Journal of Clinical Virology 101 (2018) 34–37

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Short communication

Prevalence and stability of antibodies to thirteen polyomaviruses and association with cutaneous squamous cell carcinoma: A population-based study

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ARTICLE INFO

Keywords:
Polyomavirus
Serology
Cutaneous squamous cell carcinoma

ABSTRACT

Background: Several new members of the human polyomavirus (HPyV) family that infect human skin and are potentially oncogenic have been identified in the last decade.
Objectives: To investigate prospectively the seroprevalence and stability of 13 PyVs, and possible associations with different risk factors and cutaneous squamous cell carcinoma (cSCC).
Study design: In this Australian population-based longitudinal study sera were collected at baseline in 1992 or during the next 4 years from 688 people. Of the 688, 226 developed a new cSCC between blood collection and the final follow up in 2003. The remaining 462 served as controls. Among the 462 controls, 161 had a second serum sample from 2003 analysed. Seroprevalence of 10 human PyVs (BKV, JCV, KIV, WUV, MCV, TSV, HPyV6, HPyV7, HPyV9 and HPyV10) and three non-human PyVs (SV40, LPV and ChPyV) was assessed using multiplex serology.
Results: There was no significant difference in PyV seroprevalence between people who developed cSCC during follow-up compared to those who did not. WUV and HPyV10 showed the highest serostability (93%) and JCV VP1 and SV40 VP1 the lowest (84%) over a 9-year time period (range 7–11 years).
Conclusions: We found no evidence that HPyV seroprevalence is associated with subsequent development of cSCC and observed variable stability of antibodies to polyomaviruses.

1. Background

Sun exposure is the main risk factor for cutaneous squamous cell carcinoma (cSCC), particularly in people with fair skin [1]. Organ transplant recipients on immunosuppressive therapy have considerably increased risk of cSCC [2], suggesting that viruses may also play a role. Recently several new members of the polyomavirus (PyV) family have been identified and there are indications that these may contribute to the development of cSCC [3]. The most frequently studied HPyV is Merkel cell polyomavirus (MCV), and its integration in Merkel cell carcinoma tumours is the strongest evidence for HPyV involvement in human oncogenesis [4,5].

2. Objectives

We aimed to assess if seropositivity to polyomaviruses other than MCV is associated with cSCC in a long-term population-based study. We also assessed the stability of PyV antibodies over a period of approximately nine years.

3. Study design

This study was nested within the Nambour Skin Cancer Study which has been previously described [6]. Briefly, from 1992 to 1996, 1621 randomly selected residents of Nambour, a town in Queensland, Australia, took part in a trial of daily sunscreen application and beta-carotene supplements for skin cancer prevention. At baseline in 1992, and in 1994, and 1996, dermatologists carried out full-body skin

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https://doi.org/10.1016/j.jcv.2018.01.013
Received 3 August 2017; Received in revised form 12 January 2018; Accepted 22 January 2018

examinations of participants and clinically diagnosed skin cancers were histologically confirmed. Between these skin examinations, and from 1997 to 2003, all skin cancers histologically diagnosed in participants were captured by linkage with pathology laboratories. In a nested case-control study, sera were analysed from 1992, 1993 or 1996 from 226 people who developed cSCC during follow-up to 2003 (four of the 226 cases had been diagnosed with a SCC prior to baseline) and from 462 controls with no history of cSCC. A second serum sample from 161 controls in 2003 was also analysed. The median time between samples was 8.9 years (range 7–11), and the median time between serum sample and first cSCC diagnosis was 10 years (range 0–16 years) in cases. The institutional human research ethics committee of the QIMR Berghofer Medical Research Institute approved the study (P327) and all participants gave informed written consent.

Serum samples were stored at −80° C and analysed with a multiplex assay for VP1 PyV antibodies at the German Cancer Research Center, Heidelberg, Germany, as previously described [7,8]. Sera were tested at 1:1000 dilution for the following HPyV antigens: BKV VP1, BKV large T antigen (TAg), JCV VP1, JCV large TAg, LPV VP1, LPV large TAg, TSV VP1, TSV large TAg, MCV344 VP1, MCV small T antigen (sTAg), MCV large TAg exon1, MCV large TAg exon2, MCV large TAg, KI VP1, WU VP1, HPyV6 VP1, HPyV7 VP1, HPyV7 large TAg, HPyV9 VP1, HPyV10 VP1, HPyV10 large TAg, and SV40 VP1, SV40 large TAg and ChPyV VP1 of monkey origin. Median fluorescence intensity (MFI) was measured with a Luminex analyser. Sera from the same samples have previously been tested for cutaneous HPV, BKV and JCV antibodies at a different sample dilution (1:100) [9,10].

A frequency distribution analysis with a bin width of 250 MFI was performed on the seroresponses for each HPyV tested to determine the seronegative population, which was defined by samples falling within bins with a frequency percentage above 10% as previously established for polyomavirus serology [11,12]. Cut-off values for each HPyV antigen were calculated by the mean MFI of the non-responders plus three times the standard deviation with a minimum cut-off set to 100 MFI (Suppl Table 1).

