A case-cohort study of human herpesvirus 8 seropositivity and incident prostate cancer in Tobago

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Abstract

Background: We previously reported a cross-sectional association between the presence of human herpesvirus 8 (HHV-8) serum antibodies and screen-detected prostate cancer in men living in Tobago. In the same study population, we examined the association between HHV-8 seropositivity and incident prostate cancer discovered at later screenings.

Methods: In 40-81 year-old men without prostate cancer discovered at initial digital rectal examination (DRE) and prostate-specific antigen (PSA) screening, a case-cohort design measured the association between baseline HHV-8 seropositivity (modified immunofluorescence assay for antibodies against HHV-8 lytic antigens) and incident prostate cancer detected at DRE and PSA screenings three or five years later.

Results: Analyses included 486 unique individuals, 96 incident prostate cancer cases, and 415 randomly selected subjects representing an at-risk cohort. By design, the random sub-cohort contained 25 incident prostate cancer cases. In the sub-cohort, the frequency of HHV-8 seropositivity increased across age groupings (40-49 years: 3.5%, 50-59 years: 13.6%, and ≥ 60 years: 22.9%). HHV-8 seropositivity was higher in men with elevated (≥ 4.0 ng/mL) than men with non-elevated PSA at initial screening (30.4% vs. 9.9% seropositive; crude odds ratio (OR) 3.96, 95% confidence interval (CI) 1.53-10.2; age-adjusted OR 2.42, 95% CI 0.91-6.47). HHV-8 seropositivity did not increase incident prostate cancer risk (age-adjusted hazard ratio (HR) 0.88, 95% CI 0.46-1.69).

Conclusions: Case-cohort analysis did not identify association between HHV-8 seropositivity and incident prostate cancer. However, analyses uncovered possible association between HHV-8 and PSA (a marker of prostate inflammation). Co-occurrence of HHV-8 seropositivity and PSA elevation may explain cross-sectional association between HHV-8 and PSA screen-detected prostate cancer.

Keywords: human herpesvirus 8, prostate cancer, case-cohort design

Background

In 2008, prostate cancer was the fifth most common cancer and the sixth leading cause of cancer death among men worldwide [1]. Men of African descent experience higher prostate cancer incidence and mortality than any other racial group [1-5]. Other accepted risk factors include older age and family history. The otherwise poor understanding of prostate cancer etiology motivates search for specific causal agents. Though not consistently [6], studies find prostate cancer in association with infectious disease agents, including Neisseria gonorrhoeae, Chlamydia trachomatis, human papillomavirus (HPV) type 18, and Treponema pallidum (syphilis) [7-11]. Other studies find viral DNA or evidence of viral gene expression in prostate tissues (HPV, human herpes simplex virus type 2, cytomegalovirus, Epstein-Barr virus, and human herpesvirus 8 (HHV-8) [10,12-19]), stromal fibroblasts within prostate tumors (xenotropic murine leukemia virus-related virus or XMRV [20]), or malignant prostate epithelial cells...
(XMRV [21]). These infectious agents may elicit an immune response creating a cytokine tissue environment that leads to chronic inflammation, DNA damage, cellular proliferation, angiogenesis, and ultimately prostate cancer [10,13,22,23].

HHV-8, a DNA virus, causes Kaposi’s sarcoma and primary effusion lymphoma. In a high prostate cancer risk cohort of African-Caribbean men living on Tobago [24,25], we found an association between HHV-8 seropositivity and prostate cancer discovered as a result of an initial prostate cancer screening (odds ratio [OR] 2.24, 95% confidence interval [CI] 1.29-3.90) [9]. Four studies completed later in other population settings could not confirm an association between HHV-8 and prostate cancer [6,11,26,27]. Therefore, our current study re-examines this association in our Tobago study population, through consideration of the association between HHV-8 seropositivity and prostate cancer discovered, not as a result of the initial screening, but later as a result of subsequent screenings.

