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Prevalence and risk factors of high-risk HPV and cervical abnormalities in HIV-positive women in Bali, Indonesia

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Abstract

Background Women living with Human Immunodeficiency Virus (HIV) are at higher risk of cervical cancer, particularly in regions like Indonesia where cervical cancer screening programs are limited. Bali has seen a rise in both HIV and cervical cancer cases, prompting the need for targeted interventions. This study investigates the prevalence of cervical cytological abnormalities and associated risk factors among women with HIV in Bali, focusing on their relationship with high-risk Human Papillomavirus (HR-HPV) types.

Methods A cross-sectional study was conducted from July to December 2023, recruiting 245 women from HIV outpatient clinics in Bali. Demographic and clinical data were collected via interviews and physical examinations, including cervical swabs and blood samples. HPV genotyping was performed using ThinPrep cytology followed by a two-stage PCR process. The first stage utilized universal primers (MY09/11) for HPV detection, while the second stage employed type-specific primers to identify high-risk strains, (16,18,31,33,35,39,45,51,52,56,58,59,66 and 68. Blood samples were analyzed to determine CD4 and CD8 T-cell counts.

Results Of 239 participants, 26 (10.87%) had abnormal cytology (6 cases (2.5%) of atypical squamous cells with high risk (ASC-H), 9 cases (3.8%) of atypical squamous cells of undetermined significance (ASC-US), 4 cases (1.7%) of high-grade squamous intraepithelial lesions (H-SIL), and 7 cases (2.9%) of low-grade squamous intraepithelial lesions (L-SIL)). Furthermore, 58 participants (24%) were tested positive for HPV DNA, with HPV type 18 being the most prevalent (28% in all HPV-positive samples). HPV-positive women had a seven-fold higher risk of abnormal cytology (PR = 7.022, 95% CI = 3.223–15.295). Multivariate analysis revealed HPV 18 as an independent risk factor (ExpB = 9.029, $p = 0.007$) and a history of pap smear screening reduced HR-HPV risk (ExpB = 0.358, $p = 0.013$).

Conclusion This study highlights that 10.87% of HIV-positive women in Bali had abnormal cytology, with HPV 18 significantly linked to higher risk. A history of pap smear screening reduced HR-HPV risk. These findings underscore the need for robust cervical cancer screening and HPV vaccination, particularly for younger women, to improve health outcomes in Indonesia.

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Keywords High-risk HPV, Cervical abnormalities, Cervical cytological abnormalities, HIV, Risk factors

Introduction

Cervical cancer is one of the most frequently detected cancers in women living with Human Immunodeficiency Virus (HIV) and is classified as an Acquired Immunodeficiency Syndrome (AIDS)-defining illness. From the meta-analysis of 24 studies shows that women living with HIV have six times higher risk of developing cervical cancer compared to those without HIV. Globally, an estimated 6% of new cervical cancer cases in 2018 were diagnosed among women living with HIV and 5% of all cases were attributable to HIV infection [1]. IARC 2021 data shows that in Indonesia, 0.3% of cervical cancer cases were caused by co-infection with HIV [2, 3]. Women living with HIV, who often have compromised immune systems are more prone to high-risk HPV strains that frequently lead to cervical cancer, they are also at risk of persistent HPV infection. This leads to the urgency in early detection and management of cervical cancer in the population of women living with HIV.

Cervical cancer screening in people with HIV is essential to prevent complications and mortality. Screening is the key method to reduce the occurrence of cervical cancer by finding pre-cancerous cells that can be treated before turning into cancer. Followed by adequate management, screening is one of the highly effective prevention methods recommended by the World Health Organization (WHO). Based on Centers for Disease Control and Prevention (CDC) guidelines, for woman with newly diagnosed HIV-infection, the first cervical cancer screening is recommended by the time of HIV diagnosis and repeated on 6 or 12 months after diagnosis [4]. The effectiveness of the disease's management is influenced by not only the accuracy of the screening method but also by the coverage of the cervical cancer treatment. However, in reality many countries have yet conducted established screening program for cervical cancer in this specific population, especially those of developing countries.

Indonesia was still considered at a low rate of cervical cancer screening (12%) per 2020, resulting in an emergency situation for cervical cancer until recently [5]. Many patients came to the hospital with their end-stage cancer due to the late diagnosis. On the other hand, there has also been increasing number of HIV cases. Based on the recapitulation data of the early detection of cervical cancer in Indonesia 2016, the number of suspected cervical cancer in several provinces such as Jakarta were 269 cases, Bali with 254 cases and Bangka Belitung with 227 cases. The data showed that Bali was one of the areas with high cervical cancer rates in 2016. Furthermore, this number increased in 2019 for Bali with the incidence of cervical cancer of 437 cases. On the other hand, Bali was on the sixth position for the highest HIV cases in Indonesia with 28,376 cases until June 2022 as stated by the Indonesian Ministry of Health June 2022 [6]. However, there has yet been any data on the number of cervical cancer in this HIV population.

A number of risk factors could also influence the risk of cervical cytological abnormalities and cervical cancer in women living with HIV. Besides late screening, it was thought that late Antiretroviral Therapy (ART) initiation or at lower CD4 cell counts could increase the risk of developing cervical cancer [2]. ART are expected to decrease viral load and help improve CD4 cell counts, thus enhancing clearance of HPV and reducing the incidence of cervical cytological abnormalities. Therefore, inequities in access to effective cervical cancer screening and treatment of precancerous cervical lesions are likely to be the main driver of high invasive cervical cancer rates in women living with HIV.

