Can we increase the cervical cancer screening interval with an HPV test for women living with HIV? Results of a cohort study from Maharashtra, India

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Abstract

We are reporting (a) updated incidence of cervical intraepithelial neoplasia (CIN) among women who did not have colposcopic or histopathological disease at baseline and (b) disease outcomes among women treated for CIN and their follow-up HPV status; in a cohort of women living with HIV (WHIV). The median overall follow-up was 3.5 years (IQR 2.8-4.3). The incidence of any CIN and that of CIN 2 or worse disease was 16.7 and 7.0 per 1000 person-years of observation (PYO), respectively. Compared with women who were HPV negative at baseline, women who cleared HPV infection had 23.95 times increased risk of incident CIN 2 or worse lesions (95% CI 2.40-661.07). Women with persistent HPV infection had 138.18 times increased risk of CIN 2 or worse lesions (95% CI 20.30-3300.22). Complete disease regression was observed in 65.6% of the HPV positive women with high-grade CIN and were treated with thermal ablation but HPV persistence was seen in 44.8% of those with high-grade disease. Among those who did not have any disease at baseline and were also HPV negative, about 87% (95% CI 83.79-89.48) women remained HPV negative during consecutive HPV test/s with the median interval of 3.5 years. Long-term surveillance of WHIV treated for any CIN is necessary for the prevention of cervical cancer among them. Our study provides an early indication that the currently recommended screening interval of 3 to 5 years among WHIV may be extended to at least 5 years among HPV negative women. Increasing the screening interval can be cost saving and improve scalability among WHIV to support WHO’s cervical cancer elimination initiative.

KEYWORDS

cervical cancer, CIN, HIV, HPV, posttreatment CIN

Abbreviations: ART, antiretroviral therapy; CIN, cervical intraepithelial neoplasia; CI, confidence interval; HC2, Hybrid Capture 2; HCJMRI, Hirabai Cowasji Jehangir Medical Research Institute; HPV, human papillomavirus; IARC, International Agency for Research on Cancer; LLETZ, large loop excision of the transformation zone; LMICs, low- and middle-income countries; NDMCH, Nargis Dutt Memorial Cancer Hospital; PYO, person-years of observation; RLU/PC, relative light unit to a positive control; RR, relative risks; STM, specimen transport medium; VIA, visual inspection with acetic acid; VILI, visual inspection with Lugol’s iodine; WHIV, women living with HIV; WHO, World Health Organization.

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1 | INTRODUCTION

Cervical cancer is eminently preventable by human papillomavirus (HPV) vaccination and early detection and treatment of precancerous lesions, but it is still a major public health problem in the resource limited countries. The International Agency for Research on Cancer (IARC) of the World Health Organization (WHO) estimated that there were 604,127 new cases and 341,831 deaths due to cervical cancer globally in 2020.\(^1\) China and India account for more than a third of the global burden of cervical cancer.\(^2\) About 58.5% deaths due to cervical cancer are from Asia.\(^2\)

Cervical cancer disproportionately affects women living with human immunodeficiency virus (WHIV).\(^1\) Many of the low- and middle-income countries (LMICs) bearing high cervical cancer burden are also the countries with high HIV burden. The risk factors for HIV and HPV often overlap and WLHIV have a significantly higher risk of persistent HPV infection, making them more susceptible to develop cervical cancer.\(^3,5\) WLHIV have a 6-fold increased risk of cervical cancer as compared with women in the general population.\(^5\)

Improved access to antiretroviral therapy (ART) has increased the life expectancy in HIV-infected individuals.\(^6\) Early ART initiation and sustained adherence is likely to reduce the incidence of cervical cancer in WHIV provided cervical screening and management of precancer is integrated into the ART program.\(^7\) However, compared with the general population WLHIV require more intensive screening and long-term follow-up, if they are HPV positive and/or treated for high-grade CIN.\(^8,9\)

The WHO released the guidelines for cervical cancer screening and precancer treatment in the year 2021 that also specify the screening and follow-up algorithms for WLHIV.\(^10\) However, many of the recommendations are based on “weak evidence” as not many studies have systematically followed up WLHIV, especially those positive on HPV test and/or treated for precancer.

Considering the need to advance the understanding of secondary prevention of cervical cancer in WLHIV, we initiated a cohort study in 2010 that enrolled 1153 WHIV from Maharashtra, India. Based on the cohort, we have previously reported the performance of different screening tests (visual inspection with 3-5% acetic acid [VIA], visual inspection with Lugol’s iodine [VILI], high-risk HPV testing and cytology),\(^11\) HPV genotype distribution among those with and without CIN,\(^12\) Incidence of CIN according to the HPV genotypes\(^13\) and the prevalence and predictors of bacterial vaginosis\(^14\) in WHIV. The present manuscript reports the incidence of CIN in WLHIV based on more extended follow-up data and the posttreatment follow-up outcomes of those treated for CIN.

