

Three-year persistence and clearance patterns of high-risk HPV genotype groups among women in Chirchik, Uzbekistan: A secondary analysis of a pilot screening study

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Research Article

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Abstract

Background

High-risk human papillomavirus (hrHPV) persistence is central to cervical carcinogenesis, yet longitudinal data from Central Asia remain limited. This secondary analysis assessed the distribution of hrHPV genotype groups and 3-year follow-up patterns among women in Chirchik, Uzbekistan.

Methods

From August 2021 to March 2022, 5,678 women were screened for hrHPV, of whom 547 (9.6%) tested positive. HPV genotyping was performed using the Xpert HPV assay. Single infections were defined as the detection of one genotype group, while multiple infections were defined as the detection of two or more groups. Clearance was defined as the loss of HPV16 (P1) at follow-up, regardless of the status of other genotypes.

Results

Single infections predominated at baseline (87.0%, 476/547), while double, triple, and quadruple infections accounted for 11.2%, 1.6%, and 0.2% of cases, respectively. The most frequently detected genotype groups were HPV16 (29.8%), HPV31/33/35/52/58 (19.9%), and HPV39/56/66/68 (16.5%). At 3-year follow-up, 409 baseline hrHPV-positive women were re-evaluated, and 108 remained hrHPV-positive. Single infections remained predominant among persistent hrHPV-positive cases (94.4%, 102/108). HPV16 was the most frequently detected genotype group at follow-up (38.0%), followed by HPV39/56/66/68 (24.1%) and HPV31/33/35/52/58 (19.4%). Newly detected genotype groups were observed in 36 women, with HPV39/56/66/68 being the most common (52.8%). Among women with HPV16 at baseline, clearance was observed in 66.9% (83/124) of those with single HPV16 infection, 77.8% of those with HPV16 + HPV31/33/35/52/58 (7/9) co-infection, and 83.3% of HPV16 + HPV39/56/66/68 (5/6) co-infection. A Fisher's exact test showed no statistically significant difference in clearance proportions between the three groups ($p = 0.740$).

Conclusion

This secondary analysis provides preliminary evidence on the distribution of hrHPV genotype groups and 3-year follow-up patterns in Uzbekistan. Larger studies with repeated follow-up testing and genotype-level analysis are needed to confirm these findings.

Background

Cervical cancer, primarily caused by persistent infection with high-risk human papillomavirus types, remains an urgent global health issue. As the fourth most common cancer, cervical cancer accounts for approximately 660,000 new cases and 350,000 deaths annually, with the majority occurring in low- and middle-income countries [1]. Limited resources affect the adequate distribution of the HPV vaccine, availability and quality of screening programs and treatment services.

HPV is a sexually transmitted non-enveloped DNA virus which includes more than 200 genotypes, with 40 of them having the ability to infect the genital tract. Among the hrHPV genotypes, including HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82, HPV16 and HPV18 have been identified as the most persistent, as they predominate in cervical cancer tumours. HPV16 was found to be the cause of more than half (50–60%) of cervical cancer cases, while HPV18 caused around 10–15% of cases. HPV16 is more frequently associated with high-grade precursor lesions, contributing to its distinct disease spectrum[2, 3].

In Uzbekistan, cervical cancer is a significant public health issue as it accounts for 11.9% of female cancers, making it the second most common among women in the country. hrHPV was detected in 8.5% of 44,497 women during the cervical cancer screening program conducted in Tashkent (8.8%), Andijan (9.2%), and Samarkand (6.5%) regions between 2021 and 2023 [4].

While recent large-scale studies have characterized the prevalence and genotype distribution of hrHPV in Uzbekistan, clearance rates of single versus multiple HPV infections have not been investigated in this population. Understanding whether multiple infections clear differently from single infections has important clinical implications: if co-infections clear faster, women would be managed with less intensive follow-up, reducing unnecessary procedures and therefore the health care costs. On the other hand, if co-infections are more persistent, decreasing the probability of clearance, the patients would require closer monitoring. Despite the existence of studies comparing the clearance of the same genotype in single and multiple infection, most of them assessed complete clearance, which includes clearance of all genotypes the patient is infected with. These studies do not allow isolated observation of the effect of different hrHPV genotypes on the clearance of specific hrHPV. This study aims to compare 3-year clearance proportions of HPV16 mono-infection and co-infection of HPV16 together with two groups of different hrHPV genotypes (HPV16 + HPV31/33/35/52/58 and HPV16 + HPV39/56/66/68).