Due to the reasoning above, the aim of this study was to evaluate the prevalence of high-risk HPV among women living with HIV in Bali and to determine other possible risk factors associated with cervical cytological abnormalities. This study will provide not only the actual data of the local population to define future action in eradicating cervical cancer, but also directly provide benefit in the form of early detection for women living with HIV in Bali.

Methods

Study design and setting

This research was an observational study with cross-sectional design carried out at Yayasan Kerti Praja HIV foundation Denpasar Bali clinic 26th of July 2023–11th of December 2023. The respondents were women living with HIV who had regular appointments at the HIV clinics in Bali.

Patient selection and sampling

We included women attending HIV clinics following with these inclusion criteria: (1) women living with HIV of age 18–50 years old; (2) Indonesian nationality; (3) has had sexual intercourse and willing to give their informed consent and exclusion criteria: (1) mentally unstable; (2) cervical cancer patients; (3) on menstruation during data collection; (4) pregnant women; (5) history of previous pap smear and/or HPV testing in the last 6 months; (6) on treatment of all types of pre-malignancy or malignancy and/or had hysterectomy. Patients with cervical cancer history were excluded since this study was for screening and to find the risk factors of HR-HPV and

cervical cytological abnormalities in a cross-sectional setting.

Number of samples were calculated based on the sample size formula for cross-sectional design of proportional outcome. With the level of acceptable error of 5% and the expected proportion in population of 19.05% [7], and at the 5% types I error rate, the minimum number of samples required was 237. Potential candidates were approached with consecutive sampling, a sampling method where participants are selected one after another as they fulfil the inclusion criteria, until the desired sample size is reached.

We screened 300 eligible candidates of women living with HIV, where these women had no certain symptoms, but rather concern regarding their predisposition to the disease. The screening procedure was done in a similar way to one of the algorithms (Algorithm 3) in the WHO Guidelines of Cervical Cancer Screening [8]. For research purpose, all participants in the study went through cytology screening with ThinPrep pap smear and HPV DNA testing simultaneously. This was done altogether to give a complete picture of all participants involved in this study as part of the Indonesian ethnicity.

Study procedures

Blood samples were analyzed for CD4 and CD8 cell counts using the flow cytometry method using FACSVia (R656874100022) / FASClyric tool (R659180000700).

Cytology identification

Cytological tests were performed by liquid based preparation technique (ThinPrep, Hologic, Inc., USA). All specimens were taken using ThinPrep swab kit, processed with a ThinPrep machine, stained with Papaniculaou stain, and assessed microscopically by a pathologist based on The Bethesda System criteria [9]. Abnormal cytology of cervix were those of dysplasia (Low Grade Squamous Intraepithelial Lesion (L-SIL) and High Grade Squamous Intraepithelial Lesion (H-SIL)), as well as those of not yet developing into dysplasia (Atypical Squamous Cells of Undetermined Significance (ASC-US) and Atypical Squamous Cells of High Grade (ASC-H)).

PCR for HPV DNA and genotyping

Vaginal swab samples were collected and inserted into ThinPrep cytology media (ThinPrep PreservCyt Solution1 REF 70097-005—Hologic, Inc. Marlborough, MA, USA). Subsequently, the fluid in the cytology tube was vortexed, and 1 ml of ThinPrep fluid was transferred to a microcentrifuge tube. Centrifugation was performed at 12,000 RPM for 1 min, and the pellet was washed with distilled water. DNA extraction was performed using the Zymo Quick-DNATM Miniprep Plus Kit according to the manufacturer's instructions. The extracted DNA was then stored at -20 °C until use. Next, PCR was performed with a total volume of 10 µl, consisting of 10 nM forward and reverse primers, 5 µL PCR mix (GoTaq Green, Promega), distilled water and 1 µL DNA. A single plex PCR was conducted in two stages; the first stage used the MY09/11 universal primers, and the positive samples were followed by type-specific PCR using specific primers for HPV 16,18,31,33,35,39,45,51,52,58,59,66 and 68, the specific primer used in this study was based on a previous study by van den Brule et al. [10] PCR was carried out on a VeritiTM machine (AB Biosystem) with a denaturation PCR program of 95 °C for 5 min, followed by 35 cycles of 95 °C for 1 min, 95 °C for 20 s, 55 °C for 45 s, 72 °C for 60 s, and a final extension at 72 °C for 7 min. PCR results were visualized using 1.5% agarose gel with GelRed staining under UV light.

Statistics

Initially, descriptive statistics were used to summarize the demographic and clinical characteristics of the study population. The prevalence of cervical abnormalities and HPV infection rates were calculated. Bivariate analysis was conducted to explore the association between HPV DNA positivity and abnormal cytology results. The strength of this association was quantified using odd ratio (ORs) with corresponding 95% confidence intervals (CIs). Multivariate logistic regression analysis was then performed to identify independent risk factors for cervical abnormalities, adjusting for potential confounders that had association in bivariate analysis of less than 0.25. The significance of multivariate analysis was determined with a threshold of p-value less than 0.05. We used SPSS version 29 to conduct the analysis.

