

ORIGINAL ARTICLE

Detection of human papillomavirus (HPV)-Related Cervical Cancer using a Pap smear Among Women in Basrah and Assessment of Cervical Cancer Awareness

Hayder A. Al -Hmudi*, Roaa A. Sabeeh, Mohaned A.K. Al-Atbee

Department of Biology, College of Science, University of Basrah, Basrah, Iraq

ABSTRACT

Key words:

Papillomavirus (HPV), cervical cancer, pap smear, HPV vaccine

***Corresponding Author:**

Hayder A. Al -Hmudi
Department of Biology, College
of Science, University of
Basrah, Basrah, Iraq
jinanabdulaziz3@gmail.com

Background: Cervical cancer typically develops from cervical intraepithelial neoplasia (CIN), with high-grade lesions (CIN II/III) carrying substantial risk of progression to invasive carcinoma if untreated. **Objective:** This study aims to screening of Human Papillomavirus (HPV) - related to cervical cancer in Iraqi women using Pap smear, also to conducting the level of awareness about HPV Infection and vaccination. **Methodology:** The study included a survey for HPV through cervical smear from Basrah Women's and Children's Hospital between January and March 2025, the study included 20 questions among students at the University of Basrah. **Results:** In total, nine out of 187 individuals (4.8%) presented with HPV-related epithelial abnormalities, the majority of which were low- grade lesions. Figures clarified that CIN-I involves less changes and affects the epithelial from the surface layer in comparison to CIN-II and CIN-III. The study included a survey on awareness HPV and its vaccine, it revealed that 60% of the students were in their final years of medical specialization studies, yet 57% were unaware of the sexual transmission of the virus and the same percentage were unaware that it causes cervical cancer. Furthermore, 68% had never heard of the vaccine. **Conclusion:** Combining cytological analysis and molecular diagnostics provide a more comprehensive and accurate approach to the early detection of precancerous lesions, ultimately contributing to a reduction in cervical cancer incidence rates.

INTRODUCTION

Cancer remains a major global health challenge and one of the leading causes of morbidity and mortality worldwide ¹. Cervical cancer disproportionately affects women in low- and middle-income countries, where limited screening access contributes to 94% of global deaths ². This malignancy typically develops from cervical intraepithelial neoplasia (CIN), with high-grade lesions (CIN II/III) carrying substantial risk of progression to invasive carcinoma if untreated ³. HPV is a non-enveloped virus with an icosahedral capsid and a double-stranded circular DNA genome. It is highly prevalent and primarily transmitted through sexual contact, making it one of the most common sexually transmitted infections worldwide ⁴. Among over 100 identified genotypes, approximately 15 are classified as high-risk due to their oncogenic potential. These types include 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82 ^{5,6}. Persistent infection with high-risk HPV genotypes, particularly HPV-16 and HPV-18, drives cervical carcinogenesis⁷. While global HPV vaccination and molecular screening programs have significantly reduced cervical cancer incidence, Iraq faces critical healthcare gaps. The country lacks a national HPV immunization program, and organized screening remains limited ⁸. Despite being preventable, systemic

gaps persist: the country lacks both a national screening program and HPV vaccination, resulting in abysmal early detection rates (<12% screening coverage among women aged 25–49) ⁹. The current study aims to provide an epidemiological overview of the screening outcomes of HPV - related to cervical cancer in Iraqi women using Pap smear analysis during the specified timeframe. In addition, the study aims to conducting a study on the level of awareness and knowledge about the HPV and its vaccine among students at University of Basrah.

METHODOLOGY

Sample Collection

The current study included a survey of cervical smear cases from Basrah Women's and Children's Hospital during the study period between January and March 2025. The current study included the number of women screened and their corresponding cytological diagnoses. All slides were prepared using the Papanicolaou smear technique and selected based on cytological features suggestive of HPV-related abnormalities.

Papanicolaou Staining Protocol and Microscopic Evaluation

Slides were fixed in 99% ethanol for 10–15 minutes, then stained with hematoxylin for 5 minutes, followed by eosin yellow staining for 1–2 minutes, with rinsing

between each step. Dehydration was performed using graded concentrations of ethanol for 5 minutes, followed by clearing in xylene. Finally, slides were mounted using Histo fluid to preserve cellular detail and enhance microscopic resolution. All slides were examined under a compound light microscope using 10× and 40× objective lenses. Cytological evaluation focused on features indicative of HPV infection. Lesions were classified according to the 2014 Bethesda System¹⁰.

