RESEARCH ARTICLE

HPV types 16/18 L1 E6 and E7 proteins seropositivity and cervical cancer risk in HIV-positive and HIV-negative black South African women

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Abstract

Background: In populations with high rates of human immunodeficiency virus (HIV)-coinfection, the nature of the relationship between human papillomavirus (HPV)-16 and -18 (L1, E6 and E7) antibodies and cervical cancer is still uncertain. We measured the association between seropositivity to HPV (L1, E6 and E7) proteins and cervical cancer among black South African women with and without HIV co-infection.

Methods: We used questionnaire data and serum collected from consecutively recruited patients with a newly diagnosed cancer from the Johannesburg Cancer Study from 1346 cervical cancer cases and 2532 controls (diagnosed with other non-infection related cancers). Seropositivity to HPV proteins was measured using a multiplex serological assay based on recombinant glutathione S-transferase (GST) fusion proteins. We measured associations between their presence and cervical cancer using unconditional logistic regression models and evaluated the sensitivity and specificity of these HPV biomarkers.

Results: Among controls, HIV-negative women from rural areas compared to urban had significantly higher HPV seroprevalence, HPV16 E7 (8.6% vs 3.7%) and HPV18 E7 (7.9% vs 2.0%). HPV16 E6 and E7 antibodies were positively associated with cervical cancer in HIV-positive (Adjusted Odds Ratio (AOR) = 33; 95% CI 10–107) and HIV-negative women (AOR = 97; 95% CI 46–203). In HIV-positive women, HPV E6/E7 antibodies had low sensitivity (43.0%) and high specificity (90.6%) for cervical cancer detection. In HIV-negative women, HPV E6/E7 antibodies sensitivity was 70.6% and specificity was 89.7%.

Conclusions: Our data show that HPV (L1, especially E6 and E7) antibody positivity is associated with cervical cancer in both HIV-positive and HIV-negative women. Nonetheless, being HIV-positive plays an important role in the development of cervical cancer.

Keywords: Cervical cancer, Human papillomavirus, E6 and E7, L1 proteins, Seropositivity, South Africa

Introduction

Globally, in 2018, about 70% of cervical cancers were attributable to high-risk human papillomavirus (HPV) types 16 and 18 [1]. In South Africa, HPV16 and 18 are

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important causes of cervical cancer and are included in the currently available HPV vaccine.

The HPV early (E6 and E7) oncoproteins and the late (L1) protein are encoded by the HPV genome. The HPV oncoproteins play an important role in the tumorigenesis of cervical cancer [2]. Humoral immune response against major capsid late protein (L1) is generated during infection with HPV; which is a marker of past or present infection [3]. Furthermore, integration of the HPV genome in the host cell results in overexpression of E6 and E7 oncoproteins, which are involved in the transformation and progression of cervical and other HPV-related cancers [4]. Different serological assays have been used to screen for HPV-16 and -18 (E6 and E7) [5–8] oncoprotein antibodies. Serological markers for HPV-antibodies provide useful data about past and current HPV infections and their relationship to cervical cancer [9, 10].

Previous studies have shown that HPV types 16 and 18 L1, E6 and E7 antibody proteins are associated with cervical cancer susceptibility [6, 8, 11–13] however, with varying degrees of strength of association. For example, in a case–control study from Russia, Zumbach et al. [8] utilized an enzyme-linked immunosorbent assay (ELISA) and reported odds ratios (ORs) for cervical cancer of 64.4 (95% CI 3.8–1085) for HPV16 E6 and 4.9 (95% CI 1.3–18.7) for HPV16 E7. In another case–control study from Algeria and India, Combes et al. [10] used a multiplex immunofluorescent HPV serology assay and reported ORs for cervical cancer of 37.1 (95% CI 13.4–103) for HPV16 E6, 12.5 (95% CI 6.3–24.8) for HPV16 E7 and 5.9 (95% CI 3.1–11.1) for HPV16 L1. Earlier work on the Johannesburg Cancer Study (JCS) from 946 human immunodeficiency virus (HIV)-negative women with cervical cancer and 1,342 controls using an ELISA assay and different cut-offs found ORs between moderate and high HPV-16/L1 seropositivity of 1.5 (95% CI 1.2–1.9) and 2.4 (95% CI 1.9–3.0) [14]. Thus, multiple serologic methods had been used to characterize the relationship between cervical cancer incidence and HPV.

Less is known about the role of high-risk HPV oncoproteins among cervical cancer patients in black African women who are HIV-positive and HIV-negative. In South Africa, despite high HIV prevalence (24.1%) among black South African women [15], studies on HPV16 and 18 (E6 and E7) and late (L1) seropositivity and the risk of cervical cancer are still lacking. We therefore assessed the seropositivity of HPV -16 and -18 early (E6 and E7), and late (L1) antibodies among cervical cancer cases and infection unrelated cancer controls using a multiplex HPV serology assay [16], and estimated their association with cervical cancer risk among HIV-positive and HIV-negative women.

Methods
Setting and participants
The study population were participants recruited into the Johannesburg Cancer Study (JCS) between 1995 and 2016. The aims of JCS included measuring the relative importance of known and emerging risk factors for cancer in a black African population in Johannesburg, South Africa. The details of the JCS have been described elsewhere [17]. Briefly, the JCS collected serum samples from 26,000 consecutive consenting black South African patients with newly diagnosed cancers (>90% histopathology confirmed), referred to the medical oncology and radiation therapy wards of (the main tertiary public) Charlotte Maxeke Johannesburg Academic Hospital and associated referral hospitals and clinics. Self-reported data on demographics and key lifestyle risk factors were collected using a structured questionnaire. The JCS also collected venous blood in one serum separation tube (SST) and one Ethylenediaminetetraacetic acid (EDTA) tube. SST serum was separated from whole blood within two days of sample collection and the sample was divided into a maximum of 4 aliquots. HIV testing was done using the Vironostika HIV Ag/Ab kit [18]. The study was approved by the University of the Witwatersrand Human Research Ethics Committee (Medical) (certificate number: M200252).