Results

From the targeted population, we received a list of 300 potential candidates for screening. After referred to the inclusion and exclusion criteria, we recruited 245 eligible participants, from which 239 samples (97.6%) were successfully analyzed. We had to repeat sampling 10 cases due to initial sampling inadequacy, attributed to challenging conditions such as difficult portio access or the presence of inflammatory cells. The sample was deemed inadequate for histopathological examination because it lacked the transformation zone and endocervical components necessary for accurate analysis. The research team gave significant efforts to communicate with participants requiring re-sampling, providing detailed explanations about the importance of the repeat pap smear procedure and the potential health benefits. Out of these ten needed to repeat, there were six samples excluded from the final analysis, two of these were due to suspected cervical cancer, and the other four were due to logistical issues (two participants relocated and could not return, and two

declined to participate further despite multiple contact attempts, resulting in lost to follow up). The team still provided results from the initial tests along with health education and preventive advice concerning cervical cancer (see Fig. 1).

Baseline characteristics of study participants

As reported in Table 1, the characteristics of study participants were grouped based on the presence or absence of cervical cytological abnormalities, categorized across various demographic and behavioral factors. The findings indicated that 8.8% of divorced individuals had cytological abnormalities, while 91.3% did not have these conditions. We found that individuals with higher levels of education had a slightly higher incidence of cytological abnormalities compared to those with less education. Employment status correlated with cytological abnormalities prevalence, where 15.5% of unemployed participants had cytological abnormalities compared to 8.9% of employed participants. The analysis also considered lifestyle factors such as alcohol consumption and contraception use, noting that none of the alcohol consumers in the study had cytological abnormalities, and those using contraception exhibited a higher percentage of cytological abnormalities than those who did not. Sexual behavior was also a factor, with participants who had more sexual partners or an earlier sexual debut showing slightly higher percentages of cytological abnormalities. Additional factors like gravidity, hormonal medication use, and history of abortion were examined, each contributing to the understanding of how these characteristics were associated with the presence of ASC or cervical cytological abnormalities among the participants.

As reported in Table 2, we found that participants with cytological abnormalities were on average slightly younger (35.5 years) compared to those without such conditions (38 years). However, this age difference was not statistically significant. Notably, the CD4 cell count showed a significant difference, with a median count of 415 cells/uL in those with Cytological abnormalities versus 556 cells/uL in those without, suggesting a lower immune status in the affected group. Similarly, the percentage of CD4 cells was significantly lower in participants with Cytological abnormalities, averaging 21.34%, compared to 26.62% in those without, further indicating compromised immune function in the former group. The CD8 cell count and percentage did not show significant differences. However, the CD4/CD8 ratio was significantly lower in participants with Cytological abnormalities, with a median of 0.43 compared to 0.63 in those without, underscoring a more pronounced immune system imbalance in those with cervical abnormalities.

Since there were eight non-typeable samples, we only included 231 samples to identify the risk factors of high-risk HPV infection in women living HIV. Based on Table 3, the characteristics of samples with and without high-risk HPV infection were analyzed. It was found that marital status varied, with divorced participants making up 22.1% of those with HPV and 77.9% of those without. Married participants constituted 20.3% of the HPV-positive group and 79.7% of the HPV-negative group, while single participants represented 31.3% of those with HPV and 68.8% without. Education levels also showed

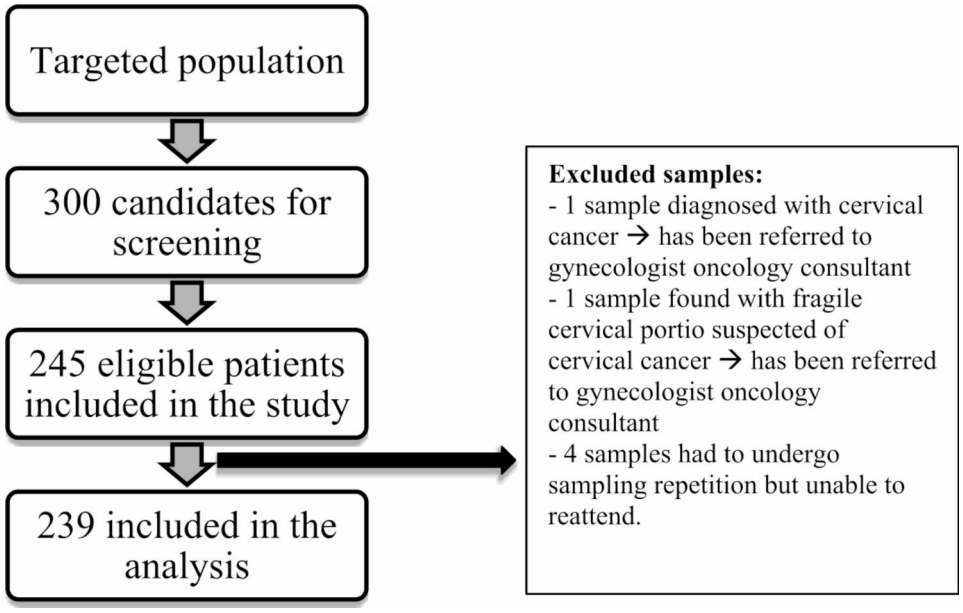

Fig. 1 Recruitment process during the study

Table 1 Characteristics of samples based on with and without cervical cytological abnormalities (categorical variables)

