

Research Article

Comparative Diagnostic Yield of HPV DNA Testing, Pap Cytology, And Colposcopy for Cervical Cancer Screening in Pregnant Women: A Cross-Sectional Study

Tania Khattak^{1*}, Shafia Zaib Mir², Hania Ifzaal Aslam³, Sobia Anwar⁴, Sana Saleem Rana⁵

^{1*}Assistant Professor Pathology, Nowshera Medical College / Qazi Hussain Ahmed Medical Complex, Nowshera, Pakistan.

²FCPS (Obstetrics and Gynaecology), Saudi National Hospital, Makkah, Saudi Arabia.

³Research Intern, Department of Public Health.

⁴FCPS, Assistant Professor Histopathology, Abu Umara Medical and Dental College, Lahore, Pakistan.

⁵Senior Demonstrator, Watim Medical and Dental College, Rawalpindi, Pakistan.

Corresponding Author: Tania Khattak

Assistant Professor Pathology, Nowshera Medical College / Qazi Hussain Ahmed Medical Complex, Nowshera, Pakistan.

Email: Mrs.khattak@gmail.com

Received: 17.07.2026, Revised: 24.09.2026, Accepted: 01.10.2026

ABSTRACT

Background: Cervical cancer screening during pregnancy provides an opportunity for early detection of cervical abnormalities. HPV DNA testing, Pap cytology, and colposcopy differ in their ability to identify clinically significant cervical lesions.

Objective: To compare the diagnostic yield of HPV DNA testing, Pap cytology, and colposcopy for detection of clinically significant cervical lesions among pregnant women.

Methods: This cross-sectional comparative study was conducted at tertiary care centers across Pakistan from March 2025 to September 2025, included 300 pregnant women. All participants underwent high-risk HPV DNA testing, Pap cytology, and colposcopic examination.

Results: The mean age of participants was 29.4 ± 5.2 years and mean gestational age was 24.6 ± 8.1 weeks. High-risk HPV DNA was positive in 54 (18.0%) women, while abnormal Pap cytology and colposcopy were found in 32 (10.7%) and 28 (9.3%), respectively. Histopathology identified CIN 2+ in 20 (6.7%) women. HPV DNA testing detected 18 (90.0%) CIN 2+ cases, while Pap cytology detected 14 (70.0%) and colposcopy detected 18 (90.0%). HPV DNA testing showed 90.0% sensitivity, 87.1% specificity, 33.3% positive predictive value, 99.2% negative predictive value, and 87.3% diagnostic accuracy. Pap cytology demonstrated 70.0% sensitivity, 93.6% specificity, 43.8% positive predictive value, 97.8% negative predictive value, and 92.0% accuracy. **Conclusion:** It is concluded that HPV DNA testing and colposcopy demonstrated the highest sensitivity for detection of CIN 2+, while colposcopy provided the highest specificity and overall diagnostic accuracy. HPV DNA testing may therefore serve as a sensitive screening method, with Pap cytology and colposcopy providing complementary assessment of pregnant women with abnormal screening results.

Keywords: HPV DNA Testing, Pap Cytology, Colposcopy, Pregnancy, Cervical Cancer Screening, CIN 2+, Diagnostic Accuracy.

INTRODUCTION

Despite these advances, cervical cancer is still a major preventable cancer disease of women worldwide, and persistent infection with high-risk Human Papillomavirus (HPV) is the major cause of cervical cancer. Recent global estimates illustrate that there is a significant ongoing burden, especially in low and middle-income countries where access to organized screening and follow-up is limited [1]. Before the onset of cervical cancer, there is a long

period of precancerous disease and thus early screening and early detection of cervical abnormalities is crucial to prevent the development of cancer [2]. An important opportunity to look for women who may not have had screening for cervical cancer before pregnancy is that of antenatal care, as this service may be one of the most regular services women receive in pregnancy [3]. However, some changes in the cervix can cause problems with interpreting the findings of cytology and

colposcopy during pregnancy. There is current evidence that the natural history of HPV infection and the risk of progression to cervical cancer is not significantly changed during pregnancy [4]. Thus, the results of routine screening during the pregnancy should not automatically be considered abnormal screening – the changes should be assessed based on the risk [5]. For many years, Pap cytology has been a significant screening tool for the cervix, and helps to identify abnormal cells that indicate potential precancerous conditions. Its performance is, however, affected by good specimen collection, and by accurate cytological interpretation, and cytology is less sensitive for clinically relevant cervical precancer than HPV testing [6]. HPV DNA testing is a molecular test that can directly detect the presence of oncogenic HPV infection, and is therefore becoming a more important tool in cervical cancer prevention. HPV DNA-based testing is now recommended as the most sensitive method for primary screening because of its increased ability to pick up cervical precancer when compared to cytology-based screening methods [7].