Cases and controls
The JCS is amenable to a case–control design and analysis by selecting controls that are unrelated to the exposures of interest. The current study was restricted to women aged 25 to 54 years because a large proportion of the older women did not have serology samples available. Cases were 1346 women with newly diagnosed cervical cancer (C53). Controls were 2532 women with newly diagnosed infection unrelated cancers (breast (C50) (n = 1953), colon (C18-20) (n = 197), oesophagus (C15) (n = 48), endometrium (C54-55) (n = 75), lung (C33-34) (n = 76), pancreas (C25) (n = 21) and minor cancer types (n = 162) (Additional file 1: Table S1)). Figure 1 outlines the criteria used to select cases and controls.

Serology data and laboratory methods
Serum aliquots were stored at −25 °C and one aliquot was shipped on dry ice to the German Cancer Research Center (Deutsches Krebsforschungszentrum (DKFZ)) in Heidelberg, for antibody testing for HPV16 and 18 (L1, E6 and E7) using a multiplex serological assay. This was based on a glutathione S-transferase (GST) capture immunoassay in combination with fluorescent beads on a Luminex platform [16]. The final net (bead graphics and key lifestyle risk factors were collected using a structured questionnaire. The JCS also collected venous blood in one serum separation tube (SST) and one Ethylenediaminetetraacetic acid (EDTA) tube. SST serum was separated from whole blood within two days of sample collection and the sample was divided into a maximum of 4 aliquots. HIV testing was done using the Vironostika HIV Ag/Ab kit [18]. The study was approved by the University of the Witwatersrand Human Research Ethics Committee (Medical) (certificate number: M200252).
Cut-offs were generated at 1:100 serum dilution [19, 20] and were thus not applicable. Using the Visual Inflection Point (VIP) method [21], we defined net MFI cut-offs of 175 for HPV16 and 18 L1, 75 for HPV16 and 18 E6, 120 for HPV16 E7, and 70 for HPV18 E7. For HPV antibodies, we generated five extra binary variables to describe high-risk combinations for the presence of either E6/E7 singly or in combination (HPV16 E6 & E7, HPV18 E6 & E7, HPV16&18 E6, HPV16 &18 E7, and HPV16/18 E6/E7).

Fig. 1 Flow chart of the case–control selection for the subject with HPV-serology results among women aged 25–54 years in the Johannesburg Cancer Study.
Statistical analysis

Data on demographic characteristics were summarized using frequencies and percentages for the categorical variables, medians and the interquartile range (IQR) for the continuous variable that was not normally distributed. A Pearson's Chi-squared test (for categorical variables) and t-test (for age) were used for the comparison of demographics between cases and controls. To assess cross-reactivity between the proteins, a tetrachoric correlation coefficient rank test [22] with Bonferroni adjustments (to take account of multiple comparisons) was used to analyze the correlation between each HPV16 and 18 (L1, E6 and E7) antibodies and HIV antibodies (See Additional file 1: Fig. S2). In the control group, we calculated the seroprevalence of antibodies to HPV16 and 18 (E6 and E7) oncoproteins and L1 protein across different demographic and lifestyle factors, such as age (25–34, 35–44, 45–54 years), place of residence (rural, urban), period of interview (1995–1999, 2000–2004, 2005–2009, 2010–2016), marital status (never married, married, previously married), educational level (none, primary, secondary and above), number of sexual partners (0–1, 2–5, 6+) HIV-status (negative and positive) and parity (0, 1, 2, 3, 4+). We have previously shown that cancer types unrelated to the exposure of interest, resemble background population prevalence for that exposure [18, 23]. We performed a test for heterogeneity on categorical variables and a score test for trends in proportions for ordinal categorical variables. On the assumption that the selected controls should have similar seroprevalences to each other, we compared heterogeneity in seroprevalence of HPV16 and 18 (L1, E6 and E7) antibodies across different control cancer types using the Cochran-Mantel–Haenszel test (See Additional file 1: Fig. S1).

We used an unconditional logistic regression model to assess the association by calculating adjusted Odds Ratios (AOR) and 95% confidence intervals between HPV16 and 18 (E6 and E7) and L1 proteins seropositivity and the risk of cervical cancer. We stratified by HIV status to assess if the seroprevalence of the HPV proteins differs in HIV-positive and HIV-negative patients. We adjusted for age, education level, number of sexual partners, marital status, period of interview and place of residence. To assess whether the clinical performance of HPV-16 and -18 antibodies as a diagnostic marker for cervical cancer we performed the same in HIV-positive and HIV-negative cancer patients. We calculated the sensitivity, specificity of the various HPV antibodies for detecting cervical cancer using the “diagti” command in STATA. In addition, the area under the ROC curve (AUC) of the receiver operator characteristics was computed to compare the performance of the best combination of HPV E6

![Fig. 2 Seroprevalence of HPV-16 and -18 (L1, E6 and E7) proteins antibodies in cervical cancer cases and controls by HIV-status](image-url)
and E7 antibodies to discriminate cervical cancer from controls (See Additional file 1: Table S4). All the statistical tests were computed using STATA software version 16.0 (Stata Corp, college station, Tx) and SAS v 9.4 (SAS Institute, Cary, NC). All tests were considered significant at a two-tailed alpha of 5%.

**Results**

Out of a total of 2,795 cervical cancer cases and 5,569 infection unrelated cancer controls participating in the JCS, 3,878 women (1,346 cases and 2,532 controls) aged 25–54 years were included (Fig. 1). Cases were relatively younger (Median age: 42 (IQR: 37–46) compared to controls (Median age: 44 (IQR: 39–50) (p value < 0.001). At least two-thirds of both cases (63.5%) and controls (Median age: 44 (IQR: 39–50) (p value < 0.001). At least two-thirds of both cases (63.5%) and controls (72.4%) had a secondary school education (Table 1).

