SEVEN
publicações acadêmicas

Early diagnosis of Cervical Cancer using tumor biomarkers P16 and Ki67: A systematic review

https://doi.org/10.56238/interdiinovationscrese-017

Ellen Nunes Feitoza
Undergraduate student in Pharmacy
Institution: Centro Universitário Fametro
E-mail: ellen.nunes15@hotmail.com

Daniel de Oliveira Oliveira
Graduating in Pharmacy
Institution: Centro Universitário Fametro
E-mail: oliverdan2016@gmail.com

Bruna Mendes Carvalho
Undergraduate student in Pharmacy
Institution: Centro Universitário Fametro
E-mail: mbrunahair26@gmail.com

Micaele Lima Andrade
Undergraduate student in Pharmacy
Institution: Centro Universitário Fametro
E-mail: micaele2022@gmail.com

ABSTRACT
INTRODUCTION: Cervical cancer is a malignant neoplasm located in the cervix (portion between the end of the vagina and the uterus) and is a disease that evolves over the years. To diagnose this disease, several tools are needed, among them is the use of biomarkers, such as the P16 and Ki67 biomarkers. OBJECTIVE: To describe the tumor biomarkers P16 and ki67 and describe their usefulness for the identification, diagnosis and early prognosis of cervical cancer. METHODS: This is a systematic review study, using the PRISMA method of systematic reviews and meta-analyses. Virtual libraries such as Scielo, PubMed and BVS were used to search for articles, with keywords in Portuguese, English and Spanish. Articles with a publication date within the last 10 years were included, within the central theme of this study. RESULTS: To support this work, 15 articles were selected to support the development of the present study. Among the biomarkers addressed, P16 stands out as a tumor suppressor protein, playing a fundamental role in the regulation of the cell cycle. Its overexpression within cervical cells indicates intraepithelial neoplasms. The biomarker Ki67 is another protein and this one related to cell proliferation that indicates the progression of cervical intraepithelial neoplasms. In normal tissue, it is found exclusively in parabasal cells, its overexposure in other layers of cervical tissue indicates dysregulation of the cell cycle caused by HPV. Scientific studies show that the biomarkers P16 and Ki67 are better when used together, because the coexpression of these two proteins result in dysregulation of the cell and are usually detectable in higher grade lesions, the higher the degree of injury, the greater the expression of these two proteins. CONCLUSIONS: Based on the studies found, tumor biomarkers p16 and Ki-67 have been shown to be valuable tools in the diagnosis and prognosis of cervical cancer. The presence of p16 is indicative of a high-grade precursor lesion, while Ki-67 expression is related to cell proliferation and tumor activity. The associated use of these biomarkers can provide more accurate information about the stage or degree of cervical injury of the disease and assist in making therapeutic decisions. Therefore, the analysis of these markers is essential for the effective clinical approach to cervical cancer.

Keywords: Tumor biomarkers, Cervical Cancer, Tumor biomarkers of Cervical Cancer, Early diagnosis of Cervical Cancer, P16, Ki-67.

1 INTRODUCTION

Cervical cancer or cervical cancer is a malignant neoplasm located in the portion between the end of the vagina and the uterus, being a disease that has a slow evolution, thus allowing its early

diagnosis and treatment of the lesions. Its development is directly linked to persistent Human Papillomavirus (HPV) infection such as HPV 16 and HPV 18 related to 70% of HPV cancers in the world (WHO, 2022). There are also some risk factors that facilitate the evolution of this type of cancer, such as smoking, early initiation of sexual intercourse, multiplicity of sexual partners, multiparity, immunosuppressants, co-infection with other sexually transmitted agents (which cause herpes simplex, chlamydia and gonorrhea), use of oral contraceptives and age, which can be a risk factor as well. (MURTA; et al., 1999; MELO, 2009; WHO, 2019).

In Brazil, cervical cancer is in third place for the malignant type of cancer that most affects women in the country, with an average of 570 thousand new cases of cervical cancer per year, with an estimated risk of 15.38 cases per 100 thousand Brazilian women, in 2020 6,627 deaths were recorded (BRASIL, 2022; INCA, 2022).

Vaccination is one of the ways to prevent cervical cancer. The vaccine is distributed free of charge by the Unified Health System (SUS) and is indicated for girls and boys aged 9 to 14 years, with a 2-dose schedule. Among the most common HPV subtypes are those with greater oncogenic potential (HPV 18 and HPV 16), most of the time cervical HPV infection is trasatory and regresses spontaneously between six months and two years after exposure (WHO, 2008). However, the vaccine does not protect against all types of HPV with oncogenic potential and needs to be in conjunction with early detection methods, such as the Pap smear, to successfully prevent and fight cancer (BRASIL, 2022; MESQUITA, et al., 2020).

It is important to say that the use of condoms prevents about 80% of HPV transmission. Because it is a disease of slow evolution, it is possible to use cervical cancer prevention treatment, since transmission occurs mainly through sexual contact. (INCA, 2020).

The early detection and identification of cervical lesions are the central objectives of cervical cancer screening and are based on three tests: gynecological cytopathology, colposcopy and biopsy. Biopsy is considered the standard for the identification of lesions.

The use of biomarkers used by pathologists in the differential diagnosis of CINs (Cervical Intraepithelial Neoplasia) and their transitions require specific biomarkers that minimize disagreement among analysts, helping in the classification of lesions and in the early identification of cervical disease.

These tumor biomarkers are proteins released by normal human cells as well as cancer cells, however, cancer cells release in greater quantities. For this reason, they serve as a screening method with analyses of various body products, such as blood, urine, and others (ONCOGUIA, 2020). The literature presents several existing tumor biomarkers for the detection of cervical cancer, including P16 and Ki67 (UTAGAWA, et al., 2021).

The biomarker P16 is a tumor suppressor, that is, it aims to reduce the incidence of tumors, preventing kinases that are cyclin-dependent, however, if this protein gene is poorly expressed, modified or inactivated, it may be linked to the appearance of malignant tumors, such as cervical cancer. In HPV, as the virus infects the cell and induces cell proliferation by inactivating the pRb (retinoblastoma protein) by the E7 gene, it causes an extremely high level expression of P16, which can be detected in immunohistochemical tests (MUNHOZ, 2009; FEBRASGO, 2018).