This transition is supported by the evidence from large-population based studies. A prospective cohort study comparing HPV testing, cytology and co-testing also showed significant improvement in the sensitivity of the HPV-based tests for detecting CIN2+ compared with cytology alone [8]. Likewise, HPV screening resulted in a higher yield of CIN2+ and CIN3+ in the real-world setting, but also referrals for colposcopy, emphasising the need for proper triage [9]. More recent and randomized proof also demonstrated an almost doubling of the number of CIN2+ lesions identified by primary HPV screening compared to primary cytology, more women needed colposcopy [10]. The evidence is also available and pregnancy specific, demonstrating the potential benefit of HPV testing. HPV prevalence among pregnant women was high, with 8.2% having a high-risk HPV infection, but only 1.5% having an abnormal Pap test, in a recent study performed in India [11]. A large group of pregnant women (8,712) also reported high risk HPV positivity (6.9%) and higher rate of HSIL+ detection by HPV testing compared to Pap testing [12]. The prevalence of oncogenic HPV has also been seen among adolescent women in the reproductive organs of the female reproductive system, with a high prevalence and significant relationship between HPV and cytological abnormalities, among

pregnant adolescents [13]. Colposcopy is important in cases of abnormal screening tests, but cervical changes that occur during pregnancy may make interpretation more difficult and may require special expertise in the examination of the cervix [14].

Objective

To compare the diagnostic yield of HPV DNA testing, Pap cytology, and colposcopy for detection of clinically significant cervical lesions among pregnant women.

METHODOLOGY

This comparative cross-sectional study was conducted at tertiary care centers across Pakistan from March 2025 to September 2025. A total of 300 pregnant women were recruited using consecutive sampling.

Inclusion Criteria

- Pregnant women aged 18–45 years.
- Women in any trimester of pregnancy.
- Women willing to undergo HPV DNA testing, Pap cytology, and colposcopy.
- Women providing written informed consent.

Exclusion Criteria

- Women with previously diagnosed cervical cancer.
- Women with a history of treatment for cervical intraepithelial neoplasia.
- Previous hysterectomy.
- Active heavy vaginal or cervical bleeding at the time of examination.
- Severe active genital tract infection.
- Women unwilling to undergo the required investigations.

Data Collection Procedure

After approval from the institutional ethical review committee, pregnant women fulfilling the eligibility criteria will be enrolled after obtaining written informed consent. Demographic and obstetric information including age, gestational age, gravidity, parity, previous cervical screening history, and relevant risk factors will be recorded on a pre-designed proforma. A cervical sample will be collected for high-risk HPV DNA testing using a standardized sampling technique. Pap cytology will subsequently be performed and reported according to the Bethesda classification system. Colposcopic examination will then be performed by an experienced gynecologist, and findings including acetowhite areas, abnormal vascular patterns, and suspected cervical lesions will be

documented. Cervical biopsy will be performed when clinically indicated, and histopathological findings will be recorded as the reference standard for clinically significant cervical lesions. The primary outcome will be the diagnostic yield of HPV DNA testing, Pap cytology, and colposcopy in detecting cervical abnormalities. Secondary outcomes will include the frequency of high-risk HPV positivity, abnormal Pap cytology, abnormal colposcopic findings, and agreement between the screening methods.

Data Analysis

Data will be entered and analyzed using SPSS version 25. Categorical variables including HPV status, Pap cytology findings, colposcopic findings, and histopathological results will be presented as frequencies and percentages, while quantitative variables including age and gestational age will be presented as mean $\pm$ standard deviation. The Chi-square test or Fisher's exact test will be used for comparison of categorical variables. Sensitivity, specificity,

positive predictive value, negative predictive value, and diagnostic accuracy will be calculated for HPV DNA testing, Pap cytology, and colposcopy using histopathology as the reference standard. Agreement between the diagnostic methods will be assessed using Cohen's kappa. A p-value ≤ 0.05 will be considered statistically significant.