Methods

Study Population

The Tobago Prostate Survey is an ongoing population-based longitudinal study of prostate cancer screening, as well as risk, in ≥ 40 year-old men living in Tobago [24]. Tobago is a small Caribbean island, 7 by 26 miles in size, with 8078 40-79 year-old men, according to a 2000 census [28]. The population as a whole is 89% African or Black and 7% mixed heritage by nationality or ethnicity [28]. Identification of study participants occurred through the agency of posters, flyers, public service announcements, public presentations, healthcare workers, private physicians, and word of mouth [24]. Prostate cancer screening occurred in three waves, Wave 1 - October 1997 to August 2003, Wave 2 - February 1999 to August 2003, and Wave 3 - May 2004 to March 2007. Although an open cohort, this report included only men screened at Wave 1 and subsequently rescreened at Waves 2 and/or 3. Study procedures included risk factor questionnaires, blood collections, and prostate cancer screening examinations, with prostate specific antigen (PSA) serum concentrations ≥ 4 ng/mL or abnormal digital rectal examinations (DRE) prompting referral for ultrasound-guided trans-rectal prostate biopsy [24].

Wave 1 enrolled 3264 40-81 year-old men (97% self-reporting African descent). The current study excluded men missing Wave 1 PSA (n = 283), men with Wave 1 PSA ≥ 4.0 ng/mL not followed by biopsy (n = 104), and men with prostate cancer detected at Wave 1 (n = 330), thereby leaving 2547 men at risk for prostate cancer at Wave 2 or Wave 3 (Figure 1). The study design excluded 756 at-risk men, including 633 at-risk men without subsequent PSA at either Wave 2 or Wave 3, 108 men with a Wave 2 or Wave 3 PSA ≥ 4.0 ng/mL not followed by biopsy, and 15 men with a prostate cancer negative Wave 2 biopsy, but no Wave 3 biopsy for Wave 3 PSA ≥ 4.0 ng/mL associated with ≥ 1.0 ng/mL PSA increase between Waves 2 and 3 (Figure 1). In the remaining 1791 at-risk men, a Wave 2 or Wave 3 biopsy completed before study closure (August 15, 2007) detected prostate cancer in 109 (Figure 1).

To measure the association between Wave 1 HHV-8 seropositivity and prostate cancer detection from a Wave 2 or Wave 3 screening, we used a case-cohort study design that compared prostate cancer cases at Wave 2 or Wave 3 (n = 109; Gleason 6 - 49%, Gleason 7 - 45%, Gleason 8 or 9 - 6%; pre-diagnostic screening PSA, mean 9.5 ng/mL, median 4.4 ng/mL) against a control group constructed as a simple random sample (n = 442) of the 1791 at-risk men screened for prostate cancer at Wave 2 or Wave 3. We used a case-cohort design because research costs prohibited determination of the HHV-8 status of all 1791 men in the at-risk group. As a result of the random selection procedure, 27 case men with prostate cancer at Wave 2 or Wave 3 entered the sub-cohort and contributed data as controls (Figure 1). The 442 and 1349 randomly selected and excluded men were statistically similar with respect to age, education, marital status, prostate cancer family history, history of smoking, personal history of cancer, history of benign prostatic hypertrophy, and Wave 1 PSA and DRE results. The 442 men selected for the sub-cohort survived a median 4.9 years (5th-95th percentile 1.7-6.8 years) between Wave 1 and the last complete post-Wave 1 visit. For the 109 cases, a median 7.2 months (25th-75th percentile 2.5-13.8 months) elapsed between pre-diagnostic screening and confirmatory biopsy.

Study participants signed an informed consent approved by the Institutional Review Boards of the Tobago Division of Health and Social Services and the University of Pittsburgh.

