Article

Not peer-reviewed version

Human Papillomavirus E6 /E7 mRNA Testing in the Follow-Up of Patients Treated for High-Grade Cervical Intraepithelial Neoplasia

Adolfo Loayza Galindo * , Alicia Hernandez Gutierrez , Ana Margarita Rodriguez Garcia , Belen Lopez Cavanillas , Cristina Gonzalez Benitez , David Hardisson , Itziar De la Peña Navarro , Maria Serrano Velasco , Rocio Arnedo Muñoz , Ignacio Zapardiel

Posted Date: 22 January 2024

doi: 10.20944/preprints202401.1576.v1

Keywords: cervical intraepithelial neoplasia; conization; human papillomavirus; E6 and E7 mRNA.

Article

Human Papillomavirus E6 /E7 mRNA Testing in the Follow-Up of Patients Treated for High-Grade Cervical Intraepithelial Neoplasia

Adolfo Loayza Galindo [1,*], Alicia Hernández Gutiérrez [1], Ana M. Rodríguez García [2], Belén López-Cavanillas [1], Cristina González-Benítez [1], David Hardisson [3], Itziar de la Peña Navarro [2], María Serrano Velasco [1], Rocío Arnedo Muñoz [1] and Ignacio Zapardiel [4]

[1] Department of Gynecology and Obstetrics. La Paz University Hospital, Madrid, Spain.
[2] Departament of Pathology. La Paz University Hospital, Madrid, Spain.
[3] Department of Pathology, Hospital Universitario La Paz, Madrid, Spain. Center for Biomedical Research in the Cancer Network (Centro de Investigación Biomédica en Red de Cáncer, CIBERONC), Instituto de Salud Carlos III, Madrid, Spain. Molecular Pathology and Therapeutic Targets Group, La Paz University Hospital (IdiPAZ), Madrid, Spain. Faculty of Medicine, Universidad Autónoma de Madrid, Spain.
[4] Gynecologic Oncology Unit. La Paz University Hospital, Madrid, Spain.
* Correspondence: address: adolfomusi@gmail.com

Abstract: In the follow-up of treated high-grade cervical intraepithelial neoplasia (HSIL/CIN) lesions, cervical cytology has a high false-negative rate, while high-risk human papillomavirus (HR-HPV) DNA testing showed high sensitivity and lower specificity. The detection of messenger RNA of the HR-HPV E6 and E7 oncoproteins (E6/E7 mRNA) seems to be an indicator of viral integration and it allows the identification of severe lesions. Our objective was to assess the clinical utility of E6/E7 mRNA determination in the follow-up of patients treated for HSIL/CIN. A retrospective observational study including 407 patients treated for HSIL/CIN was performed. The recurrence rate and the validity of E6/E7 mRNA testing were evaluated. The recurrence rate of high-grade lesions was 1.7%. The sensitivity of the test was 88% in the first clinical revision and 100% in the second and third revisions. Specificity was 91% in the first revision, 92% in the second revision, and 85% in the third revision. The positive predictive value was 18% in the first medical revision, 10% in the second and 14% in the third. The negative predictive value was 100% in all follow-up visits. In conclusion, the E6/E7 mRNA test seems to be effective in ruling out recurrence after treatment for HSIL/CIN lesions.

Keywords: cervical intraepithelial neoplasia; conization; human papillomavirus; E6 and E7 mRNA

1. Introduction

High-grade cervical intraepithelial neoplasia (HSIL/CIN) is the precursor lesion of cervical cancer (CC). Human papillomavirus (HPV) is responsible for 98.7% of CC (1) . Patients treated for pre-invasive lesions of the cervix have an elevated risk (5% to 30%) of having a persistence or recurrence of the lesion and, therefore, an increased risk of invasive cancer (2). In general, persistent disease is considered when HPV is detected at the first check-up after treatment (during the first 6-12 months); however, recurrent disease is defined when it re-appears after the first normal check-up.

