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Molecular diagnosis of HPV by PCR combined with cervical cytology in the prevention of cervical cancer

Diagnóstico molecular do HPV por PCR associado à citologia cervical na prevenção do câncer do colo do útero

Diagnóstico molecular del VPH mediante PCR asociado con la citología cervical en la prevención del cáncer de cuello uterino

Maria Clara Cremonesi Nastari – Centro Universitário Module. E-mail: mariaclaracremo@gmail.com
Jessieli Santos Trindade – Centro Universitário Module. E-mail: jessielitrindade21@gmail.com
Luiz Carlos Barone – Advisor.

Abstract: Cervical cancer is an important public health problem and is causally associated with persistent infection by Human Papillomavirus (HPV), particularly high-risk oncogenic types. This study aimed to analyze the contribution of molecular HPV diagnosis through Polymerase Chain Reaction (PCR), combined with cervical cytology, to the early detection of viral infection and cervical abnormalities associated with cervical cancer in women aged 25 to 35 years. A qualitative and descriptive analysis was conducted based on scientific studies published between 2020 and 2026, as well as national and international documents and guidelines related to HPV screening and diagnosis. The studies analyzed indicate that PCR has a high capacity to detect HPV DNA, including in situations in which significant cellular abnormalities are not yet identified by cytology, while cytopathological examination enables the identification of morphological changes and intraepithelial lesions. The results also highlight the relevance of identifying high-risk genotypes and combining molecular and cytological methods to broaden risk assessment and guide the follow-up of women with positive results. It is concluded that the complementary use of PCR and cervical cytology can contribute to improving cervical cancer screening strategies by facilitating the early identification of HPV infection and precursor lesions, enabling timely interventions and contributing to the prevention of disease progression.

Keywords: Human Papillomavirus; Cervical cytology; Cervical cancer; Polymerase Chain Reaction.

Resumo: O câncer do colo do útero constitui um importante problema de saúde pública, apresentando relação causal com a infecção persistente pelo Papilomavírus Humano (HPV), especialmente pelos tipos de alto risco oncogênico. Este estudo teve como objetivo analisar a contribuição do diagnóstico molecular do HPV, por meio da Reação em Cadeia da Polimerase (PCR), associado à citologia cervical na detecção precoce da infecção viral e de alterações cervicais relacionadas ao câncer do colo do útero em mulheres de 25 a 35 anos. Foi realizada uma análise qualitativa e descritiva a partir de estudos científicos publicados entre 2020 e 2026, além de documentos e diretrizes nacionais e internacionais relacionados ao rastreamento e diagnóstico do HPV. Os estudos analisados indicam que a PCR apresenta elevada capacidade de detectar o DNA do HPV, inclusive em situações nas quais ainda não são observadas alterações celulares significativas pela citologia, enquanto o exame citopatológico possibilita a identificação de alterações morfológicas e lesões intraepiteliais. Os resultados também evidenciam a relevância da identificação dos genótipos de alto risco e da associação entre métodos moleculares e citológicos para ampliar a avaliação do risco e orientar o acompanhamento de mulheres com resultados positivos. Conclui-se que a utilização complementar da PCR e da citologia cervical pode contribuir para o aprimoramento das estratégias de rastreamento do câncer do colo do útero, favorecendo a identificação precoce da

infecção pelo HPV e de alterações precursoras, além de possibilitar intervenções oportunas e contribuir para a prevenção da progressão da doença.

Palavras-chave: Papilomavírus Humano; Citologia cervical; Câncer do colo do útero; Reação em Cadeia da Polimerase.

Resumen: El cáncer de cuello uterino constituye un importante problema de salud pública, presentando relación causal con la infección persistente por el Papilomavirus Humano (VPH), especialmente por los tipos de alto riesgo oncogénico. Este estudio tuvo como objetivo analizar la contribución del diagnóstico molecular del VPH, por medio de la Reacción en Cadena de la Polimerasa (PCR), asociado a la citología cervical en la detección precoz de la infección viral y de alteraciones cervicales relacionadas al cáncer del cuello uterino en mujeres de 25 a 35 años. Se realizó un análisis cualitativo y descriptivo a partir de estudios científicos publicados entre 2020 y 2026, además de documentos y directrices nacionales e internacionales relacionados al rastreo y diagnóstico del VPH. Los estudios analizados indican que la PCR presenta elevada capacidad de detectar el ADN del VPH, inclusive en situaciones en las cuales todavía no son observadas alteraciones celulares significativas por la citología, mientras que el examen citopatológico posibilita la identificación de alteraciones morfológicas y lesiones intraepiteliales. Los resultados también evidencian la relevancia de la identificación de los genotipos de alto riesgo y de la asociación entre métodos moleculares y citológicos para ampliar la evaluación del riesgo y orientar el acompañamiento de mujeres con resultados positivos. Se concluye que la utilización complementar de la PCR y de la citología cervical puede contribuir para el perfeccionamiento de las estrategias de rastreo del cáncer del cuello del útero, favoreciendo la identificación precoz de la infección por el VPH y de alteraciones precursoras, además de posibilitar intervenciones oportunas y contribuir para la prevención de la progresión de la enfermedad.