Laboratory Methods

Laboratory assays used frozen serum samples (thawed once and never re-frozen) stored temporarily in a -20°C freezer at the Tobago Health Studies office in Scarborough, Tobago, and stored later in a -80°C freezer at the University of Pittsburgh, Department of Epidemiology. PSA measurements used either Abbott Diagnostics AxSYM® or Siemens Healthcare Diagnostics ADVIA Centaur® immunoassays. To detect serum antibodies against HHV-8 lytic antigens, an indirect immunofluorescence assay, as described elsewhere [29], used BCBL-1 cells containing the HHV-8 genome with a modified Rta gene inducible by doxycyclin [30]. Targeting fixed and
permeabilized B cells that have been induced to replicate HHV-8, this assay potentially identifies any of the lytic proteins involved in HHV-8 replication. A single reader (FJJ), blinded to samples’ prostate cancer case status, examined microscopic slides for fluorescence. A positive assay result required specific fluorescence at a 1:100 dilution. Each assay run included prostate cancer case and non-case subject sera and known HHV-8 positive and negative control sera. We tested each sample in duplicate on separate days with disagreements resolved by retesting on a third day. Estimates of assay sensitivity and specificity range between 53.4-89.9% and 96.6-97.5%, respectively [31]. Kappa agreement between first and second assay results was 0.76 (95% CI 0.71-0.81).

**Data Analysis**
We used the chi-square test to evaluate 1) the statistical significance of differences in the sub-cohort rates of HHV-8 seropositivity with respect to the same baseline factors. In analyses restricted to sub-cohort members, logistic regression estimated odds ratios to express strengths of association between Wave 1 PSA elevation and Wave 1 HHV-8 seropositivity, two factors determined at the same point in time. We used Cox proportional hazards models (Breslow weighted denominator method) for case-cohort designs to estimate hazard ratio [HR] measures of unadjusted and age-adjusted association between HHV-8 seropositivity measured at baseline (Wave 1) and prostate cancer detected later in time, at Wave 2 or Wave 3 [32]. These models compare prostate cancer cases detected at one or the other points in time (Wave 2 or Wave 3) with the appropriate risk set, constructed from sub-cohort members disease-free and available at Wave 2 or Wave 3. For prostate cancer cases in the sub-cohort, Cox models started follow-up at the Wave 1 screening date and censored follow-up at the Wave 2 or Wave 3 screening date that led to prostate cancer diagnosis. For non-cases in the sub-cohort, Cox models started follow-up at the Wave 1 screening date and censored follow-up at the date of the last
completed Wave 2 or Wave 3 screening. Calculating age on the date of the Wave 1 PSA blood collection, age adjustments used either two age categories (40-59 and ≥ 60 years), three age categories (40-49, 50-59, or ≥ 60 years), four age categories (40-44, 45-49, 50-59, and ≥ 60 years), or age (integer years) modeled as a continuous variable. Statistical inferences used a p = 0.05 two-sided significance level.

Results
Table 1 summarizes the characteristics of all 3264 40-81 year-old men enrolled at Wave 1. The current study excluded 717 men missing a Wave 1 PSA, men with Wave 1 PSA ≥ 4.0 ng/mL not followed by prostate biopsy, and men with prostate cancer detected at Wave 1, leaving 2547 screen-negative men at risk for prostate cancer at Wave 2 or Wave 3 (Figure 1). Table 1 compares these 2547 at-risk men with the 717 men who were either inadequately screened at Wave 1 or discovered to have prostate cancer at Wave 1. At-risk men were younger and better educated (Table 1). At-risk men less often reported a history of smoking, cancer, or benign prostatic hypertrophy (Table 1). PSA values were lower and DRE results positive less often in at-risk men, as expected, since the group not at risk included men with prostate cancer detected as direct result of PSA elevation or DRE abnormality (Table 1). The study design excluded 756 men from the group of 2547 at-risk men eligible for follow-up (Figure 1). As shown in Table 1 the 756 at-risk men with incomplete follow-up differed from the 1791 men with complete follow-up, as follows, age (35.6% vs. 26.0% ≥ 60 years), smoking history (48.5% vs. 39.6%), personal cancer history (1.3% vs. 0.3%), Wave 1 PSA ≥ 4 ng/mL (13.5% vs. 5.5%), and Wave 1 DRE (64.7% vs. 76.3% negative and 20.0% vs. 10.3% missing).