Overall, 50.2% of women with cervical cancer were HIV positive vs. 26.3% HIV positive controls (Table 1). Furthermore, 13.5% of HPV16 L1 seropositive women were also HPV18 L1 seropositive (Additional file 1: Table S3).

Stratifying by HIV-status, HPV16 E6 & E7 and HPV18 E6 & E7 seropositivities were higher among HIV-negative women amongst both cases HPV16 E6 (42.7%) and controls HPV16 E6 (3.0%) as compared to HIV-positive women cases HPV16 E6 (26.8%) and controls HPV16 E6 (2.8%) (Fig. 2).

Among HIV-negative controls, the overall antibody seroprevalence for HPV16 L1 was 14.3% and 0.5% for combined HPV16 E6&E7. Similar observations were made for HPV18 L1 (16.3%) and for combined HPV18 E6&E7 (0.2%) (Table 2). In HIV-positive controls, the overall antibody seroprevalence was 15.9% for HPV16 L1, HPV18 L1(15.7%), 0.5% for HPV16 E6 and E7 and 0.2% for HPV 18 E6 and E7 (Table 3).

In general, we did not observe differences in seroprevalence to HPV types 16 and 18 L1 and (E6 and E7) antibodies by demographic and sexual/reproductive factors (p value > 0.05). Among HIV-negative controls, the prevalence of HPV16 E7 antibody was higher among women who lived in rural areas (8.6%) as compared to women who lived in urban areas (3.7%) (p value for heterogeneity = 0.005) (Table 2). A similar pattern was observed for HPV18 E7 (rural (7.9%) vs urban (2.0%); p value = 0.001). Among HIV-positive controls, the prevalence of HPV16 E6 & E7 antibodies decreased with an increase in number births up to 2 births (p value = 0.006) (Table 3).

Being seropositive to HPV16 antibodies was significantly associated with cervical cancer: combined HPV16 E6 and 7 (AOR=69.20, 95% CI 37.07–129.18), HPV16 E6 (AOR = 21.96, 95% CI 16.61–29.02), HPV16 E7 (AOR = 7.93, 95% CI 6.16–10.22) and HPV16 L1 (AOR = 1.74, 95% CI 1.45–2.07). For HPV18, antibody seroprevalence ORs for cervical cancer ranged from AOR=38.61 (95% CI 15.13–98.53) for combined HPV18 E6&E7, AOR=8.94 (95% CI 6.64–12.03) for HPV18 E7, AOR=4.67 (95% CI 3.30–6.50) for HPV18 E6 and AOR=1.31 (95% CI 1.10–1.56) for HPV18 L1 (Table 4). In general, HPV L1 cervical cancer ORs were higher in HIV-positive women but lower in HIV-negative women. The AORs of cervical cancer for combined HPV16 E6 & E7 seropositivity were 97.40 (95% CI 46.68–203.23) in HIV-negative, and 33.10 (95% CI 10.22–107.20) in HIV-positive women (Fig. 3).

Among HIV-positive women, HPV16/18 E6/E7 antibodies had a sensitivity of 43.0%, specificity of 90.6% and AUC of 67% to detect cervical cancer. Among HIV-negative women, the sensitivity, specificity and AUC for HPV16/18 E6/E7 antibodies was 70.6%, 89.7% and 80% respectively for detection of cervical cancer. The sensitivity, specificity and AUC for discriminating cervical cancer based on HPV16 E6 positivity was 26.8%, 97.2% and 62% respectively in HIV-positive women. For HIV-negative women who were HPV E6 seropositive, the sensitivity was 42.7%, specificity was 95.6% and the AUC 69% (Table 5).

**Discussion**

In this study, we assessed antibody seropositivity to each of HPV-16 and -18 oncoproteins (E6 and E7), and L1 protein and calculated their association with cervical cancer. While the seroprevalence of HPV16 L1 in the previous JCS study among HIV-seronegative women was higher [14] than in this study, ORs between HPV16 L1 and cervical cancer were about the same (overall crude OR 2.2; 95% CI 1.8–2.6, vs 2.1; 1.5–2.7, Fig. 3). Previous data on HPV16 L1 seroprevalence in controls reflect different assay methods and cutoffs used, and perhaps some cross-reactivity with other HPV types. Our key findings, using larger sample size, more recent serological methods measuring a range of high-risk HPV types showed the important role of HPV-16 and -18 (E6 and E7) oncoproteins in cervical cancer risk in both HIV-positive and HIV-negative cancer patients. HPV-16 and -18 (E6 and E7) oncoproteins were associated in cervical cancer risk in both HIV-positive and HIV-negative cancer patients. HIV-positive patients compared to HIV-negative patients had lower sensitivity but with both high specificity for HPV16 and HPV 18(E6 and E7). Findings from our study confirmed that, in HIV-negative controls, HPV16 E7 and HPV18 E7 seroprevalence differed by place of residence. Among HIV-positive women, HPV16 E6&E7 seroprevalence increased with an increase in number of births.

The higher seroprevalence of HPV16 E6, HPV16 E7 and HPV18 E7 in women from rural areas might indicate a greater number of women with early undetected cervical or related cancer lesions. In rural areas of...
South Africa, women have poorer access to health care services, are less likely to present themselves to cervical cancer screening services and have less access to cancer diagnostic facilities, which are mainly found in tertiary hospitals in urban areas [24].

