

RESEARCH ARTICLE

Cancer Epidemiology

Antibodies against high-risk human papillomavirus proteins as markers for noncervical HPV-related cancers in a Black South African population, according to HIV status

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Abstract

Human papillomavirus (HPV) proteins may elicit antibody responses in the process toward HPV-related malignancy. However, HPV seroepidemiology in noncervical HPV-related cancers remains poorly understood, particularly in populations with a high prevalence of human immunodeficiency virus (HIV). Using a glutathione S-transferase-based multiplex serology assay, antibodies against E6, E7 and L1 proteins of HPV16 and HPV18 were measured in sera of 535 cases of noncervical HPV-related cancers (anal ($n = 104$), vulval ($n = 211$), vaginal ($n = 49$), penile ($n = 37$) and oropharyngeal ($n = 134$)) and 6651 non-infection-related cancer controls, from the Johannesburg Cancer Study that recruited Black South African with newly diagnosed cancer between 1995 and 2016. Logistic and Poisson regression models were used to calculate adjusted odds ratios (aOR) and prevalence ratios (aPR) and 95% confidence intervals (CI) in cases versus controls. HPV16 E6 was more strongly associated with noncervical HPV-related cancers than HPV16 L1 or E7, or HPV18 proteins: anal (females (HPV16 E6 aOR = 11.50;95%CI:6.0–22.2), males (aOR = 10.12;95%CI:4.9–20.8), vulval (aOR = 11.69;95%CI:7.9–17.2), vaginal (aOR = 10.26;95%CI:5.0–21), penile (aOR = 18.95;95%CI:8.9–40), and oropharyngeal (females (aOR = 8.95;95%CI:2.9–27.5), males (aOR = 3.49;95%CI:1.8–7.0)) cancers. HPV16-E6 seropositivity

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ranged from 24.0% to 35.1% in anal, vulval, vaginal and penile cancer but was significantly lower (11.2%) in oropharyngeal cancer. After adjustment for HIV, prevalence of which increased from 22.2% in 1995–2005 to 54.1% in 2010–2016, HPV16 E6 seropositivity increased by period of diagnosis (aPR for 2010–2016 vs. 1995–2006 = 1.84; 95%CI:1.1–3.0). Assuming HPV16 E6 seroprevalence reflects HPV attributable fraction, the proportion of certain noncervical-HPV-related cancers caused by HPV is increasing over time in South Africa. This is expected to be driven by the increasing influence of HIV.

KEYWORDS

Black South African, HIV status, human papillomavirus, noncervical HPV-related cancers, proteins

What's new?

Human papillomavirus seroepidemiology in non-cervical HPV-related cancers is still poorly understood, particularly in populations with a high prevalence of HIV. Here, the authors investigated seropositivity for HPV16 and HPV18 antibodies in non-cervical HPV-related cancer cases and cancer controls, according to HIV status. The data suggested that the proportion of non-cervical HPV-related cancers caused by HPV, particularly vulval cancer, is raising in South Africa, a trend likely driven by the increasing influence of HIV. The findings may inform prevention strategies through HPV vaccination and early detection in populations with a high prevalence of both HPV and HIV.

1 | INTRODUCTION

While cervical cancer is the most common human papillomavirus (HPV)-related cancer, with HPV as a necessary cause (690,000 attributable cancers annually), a variable proportion of cancer at noncervical sites, including the anus (29,000 HPV-attributable cases diagnosed annually), penis (18,000), vulva (11,000), vagina (14,000), and oropharynx (42,000), (total of 114,000 cancers worldwide) are also caused by HPV.¹ HPV16 is by far the most oncogenic type, responsible for the large majority of all HPV-related cancers, particularly those arising outside the cervix.² Human immunodeficiency virus (HIV) coinfection increases the risk of all HPV-related cancers,³ through immunosuppression.⁴ It can also influence the proportion of noncervical cancers attributable to HPV,^{5–8} but it may also influence the immune response to HPV proteins.⁹

Different HPV proteins are expressed during the development process toward HPV-related malignancy and can produce antibody responses. The late protein L1 forms the virus capsid, and antibodies against HPV L1 are considered markers of cumulative exposure to the virus, even though many HPV-infected women are known to not seroconvert.¹⁰ Early HPV oncoproteins E6 and E7 interact with cellular proteins involved in cell cycle control and DNA repair, most notably p53 and pRb, and are implicated in malignant cellular transformation. Indeed, antibodies against E6 of HPV16 and HPV18 have been found to be highly specific markers of cervical cancer in comparison to controls,^{9–13} as well as of oropharyngeal cancer.^{14–18} However, there remains a limited understanding of E6 and E7 antibodies as potential biomarkers for other HPV-related cancers,^{19–23} which were performed in European or North American populations only.