Variables	With cytological abnormalities (*n*, %)	Without cytological abnormalities (*n*, %)	*p*-value	Odd ratio (95%CI)
Marital Status (n = 239)				
Divorced	7 (8.8%)	73 (91.3%)	0.492	-
Married	18 (12.8%)	123 (87.2%)		
Single	1 (5.6%)	17 (94.4%)		
Education (n = 239)				
< High School	8 (9.1%)	80 (90.9%)	0.498[a]	-
≥High School	18 (11.9%)	133 (88.1%)		
Occupation				
Unemployed	11 (15.5%)	60 (84.5%)	*0.136*[a]	0.535 (0.232–1.230)
Employed	15 (8.9%)	153 (91.1%)		
Alcohol (n = 239)				
Yes	0 (0%)	1 (100.0%)	1.000[b]	-
No	26 (10.9%)	212 (89.1%)		
Contraception (n = 239)				
Yes	17 (14.5%)	100 (85.5%)	*0.076*[a]	2.134 (0.911–5.002)
No	9 (7.4%)	113 (92.6%)		
Number of Sexual Partner (n = 239)				
≥5	6 (8.5%)	65 (91.5%)	0.433[a]	-
< 5	20 (11.9%)	148 (88.1%)		
Sexual debut year (n = 239)				
< 21	17 (10.7%)	142 (89.3%)	0.896[a]	-
≥21	9 (11.3%)	71 (88.8%)		
Viral load (n = 182)				
Detected	5 (16.1%)	26 (83.9%)	0.329[a]	-
Not detected	14 (9.3%)	137 (90.7%)		
HIV Duration (years) (n = 239)				
≥5	15 (9.4%)	145 (90.6%)	0.288[a]	-
< 5	11 (13.9%)	68 (86.1%)		
ARV Treatment Duration (Years) (n = 239)				
≤1	6 (15.0%)	34 (85.0%)	*0.242*	-
2–4	7 (15.9%)	37 (84.1%)		
≥5	13 (8.4%)	142 (91.6%)		
Smoking status (n = 239)				
Yes	3 (9.7%)	28 (90.3%)	1.000[b]	-
No	23 (11.1%)	185 (88.9%)		
HPV infection history (n = 239)				
Yes	4 (18.2%)	18 (81.8%)	0.274[b]	-
No	22 (10.1%)	195 (89.9%)		
Pap smear history (n = 239)				
Yes	7 (5.6%)	119 (94.4%)	0.005**[a]	0.291 (0.117–0.721)
No	19 (19.8%)	94 (83.2%)		
HPV Vaccine Status (n = 239)				
No	26 (11.1%)	208 (88.9%)	1.000[b]	-
Yes	0 (0%)	5 (100%)		
Gravidity (n = 239)				
No gravidity	2 (7.7%)	24 (92.3%)	0.420	-
≤ 2	12 (9.2%)	118 (90.8%)		
> 2	12 (14.5%)	71 (85.5%)		
Hormonal medication (n = 239)				
Yes	1 (25%)	3 (75%)	0.371[b]	-
No	25 (10.6)	210 (89.4%)		
Abortion (n = 239)				

Table 1 (continued)

Variables	With cytological abnormalities (*n*, %)	Without cytological abnormalities (*n*, %)	*p*-value	Odd ratio (95%CI)
Yes	7 (8%)	81 (92%)	0.268[a]	-
No	19 (12.6%)	132 (87.4%)		
History of STDs (n = 239)				
Yes	4 (19%)	17 (81%)	0.260[b]	-
No	22 (10.1%)	196 (89.9%)		
Menopause (n = 239)				
Yes	1 (4%)	24 (96%)	0.328[b]	-
No	25 (11.7%)	189 (88.3%)		
Universal HPV DNA results (n = 239)				
HPV (+)	18 (31.0%)	40 (69.0%)	0.000[a]**	9.731 (3.952–23.959)
HPV (-)	8 (4.4%)	173 (95.6%)		

[a]analysed by chi-square test

[b]analysed by fisher's exact test

Table 2 Characteristics of samples based on with and without cervical cytological abnormalities

Variables	With cytological abnormalities (mean ± SD or median (min-max)	Without cytological abnormalities (mean ± SD or median (min-max)	*p*-value (CI95%)
Age (years)	35.5 (27–47)	38 (24–50)	0.330[c]
CD4# (cells/uL)	415 (65-1132)	556 (103–1680)	0.011*[c]
CD8# (cells/uL)	1009 (299–2268)	857 (277–1839)	0.398[c]
CD4%	21.34 ± 9.87	26.62 ± 8.63	0.004** (1.6918–8.8726)[d]
CD8%	46.86 (18.42–68.18)	39.62 (20.28–74.31)	0.013*[c]
CD4/CD8 Ratio	0.43 (0.13–1.41)	0.63 (0.07–1.75)	0.005**[c]

[c]analysed by mann-whitney test

[d]analysed by independent T-test

*$p < 0.05$

differences; those with less than high school education made up 19.8% of the HPV-positive group and 80.2% of the HPV-negative group. Regarding contraception use, 25.2% of those using contraception were HPV-positive compared to 74.8% who were negative. The number of sexual partners also correlated with HPV status; 27.3% of those with more than five partners were HPV-positive.

