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Original Research Article

Evaluation of HPV Genotyping as a Triage Tool in Women with Atypical Squamous Cells of Undetermined Significance (ASC-US)

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Abstract: ***Background***: Atypical squamous cells of undetermined significance (ASC-US) remain the most common borderline abnormality in cervical cytology, presenting a clinical challenge for risk stratification. High-risk human papillomavirus (HPV) genotyping offers a potential triage solution, particularly for identifying women at greatest risk of underlying high-grade cervical lesions. This study aimed to evaluate the performance of HPV genotyping as a triage tool in women with ASC-US cytology. ***Methods***: Our study conducted a prospective observational study from January 2024 to January 2025, enrolling 72 women with ASC-US. All participants underwent high-risk HPV genotyping using the Cobas 4800 assay, with classification into HPV16, HPV18, other high-risk (HR) HPV, or HPV-negative groups. Colposcopy and histopathology were performed in HPV-positive cases to confirm cervical intraepithelial neoplasia (CIN). Diagnostic accuracy measures (sensitivity, specificity, PPV, NPV) for detecting CIN2+ were calculated. ***Results***: HPV positivity was found in 52.8% of women, with HPV16 in 16.7% and HPV18 in 11.1%. CIN2+ was present in 83.3% of HPV16-positive, 75.0% of HPV18-positive, and 44.4% of other HR-HPV-positive women, but only 5.9% of HPV-negative women ($p<0.001$). HPV genotyping showed a sensitivity of 91%, specificity of 68%, positive predictive value of 58%, and negative predictive value of 94% for CIN2+ detection. ***Conclusion***: HPV genotyping is a reliable triage strategy for ASC-US cytology, with HPV16/18 positivity strongly predicting high-grade lesions. Reflex testing safely reduces unnecessary colposcopies while effectively identifying women who need immediate evaluation.

Keywords: HPV genotyping; ASC-US; cervical cytology; CIN2+; HPV16; cervical cancer screening; triage strategy; high-risk HPV.

INTRODUCTION

Cervical cancer remains one of the leading causes of cancer-related morbidity and mortality in women worldwide, particularly in low- and middle-income countries. Although the number of new cases and fatalities caused by this disease has decreased due to the advancement of screening and vaccination, the World Health Organization (WHO) estimates that in 2020 alone, there were more than 600,000 new cases and 340,000 deaths worldwide. Cervical cytology has been a legacy of early detection, and the Bethesda classification is very popular in reporting

abnormalities of the epithelium. Of these, atypical squamous cells of undetermined significance (ASC-US) is the most widely reported borderline lesion, having been reported in the range of 3-5 percent of all Pap test findings. In spite of the fact that ASC-US is usually benign, it may also be the early signs of high-grade cervical intraepithelial neoplasia (CIN), especially in case of persistent high-risk human papillomavirus (HPV) infection(1-3).

The problem of ASC-US management has been a clinical dilemma since it is highly non-specific and presents an unpredictable natural progression. Various guidelines including those 'by the American Society Colposcopy and Cervical Pathology (ASCCP) and WHO have recommended use of reflex high-risk HPV testing as triage method of choice in ASC-US'. Women who test positive with high-risk HPV should proceed to colposcopy immediately whereas HPV-negative women can resume normal surveillance. Notably, the most recent developments have made it possible to partially genotype HPV and differentiate between HPV16 and HPV18 the two most carcinogenic types of HPV and the rest of the high-risk strains. This enables further risk stratification: HPV16/18 positivity is a risk factor that greatly increases the likelihood of CIN2+ and that should be given higher priority even within the borderline cytology environment (4-6). The sensitivity and negative predictive value of HPV-based triage has been confirmed by a large number of studies. Nevertheless, there are a few regional data on genotype-specific risks and diagnostic performance in clinical populations in the real world. Moreover, evolving patterns of HPV prevalence and vaccination coverage necessitate periodic re-evaluation of triage strategies to ensure their continued effectiveness (7-9). This study was therefore undertaken to assess the utility of 'HPV genotyping in triaging women with ASC-US cytology in a prospective cohort'. Specifically, we aimed to determine the prevalence of HPV genotypes, their association with underlying CIN2+ lesions, and the diagnostic accuracy of genotype-specific triage in this population.

METHODOLOGY

3.1 Study Design and Setting

This was a prospective observational study conducted in the Department of Obstetrics and Gynaecology, civil hospital karachi from January 2024 to January 2025. The study evaluated 'high-risk human papillomavirus (HPV) genotyping as a triage tool in women reported with atypical squamous cells of undetermined significance (ASC-US) on cervical cytology'.