The high seroprevalence of HPV16 E6 (35.9%) antibodies in cervical cancer cases found in our study are similar to the seroprevalence of HPV16 E6 (32%) in cervical cancer cases reported from the United Kingdom (35%) and India and Algeria (32%) [5, 10]. We found a relatively low

### Table 1 Comparison of demographic characteristics of study participants from the JCS (1995 – 2016)

| Characteristics          | Total (N = 3878) | Cervical cancer Cases (N = 1346) | Controls (N = 2532) | Comparison of cases and controls p value |
|--------------------------|------------------|-------------------------------|---------------------|---------------------------------------|
|                          | N (% )           | n (%)                         | n (%)               |                                       |
| **Age**                  |                  |                               |                     |                                       |
| Median (IQR)             | 44 (38–48)       | 42 (37–46)                    | 44 (39–50)          | <0.001†                               |
| 25–34                    | 528 (13.6)       | 186 (13.8)                    | 342 (13.5)          |                                       |
| 35–44                    | 1,631 (42.1)     | 693 (15.1)                    | 938 (37.1)          | <0.001†                               |
| 45–54                    | 1,719 (44.3)     | 467 (34.7)                    | 1,252 (49.5)        |                                       |
| **Period of interview**  |                  |                               |                     |                                       |
| 1995–1999                | 255 (6.6)        | 133 (9.9)                     | 122 (4.8)           | <0.001                               |
| 2000–2004                | 717 (18.5)       | 254 (18.9)                    | 463 (18.3)          |                                       |
| 2005–2009                | 1,431 (36.9)     | 538 (40.0)                    | 893 (35.3)          |                                       |
| 2010–2016                | 1,475 (38.0)     | 421 (31.3)                    | 1,054 (41.6)        |                                       |
| **Number of sexual partners** |               |                               |                     |                                       |
| 0–1                      | 269 (6.9)        | 78 (5.8)                      | 191 (7.5)           | <0.001                               |
| 2–5                      | 2,400 (61.9)     | 929 (69.0)                    | 1,471 (58.1)        |                                       |
| 6+                       | 505 (13.0)       | 192 (14.3)                    | 313 (12.4)          |                                       |
| Unknown                  | 704 (18.2)       | 147 (10.9)                    | 557 (22.0)          |                                       |
| **Parity**               |                  |                               |                     |                                       |
| 0                        | 68 (1.8)         | 12 (0.9)                      | 56 (2.2)            | <0.001                               |
| 1                        | 632 (16.3)       | 174 (12.9)                    | 458 (18.1)          |                                       |
| 2                        | 1,008 (26.0)     | 335 (24.9)                    | 673 (26.6)          |                                       |
| 3                        | 922 (23.8)       | 325 (24.2)                    | 597 (23.6)          |                                       |
| 4+                       | 1,090 (28.1)     | 473 (35.1)                    | 617 (24.4)          |                                       |
| Missing data             | 158 (4.1)        | 17 (2.0)                      | 131 (5.2)           |                                       |
| **Marital Status**       |                  |                               |                     |                                       |
| Never married            | 1,009 (26.0)     | 356 (26.5)                    | 653 (25.8)          | <0.001                               |
| Married                  | 1,941 (50.1)     | 682 (50.7)                    | 1,259 (49.7)        |                                       |
| Previously married       | 919 (23.7)       | 303 (22.5)                    | 616 (24.3)          |                                       |
| Missing data             | 9 (0.2)          | 5 (0.4)                       | 4 (0.2)             |                                       |
| **Place of Residence**   |                  |                               |                     |                                       |
| Rural                    | 317 (8.2)        | 150 (11.1)                    | 167 (6.6)           | <0.001                               |
| Urban                    | 3,556 (91.7)     | 1,195 (88.8)                  | 2,361 (93.3)        |                                       |
| Missing data             | 5 (0.1)          | 1 (0.1)                       | 4 (0.2)             |                                       |
| **HIV-status**           |                  |                               |                     |                                       |
| Negative                 | 2,535 (65.4)     | 670 (47.8)                    | 1,865 (73.7)        | <0.001                               |
| Positive                 | 1,345 (34.6)     | 676 (50.2)                    | 667 (26.3)          |                                       |
| **Education Level**      |                  |                               |                     |                                       |
| None                     | 278 (7.2)        | 128 (9.5)                     | 150 (5.9)           | 0.016                                 |
| Primary                  | 903 (23.3)       | 358 (26.6)                    | 545 (21.5)          |                                       |
| Secondary and above      | 2,688 (69.3)     | 854 (63.5)                    | 1,834 (72.4)        |                                       |
| Missing data             | 9 (0.2)          | 6 (0.5)                       | 3 (0.1)             |                                       |

Controls are cancers unrelated to infection, Ever married includes widowed and divorced, IQR = Interquartile range, †p value for a t-test, ‡p value for the chi-squared test
Table 2 Seroprevalence of antibodies to HPV-16 and-18 L1, E6 and E7 proteins in infection unrelated cancer controls in HIV-negative women