From the analysis presented in Table 4, key findings regarding the characteristics of samples with and without high-risk HPV infection were observed. The age of participants did not show a significant difference, with both HPV-positive and HPV-negative groups having a median age of 38 years. However, there was a significant difference in CD4 cell counts; participants with HPV had a median CD4 count of 407 cells/uL, while those without HPV had a higher median of 594 cells/uL. This suggested a lower immune status in the HPV-positive group. Additionally, the percentage of CD4 cells was significantly lower in the HPV-positive participants, averaging 21% compared to 27.44% in the HPV-negative group. The CD8 cell counts were similar between the two groups, but the CD4/CD8 ratio was significantly lower in the HPV-positive group, with a median of 0.44 compared to 0.68 in the HPV-negative group, indicating a more pronounced immune system imbalance in those with high-risk HPV infection.

HPV genotyping and cytology results

In the study, Tables 5 and 6 provided results of Pap smear and HPV genotyping results. The Pap smear results showed that various abnormalities were detected: 2.5% had ASC-H, 3.8% had ASC-US, 1.7% had H-SIL, and 2.9% had L-SIL. The HPV genotyping revealed that HPV 18 was the most prevalent, found in 56.2% of the samples with cytological abnormalities, significantly higher than other types. Other HR-HPV were also detected but with lower frequencies. A single individual could be detected for two genotypes, which made it appear as though they were counted twice. However, in reality, there were just 58 unique individuals tested positive for HR-HPV. In terms of 8 untypable samples were those that could not be determined, possibly because they were not detected by the 14 specific primers used in this study or that they belonged to low-risk HPV types. Confirmation is indeed necessary; however, in this study, we have not yet conducted confirmation study for those untypable samples.

Multivariate analysis of cervical cytological abnormalities' risk factors

Table 7 provided a multivariate analysis of the risk factors for cervical cytological abnormalities in women living with HIV. The analysis revealed that HPV 18 was a significant independent risk factor, with an exponent (B) of 9.029 and a *p*-value of 0.007, indicating a strong association between HPV 18 infection and the presence of

Table 3 Characteristics of samples with and without high-risk HPV infection

Variables	HPV (+) (n, %)	HPV (-) (n, %)	*p*-value	Odd ratio (95%CI)
Marital Status (n = 231)				
Divorced	17 (22.1%)	60 (77.9%)	0.598	-
Married	28 (20.3%)	110 (79.7%)		
Single	5 (31.3%)	11 (68.8%)		
Education (n = 231)				
< High School	17 (19.8%)	69 (80.2%)	0.594[a]	-
≥High School	33 (22.8%)	112 (77.2%)		
Occupation (n = 231)				
Unemployed	17 (23.9%)	54 (76.1%)	0.572[a]	-
Employed	33 (20.6%)	127 (79.4%)		
Alcohol (n = 231)				
Yes	1 (100%)	0 (0%)	*0.216*[b]	4.694 (3.662–6.017)
No	49 (21.3%)	181 (78.7%)		
Contraception (n = 231)				
Yes	28 (25.2%)	83 (74.8%)	*0.204*[a]	1.376 (0.839–2.258)
No	22 (18.3%)	98 (81.7%)		
Number of Sexual Partner (n = 231)				
≥5	18 (27.3%)	48 (72.7%)	*0.189*[a]	1.406 (0.851–2.323)
< 5	32 (19.4%)	133 (80.6%)		
Sexual debut year (n = 231)				
< 21	38 (24.7%)	116 (75.3%)	*0.114*[a]	0.632 (0.351–1.138)
≥21	12 (15.6%)	65 (84.8%)		
Viral load (n = 176)				
Detected	7 (23.3%)	23 (76.7%)	0.672[a]	-
Not detected	29 (19.9%)	117 (80.1%)		
HIV Duration (years) (n = 231)				
≥5	21 (13.6%)	133 (86.4%)	*0.000***[a]	0.362 (0.222–0.591)
< 5	29 (37.7%)	48 (62.3%)		
ARV Treatment Duration (Years) (n = 231)				
≤1	20 (52.6%)	18 (47.4%)	*0.000***	-
2–4	11 (25.6%)	32 (74.4%)		
≥5	19 (12.7%)	131 (87.3%)		
Smoking status (n = 231)				
Yes	8 (26.7%)	22 (73.3%)	0.480[a]	-
No	42 (20.9%)	159 (79.1%)		
HPV infection history (n = 231)				
Yes	6 (27.3%)	16 (72.7%)	0.586[b]	-
No	44 (21.1%)	165 (78.9%)		
Pap smear history (n = 231)				
Yes	17 (14.2%)	103 (85.8%)	*0.004***[a]	0.477 (0.282–0.806)
No	33 (29.7%)	78 (70.3%)		
HPV Vaccine Status (n = 231)				
No	50 (22.1%)	176 (77.9%)	0.588[b]	
Yes	0 (0%)	5 (100%)		
Gravidity (n = 231)				
No gravidity	10 (40%)	15 (60%)	*0.060*	
≤ 2	25 (20%)	100 (80%)		
> 2	15 (18.5%)	66 (81.5%)		
Hormonal medication (n = 231)				
Yes	3 (75%)	1 (25%)	*0.033**[b]	3.622 (1.948–6.737)
No	47 (20.7%)	180 (79.3%)		
Abortion (n = 231)				
Yes	15 (17.4%)	71 (82.6%)	*0.232*[a]	0.723 (0.420–1.244)