RESULTS

The mean age of the 300 pregnant women was 29.4 ± 5.2 years. Among them, 164 (54.7%) were aged <30 years and 136 (45.3%) were ≥ 30 years. Primigravida women accounted for 112 (37.3%) and multigravida women for 188 (62.7%). Mean gestational age was 24.6 ± 8.1 weeks, with 54 (18.0%) in the first, 132 (44.0%) in the second, and 114 (38.0%) in the third trimester. Previous cervical screening was reported by 96 (32.0%), while 204 (68.0%) had no previous screening. Previous sexually transmitted infection and smoking history were present in 24 (8.0%) and 18 (6.0%), respectively.

Table 1. Demographic and Obstetric Characteristics of Pregnant Women (N = 300)

Variable	n (%) / Mean $\pm$ SD
Age (years)	29.4 $\pm$ 5.2
Age <30 years	164 (54.7%)
Age ≥ 30 years	136 (45.3%)
Primigravida	112 (37.3%)
Multigravida	188 (62.7%)
Gestational age (weeks)	24.6 $\pm$ 8.1
First trimester	54 (18.0%)
Second trimester	132 (44.0%)
Third trimester	114 (38.0%)
Previous cervical screening	96 (32.0%)
No previous cervical screening	204 (68.0%)
Previous sexually transmitted infection	24 (8.0%)
History of smoking	18 (6.0%)

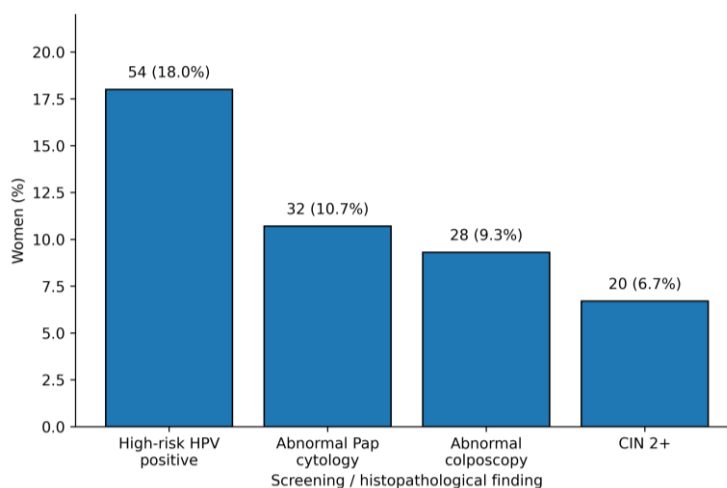


Figure 1. Cervical Screening and Histopathological Findings among Pregnant Women

Tania Khattak et al / Comparative Diagnostic Yield of HPV DNA Testing, Pap Cytology, And Colposcopy for Cervical Cancer Screening In Pregnant Women: A Cross-Sectional Study

High-risk HPV DNA was positive in 54 (18.0%) women and negative in 246 (82.0%). Pap cytology was normal in 268 (89.3%), while ASC-US, LSIL, HSIL, and AGC were found in 15 (5.0%), 9 (3.0%), 5 (1.7%), and 3 (1.0%),

respectively. Colposcopy showed normal or benign findings in 272 (90.7%) and abnormal findings in 28 (9.3%). Histopathology demonstrated CIN 1 or less in 280 (93.3%) and CIN 2+ in 20 (6.7%).