The biomarker ki67 is a non-histone protein, that is, a protein that acts efficiently in the repair and expression of DNA, assiduously active in meiosis and mitosis, for this reason it is an excellent marker of cell proliferation being linked to malignant neoplasms. However, this marker is only commonly seen in the cells of the basal layer in the uterine cervix, which is the most superficial layer and is in successive renewal, but when it is detected at high levels in cells of the deeper layers of the cervix, it acts as an alert to the incidence of cervical cancer caused by HPV, classifying them into types of lesions and degrees according to the deepening and expression of this marker in the layers of the uterine cervix (MUNHOZ, 2009; SILVA, 2017).

This study will use the existing literature to demonstrate the usability of detecting these biomarkers in cervical cancer investigation and diagnostic exams, since these biomarkers are promising for the diagnosis of cervical cancer in early stages, facilitating and anticipating the treatment of affected women, causing the incidence of deaths to decrease over the years.

Thus, the general objective of this study is the tumor biomarkers P16 and ki67 used for the diagnosis of cervical cancer, specifying important points, such as:

- Describe the characteristics and usability of tumor biomarkers P16 and ki67 used in the diagnosis of cervical cancer;
- To associate the degree of injury with the tumor biomarkers P16 and ki67 used in the diagnosis and prognosis of cervical cancer.
- To compare the efficacy of the biomarkers P16 and Ki-67 in the diagnosis of cervical cancer.

2 METHODOLOGY

This is a systematic review study, based on the PRISMA form. The search for scientific articles was carried out in the PUBMED, VHL, and SCIELO virtual libraries. The literature search was carried out using the following keywords: in Portuguese, the words were as follows: Tumor biomarkers of cervical cancer; early diagnosis of cervical cancer; tumor biomarker P16; tumor biomarker Ki-67; tests that detect the tumor biomarker P16; tests that detect the tumor biomarker Ki-67. Cervical cancer tumor biomarkers; early diagnosis of cervical cancer; tumor biomarker P16; tumor biomarker Ki-67; tests that detect the tumor biomarker P16; tests that detect the tumor biomarker Ki-67. E in Spanish: Tumor

biomarkers of cancer of uterine cancer; Diagnosis of uterine cancer cancer; tumor biomarker P16; tumor biomarker Ki-67; pruebas that detect the tumor biomarker P16; pruebas that detect the tumor biomarker Ki-67.

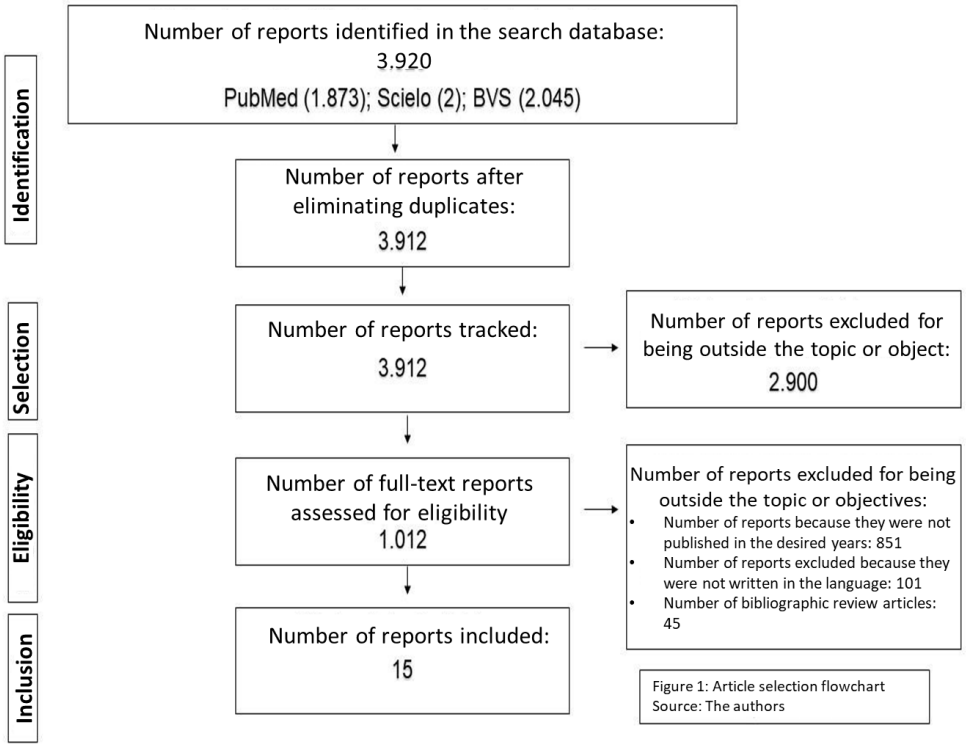
The inclusion criteria chosen to be part of this systematic review are: observational studies, experimental studies, case reports, and cohort studies; articles published from January 1, 2013 to May 2023; written in Portuguese, English and Spanish and articles related to the early diagnosis of cervical cancer using tumor biomarkers P16 and ki67.

The exclusion criteria were: systematic review articles, literature review, meta-analysis, and opinion articles; articles published until December 2012; written in languages other than Portuguese, English and Spanish and outside the chosen theme.

3 RESULTS

To support this work, a search was carried out within the digital platforms, in the first stage, 3,920 articles were initially registered, in which the platform that stood out was the virtual health library-VHL of this screening 2,045 articles found, following on the platforms with the keywords PubMed presented 1,873 articles and Scielo, 2 articles, using the keywords in the three languages, Portuguese, English and Spanish, most of the articles were in the English language. The second stage was the organization of the process of choosing the articles in a reliable way, where some stages were established that mark the quantitative aspects of this process, expressed in Figure 1. The third stage was the selection of the 15 articles selected for the theoretical development of the present study, the flowchart below was used, where all the steps were performed so that all relevant articles were reported in this study, within the inclusion and exclusion criteria already mentioned.