Little is known about HPV E6 serology in noncervical HPV-related cancers in sub-Saharan Africa, where the fraction of these cancers attributable to HPV may differ²⁴ due to the higher population burden of both HPV and HIV. In South Africa, where HIV is common, noncervical HPV-related cancers are emerging as a significant health burden, particularly among Black populations.^{25,26} Unlike cervical cancer, for which effective screening methods exist, noncervical HPV-related cancers lack early detection measures. Therefore, identifying potential biomarkers for HPV-related cancers would facilitate early detection and help to develop targeted prevention and treatment strategies.

Leveraging data from the Johannesburg Cancer Study (JCS), which collected serum samples and demographic information from newly diagnosed cancer patients in the Black South African population between 1995 and 2016,² we investigated the seropositivity of HPV16 and HPV18 antibodies, most notably HPV16 E6, in noncervical HPV-related cancers cases and cancer controls, according to HIV status and other predictors. We were particularly interested in using HPV16 E6 seroprevalence as a surrogate for changes in HPV attributable fraction.

2 | MATERIALS AND METHODS

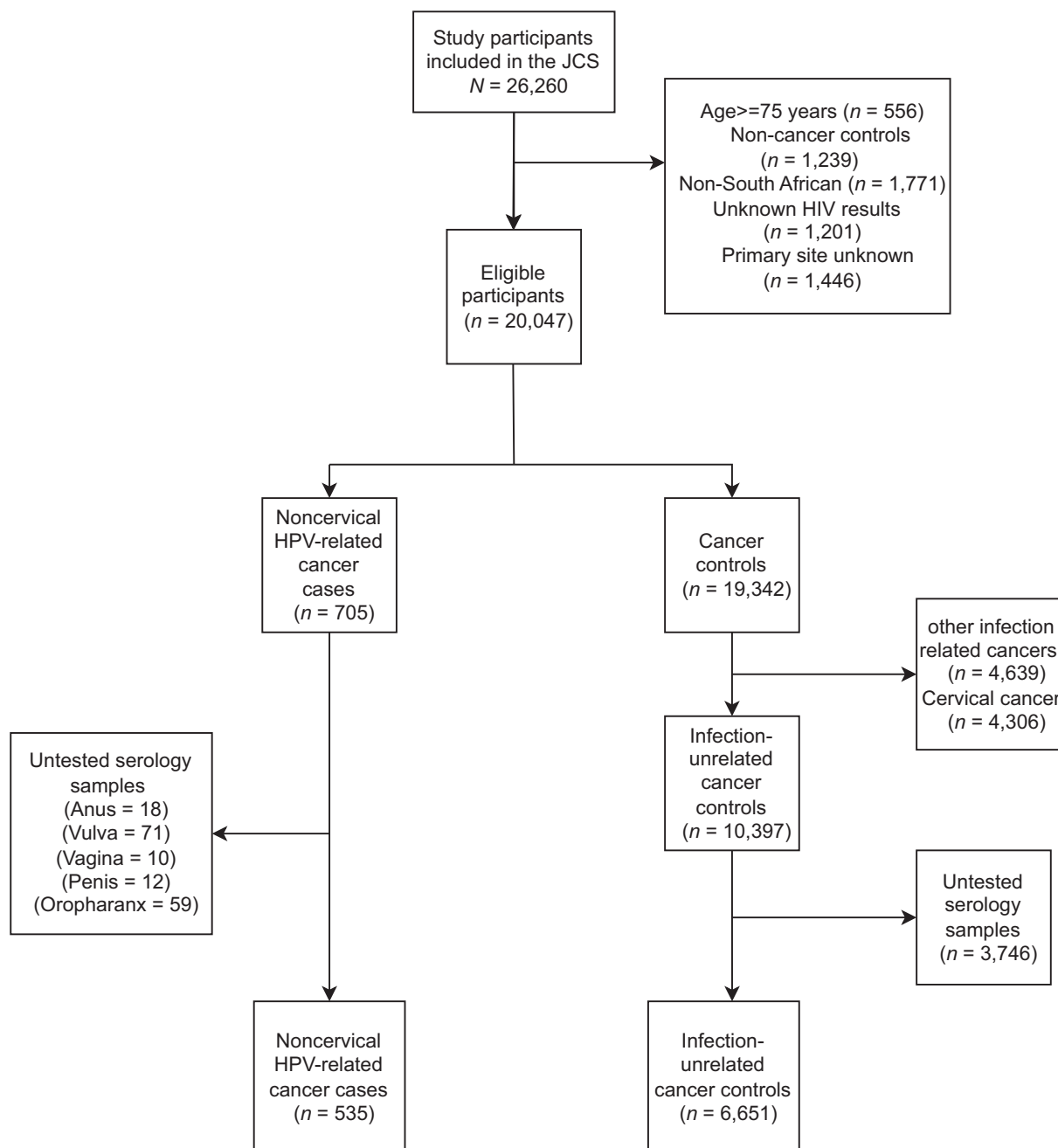
2.1 | Data source

For our analysis, we used data from the JCS, the details of which have been described previously.^{3,27,28} From 1995 to 2016, the JCS collected information from ~25,000 Black South African patients (male

and female). Eligibility criteria included being 18 years and above, having a newly diagnosed cancer and being referred to medical oncology and radiation therapy wards at hospitals and clinics in Johannesburg.

Trained nurses conducted one-off interviews in English or the patient's preferred South African language, at the time of cancer diagnosis. Patients provided self-reported demographic and key lifestyle risk factor information. Blood samples were collected in serum separator tubes and EDTA (for DNA analyses-not reported here) before receiving chemo- or radiation therapy. Sera were separated from whole blood on the same day of collection and divided into up to four