| Characteristics                       | Total N = 1865 | Serology markers |
|----------------------------------------|----------------|-----------------|
|                                        | HPV-16 L1 (%)  | HPV-18 L1 (%)   | E6 (%) | E7 (%) | E6 & E7 (%) | E6 (%) | E7 (%) | E6 & E7 (%) |
| Overall seroprevalence                | 14.3           | 16.3            | 2.9    | 4.0    | 0.5         | 2.4    | 2.5    | 0.2         |
| Demographic                           |                |                 |        |        |             |        |        |             |
| Age                                    |                |                 |        |        |             |        |        |             |
| 25–34                                  | 206            | 25 (12.1)       | 34 (16.5) | 2 (1.0) | 5 (2.4) | 1 (0.5) | 6 (2.9) | 3 (1.5) | 0 (0.0)  |
| 35–44                                  | 638            | 86 (13.5)       | 93 (14.6) | 19 (3.0) | 21 (3.3) | 4 (0.7) | 17 (2.7) | 12 (1.9) | 1 (0.2)  |
| 45–54                                  | 1021           | 156 (15.3)      | 177 (17.3) | 33 (3.2) | 49 (4.8) | 3 (0.3) | 21 (2.1) | 32 (3.1) | 3 (0.3)  |
| Chi-square trend (p value)             | 0.166          | 0.352           | 0.133  | 0.053  | 0.472      | 0.345  | 0.067  | 0.358       |
| Place of residence                     |                |                 |        |        |             |        |        |             |
| Rural                                  | 139            | 18 (13.0)       | 20 (14.4) | 9 (6.5) | 12 (8.6) | 2 (1.6) | 3 (2.2) | 11 (7.9) | 1 (0.8)  |
| Urban                                  | 1772           | 248 (14.4)      | 284 (16.5) | 44 (2.6) | 63 (3.7) | 6 (0.4) | 41 (2.4) | 35 (2.0) | 3 (0.2)  |
| Chi-square heterogeneity age-adjusted (p value) | 0.406 | 0.512 | 0.202 | 0.005 | 0.042 | 0.886 | <0.001 | 0.184 |
| Period of interview                    |                |                 |        |        |             |        |        |             |
| 1995–1999                              | 115            | 22 (19.1)       | 26 (22.6) | 5 (4.4) | 7 (6.1) | 1 (1.0) | 4 (3.5) | 4 (3.5) | 0 (0.0)  |
| 2000–2004                              | 373            | 61 (16.4)       | 65 (17.4) | 15 (4.0) | 17 (4.6) | 4 (1.2) | 5 (1.3) | 7 (1.9) | 2 (0.6)  |
| 2005–2009                              | 663            | 86 (13.0)       | 95 (14.3) | 19 (2.9) | 27 (4.1) | 1 (0.2) | 15 (2.3) | 14 (2.1) | 1 (0.2)  |
| 2010–2016                              | 714            | 98 (13.7)       | 118 (16.5) | 15 (2.1) | 24 (3.4) | 2 (0.3) | 20 (2.8) | 22 (3.1) | 1 (0.16) |
| Chi-square heterogeneity age-adjusted (p value) | 0.1757 | 0.123 | 0.202 | 0.455 | 0.142 | 0.368 | 0.538 | 0.454 |
| Marital Status                         |                |                 |        |        |             |        |        |             |
| Never Married                          | 422            | 56 (13.3)       | 67 (15.9) | 11 (2.6) | 12 (2.4) | 2 (0.5) | 5 (1.2) | 11 (2.6) | 0 (0.0)  |
| Married                                | 1002           | 134 (13.4)      | 161 (16.1) | 30 (3.0) | 47 (4.7) | 5 (0.5) | 34 (3.4) | 23 (2.3) | 4 (0.4)  |
| Ever married                           | 437            | 75 (17.2)       | 75 (17.2) | 13 (3.0) | 16 (3.7) | 1 (0.2) | 5 (1.1) | 13 (3.0) | 0 (0.0)  |
| Chi-square heterogeneity age-adjusted (p value) | 0.227 | 0.926 | 0.976 | 0.278 | 0.814 | 0.007 | 0.798 | 0.180 |
| Education Level                        |                |                 |        |        |             |        |        |             |
| None                                   | 123            | 22 (17.9)       | 21 (17.1) | 6 (4.9) | 2 (1.6) | 0 (0.0) | 4 (3.3) | 5 (4.1) | 0 (0.0)  |
| Primary                                | 411            | 54 (13.1)       | 61 (14.8) | 9 (2.2) | 20 (4.9) | 1 (0.3) | 10 (2.4) | 8 (2.0) | 1 (0.3)  |
| Secondary and above                    | 1329           | 191 (14.4)      | 222 (16.7) | 38 (2.9) | 52 (3.9) | 7 (0.6) | 30 (2.3) | 34 (2.6) | 3 (0.2)  |
| Chi-square adjusted for age trend (p value) | 0.406 | 0.543 | 0.298 | 0.276 | 0.630 | 0.654 | 0.400 | 0.838 |
| Sexual/ reproductive history           |                |                 |        |        |             |        |        |             |
| Parity                                 |                |                 |        |        |             |        |        |             |
| 0                                      | 34             | 4 (11.8)        | 5 (14.7) | 2 (5.9) | 1 (2.9) | 1 (3.0) | 0 (0.0) | 1 (2.9) | 0 (0.0)  |
| 1                                      | 316            | 30 (9.5)        | 44 (13.9) | 10 (3.2) | 15 (4.8) | 4 (1.3) | 11 (3.5) | 9 (2.9) | 1 (0.3)  |
| 2                                      | 485            | 68 (14.0)       | 77 (15.9) | 18 (3.7) | 15 (3.1) | 1 (0.2) | 13 (2.7) | 11 (2.3) | 0 (0.0)  |
| 3                                      | 449            | 81 (18.0)       | 70 (15.6) | 14 (3.1) | 18 (4.0) | 2 (0.5) | 6 (1.3) | 11 (2.6) | 0 (0.0)  |
| 4+                                     | 486            | 67 (13.8)       | 88 (18.1) | 9 (1.9) | 23 (4.7) | 0 (0.0) | 12 (2.5) | 14 (2.9) | 3 (0.6)  |
| Chi-square adjusted for age trend (p value) | 0.143 | 0.181 | 0.031 | 0.931 | 0.006 | 0.610 | 0.738 | 0.356 |
| Number of sexual partners              |                |                 |        |        |             |        |        |             |
| 0–1                                    | 170            | 22 (12.9)       | 25 (14.7) | 6 (3.5) | 6 (3.5) | 0 (0.0) | 1 (0.6) | 2 (1.2) | 0 (0.0)  |
| 2–5                                    | 1116           | 161 (14.4)      | 182 (16.3) | 36 (3.2) | 53 (4.8) | 7 (0.7) | 28 (2.5) | 31 (2.8) | 3 (0.3)  |
| 6+                                     | 204            | 32 (15.7)       | 32 (15.7) | 5 (2.6) | 6 (2.9) | 1 (0.5) | 9 (4.4) | 5 (2.5) | 0 (0.0)  |
| Chi-square adjusted for age trend (p value) | 0.908 | 0.522 | 0.133 | 0.332 | 0.796 | 0.850 | 0.862 |
| Unknown                                | 375            | 52 (13.9)       | 65 (17.3) | 7 (1.9) | 10 (2.7) | 0 (0.0) | 6 (1.6) | 9 (2.4) | 1 (0.3)  |
Table 3  Seroprevalence of antibodies to HPV-16 and- 18 L1, E6 and E7 proteins in infection unrelated cancer controls in HIV-positive women