HPV Awareness Survey

The study included a survey on the level of awareness and knowledge of the human papillomavirus (HPV) and its vaccine, which included 20 questions of 250 female students at the University of Basrah.

RESULTS

Screening Data

During the study period, a total of 187 women underwent cervical cytological screening at Basrah Women and Children's Hospital. In January, 49 women were screened, 4 cases (8.2%) showing abnormal cytological findings-3 diagnosed as CIN I and 1 as CIN II. During February recorded the highest screening rate with 108 women, among whom five cases (4.6%) exhibited abnormalities-3 CIN I and 2 CIN II). During March, 30 women were screened, without cytological abnormalities were detected. At all, 9 out of 187 individuals (4.8%) presented with HPV-related epithelial abnormalities, the majority of which were low- grade lesions (Table 1).

Table 1. Distribution of abnormal cytological findings across three months

Month	No. of women screened	Positive cases	CIN I	CIN II	Positive %
January	49	4	3	1	8.2 %
February	108	5	3	2	4.6 %
March	30	0	0	0	0 %
Total	187	9	6	3	4.8 %

Cytological Observations

Figures clarified that CIN-I involves less changes and affects the epithelial from the surface layer in comparison to CIN-II and CIN-III. These, which mean high-grade lesions, progress to include the entire part of the

epithelium. This is associated with bleeding and proliferation of inflammatory cells (lymphocytes), which may form nodes and irregular cells with granular cytoplasm and tissue debris (Figure. 1, 2, 3a, 3b).

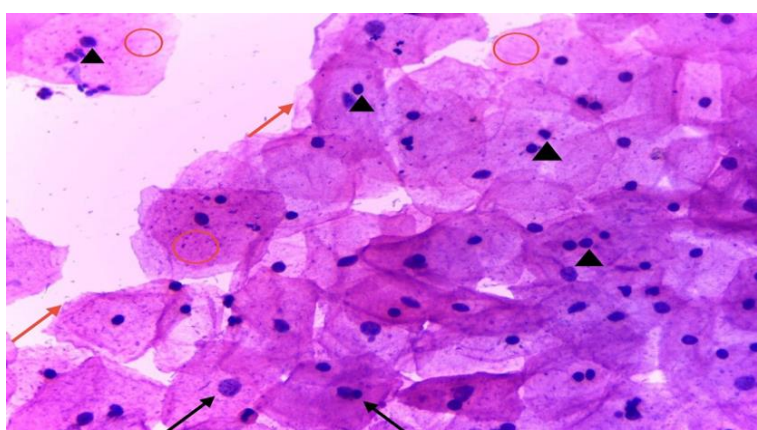


Fig. 1: Pap smear in cervix CIN-I showed pre-cancerous, irregular cells (red arrow), Changes in cytoplasm (hollow red circle), mitotic figures (black triangle) and giant cells also observed (black arrow). H&E stain (40X)

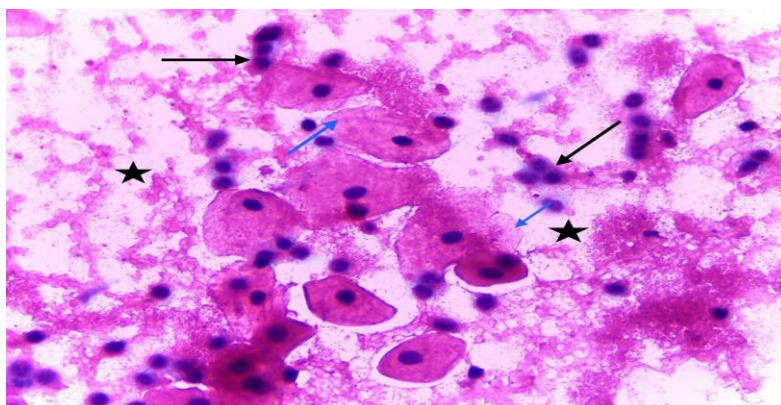


Fig. 2: Pap smear in cervix carcinoma CIN-2 Showed variable degree in nuclei shape, size, irregular and intensity of staining (hyperchromasia) (black arrow), invasive stroma (black star) and cell with vacuolated cytoplasm (blue arrow). H&E stain (40X)