aliquots. HIV testing was performed using the Vironostika (HIV Uni-form II plus O) micro-enzyme-linked immunosorbent assay (ELISA) antibody test. Since histological confirmation is a requirement before cancer treatment at the medical oncology and radiation therapy department (where recruitment took place), we had access to the pathology forms, of which >90% of HPV-related cancer cases were histologically verified as squamous cell carcinoma or adenocarcinoma (Supplementary Table S1). All cancer types were classified based on the International Classification for Diseases for Oncology (Third Edition).



HIV: human immunodeficiency virus, HPV: human papillomavirus, JCS: Johannesburg Cancer Study

FIGURE 1 Selection of cancer cases and cancer controls.

2.2 | Cancer cases and cancer controls

Over a period of 21 years, JCS enrolled 705 individuals with noncervical HPV-related cancers, comprising cancers of the anus (C21) ($n = 122$), vulva (C51) ($n = 282$), vagina (C52) ($n = 59$), penis (C60) ($n = 49$),²⁷ and oropharynx ($n = 193$) (C01.9, C02.4, C05.1, C05.2, C09.0–10.9).²⁹

For our study, and after excluding cancers without serum samples, we included a total of 535 noncervical HPV-related cases, consisting of anal (C21) ($n = 104$, [HIV+ = 33, HIV− = 71]), vulval (C51) ($n = 211$, [HIV+ = 105, HIV− = 106]), vaginal (C52) ($n = 49$, HIV+ = 26, HIV− = 23), penile (C60) ($n = 37$, HIV+ = 18, HIV− = 19),

and oropharyngeal (C01.9, C02.4, C05.1, C05.2, C09.0–10.9) ($n = 134$, HIV+ = 28, HIV− = 106) cancers.

The JCS is amenable to a case-control study design and analysis, where the selection of cancer controls can be used to examine exposures of interest.⁹ During the same period, after excluding other infection-related cancers (including 4306 cervical cancers for which HPV serology results have been reported previously),⁹ 10,397 participants with non-infection-related cancers were recruited in the JCS (the distribution of the cancers are in Supplementary Table S2). Of these 10,397 participants with non-infection-related cancers, 6651 (males ($n = 2308$) and females ($n = 4343$)) had their serology samples tested (Figure 1).

TABLE 1 Characteristics of Johannesburg cancer study participants in the analytic population.