Currently, the follow-up tests after treatment for HSIL/CIN include cervical cytology, HPV testing and colposcopy. Cervical cytology has a low sensitivity (62%), which limits its effectiveness (3). HPV DNA testing detects the L1 region, the structural protein of the virus. DNA testing detects the virus in patients who present a high prevalence, such as young women. It is known that most infections are transient, without clinical significance. This would explain the low specificity (75%) of the DNA test for the detection of HSIL/CIN or cancer (4) . In severe lesions, the entire viral genome or fragments thereof are integrated into the chromosomal DNA of the host cell. The most important

 doi:10.20944/preprints202401.1576.v1

consequence of this process is the overexpression of the HPV oncoproteins E6 and E7. These play a crucial role in carcinogenesis since they lead to increased genomic instability, acquisition of oncogenic mutations and eventual malignant transformation (5). The detection of E6 and E7 messenger RNA (E6/E7 mRNA) of high-risk HPV (HR-HPV) is an indicator, not only of infection, but also of viral integration, allowing the identification of more severe lesions. Follow-up studies in patients with low-risk cervical lesions (LSIL/CIN) conclude that the E6/E7 mRNA test has a higher specificity and the same sensitivity compared to DNA test, allowing greater accuracy in the selection of women at risk of LSIL/CIN progression (6,7).

Our objective was to analyze the recurrence rates after treatment for HSIL/CIN and the clinical utility of the HR-HPV E6/E7 mRNA test in the follow-up of these patients.

2. Materials and Methods

After IRB approval, a retrospective observational study was conducted to evaluate the clinical utility of the diagnostic tests detecting HPV mRNA. We reviewed 407 patients treated for CIN grade 2-3 between June 2015 and June 2018. All patients were older than 18 years of age. They were tested for E6/E7 HR-HPV mRNA at all clinical controls after treatment.

Data on the following variables were collected: age of the patient at the time of diagnosis, cytological and histological diagnosis, therapeutic procedure, pathological outcome, margins in case of conization or LLETZ (large loop excision of the transformation zone), number of revisions, follow-up time, and results of the tests performed at each revision. The first revision was performed at 6 months, 4 months if the margin was positive after conization, and at 18, 42 and 78 months after treatment. A cervical cytology, HPV mRNA test and colposcopy, with directed biopsy if required, were performed at each follow-up visit.

Any abnormal cervical cytology was considered as recurrence. Treatment failure was defined as the presence of residual or recurrent high-grade lesion confirmed by cervical cytology or biopsy at first clinical visit.

A complete gynecological examination was performed at each control. The liquid-based cervical cytology sample was taken with both endocervical brush and spatula. The sample was processed by the Pathology Department. The fixed sample was processed in ThinPrep 5000 (Thinprep Hologic, Bedford, MA), following the manufacturer´s recommendations. The presence of viral RNA was evaluated using the Aptima HPV Assay (Hologic Iberia S.L., Madrid, Spain). It is a qualitative molecular test for the detection of 14 HPV genotypes (16/18/31/33/35/39/45/51/52/56/58/59/66/68) that later allows differentiation between types 16 and 18/45, following the manufacturer´s recommendations. In the processing of cervical biopsies the samples fixed in 10% buffered formalin were embedded in paraffin and cut into 4-micron sections and stained with hematoxylin-eosin.

Qualitative data were described using absolute frequencies and percentages. Quantitative data were described using mean ± standard deviation. Comparison between groups of qualitative variables was performed with the Chi-square test. The usefulness of the mRNA test was estimated by calculating the sensitivity, specificity, and predictive values, with their corresponding 95% confidence intervals. The alpha error was set at 5%. Statistical analyses were performed with the SPSS v15 software (IBM corp., Armouk, USA).

3. Results

A total of 407 patients were included: 401 (97.4%) women with histological diagnosis of HSIL/CIN, 297 (73%) CIN 2 and 104 (25.6%) of CIN 3; and 6 (2.6%) patients treated without histological confirmation. The mean age was 37.6 ± 9.08 years. The mean follow-up time of the patients was 3.78 ± 1.87 years.

The treatment performed was conization in 362 (88.9%) cases. 253 (69.8%) were performed due to CIN 2, 103 (28.4%) due to CIN 3 and 6 (1.6%) with no previous histological diagnosis. Laser vaporization was performed as treatment in 45 (11.1%) women. Among them, 43 (95.6%) for CIN 2 and 2 (4.4%) for CIN 3.

 doi:10.20944/preprints202401.1576.v1

The histological results of the conization specimen were CIN 2 in 220 (60.7%) patients, CIN 3 in 96 (26.6%) and no residual lesion in 46 (12.7%) cases. The margins of the conization specimen were free in 266 (84.0%) cases and affected in 50 (16.0%).