Materials and Methods

Study design and setting

This study is a secondary analysis of data from a pilot study examining the value of HPV testing for primary cervical cancer screening in Uzbekistan. The original pilot study was conducted in response to Presidential Decree № 2866 (04.04.2017) "On measures to further develop and improve oncological assistance to the population of the Republic of Uzbekistan for 2017–2021" and was supported by

UNFPA, the Government of Japan, the French Embassy in Uzbekistan, and the World Health Organization (WHO) at the end of 2019, aligning with targets of the WHO global strategy for elimination of cervical cancer.

For this analysis, the data from a subset of the pilot population were used. The overall pilot study was conducted between 1 April 2021 and 1 March 2022 across 4 primary healthcare clinics (PHC) in Chirchik and 11 PHC clinics in the Republic of Karakalpakstan. Training for the Chirchik site was conducted in June 2021, and the recruitment in Chirchik began in August 2021 and continued until March 2022. A total of 5,678 women were recruited from the Chirchik clinics. Women were eligible if they were aged 30–55 or if they had two or more children, which allowed the inclusion of younger women aged 20–29. Recruitment was conducted using two strategies: clinic-based enrollment, where women attending routine visits were identified by PHC providers and invited to participate; and community-based enrollment, where patronage nurses and local neighbourhood leaders selected women based on their eligibility within the communities and invited them to participate [5].

Women were considered eligible for inclusion in the original pilot testing if they were residents of Uzbekistan, had no prior diagnosis or treatment for invasive cervical cancer, and were able to provide informed consent. Women were excluded if they were pregnant, had abnormal vaginal bleeding, had experienced a hysterectomy, had vaginal discharge indicative of a sexually transmitted infection (STI), or were in conditions preventing them from enrollment. From the Chirchik cohort, women who tested positive for high-risk HPV at baseline and returned for the 3-year follow-up were included in the present analysis. The original pilot study obtained written informed consent from all participants. For this secondary analysis, data were provided by the participating clinics following written authorisation [5]. Baseline and follow-up records were linked using the Cepheid ID. No patient identifiers were included in the analysis dataset.

Sample collection, HPV detection, and genotyping

Cervical specimens were collected by trained gynaecologists using an endocervical brush and suspended in PreservCyt solution (Hologic, Inc., Marlborough, MA, USA) for transport and storage. Cepheid Xpert HPV test (Cepheid, Sunnyvale, CA, USA) was used in a pilot. It is a fully automated cartridge-based hrHPV DNA assay, which contains a sample adequacy control and detects the E6/E7 region of the viral DNA of 14 hrHPV genotypes. The Xpert HPV Assay has a sensitivity of 98.73% for CIN 2 lesions or higher and 100% for CIN3 or higher. The Xpert HPV assay reports HPV16 and HPV18/45 individually and provides three separate pooled results for remaining high-risk genotypes: P3 (HPV31/33/35/52/58), P4 (HPV51/59), and P5 (HPV39/56/66/68). Individual genotypes within each pool cannot be distinguished [6, 7, 8, 9].

Data Collection and Statistical Analysis

- All statistical analyses performed for this study used jamovi (version 2.6.44) for Windows. Prevalence, genotype distribution, co-infections and clearance proportions were compared using Fisher's exact test with Monte Carlo simulations for categorical variables, as appropriate for small sample sizes.
- For analysis, HPV types were assigned letter codes: P1 represents HPV16, P2 represents HPV18/45, P3 represents HPV31/33/35/52/58, P4 represents HPV51/59, and P5 represents HPV39/56/66/68. Infections were categorized as single (only one of the five letter codes) or multiple (two or more codes, e.g., P1 + P3, P1 + P5).
- Clearance was defined as the loss of HPV16 (P1) at follow-up, regardless of the status of other genotype groups. Women who cleared P1 but remained positive for another genotype group were classified as cleared for P1.