Characteristics	Noncervical HPV-related cases					Controls ($N = 6651$) n (%)
	Anus ($N = 104$)	Vulva ($N = 211$)	Vagina ($N = 49$)	Penis ($N = 37$)	Oropharynx ($N = 134$)	
	n (%)	n (%)	n (%)	n (%)	n (%)	
Age (years)						
<45	39 (37.5)	82 (38.9)	18 (36.7)	10 (27.0)	10 (7.5)	1911 (28.7)
45–54	30 (28.8)	71 (33.6)	9 (18.4)	14 (37.8)	51 (30.1)	1997 (30.0)
55–74	35 (33.7)	58 (27.5)	22 (44.9)	13 (35.1)	73 (54.5)	2743 (41.2)
Age (years)-median (IQR)	48.5 (39–57)	47.0 (40–56)	54.0 (40–63)	51.0 (43–57)	55 (50–61)	52 (43–60)
Gender						
Males	50 (48.1)	0 (0.0)	0 (0.0)	37 (100.0)	115 (85.8)	2303 (34.7)
Females	54 (51.9)	211 (100.0)	49 (100.0)	0 (0.0)	19 (14.2)	4343 (65.3)
Period of diagnosis						
1995–2004	28 (26.9)	85 (40.3)	11 (22.4)	12 (32.4)	8 (6.0)	1764 (26.5)
2005–2009	30 (28.8)	40 (19.0)	8 (16.3)	12 (32.4)	59 (44.0)	2115 (31.8)
2010–2016	46 (44.2)	86 (40.8)	30 (61.2)	13 (35.1)	67 (50.0)	2772 (41.7)
Marital status						
Never married	25 (24.0)	74 (35.1)	13 (26.5)	7 (18.9)	19 (14.2)	1256 (19.0)
Married	51 (49.0)	66 (31.3)	15 (30.6)	20 (54.1)	72 (53.7)	3450 (52.1)
Ever married	27 (26.0)	71 (33.6)	21 (42.9)	10 (27.0)	43 (32.1)	1922 (29.0)
Missing	1 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Education level						
No education/primary	38 (36.5)	118 (55.9)	17 (34.7)	19 (51.4)	60 (44.8)	2676 (40.3)
Secondary and above	66 (63.5)	93 (44.1)	32 (65.3)	17 (45.9)	73 (54.5)	3965 (59.7)
Missing	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.8)	0 (0.0)
HIV-status						
Negative	71 (68.3)	106 (50.2)	23 (46.9)	19 (51.4)	106 (79.1)	5457 (82.0)
Positive	33 (31.7)	105 (49.8)	26 (53.1)	18 (48.6)	28 (20.9)	1194 (18.0)
^a ART use						
Never	6 (20.7)	38 (39.6)	5 (27.8)	4 (36.4)	7 (36.4)	353 (46.9)
Ever	23 (79.3)	58 (60.4)	13 (72.2)	7 (63.6)	13 (63.6)	399 (53.1)
Missing	4	9	8	7	8	442

Abbreviations: ART, antiretroviral therapy; HIV, human immunodeficiency virus; IQR, interquartile range.

^aART self-reported use among HIV-positive cancer cases only.

3.2 | HPV16 protein seropositivity among noncervical HPV-related cases and non-infection-related cancer controls

In non-infection-related cancer controls, HPV16 protein seropositivity was 13.9% and 6.2% for L1, 2.9% and 2.8% for E6, and 6.4% and 4.9% for E7, among females and males respectively (Table 2). Compared to the non-infection-related cancer controls, there was a strong association between HPV16 E6 seropositivity and the risk of noncervical HPV-related cancers. In anal cancer, HPV16 E6 seropositivity in both females (24.1%) and males (24.0%) was significantly elevated versus controls, [aOR = 11.50 (95% CI: 5.96–22.2) and 10.12 (95% CI: 4.91–20.8), respectively]. Similarly, HPV16 E6 seropositivity was significantly associated with vulval (23.7%; aOR = 11.69, 95% CI: 7.92–17.2), vaginal (22.5%; aOR = 10.26, 95% CI: 4.98–21.3), penile (35.1%; aOR = 18.95, 95% CI: 8.87–40.5), and oropharyngeal cancer, both among females (21.1%;

aOR = 8.95, 95% CI: 2.91–27.5) and males (9.6%; aOR = 3.49, 95% CI: 1.76–6.95). Among oropharyngeal cancers, there were no differences in E6 seropositivity between tonsillar ($n = 71$, 11.3%) and non-tonsillar ($n = 63$, 11.1%) sites (data not shown). There were no HPV16 E6 seropositivity associations with variables among control cancers (Supplementary Table S5).