Figure 1 – Flowchart of the process of choosing eligible articles.



Source: Prepared by the authors.

Table 1 – Summary of the information of the main articles selected by the search.

No.	Authors/Year	Title/Article	Type of Study	Objective	Conclusions
	MATA, S., FERREIRA, J., NICOLÁS, I., ESTEVES, S., et al. 2021.	P16 and HPV Genotype Significance in HPV-Associated Cervical Cancer-A Large Cohort of Two Tertiary Referral Centers.	Cohort	To verify the prognostic significance of the P16 biomarker; to analyze the factors that lead to the meanings of overexpression of the P16 gene, to observe the behavior of the HPV genotype and genes in the outcome of patients with HPV-associated cervical cancer (CHD).	Overexpression of HPV16, α-9, and P16 genera has been associated with better survival in patients with HPV-associated cervical cancer. The results confirm the existence of a small number of HPV-associated cervical cancers that do not overexpress P16 (5%). These patients tend to be older and have advanced disease, both of which are poor prognostic factors.
	TSAKOGIANNIS D., MOSCHONAS G. D., BELA E., et al. 2018	Association of p16 (CDKN2A) polymorphisms with the development of HPV16-related precancerous lesions and cervical cancer in the Greek population	Transverse	To clarify whether P16 genotypes/haplotypes have the potential to emerge as essential biomarkers for the prognosis of cervical disease in the Greek population.	The P16 C540G polymorphism influences the susceptibility of patients to more severe dysplasia. This polymorphism may emerge as a valuable biomarker for the development of high-grade Squamous

					Intraepithelial Lesion (HSIL).
	ZUBERI Z., MREMI A., CHILONGOLA J. O., et al. 2021.	Expression analysis of p16 and TOP2A protein biomarkers in cervical cancer lesions and their correlation with clinic-histopathological characteristics in a referral hospital, Tanzania	Cross-sectional retrospective	To evaluate the usefulness of P16 and TOP2A as potential biomarkers in dysplastic and malignant changes of the cervical epithelium by analyzing a range of benign, precancerous, and cancerous cervical lesions, so as to assess whether their expression may be useful for prognosis in cervical carcinogenesis in Tanzania.	Overexpression of TOP2A is related to the degree of cervical intraepithelial neoplasia, but does not predict the prognosis of cervical cancer. Similarly, the expression of P16 is related to the degree of histological dysplasia and malignancy, suggesting its prognostic and preventive value in the treatment of cervical cancers.
	ISHIKAWA M., NAKAYAMA K., NAKAMURA K., et al. 2021	P16(INK4A) expression might be associated with a favorable prognosis for cervical adenocarcinoma via dysregulation of the RB pathway	Transverse	To investigate the relationship between P16 expression and patient prognosis. In addition, to evaluate the potential relationship between P16 expression and immune checkpoint inhibitor-related therapy in cervical adenocarcinoma.	P16 expression can be used as a biomarker to improve the prognosis of patients with cervical adenocarcinoma. The findings suggest that the status of P16 expression may influence prognosis.
	Li, C., Zheng, M., Zheng, X., et al.	Predictive Ki-67 Proliferation Index of Cervical Squamous Cell Carcinoma Based	Diagnostic Test Validation	Determine IVIM-DWI (Diffusion Weighted Image	Texture analysis in IVIM-DWI (Motion Diffusion Weighted Image)
	2021	on IVIM-DWI Combined with Texture Features		Incoherent Motion (Intravoxel) combined with preoperative IVIM-DWI-based texture features can be used to predict Ki-67, which is a widely used biomarker of cell proliferation in cervical cancer (CC).	Incoherent Intravoxel) and its parameters was useful for predicting Ki-67. This may provide a non-invasive method to investigate important imaging biomarkers for cervical cancer.
	WANG X., LI S., LU Y., et al. 2022	Evaluation of tracer kinetic parameters in cervical cancer using dynamic contrastenhanced MRI as biomarkers in terms of biological relevance, diagnostic performance and inter-center variability	Cross-Sectional Retrospective	To evaluate the clinical value of immunohistochemical test parameters of the biomarkers Ki67 and CD34 dynamic contrast MRI on the correlation with angiogenesis and proliferation of cervical cancer, diagnostic performance and	CD34 and Ki67 counts in cervical cancer tissue were significantly larger than in normal cervical tissue. The extracellular volume (Ve) of each of the five tracer kinetic models was significantly lower in cervical cancer tissue than in normal

				reproducibility of dynamic contrast MRI parameters.	cervical tissue, indicating greater proliferation of cervical cancer cells.
	SARMA U., OF C. G., SARMAH B. 2021	Predictive Value of Marker of Proliferation Ki-67 and Cell Cycle Dependent Protein kinase Inhibitor P16INK4a in Cervical Biopsy to Determine Its Biological Behavior.	Cross-Sectional Retrospective	The aim of the study is to analyze the immunohistochemical expression of Ki67 and P16 in cases of CIN and cervical cancer and their usefulness to determine the accuracy of the histological diagnosis and predict the biological behavior of the cervical lesion.	Histopathology remains the "gold standard" for diagnosing low- and high-grade CIN. Biomarkers such as Ki67 and P16 have emerged as useful adjuvants. Its combined use can aid in the histopathological classification of pre-invasive lesions.
	CLARKE, M., A.; CHEUNG, L.,C.; CASTELO, P.,E., et al. 2019	Five-Year Risk of Cervical Precancer Following p16/Ki-67 Dual-Stain Triage of HPV-Positive Women	Prospective cohort	To evaluate the longitudinal performance of P16 and Ki67 using dual-stain screening for detection of cervical precancer in HPV-positive women over 5 years of follow-up in the context of clinical management thresholds	Screening with the P16 and Ki67 biomarkers provides better long-term risk stratification than 5-year cytology. Low risk of cervical precancer allows for safe extension of follow-up intervals for 3 years. It also reduces the risk of cervical cancer recurrence in women.
	KANTHIYA K., KHUNNARONG J., TANGJITGAMOL S., et al. 2016.	Expression of the p16 and Ki67 in Cervical Squamous Intraepithelial Lesions and Cancer	Transverse	To evaluate the expression of P16 and Ki67 in cervical intraepithelial neoplasia (CIN) and cancer.	Both markers had high sensitivity and specificity in the determination of >CIN2. The expression rates of P16 and Ki67 were directly associated with the severity of cervical injuries. Significant differences in the expression of these markers may be useful in cases with doubtful histological features between cervical intraepithelial lesions and non-dysplastic lesions.