What’s new?

Women living with HIV (WHIV) are at disproportionately high risk of human papillomavirus (HPV) infection and subsequent cervical cancer. The extent to which cervical intraepithelial neoplasia (CIN) affects WHIV and outcomes among those treated for CIN are not fully known. Here, among women in India, WHIV with persistent HPV infection had a 138.18-fold increase in risk of CIN2 or worse. Two-thirds of HPV-positive WHIV treated with thermal ablation for high-grade CIN experienced complete disease regression. Nonetheless, HPV persisted in nearly 45% of ablation-treated WHIV. A significant majority of WHIV who were HPV-negative at baseline remained HPV-free in subsequent screenings.

2 | METHODS

The study was conducted at a designated cervical cancer screening clinic at Prayas, Pune, India, a nongovernmental, nonprofit organization. We enrolled 1153 WLHIV between 2010 and 2011 in a longitudinal cohort study and the study procedures and the baseline results have been published earlier.\(^11-16\)

The study procedures at baseline and subsequent follow-up visits are explained in Figure 1. Consecutive, serologically confirmed WHIV, regardless of their CD4+ cell counts and ART status, were recruited after a written informed consent if they were aged between 21 and 60 years, had an intact uterus, did not have significant utero-vaginal prolapse, who were not pregnant and were not diagnosed with CIN or cervical cancer in the past. The enrolled women were interviewed by a female social worker and a structured questionnaire was used to collect information on sociodemographic, sexual, reproductive, medical and HIV infection related characteristics. History regarding the time since diagnosis of HIV infection, nadir CD4 count and time since ART was noted based on their available medical records.

Then a trained nurse collected a cervical specimen in the Digene specimen transport medium (STM) for Hybrid Capture 2 (HC2) assay, another for HPV genotyping in Preservcyt medium and for cytology. The nurse also performed VIA and recorded the findings. A trained colposcopist performed colposcopy on all the women at baseline. When colposcopy was suggestive of a low-grade or a high-grade lesion and the woman was eligible for ablative treatment, thermal ablation was performed in the same sitting after obtaining punch biopsies. If the lesion was not eligible for thermal ablation, large loop excision of the transformation zone (LLETZ) was performed later.

Women treated with thermal ablation or LLETZ were recalled after 6 to 12 weeks to exclude treatment complications and to review the histopathology report and then after 1 year. Women who were not treated at baseline were called initially after 6 months (to review the HPV and cytology reports) and every year thereafter for the first 3 years. Both the treated and the untreated women underwent...
colposcopy at the yearly visits. If colposcopy was suggestive of any low-grade or worse lesion, directed punch biopsies were collected from abnormal areas and immediate treatment with thermal ablation was offered when eligible or LLETZ at a later date.

All the women were followed up with yearly colposcopy for the first three consecutive years, following which yearly follow-up was replaced by 3-yearly HPV DNA test (HC2) at least for additional two rounds. Women who tested HPV negative, were called to the clinic for a repeat HPV test after 3 years. If the HPV test was positive at any of the visits, the women were called for colposcopy, biopsy and treatment when indicated. Women with persistent HPV and no lesion on colposcopy had random biopsies. Repeat treatment with thermal ablation was offered when eligible. An endocervical curettage was done when the squamocolumnar junction was not visible and there was no visible lesion on colposcopy.

2.1 | HPV DNA testing

The cervical specimens in STM were tested at the Nargis Dutt Memorial Cancer Hospital (NDMCH), Barshi, India by HC2 assay for 13 high-risk HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68). Specimens with a ratio of relative light unit to a positive control (RLU/PC) of 1 or more (corresponding to 5000 or more viral copies) were considered to be HC2 positive. HPV test in this manuscript refers to the HC2 test unless specified.

2.2 | HPV genotyping

At baseline, HPV genotyping was done for the high-risk HPV types (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68a, 68b, 70, 73 and 82), and two low-risk HPV types (HPV-6 and -11) by HPV type-specific E7 PCR bead-based multiplex genotyping (TS-MPG) and the procedure has been described earlier.