Associations for HPV16 L1 and E7 were weaker than for HPV16 E6. HPV16 E7 seropositivity was associated with anal (aOR = 4.54, 95% CI: 2.17–9.48) and oropharyngeal cancer (aOR = 5.74, 95% CI: 1.87–17.6) among females but not among males, as well as with vulval (aOR = 4.67, 95% CI: 3.11–7.04), vaginal (aOR = 5.32, 95% CI: 2.50–11.3), and penile cancer (aOR = 4.66, 95% CI: 2.22–12.1). HPV16 L1 seropositivity was associated with anal cancer in females (aOR = 2.29, 95% CI: 1.25–4.19) and males (aOR = 6.44, 95% CI: 3.36–12.3), with vulval (aOR = 3.48, 95% CI: 2.57–4.69), vaginal (aOR = 1.97, 95% CI: 1.03–3.78) and penile cancer (aOR = 4.68, 95% CI: 2.11–10.3), but not with oropharyngeal cancer (Table 2).

TABLE 3 Factors associated with HPV16 antibody seropositivity among noncervical HPV-related cancers.

		HPV16					
		L1		E6			
Characteristics	N = 535	Seropositive n (%)	aPR [95% CI]	Seropositive n (%)	aPR [95% CI]	Seropositive n (%)	aPR [95% CI]
Cancer types							
Anal	104	30 (28.9)	1.00	25 (24.0)	1.00	12 (11.5)	1.00
Vulval	211	79 (37.4)	1.19 [0.78–1.85]	50 (23.7)	1.17 [0.72–1.95]	35 (16.6)	1.08 [0.98–1.17]
Vaginal	49	13 (26.5)	0.81 [0.41–1.54]	11 (22.5)	1.05 [0.49–2.11]	9 (18.4)	1.09 [0.97–1.23]
Penile	37	9 (24.3)	0.77 [0.34–1.58]	13 (35.1)	1.75 [0.86–3.43]	8 (21.6)	1.12 [0.98–1.28]
Oropharyngeal	134	9 (6.7)	0.26 [0.11–0.53]	15 (11.2)	0.40 [0.21–0.77]	13 (9.7)	0.96 [0.87–1.05]
Age (years)							
<45	159	63 (39.6)	1.00	29 (18.2)	1.00	19 (12.0)	1.00
45–54	175	38 (21.7)	0.79 [0.51–1.20]	45 (25.7)	1.31 [0.80–2.19]	27 (15.4)	1.03 [0.95–1.11]
55–74	201	39 (19.4)	0.92 [0.57–1.46]	40 (19.9)	0.90 [0.52–1.57]	31 (15.4)	1.01 [0.93–1.10]
Year of diagnosis							
1995–2004	144	39 (27.1)	1.00	30 (20.8)	1.00	21 (14.6)	1.00
2005–2009	149	35 (23.5)	1.12 [0.69–1.80]	27 (18.1)	1.29 [0.74–2.22]	24 (16.1)	1.07 [0.98–1.16]
2010–2016	242	66 (27.3)	0.98 [0.64–1.52]	57 (23.6)	1.84 [1.14–3.01]	32 (13.2)	1.04 [0.96–1.13]
HIV-status							
Negative	325	58 (17.9)	1.00	80 (24.6)	1.00	54 (16.6)	1.00
Positive	210	82 (39.5)	1.76 [1.16–2.69]	34 (16.2)	0.45 [0.27–0.74]	23 (11.0)	0.92 [0.85–0.99]

Note: Prevalence ratios adjusted for noncervical HPV-related cancers, Age, HIV status, and year of diagnosis. The bold values of aPR and aOR are significantly different from the reference group.

Abbreviations: aPR, adjusted prevalence ratio; CI, confidence interval; HIV, human immunodeficiency virus; HPV, human papillomavirus.

TABLE 4 HPV16 E6 seroprevalence by cancer site and period of cancer diagnosis.