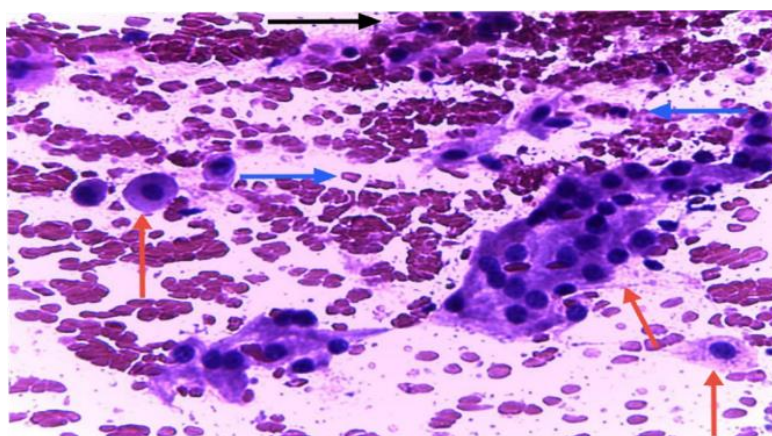


Fig. 3a. Photomicrograph in cervical intraepithelial neoplasia CIN-3 showing heavy inflammatory cells (black arrow) proliferation, bleeding (blue arrow), numerous dysplastic cells (red arrow). H&E stain (40X)

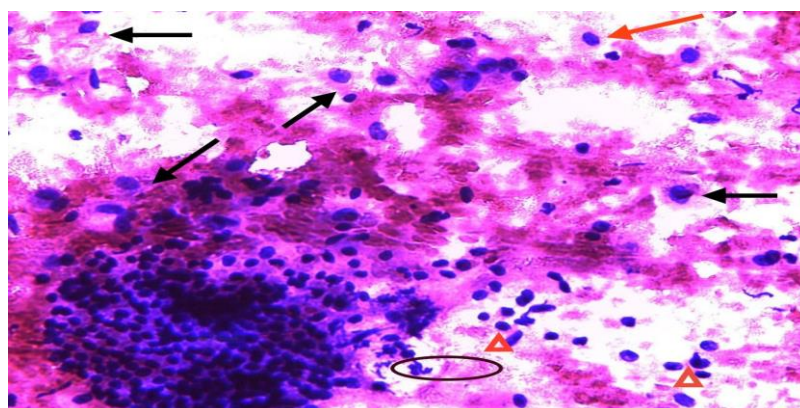


Fig. 3b: photomicrograph in cervical intraepithelial neoplasia CIN-3 showing most inflammatory cells was plasma cells (red triangle), numerous dysplastic cells (hollow black oval), macrophage (red arrow) and changes in most epithelial cells (black arrow). H&E stain (40X)

HPV Awareness Survey

Table (2) summarizes the results of the human papillomavirus (HPV) awareness survey. It revealed that 60% of the students were in their final years of

medical specialization studies, yet 57% were unaware of the sexual transmission of the virus and the same percentage were unaware that it causes cervical cancer. Furthermore, 68% had never heard of the vaccine.

Table 2. Summary with Results of HPV Awareness Survey

Section	Question Theme	Purpose	Questions	Results
Demographics	Age	Understand participants' age groups	Q1	18-21: 31% 21-25 : 69% >25 :2%
Demographics	Academic Major	Compare awareness by field of study	Q2	Medical: 60% non-medical; 40%
Demographics	Study Level	Compare awareness by academic level	Q3	Fourth stage:60% Below fourth:39%
General Awareness	Heard of HPV	Measure general awareness	Q4	Yes: 66% No : 33%
General Awareness	Transmission knowledge	Check if students know it's sexually transmitted	Q5	Yes:43% No: 57%
General Awareness	Cancer risk awareness	Check if students know it can cause cervical cancer	Q6	Yes: 43% no: 57%
General Awareness	Genital warts knowledge	Assess broader knowledge	Q7	Yes: 49% No: 45%
General Awareness	Asymptomatic nature	Identify knowledge of HPV being latent	Q8	Yes:61 % No : 36%
Information Sources	HPV Info Sources	Identify how students learned about HPV	Q9	Internet :47% College : 58% Doctors : 13% Media: 6% family and friends :12%
Vaccine Awareness	Heard of vaccine	Measure awareness of HPV vaccine	Q10	Yes: 32% No: 68%
Vaccine Awareness	Vaccine prevents cancer	Check knowledge of vaccine benefit	Q11	Yes: 51 % No: 46%
Vaccine Awareness	Timing of vaccine	Check if students know when it's given	Q12	Yes : 30% No:67%
Vaccine Awareness	Vaccine availability	Understand awareness of access	Q13	Yes : 39% No:48%
Information Sources	Vaccine Info Sources	Identify sources of vaccine knowledge	Q14	Internet:47% College: 58% Doctors : 13% Media: 6% family and friends:12%
Vaccine Attitude	Willingness to vaccinate	Measure acceptance of HPV vaccine	Q15	Yes:15% No : 18% Not sure :71%
Vaccine Attitude	Reasons for refusal	Understand barriers to vaccination	Q16	Lack of knowledge: 56% Other reasons: 26% Anxiety: 44% Hige cost: 3 % Religious reasons:2% No need : 35%
Awareness Needs	Need for more education	Determine need for more HPV campaigns	Q17	Yes :100% No :0%
Health Practices	Screening practices	Measure health behavior	Q18	No :74% Yes:1% Sometimes :25%
University Role	University awareness role	Check if students want university involvement	Q19	Yes:67% Not sure :21% No:12%
Social Perception	Social stigma	Understand perception and stigma	Q20	Yes :55% Not sure :1% No: 44 %