Palabras clave: Papilomavirus Humano; Citología cervical; Cáncer de cuello uterino; Reacción en cadena de la polimerasa.

1 Introduction

Cervical cancer is an important public health problem, presenting a causal relationship with persistent infection by Human Papillomavirus (HPV), especially due to the types of high oncogenic risk. Although it is a potentially preventable neoplasm through vaccination, screening and appropriate treatment of precursor lesions, the disease still poses a challenge for health systems. In 2022, approximately 662,000 new cases and 349,000 deaths from cervical cancer in the world were estimated, highlighting the relevance of expanding prevention and early detection strategies (WORLD HEALTH ORGANIZATION, 2024a).

HPV infection is frequent and in most cases can be eliminated by the body without causing persistent changes. However, prolonged infection by high-risk genotypes may promote progressive changes in the cervical epithelium, favoring the development of precursor lesions and later invasive cancer. In this context, the identification of HPV presence and early

recognition of cervical changes are relevant for risk stratification and for adequate follow-up of women.

Historically, cervical cytology has played an important role in screening cervical cancer, allowing the identification of morphological changes in cervical cells. However, because it mainly evaluates cellular alterations, cytology does not directly identify the presence of viral genetic material. Thus, molecular methods capable of detecting HPV DNA have become more important in screening strategies, especially due to the possibility of identifying infection before the development of significant cytological changes. The World Health Organization (WHO) has prioritized the use of tests for HPV detection in screening, highlighting the need for molecular methods with adequate performance, accessibility and possibility of implementation in different health contexts (WORLD HEALTH ORGANIZATION, 2024b).

Among the available molecular methodologies, Polymerase Chain Reaction (PCR) stands out for the ability to amplify specific sequences of viral DNA, enabling HPV identification even when the amount of genetic material present in the sample is reduced. The use of molecular methods also allows the identification and, depending on the methodology used, genotyping of HPV types associated with different levels of oncogenic risk. The most recent WHO recommendations highlight the importance of HPV genotyping in the stratification of the risk of women with positive test, allowing the direction of follow-up according to the viral type identified (WORLD HEALTH ORGANIZATION, 2026).

In Brazil, this discussion gained relevance with the publication, in 2025, of the new Brazilian Guidelines for the Tracking of Cervical Cancer by the National Cancer Institute (INCA). The document recommends the oncogenic DNA-HPV test, with partial or extended genotyping, as a primary screening method, basing this recommendation on evidence related to the reduction of incidence and mortality by the disease (NATIONAL CANCER INSTITUTE, 2025).

Although molecular tests have gained greater prominence in contemporary screening strategies, cervical cytology remains relevant for the evaluation of morphological changes of the epithelium. The complementary use of molecular and cytological methods may provide distinct information on the presence of the infectious agent and on the cellular changes resulting from the infection. Thus, the association between molecular HPV detection and cytological evaluation may contribute to a broader approach to cervical health, especially in the identification of women who need further investigation or follow-up.

Given this scenario, it is relevant to analyze the contribution of the molecular diagnosis of HPV through PCR associated with cervical cytology in the detection of viral infection and

cervical changes related to the development of cervical cancer. Understanding the contribution of these methodologies can help in discussing screening strategies and early identification of women at higher risk, contributing to disease prevention and control actions.

2 Theoric mark

Human Papillomavirus (HPV) is the main etiological agent associated with cervical cancer. Infection occurs predominantly through sexual contact and in most cases is eliminated by the body within 24 months. However, some women have persistent infection with oncogenic genotypes, increasing the risk of developing cervical intraepithelial lesions and later cancer. Progression usually occurs slowly and gradually, and may start with low-grade lesions (LSIL), which may regress or progress to high-grade lesions (HSIL) and, in certain cases, to invasive cancer. This period allows the identification and treatment of precursor lesions through screening.