Wave 1 HHV-8 serologic status was available for 415 (93.9% of 442) men in the sub-cohort and for 96 (88.1% of 109) men in the case group (Gleason 6 - 49%, Gleason 7 - 45%, Gleason 8 or 9 - 6%; pre-diagnostic PSA, mean 10.0 ng/mL and median 4.4 ng/mL). Table 2 summarizes the baseline characteristics for these men with non-missing HHV-8. In addition, Table 2 compares sub-cohort rates of HHV-8 seropositivity according to the same baseline characteristics. Referenced against the sub-cohort, characteristics of the case group included older age, less frequent smoking history, more frequent benign prostatic hypertrophy history, more frequently elevated Wave 1 PSA (27.1% vs. 5.5% PSA ≥ 4 ng/mL), and more frequently positive Wave 1 prostate cancer screening (43.8% vs. 16.9% DRE or PSA positive). Case and sub-cohort HHV-8 seropositivity rates were 17.7% and 11.1%, respectively. When compared with 40-49 year-old sub-cohort men (3.5% HHV-8 seropositive), HHV-8 seropositivity was higher in 50-59 year-old sub-cohort men (13.6% HHV-8 seropositive) and higher yet in ≥ 60 year-old sub-cohort men (22.9% HHV-8 seropositive). HHV-8 seropositivity rates were lower in sub-cohort men with a history of smoking than those without (7.1% vs. 13.9%) and higher in sub-cohort men with a history of benign prostatic hypertrophy than those without (24.0% vs. 10.5%). HHV-8 seropositivity increased with Wave 1 PSA (7.6%, 12.1%, 12.7%, and 30.4% for PSA 0.0-0.9, 1.0-1.9, 2.0-3.9, and ≥ 4 ng/mL, respectively). HHV-8 seropositivity was higher in men with elevated (≥ 4.0 ng/mL) than men with non-elevated PSA (30.4% vs. 9.9% seropositive; crude OR 3.96, 95% CI 1.53-10.2; age-adjusted OR 2.42, 95% CI 0.91-6.47; data not shown). HHV-8 seropositivity was higher in sub-cohort men with a positive than in men with a negative Wave 1 prostate cancer screen result (20.0% vs. 9.3% seropositive).

Table 3 compares Wave 1 HHV-8 seropositivity between the case and sub-cohort groups, according to age and Wave 1 PSA. Age-specific HHV-8 seropositivity rates were lower in case than sub-cohort men (40-49 years: 0.0% vs. 3.5% and 50-59 years: 10.8% vs. 13.6%), except in the oldest age group (≥ 60 years: 27.7% vs. 22.9%). In men with a non-elevated (< 4 ng/mL) Wave 1 PSA, the HHV-8 seropositivity rate was higher in the case group (17.1% vs. 9.9%). In men with an elevated (≥ 4 ng/mL) Wave 1 PSA, however, the HHV-8 seropositivity rate was lower in the case group (19.2% vs. 30.4%). In the two age sub-groups with appreciable HHV-8 seropositivity, age-specific HHV-8 seropositivity rates were not consistently higher or lower in case than sub-cohort men with non-elevated Wave 1 PSA (50-59 years: 11.5% vs. 13.7% and ≥ 60 years: 28.1% vs. 20.4%) and consistently lower in case than sub-cohort men with elevated Wave 1 PSA (50-59 years: 9.1% vs. 12.5% and ≥ 60 years: 26.7% vs. 41.7%).

