustment for these factors did not alter the magnitude of the observed association between MCV seroreactivity and SCC, suggesting that UV-induced cutaneous immunosuppression is not a confounder.

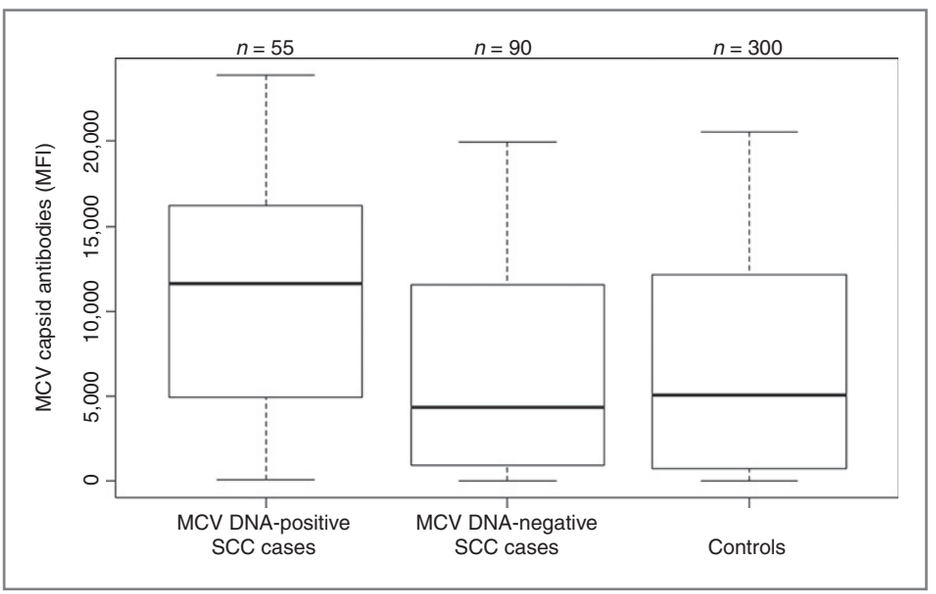

Figure 1. MCV seroreactivity in SCC cases and controls. MCV seroreactivity is plotted for 55 MCV DNA-positive SCC cases, 90 MCV DNA-negative SCC cases, and 300 controls. As compared with controls (mean = 6,495 MFI, SD = 5,891 MFI), MCV antibody levels were statistically significantly higher among SCC patients with MCV DNA in their tumor tissues (mean = 11,195 MFI, SD = 7,150 MFI; $P < 0.0001$), but not among SCC cases without MCV DNA in their tumor tissues (mean = 6,413 MFI, SD = 5,736 MFI; $P = 0.85$).

Table 4. Associations between polyomavirus seroreactivity and cutaneous SCC stratified by the presence or absence of MCV DNA in the SCC tumor tissues

Polyomavirus	Controls ($n = 300$)		MCV DNA-positive SCC cases ($n = 55$)				MCV DNA-negative SCC cases ($n = 90$)				MCV DNA-positive versus DNA-negative SCC cases	
Seroreactivity	***n***	**%**	***n***	**%**	**OR[a]**	**95% CI[a]**	***n***	**%**	**OR[a]**	**95% CI[a]**	**OR[a]**	**95% CI[a]**
MCV												
Seronegative	80	26.7	7	12.7	1.00	Reference	20	22.2	1.00	Reference	1.00	Reference
Seropositive	220	73.3	48	87.3	2.49	1.03–6.04	70	77.8	1.38	0.76–2.48	1.96	0.76–5.08
Quartile 1	75	25.0	6	10.9	1.00	Reference	19	21.1	1.00	Reference	1.00	Reference
Quartile 2	75	25.0	8	14.6	1.83	0.57–5.91	27	30.0	1.65	0.81–3.35	0.86	0.25–2.95
Quartile 3	75	25.0	15	27.3	2.27	0.78–6.61	29	32.2	1.63	0.81–3.28	1.62	0.52–5.04
Quartile 4	75	25.0	26	47.3	3.93	1.43–10.76	15	16.7	0.80	0.37–1.76	5.76	1.82–18.28
P_{trend}						0.01				0.67		0.0004
JCV												
Seronegative	108	36.0	14	25.5	1.00	Reference	27	30.0	1.00	Reference	1.00	Reference
Seropositive	192	64.0	41	74.6	1.30	0.64–2.66	63	70.0	1.23	0.72–2.11	1.24	0.57–2.69
Quartile 1	75	25.0	8	14.6	1.00	Reference	17	18.9	1.00	Reference	1.00	Reference
Quartile 2	75	25.0	15	27.3	2.15	0.78–5.95	22	24.4	1.61	0.76–3.44	1.60	0.54–4.77
Quartile 3	75	25.0	11	20.0	1.37	0.48–3.93	19	21.1	1.09	0.50–2.34	1.18	0.38–3.69
Quartile 4	75	25.0	21	38.2	1.45	0.55–3.81	32	35.6	1.43	0.70–2.90	1.24	0.44–3.50
P_{trend}						0.82				0.55		0.93

[a]ORs and 95% CIs adjusted for age and sex.

MCV could be a "passenger virus" in SCC, present in tumor tissues but not causally associated, in which case antibodies would simply be a marker of the disease. Additional serologic markers of MCV infection could be measured, such as antibodies against the MCV LT. However, at least 1 study has reported the absence of T-antigen expression in SCC tumors (25), indicating that LT antibodies may not be present in SCC cases. A final possible explanation for the observed findings is that MCV infection plays a role in SCC pathogenesis. To clearly distinguish whether MCV is a risk factor or a marker of disease, a prospective study is needed, investigating the presence of MCV infection prior to SCC development.

This study was clinic based and may have been subject to selection bias. However, the clinics from which cases and controls were recruited largely serve the same underlying population, with an estimated 28% of the SCC cases having records in the family medicine clinic from which the controls were recruited. Furthermore, MCV seropositivity was not associated with any of the demographic or skin cancer risk factors assessed, and therefore, there is no reason to think that either comparison group was biased with respect to MCV seropositivity. Because SCC cases were eligible for the study if they had a history of any other type of cancer, whereas potential controls with a history of cancer were excluded, the observed findings could have been biased if MCV is associated with those cancers. However, there was no significant difference in MCV seroprevalence between SCC cases with or without a self-reported history of cancer prior to their SCC diagnosis. The advantages to the clinic-based design included the ability to conduct physical exams to rule out prevalent disease among the controls, and the accessibility of fresh-frozen tumor tissues from the cases. The use of 2 different laboratories for the measurement of MCV DNA in tumor tissues and serum antibodies is another study strength, given that the personnel in each laboratory were masked to the results generated from the other laboratory.

MCV DNA was observed in 38% of fresh-frozen SCC tumor tissues, a higher proportion than observed in previous studies. However, all previous studies investigated formalin-fixed, paraffin-embedded tissues. It is possible that DNA degradation may have contributed to lower MCV DNA prevalence estimates in some of these studies, particularly when larger PCR amplicons were used. Compared with SCC, MCV DNA has been reported in a much higher proportion of MCC (~75%) in most published case series. Although MCV is more prevalent in MCC than in SCC, the number of SCC cases potentially attributed to MCV in the general

population is far greater than that of MCC, given that close to 1 million SCC cases are diagnosed each year in the United States in contrast to 1,500 cases of MCC. If MCV is shown to play a role in SCC carcinogenesis, then this polyomavirus may be a novel target for primary prevention through vaccination.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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