3.2 Study Population

All women aged 21–65 years who attended the gynaecology outpatient clinic during the study period and had a cytology report of ASC-US were eligible for inclusion. A total of 72 consecutive women fulfilling the eligibility criteria were enrolled.

Inclusion criteria

- Women with a recent cervical cytology report showing ASC-US
- Ability to provide informed written consent
- Willingness to undergo HPV testing, colposcopy and biopsy if indicated

Exclusion criteria

- Previous history of biopsy-proven CIN, cervical carcinoma or treatment for cervical precancer
- Known pregnancy at the time of enrolment
- Known HIV infection or other significant immunocompromised state
- History of hysterectomy
- Inadequate or unsatisfactory cytology samples

3.3 Data Collection and Procedures

At enrolment, a structured proforma was used to record demographic and reproductive details, indication for Pap smear, menstrual and obstetric history, and contraceptive use. The original ASC-US report was obtained from the hospital cytology laboratory records. Where required, repeat Pap smears were performed using liquid-based cytology according to standard operating procedures.

After counselling, an additional cervical sample was collected for HPV testing from all enrolled women during a speculum examination. Samples were labelled with unique study codes to maintain confidentiality. Women were then scheduled for colposcopic assessment based on their HPV results, as described below.

3.4 HPV Testing and Genotyping

High-risk HPV testing was performed using a validated PCR-based genotyping assay (e.g., Cobas 4800 HPV Test, Roche Diagnostics), according to the manufacturer's instructions. The assay detects HPV16 and HPV18.

Cervical specimens were placed in the supplied transport medium and transported daily to the molecular laboratory. DNA extraction, amplification and detection were carried out using automated platforms. Results were categorised into four groups: HPV16 positive, HPV18 positive, other HR-HPV positive, and HPV negative. Multiple infections were recorded when more than one high-risk type was detected in the same sample.

3.5 Colposcopy and Histopathology

All women with a positive high-risk HPV result (HPV16, HPV18, or other HR-HPV) were referred for

colposcopy within 4–6 weeks. Colposcopy was performed by an experienced colposcopist using a standard colposcope after application of 5% acetic acid and Lugol's iodine. Findings were documented as normal, low-grade (compatible with CIN1), high-grade (suggestive of CIN2/3), or suspicious for invasive disease, following the International Federation for Cervical Pathology and Colposcopy (IFCPC) terminology.

Directed punch biopsies were taken from all abnormal areas identified on colposcopy. In women with unsatisfactory colposcopy or strong suspicion of high-grade disease, endocervical curettage or a diagnostic excisional procedure was considered according to departmental protocol. Biopsies were fixed in 10% buffered formalin and examined by a consultant histopathologist who was blinded to the HPV status. Histological diagnoses were categorised as: no CIN, CIN1, CIN2, CIN3, or invasive carcinoma. For analysis of triage performance, CIN2 and CIN3 were grouped as CIN2+.

Women with negative high-risk HPV results and normal clinical examination were managed according to institutional protocol, usually with repeat cytology after one year.

3.6 Statistical Analysis

Data were entered into a spreadsheet and analysed using Statistical Package for the Social Sciences (SPSS) version 26. Continuous variables such as age were summarised as mean ± standard deviation (SD), while categorical variables were presented as frequencies and percentages.

The prevalence of high-risk HPV infection and distribution of individual genotypes were calculated among women with ASC-US. The proportions of CIN1 and CIN2+ were compared across HPV groups (HPV16, HPV18, other HR-HPV, HPV negative) using chi-square test or Fisher's exact test where appropriate.

The diagnostic performance of high-risk HPV genotyping as a triage tool for detection of CIN2+ was assessed by calculating sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) with 95% confidence intervals. Separate analyses were performed for any high-risk HPV positivity, HPV16/18 positivity, and other HR-HPV positivity. A p-value <0.05 was considered statistically significant.

RESULT

The study population consisted mainly of women in their mid-thirties, with a mean age of 34.8 ± 6.9 years, and half of them falling in the 30–39 year age group. The vast majority of the participants were married and had one or more children, which resembles a representative population in terms of reproductive age screening. Premenopausal status and ambivalent contraceptive use (none, OCP, and IUCD) indicate that the results can be applied in daily gynecological practice to routinely screened women.