Table 2. Findings of HPV DNA Testing, Pap Cytology and Colposcopy (N = 300)

Screening method	Finding	n (%)
HPV DNA testing	High-risk HPV positive	54 (18.0%)
	High-risk HPV negative	246 (82.0%)
Pap cytology	Normal	268 (89.3%)
	ASC-US	15 (5.0%)
	LSIL	9 (3.0%)
	HSIL	5 (1.7%)
	AGC	3 (1.0%)
Colposcopy	Normal/benign findings	272 (90.7%)
	Abnormal findings	28 (9.3%)
Histopathology	CIN 1 or less	280 (93.3%)
	CIN 2+	20 (6.7%)

Among women with CIN 2+, HPV DNA testing identified 18 (90.0%) as positive and 2 (10.0%) as negative, while among those without CIN 2+, 36 (12.9%) were HPV positive and 244 (87.1%) were HPV negative. Abnormal Pap cytology detected 14 (70.0%) women with CIN 2+, compared with 6 (30.0%) with normal

cytology; among women without CIN 2+, 18 (6.4%) had abnormal cytology and 262 (93.6%) had normal results. Abnormal colposcopy was present in 18 (90.0%) women with CIN 2+ and 10 (3.6%) without CIN 2+, whereas normal colposcopy was observed in 2 (10.0%) and 270 (96.4%), respectively.

Table 3. Detection of Clinically Significant Cervical Lesions by Screening Method (N = 300)

Method	CIN 2+ detected, n (%)	No CIN 2+, n (%)	Total
HPV DNA positive	18 (90.0%)	36 (12.9%)	54
HPV DNA negative	2 (10.0%)	244 (87.1%)	246
Abnormal Pap cytology	14 (70.0%)	18 (6.4%)	32
Normal Pap cytology	6 (30.0%)	262 (93.6%)	268
Abnormal colposcopy	18 (90.0%)	10 (3.6%)	28
Normal colposcopy	2 (10.0%)	270 (96.4%)	272

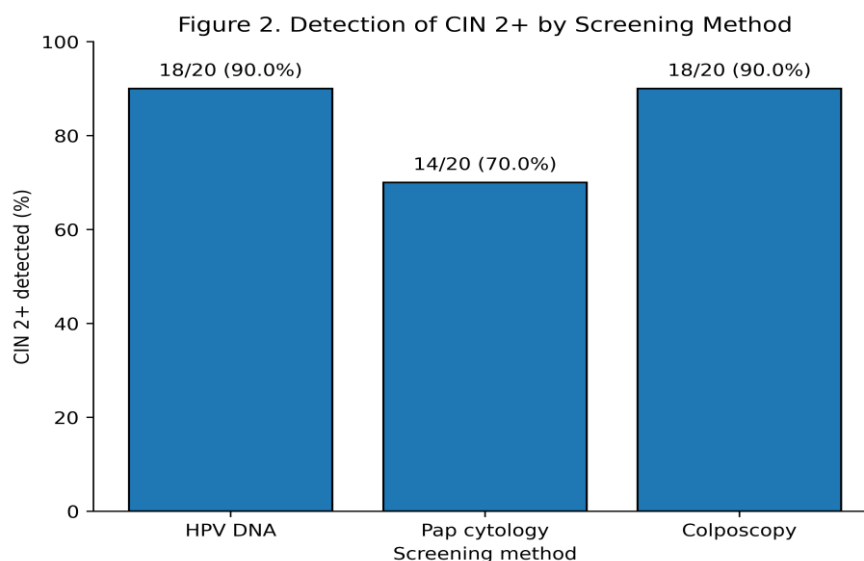


Figure 2. Detection of CIN 2+ by HPV DNA Testing, Pap Cytology and Colposcopy

HPV DNA testing showed 90.0% sensitivity, 87.1% specificity, 33.3% positive predictive value, 99.2% negative predictive value, and 87.3% diagnostic accuracy. Pap cytology demonstrated 70.0% sensitivity, 93.6% specificity, 43.8% positive predictive value,

97.8% negative predictive value, and 92.0% accuracy. Colposcopy showed 90.0% sensitivity and the highest specificity (96.4%), positive predictive value (64.3%), negative predictive value (99.3%), and diagnostic accuracy (96.0%).