	HAMMER A., Gustafsono L. W., Christensen P. N., et al. 2020	Implementation of p16/Ki67 dual stain cytology in a Danish routine screening laboratory: Importance of adequate training and experience	Diagnostic Test Validation	To describe the authors' experience with the implementation of P16/Ki67 double staining for screening of elderly women with abnormal screening results in a Danish routine screening laboratory.	When interpreting older women's double-stained slides, the agreement increased slightly as novice raters received more training and experience. While further evaluation is needed, these findings indicate that a significant amount of training may be required to ensure accurate interpretation of dual staining in this age group.
	HAMASHIMA C. 2021	Emerging technologies for cervical cancer screening	Literature review	Compare the sensitivity and specificity of the p16/Ki67 double staining technique with cytology, and estimate them.	When P16/Ki67 double staining was used for screening for human papillomavirus testing, the sensitivity of 2 or more detection of cervical intraepithelial neoplasia (CIN2+) was higher than that of cytology without decreased specificity.
	ZHANG S. K., JIA M.M., ZHAO D.M., ET AL. 2019	Evaluation of p16/Ki-67 dual staining in the detection of cervical precancer and cancer in China	Diagnostic Test Validation Study	To evaluate the clinical performance of P16/Ki67 double staining in the detection of grade 2 or 3 cervical intraepithelial neoplasia (CIN2+/CIN3+) in Chinese women.	P16/Ki-67 double staining could probably provide an optional method for China's national cervical cancer screening and could also be considered an efficient screening method for the management of women with ASC-US (Atypical Cells of Undetermined Significance).
	LOOKING FOR A M., MENTZELOPOULOU, P., MAGKANA, E., et al. 2021	The p16/ki-67 assay is a safe, effective and rapid approach to triage women with mild cervical lesions	Diagnostic Test Validation Study	The aim of this study was to evaluate the diagnostic accuracy and efficiency of P16/ki-67 double staining in the identification of cervical intraepithelial neoplasia (CIN2+) in Greek women	The results of the study indicate that p16/ki-67 is a safe and rapid assay that can be used to detect cervical intraepithelial neoplasia (CIN2+) among women with mild cervical lesions, with high sensitivity
				with cytology: atypical cells of undetermined significance or low-grade intraepithelial lesion.	and specificity and can minimize the psychological and economic burden of HPV screening.

	Ahmad A., Raish M., Shahid M., et al. 2017	The synergic effect of HPV infection and epigenetic anomaly of the p16 gene in the development of cervical cancer	Prospective cohort	To evaluate the expression and methylation of the P16 promoter in squamous cell carcinoma of the uterine cervix infected with HR-HPV.	An association between P16 methylation, expression, and HR-HPV infection suggested that compliance of HPV infection and alteration of the P16 gene have a synergistic effect on cervical carcinoma initiation and progression.
	JIN M., WANGL., ZHENG T., ET AL. 2021	MiR-195-3p inhibits cell proliferation in cervical cancer by targeting BCDIN3D	Clinical Trial	To explore the potential role of mir-195-3p in cervical cancer progression and the use of the Ki67 biomarker in the tracking control of this progression.	The present research led us to a conclusion that mir-195-3p can inhibit cervical cancer cell proliferation and was inversely regulated by BCDIN3D. Ki67 protein expression was inhibited by mimickers of mimicry or BCDIN3D silence. This suggests that miR-195-3p/BCDIN3D si-RNA mimics may be used in the treatment of cervical cancer in the future, following several animal trials and clinical trials.

Source: Prepared by the authors.

4 DISCUSSION

4.1 DEFINITION OF P16 AND KI67 BIOMARKERS

According to the authors MELO and MELO (et al, 2021, 2018), the P16 protein has received special attention as an immunohistochemical biomarker for the diagnosis of cervical cancer, due to the overexpression of the P16 protein in cervical neoplasms having been associated with HPV infection. Since the inactivity of the pRb protein, by association with the HPV E7 protein, increases the release of the transcription factor E2F, that is, it is determinant in the increase of the expression levels of the P16 protein. In a range of 10 to 100%, hypermethylation occurs in the tumor cell community in precursor or invasive squamous cervical lesions. What nullifies the negative control of the P16 protein is the loss of transcription of the P16 gene, causing a deviation of cells with damaged DNA from the cell cycle, causing it to facilitate disordered cell proliferation.

In the case of the immunohistochemical biomarker ki67, it is used for the diagnosis of cervical cancer because the nuclear antigen Ki67 has been associated with cell proliferation, since the P53 protein, which regulates the cell cycle in G1/S and G2/M, is degraded by the E6 oncoprotein, influencing the increase in viral DNA replication. Without P53, the diseased cells do not break down and die, causing the cancerous tissue to continue growing. The Ki67 biomarker is expressed during the

G1/S and G2/M cell cycle, which demonstrates confidence to be used in the diagnosis of cervical injuries (PAIVA, 2016; MELO et al., 2021; SILVA, 2016).

The association of these two biomarkers can demonstrate a more comprehensive and clear efficacy in the diagnosis of cervical cancer and define the degree of injury, both are protein biomarkers that are revealed through immunohistochemistry stains also called immunocytochemistry. This staining is used in slide smears collected during the Pap smear and tissues removed in biopsies (MELO et al., 2021; SILVA, 2016).