2.3 | Treatment of cervical abnormalities

The eligibility criteria for treatment with thermal ablation included ectocervical lesion involving <3/4th of a Type 1 Transformation Zone, no endocervical or vaginal extension, and no clinical/colposcopic suspicion of invasive cancer. Thermal ablation was performed by trained nurses or physicians without any local anesthesia. Treatment involved application of the flat probe heated to 105°C centigrade for 45 s. Multiple overlapping applications were made when indicated to cover the
entire transformation zone. Women not eligible for ablation were treated with LLETZ. Women diagnosed with invasive cervical cancer were referred for appropriate anticancer treatment.

2.4 | Histopathology reporting

Cervical biopsy specimens were processed at a local laboratory, and the histopathologic findings were reported according to the CIN terminology.15

2.5 | Statistical analysis

Data were entered using Access 2000 software and analyzed using STATA software, version 17.0 (Stata-Corp, College Station, TX). The final diagnosis was based on either normal colposcopy or histopathology for those undergoing cervical biopsies, endocervical curettage or LLETZ. The distribution of the outcomes derived from the different endpoints was presented as proportions. For each participant, person-years of observation (PYO) were calculated from the date of baseline screening to the date of their first instance of the endpoint or the last date of follow-up for those who attained the endpoint during the follow-up. Relative risks (RRs) and 95% confidence intervals (CIs) obtained from the exact Poisson regression models were used to assess the effect of the sociodemographic, sexual, reproductive, medical and HIV infection-related characteristics on the CIN incidence. Adjusted regression analysis was carried out only on variables significant in the univariate regression analyses and 5% level of significance was used to infer statistical significance using mid p values from the regression models. Two endpoints (disease endpoint and HPV endpoint) were evaluated in this analysis. The disease endpoint was graded as CIN1, CIN 2, CIN 3 and invasive cancer. For each participant, the final disease endpoint during follow-up was defined as the highest disease grade diagnosed during the follow-up visits. Any CIN diagnosed during follow-up among women normal at baseline was further termed as incident CIN. Among women diagnosed with and treated for CIN at baseline, the disease endpoint was further categorized into no evidence of disease (for those regressed completely); persistent CIN for those diagnosed with the same or a lower grade CIN; and progressed CIN if a higher grade CIN was diagnosed at follow-up.

The HPV endpoint was graded as negative or positive. Women HPV negative at baseline but HPV positive during the follow-up were deemed to have an incident HPV infection. Those with a baseline positive and follow-up negative HPV result were considered to have cleared HPV infection. Those positive at both baseline and follow-up were referred to as having persistent HPV infection.

3 | RESULTS

Between 2010 and 2011, 1153 WHIV were enrolled and the time of follow-up since baseline diagnosis (in years) is presented in Table 1. The median duration of follow-up was 3.5 years (IQR 2.8-4.3). Figure 2 (flow-chart) describes the disease outcomes of women enrolled in the study. Out of the 1153 women enrolled, 12 women were excluded from the analysis since they had abnormal colposcopy but histopathology report was not available. Thus, there were 1141 women with final confirmed diagnosis at baseline. Of them, 1040 (91.1%) did not have any colposcopically and or histopathologically detected CIN or cancer at baseline. At least one follow-up was performed on 869 women and of them 814 (93.7%) did not have any disease at the last follow-up visit. Total 54 cases of incident CIN (35 CIN 1, 12 CIN 2 and 7 CIN 3) and one case of invasive cancer were detected during follow-up of these women.

Table 2 provides the follow-up of women with no evidence of CIN or cervical cancer at baseline with their disease outcomes at the last follow-up visits. The left panel of Table 2 provides the follow-up of women who had a repeat HC2 test (along with colposcopy) at follow-up. Among the 577 women who were HPV negative at baseline, 501 (86.8%, 95% CI 83.7-89.5) remained HPV negative throughout. Among the 499 cases who remained HPV negative throughout and who had an assessment for CIN by colposcopy and directed biopsy when indicated (CIN endpoint), only 14 (2.8%) cases of incident CIN 1 and no CIN 2 or worse lesions (CIN 2+) were detected. Of the 76 (13.2%, 95% CI 10.5-16.2) cases with incident HPV infection, six (7.9%) had CIN 1 and none developed any high-grade disease during the follow-up.