Table 4. Diagnostic Performance of HPV DNA Testing, Pap Cytology and Colposcopy For Detection of CIN 2+ (N = 300)

Diagnostic method	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
HPV DNA testing	90.0	87.1	33.3	99.2	87.3
Pap cytology	70.0	93.6	43.8	97.8	92.0
Colposcopy	90.0	96.4	64.3	99.3	96.0

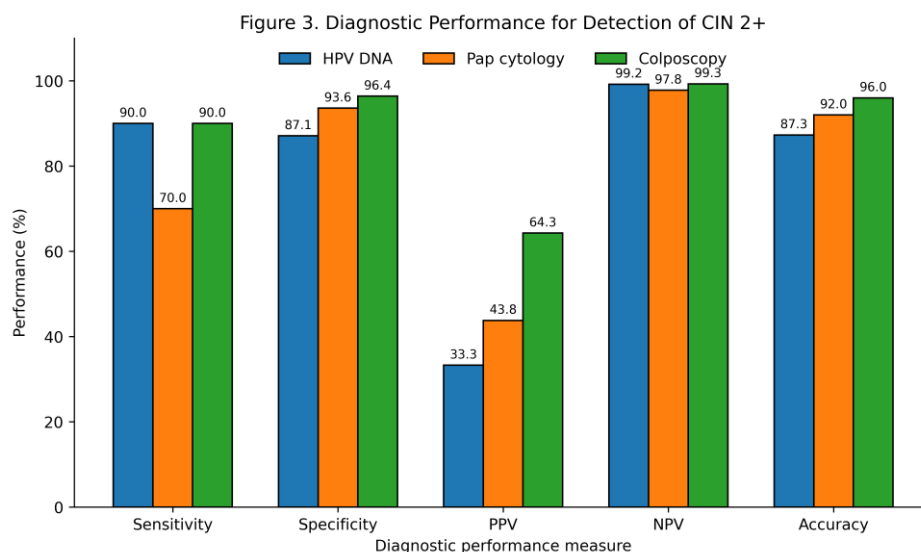


Figure 3. Diagnostic Performance of HPV DNA Testing, Pap Cytology and Colposcopy for Detection of CIN 2+

DISCUSSION

The present study showed that HPV DNA testing provided a higher number of positive results than Pap cytology and colposcopy, which can be considered a high-risk HPV test. Fifty-four (18.0%) women were high risk HPV positive, 32 (10.7%) had abnormal pap cytology, and 28 (9.3%) had abnormal colposcopy. This higher positivity rate for HPV testing was in line with previous studies with HPV testing in pregnant women, with 601 of 8712 (6.9%) women testing positive for HPV infection and 38 of 8712 (0.44%) of women with abnormal cytology. The same study revealed that the rate of detection of high-grade lesions by cytology alone was the lowest, which was further indication of the increased sensitivity of HPV-based screening [15]. In this study 20 (6.7%) women had CIN 2+. Eighteen (90.0%) of these cases were identified by HPV DNA testing, 14 (70.0%) by abnormal Pap cytology and 18 (90.0%) by abnormal

colposcopy. The high sensitivity of this HPV test is comparable with previous studies that used HPV DNA test in pregnant women, with a reported 90.5% sensitivity for significant cervical lesions and a negative predictive value of 96.4%. The results are consistent with the potential of HPV DNA testing to detect women at risk before the appearance of any cytological abnormalities [16]. Lower sensitivity was found by pap cytology in the present study with 70.0% of detection for CIN 2+ lesions and a relatively high specificity of 93.6%. The finding is in agreement with previous studies demonstrating that cytology might be less sensitive than HPV, but can provide useful triage information in HPV positive women. More recent comparisons have shown that HPV screening can detect significant numbers more lesions of CIN2+ than cytology, while HPV screening may lead to more women being referred for colposcopy [17]. Colposcopy was the most specific (96.4%) and accurate

(96.0%) method in this analysis, but also had the lowest sensitivity (90.0%). Previous studies with pregnant women with abnormal cytology and/or positive HPV testing demonstrated good agreement between colposcopy and histopathology ($\kappa=0.82$, $p<0.001$) highlighting the clinical relevance of colposcopy for pregnant women with abnormal cytology and/or HPV positivity [7].