Table 3 (continued)

Variables	HPV (+) (n, %)	HPV (-) (n, %)	*p*-value	Odd ratio (95%CI)
No	35 (24.1%)	110 (75.9%)		
History of STDs (n = 231)				
Yes	6 (28.6%)	15 (71.4%)	0.412[b]	
No	44 (21%)	166 (79%)		
Menopause (n = 231)				
Yes	4 (16%)	21 (84%)	0.468[b]	
No	46 (22.3%)	160 (77.7%)		

[a]analysed by chi-square test

[b]analysed by fisher's exact test

Table 4 Characteristics of samples with and without high-risk HPV infection

Variables	HPV (+) (mean ± SD or median (min-max)	HPV (-) (mean ± SD or median (min-max)	*p*-value (CI95%)
Age (years)	38 (24–48)	38 (24–50)	*0.178*[c]
CD4# (cells/uL)	407 (65-1214)	594 (103–1680)	*0.000***[c]
CD8# (cells/uL)	881 (299–2268)	854 (277–1839)	0.602[c]
CD4%	21 ± 10.53	27.44 ± 7.86	*0.000*** (-9.6311-(-3.2436))[d]
CD8%	45.72 ± 12.08	39.16 (20.28–70.13)	*0.002***[c]
CD4/CD8 Ratio	0.44 (0.11–1.75)	0.68 (0.07–1.54)	*0.000***[c]

[c]analysed by mann-whitney test

[d]analysed by independent T-test

*$p < 0.05$

**$p < 0.01$

Table 5 Cytological abnormalities and HPV genotyping

Variables	Frequency (*n* = 239)
Cytological abnormalities	26 (10.87%)
Atypical squamous cells high grade (ASC-H)	6 (2.5%)
Atypical squamous cells of undetermined significance (ASC-US)	9 (3.8%)
High Grade Squamous Intraepithelial Lesion (H-SIL)	4 (1.7%)
Low Grade Squamous Intraepithelial Lesion (L-SIL)	7 (2.9%)
HPV DNA universal analysis	
Positive	58 (24.3%)
Negative	181 (75.7%)
HPV genotyping (*n* = 58)	
Single genotyping	40 (69%)
Double genotyping	
16, 18	2 (3.4%)
16, 52	1 (1.7%)
18, 45	3 (5.2%)
18, 52	2 (3.4%)
51, 58	1 (1.7%)
56, 58	1 (1.7%)
Untypable	8 (13.8%)
HPV genotypes (*n* = 58)	
HPV 16	7 (12.1%)
HPV 18	16 (27.6%)
HPV 31	4 (6.9%)
HPV 33	1 (1.7%)
HPV 45	3 (5.2%)
HPV 51	2 (3.4%)
HPV 52	9 (15.5%)
HPV 56	9 (15.5%)
HPV 58	9 (15.5%)
HPV 35,39,59,66,68	0 (0%)

cervical abnormalities. Other HPV types such as HPV 16, 31, 45, 51, 52 and 56 were also analyzed, but they did not show significant associations, suggesting less impact on the risk of cervical cytological abnormalities. Additionally, a history of Pap smear screening showed a trend towards reducing the risk of cervical abnormalities, although it was not statistically significant with a *p*-value of 0.083. The analysis underscored the critical role of HPV 18 in contributing to cervical cytological abnormalities among women with HIV and highlighted the potential protective effect of regular Pap smear screenings.

Multivariate analysis of high-risk HPV infection risk factors

Table 8 presented a multivariate analysis of the risk factors for high-risk HPV infection in women living with HIV. The findings indicated that a history of contraception use was marginally significant as a risk factor, with an exponent (B) of 2.189 and a *p*-value of 0.053, suggesting a potential association with increased risk of HPV infection. The number of sexual partners and the age at sexual debut did not show significant associations with HPV infection, as indicated by their *p*-values. Notably, a history of Pap smear screening was significantly associated with a reduced risk of high-risk HPV infection, with an exponent (B) of 0.358 and a *p*-value of 0.013, highlighting the protective effect of regular screening. The duration of ARV treatment and other factors like gravidity, abortion, and hormonal treatment did not show significant associations with HPV risk. This analysis underscored the importance of Pap smear history in reducing the risk of HPV infection among women with HIV.

Table 6 Distribution of samples into cytology groups based on HPV genotypes ($n = 239$)