Among the 149 women who were HPV positive at baseline and had no CIN, 85 (57.0%) cleared HPV infection and 64 (43.0%) had persistent HPV infection during the repeat HPV test at follow-up. Among the 85 women who cleared HPV infection, incident CIN 1 was detected during follow-up in 5 (5.9%) women and CIN 2/3 disease in 3 (3.5%) women.

| Baseline final diagnosis | Baseline HPV result | No  | Mean (SD; Range) | Median (IQR) |
|--------------------------|--------------------|-----|-----------------|--------------|
| Normal                   | Negative           | 691 | 3.6 (1.5; 0.5-9.1) | 3.5 (2.9-4.3) |
| Normal                   | Positive           | 178 | 3.4 (1.6; 0.4-8.8) | 3.4 (2.7-4.3) |
| CIN 1                    | Negative           | 18  | 3.8 (1.8; 0.6-8.2) | 3.5 (2.9-4.8) |
| CIN 1                    | Positive           | 20  | 3.3 (1.4; 0.5-5.7) | 3.4 (2.8-4.2) |
| CIN 2/3                  | Negative           | 2   | 4.3 (0.0-4.3-4.4) | 4.3 (4.3-4.4) |
| CIN 2/3                  | Positive           | 37  | 3.2 (1.5; 0.6-8.3) | 3.1 (2.6-3.7) |
| Overall                  |                    | 946 | 3.5 (1.5; 0.4-9.1) | 3.5 (2.8-4.3) |

**TABLE 1** Baseline final diagnosis, HPV test outcome and time of follow-up since baseline final diagnosis (in years)
The women with persistent HPV infection (n = 64) had much higher number of CIN cases detected at follow-up with 9 (14.3%) cases of CIN 1 and CIN 2 each; and 4 (6.3%) cases of CIN3. Among the nine cases with persistent HPV infection who developed incident CIN 1 lesions, HPV was not detected among two of them by HPV genotyping, two were HPV 16/18 positive and five had other high-risk HPV infection (other than 16/18) at baseline. High-grade CIN lesions were detected in 13 (20.6%) cases who had persistent HPV infection. No invasive cancer was detected during follow-up in the HPV positive women without any CIN at baseline.

The Table 2 (right panel) provides the follow-up outcomes of women with no evidence of disease at baseline and who had colposcopy but no HPV test at follow-up. Of the 238 women who were HPV negative at baseline, 116 (48.7%) had assessment for CIN endpoint and none developed any CIN or cancer. Among 76 women who were HPV positive at baseline, 30 (39.5%) had an assessment for CIN endpoint with colposcopy and histopathology. Total five cases (16.7%) of incident CIN or cancer were detected with 1 (3.3%) case each of CIN 1 and CIN 2, 2 (6.7%) cases of CIN 3 and 1 (3.3%) case of invasive cancer. The total number of CIN

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**TABLE 2** Follow-up of women with no evidence of CIN or cervical cancer at baseline

| Follow-up with colposcopy and HPV test | HPV negative at baseline n = 577 | HPV positive at baseline n = 149 | Follow-up with colposcopy only |
|----------------------------------------|---------------------------------|---------------------------------|--------------------------------|
| Participants diagnosed with no evidence of disease at baseline | 501 (97.2) | 76 (92.1) | 85 (90.6) | 64 (65.1) | 238 (100.0) |
| Participants with assessment of CIN endpoint at follow-up | 499 (99.6) | 76 (100.0) | 85 (100.0) | 63 (98.4) | 116 (48.7) |
| Final diagnosis at last follow-up | | | | | |
| Normal | 485 (97.2) | 70 (92.1) | 77 (90.6) | 41 (65.1) | 116 (100.0) |
| Incident CIN/cancer | 14 (2.8) | 6 (7.9) | 8 (9.4) | 22 (34.9) | 0 (0.0) |
| CIN 1 | 14 (2.8) | 6 (7.9) | 5 (5.9) | 9 (14.3) | 0 (0.0) |
| CIN 2 | 0 (0.0) | 0 (0.0) | 2 (2.4) | 9 (14.3) | 0 (0.0) |
| CIN 3 | 0 (0.0) | 0 (0.0) | 1 (1.2) | 4 (6.3) | 0 (0.0) |
| CIN 2/3 | 0 (0.0) | 0 (0.0) | 3 (3.5) | 13 (20.6) | 0 (0.0) |
| Invasive cancer | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |

Abbreviations: CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus.

*HPV negative at baseline and during follow-up.

*HPV negative at baseline and HPV positive during follow-up.

*HPV positive at baseline and HPV negative during follow-up.

*HPV positive both at baseline and during follow-up.
2/3 cases detected among those who were positive for HPV at baseline were 3/30 (10%).