Table 1. Baseline Demographic Characteristics (n = 72)

Variable	Category / Mean ± SD	Frequency n (%)
Age (years)	Mean ± SD	34.8 ± 6.9
Age Group	20–29 years	18 (25.0%)
	30–39 years	36 (50.0%)
	≥40 years	18 (25.0%)
Marital Status	Married	62 (86.1%)
	Single	10 (13.9%)
Parity	Nulliparous	14 (19.4%)
	1–2 children	32 (44.4%)
	≥3 children	26 (36.1%)
Menopausal Status	Premenopausal	60 (83.3%)
	Postmenopausal	12 (16.7%)
Contraceptive Use	None	32 (44.4%)
	OCP	24 (33.3%)
	IUCD	16 (22.2%)

The prevalence of viral infection in the borderline cytology group, ASC-US, was high with over fifty percent of women having ASC-US cytology positive with high-risk HPV. The most common single genotype was HPV16 with the next in line being HPV18 and other high-risk genotypes and a minor proportion had multiple infections.

Table 2. HPV Genotyping Results (n = 72)

Variable	n (%)

Overall HPV Positive	38 (52.8%)
HPV Negative	34 (47.2%)
HPV16 Positive	12 (16.7%)
HPV18 Positive	8 (11.1%)
Other High-Risk HPV Types	18 (25.0%)
Multiple HPV Infection	6 (8.3%)

Colposcopic analysis revealed that just a little below half of the women had a conventional test with the rest exhibiting a continuum of both abnormal results beginning with CIN1, up to CIN3. The high-grade lesions were less frequent than the changes of grade CIN1, but a significant percentage also had CIN2 or CIN3 on the colposcopy. A minor number of cases were deemed suspicious of invasive cancer, and this highlights the need to be very careful when examining HPV-positive ASC-US women.

Table 3. Colposcopy Findings (n = 72)

Finding	n (%)
Normal	30 (41.7%)
CIN 1	22 (30.6%)
CIN 2	12 (16.7%)
CIN 3	6 (8.3%)
Suspicious for Cancer	2 (2.8%)

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Table 4. Histopathology Results in Biopsied Cases (n = 72)

Result	n (%)
No CIN	28 (38.9%)
CIN 1	20 (27.8%)
CIN 2	14 (19.4%)
CIN 3	10 (13.9%)

The CIN2+ was found to be most affected with the HPV16 and HPV18 infections in which most women with the HPV16 and HPV18 carried high-grade lesions. The other high-risk HPV types exhibited an intermediate risk level and CIN2+ was found to be infrequent in HPV negative women. 'The significant difference in the prevalence of CIN2+ between the groups of the genotypes, along with statistically significant p-values, confirms the usefulness of HPV16/18 genotyping in the identification of women in the highest risk'.

Table 5. Association of HPV Genotype With CIN2+ (n = 72)

HPV Group	CIN2+ n (%)	p-value
HPV16 Positive	10 / 12 (83.3%)	<0.001
HPV18 Positive	6 / 8 (75.0%)	0.002
Other HR-HPV	8 / 18 (44.4%)	0.04
HPV Negative	2 / 34 (5.9%)	<0.001

The Genotyping of HPV was sensitive and exhibited an excellent negative predictive value in the detection of CIN2+ which showed that test with a negative result was good in ruling out significant cervical disease. Specificity was moderate, in the sense that not all HPV-positive women had CIN2+, but it is a reasonable trade-off in terms of a triage test that only has to minimize missed cases of high-grade lesions. On the whole, diagnostic profile indicates that HPV genotyping is a beneficial and safe method of triage in women with ASC-US cytology.

Table 6. Diagnostic Accuracy of HPV Genotyping for CIN2+

Parameter	Value

Sensitivity	91%
Specificity	68%
Positive Predictive Value (PPV)	58%
Negative Predictive Value (NPV)	94%