Although the HPV DNA test had a high sensitivity and negative predictive value, its positive predictive value was relatively low at 33.3%. This is anticipated as HPV infection does not necessarily mean there is a high-grade cervical lesion present. Previous studies have also shown that the positive predictive value for HPV testing as a primary screening test is relatively low in the context of HPV infection in women, highlighting the need for appropriate triage. The risk of CIN2+ was highly variable with HPV genotype and associated cytology in a large clinical audit, highlighting the need for HPV testing to be linked to suitable triage rather than assumed to be "significant disease" [16]. The present results further demonstrated that although 268 (89.3%) women had normal Pap lab results, 6 (30.0%) women with CIN 2+ had normal Pap lab results. In contrast, only 2 (10.0%) cases of HPV DNA negative had CIN 2+. The difference suggests that there is a potential for a clinically important lesion to be overlooked using cytology alone. The results of previous studies also demonstrate that women with HPV who have either negative or minor cytologic abnormalities are also at risk of having a clinically significant HPV infection regardless of how they are classified in the context of HPV testing, especially when they have high-risk HPV types [19]. The prevalence of high risk HPV in the present study 18.0% was higher than the 6.9% HPV prevalence reported with positive HPV DNA in a large cohort of pregnant women, and the 13.4% HPV DNA positivity reported in pregnant women in another cross-sectional study. Differences could be due to population differences, to the different epidemiology of HPV, age differences, sexual and obstetric risk factors, and due to the different methods of HPV detection. This latter study also identified 26.6% of the pregnant women who were found to have abnormal cytology, which again shows the range of variation for the existence of cervical abnormalities among different pregnant populations [20]. The cross-sectional design, single center study and relative small sample size of 300 pregnant women in this study may

limit its generalizability. The interpretation of cytology and colposcopy can also be affected by changes in the cervix that occur during pregnancy. Furthermore, only for clinical indications was there a histopathological confirmation; and the pathological results were obtained with one study population.

CONCLUSION

It is concluded that HPV DNA testing demonstrated high sensitivity (90.0%) and negative predictive value (99.2%) for detection of CIN 2+, while Pap cytology showed comparatively lower sensitivity (70.0%) but good specificity (93.6%). Colposcopy demonstrated the highest specificity (96.4%) and diagnostic accuracy (96.0%). These findings support HPV DNA testing as a sensitive screening approach, with Pap cytology and colposcopy providing valuable complementary assessment for pregnant women with abnormal screening results.

REFERENCES

1. Cianfrini F, d'Amati A, Molinaro C, Padial Urteta B, Boccaccini C, Donato ABM, et al. Cervical cytological findings and vaginal microbiota alterations during pregnancy: a retrospective analysis. *Sage Open Pathol.* 2026; 19:30502098261471115. doi:10.1177/30502098261471115.
2. Yalcin N, Alci A, Gokkaya M, Goksu M, Toptas T, Ureyen I. Evaluation of the outcomes of patients undergoing colposcopy for high-risk human papillomavirus positivity and/or abnormal cervical cytology. *Diagnostics (Basel).* 2026;16(3):465. doi:10.3390/diagnostics16030465.
3. Beira Salvador PM, García Grandal T, Iglesias Méndez R. Risk of HSIL/CIN2+ lesions in women with negative cytology and high-risk HPV referred to colposcopy: experience in a population-based screening program. *Med Clin (Barc).* 2026;166(7):107451. doi: 10.1016/j.medcli.2026.107451.
4. Nowakowski A, Macios A, Glinska P, Chmielewska A, Janik B, Jędrzejewska I, et al. Primary human papillomavirus screening versus cytology for detection of cervical intraepithelial neoplasia grade 2 or higher in Poland: a parallel-group, randomized controlled trial. *Lancet Reg Health Eur.* 2026; 66:101697. doi: 10.1016/j.lanepe.2026.101697.
5. Stuebs FA, Dietl AK, Schulmeyer CE, Behrens AS, Seibold A, Brueckner L, et