Table 3 provides the risk factors (particularly related to HIV) for incident CIN 2 or worse disease among women with no evidence of disease at baseline. The incidence of CIN 2 or worse disease was 7.0 per 1000 person-years of observation (PYO). In the crude multinomial logistic regression analysis, women who cleared HPV infection had 23.46 times increased risk of CIN 2 or worse disease (95% CI 2.50-617.78) and women with persistent HPV infection had 146.32 times increased risk of CIN 2 or worse disease (95% CI 25.65-3139.25) compared with the HPV negative women at baseline. Similarly, women with HPV 16 and or HPV 18 infection at baseline had 15.22 times increased risk of CIN 2 or worse disease (95% CI 5.00-55.26) and women who had other high-risk HPV infection (other

**TABLE 3** HIV related risk factors for incident CIN 2 or worse disease in a cohort of women living with HIV

| Baseline characteristics | No. with follow-up | Person-years of observation (PYO) | Incident CIN 2 or worse | CIN 2 + incidence rate per 1000 PYO | Crude relative risk (95% CI) | Adjusted relative risk (95% CI) |
|--------------------------|--------------------|-----------------------------------|------------------------|-------------------------------------|-----------------------------|-------------------------------|
| Women assessed           | 834a               | 2990                              | 21                     | 7.0                                 |                             |                               |
| Age at recruitment (in years) |                |                                   |                        |                                     |                             |                               |
| 21-29                    | 134                | 478                               | 4                      | 8.4                                 | 1.00                        |                               |
| 30-39                    | 519                | 1896                              | 14                     | 7.4                                 | 0.88 (0.30-3.11)            |                               |
| 40-49                    | 148                | 518                               | 3                      | 5.8                                 | 0.69 (0.13-3.35)           |                               |
| 50-62                    | 33                 | 98                                | 0                      | 0.0                                 | 0.92 (0.00-5.42)           |                               |
| Time since diagnosis of HIV infection (in years) | |                                   |                        |                                     |                             |                               |
| 1-5                      | 412                | 1438                              | 12                     | 8.3                                 | 1.00                        |                               |
| 6+                       | 419                | 1543                              | 9                      | 5.8                                 | 0.70 (0.28-1.68)           |                               |
| Baseline absolute CD4 count (cells/mm³) |             |                                   |                        |                                     |                             |                               |
| <200                     | 68                 | 233                               | 2                      | 8.6                                 | 1.00                        |                               |
| 200-499                  | 313                | 1069                              | 9                      | 8.4                                 | 0.98 (0.23-6.65)           |                               |
| 500+                     | 432                | 1616                              | 10                     | 6.2                                 | 0.72 (0.18-4.83)           |                               |
| Baseline ART status      |                    |                                   |                        |                                     |                             |                               |
| Not on ART               | 187                | 650                               | 6                      | 9.2                                 | 1.00                        |                               |
| On ART                   | 637                | 2311                              | 15                     | 6.5                                 | 0.70 (0.28-1.97)           |                               |
| Duration on ART (in years) |                  |                                   |                        |                                     |                             |                               |
| <2                       | 214                | 744                               | 4                      | 5.4                                 | 0.58 (0.14-2.13)           |                               |
| 2+                       | 403                | 1493                              | 9                      | 6.0                                 | 0.65 (0.23-1.97)           |                               |
| Absolute CD4 count at start of ART (cells/mm³) |             |                                   |                        |                                     |                             |                               |
| <200                     | 347                | 1237                              | 10                     | 8.1                                 | 1.00                        |                               |
| 200+                     | 211                | 782                               | 2                      | 2.6                                 | 0.32 (0.05-1.30)           |                               |
| WHO HIV clinical stage   |                    |                                   |                        |                                     |                             |                               |
| I                        | 532                | 1893                              | 11                     | 5.8                                 | 1.00                        |                               |
| II                       | 189                | 666                               | 7                      | 10.5                                | 1.81 (0.66-4.69)           |                               |
| III-IV                   | 112                | 428                               | 3                      | 7.0                                 | 1.21 (0.27-4.07)           |                               |
| HPV status               |                    |                                   |                        |                                     |                             |                               |
| Negative                 | 671                | 2422                              | 1                      | 0.4                                 | 1.00                        | 1.00                          |
| Cleared                  | 80                 | 310                               | 3                      | 9.7                                 | 23.46 (2.50-617.78)        | 23.95 (2.40-661.07)           |
| Persistent               | 54                 | 215                               | 13                     | 60.4                                | 146.32 (25.65-3139.25)     | 138.18 (20.30-3300.22)        |
| HPV genotyping results   |                    |                                   |                        |                                     |                             |                               |
| No oncogenic types       | 523                | 1926                              | 4                      | 2.1                                 | 1.00                        | 1.00                          |
| HPV 16 and/or 18         | 101                | 348                               | 11                     | 31.6                                | 15.22 (5.00-55.26)         | 1.59 (0.43-6.69)              |
| Other oncogenic types    | 178                | 620                               | 6                      | 9.7                                 | 4.66 (1.28-18.74)          | 0.80 (0.21-3.43)              |

Note: Only variables significant in the univariate analysis were included in the multivariate regression analysis.
Abbreviations: ART, antiretroviral therapy; CD4, cluster of differentiation 4; CI, confidence interval; CIN, cervical intraepithelial neoplasia; HIV, human immunodeficiency virus; HPV, human papillomavirus; WHO, World Health Organization.