4.2 USABILITY, DEGREE OF INJURY, AND EFFICACY OF THE P16 BIOMARKER IN THE EARLY DIAGNOSIS OF CERVICAL CANCER

In studies conducted by MATA, et al (2021), it was reported in a cohort study with 348 women with a mean age of 47.5 years, affected by HPV-associated cervical cancer, that 80% were squamous cell carcinoma, 19% of the women had adenocarcinoma, and four other rarer subtypes (three adenosquamous carcinomas and one neuroendocrine carcinoma). Among these 348 women, 241 were infected with HPV16 aged between 46 and 49 years, among these 241 women, 70% had invasive cervical cancer (CCI), HPV18 was also associated with ICC, but represented only 9.7% of the percentage. Another interesting number was that 330 were P16-positive, that is, only 18 women out of 348 were P16-negative. According to the authors, the number of P16-negative women was higher in older women, about 59 years old, because in them the stage of the disease was more advanced and there is an increased risk of mortality. The study also explains that the reasons for the overexpression of P16 associated with HPV in women under 46 years of age may be due to several factors, such as the loss of heterozygosity and the mutation of the CDKN2A gene located on chromosome 9, p21 band of these patients.

Thus, the overexpression of the biomarker in the immunohistochemical test, which would be feasible for identification and differentiation of cervical cancer, prevailed in younger women.

In the same study, it was observed that 241 women infected with HPV16 had a better clinical result than those infected with other types of HPVs, but a curiosity was observed, a patient with CHD infected with HPV6 was associated with P16 overexpression, and the patient was living considerably well, even after 15 years of diagnosis. It is known that this type of HPV was already commonly known to offer a low risk of invasive lesions and slow progression (MATA, et al., 2021).

In a comparative study of 146 HPV1-positive women, conducted between 2013 and 2015, including 42 with low-grade squamous intraepithelial lesions (LSIL), 44 high-grade squamous intraepithelial lesions (HSIL), 10 with cervical cancer, and 50 in the control group with normal cervical samples and without HPV16, it was shown that women who have P16 540 CG/GG genotype are 2.7 times more likely to develop HSIL in association with HPV16 infection; the 540G/580C haplotype

was also 3.67 times more likely to be associated with the development of HSIL, i.e., having genotypes with the G allele confers a higher risk of developing high-grade squamous intraepithelial lesions, a precursor lesion of cervical cancer. The results further demonstrate that P16 C540G polymorphisms can lead patients to develop more severe dysplasia and suggest that this polymorphism can be used as a biomarker for HSIL. However, another polymorphism investigated in the study, P16 C580T, was not associated with the development of cervical lesion in the patients studied (TSAKOGIANNIS, et al., 2018).

Following this literature review of P16 as a biomarker for cervical cancer, a retrospective study conducted by ISHIKAWA (et al., 2021) that used 82 samples from patients from a hospital in Japan, observed that of the 82 samples, 60 (73.1%) of the patients had a strong expression of P16, it was also possible to observe that 48 (58.5%) of the 82 women had adenocarcinomas larger than the 2 previous stages but that had not yet spread to the lymph nodes; 49 (59.6%) women had tumors smaller than 4 cm and 19 (23.2%) of them died as a result of cervical adenocarcinoma. Of the 82 patients analyzed with cervical adenocarcinoma, they had extremely better progression-free survival and overall survival with positive P16, when compared to those with negative P16.

The study also confirms that the overexpression of P16 is positively associated with the prognosis, suggesting that the possibility of P16 also functioning as a tumor suppressor gene should be explored, since the loss of P16 accentuates the phosphorylation of the retinoblastoma (RB) protein (pRB), so cell proliferation is suppressed and tumors that express P16 would advance with a satisfactory prognosis by inducing invasive cells of the patient's immune system (ISHIKAWA et al., 2021).

We can affirm that the P16 protein can be a biomarker of satisfactory results, since its overexpression is always seen in most cases and in patients. The studies analyzed about P16 are studies from different places, which demonstrate that it is not only in some populations that there is an expression of P16 in precancerous and cancerous lesions, but that there is a globalization regarding this protein expression, which indicates that this marker has the potential to be used in the future as a tumor suppressor.

4.3 USABILITY, DEGREE OF INJURY, AND EFFICACY OF THE KI67 BIOMARKER IN THE EARLY DIAGNOSIS OF CERVICAL CANCER

According to LI (et al., 2021) in a study conducted in China, with 70 patients, 16 patients had a Ki67 proliferation index <50% and 54 patients had a Ki67 proliferation index >50%, but there was no noticeable difference between the degree of lesion and/or age of the patients, i.e., the higher the histological grade of the tumor, The more potent is the multiplication of tumor cells. And also the higher the Ki 67 level, the greater the correlation with the histological grade of the tumor. The study

also points out that Ki67 is not only related to the multiplicability of tumor cells and their ability to invade adjacent tissues, but also to the control of efficacy and quality of the therapy being used in the patient, be it radiotherapy or chemotherapy. However, due to the number of patients analyzed by the study, it was insufficient to define other parameters with greater precision.

In another retrospective study with 113 patients with a mean age of 56 years, 95 of whom had been diagnosed with cervical cancer and 18 with cervical fibroids, it used the comparison between three different techniques, imaging with histological analysis of dynamic contrast magnetic resonance imaging (DMMR) with immunohistochemical testing with the biomarker Ki67, and imaging with histological analysis of dynamic contrast magnetic resonance imaging (CDMR) with immunohistochemical testing with the CD34 biomarker. It was not possible to perform immunohistochemical analysis in all women because there were clinical treatment recommendations that prevented them from being eligible for this test, so 26 women were eligible for this technique, of which 14 were cervical cancer positive and 12 were positive for cervical fibroids (WANG, et al., 2022).

Of these 26 women, it was only possible to perform the CD34 immunohistochemical test on samples from 6 women with cervical cancers and 5 women with cervical fibroids. For the immunohistochemical analysis of the Ki67 biomarker, it was possible to analyze more samples, 13 women with cervical cancers and 10 women with cervical fibroids. The result was: in both, there was greater quantification of expression in tissues with cervical cancer, with CD34 (20.35±5.82) being higher than in normal cervical tissue (5.98±2.77) (<0.05) and Ki67 (65%±29%) in cervical cancer (WANG, et al., 2022).