During the follow-up period, 55 (13.5%) patients of the total presented an abnormal cervical result of any grade; among them, 7 (1.7%) were high grade. Of the 45 patients treated with laser vaporization, 7 (15.5%) low-grade recurrences and none high-grade recurrence were detected.

Table 1 shows the results of mRNA testing in each clinical visit and the cases of persistence and recurrence. A total of 986 tests were performed. Table 2 shows the mRNA test results and recurrences according to the type of treatment, including patients who underwent conization.

During the follow-up, among the 55 patients who had an abnormal result of any grade, 33 (60%) had a positive mRNA test result. Of the 352 patients who had normal results, 49 (13.9%) had a positive mRNA test during follow-up. These differences were statistically significant ($p<0.001$).

Among the 266 cases with negative margins in the conization specimen, 32 (12%) had abnormal results of any grade during follow-up; of the 50 cases with positive margins, 11 (22%) were pathologic results, these differences were not statistically significant ($p=0.064$).

Among the 7 high-grade recurrences, 3 (1.1%) were diagnosed among the 266 patients with negative margins, and 4 (8%) among the 50 patients with positive margins. These 4 cases had positive mRNA testing. Considering the 17 patients with positive margins and positive mRNA test, only 4 had high-grade recurrence.

The sensitivity, specificity and predictive values of the mRNA test are shown in Table 3. Due to the absence of high-grade recurrences in the fourth, fifth and sixth revisions, the validity and safety of the test could not be determined for these clinical visits.

Table 1. Results of mRNA testing, persistence and recurrence. Data is n and %.

Clinical Visit	First	Second	Third	Fourth	Fifth	Sixth
Negative mRNA	355 (89.9%)	311 (91,2%)	141 (83.4%)	48 (78.7%)	11 (78.6%)	5 (83,3%)
Positive mRNA	40 (10.1%)	30 (8.8%)	28 (16.6%)	13 (21.3%)	3 (21.4%)	1 (16.7%)
Any Grade Recurrence	26 (6,6%)	15 (4.4%)	10 (6%)	6 (10%)	2 (14.3%)	0 (0%)
High Grade Recurrence	3 (0.6%)	1 (0.3%)	3 (1,8%)	0 (0%)	0 (0%)	0 (0%)

Table 2. Positive mRNA test and recurrences according to treatment.

	Positive mRNA	Any Grade Recurrence	High Grade Recurrence	Total
Laser vaporization	9 (20 %)	7 (15.5%)	0 (0%)	45
Conization	73 (20.1%)	48 (13.2%)	7 (1.9%)	362
[a]No residual lesion	6 (13%)	5 (10.8%)	0 (0%)	46
[a]HSIL/CIN	67 (21.2%)	43 (13.6%)	7 (2.2%)	316
[b]Free	50 (18.7)	32 (12%)	3 (1.1%)	266
[b]Affected	17 (34%)	11 (22%)	4 (8%)	50

 doi:10.20944/preprints202401.1576.v1

Abbreviations: HSIL/CIN, high-grade cervical intraepithelial neoplasia, Data is n or n (%). [a]After conization. [b]Margins after conization.

Table 3. Validity and safety of the mRNA test.

	First Review		Second Review		Third Review	
	Any Grade	**High Grade**	**Any Grade**	**High Grade**	**Any Grade**	**High Grade**
Sensitivity	41 (26-55)	88 (65-110)	56 (37-75)	100 (100-100)	67 (40-93)	100 (100-100)
Specificity	94 (91-96)	91 (89-94)	95 (93-97)	92 (89-95)	87 (82-92)	85 (80-91)
PPV	45 (30-60)	18 (6-29)	47 (29-65)	10 (2-16)	29 (12-45)	14 (1-27)
PNV	93 (90-95)	100 (100-100)	100 (94-99)	100 (100-100)	97 (94-100)	100 (100-100)

Abbreviations: VPP, positive predictive value; VPN, negative predictive value; CI: confidence interval. Data is % (95% CI).

4. Discussion

The sensitivity of the mRNA test for the detection of cervical dysplasia of any grade is low: 41% at the first review, 56% at the second review and 67% at the third review. The explanation for these results is because it is designed to detect high-grade lesions. Since low-grade lesions express fewer oncogenes, the accuracy of these tests decrease. Several studies with different mRNA detection tests, have shown that the level of expression of this molecule is related to the degree of the lesion to detect (8,9). This justifies the improved diagnostic yield values in our study when only high-grade lesions are considered: 70% in the first review and 100% in the second and third review.