*aNumber of women detected with CIN 1 have not been considered for this table therefore the number of women assessed is 834.*
than HPV 16 and or 18) had 4.66 times increased risk of high-grade disease (95% CI 1.28-18.74) compared with the HPV negative at baseline. In the crude multinomial logistic regression analysis, the demographic, personal history, sexual, reproductive history and HIV-related factors, duration of ART, etc. did not increase or decrease the risk of incident CIN 2 or worse disease [Data not shown].

Factors found significant in the crude multinomial logistic regression analysis were used in the multivariate multinomial logistic regression analysis. Compared with the women who were HPV negative at baseline, significantly higher risk of CIN 2+ disease was observed in women who cleared HPV infection (RR 23.95, 95% CI 2.40-661.07) and in those with persistent HPV infection (RR 138.18, 95% CI 20.30-3300.22).

The incidence of any CIN in the study cohort was 16.7 per 1000 PYO. When we analyzed the risk factors for any CIN, women who were on ART for more than 2 years at the time of recruitment had 55% lower risk in the univariate and multivariate analysis (adjusted relative risk 0.45, 95% CI 0.22-0.89) [Data not shown].

The follow-up outcomes of women diagnosed with any CIN according to their HPV status at baseline are presented in Table 4. Of the 101 participants detected with any HPV, 75 were HPV positive and 26 were HPV negative at baseline. Of the 20 HPV positive participants with CIN 1 treated with thermal ablation, 17 completed follow-up visits. Of them, 12 (70.6%) had no evidence of disease, 3 (17.6%) had persistent CIN 1 and 2 (11.8%) progressed to CIN 2. All the 17 participants treated with thermal ablation for CIN 1, had a repeat HPV test at follow-up and only 3 (17.6%) had persistent HPV infection while 14 (82.4%) cleared HPV infection.

Among 52 HPV positive participants who had CIN 2/3 at baseline, 51 (98.1%) received treatment; 41 (80.4%) were treated with thermal ablation, 7 (13.7%) by LLETZ and 3 (5.9%) underwent hysterectomy. Of the 41 participants treated with thermal ablation, 32 (78.0%) attended follow-up and 21 (65.6%) had no evidence of disease. CIN 1, CIN 2 and CIN 3 were detected at follow-up in 2 (6.3%), 4 (12.5%) and 5 (15.6%) cases, respectively. Of the 32 women treated with thermal ablation for CIN 2/3, 29 had their follow-up HPV test

### Table 4 Follow-up outcomes among women with cervical intraepithelial neoplasia (CIN) diagnosis at baseline

| Baseline diagnosis | HPV positive at baseline (n = 75) | HPV negative at baseline (n = 26) |
|--------------------|----------------------------------|----------------------------------|
|                    | CIN 1 n (%)                      | CIN 2/3 n (%)                    | CIN 1 n (%)                  | CIN 2/3 n (%)                  |
| Participants recruited | 23 (13.0)                        | 52 (87.0)                        | 23 (8.7)                     | 0 (0.0)                       |
| Treatment received at baseline | 20 (87.0)                        | 51 (98.1)                        | 21 (91.3)                    | 3 (100.0)                     |
| Thermal ablation | 20 (100.0)                       | 41 (80.4)                        | 20 (95.2)                    | 3 (100.0)                     |
| LLETZ | 0 (0.0)                          | 7 (13.7)                         | 1 (4.8)                      | 0 (0.0)                       |
| Hysterectomy | 0 (0.0)                          | 3 (5.9)                          | 0 (0.0)                      | 0 (0.0)                       |