Year of diagnosis	Anus		Vulva		Vagina		Penis		Oropharynx	
	N	HPV16 E6+	N	HPV16 E6+	N	HPV16 E6+	N	HPV16 E6+	N	HPV16 E6+
		n (%)		n (%)		n (%)		n (%)		n (%)
1995–2004	28	9 (32.1)	85	14 (16.5)	11	1 (9.1)	12	6 (50.0)	8	0 (0.0)
2005–2009	30	5 (16.7)	40	10 (25.0)	8	3 (37.5)	12	3 (25.0)	59	6 (10.2)
2010–2016	46	11 (23.9)	86	26 (30.2)	30	7 (23.3)	13	4 (30.8)	69	9 (13.4)

Abbreviation: HPV, human papillomavirus.

Similar patterns but generally weaker associations were observed in L1, E6 and E7 for HPV18 and HPV16/18 combined (Supplementary Tables S6 and S7). The correlation of seropositivity for L1, E6 and E7 antibodies against HPV16 and HPV18 is shown in Supplementary Figure S1.

3.3 | Factors associated with HPV16 antibody seropositivity in noncervical HPV-related cancers

HPV16 L1 seroprevalence was comparable across anal, vulval, vaginal and penile cancer sites but was significantly lower in oropharyngeal cancer (aPR vs. anal cancer = 0.26, 95% CI: 0.11–0.53) and was higher in PLWH than HIV-negative cancers (aPR = 1.76, 95% CI: 1.16–2.69) (Table 3). HPV16 E6 seroprevalence was also comparable across anal, vulval, vaginal and penile cancer sites but significantly lower in oropharyngeal cancer (aPR vs. anal cancer = 0.40, 95% CI: 0.21–0.77). Conversely to HPV16 L1, however, HPV16 E6 seroprevalence was lower in PLWH than in HIV-negative cancers (aPR = 0.45, 95% CI: 0.27–0.74). There was also a significant increase in HPV16 E6 seroprevalence by period of cancer diagnosis (aPR for 2010–2016 vs. <2005 = 1.84, 95% CI: 1.14–3.01). (Table 3). Among PLWH and cancer for whom there was information on ART use, seroprevalence of HPV16 L1 (aPR = 0.31, 95% CI: 0.14–0.61) and E6 (aPR = 0.34, 95% CI: 0.09–0.92) was lower among never ART users (data not shown).

HPV16 E6 seropositivity is shown for individual noncervical HPV-related cancer sites by period of cancer diagnosis in Table 4; increases in E6 seroprevalence by period were particularly apparent for vulval cancer (Table 4), even when restricted to cases of PLWH only (0/23 (0%) for <2005 vs. 16/64 (25%) for 2010/2016; data not shown). No significant differences were observed for E7 seroprevalence in noncervical HPV-related cancers, nor were any significant differences observed in L1, E6 or E7 seroprevalence by age (Table 3). Similar patterns of associations were observed in L1, E6 and E7 seropositivity for HPV18 and HPV16/18 combined (Supplementary Tables S8 and S9).

4 | DISCUSSION

This study contributes to the understanding of HPV seroepidemiology in noncervical HPV-related cancers in a Black South African

population. Our findings show a significant association between seropositivity of HPV16 and HPV18 antibodies and anal, vulval, vaginal, penile and oropharyngeal cancers, confirming the role of HPV16/18 in the etiology of these cancers in high HIV prevalence settings. For all cancer types tested for the immune marker, positivity for HPV16 E6 antibodies best distinguished noncervical HPV-related cancers from cancer controls. Interestingly, HPV16 E6 seropositivity was significantly lower in noncervical HPV-related cancers diagnosed among PLWH than among HIV-negative persons (and particularly among those who never used ART), even if the fraction of cancers attributable to HPV is expected to be higher among PLWH. This phenomenon may be due to HPV16 E6 immune response impairment due to severe immunodeficiency from HIV. Furthermore, HPV16 E6 seropositivity increased with period of cancer diagnosis over time, particularly for vulval cancer. This suggests relative increases in the fraction of these noncervical cancers, particularly vulval cancer that are attributable to HPV over time in this setting. However, E6 seropositivity is still expected to underestimate the absolute fraction attributable to HPV due to lack of sensitivity for some of these cancer sites.