The objective of the follow-up of patients treated for HSIL/CIN is to rule out new or persistent precancerous lesions. A diagnostic test with high sensitivity and NPV should be available (10). The test evaluated in the present study presents these characteristics. In the first clinical visit one HSIL was detected by cytology; but in the second and third visits all high-grade recurrences were detected by the mRNA test.

Tropé et al. reported a low sensitivity, 45.5 % (95% CI: 26.8-65.5) and high NPV, 96.2 % (95 % CI: 93.5-97.8) of the mRNA test for predicting high-grade CIN at 6 months after conization. The test used was based on detection of full-length E6 and E7 mRNA of HR-HPV types 16, 18, 31, 33, and 45. The follow-up of that study was 18 months. The authors concluded that the low sensitivity of mRNA testing could be explained by the low expression of oncogenes in the new infections (10).

In another study of 143 cases, with a mean follow-up of 3.6 years, Persson M et al. reported a low sensitivity, 57.1 % (95% CI 25.0-84.2), and a high specificity, 93.4 % (95% CI 87.9-96.5). The same mRNA test was used as in our study. In addition, the test was performed from liquid-based recovered samples that were used for HPV DNA analysis. The authors concluded that the false-negative rate of HPV E6 and E7 could caused by three reasons: the distribution of HR-HPV types in the stored samples; the quality of the samples stored; and the period between collection and testing, between 6 and 12 months (11).

In a larger study, 475 patients treated by conization between 2003 and 2010, Frega A et al. detected a higher sensitivity of 73.5% and a NPV of 97% for the detection of recurrences. E6 and E7 mRNA testing of HPV types 16, 18, 31, 33, and 45, was based on a qualitative technique of nucleic acid real-time amplification (NASBA). The recurrence rate was 21%. The study differentiated between residual and recurrent disease in order to analyze the accuracy of the test as a predictive

 doi:10.20944/preprints202401.1576.v1

value for recurrent disease. Recurrence was defined as the appearance of LSIL or HSIL after negative colposcopy and cytology at 3 and 6 months after conization. The authors concluded that the HPV mRNA test has a higher sensitivity and higher NPV in predicting recurrent disease and should be used in the follow-up of patients treated for HSIL/CIN with conization (12).

The sensitivity and NPV results of our study seem to be consistent since almost 1,000 mRNA tests were analyzed. Based on it, from our point of view in the first visit, with the aim of ruling out a persistent lesion, the mRNA detection test and cytology should be included. In subsequent clinical visits, with the aim of ruling out recurrence, only the mRNA test could be performed and if it is negative, the other tests could be applied (cytology, HPV DNA test or colposcopy). The results of screening studies reinforce this conclusion: the HR-HPV E6 and E7 mRNA test used in our center showed similar sensitivity and slightly higher specificity for the detection of HSIL/CIN compared to the standard HPV DNA-based test (13–15).

On the other hand, our results showed a high specificity and a low PPV in the follow-up of patients treated for HSIL/CIN with conization, the positivity of the studied mRNA test would not predict HSIL/CIN recurrences. However, the high-grade recurrence rate in our study is low, 1.72%, compared to other studies (10,11). This low probability would justify the PPV data. The study by Zappacosta R. et al. (16) included 116 patients treated with conization between 2008 and 2010 reporting a recurrence rate of 8.6%. The HPV mRNA test used, based on real-time amplification of E6 and E7 of the 5 most frequent HPV types, presented a specificity of 100% and a PPV 100%, higher than those detected with the combination of cytology and HPV DNA test. They concluded that the inclusion of mRNA in the follow-up protocol could predict earlier the risk of recurrent lesions after conization; consequently, reducing overtreatment, especially in women older than 30 years. According to our results and the demonstrated association between the affected margin of the conization specimen and the mRNA test, closer monitoring should be indicated in patients with a positive mRNA test during follow-up, regardless of the low PPV detected.

The meta-analysis by Arbyn M et al. demonstrated that the risk of residual or recurrent HSIL/CIN is significantly higher when the margins of the conization specimen are affected (17). Our study also detected that the likelihood of recurrence was higher when margins were affected (22% of recurrences of any grade with affected margins and 12% of all-grade recurrences with free margins), and in the case of high-grade recurrences (8.0% compared to 1.1% with free margins).