DISCUSSION

HPV infection causes distinct cytological alterations in cervical epithelial cells. Viral replication occurs across the epithelial layers without inducing cell lysis, though it may increase epithelial thickness¹¹. The key cytological marker is koilocytosis, seen mainly in LSIL, characterized by nuclear enlargement, irregular nuclear membranes, hyperchromasia, and perinuclear clearing^{12,13}. In HSIL, cells display a higher nuclear-to-cytoplasmic ratio, nuclear pleomorphism, and chromatin irregularities, facilitating accurate cytological grading and diagnosis^{14,15}.

The observed cytological features: particularly koilocytosis, nuclear enlargement, and hyperchromasia are consistent with early dysplastic changes associated with HPV infection, in line with the Bethesda classification system¹⁶. These findings underscore the diagnostic value of Pap smear cytology in detecting early-stage cervical lesions. However, as noted by Sherman *et al.*¹⁷, latent HPV infections may go undetected by cytological methods alone, especially in early stages when viral gene expression is minimal. This highlights the need to integrate molecular diagnostic tools such as HPV DNA testing to improve detection sensitivity and reduce false-negative rates.

HPV drives carcinogenesis mainly through its oncoproteins E6 and E7, which interfere with key tumor suppressors. E7 inactivates pRb, releasing E2F and promoting uncontrolled cell cycle progression. In turn, the cell upregulates p16 as a compensatory response, making it a useful marker in HPV-related lesions¹⁸. Meanwhile, E6 promotes the degradation of p53, suppressing apoptosis and enhancing genomic instability. Together, these disruptions enable infected epithelial cells to bypass normal growth controls, laying the foundation for malignant transformation¹⁹.

This study reported a low prevalence of cervical abnormalities, with only 9 out of 187 women (4.8%) testing positive predominantly CIN I (66.6%). In contrast, Al-Asadi documented significantly higher rates of HSIL (53%) and cervical carcinoma (22%) over a two-year period²⁰. The discrepancy may be attributed to differences in study duration, sample size, screening coverage, or population characteristics. A shorter study period, such as the three-month window in the present work, may reduce the likelihood of capturing advanced lesions.

While the Pap smear's sensitivity for detecting high-grade lesions (CIN3+) ranges from 50–70%, its effectiveness significantly improves with repeated testing and structured follow-up. Organized national programs have demonstrated mortality reductions of up to 80%²¹. In a Baghdad-based study, 86% of screened women showed abnormal cytological findings - primarily LSIL and HSIL-reflecting the value of cytological screening, even in the absence of widespread molecular diagnostics²².

Since HPV infection is most common soon after sexual debut, the majority of participants were between the ages of 21 and 25, reflecting a population at heightened epidemiological relevance²³. Given that prompt education and vaccination during early adulthood are linked to a significant decrease in the incidence of cervical cancer later in life, university students represent a strategic target group for HPV prevention. Despite making up a sizable portion of the sample, medical students' knowledge levels were not significantly higher than those of non-medical students. This result is consistent with earlier research indicating that HPV-related material is frequently not given enough attention, even in medical education programs²⁴.