Results

During phase 1 of the pilot study, conducted from August 2021 to March 2022, 5,678 women from Chirchik, Tashkent Region, Uzbekistan, were screened for high-risk human papillomavirus (hrHPV). Overall, 547 women tested positive, corresponding to an hrHPV positivity rate of 9.6%. Among hrHPV-positive women, single infections were predominant and accounted for 87.0% of cases (476/547). Double, triple, and quadruple infections were detected in 11.2% (61/547), 1.6% (9/547), and 0.2% (1/547) of cases, respectively. At baseline, the most frequently detected single genotype groups were P1 (29.8%, 163/547), P3 (19.9%, 109/547), P5 (16.5%, 90/547), P4 (12.4%, 68/547), and P2 (8.4%, 46/547). P1 was the leading genotype group among women aged 30–39 years and 40–49 years, accounting for 28.7% and 35.3% of infections, respectively. Among women aged 50–59 years, P1, P3, and P5 were detected at comparable frequencies. In the 20–29 age group, P3 was the most frequent genotype group (40.0%, 4/10). The most common double-infection combinations were P1 + P3 (2.7%, 15/547), P1 + P5 (2.6%, 14/547), and P3 + P5 (1.6%, 9/547). Triple and quadruple infections were uncommon, with each specific combination detected in 0.5% or fewer of hrHPV-positive women. The overall distribution of genotype combinations did not differ across age groups (Fisher's exact test, $p = 0.347$; Table 1).

Table 1
Age-specific distribution of high-risk HPV genotype groups among hrHPV-positive women at baseline, Chirchik, Uzbekistan, August 2021 to March 2022

HPV Genotype	Age				Total (n = 547)
	20–29	30–39	40–49	50–59	
	(n = 10)	(n = 303)	(n = 153)	(n = 81)	
Single Infection					
P1	1(10.0%)	87(28.7%)	54(35.3%)	21(25.9%)	163(29.8%)
P2	1(10.0%)	30(9.9%)	10(6.5%)	5(6.2%)	46(8.4%)
P3	4(40.0%)	57(18.8%)	28(18.3%)	20(24.7%)	109(19.9%)
P4	1(10.0%)	39(12.9%)	15(9.8%)	13(16.0%)	68(12.4%)
P5	0	42(13.9%)	28(18.3%)	20(24.7%)	90(16.5%)
Double Infection					
P1 + P2	0	2(0.7%)	0	0	2(0.4%)
P1 + P3	0	11(3.6%)	4(2.6%)	0	15(2.7%)
P1 + P4	0	4(1.3%)	2(1.3%)	0	6(1.1%)
P1 + P5	2(20.0%)	8(2.6%)	4(2.6%)	0	14(2.6%)
P2 + P3	0	1(0.3%)	2(1.3%)	0	3(0.5%)
P2 + P5	0	2(0.7%)	0	0	2(0.4%)
P3 + P4	0	3(1.0%)	1(0.7%)	0	4(0.7%)
P3 + P5	1(10.0%)	7(2.3%)	0	1(1.2%)	9(1.6%)
P4 + P5	0	2(0.7%)	3(2.0%)	1(1.2%)	6(1.1%)
Triple Infection					
P1 + P2+P4	0	1(0.3%)	0	0	1(0.2%)
P1 + P3+P5	0	2(0.7%)	1(0.7%)	0	3(0.5%)
P1 + P3+P4	0	2(0.7%)	0	0	2(0.4%)
P1 + P4+P5	0	1(0.3%)	1(0.7%)	0	2(0.4%)
P2 + P3+P5	0	1(0.3%)	0	0	1(0.2%)
Quadruple Infection					
P1 + P2+P3 + P4	0	1(0.3%)	0	0	1(0.2%)