Our study also detected that the probability of any-grade recurrence is higher in patients with an affected margin compared with free-margin specimens (22% vs. 12%, respectively). Same findings were observed for high-grade recurrence (8% vs. 1.1%, respectively). In addition, 4 of the 7 high-grade recurrences diagnosed had had an affected margin in the specimen and a positive mRNA test.

Recurrence rates of any grade were similar regardless of the treatment performed, (laser vaporization or conization). In the group of patients treated with laser vaporization, no high-grade recurrence was detected. This could be justified by the selection of patients since they were mostly small lesions, CIN 2 and with transformation zone type 1. The absence of high-grade recurrences in the group of patients without lesion in the conization specimen could be justified by the small extension of the initial lesion which probably was completely excised with the diagnostic biopsy.

The recurrence rate of HSIL/CIN in our population was low compared to other series. Persson et al, detected a 4.9% recurrence rate in 143 treated between 1999 and 2009; and Tropé et al, reported 6.4% recurrence rate in 344 treated between 2005 and 2006 (10,11). The low rate found in our study could be related to HPV vaccination. This would be consistent with the conclusions of both meta-analysis by Lichter K and Kechagias KS, published in 2020 and 2022, respectively, which concluded that adjuvant HPV vaccination in the context of surgical excision for CIN 2 or 3 is associated with a lower risk of recurrent CIN (18,19).

5. Conclusions

In the follow-up of patients treated for HSIL/CIN, the mRNA detection test shows high sensitivity and high NPV in the detection of persistent disease. Patients could be monitored at the first visit after treatment with HPV mRNA test and cytology.

In the following visits the mRNA test alone seems to be appropriate; if it is negative, no further testing would be required. Patients with positive margins on the conization piece and positive mRNA test should be closely monitored. Recurrence rates are similar regardless of the therapy used, conization and laser vaporization.

Author Contributions: ALG and IZ designed the project; ALG, AMRG, IPN, and RAM acquired the data; ALG, IZ, and CGB analyzed and interpreted the data; ALG, AMRG, BLC, RAM, and IZ wrote and edited the paper; MSV, AHG, and DHH supervised the work and contributed to the design of the manuscript. The authors have not received any form of payment to produce the paper. All authors have read and accepted the final version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The protocol of the present study was approved by the Ethics Committee for Research with Medicines of the La Paz University Hospital of Madrid on April 7, 2022. HULP internal code: PI-5227. 2022-020.

Informed Consent Statement: The need for informed consent was waived as part of the ethics approval of our study due to the retrospective design and low risk to the subjects.

Data Availability Statement: This study is based on real-world patient data, including demographics and comorbidity factors, that cannot be communicated due to patient privacy concerns.

Acknowledgments: To Mariana Díaz Almirón, coordinator of the Biostatistics Section of the Research Institute (IdiPAZ) of the La Paz University Hospital, Madrid.

Conflicts of Interest: The authors declare that they have no conflict of interest with respect to funding, authorship and/or publication of this article.