During the persistence of infection by high-risk genotypes, the integration of viral genetic material to host cell DNA may favor the expression of oncoproteins E6 and E7. These proteins interfere with cell cycle regulation, contributing to cell transformation and development of neoplastic changes. Thus, viral persistence is relevant in the evolution of cervical changes and reinforces the need for early identification of infection.

Molecular diagnostic methods enable direct identification of HPV genetic material in cervical samples. Among them, the Polymerase Chain Reaction (PCR) allows amplifying specific DNA sequences, enabling the detection of the virus even when present in low amounts. Molecular tests have greater ability to identify infection when compared to isolated cytology, and may detect viral genetic material before the appearance of morphological changes. Some methodologies also allow identifying high-risk genotypes, especially types 16 and 18, in addition to evaluating viral activity through messenger RNA of oncoproteins E6 and E7. According to Liu et al. (2021), this approach has greater clinical specificity in certain situations, because it is related to the transcription activity of viral oncogenes.

In the study by Liu et al. (2021), women with negative cytology for intraepithelial injury or malignancy (NILM) and positive test for high-risk HPV were evaluated. Even without identifiable cytological changes, relevant cervical lesions were observed. Among women positive for HPV 16, the prevalence of HSIL or more severe lesions was 19.1%, while among those positive for HPV 18/45, the prevalence of adenocarcinoma in situ (AI) was 3.3%. In the cytology group NILM and high-risk HPV, approximately 7.6% had HSIL or more severe

lesions and 0.7% AIS. The results also showed higher frequency of HSIL or more severe lesions among women positive for HPV 16, while the other 11 genotypes had lower values, highlighting the importance of genotyping in risk assessment.

The analysis of the selected articles demonstrates the importance of molecular methods in cervical cancer screening. HPV DNA research has high sensitivity to identify infection and precursor lesions, while cytology remains relevant in the evaluation of cellular changes and screening of women with positive molecular results. In the study analyzed, the presence of high-risk HPV occurred even in women with NILM cytology, demonstrating that the absence of cytological alterations does not exclude the possibility of precursor lesions.

In addition, Liu et al. (2021) demonstrated that the test based on the detection of E6/E7 mRNA associated with cytology could reduce referrals to colposcopy by more than 30% when compared to the combination between cytology and HPV DNA tests, in addition to presenting reduction of false-positive results. Thus, the results reinforce the relevance of integration between molecular methods and cervical cytology in cervical cancer screening and prevention.

3 Material and method

The present study was developed through a qualitative and descriptive study with the aim of analyzing the contribution of the molecular diagnosis of HPV through polymerase chain reaction (PCR), associated with cervical cytology, in the prevention of cervical cancer.

For the study, 15 scientific articles published from 2020 were selected, obtained from the Google Academic and PubMed databases, as well as four institutional documents. Studies related to the diagnosis of HPV infection, the application of the PCR technique, cervical cytology and the prevention and early detection of cervical cancer were considered.

After selection, the studies were submitted to reading and analysis, considering information regarding diagnostic methods, HPV identification and associated use of molecular and cytological methods. Relevant information was organized and compared according to the proposed objectives, allowing the identification of the main findings related to HPV diagnosis and the detection of cervical changes.

4 Results and discussion

The analysis of the selected studies showed the relevance of the diagnosis of Human Papillomavirus (HPV) infection in cervical cancer screening and prevention strategies. The studies analyzed showed advances in the use of molecular methods. Polymerase Chain Reaction

(PCR) stands out among the molecular methodologies used to identify HPV by allowing the amplification of specific sequences of viral genetic material. Silva (2024) describes the application of CRP in the molecular diagnosis of HPV and highlights its use in the detection of viral genetic material and in the identification of different genotypes. Similarly, Williams, Kostiuk and Biron (2022) describe the use of PCR and other molecular techniques for HPV detection, highlighting its application in the identification of oncogenic types and in the evaluation of samples related to diseases associated with the virus.

The literature also shows that molecular methods have different characteristics in relation to conventional cytology. Martins and Levi (2025) describe the evolution of molecular methods applied to cervical cancer screening, highlighting the use of tests for HPV detection as part of contemporary screening strategies. In this context, cytology remains a method capable of identifying morphological changes in cervical cells, while molecular tests allow identifying the presence of HPV and, depending on the methodology employed, determining specific genotypes of greater clinical relevance.