Table 4 shows associations, unadjusted and age-adjusted, between Wave 1 HHV-8 seropositivity and prostate cancer at Wave 2 or Wave 3, overall and in sub-groups defined by Wave 1 screen results. Though not statistically significant, HR point estimates indicate lower prostate cancer risk in HHV-8 seropositive men, overall (age-adjusted HR 0.88, 95% CI 0.46-1.69) and in HHV-8 seropositive men with elevated Wave 1 PSA (age-adjusted HR 0.39, 95% CI 0.10-1.63), and equivalent risk in HHV-8 seropositive men with non-elevated Wave 1 PSA (age-adjusted HR 1.03, 95% CI 0.49-2.16). In men eligible for prostate biopsy at Wave 1 (DRE or PSA positive), HHV-8 seropositivity reduced risk (age-adjusted HR 0.59, 95% CI 0.18-1.91) to a statistically insignificant level. In perhaps the most meaningful sub-group, men not eligible for prostate biopsy at Wave 1 (DRE not positive and PSA < 4.0 ng/mL), analyses supplied no evidence of association between seropositivity and prostate cancer risk (age-adjusted HR: 1.02, 95% CI
In men with non-elevated Wave 1 PSA, single-year-of-age-adjusted (continuous) risk estimates were HR 0.80 (95% CI 0.19-3.34) and HR 1.27 (95% CI 0.50-3.25) for the 50-59 and ≥ 60 year-old men, respectively (data not shown). Using all (96 case and 415 sub-cohort) men or only ≥ 45 year-old (95 case and 312 sub-cohort) men made no meaningful difference in the age-adjusted risk estimates (data not shown).

Table 1 Characteristics of all men enrolled at Wave 1, men not at risk and at risk for prostate cancer at Wave 2 or Wave 3 based on Wave 1 screen results, and at-risk men with incomplete and complete follow-up.

| Characteristic | All n = 3264 | Not at risk n = 717 | At risk n = 2547 | Incomplete n = 756 | Complete n = 1791 | p-value
|---------------|-------------|---------------------|------------------|-------------------|-------------------|---------
| Age (years) | <.0001 | | | | | |
| 40-44 | 624 19.1 | 59 8.2 | 565 22.2 | 151 20.0 | 414 23.1 | |
| 45-49 | 571 17.5 | 71 9.9 | 500 19.6 | 115 15.2 | 385 21.5 | |
| 50-59 | 914 28.0 | 166 23.2 | 748 29.4 | 221 29.2 | 527 29.4 | |
| 60-81 | 1155 35.4 | 421 58.7 | 734 28.8 | 269 35.6 | 465 26.0 | |
| Education ≤11 years | (23) | (7) | (16) | (7) | (9) | 0.76 |
| ≥12+ years | 2411 74.4 | 553 77.9 | 1858 73.4 | 553 73.8 | 1305 73.2 | |
| Marital status | 2699 82.4 | 909 12.2 | 2090 79.5 | 602 80.2 | 1477 82.9 | 0.13 |
| ever married | 2669 82.4 | 909 12.2 | 2090 79.5 | 602 80.2 | 1477 82.9 | |
| never married | 569 17.6 | 117 16.5 | 452 17.9 | 147 19.6 | 305 17.1 | |
| Family history of ... | 0.02 | | | | | |
| Prostate cancer missing | 323 9.9 | 77 10.7 | 246 9.7 | 86 11.4 | 160 89 | |
| yes | 218 6.7 | 40 5.6 | 178 7.0 | 64 8.5 | 114 64 | |
| no | 2723 83.4 | 600 83.7 | 2123 83.4 | 606 80.2 | 1517 84.7 | |
| History of ... | <.0001 | | | | | |
| Smoking yes | 1410 43.5 | 341 48.0 | 1069 42.2 | 365 48.5 | 704 39.6 | |
| no | 1833 56.5 | 369 48.0 | 1464 57.8 | 388 51.5 | 1076 60.4 | |
| Cancer yes | 26 0.8 | 11 1.6 | 15 0.6 | 10 1.3 | 5 0.3 | |
| no | 3195 99.2 | 693 98.4 | 2502 99.4 | 737 98.7 | 1765 99.7 | |
| History of ... | 0.67 | | | | | |
| Benign prostatic hypertrophy yes | 248 7.8 | 78 11.3 | 170 6.8 | 53 7.2 | 117 6.7 | |
| no | 2932 92.2 | 615 88.7 | 2317 93.2 | 686 92.8 | 1631 93.3 | |
| Entry prostate cancer screen PSA (ng/mL) | 0.0001 | | | | | |
| 0.0-0.9 | (190) | (190) | (190) | (190) | (190) | |
| 1.0-1.9 | 1213 39.5 | 43 8.2 | 1170 45.9 | 306 40.5 | 864 48.2 | |
| 2.0-2.9 | 850 27.7 | 45 8.5 | 805 31.6 | 206 27.2 | 599 33.4 | |
| 3.0-3.9 | 268 8.7 | 21 4.0 | 247 9.7 | 84 11.1 | 163 9.1 | |
| 4.0-9.9 | 150 4.9 | 26 4.9 | 124 4.9 | 58 7.7 | 66 3.7 | |
| 10+ | 354 11.5 | 199 37.8 | 155 6.1 | 73 9.7 | 82 4.6 | |
| DRE | <.0001 | | | | | |
| missing | 555 17.0 | 220 30.7 | 335 13.2 | 151 20.0 | 204 10.3 | |
| positive | 636 19.5 | 279 38.9 | 357 14.0 | 116 15.3 | 241 13.5 | |
| negative | 2073 63.5 | 218 30.4 | 1855 72.8 | 489 64.7 | 1366 76.3 | |