During phase 2, conducted from October to December 2024, 409 of the 547 baseline hrHPV-positive women were followed up, yielding a follow-up rate of 74.8%. Among these participants, 108 remained hrHPV-positive and were included in the follow-up genotype distribution analysis. Single infections remained predominant, accounting for 94.4% of persistent hrHPV-positive cases (102/108), whereas double infections accounted for 5.6% (6/108). No triple or quadruple infections were detected at follow-up. At follow-up, P1 remained the most frequently detected genotype group (38.0%, 41/108), followed by P5 (24.1%, 26/108) and P3 (19.4%, 21/108). Among women aged 30 to 39 years, P1 was the leading genotype group (48.5%, 24/50), followed by P5 (22.0%, 11/50). Among women aged 40 to 49 years, P1 was most common (33.3%, 13/39), followed by P3 and P5 (both 25.6%, 10/39). In women aged 50 to 59 years, P1, P3, and P5 showed comparable frequencies. The 20 to 29 year age group included only two women, both of whom tested positive for P3. Double infections at follow-up were rare. Six double-infection combinations were observed, including P1 + P3, P1 + P5, P1 + P4, P2 + P3, P2 + P5, and P3 + P5. Each combination was detected in one case only (0.9%). No significant association was observed between age group and HPV genotype distribution at follow-up (Fisher’s exact test, $p = 0.358$; Table 2).

Table 2
Age-specific distribution of persistent high-risk HPV genotype groups among women with hrHPV positivity at 3-year follow-up, Chirchik, Uzbekistan, October to December 2024

HPV Genotype	Age				Total (n = 108)
	20–29	30–39	40–49	50–59	
	(n = 2)	(n = 50)	(n = 39)	(n = 17)	
Single Infection					
P1	0	24(48.5%)	13(33.3%)	4(23.5%)	41(38.0%)
P2	0	4(8.0%)	2(5.2%)	2(11.8%)	8(7.4%)
P3	2(100%)	5(10.0%)	10(25.6%)	4(23.5%)	21(19.4%)
P4	0	3(6.0%)	2(5.2%)	1(5.9%)	6(5.6%)
P5	0	11(22.0%)	10(25.6%)	5(29.4%)	26(24.1%)
Double Infection					
P1 + P3	0	1(2.0%)	0	0	1(0.9%)
P1 + P5	0	0	0	1(5.9%)	1(0.9%)
P1 + P4	0	1(2.0%)	0	0	1(0.9%)
P2 + P3	0	0	1(2.7%)	0	1(0.9%)
P2 + P5	0	0	1(2.7%)	0	1(0.9%)
P3 + P5	0	1(2.0%)	0	0	1(0.9%)

In addition to persistent hrHPV positivity, 36 women had newly detected hrHPV genotype groups at the 3-year follow-up that were not detected at baseline (Table 3). Among these cases, P5 was the most frequent genotype group (52.8%, 19/36), followed by P1 and P3, each detected in 16.7% of cases (6/36). P2 and P4 were detected in 8.3% (3/36) and 5.6% (2/36) of cases, respectively. P5 was also the most common newly detected genotype group among women aged 30 to 39 years (68.4%, 13/19) and 40 to 49 years (41.7%, 5/12). Among women aged 50 to 59 years, newly detected genotype groups included P1 (50.0%, 2/4), P4 (25.0%, 1/4), and P5 (25.0%, 1/4). The 20 to 29 year age group included only one newly detected genotype group, which was P3.