The results of the meta-analysis performed by Rocha et al. (2026) reinforce this difference between the methodologies. The study evaluated eight clinical trials, totaling 414,846 participants, and found a detection rate of cervical cancer grade 2 or higher (CIN2+) 61% higher in groups submitted to HPV-based tests compared to groups submitted to conventional or liquid cytology (RR = 1.61; 95%CI: 1.30– 1.98). The authors also highlighted that the use of HPV tests may be associated with the identification of a greater number of lesions, requiring the adoption of risk stratification strategies and follow-up of women with positive results.

The association between cytology in liquid medium and CRP for high-risk HPV was also investigated by de Lima et al. (2021). The study evaluated the joint use of these methodologies in cervical cancer screening, demonstrating the real-time application of CRP for the detection of high-risk HPV in samples used for cytological evaluation.

The identification of viral genotype is another relevant aspect observed in the studies analyzed. Liu et al. (2021) evaluated 26,228 women with negative cytological results for intraepithelial injury or malignancy (NILM) and positive test for high-risk HPV. Among these women, 5,893 presented biopsy results or histological evaluation of immediate follow-up. We identified 7.6% cases of HSIL or more severe squamous lesions and 0.7% of adenocarcinoma in situ (AI). The prevalence of HSIL+ lesions was significantly higher among women positive for HPV 16, while glandular AIS+ lesions showed a higher prevalence among women positive for HPV 18/45.

These findings are relevant because they demonstrate that a cytological result NILM does not necessarily mean absence of HPV infection or absence of changes that can be identified later. The importance of integration between different methods is also observed in the study by Varesano et al. (2025), which evaluated a molecular diagnostic flow using samples of cervical tissue fixed in formalin and included in paraffin. In the study, HPV DNA was identified in 56.4% of the 55 samples analyzed, and 21 genotypes were detected. The authors also observed an association between higher viral load and high-grade lesions, indicating that molecular information, such as genotyping and viral load, can complement morphological evaluation and contribute to risk stratification. However, the authors themselves point out that the sample size was limited, requiring additional evaluations to validate the flow in different contexts.

The studies analyzed also indicate that the use of molecular tests does not eliminate the need for complementary evaluation strategies. The HPV detection identifies the presence of the viral agent, but the interpretation of the result should consider the genotype identified, the patient's history and the methods used to assess cervical changes. In this sense, the association between molecular testing and cytology may provide additional information, especially in women with high-risk HPV and initially negative cytology.

In general, the results found in the studies analyzed indicate an increase in the use of molecular methods in cervical cancer screening. PCR presents application in the detection of HPV genetic material and in the identification of genotypes, while cytology allows the evaluation of morphological changes of cervical cells. Data from Liu et al. (2021), from Lima et al. (2021), Martins and Levi (2025), Varesano et al. (2025) and Rocha et al. (2026) demonstrate different applications of these methodologies and provide evidence on the complementarity between molecular identification of infection and cell evaluation.

Thus, considering the studies evaluated, the association between molecular HPV diagnosis and cervical cytology allows to gather different information on the presence of infection, the viral type identified and the occurrence of cellular alterations. This approach can be applied as part of the strategies for screening and monitoring cervical cancer, especially given the results that demonstrate the presence of precursor lesions in women with high-risk HPV even when the initial cytology does not present changes.

5 Final considerations

This study allowed us to understand the contribution of using different methods for the screening and diagnosis of HPV infection, especially given the relationship between the types of high oncogenic risk and the development of cervical cancer. Early identification of the infection and the changes caused by the virus may favor adequate monitoring and contribute to the reduction of lesion progression.

Cervical cytology remains a relevant method for the identification of cellular alterations, while its association with molecular biology techniques, such as PCR, enables the collection of complementary information. PCR allows us to detect HPV DNA and identify high-risk types even when there are still no evident cellular changes in cytology. Thus, the joint use of these methodologies can provide additional information for risk assessment and follow-up of women with positive results.

The identification of genotypes with higher oncogenic potential may help to define women who need greater attention and follow-up. The prevention of cervical cancer depends on the articulation between screening strategies, early diagnosis, adequate follow-up and preventive measures against HPV infection.

In this context, the association between CRP and cervical cytology can contribute to the improvement of screening strategies, enabling the identification of women at higher risk for the development of cervical lesions. It is concluded that the integration between molecular PCR diagnosis and cervical cytology has the potential to increase the detection of HPV infection and associated alterations, especially when the results are interpreted according to viral genotype, cytological findings and clinical context. The continuity of research and the improvement of diagnostic techniques can contribute to more efficient and accessible screening strategies, strengthening prevention actions and contributing to the reduction of the occurrence and mortality related to cervical cancer.

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