HPV genotype	With Cytological abnormalities (*n*, %)	Without Cytological abnormalities (*n*, %)	*p*-value
HPV 16 (+)	3 (42.9%)	4 (57.1%)	0.000**
Other than HPV 16	15 (29.4%)	36 (70.6%)	
Negative for HPV	8 (4.4%)	173 (95.6%)	
HPV 18 (+)	9 (56.2%)	7 (43.8%)	0.000**
Other than HPV 18	9 (21.4%)	33 (78.6%)	
Negative for HPV	8 (4.4%)	173 (95.6%)	
HPV 31 (+)	2 (50.0%)	2 (50.0%)	0.000**
Other than HPV 31	16 (29.6%)	38 (70.4%)	
Negative for HPV	8 (4.4%)	173 (95.6%)	
HPV 33 (+)	0 (0.0%)	1 (100.0%)	-
Other than HPV 33	18 (31.6%)	39 (68.4%)	
Negative for HPV	8 (4.4%)	173 (95.6%)	
HPV 45 (+)	1 (33.3%)	2 (66.7%)	0.000**
Other than HPV 45	17 (30.9%)	38 (69.1%)	
Negative for HPV	8 (4.4%)	173 (95.6%)	
HPV 51 (+)	1 (50.0%)	1 (50.0%)	0.000**
Other than HPV 51	17 (30.4%)	39 (69.6%)	
Negative for HPV	8 (4.4%)	173 (95.6%)	
HPV 52 (+)	4 (44.4%)	5 (55.6%)	0.000**
Other than HPV 52	14 (28.6%)	35 (71.4%)	
Negative for HPV	8 (4.4%)	173 (95.6%)	
HPV 56 (+)	7 (77.7%)	2 (22.2%)	0.000**
Other than HPV 56	11 (22.4%)	38 (77.5%)	
Negative for HPV	8 (4.4%)	173 (95.6%)	
HPV 58 (+)	0 (0.0%)	9 (100.0%)	-
Other than HPV 58	18 (36.7%)	31 (63.3%)	
Negative for HPV	8 (4.4%)	173 (95.6%)	

Table 8 The effects of all the potential risk factors on the risk of high-risk HPV infection in women with HIV in this study ($n = 231$)

Covariates	Exponent (B)*	*P*-value	95% CI
Contraception history	2.189	0.053	0.989–4.845
Number of sexual partner (≥ 5 people)	1.368	0.463	0.592–3.161
Sexual debut year (≥ 21 years old)	0.573	0.218	0.236–1.390
HIV duration (≥ 5 years)	0.542	0.388	0.135–2.175
ARV treatment duration (≤ 1 year)	2.504	0.239	0.544–11.519
Pap smear History	0.358	**0.013***	0.160–0.802
Gravidity (> 2 times)	0.481	0.239	0.142–1.628
Abortion	0.682	0.360	0.301–1.546
Hormonal treatment	8.319	0.106	0.637–108.736
Age	1.020	0.522	0.960–1.084
CD4:CD8	0.349	0.093	0.103–1.190

*The natural logarithm of the odds ratio

Table 7 The effects of all the potential risk factors on the risk of cervical cytological abnormalities in women with HIV in this study ($n = 239$)

Covariates	Exponent (B)*	*P*-value	95% CI
HPV DNA	2.800	0.177	0.629–12.45
HPV 16	1.99	0.492	0.280–14.155
HPV 18	9.029	**0.007****	**1.835–44.425**
HPV 31	3.908	0.300	0.298–51.317
HPV 45	0.617	0.745	0.034–11.327
HPV 51	8.295	0.200	0.327–210.262
HPV 52	3.277	0.207	0.519–20.680
HPV 56	3.200	0.310	0.501–20.013
Papsmear history	0.374	0.083	0.123–1.138
ARV treatment duration (≤ 1 year)	0.388	0.212	0.088–1.718
Contraception history	2.710	0.065	0.940–7.809
Occupation (unemployed)	1.845	0.250	0.650–5.235
CD4:CD8 ratio	0.378	0.234	0.076–1.876

*The natural logarithm of the odds ratio

Discussion

HR-HPV 18 as a risk factor of cervical abnormalities in women living with HIV

Our results underscored the prevalence of high-risk HPV types among women with HIV in Bali and their association with various grades of cervical abnormalities. Among these, HR-HPV 18 emerged as the most significant risk factor for cervical cytological abnormalities, while other HR-HPV types were less commonly detected. This differs from previous findings, such as a 2012 meta-analysis that reported HPV 16 as the most prevalent genotype in regions like India, Thailand and China [11]. Similarly, another meta-analysis about HPV genotypes in Kenya also found HPV 16 to be the most common infection, followed by HPV 18. HPV 16 was consistently identified as the most prevalent genotype in women with abnormal cytology and invasive cervical cancer (ICC). For instance, in women with abnormal cytology, HPV 16 was found to be present in 26% of cases, and in women with ICC, its prevalence increased to 37% [12]. This indicates a clear escalation in the prevalence of HPV 16 as the severity of cervical disease increases. Following HPV 16, HPV 18 is the second most common genotype in ICC cases, with a prevalence of 24%. This discrepancy highlights the geographical and demographic variations in HPV genotype distribution, which is a crucial factor in understanding the epidemiology of HPV and its associated risks for cervical cancer. Our findings suggest a potential role of HR-HPV 18 in cervical cancer progression among this population.

HPV 18, along with HPV 16, belongs to the high-risk category of HPV genotypes that are strongly associated with the development of cervical cancer. These genotypes are known for their ability to integrate into the host cell DNA, disrupting normal cell functions and leading to the progression of precancerous lesions to invasive cancer [13]. The variation in prevalence between our findings and the previous studies could be attributed to differences in study populations, methodologies, or regional public health practices such as the prevalence of vaccination and screening.