Table 3
Age-specific distribution of newly detected high-risk HPV genotype groups at 3-year follow-up among women in Chirchik, Uzbekistan

Newly detected genotype group	Age				Total (n = 36)
	20–29	30–39	40–49	50–59	
	(n = 1)	(n = 19)	(n = 12)	(n = 4)	
P1	0	2(10.5%)	2(16.7%)	2(50.0%)	6(16.7%)
P2	0	1(5.3%)	2(16.7%)	0	3(8.3%)
P3	1(100%)	3(15.8%)	2(16.7%)	0	6(16.7%)
P4	0	0	1(8.3%)	1(25.0%)	2(5.6%)
P5	0	13(68.4%)	5(41.7%)	1(25.0%)	19(52.8%)

HPV clearance was further assessed among women with single P1 infection and the two most common P1-containing co-infections, P1 + P3 and P1 + P5. Clearance was observed in 66.9% (83/124) of women with single P1 infection, compared with 77.8% (7/9) of women with P1 + P3 co-infection and 83.3% (5/6) of women with P1 + P5 co-infection. The difference in clearance proportions between the three groups was not statistically significant (Fisher's exact test, $p = 0.740$, Table 4).

Table 4
Clearance of HPV16 (P1) by baseline infection group

Group	Cleared (n)	Persisted (n)	Total (n)	Clearance (%)
P1	83	41	124	66.9
P1 + P3	7	2	9	77.8
P1 + P5	5	1	6	83.3
Total	95	44	139	68.3

Fisher's exact test, $p = 0.740$. The number of women with persistent P1 + P3 or P1 + P5 in Table 4 differs from the number in Table 2, because some women cleared P1 but remained positive for another

genotype group.

Discussion

This secondary analysis examined the age-dependent distribution of high-risk HPV (hrHPV) genotype groups, newly detected infections, and 3-year clearance patterns among women in Chirchik, Uzbekistan. At baseline, 9.6% of screened women were hrHPV-positive, with single infections predominating. P1, P3, and P5 were the most frequently detected genotype groups. At follow-up, 108 women remained hrHPV-positive, with P1 remaining the most common genotype group. In addition, 36 women had genotype groups detected at follow-up that were not present at baseline, among which P5 was the most frequent. There was no significant association between age group and genotype distribution ($p = 0.347$). Clearance proportions were relatively high across all analyzed groups, ranging from 66.9% in women with single P1 infection to 83.3% in women with P1 + P5 co-infection ($p = 0.740$).

The finding of no significant difference in clearance proportions between single and multiple infections is consistent with most prospective studies [10, 11]. One study that did report a difference defined co-infection as the detection of additional types at any point during follow-up. As Campos et al. (2011) noted, this approach can introduce bias: infections that persist longer have more opportunity to acquire new genotype groups, making persistent infections more likely to be labelled as co-infections. By defining co-infection at baseline, the current study avoids this bias, and the results support the view that having multiple types does not necessarily prolong infection duration [12]. Overall, no statistically significant difference in P1 clearance was observed between single P1 infection and the two P1-containing co-infection groups. The co-infection groups were very small (P1 + P3, $n = 9$; P1 + P5, $n = 6$), and the study was underpowered to detect small differences. Therefore, this non-significant result should not be interpreted as evidence that co-infection has no effect on HPV persistence.

Co-infection with multiple hrHPV types may theoretically reflect a weaker immune response, allowing individual infections to reach detectable viral loads and persist longer. However, immune status and other important confounders were not measured in this study, so this explanation remains speculative. The present study found no difference in clearance proportions between single and multiple infections, suggesting that in this population, co-infection did not prolong infection duration.

A large cross-sectional study conducted in Uzbekistan reported an 8.5% hrHPV prevalence, with mixed genotype infections comprising 52.8% of positive cases—substantially higher than the 11.2% double infections observed in our baseline cohort [4]. This discrepancy may be explained by differences in population selection, testing methods, and regional genotype circulation. The Uzbekistan study also found the highest HPV prevalence in women aged 20–29 years (13.0%); however, direct comparison with our age-stratified findings is limited by the small sample size in this age group ($n = 10$), making firm conclusions difficult.

The predominance of P5 among newly detected genotype groups is noteworthy but should be interpreted cautiously. Because only one baseline and one follow-up test were available, these detections

cannot be definitively classified as incident infections. They may reflect true acquisition, intermittent detection, low viral load at baseline, or reactivation of a previously undetected infection. Therefore, this finding should be considered hypothesis-generating rather than conclusive. The baseline hrHPV positivity rate was broadly comparable with recent data from Uzbekistan, although differences in study population, region, testing method, and genotype classification limit direct comparison. The lower proportion of mixed infections in the present cohort may partly reflect differences in assay design and HPV genotype grouping.