| Characteristics                        | Total Serology markers | HPV-16 | HPV-18 | HPV-16 | HPV-18 |
|----------------------------------------|------------------------|--------|--------|--------|--------|
|                                        | N = 667                | L1     | L1     | E6     | E7     |
|                                        |                        | (%)    | (%)    | (%)    | (%)    |
| Overall seroprevalence                 | 15.9                   | 15.7   | 2.4    | 3.3    | 0.5    |
| Demographic                            |                        |        |        |        |        |
| Age                                    |                        |        |        |        |        |
| 25–34                                  | 136                    | 24 (17.7) | 15 (11.0) | 5 (3.7) | 5 (3.7) | 1 (0.8) | 1 (0.7) | 0 (0.0) |
| 35–44                                  | 300                    | 42 (14.0) | 50 (16.7) | 7 (2.3) | 12 (4.0) | 0 (0.4) | 5 (1.7) | 10 (3.3) | 0 (0.0) |
| 45–54                                  | 231                    | 40 (17.3) | 40 (17.3) | 4 (1.7) | 5 (2.2) | 1 (0.5) | 4 (1.7) | 10 (4.3) | 1 (0.5) |
| Chi-square trend (p value)             |                        | 0.896  | 0.137  | 0.254  | 0.191  | 0.719  | 0.490  | 0.067  | 0.236  |
| Place of residence                     |                        |        |        |        |        |        |        |        |        |
| Rural                                  | 28                     | 2 (7.1) | 4 (14.3) | 0 (0.0) | 1 (3.6) | 0 (0.0) | 1 (3.6) | 1 (3.6) | 0 (0.0) |
| Urban                                  | 639                    | 104 (16.3) | 101 (15.8) | 16 (2.5) | 21 (3.3) | 3 (0.5) | 9 (1.4) | 20 (3.1) | 1 (0.2) |
| Chi-square heterogeneity age-adjusted (p value) |            | 0.218  | 0.797  | 0.399  | 0.982  | 0.724  | 0.376  | 0.912  | 0.856  |
| Period of interview                    |                        |        |        |        |        |        |        |        |        |
| 1995–1999                              | 7                      | 1 (14.3) | 1 (14.3) | 1 (14.3) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| 2000–2004                              | 90                     | 9 (10.0) | 11 (12.2) | 4 (4.4) | 4 (4.4) | 1 (1.2) | 3 (3.3) | 1 (1.1) | 1 (1.1) |
| 2005–2009                              | 230                    | 32 (13.9) | 36 (15.7) | 5 (2.2) | 7 (3.0) | 0 (0.0) | 4 (1.7) | 7 (3.0) | 0 (0.0) |
| 2010–2016                              | 340                    | 64 (18.8) | 57 (16.8) | 6 (1.8) | 11 (3.2) | 2 (0.6) | 3 (0.8) | 13 (3.8) | 0 (0.0) |
| Chi-square heterogeneity age-adjusted (p value) |            | 0.138  | 0.872  | 0.109  | 0.898  | 0.572  | 0.287  | 0.701  | 0.036  |
| Marital Status                         |                        |        |        |        |        |        |        |        |        |
| Never Married                          | 231                    | 38 (16.5) | 36 (15.6) | 4 (1.7) | 4 (1.7) | 0 (0.0) | 1 (0.4) | 5 (2.2) | 0 (0.0) |
| Married                                | 257                    | 32 (12.5) | 35 (13.6) | 8 (3.1) | 12 (4.7) | 1 (0.4) | 4 (1.6) | 8 (3.1) | 0 (0.0) |
| Ever married                           | 179                    | 36 (20.1) | 34 (19.0) | 4 (2.2) | 6 (3.4) | 2 (1.2) | 5 (2.8) | 8 (4.5) | 1 (0.6) |
| Chi-square heterogeneity age-adjusted (p value) |            | 0.101  | 0.448  | 0.573  | 0.171  | 0.178  | 0.188  | 0.673  | 0.488  |
| Education Level                        |                        |        |        |        |        |        |        |        |        |
| None                                   | 24                     | 2 (7.4) | 1 (3.7) | 3 (1.7) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Primary                                | 134                    | 21 (15.7) | 23 (17.2) | 2 (1.5) | 3 (2.2) | 0 (0.0) | 4 (3.0) | 4 (3.0) | 0 (0.0) |
| Secondary and above                    | 505                    | 83 (16.4) | 81 (16.0) | 13 (2.6) | 19 (3.8) | 3 (0.6) | 6 (1.2) | 17 (3.4) | 1 (0.2) |
| Chi-square adjusted for age trend (p value) |            | 0.380  | 0.169  | 0.731  | 0.543  | 0.611  | 0.339  | 0.416  | 0.701  |
| Sexual/ reproductive history           |                        |        |        |        |        |        |        |        |        |
| Parity                                 |                        |        |        |        |        |        |        |        |        |
| 0                                      | 22                     | 9 (40.9) | 5 (22.7) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| 1                                      | 142                    | 20 (14.1) | 15 (10.6) | 3 (2.1) | 7 (4.9) | 0 (0.0) | 0 (0.0) | 1 (0.7) | 0 (0.0) |
| 2                                      | 188                    | 38 (20.2) | 30 (16.0) | 6 (3.2) | 8 (4.3) | 2 (1.1) | 3 (1.6) | 8 (4.3) | 1 (0.6) |
| 3                                      | 148                    | 17 (11.5) | 27 (18.2) | 3 (2.0) | 5 (3.4) | 1 (0.7) | 4 (2.7) | 7 (4.7) | 0 (0.0) |
| 4+                                     | 131                    | 17 (13.0) | 21 (16.0) | 3 (2.3) | 2 (1.5) | 0 (0.0) | 2 (1.5) | 4 (3.1) | 0 (0.0) |
| Chi-square adjusted for age trend (p value) |            | 0.022  | 0.688  | 0.614  | 0.340  | 0.952  | 0.294  | 0.347  | 0.373  |
| Number of sexual partners              |                        |        |        |        |        |        |        |        |        |
| 0–1                                    | 21                     | 3 (14.3) | 4 (19.1) | 1 (4.8) | 2 (9.5) | 0 (0.0) | 0 (0.0) | 1 (4.8) | 0 (0.0) |
| 2–5                                    | 355                    | 55 (15.5) | 52 (14.7) | 5 (1.4) | 9 (2.5) | 0 (0.0) | 8 (2.3) | 8 (2.3) | 1 (0.3) |
| 6+                                     | 109                    | 20 (18.4) | 19 (17.4) | 5 (4.6) | 3 (2.8) | 1 (1.0) | 1 (0.9) | 3 (2.8) | 0 (0.0) |
| Chi-square adjusted for age trend (p value) |            | 0.869  | 0.875  | 0.260  | 0.518  | 0.041  | 0.143  | 0.291  | 0.602  |
| Unknown                                | 182                    | 28 (15.4) | 30 (16.5) | 5 (2.8) | 8 (4.4) | 2 (1.2) | 1 (0.6) | 9 (5.0) | 0 (0.0) |
seroprevalence of HPV16 E6 & E7 and HPV18 E6 & E7 antibodies among controls in keeping with a low prevalence of HPV oncoprotein antibodies among women without cervical cancer [25]. Our study demonstrates that the high seroprevalence of HPV16 E6 and E7 antibodies in cervical cancer development is in accordance with other studies from Russia and Italy [8, 26].