In this study, 66% of participants have knowledge of HPV before. This figure is still much lower than the awareness levels found in countries with organized HPV education and vaccination programs, where rates frequently exceed 80%, even though it is higher than those found in several low- and middle-income countries²⁵. This discrepancy demonstrates how national public health policies affect awareness at the population level. Just 43% of respondents correctly identified HPV as a sexually transmitted infection, and a comparable percentage acknowledged that it causes cervical cancer. These results point to a significant gap between accurate biomedical understanding and general awareness. Research conducted worldwide has demonstrated that the identification of HPV as the primary cause of cervical cancer is a strong predictor of preventive behaviours, such as screening and vaccination uptake²⁶. This study's low awareness might have been partly explained by sociocultural barriers that prevent candid conversations about sexual health issues; this phenomenon has been documented in conservative societies²⁷.

Only 32% of people were aware of the HPV vaccine. This result stands in stark contrast to data from nations that have incorporated HPV vaccination into national immunization programs, where awareness frequently surpasses 80% and has resulted in significant drops in HPV prevalence and cervical precancers²⁵. This deficit is probably mostly caused by Iraq's lack of a national HPV vaccination program. There was little acceptance of the vaccine; many participants expressed uncertainty rather than outright rejection. According to global data, the main obstacles to acceptance were fear of negative consequences and a lack of knowledge²⁶. Significantly, there were few financial and religious concerns, indicating that focused educational interventions could greatly increase uptake. In a similar vein, the low rate of cervical cancer screening found in this study is consistent with patterns documented in other low-resource environments, where social stigma and ignorance continue to be major barriers²⁸. The combination of poor screening practices, low vaccine acceptance, inadequate detailed knowledge, and

moderate awareness points to a persistent risk of HPV transmission and future cervical cancer burden among young women in Basrah. Improving HPV vaccination coverage and screening participation could prevent most cases of cervical cancer within a generation, according to data from global modelling studies ²⁵.

CONCLUSION

Although the detection rate in this study was relatively low, the results underscore the importance of implementing routine cervical screening programs. Combining cytological analysis and molecular diagnostics provides a more comprehensive and accurate approach to the early detection of precancerous lesions, ultimately contributing to a reduction in cervical cancer incidence rates. Also, this study offers a thorough evaluation of female University of Basrah students' awareness, knowledge, attitudes, and preventive behaviors regarding the Human Papillomavirus (HPV). The results show significant gaps in our understanding of HPV, especially with regard to vaccination, transmission, and the connection between HPV and cervical cancer. When compared to both regional and global evidence, these gaps have important implications for public health.

Acknowledgement

The researchers extend their thanks to the undergraduate student Fatima Shari Bahedh in the Department of Biology, College of Science, University of Basrah, for her effective contribution to the completion of this research.

Ethical Approval

The study was conducted after obtaining approval from the Institutional Ethics Committee at the University of Basrah, in accordance with protocol number (EC-08, dated January 3, 2025). Informed verbal consent was also obtained from all participating patients.

Conflict of Interest

No conflict of interest exists.

Funding

None

REFERENCES

- Karidio I, Sanlier S. Reviewing cancer's biology: an eclectic approach. *J Egypt Natl Canc Inst* 2021;33(1):32.
- World Health Organization. Cervical cancer. Available at: <http://www.who.int/news-room/fact-sheets/detail/cervical-cancer>; 2024 [accessed 08.05.25].
- Tao L, Han L, Li X, Gao Q, Pan L, Wu L, Luo Y, Wang W, Zheng Z, Guo X. Prevalence and risk factors for cervical neoplasia: a cervical cancer screening program in Beijing. *BMC Public Health* 2014;14:1185.
- Smith H. What causes cervical cancer besides HPV? *Medical News Today*; 2023.
- Muñoz N, Bosch FX, de Sanjosé S, Herrero R, Castellsagué X, Shah KV, Snijders PJ, Meijer CJ. Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med* 2003;348(6):518–527.
- De Villiers EM, Fauquet C, Broker TR, Bernard HU, zur Hausen H. Classification of papillomaviruses. *Virology* 2004;324(1):17–27.
- Ferchichi M, Satouri L, Ghoul F, Malek-Mellouli M, Derbel AMS, Makni MK, Reziga H, Baba A, Zili M, Segondy M, Khelifa R. Phylogeny and classification of human papillomavirus (HPV)16 and HPV18 variants based on E6 and L1 genes in Tunisian women with cervical lesions. *Asian Pac J Cancer Prev* 2018;19(12):3361–3366.
- Daniel S, Mohammed AS, Ibrahim N, Hussein NR, Balatay AA, Naqid IA, Shekko CK, Musa DH, Saleem ZSM. Human papillomavirus (HPV) genotype prevalence and impact of COVID-19 on the HPV prevention program in Duhok city. *Dialogues Health* 2022;1:100055.
- Hasan TN, Taher TMJ, Ghazi HF. Awareness regarding pap smear among women in Baghdad City, Iraq. *Wiad Lek* 2021;74(9 Pt 2):2287–2292.
- Nayar R, Wilbur DC. The Bethesda System for Reporting Cervical Cytology. 3rd ed. New York: Springer; 2015.
- Sanyal S, Hazra A, Guchhait P, Das S. Early cervical cytological changes in human papilloma virus (HPV) positive cases. *Int J Clin Diagn Pathol* 2023;6(4):12–19.
- Miller SB, Zhao C. The evolving management of LSIL in Pap tests. *CAP TODAY*; August 2016.
- Jager L, Choy B, Pusztaszeri M, Bennett JA. Cervix: Premalignant/preinvasive lesions – cytology: LSIL (cytology). PathologyOutlines.com. Available at: <https://www.pathologyoutlines.com>; 2023 [accessed 08.05.25].
- Khieu M, Butler SL. High-grade squamous intraepithelial lesion of the cervix. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2023. Available at: <https://www.ncbi.nlm.nih.gov/books/NBK430728/> [accessed 08.05.25].
- El-Wakil DM, Shaker OG, Rashwan ASSA, Elesawy YF, Samir N. High-risk human papillomavirus infection and cervical cytopathology: relationship with cervical nitric oxide levels. *Virology* 2024;21:173.