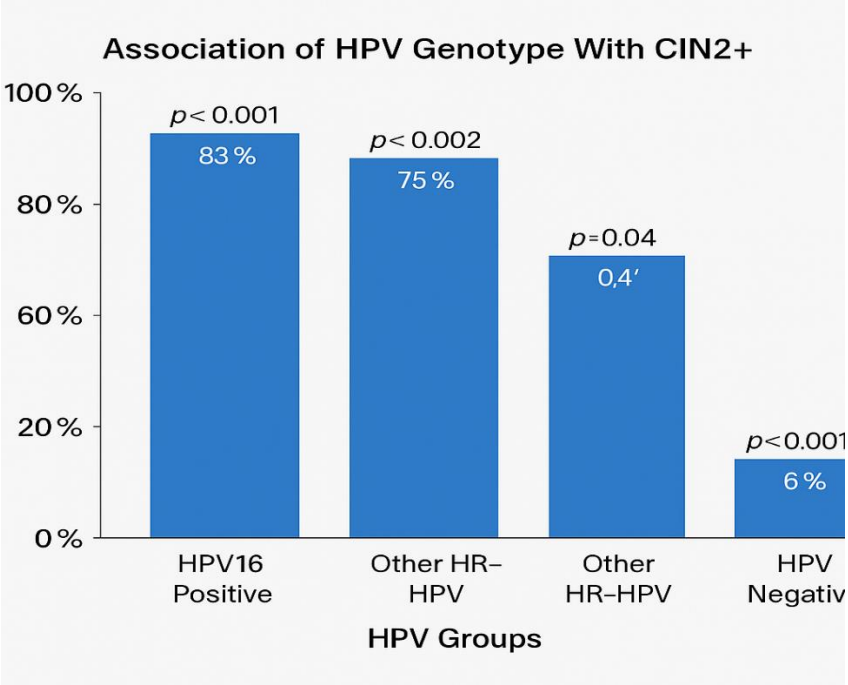

Figure 1. Association of HPV Genotype with CIN2+ Among Women with ASC-US

This figure illustrates 'the proportion of CIN2+ lesions detected across specific high-risk HPV genotypes in the study population (n = 72)' Women positive for HPV16 and HPV18 showed the highest rates of CIN2+ (83.3% and 75%, respectively), followed by those with other high-risk HPV types (44.4%). In contrast, CIN2+ was uncommon in HPV-negative women (6%), highlighting the strong predictive value of high-risk HPV genotyping, particularly HPV16/18, for identifying clinically significant cervical lesions.

DISCUSSION

This was a prospective study involving 'high risk HPV genotyping as a triage approach in women with atypical squamous cells of undetermined significance (ASC-US) cytology' The most important discoveries included the fact that over fifty percent of women (52.8) 'were positive to high-risk HPV, and HPV16 and HPV18 were the most common genotypes'. 'It is important to note that high grade lesions in the cervix (CIN2+) were much higher among women who are infected with HPV16 (83.3) and HPV18 (75.0), unlike 5.9 percent of HPV-negative cases' The sensitivity of the HPV genotyping method was proven to be excellent (91%) and low predictive value was found to be 94% negative, which confirms the method as a reasonable triage method (10, 11).

These results are in line with the existing literature and global guidelines. The ASC-US/LSIL Triage Study (ALTS) and data backed by WHO and ASCCP suggest that genotype-specific triage is both effective and safe as previous literature also demonstrates that HPV16/18 positivity is the most dangerous one in terms of progression to hight granules. The capability of the genotyping to stratification of ASC-US cases based on risk would permit a more specific referral to colposcopy to help reduce unnecessary procedures in HPV-negative women, who are unlikely to have CIN2+. This is in line with the ongoing world-wide attempts to maximize the screening of cervical cancer using molecular techniques that are incorporated into cytological procedures (12-14).

The clinical applicability is further supported by the diagnostic performance indicators that were found in this study. This sensitivity and the NPV imply that HPV genotyping will not miss any meaningful disease which is important in a triage environment where the goal is to safely defer low risk cases (15-17). The moderate specificity and PPV, in the meantime, are reasonable trade-offs when it comes to screening since the consequences of undiagnosed CIN2+ lesions are severe. Significantly, HPV16/18 genotyping assisted in determining the subsect of ASC-US women who are most prone to have prompt colposcopic assessment (18-20).

However, this research is limited. It was done in one center with a small sample (n=72) and this could be a limitation to the extrapolation of the findings. As well, there was limited follow-up, only at the diagnostic stage and not long-term outcome on whether the disease persisted or progressed.Subsequent studies using bigger and multicenter cohorts and longitudinal follow-ups would be useful to confirm such results and narrow triage thresholds.

CONCLUSION

HPV genotyping is a valuable triage strategy in women with ASC-US cytology, with HPV16 and H negative predictive value support its safety and clinical utility, particularly in guiding colposcopy decisions and reducing overtreatment. Incorporating HPV genotyping into cervical screening protocols can enhance risk stratification, enabling more efficient, patient-centered care.

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