We used Chi-squared tests to compare the characteristics of cases and controls. Associations between seropositivity for individual antigens and cSCC were assessed using logistic regression adjusting for age and sex. Receiver operating characteristic (ROC) analysis (sensitivity analysis) was performed to determine if MFI to individual antigens predicted cSCC status. Differences and ratios in MFI over time were assessed using simple descriptive statistics. Cohen's kappa was used to measure agreement between VP1 and TAg agreement for the same polyomavirus. To analyse serostability over time we classified control participants as stably seropositive or stably seronegative at both time points, seroconverting from seronegative to seropositive, or seroreverting from seropositive to seronegative. We used McNemar's test to assess whether significantly more people changed than did not. To determine if changes in the MFI values were correlated between viruses we performed a series of two-way correlations using Spearman's rho.

4. Results

The mean ages of cases and controls were 60 and 58 years respectively. More cases than controls were male and had fair skin while fewer cases were never-smokers (Table 1).

The proportions seropositive for VP1 for the different PyVs ranged from 11% for HPyV9 to 99% for HPyV10 with no statistically significant differences between cases and controls (Table 2).

The proportions seropositive for large and small T antigens varied from 0.4% to 20% (Suppl Table 2). The correlation between seropositive TAg and seropositive VP1 for the same virus was for BKV (96%; kappa 0.22), HPyV6 (93%; kappa 0.03), HPyV7 (79%; kappa 0.04) and HPyV10 (97%; kappa 0.02), MCV (60%; kappa −0.01), JCV (44%; kappa −0.01), SV40 (42%; kappa 0.05), and LPV (17%; kappa 0.03).

Table 1
Characteristics of the 462 controls and 226 cases. (Values in bold highlights statistically significant P values.)

		Controls N (%)	Cases N (%)	P value[1]
Age distribution	< 40	106 (23)	43 (19)	.346
	40–49	120 (26)	55 (24)	
	50 and older	236 (51)	128 (57)	
Sex	Female	249 (54)	103 (46)	**.040**
	Male	213 (46)	123 (54)	
Year serum collected	1992	249 (54)	114 (50)	.098
	1993	0 (0)	2 (1)	
	1996	213 (46)	110 (49)	
Hair colour	Blonde/Light brown	236 (51)	118 (53)	< **.001**
	Red	25 (5)	30 (13)	
	Dark brown/ Black	198 (43)	76 (34)	
Skin colour	Fair	228 (49)	149 (66)	< **.001**
	Medium	188 (41)	71 (31)	
	Olive, brown or black	46 (10)	6 (3)	
Skin response after exposure to strong sun	Burn only	74 (16)	80 (35)	< **. 001**
	Burn, then tan	310 (67)	131 (58)	
	Tan only	78 (17)	15 (7)	
Type of occupation	Mainly outdoors	89 (19)	55 (24)	.281
	Outdoors and indoors	189 (41)	90 (40)	
	Mainly indoors	184 (40)	81 (36)	
Smoking	Never smoked	254 (58)	108 (48)	**.014**
	Current smoker	50 (12)	24 (11)	
	Ex-smoker	131 (30)	93 (41)	

[1] P-value calculated using Chi-squared test.

Table 2
Seroprevalence and odds ratios with 95% confidence intervals for 12 polyomaviruses (VP1) in cSCC cases and controls.

	Control N (%)		Case N (%)		Adjusted OR*	(95% CI)
	+	−	+	−		
BK VP1	412 (89)	49 (11)	201 (89)	25 (11)	0.96	(0.57–1.59)
JC VP1	220 (48)	240 (52)	101 (45)	125 (55)	0.88	(0.64–1.21)
LPV VP1	43 (9)	418 (91)	20 (9)	206 (91)	0.94	(0.54–1.65)
TSV VP1	349 (76)	112 (24)	176 (78)	50 (22)	1.13	(0.77–1.65)
MCV344 VP1	205 (45)	256 (55)	103 (46)	123 (54)	1.05	(0.76–1.44)
KI VP1	340 (74)	121 (26)	157 (70)	69 (30)	0.81	(0.57–1.15)
WU VP1	445 (96)	16 (4)	220 (97)	6 (3)	1.32	(0.51–3.42)
HPYV6 VP1	364 (79)	97 (21)	168 (74)	58 (26)	0.77	(0.53–1.12)
HPYV7 VP1	253 (55)	208 (45)	138 (61)	88 (39)	1.29	(0.93–1.78)
HPYV9 VP1	52 (11)	409 (89)	26 (12)	200 (88)	1.02	(0.62–1.69)
HPYV10 VP1	446 (97)	15 (3)	224 (99)	2 (1)	3.77	(0.85–16.6)
SV40 VP1	98 (21)	363 (79)	45 (20)	181 (80)	0.92	(0.62–1.37)

* Adjusted for age and sex.

The median number of PyV antigens cases and controls tested positive for (VP1 and TAg for most PyVs) was 7, with maximum 15 in cases and 18 in controls of a total of 25 antigens tested (Table 3). We found no differences between cases and controls in positivity to any particular phylogenetic group. The median of the% CVs across the VP1