Follow-up diagnosis among participants treated with thermal ablation at baseline

| Final diagnosis category | HPV positive at baseline (n = 75) | HPV negative at baseline (n = 26) |
|--------------------------|----------------------------------|----------------------------------|
| No evidence of disease | 12 (70.6)                        | 21 (65.6)                        | 12 (80.0)                    | 2 (66.7)                      |
| Persistent CIN | 3 (17.6)                          | 11 (34.4)                        | 3 (20.0)                     | 0 (0.0)                       |
| Progressed CIN | 2 (11.8)                          | 0 (0.0)                          | 0 (0.0)                      | 1 (33.3)                      |
| Final diagnosis | 12 (70.6)                        | 21 (65.6)                        | 12 (80.0)                    | 2 (66.7)                      |
| CIN 1 | 3 (17.6)                          | 2 (6.3)                          | 3 (20.0)                     | 0 (0.0)                       |
| CIN 2 | 2 (11.8)                          | 4 (12.5)                          | 0 (0.0)                      | 0 (0.0)                       |
| CIN 3 | 0 (0.0)                          | 5 (15.6)                          | 0 (0.0)                      | 1 (33.3)                      |

Follow-up HPV test among participants treated with thermal ablation at baseline

| No. with HPV test at follow-up | 17 | 29 | 12 | 3 |
|-------------------------------|----|----|----|---|
| HPV negative | 9 (75.0) | 2 (66.7) | 3 (25.0) | 1 (33.3) |
| Incident HPV | | | | |
| Cleared HPV | 14 (82.4) | 16 (55.2) | | |
| Persistent HPV | 3 (17.6) | 13 (44.8) | | |

Abbreviations: CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; LLETZ, lower loop excision of transformation zone.

*Median time between thermal ablation treatment and follow-up HPV test: 3.21 years (IQR 2.74-4.31).
and of them 13 (44.8%) had persistent HPV infection. The median time between treatment and follow-up HPV test among those treated by thermal ablation was 3.21 years (IQR 2.74-4.31).

Among the 23 HPV negative participants with CIN 1 at baseline, 20 (95.2%) were treated with thermal ablation and 1 (4.8%) was treated with LLETZ. Of the 20 participants receiving thermal ablation, 15 (75%) were followed for disease endpoint. Except 3 (20%) women having persistent CIN 1, the rest were cured of the disease. All the HPV negative participants with CIN 2/3 at baseline (N = 3) were treated with thermal ablation and a single case of CIN 3 was detected among them at follow-up and she had incident HPV infection.

Of the total eight participants treated with LLETZ at baseline, seven were HPV positive and had CIN 2/3 disease. Of these eight participants, six were followed up and two of them (both HPV positive at baseline) were detected to have invasive cancer at follow-up [Data not shown].

4 | DISCUSSION

Significant improvement in access to highly active antiretroviral treatment and systematic HIV disease monitoring has resulted in significant gains in the life expectancy of people living with HIV in the last 15 years. But most WHIV in the LMICs lack access to cervical cancer screening, which can be lifesaving. The WHO’s recently published guidelines for screening and management of cervical pre-cancer have a major focus on WHIV, but many of these guidelines for WHIV are based on low-certainty evidence due to the lack of longitudinal data. Our study involving a large cohort of WHIV followed systematically over several years in India has addressed some of these knowledge gaps.

Our analysis shows that the incidence of CIN 2 or worse disease in women who were free of disease at baseline is 7 per 1000 PYO. Although the number of person-years of observations has increased after we published the incidence of CIN 2 or worse disease earlier, there has not been any change in the incidence with additional follow-up. Women with persistent HPV infection had 138.18 times increased risk of CIN 2+ lesions than those who were HPV negative at baseline. In our cohort, about 20% of the women with persistent HPV infection had incident CIN 2 or worse disease at follow-up. We have previously reported that women who were HPV 16/18 positive at baseline had almost nine times increased risk of CIN 2+ disease (95% CI 2.71-34.25) compared with 3.33 (95% CI 0.83-14.18) times risk in women positive for other high-risk types. HPV positive women without any disease at baseline who cleared HPV infection were also at a considerable risk of incident CIN 2 or worse lesions (RR 23.95) compared with those who were HPV negative at baseline. This can be explained by HPV latency and individual-level natural history of HPV infection over the course of a lifetime of a woman.

Among HPV positive women without any disease at baseline, HPV clearance was seen in 57.0% women in our cohort with the median follow-up of 3.4 years. Previous metaanalysis among HIV-infected men as well as women has suggested that both HIV and HPV have a synergistic relationship and clearance rate of high-risk HPV is approximately halved among individuals with HIV compared with those without HIV (pooled RR adjusted for possible confounders = 0.59, 95% CI: 0.33 to 1.05). Early initiation of ART and sustained adherence can reduce the incidence of CIN and progression to cervical cancer provided screening and treatment of precancer is integrated into the program. In our study, although ART for more than 2 years had a protective effect on incidence of any CIN, we did not observe the same protective effect on incident CIN 2 or worse disease and it is possibly because of small number of observations. In addition, when we initiated the study in 2010, about 24% of women were not on ART and the ART guidelines have changed many times since we enrolled the cohort. We initiated our study in 2010 while the WHO’s “test and treat” strategy for HIV-infected individuals was introduced in 2016.