References

1. Arbyn M, Tommasino M, Depuydt C, Dillner J. Are 20 human papillomavirus types causing cervical cancer? J Pathol. 2014 Dec;234(4):431–5.
2. Arbyn M, Sasieni P, Meijer CJLM, Clavel C, Koliopoulos G, Dillner J. Chapter 9: Clinical applications of HPV testing: a summary of meta-analyses. Vaccine. 2006 Aug 31;24 Suppl 3:S3/78-89.
3. Nobbenhuis MA, Meijer CJ, van den Brule AJ, Rozendaal L, Voorhorst FJ, Risse EK, et al. Addition of high-risk HPV testing improves the current guidelines on follow-up after treatment for cervical intraepithelial neoplasia. Br J Cancer. 2001 Mar 23;84(6):796–801.
4. Kocken M, Uijterwaal MH, de Vries ALM, Berkhof J, Ket JCF, Helmerhorst TJM, et al. High-risk human papillomavirus testing versus cytology in predicting post-treatment disease in women treated for high-grade cervical disease: a systematic review and meta-analysis. Gynecol Oncol. 2012 May;125(2):500–7.
5. Duensing S, Münger K. Mechanisms of genomic instability in human cancer: insights from studies with human papillomavirus oncoproteins. Int J Cancer. 2004 Mar 20;109(2):157–62.
6. Benevolo M, Vocaturo A, Caraceni D, French D, Rosini S, Zappacosta R, et al. Sensitivity, specificity, and clinical value of human papillomavirus (HPV) E6/E7 mRNA assay as a triage test for cervical cytology and HPV DNA test. J Clin Microbiol. 2011 Jul;49(7):2643–50.
7. Macedo ACL, Gonçalves JCN, Bavaresco DV, Grande AJ, Chiaramonte Silva N, Rosa MI. Accuracy of mRNA HPV Tests for Triage of Precursor Lesions and Cervical Cancer: A Systematic Review and Meta-Analysis. J Oncol. 2019;2019:6935030.
8. Ratnam S, Coutlee F, Fontaine D, Bentley J, Escott N, Ghatage P, et al. Aptima HPV E6/E7 mRNA test is as sensitive as Hybrid Capture 2 Assay but more specific at detecting cervical precancer and cancer. J Clin Microbiol. 2011 Feb;49(2):557–64.
9. Andersson E, Kärrberg C, Rådberg T, Blomqvist L, Zetterqvist BM, Ryd W, et al. Type-specific human papillomavirus E6/E7 mRNA detection by real-time PCR improves identification of cervical neoplasia. J Clin Microbiol. 2011 Nov;49(11):3794–9.
10. Tropé A, Jonassen CM, Sjøborg KD, Nygård M, Dahl FA, Alfsen GC, et al. Role of high-risk human papillomavirus (HPV) mRNA testing in the prediction of residual disease after conisation for high-grade cervical intraepithelial neoplasia. Gynecol Oncol. 2011 Nov;123(2):257–62.
11. Persson M, Brismar Wendel S, Ljungblad L, Johansson B, Weiderpass E, Andersson S. High-risk human papillomavirus E6/E7 mRNA and L1 DNA as markers of residual/recurrent cervical intraepithelial neoplasia. Oncol Rep. 2012 Jul;28(1):346–52.
12. Frega A, Sesti F, Lombardi D, Votano S, Sopracordevole F, Catalano A, et al. Assessment of HPV-mRNA test to predict recurrent disease in patients previously treated for CIN 2/3. J Clin Virol. 2014 May;60(1):39–43.

13. Haedicke J, Iftner T. A review of the clinical performance of the Aptima HPV assay. J Clin Virol. 2016 Mar;76 Suppl 1:S40–8.
14. Iftner T, Neis KJ, Castanon A, Landy R, Holz B, Woll-Herrmann A, et al. Longitudinal Clinical Performance of the RNA-Based Aptima Human Papillomavirus (AHPV) Assay in Comparison to the DNA-Based Hybrid Capture 2 HPV Test in Two Consecutive Screening Rounds with a 6-Year Interval in Germany. J Clin Microbiol. 2019 Jan;57(1):e01177-18.
15. Arbyn M, Simon M, de Sanjosé S, Clarke MA, Poljak M, Rezhake R, et al. Accuracy and effectiveness of HPV mRNA testing in cervical cancer screening: a systematic review and meta-analysis. Lancet Oncol. 2022 Jul;23(7):950–60.
16. Zappacosta R, Ianieri MM, Tinelli A, Gustapane S, D'Angelo C, Gatta DMP, et al. Detection of residual/recurrent cervical disease after successful LEEP conization: the possible role of mRNA-HPV test. Curr Pharm Des. 2013;19(8):1450–7.
17. Arbyn M, Redman CWE, Verdoodt F, Kyrgiou M, Tzafetas M, Ghaem-Maghami S, et al. Incomplete excision of cervical precancer as a predictor of treatment failure: a systematic review and meta-analysis. Lancet Oncol. 2017 Dec;18(12):1665–79.
18. Lichter K, Krause D, Xu J, Tsai SHL, Hage C, Weston E, et al. Adjuvant Human Papillomavirus Vaccine to Reduce Recurrent Cervical Dysplasia in Unvaccinated Women: A Systematic Review and Meta-analysis. Obstet Gynecol. 2020 May;135(5):1070–83.
19. Kechagias KS, Kalliala I, Bowden SJ, Athanasiou A, Paraskevaidi M, Paraskevaidis E, et al. Role of human papillomavirus (HPV) vaccination on HPV infection and recurrence of HPV related disease after local surgical treatment: systematic review and meta-analysis. BMJ. 2022 Aug 3;378:e070135.