Antibodies to the E6 and E7 oncoproteins are late markers of invasive cervical cancer [27]. In our study, seropositivity to each of the HPV-16 and -18 (E6 and E7) antibodies was associated with high risks of cervical cancer. Notably, seropositivity to HPV16 (E6 & E7) and HPV18 (E6 & E7) exhibited the highest ORs for cervical cancer (Fig. 3). Similar findings were reported in a case–control study of patients recruited from India and Algeria [10]. Our findings support those from other cross-sectional, case–control and prospective studies, albeit each using different assays [27–30].

Individuals with suppressed immune systems are susceptible to chronic infection, including HPV [31]. Thus, HIV infection increases the risk of HPV persistence [32]. Existing evidence of the association between HPV (E6 and E7) and L1 proteins antibodies and HIV has come from studies on men who have sex with men [10, 33, 34]. In contrast, most of the existing studies on the association between antibodies to HPV-16 and -18 L1, E6 and E7 proteins and cervical cancer risk have been conducted in low HIV prevalence settings [6, 8, 13, 27]. In our study, access to HIV data made it possible to stratify our analysis by HIV status. Our finding showed that antibody seropositivity to each of HPV-16 and -18 (E6 and E7) oncoproteins was strongly associated with the risk of cervical cancer among HIV-positive women. Our data support the hypothesis that HIV plays an important role in the persistence of HPV infection.

Unexpected findings in our study were that HPV-16 and -18 (E6 and E7) oncoproteins seropositivity and cervical cancer risk were higher in HIV-negative cancer patients compared to HIV-positive cancer patients. Similar findings have been observed in previous studies of HPV antibodies among HIV-positive and HIV-negative patients [35–38]. In our study, the possible explanation could be that cervical cancer risk in HIV positive women may be related to more than the two main types HPV16 and HPV18 that we tested for so their relative contribution appears attenuated (Additional file 1: Table S3). Other studies on HPV genotype distribution by HIV-status that were conducted in South Africa reported that HPV types 35, 58 and 33 were more commonly identified [39, 40] in addition to types 16 and 18. However, if this is indeed true, then the current HPV vaccine (16/18) may be less effective for HIV positive women. Another possible explanation is that in HIV-positive patients, antibodies could be an indication of reactivation of a latent HPV while in HIV-negative patients antibodies could be reinfection with a new type [35]. Another unexpected finding in our study was that the number of sexual partners was not associated with HPV L1 and (E6 and E7) antibodies. The plausible explanation could be that peak acquisition of HPV occurs at an age earlier than the age of participants in our study [41].

In the new guidelines for screening cervical cancer, the World Health Organization (WHO) has included HPV antibodies and oncoproteins as a future potential screening test [42]. We, therefore, assessed the clinical performance of HPV E6/E7 antibodies as possible

| Serology markers | Cases (N = 1,346) | Controls (N = 2,532) | Adjusted OR (95% CI) |
|------------------|------------------|---------------------|---------------------|
| HPV16 L1 | 332 (24.4) | 373 (14.7) | 1.74 (1.45–2.07) |
| HPV18 L1 | 292 (21.7) | 409 (16.2) | 1.31 (1.10–1.56) |
| HPV type 16 | | | |
| HPV16 E6 | 467 (35.9) | 70 (2.8) | 21.96 (16.61–29.02) |
| HPV16 E7 | 309 (24.7) | 97 (3.8) | 7.93 (6.16–10.22) |
| HPV16 E6 & E7 | 209 (23.3) | 11 (0.5) | 69.20 (37.07–129.18) |
| HPV type 18 | | | |
| HPV18 E6 | 120 (8.9) | 54 (2.1) | 4.67 (3.30–6.59) |
| HPV18 E7 | 235 (18.6) | 68 (2.7) | 8.94 (6.64–12.03) |
| HPV18 E6 & E7 | 64 (5.9) | 5 (0.2) | 38.61 (15.13–98.53) |
| HPV types 16 or 18 | | | |
| HPV16/18 E6/E7 | 746 (56.8) | 255 (10.1) | 13.63 (11.32–16.41) |