Limitations

This study has several limitations. The Xpert Cepheid system detects HPV16 and HPV18/45 individually and provides three separate pooled results for the remaining high-risk genotypes (P3, P4, P5). Therefore individual genotypes within each pool cannot be distinguished, limiting the precision of genotype-specific analyses. Several subgroup analyses had limited statistical power, particularly the co-infection groups (P1 + P3, $n = 9$; P1 + P5, $n = 6$) compared with single P1 infection ($n = 124$). Loss to follow-up (74.8%, 409/547) may have introduced selection bias. Clearance was assessed using only one follow-up time point, which may not capture intermittent clearance and reinfection. Because women were tested only at baseline and at follow-up, the exact timing of clearance is unknown, producing interval-censored data that cannot be analysed using standard survival methods. The potential confounders, such as smoking, oral contraceptive use, HPV vaccination history, cervical cytology, immune status, and sexual behaviour, were not available for adjusted analysis. Finally, the small sample size in certain age groups (e.g., 20–29 years, $n = 10$) weakens the ability to detect small differences between groups.

Conclusion

In conclusion, this secondary analysis of a pilot screening study provides preliminary data on hrHPV genotype group distribution and 3-year clearance patterns among women in Chirchik, Uzbekistan. Single infections predominated at both baseline and follow-up, and P1 was the most frequently detected genotype group. Clearance proportions were high across all groups, and no statistically significant difference was observed between single P1 infection and P1-containing co-infections ($p = 0.740$). The predominance of P5 among newly detected genotype groups is hypothesis-generating and warrants further investigation. Larger longitudinal studies with repeated testing, individual genotype-level analysis, and adjustment for confounders are needed to confirm these findings and better understand the clinical significance of hrHPV co-infections.

Abbreviations

HPV
Human Papillomavirus
hrHPV
High-risk HPV

CIN

Cervical Intraepithelial Neoplasia

CIN 2

Cervical Intraepithelial Neoplasia grade 2

CIN 3

Cervical Intraepithelial Neoplasia grade 3

DNA

Deoxyribonucleic acid

PCR

Polymerase Chain Reaction

WHO

World Health Organization

UNFPA

United Nations Population Fund

Declarations

Ethics approval and consent to participate

The original HPV screening pilot study was conducted under the authority of the Ministry of Health of the Republic of Uzbekistan, as established by Presidential Decree № 2866 (04.04.2017) and the subsequent directives of 11 June 2021 (Ref: 11-4-02/576) and 30 November 2024 (Ref: 05–11/28007). The study was conducted in accordance with Article 34 of the Law of Republic of Uzbekistan ‘On protection of Citizens’ Health’ (No. 265-I, 29 August 1996), which permits biomedical research involving human participants in state healthcare institutions upon obtaining written informed consent. As a national public health program, the pilot was exempt from separate ethics committee review according to national regulations. All participants provided written informed consent at enrollment, including consent for follow-up testing. This secondary analysis used de-identified data from the original pilot study. As the analysis used existing data and involved no direct contact with human participants, it was exempt from separate ethics committee review according to national regulations. For this secondary analysis, data were provided by Family Polyclinic No2 in Chirchik following written authorization. No patient identifiers were included in the analysis dataset. All procedures were conducted in accordance with the Declaration of Helsinki.

Consent for publication

Not applicable.

Competing interests

The author declares that she has no competing interests.

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Author Contribution

D.A. carried out the study, performed the data analysis, interpreted the results, drafted and revised the manuscript. The author read and approved the final manuscript.

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Data Availability

The dataset analysed during this study is not publicly available due to the original data-sharing agreements with the screening program organisers. However, aggregated data are presented in the tables and figures of this manuscript. Requests for further information may be directed to the corresponding author.

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