It is noteworthy that the analysis of both biomarkers was impaired, especially the CD34 biomarker, because there was an exaggeration of tissue in the slide making it difficult to read it, this demonstrates, within the theme of the present study, that compared to the CD34 biomarker and magnetic resonance imaging with dynamic contrast. The biomarker Ki67 obtained a higher quantified expression in tumor tissues, thus being a more efficient biomarker than these other 2 techniques for diagnosing cervical cancer (WANG, et al., 2022).

In the study by SILVA (2017), it is possible to observe that the degree of lesion is related to the labeling pattern of P16 and Ki67: when the patient did not have intraepithelial neoplasia, the staining test was negative, and the higher the level of injury, the greater the immunohistochemical expression of the two biomarkers. In the samples analyzed, there was a prevalence of positive staining for CIN3 (P16 62.2% and Ki 67 85.66%) and invasive carcinoma (P16 71.8% and Ki67 87.2%) for CIN1 lesions there was a prevalence of negative cases (P16 91.7% and Ki67 85.8%), while for CIN2 there was a dominance of positive results for Ki67 and dominance of negative results for P16, This fact would indicate that there is no adequate cut-off point or that this class includes high-grade lesions, which could advance to neoplasms or low-grade lesions that would have the possibility of regression. There

is no specific limit for the Ki67 biomarker to distinguish between CIN2 cases and upper and lower lesions, but the behavior appears to be similar to P16, perhaps it can be combined or replaced, as the use of Ki67 is expanding in the diagnostic and prognostic system of many other neoplasms. This finding requires further investigation, since the use of Ki67 did not improve the quality of research results in this study.

4.4 COMPARATIVE STUDIES BETWEEN EFFICACY OF KI67 AND BIOMARKERS P16

In 2021, a retrospective cross-sectional study conducted by SARMA (et al., 2021), used 110 biopsy cases from the Gauhati Medical College hospital, archived from June 2014 to May 2016. Of these 110 cases, 27 had cervical cancer, 10 cases had chronic inflammation of the cervix, 5 had metaplastic alterations, and 68 of them had an initial diagnosis of cervical intraepithelial neoplasia in varying degrees.

In 57 (51.8%) of the 110 cases, the study revealed the P16 expression level, according to the diagnostic situation as follows: chronic cervical inflammation/cervitis: 0 (0%) cases; metaplasia: 2 (1.8%) cases; Non-dysplastic cases: 55 (50%) cases. In 61 (55.5%) of the 110 cases, the study revealed the level of Ki67 expression, according to the diagnostic situation, as follows: chronic cervical inflammation/cervitis: 2 (1.8%) cases; metaplasia: 2 (1.8%) cases; non-dysplastic cases: 57 (51.8%) cases (SARMA et al., 2021).

Thus, the efficiency parameters of both were compared as follows: the sensitivity of Ki67 was 84.0% and the sensitivity of P16 was 92.0%. The specificity of Ki67 was 67.1% and the specificity of P16 was 64.7%. The sensitivity of the two markers when used together was 92.45% and the specificity was 100% (SARMA et al., 2021).

Previously, in 2019, a prospective cohort study was published that aimed to evaluate the risk of precancer after screening using P16/Ki67, in this study 1,549 women with an average age of 42.2 years were included, in order to determine the risk of acquiring cancer from being infected with HPV, the follow-up lasted 3.7 years (with intervals of 0/2/5.4 years). The results were: 1,308 women had a grade lower than grade 2 cervical intraepithelial neoplasia (CIN2); 110 women were diagnosed with grade 3 cervical intraepithelial neoplasia (CIN3); 108 had grade 2 cervical intraepithelial neoplasia (CIN2); 12 women had adenocarcinoma in situ and 11 women were diagnosed with cervical cancer (CLARKE et al, 2019).

In KANTHIYA (et al., 2016) described in their cross-sectional study that aimed to respond to the expression of P16 and Ki67 in intraepithelial lesions of cervical squamous cells and cancer, with 243 clinical cases, with women with a mean age of 40.4 years and with a primary diagnosis of: 106 with grade 1 cervical intraepithelial neoplasia; 61 with grade 2/3 cervical intraepithelial neoplasia; 53 women with non-dysplastic lesions and 23 invasive carcinomas. This study revealed a result of P16

expression in 85 cases with a percentage of 35.0% and Ki67 expression in 99 cases with a percentage of 40.7%.

In the same study, the expression of P16 in each lesion grade was: carcinoma 91.3%, CIN2/3 78.7%, CIN1 10.4%, non-dysplastic lesions 9.4%. The expression of Ki67 was: 100% of all invasive carcinomas, CIN2/3 75.4%, CIN1 22.6% and 11.3% non-dysplastic, reaching the conclusion that expression of P16 and Ki67 was significantly different between CIN2/3 and CIN1, where for CIN2/3 P16 84.5% and Ki 67 90.5%, for CIN1 P16 82.1% and Ki67 88.6%. Therefore, the expressions of both biomarkers were totally associated with the degrees of cervical lesions, especially in the higher degrees such as carcinoma and CIN2/3, i.e., the greater the degree of injury, the greater its expressions, thus being able to obtain false-negatives in the diagnosis of low-grade cervical lesions, but the association of the 2 biomarkers can define the diagnosis regarding the degree of lesion with more clarity and objectivity (KANTHIYA et al., 2016).

According to the articles studied, the P16/Ki67 double staining test proved to be effective in the detection and prognosis of cervical cancer, but here are some aspects about its usability: through the studies it was observed that in fact the usability of the double staining of P16 and Ki67 was effective in answering whether in 5 years women would progress to cancer, since 705 women with a percentage of 45.5% were positive for double staining, i.e., they had both P16 and Ki67 expression, and 785 with a percentage of 50.7% had ASC-US cytology (lesions likely to become cancer) without expressing P16 and Ki67 at the beginning of the study. As the studies progressed, it was observed that the positivity of the double staining of P16 and Ki67 was increased along with the observation of cytological worsening in 32% of women negative for intraepithelial lesion and malignancy, to 83% in women with high-grade intraepithelial squamous lesions; It also increased double staining positivity by 22% in women without biopsy; to 77% in women with CIN3 and 91% in women with cancer (CLARKE et al., 2019).