The lower prevalence of HPV 16 observed in women with HIV in Bali, despite the low vaccination coverage, can be attributed to a combination of factors. Firstly, Indonesia's HPV vaccination program, introduced in 2016, targets HPV types 16 and 18 [14]. Although vaccination rates remain low, the program may have had some early impact in reducing the prevalence of HPV 16 in certain sub-populations. Regional differences in HPV distribution, influenced by local behavior, cultural practices, and healthcare access, may also play a role. Furthermore, our current study focused on a specific population which is women with HIV, who may exhibit different HPV prevalence patterns compared to the general population. The interplay of these factors likely accounts for the observed lower prevalence of HPV 16 in Bali, even with limited vaccination coverage. This underlines the importance of localized data to inform public health strategies effectively and further research is required to fully understand the dynamics at play. For regions where HPV 18 is more prevalent, targeted interventions to address this specific risk factor are crucial [12]. This includes promoting HPV vaccination that covers HPV 18, enhancing screening programs to detect and treat precancerous changes early, and educating the public and healthcare providers about the risks associated with HPV 18.

Pap smear as an effective preventive measure of cervical cancer

The Pap smear is a critical preventive measure for cervical cancer, particularly significant for HIV-seropositive women in developing countries. As a cytological-based test, the Pap smear has been extensively utilized in developed countries for many years, demonstrating substantial success in reducing both the incidence and mortality of cervical cancer [15].

One of the primary benefits of the Pap smear is its ability to detect precancerous changes in the cervix, known as cervical intraepithelial neoplasia (CIN), at an early stage. Early detection allows for the effective treatment of these changes before they have the chance to progress to cancer. Consequently, this early intervention significantly contributes to the reduction in mortality associated with cervical cancer, as treatments tend to be more effective at earlier stages [5, 16].

Moreover, the Pap smear is a cost-effective procedure. It does not require highly specialized equipment, which makes it a viable option for many healthcare settings, including those with limited resources typical of developing countries. The success of the Pap smear in developed countries highlights its potential effectiveness when implemented with regular screenings and proper follow-up care [5].

Despite its benefits, the implementation of the Pap smear in developing countries faces several challenges. These include a lack of proper infrastructure, insufficient qualified personnel, and limited resources, which can impede the utilization and effectiveness of the Pap smear [5]. Nonetheless, it remains a cornerstone of cervical cancer prevention strategies. There is a pressing need to improve its accessibility and integration into healthcare systems, especially for populations at higher risk, such as HIV-seropositive women.

This study is not without limitation. The cross-sectional nature of this research did indeed restrict the ability to establish causal relationships between the possible risk factors and cervical cytological abnormalities. The reliance on self-reported data for certain demographic and behavioral factors might also affect the accuracy of the information collected. This study was conducted in Bali, a specific region in Indonesia, which may not account for regional variations in HPV prevalence in the country. Moreover, while HR-HPV genotyping was unsuccessful in about 14% of positive samples (8 out of 58 samples), these 14% untypable samples were most likely due to non-high-risk types but low-risk types. Low-risk HPV types, such as HPV 6 and 11, are generally linked to benign conditions like genital warts and are not typically associated with cervical abnormalities, thus remaining undetected by the type-specific primers used in the genotyping process in this study. Multiple HPV infections were observed but not explored in terms of their relationship to cervical precancerous lesions due to the low number of cases found. However, a previous study of 400 cervical cancer patients found that while multiple infections were common (32.29% of HPV-positive cases), they were not significantly correlated with increased cancer severity (stage, tumor size, metastasis) compared to single infections. The primary risk driver was the HPV subtype rather than infection multiplicity, with HPV 16 dominating in 67.7% of cases (both single and co-infections) alongside other high-risk strains (HPV 18, 58, 33, 52) [17]. Nonetheless, this study proved that in Bali HPV18 was found to be the most prevalent HR-HPV type unlike what is found in other areas in the world, where HPV16 is the prevalent HR-HPV type. Our study underscores the need for further investigation regarding

this, as this unique distribution could provide valuable insights into local epidemiological patterns and inform targeted prevention strategies to address cervical cancer in this region.

Conclusions

Our research found HR-HPV 18 as a significant risk factor for cervical cytological abnormalities and potentially, cervical cancer in women living with HIV, supporting the indicative measure for a routine pap-smear screening in this specified population. Along with the pap smear, vaccination for HR-HPV 18 also becomes urgently important to be readily supplied in Indonesia.

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Author contributions
IKAS contributed to the conception and design of research; MLAP contributed to the conception, design of research, samplings, as well as wrote the main manuscript text; NWW acted as clinical pathology specialist that confirmed the results of the samples lab; IBNPD contributed to the processing of samples; DMPP, AAAYG and INGB contributed to the selection of sample candidates; AASS contributed to the statistical analysis; KJPP and KTPM acted as supervisors of the research; all authors reviewed the manuscript.

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Data availability
Data cannot be shared publicly because of patients confidentiality. Data are available from the Institutional Data Access / Ethics Committee (contact via first author) for researchers who meet the criteria for access to confidential data.

Declarations

Ethics approval and consent to participate
This study reported data of human tissue samples, which were performed in accordance with relevant guidelines and regulations from established laboratory. All participants gave their consent by signing on a written informed consent. This study has been granted an ethical statement from the Ethic Committee of Faculty of Medicine, Udayana University with the ethical clearance number: 1662/UN14.2.2.VII.14/LT/2023.

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Competing interests
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