Relatively few studies have evaluated HPV16 antibodies in noncervical HPV-related cancers, and there are no studies to date in a high HIV prevalence setting such as sub-Saharan Africa. Our finding of HPV16 E6 seropositivity in anal cancer (~24% in both men and women) is similar to findings from a European prospective study in HIV-negative persons (29.2%),¹⁹ a case-control study from the United States (29.3%),²³ and in PLWH in the Swiss HIV cohort (23.3%),²⁰ which all confirmed significant associations in cases versus controls. This consistency in HPV seropositivity across studies may be partly due to the expected consistency in HPV attributable fraction in anal cancer, for which HPV is considered an almost necessary cause, irrespective of HIV status.¹ These findings confirm a lack of sensitivity of HPV16 E6 antibodies to detect HPV-related anal cancer, even at the time of cancer diagnosis. This is similar to the lack of sensitivity for cervical cancer in which HPV16 is also considered a cause of the majority of cases.⁹ Of note, in South Africa, a significant upward trend in anal cancer incidence has been reported between the period 2005–2012.²⁵

For other noncervical HPV-related cancer sites, HPV is known to cause only a fraction of cancers, and this fraction may vary geographically, given the balance with other risk factors. Most previous data have been generated for oropharyngeal cancer, observing a strong association with HPV16 E6 seropositivity.^{14,16–18,35} In these studies,

HPV16 E6 seropositivity in oropharyngeal cancer, which was performed in high- and low-income countries, ranged from 2.5%¹⁶ to 63.5%.¹⁵ In studies that were able to confirm HPV16 DNA in the tumor, E6 seropositivity was shown to show very high sensitivity for HPV-related oropharyngeal cancer,³⁶ so HPV16 E6 seropositivity in oropharyngeal cancer can be considered a good surrogate of HPV16 attributable fraction. Our finding of 11.2% HPV16 E6 seropositivity in oropharyngeal cancer would thus suggest only a relatively small HPV-attributable fraction in South Africa, with the majority of oropharyngeal cancers in this setting being caused by other risk factors. Indeed, this confirms evidence from a number of studies of HPV DNA in oropharyngeal tumor series in South Africa.^{37,38} No significant change in oropharyngeal cancer incidence have been reported in South Africa.²⁵ However, assuming people in South Africa are diagnosed at a later stage than in high-income countries, some oropharyngeal cancers may be cancers of adjoining tissues, thus diluting the true relationship.

With respect to penile cancer, significant associations between HPV16 E6 seropositivity and penile cancer have been found in the Netherlands,²² for which HPV16 E6 seropositivity was 15%, and in a prospective European cohort study (14% within 5 years of cancer diagnosis).¹⁹ While we confirmed this association in South Africa, our study showed that HPV16 E6 seropositivity in penile cancer was considerably higher (35.1%), suggesting a higher HPV-attributable fraction in penile cancer in South Africa than in Europe, where incidence is reported to be significantly increasing from 1994 to 2012.²⁵ However, HPV16 E6 seropositivity in penile cancer is still expected to be an under-representation of the fraction of tumors that are truly HPV16-related.²²

Few studies have reported on HPV16 E6 seropositivity in vulval and vaginal cancer.^{19,21} A prospective European cohort study on HPV16 E6 seropositivity and anogenital cancers showed that HPV16 E6 seropositivity for anogenital cancers was 8.3% for 12 vaginal cancers and 1.5% for 67 vulval cancers.¹⁹ A case-control study that used radioimmunoprecipitation assay for serology testing showed that HPV16 E6 seropositivity for vulval cancer was 14.3%.²¹ Our findings on 211 cases of vulval cancers and 49 cases of vaginal cancer, which are relatively more common in South Africa than elsewhere in the world,³⁹ thus add considerably to the evidence base, and the higher HPV16 E6 seropositivity (23.7% and 22.5%, respectively), suggests a higher HPV attributable fraction in this South African population with high HIV prevalence than in Europe.⁴⁰

Our study showed an increase in E6 seroprevalence in vulval cancer over time in South Africa. Assuming HPV16 E6 seroprevalence to be a reflection of HPV causality, our data suggest that the proportion of vulval cancer related to HPV16 is increasing. Additionally, the increase in E6 seroprevalence could be due to increasing HIV, and improved immunocompetency due to wider use of ART. Chikandiwa et al. observed important increases in the incidence of vulval cancer in South Africa from the period 1994–2012 with an annual period change of 16.1%, p -value = .01.²⁵ This is expected to reflect increases in HIV prevalence.⁴¹