HAMMER (et al., 2020) discusses the usability and implementation of the double staining of P16 and Ki67 in a laboratory routine in Denmark, emphasizing the importance of having adequate training to perform this technique, which so far has shown promise in terms of accuracy in the diagnosis of cervical cancer. This study obtained as a result of its analysis, in 50 slides randomly chosen from the 600 slides available, 45 of them belonged to women aged between 65 and 69 years with a positive HPV test and 5 from women aged around 45 years referred for colposcopy with abnormal screening test. The 50 slides were analyzed by cytotechnicians and specialists previously trained and with experience in reading the cervical slide, but without experience in double staining, however, they were trained before the analysis began. For the results of the double staining, both cytotechnicians reported exactly the same results. Among cytotechnicians and specialists, there was 95% agreement and 84.0% overall agreement.

Novice evaluators, when submitted to training, are extremely important in the development of the safe implementation of double staining, increasing the agreement of results between novice evaluators and specialists, as disagreement could lead to erroneous results. It is important that a training network is established with dual staining specialists and cytotechnicians from routine screening laboratories to achieve slide interpretation skills. Only with additional training and time of experience in double staining, the level of analysis agreement among professionals is improved and perfected, making it fully possible to use double staining in cervical slide screening to reach the final diagnosis (HAMMER et al., 2020).

Regarding the usability of double staining tests, another important aspect is the cost associated with the use of the test, which could allow cost reduction when compared to cytology. Because the double staining test has high sensitivity, this could lead to a decrease in medical visits, unnecessary follow-up, referral of women for colposcopy and biopsies, in addition to reducing psychological damage, reducing overdiagnosis and overtreatment. For these reasons, double staining has been studied as an alternative for use in the screening of HPV-positive women.

In view of the comparative results between the P16 and Ki67 proteins as biomarkers, it can be observed that there is no better or worse between them, as both complement each other, making their association effective at high levels, leading to the diagnosis of lesions.

5 CONCLUSION

In view of the findings of this systematic review, it can be concluded that the biomarker P16 is a promising biomarker in the diagnosis of cervical cancer in high-grade lesions. P16 positivity for high-grade lesions is more common, whereas in lesions of lower grades there is a high probability of non-positive, leading to the belief that the result is a false negative, following this same rationale with lesions of older women.

The ki67 biomarker demonstrates a clearer specificity regarding the degree of injury. However, more studies are needed to point out the efficacy of this biomarker in the diagnosis of cervical cancer. From the results of the studies using double immunohistochemical staining, it can be concluded that the sensitivity and specificity of both biomarkers together seem to be higher and more comprehensive.

REFERENCES

BRASIL. Saúde amplia vacinação contra meningite e HPV; entenda o que muda. 2022. Disponível em:https://www.gov.br/saude/pt-br/assuntos/noticias/2022/setembro/saudeamplia-vacinacao-contra-meningite-e-hpv-entenda-o-que-muda. Acesso em: 31 outubro 2022.

1 Saúde amplia vacinação contra meningite e HPV; entenda o que muda. 2022. Disponível em: https://www.gov.br/saude/pt-br/assuntos/noticias/2022/setembro/saudeamplia-vacinacao-contra-meningite-e-hpv-entenda-o-que-muda. Acesso em 01 novembro 2022.

CLARKE, M. A.; CHEUNG, L. C.; CASTELO, P. E. et al. Five-Year Risk of Cervical Precancer Following p16/Ki-67 Dual-Stain Triage of HPV-Positive Women. 2019. Jornal Oncologia JAMA. vol. 5. ed. 2, p. 181-186.

1 Five-Year Risk of Cervical Precancer Following p16/Ki-67 Dual-Stain Triage of HPV-Positive Women. 2019. JAMA oncology. ed.2. vol.5.

FEBRASGO. Biomarcadores moleculares na progressão da lesão precursora para o câncer do colo uterino. 2018. Disponível em: https://www.febrasgo.org.br/pt/noticias/item/327biomarcadores-moleculares-na-progressao-da-lesao-precursora-para-o-cancer-do-colouterino. Acesso em: 17 outubro 2022.

HAMMER, A.; GUSTAFSONO, L. W.; CHRISTENSEN, P. N.; et al. Implementation of p16/Ki67 dual stain cytology in a Danish routine screening laboratory: Importance of adequate training and experience. 2020. Journal Cancer medicine. ed.21. vol.9. p. 8235-8242.

INCA – Instituto Nacional do Câncer. Conceito e Magnitude: Entenda o conceito do câncer do colo do útero e sua magnitude no Brasil. 2022. Disponível em: https://www.gov.br/inca/ptbr/assuntos/gestor-e-profissional-de-saude/controle-do-cancer-do-colo-do-utero/conceito-emagnitude. Acesso em: 03 outubro de 2022.

INCA. CAUSAS E PREVENÇÃO- Estatísticas de câncer. 2020. Disponível em: https://www.inca.gov.br/numeros-de-cancer. Acesso em: 20 de setembro de 2022.

ISHIKAWA, M.; NAKAYAMA, K.; NAKAMURA, K.; et al. P16(INK4A) expression might be associated with a favorable prognosis for cervical adenocarcinoma via dysregulation of the RB pathway. 2021. Scientific reports. ed.1. vol.11. p. 18236.

KANTHIYA, K.; KHUNNARONG, J.; TANGJITGAMOL, S.; et al. Expression of the p16 and Ki67 in Cervical Squamous Intraepithelial Lesions and Cancer. 2021. Asian Pacific journal of cancer prevention: APJCP. ed.7. vol.17.

LI, C.; ZHENG, M.; ZHENG, X.; et al. Predictive Ki-67 Proliferation Index of Cervical Squamous Cell Carcinoma Based on IVIM-DWI Combined with Texture Features. 2021. Contrast media & molecular imaging. vol. 2021 8873065.

MATA, S., FERREIRA, J., NICOLÁS, I., ESTEVES, S. et. P16 and HPV Genotype Significance in HPV-Associated Cervical Cancer-A Large Cohort of Two Tertiary Referral Centers. 2021. International Journal of Molecular Sciences.