PSA - prostate-specific antigen, DRE - digital rectal examination
1. Numbers in parentheses indicate missing data
2. Statistical significance (chi-square) of differences between at-risk men with incomplete and complete follow-up
Discussion

Our previous study used an immunofluorescence assay to measure HHV-8 antibodies in 138 prostate cancer cases and in 140 age-matched controls [9]. HHV-8 sero-positivity was significantly more frequent in cases than controls (39.9% vs. 22.9%, OR 2.24, 95% CI 1.29-3.90) [9]. Our previous study compared Wave 1 screen-detected (DRE positive and/or PSA elevated) prostate cancer cases with DRE negative and PSA non-elevated controls. In the same Tobago study population, using a
similar assay, the current prospective case-cohort study offered an opportunity to evaluate temporal relationships between HHV-8 seropositivity and prostate cancer, in men with and without elevated PSA at baseline. Including men with non-elevated (< 4.0 ng/mL) Wave 1 PSA and men with elevated (≥ 4.0 ng/mL) Wave 1 PSA, but prostate cancer not seen on Wave 1 biopsy, case-cohort analysis did not observe HHV-8-related incident prostate cancer risk in men overall (age-adjusted HR 0.88, 95% CI 0.46-1.69), in men with Wave 1 PSA < 4 ng/mL (age-adjusted HR 1.03, 95% CI 0.49-2.16), or in men without a positive Wave 1 prostate cancer screen result (age-adjusted HR 1.02, 95% CI 0.44-2.39; Table 4).

A positive association between HHV-8 seropositivity and prevalent prostate cancer in a cross-sectional study [9] and an inverse (though not statistically significant) association in a prospective study, an inverse association most evident in men sent for biopsy (e.g., PSA ≥ 4 ng/mL, age-adjusted HR 0.39, 95% CI 0.10-1.63; Table 4), lead to the following speculation. HHV-8 may associate with factors, such as elevated PSA, that prompt biopsy and subsequent recognition of prostate cancer. In effect, HHV-8 may segregate men with manifest and emergent prostate cancer into two groups, HHV-8 seropositive prostate cancer detected immediately and HHV-8 seronegative prostate cancer detected later. This selection bias may explain opposing positive and negative HHV-8 associations seen with prevalent and incident prostate cancer, respectively. A similar selection bias may explain inverse associations between HHV-8 and prostate cancer observed in other prospective studies, as described below.