16. Moriarty A, Davey D, O'Connor D, Solomon D, Kurman R, Prey M, Raab S, Sherman M, Wilbur D, Wright T Jr, Young N. Terminology for reporting results of cervical cytology: Bethesda 2001 workshop. *JAMA* 2002;287(15):2114–2119.
17. Sherman ME, Castle PE, Wacholder S. Absolute risk of a subsequent abnormal Pap among oncogenic human papillomavirus DNA-positive cytologically negative women. *Cancer* 2002;95(4):2145–2151.
18. Wai KC, Strohl MP, van Zante A, Ha PK. Molecular diagnostics in human papillomavirus-related head and neck squamous cell carcinoma. *Cancers* 2020;12(5):1335.
19. Gupta SM, Mania-Pramanik J. Molecular mechanisms in progression of HPV-associated cervical carcinogenesis. *J Biomed Sci* 2019;26:28.
20. Al-Asadi SYR. Molecular and immunohistochemical study of HPV associated with cervical carcinoma and cervical intraepithelial neoplasia (CIN) with expression of p16INK4a. PhD thesis. Basrah: University of Basrah, College of Science; 2020.
21. Nanda K, McCrory DC, Myers ER, Bastian LA, Hasselblad V, Hickey JD, Matchar DB. Accuracy of the Papanicolaou test in screening for and follow-up of cervical cytologic abnormalities: a systematic review. *Ann Intern Med* 2000;132(10):810–819.
22. Abdulla KN, Alheshimi SJ, Aljebory HDS, Altaei TJK. Evaluation of Pap smear data in Baghdad province. *Int J Sci Res Publ* 2016;6(5).
23. World Health Organization. Human papillomavirus (HPV) and cervical cancer. Geneva: World Health Organization; 2023. Available at: [https://www.who.int/news-room/fact-sheets/detail/human-papillomavirus-\(hpv\)-and-cervical-cancer](https://www.who.int/news-room/fact-sheets/detail/human-papillomavirus-(hpv)-and-cervical-cancer).
24. Kessels SJM, Marshall HS, Watson M, Braunack-Mayer AJ, Reuzel R, Tooher RL. Factors associated with HPV vaccine uptake in teenage girls: a systematic review. *Prev Med* 2019;122:124–134.
25. Drolet M, Bénard É, Pérez N, Brisson M. Population-level impact and herd effects following human papillomavirus vaccination programmes: a systematic review and meta-analysis. *Lancet Infect Dis* 2019;19(5):565–580.
26. Holman DM, Benard V, Roland KB, Watson M, Liddon N, Stokley S. Barriers to human papillomavirus vaccination among US adolescents: a systematic review of the literature. *Am J Prev Med* 2019;56(4):591–602.
27. McBride KR, Singh S. Predictors of adults' knowledge and awareness of human papillomavirus (HPV), HPV-associated cancers, and HPV vaccines: a cross-sectional study. *Prev Med* 2018;111:95–102.
28. World Health Organization. WHO guideline for screening and treatment of cervical pre-cancer lesions for cervical cancer prevention. 2nd ed. Geneva: World Health Organization; 2021.