1 P16 and HPV Genotype Significance in HPV-Associated Cervical Cancer-A Large Cohort of Two Tertiary Referral Centers. 2021. International journal of molecular sciences. ed.05. vol.22.

MELO, C. A. F; UTAGAWA, M. L. Expressão imuno-histoquímica dos biomarcadores p16 e Ki-67 na lesão intraepitelial cervical de alto grau: revisão de estudos. 2021. Disponível em: https://www.rbac.org.br/artigos/expressao-imuno-histoquimica-dos-biomarcadores-p16-e-ki67-na-lesao-intraepitelial-cervical-de-alto-grau-revisao-de-estudos/. Acesso em: 25 abril 2023.

MELO, Y. L. M. F. Biomarcadores moleculares na progressão da lesão precursora para o câncer do colo uterino. 2018. Disponível em: https://www.febrasgo.org.br/pt/noticias/item/327biomarcadores-moleculares-na-progressao-da-lesao-precursora-para-o-cancer-do-colouterino. Acesso em: 25 abril 2023.

MELO. Simone Cristina Castanho Sabaini; et al. Alterações citopatológicas e fatores de risco para a ocorrência do câncer de colo uterino. Rev. Gaúcha Enferm. (Online) vol.30 no.4 Porto Alegre Oct./Dec. 2009. p. 604-607 Disponível em: https://www.scielo.br/scielo.php?script=sci_arttext&pid=S198314472009000400004&lang=p t#top. Acesso em: 26 de setembro de 2022.

MESQUITA, A. D., TELES, K. K. N., SILVA, S. C. B., et al. Conhecimentos, atitudes e práticas de mulheres frente ao exame preventivo do câncer do colo uterino. Journal Health NPEPS. vol.5. no1. p. 261–275.

MUNHOZ, N. G. O uso dos marcadores moleculares (p16, Ki-67 eE-caderina) em biópsias uterinas cervicais. 2009. Disponível em: https://bdtd.famerp.br/bitstream/tede/525/2/NataliaGasparMunhoz_dissert.pdf. Acesso em: 25 outubro 2022.

MURTA. Edie Fernando Candido; et al. Câncer do Colo Uterino: Correlação com o Início da atividade Sexual e Paridade. Rev. Bras. Ginecol. Obstet. v.21 n.9 Rio de Janeiro out. 1999. p. 556-558. Disponível em: https://www.scielo.br/scielo.php?script=sci_arttext&pid=S010072031999000900009&lng=pt &nrm=iso&tlng=pt. Acesso em: 26 de setembro de 2022.

ONCOGUIA. Marcadores tumorais. 2020. Disponível em: http://www.oncoguia.org.br/conteudo/marcadores-tumorais/4011/1/. Acesso em: 17 outubro 2022.

PAIVA, C. B. Associação da expressão do ki67 e linfonodos axilares metastáticos com a sobrevida livre de doença no câncer de mama invasivo. 2016. Disponível em: https://repositorio.ufpe.br/bitstream/123456789/20411/1/Disserta%c3%a7ao_Carina_Paiva_P PG_MESTRADO_PATOLOGIA_TURMA2014-2016.pdf. Acesso em: 25 abril 2023.

SARMA, U.; DO, C. G.; SARMAH, B. Predictive Value of Marker of Proliferation Ki-67 and Cell Cycle Dependent Protein kinase Inhibitor P16INK4a in Cervical Biopsy to Determine Its Biological Behaviour. 2021. Asian Pacific journal of cancer prevention: APJCP. ed.7. vol.22. p. 2237-2241.

SILVA, V. R. O impacto da marcação imuno-histoquímica para p16 e ki-67 na reprodutibilidade diagnóstica das biópsias do colo uterino. 2017. Disponível em: https://www.arca.fiocruz.br/bitstream/handle/icict/25222/viviane_silva_iff_mest_2017.pdf?se quence=2&isAllowed=y. Acesso em 25 outubro de 2022.

1 O impacto da marcação imuno-histoquímica para p16 e ki-67 na reprodutibilidade diagnóstica das biópsias do colo uterino. 2017. Disponível em: https://www.arca.fiocruz.br/bitstream/handle/icict/25222/viviane_silva_iff_mest_2017.pdf?se quence=2&isAlloisA=y. Acesso em: 25 abril 2023.

TSAKOGIANNIS, D.; MOSCHONAS, G. D.; BELA, E.; et al. Association of p16 (CDKN2A) polymorphisms with the development of HPV16-related precancerous lesions and cervical cancer in the Greek population. 2018. Journal of medical virology. ed.5 vol.90. p. 965-971.

UTAGAWA, M. L., MELO, C. A. F. Expressão imuno-histoquímica dos biomarcadores p16 e Ki-67 na lesão intraepitelial cervical de alto grau: revisão de estudos. 2021. Disponível em: http://www.rbac.org.br/artigos/expressao-imuno-histoquimica-dos-biomarcadores-p16-e-ki67-na-lesao-intraepitelial-cervical-de-alto-grau-revisao-de-estudos/. Acesso em: 20 outubro 2021.

WANG, X.; LI, S.; LU, Y.; et al. Evaluation of tracer kinetic parameters in cervical cancer using dynamic contrast-enhanced MRI as biomarkers in terms of biological relevance, diagnostic performance and inter-center variability. 2022. Frontiers in oncology. vol.12.

WHO. Câncer cervical. Disponível em: https://www.who.int/news-room/factsheets/detail/cervical-cancer. Acesso em: 01 de novembro de 2022.

WHO. Human papillomavirus (HPV) and cervical câncer. 2019. Disponível em: https://www.who.int/news-room/fact-sheets/detail/human-papillomavirus-(hpv)-and-cervicalcancer. Acessado em: 23 de setembro de 2022.

ZUBERI, Z.; MREMI, A.; CHILONGOLA, J. O.; et al. Expression analysis of p16 and TOP2A protein biomarkers in cervical cancer lesions and their correlation with clinicohistopathological characteristics in a referral hospital, Tanzania. 2021. Journal PloS one. ed.10. vol.16.