Four comparative studies of HHV-8 and prostate cancer have appeared [6,11,26,27] since our 2004 publication [9]. In a prospective study from Finland, ELISA detected serum antibodies against the HHV-8 ORF65 protein in 3 (1.8%) of 163 men with incident prostate cancer and in 7 (2.4%) of 288 age-matched men without cancer (OR 0.74, 95% CI 0.19-2.88; [27]). In a U.S. population-based case-control study, the immunofluorescence assay detected serum antibodies against HHV-8 lytic antigens less often in cases than controls (95 African-American cases and 75 controls: OR 0.56, 95% CI 0.28-1.14; 104 white cases and 80 controls: OR 0.71, 95% CI 0.36-1.43; [26]). In a study of 691 individually matched case-control pairs nested within the U.S. Health Professional Follow-up Study, the immunofluorescence assay detected plasma antibodies against HHV-8 lytic antigens less often in men diagnosed with prostate cancer, on average, 3.1 years later (OR 0.70, 95% CI 0.52-0.95; [6]). Finally, in the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial, ELISA detected IgG antibodies against the HHV-8 K8.1 structural protein in study entry serum samples from 103 (13.5%) of 765 and 103 (11.3%) of 915 white prostate cancer cases and age-matched controls, respectively (OR 1.3, 95% CI 0.9-1.7).

Table 3 Sub-cohort and case group Wave 1 HHV-8 seropositivity, by age and Wave 1 PSA result

| Age years | PSA ng/mL | Cases | Sub-cohort |
|-----------|-----------|-------|------------|
|           |           | HHV-8 positive | HHV-8 positive |
|           | n | n | % | n | n | % |
| 40-49     | All | 12 | 0 | 0.0 | 200 | 7 | 3.5 |
| 50-59     | All | 37 | 4 | 10.8 | 110 | 15 | 13.6 |
| ≥ 60      | All | 47 | 13 | 27.7 | 105 | 24 | 22.9 |
| All < 4   | 70 | 12 | 17.1 | 392 | 39 | 9.9 |
| All ≥ 4   | 26 | 5 | 19.2 | 23 | 7 | 30.4 |
| 40-49     | < 4 | 12 | 0 | 0.0 | 197 | 6 | 3.0 |
| 50-59     | < 4 | 26 | 3 | 11.2 | 102 | 14 | 13.7 |
| ≥ 60      | < 4 | 32 | 9 | 28.1 | 93 | 19 | 20.4 |
| 40-49     | ≥ 4 | 10 | 0 | 0.0 | 197 | 6 | 3.0 |
| 50-59     | ≥ 4 | 11 | 1 | 9.1 | 8 | 1 | 12.5 |
| ≥ 60      | ≥ 4 | 15 | 4 | 26.7 | 12 | 5 | 41.7 |

PSA - prostate-specific antigen, DRE - digital rectal examination, HHV-8 - human herpesvirus 8

Table 4 Unadjusted and age-adjusted associations (hazard ratio) between Wave 1 HHV-8 seropositivity and prostate cancer at Wave 2 or Wave 3, overall and within strata defined by Wave 1 prostate cancer screening test results

| Wave 1 prostate cancer screening test result | Cases | Sub-cohort | Unadjusted | Age-adjusted |
|-------------------------------------------|-------|------------|------------|--------------|
|                                            | Pos   | n | Pos | n | HR | 95% CI | HR | 95% CI |
| Overall                                   | 17    | 96 | 46  | 415 | 1.46 | 0.78-2.74 | 0.881 | 0.46-1.69 |
| PSA (ng/mL)                               |       |    |     |     |     |         |     |        |
| < 4                                       | 12    | 70 | 39  | 392 | 1.61 | 0.77-3.38 | 1.031 | 0.49-2.16 |
| ≥ 4                                       | 5     | 26 | 7   | 23  | 0.44 | 0.11-1.71 | 0.392 | 0.10-1.63 |
| DRE positive or PSA ≥ 4 ng/mL              |       |    |     |     |     |         |     |        |
| no                                        | 9     | 54 | 32  | 345 | 1.67 | 0.72-3.86 | 1.021 | 0.44-2.39 |
| yes                                       | 8     | 42 | 14  | 70  | 0.85 | 0.52-2.38 | 0.593 | 0.18-1.91 |