- al. Prognostic significance of the detection of human papilloma virus L1 protein in smears of cervical intraepithelial neoplasia grade 3 in pregnant women. *Anticancer Res.* 2025;45(2):677-683. doi:10.21873/anticancer.17455.
6. Mekuria S, Assiged N, Biazin H, Borgfeldt C, Abebe T, Mihret A, et al. Assessing the feasibility of HPV screening for cervical cancer in pregnant women in Ethiopia. *Sci Rep.* 2025; 15:31771. doi:10.1038/s41598-025-15957-y.
7. Trojnarowska D, Wielgus M, Górniewicz K, Kot J, Jach R. Challenges and opportunities in managing pregnant patients with abnormal cervical cytology and positive HPV result in Poland: a single-center retrospective analysis. *Folia Med Cracov.* 2025;65(2):101-111. doi:10.24425/fmc.2025.156128.
8. Mi X, Rashmi M, Pan Z, Wu D, Miao J. The significance of biological samples from pregnant women in cervical intraepithelial neoplasia. *Front Med (Lausanne).* 2025; 12:1645567. doi:10.3389/fmed.2025.1645567.
9. Verma R, Sharma R, Mishra P, Mishra N. Opportunistic cervical cancer screening during antenatal visits: a small step toward early detection. *Cureus.* 2025;17(8): e89433. doi:10.7759/cureus.89433.
10. Lindquist S, Kjær SK, Frederiksen K, Ørnskov D, Munk C, Waldstrøm M. Comparative analysis of HPV testing versus cytology in Danish cervical cancer screening: insights from a large-scale implementation study. *Gynecol Oncol.* 2024; 191:45-55. doi: 10.1016/j.ygyno.2024.09.013.
11. Gu L, Hu Y, Wei Y, Hong Z, Zhang Y, Lin J, et al. Optimising cervical cancer screening during pregnancy: a study of liquid-based cytology and HPV DNA co-test. *Epidemiol Infect.* 2024;152: e25. doi:10.1017/S095026882400013X.
12. Chen M, Ye Z, Wang H, Cui X, Seery S, Wu A, et al. Genotype, cervical intraepithelial neoplasia, and type-specific cervical intraepithelial neoplasia distributions in hrHPV+ cases referred to colposcopy: a multicenter study of Chinese mainland women. *J Med Virol.* 2024;96(3): e29475. doi:10.1002/jmv.29475.
13. Dorismond VG, Saraiya M, Gopalani SV, Soman A, Kenney K, Miller J, et al. Variation in cervical cancer screening test utilization and results in a United States-based program. *Gynecol Oncol.* 2024; 184:96-102. doi: 10.1016/j.ygyno.2024.01.020.
14. Stuebs FA, Koch MC, Dietl AK, Schulmeyer CE, Behrens AS, Seibold A, et al. Management of cervical intraepithelial neoplasia in pregnant women. *Anticancer Res.* 2023;43(7):3153-3158. doi:10.21873/anticancer.16488.
15. Li X, Xiang F, Zhao Y, Li Q, Gu Q, Zhang X, et al. Detection of cervical high-grade squamous intraepithelial lesions and assessing diagnostic performance of colposcopy among women with oncogenic HPV. *BMC Womens Health.* 2023; 23:411. doi:10.1186/s12905-023-02538-2.
16. Effah K, Tekpor E, Klutsey GB, Bannor HT, Amuah JE, Wormenor CM, et al. Antenatal and postnatal cervical precancer screening to increase coverage: experience from Battor, Ghana. *Ecancermedicalscience.* 2023; 17:1616. doi:10.3332/ecancer.2023.1616.
17. Sudhakaran S, Bhatla N, Mathur SR, Mahey R, Vashist S, Natarajan J, et al. Evaluation of cervical cancer screening during pregnancy in India: human papillomavirus testing can change the paradigm. *Natl Med J India.* 2023;36(1):17-21. doi:10.25259/NMJI_768_20.
18. de Souza HD, Waissman AL, Diório GR, Peres SV, Francisco RP, Galletta MAK. Prevalence of oncogenic human papillomavirus in pregnant adolescents, association with colposcypological changes, risk factors and obstetric outcomes. *Clinics (Sao Paulo).* 2022; 77:100127. doi: 10.1016/j.clinsp.2022.100127.
19. Freudenreich R, Weiß M, Engler T, et al. Characterization and clinical management of abnormal cytology findings in pregnant women: a retrospective analysis. *Arch Gynecol Obstet.* 2022;306(6):2017-2026. doi:10.1007/s00404-022-06699-7.
20. Luo D, Peng M, Wei X, Pan D, Xue H, Xu Y, et al. Prevalence of human papillomavirus and genotype distribution in pregnant and non-pregnant women in China. *Risk Manag Healthc Policy.* 2021; 14:3147-3157. doi:10.2147/RMHP.S288838.