With respect to HIV status, HPV16 L1 seropositivity was significantly higher in PLWH than in HIV-negative noncervical HPV-related

cancers, suggesting increased exposure to HPV in PLWH with these cancers.⁴² HPV16 E6 seropositivity, however, was significantly lower in (particularly vulva) cancers diagnosed among PLWH than among HIV-negative persons, even though the fraction of cancers attributable to HPV was expected to be higher among PLWH. This phenomenon was also observed for HPV18 E6 and is similar to that seen previously for cervical cancer in this same population.⁹ It may be due to E6 immune response impairment in the presence of HIV infection. This was further supported by the observation that E6 seroprevalence was lower among never-than-ever ART users, which may be a surrogate for the degree of HIV-related immunodeficiency.

Our study has several limitations. First of all, we lacked HPV DNA results in tumor samples to estimate the sensitivity of HPV seropositivity for the HPV-related fraction of these noncervical anogenital cancers. We also lacked information on tumor staging. We did not have a sufficient sample size to analyze determinants of HPV seropositivity by individual cancer site, so we relied on a multivariate model adjusted for cancer sites. The odds ratios for HPV16 E6 seropositivity in noncervical HPV-related cancers tended to be lower than that reported in the EPIC study.¹⁹ This is partly explained by a higher prevalence of antibodies against HPV16 E6 (2.8%;189/6651) and E7 (5.2%; 348/6651) observed in controls compared to other large case-control studies.^{32,43} However, similar findings were seen in the United States among anal cancer patients.²³

In our study, we focused on HPV16 as by far the most important HPV type in noncervical HPV-related cancers. However, similar results were seen for HPV18 (albeit with lower power due to lower seropositivity) or HPV16/18 combined. In addition, HPV16 E6 was the most discriminant marker over E7 and L1, as shown in previous studies of HPV-related cancers.^{12,14,44}

The strengths of our study include using the multiplex serology assay,³⁰ which assesses different antigens simultaneously at the same laboratory, a large sample size for cases and cancer controls, the representativeness of the JCS of cancers among a Black sub-Saharan African population, and the ability to assess HIV-positive and HIV-negative cancer patients simultaneously. The study also used a stringent oropharyngeal cancer definition by state-of-the-art international classification of diseases coding.

In conclusion, HIV infection appears to influence HPV antibody response in noncervical HPV-related cancers. Assuming HPV16 E6 seroprevalence to be a reflection of HPV causality, our data suggests that the proportion of noncervical-HPV-related cancer caused by HPV, particularly vulval cancer, is increasing over time in South Africa, expected to be driven by increasing influence of HIV. These findings can inform prevention strategies, most notably through HPV vaccination and early detection, in populations with a high prevalence of both HPV and HIV, to reduce the burden of HPV-related cancers and prevent comorbidity among PLWH.

AUTHOR CONTRIBUTIONS

Mwiza Gideon Singini conceptualized the study, performed data analysis and wrote the first draft of the manuscript. Gary M. Clifford conceptualized the study and supervised the writing of the manuscript.

Freddy Sitas, Mazvita Muchengeti, Wenlong Carl Chen, Jean-Damien Combes, and Tim Waterboer edited the manuscript. Tim Waterboer conducted the laboratory analyses. Wenlong Carl Chen was responsible for data acquisition, data curation quality controls, and management. All authors read and provided feedback to improve the final version of the manuscript. The work reported in the paper has been performed by the authors, unless clearly specified in the text.

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CONFLICT OF INTEREST STATEMENT

TW serves on advisory boards for Merck (MSD), Sharp & Dohme. All other authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data are available from the National Cancer Registry of South Africa (contact: Dr Mazvita Muchengeti mazvitam@nicd.ac.za) for researchers who meet the relevant ethics criteria for access to these data. Further information is available from the corresponding author upon request.

ETHICS STATEMENT

The Johannesburg Cancer Study (JCS) and the present study were approved by the University of the Witwatersrand Human Research Ethics Committee (Medical) (certificate number for the current study. M2210104). In the JCS, participants gave written informed or witnessed consent to a one-off interview and optional blood draw and to have their information and blood sample anonymized. Any future investigations require approval of the University of the Witwatersrand Human Research Ethics Committee (HREC).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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