Pos - number HHV-8 positive, n - case or sub-cohort count, HR - hazard ratio, CI - confidence interval, PSA - prostate-specific antigen, DRE - digital rectal examination

1. Adjusted across four age categories: 40-44, 45-49, 50-59, ≥ 60 years
2. Adjusted across two age categories: 40-59, ≥ 60 years
3. Adjusted across three age categories: 40-49, 50-59, ≥ 60 years

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and in 2 (1.9%) of 103 and 22 (6.0%) of 367 black cases and age-matched controls, respectively (OR 0.3, 95% CI 0.1-1.4; [11]). On balance, these studies suggest that HHV-8 does not influence prostate cancer risk.

Analyses restricted to the sub-cohort showed strong association 1) between HHV-8 seropositivity and increasing age, a result also seen in Tobago women [33] and many other populations [34,35], and 2) between HHV-8 seropositivity and PSA elevation ≥ 4.0 ng/mL. Though not statistically significant (p = 0.17), age-adjusted geometric mean Wave 1 PSA was 18% higher in HHV-8 seropositive than seronegative sub-cohort men (data not shown). The age-adjusted odds of HHV-8 seropositivity was more than two-fold higher in sub-cohort men with elevated PSA than men with non-elevated PSA. Personal or environmental factors related to HHV-8 exposure or immune function may explain the age association with HHV-8 [33]. Accepting PSA as a marker of prostate inflammation, we postulate that the association between HHV-8 seropositivity and elevated PSA signifies either the effects of HHV-8 infection on prostate inflammation [19] or the effects of prostate inflammation on HHV-8 reactivation. PSA elevation has been observed in relation to other infectious disease agents [36,37].

Study strengths include unique population and setting (predominantly African ancestry Tobago residents [38]) and a control group large enough to estimate age-specific HHV-8 seroprevalence rates with acceptable precision. Study limitations include unavoidable misclassification according to prostate cancer outcome. DRE and PSA invariably miss instances of biopsy detectable prostate cancer. The Prostate Cancer Prevention Trial, for example, observed a 15% prostate cancer biopsy prevalence in men with PSA ≤4 ng/mL [39]. In addition, our study can not define the prostate cancer risk experience of men who did not return for repeat screening. Follow-up intervals much longer than our five-year interval between initial and final screening may be needed to detect a prostate cancer effect from any chronic inflammation caused by HHV-8 infection. Also, HHV-8 may cause inflammation and prostate cancer only in a relatively small genetically susceptible subgroup. Finally, a small case count limits, especially in subgroups, the precision of our risk estimates. For example, in men not eligible for prostate biopsy at Wave 1, the 95% confidence interval embraced both 50% lower and 200% higher prostate cancer risks in relation to HHV-8 seropositivity.

Conclusions
Our prospective study could not demonstrate an association between HHV-8 seropositivity and incident prostate cancer. However, analyses uncovered a strong relationship between elevated HHV-8 seropositivity and PSA. The HHV-8 association previously observed with prevalent prostate cancer may signify enhanced detection of prostate cancer possibly caused by the effects of HHV-8 on PSA. In this context, the association we observed between HHV-8 seropositivity and PSA elevation deserves further study.

List of Abbreviations
HHV-8: human herpesvirus 8; DRE: digital rectal examination; PSA: prostate-specific antigen; OR: odds ratio; CI: confidence interval; HR: hazards ratio; HPV: human papillomavirus; PLCO: Prostate Lung Colorectal, and Ovarian Cancer Screening Trial.

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Authors’ contributions
ACM participated in study design, data analysis and interpretation, prepared a first draft of the manuscript, and helped revise the final manuscript. FJJ completed immunofluorescence assays and helped revise the final manuscript. CHB conceived the study, acquired data, provided study coordination, and helped revise the final manuscript. JWW selected analytic methods and directed statistical analysis. ALP acquired data and provided study coordination. JLW participated in study design, data analysis and interpretation and helped revise the final manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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