The high negative predictive value of the HPV test provides reassurance while allowing the screening interval to be increased among women in the general population, even beyond 5 years. The current WHO guidelines mention repeat screening of HPV negative WHIV after 3 to 5 years. In our study, almost 87% women remained HPV negative on at least two consecutive HPV tests with the median interval of 3.5 years. In addition, 13% of those who tested HPV negative at baseline and positive during the second round of screening after 3 years (incident HPV), none developed any high-grade cervical disease or cancer in the intervening period. Therefore, our study supports the WHO recommendation to extend the screening interval beyond 3 years for the HPV negative women.

Several studies have shown that despite treatment of CIN, WHIV have an increased risk of failure compared with non-HIV infected women. Our cohort of treated women has been very rigorously evaluated with HPV detection test combined with colposcopy at follow-up and the follow up duration (median IQR 3.1, 95% CI 2.6-3.7) was longer than most of the studies evaluating treatment of CIN 2/3. Among the HPV positive women at baseline treated for CIN 1 with thermal ablation, 11.8% women in our cohort progressed to a higher grade abnormality. Complete regression of disease on colposcopy and histopathology was observed in 65.6% of the HPV positive WHIV who had high-grade CIN and were treated with thermal ablation. Randomized studies have demonstrated significantly better cure rate of excisional treatment of CIN 2-3 disease compared with ablation in WLHIV. Unfortunately, the access to high quality excisional treatment in the LMICs having high burden of HIV and cervical cancer is quite limited. In the high-income countries, ART with adequate control of HIV (HIV PVL <50 copies/ml) was shown to reduce the subsequent risk of CIN 2+ disease but the same was not seen in low- and middle-income countries.

The strongest risk factor for CIN 2 or worse disease was persistent HPV infection. Women who cleared HPV infection were also at a substantial risk of developing CIN 2+ lesions. Posttreatment HPV clearance among the women was low (44.8%) in our study and was very similar to that observed in a Zambian study. Persistence of HPV infection following treatment which increases the risk of subsequent CIN is a concern among WHIV.
Our study has a few limitations. When the study was initiated in 2010, HIV viral load testing was not available at the National AIDS Control Organization’s ART centers in India and was unaffordable to the majority of the WHIV outside the public health settings. Therefore, majority of the participants were monitored immunologically with CD4 counts as per the national guidelines at the ART centers prevailing time to time. The long-term follow-up was particularly affected by the first wave of Covid-19 pandemic and extended lockdown in India. Even then, the outcomes of our study are highly reliable. Based on rigorous evaluation of the cohort with multiple tests at baseline as well as subsequent follow-up visits, our study provides the updated estimates of the incidence of CIN among WHIV and outcomes after treatment of CIN. The observation that the HPV negative women have very low risk of CIN 3 or invasive cancer within a follow-up period exceeding 5 years provides us confidence to increase the HPV screening interval safely to 3 years or more pragmatically to 5 years. The cost of the HPV test is a major factor driving the cost-effectiveness analysis of different screening tests for cervical cancer in the low- and middle-income countries. Increasing the screening interval when screened with an HPV test among WHIV will have a huge impact on the cost-effectiveness and scaling-up of the cervical cancer prevention.

AUTHOR CONTRIBUTIONS
Smita Joshi: Participated in the conception of the study and its design, conduct of the study, monitoring, supervision, acquisition and interpretation of the data and the provision of clinical services in the study. Richard Muwonge: was responsible for the statistical analysis of the data, monitoring the study and the interpretation of the data. Vinay Kulkarni: participated in the conception of the study, conduct of the study and the acquisition and interpretation of the data. Mahesh Mandolkar: was responsible for histopathology reporting, analysis and interpretation of the data. Eric Lucas: monitoring the study, and the interpretation of the data. Sanjay Pujari: acquisition and interpretation of the data. Rengaswamy Sankaranarayanan: had the initial idea and was responsible for the conception, study design and the conduct, monitoring and supervision of the study, acquisition, analysis and interpretation of the data. Partha Basu: acquisition, analysis and interpretation of the data. The work reported in the paper has been performed by the authors, unless clearly specified in the text.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT
The study was approved by the scientific review and ethics committees of Hirabai Cowasji Jehangir Medical Research Institute (HCJMRI), Prayas, Pune, India and the International Agency for Research on Cancer (IARC), Lyon, France. All participants provided a written informed consent before study enrolment and then for the follow-up study.

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