OR adjusted for age, HIV antibodies, education, number of sexual partners, place of residence, marital status and period of interview.
screening tests. We found that the sensitivity of the HPV antibodies for detection of cervical cancer was low but the specificity was high among HIV-positive women. In contrast, sensitivity and specificity for the detection of cervical cancer were high among HIV-negative women. Predictive values would thus vary considerably depending on the background prior likelihood of disease. Our finding is in line with the previous findings on the sensitivity and specificity of HPV16/18 E6/E7 antibodies as a screening test [28, 43].

There are strengths to our study. The seroprevalence of HPV antibodies was conducted on a large sample size, and we used a high throughput multiplex serology so all tests were done under the same laboratory conditions. The laboratory tests were conducted ‘blind’ without prior knowledge of the case/control status of the participants. Nonetheless, our study has some limitations. Serology data on exposure and cervical cancer diagnoses (of all stages) were collected at the same time. We did not have tumor HPV Deoxyribonucleic

Table 5  Clinical performance of HPV16 and 18 antibody positivity as diagnostic markers for cervical cancer by HIV-status

| Serology markers | HIV-positive | HIV-negative |
|------------------|--------------|--------------|
|                  | Sensitivity (95% CI) | Specificity (95% CI) | AUC | Sensitivity (95% CI) | Specificity (95% CI) | AUC |
| HPV16 E6         | 26.8 (23.5–30.3) | 97.6 (96.1–98.6) | 62  | 42.7 (38.9–46.5) | 95.6 (94.5–96.4) | 69  |
| HPV16 E7         | 15.5 (12.9–18.5) | 96.7 (95.0–97.9) | 56  | 30.4 (27.0–34.1) | 96.0 (95.0–96.8) | 63  |
| HPV16 E6 & E7    | 12.4 (9.6–15.5)  | 99.5 (98.6–99.9) | 56  | 30.9 (26.7–35.2) | 99.5 (99.1–99.8) | 65  |
| HPV18 E6         | 7.1 (5.3–9.3)    | 98.5 (97.3–99.3) | 53  | 10.7 (8.5–13.3)  | 97.6 (96.8–98.3) | 54  |
| HPV18 E7         | 11.5 (9.2–14.2)  | 97.9 (95.2–98.0) | 54  | 23.4 (20.3–26.8) | 97.1 (96.4–97.7) | 60  |
| HPV18 E6 & E7    | 3.4 (2.1–5.2)    | 99.8 (99.1–100)  | 52  | 8.3 (6.1–11.0)   | 99.9 (99.5–99.9) | 54  |
| HPV16/18 E6/E7   | 43.0 (39.3–46.9) | 90.6 (88.1–92.7) | 67  | 70.6 (67.0–74.0) | 89.7 (88.2–91.0) | 80  |
Acid (DNA) data to determine the causative type(s). Since only 50–70% of women with detectable HPV DNA in the cervix seroconvert [44], our study underestimates the true prevalence of HPV infection. The JCS did not recruit women with cervical pre-cancerous lesions. Study results are based on black women aged 25–54 years who were recruited from the catchment area of the largest public tertiary hospital in Johannesburg. Therefore, our findings might not be generalizable to other South African regions and women aged 55 years and above.

Conclusions
Our data contribute to the evidence on the importance of HPV-16 and -18 E6 and E7 oncoproteins antibodies in discriminating cervical cancer from controls in a black South African population. HPV L1 antibodies show to be exposure markers of past HPV infection. HPV E6 and E7 show to be important markers of invasive cervical cancer. Furthermore, antibodies to HPV-16 and-18 E6 and E7 seropositivity are strongly associated with cervical cancer in both HIV-positive and HIV-negative patients.

Abbreviations
AOR: Adjusted odds ratio; AUC: Area under the curve; CI: Confidence interval; DFKZ: Deutsches Krebsforschungszentrum; DNA: Deoxyribonucleic acid; ELISA: Enzyme-linked immunosorbent assay; EDTA: Ethylenediaminetetraacetic acid; GST: Glutathione S-transferase; HIV: Human immunodeficiency virus; HPV: Human papillomavirus; IQR: Interquartile range; JCS: Johannesburg Cancer Study; SST: Serum separation tube; MFI: Median fluorescence intensity; ROC: Receiver operating characteristic; USA: United States of America; VIP: Visual inflection point; WHO: World Health Organization.

Supplementary Information
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Authors’ contributions
MGs conceptualised the study. MGs performed data analysis and drafted the manuscript. FS, TW, CBDeV and ES edited the manuscript. NB and TW conducted the laboratory analyses. WCC was responsible for data acquisition, data curation quality controls, and management. DB, TW, CGM, FS, ES, MMo, MMu, ABK and RN are the members of the ERICA_SA collaborative study. All authors read and provided feedback to improve the final version of the manuscript.

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Availability of data and materials
Data cannot be shared publicly because of ethics policy at University of Witwatersrand, whereby any new analyses require Human Research Ethics Committee approval. Data are available from the SA-NCR /National Health Laboratory Services. (contact: adrianp@nicd.ac.za) for researchers who meet the relevant ethics criteria for access to these data.

Competing interests
Freddy Sitas is a member of the Editorial Board of Infectious Agents and Cancer journal but had no involvement in the review process of this manuscript.

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