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The Role of Immunohistochemical P16 Tissue Expression in Determining the Presence of the Main Oncoproteins E6 and E7 of Human Papilloma Virus: A Systematic Review

[1] Gizem Uysal Yantur, [2] Nurbanu Sezak, [3] Gulden Diniz

[1] Izmir Democracy University School of Medicine, Institute of Health Sciences, Izmir, Turkiye

[2] Department of Infectious Disease and Medical Microbiology, Izmir Democracy University School of Medicine, Izmir, Turkiye

[3] Department of Medical Pahtology, Izmir Democracy University School of Medicine, Izmir, Turkiye

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Corresponding Author: **Nurbanu Sezak**

Abstract

Objective

This systematic review was planned to examine the predictive effect of tissue expression of p16, a cycle inhibitor, in predicting the presence of E6 and E7 proteins, which are the main oncogenes that play a role in the cancer development of Human Papilloma Virus (HPV) and are found only in high-risk HPV types.

Materials and Methods

The 1570 articles that made up the study's population were found by searching the "Pubmed," "Medline," "Dergipark," "Elsevier," "Google Scholar," and "Networked Digital Library of Theses and Dissestations" databases between January and March 2024. In the review, articles published in the last 20 years with the keywords "carcinogenesis", "Human papilloma virus", "E6", "E7" and "p16 tissue expression", published in Turkish or English and with full text were selected. The study's sample consisted of 20 papers in total that satisfied the research requirements from these publications.

Results

The main oncogenes of HPV, E6 and E7, which are thought to be effective in the development of carcinogenesis, were found to be mostly positive in anogenital cancers. In most systems, p16 tissue expression correlated with HPV positivity (P=0,001, r=0,723).

Conclusions

The reviewed literature indicates that there is a strong correlation between cervical squamous cell lesions and high-risk HPV. Particularly in urogenital cancers, p16 tissue expression is also associated with HPV positivity and prognosis. Thus, it was determined that routine use of immunohistochemical examination to investigate the presence of p16 is both required and beneficial. However, considering that the correlation between p16 expression and HPV infection is quite low in head and neck cancers, except for the oropharynx, molecular tests should also be performed to detect HPV infection in HNSCCs.

Keywords: HPV, Carcinogenesis, E6, E7, p16 Tissue Expression

Introduction

HPV is a carcinogenic virus that has been linked to a number of squamous cell carcinomas (SCC), particularly cervical carcinoma. Cervical cancer ranks third globally in terms of incidence, accounting for more than 10% of all cancer cases in women. It is significantly higher in underdeveloped nations. According to reports, women are 9.9–13.5% likely to have a cervical HPV infection, and 5.4–22.9% likely to have high-risk HPV (hr-HPV) [1-3]. At some time in their lives, almost 80% of individuals of both sexes will get infected with HPV [4, 5]. The development of koilocytes, which are characterized by inflammatory infiltration and a clear space around the nucleus in the squamous cells, is the only indication of a persistent chronic infection that first appears at the squamocolumnar junction of the cervical epithelium in women. The presence of koilocytes in an infection is typically a sign of low-risk HPV (lr-HPV) infection. Koilocytes are dead epidermal cells that do not exhibit malignant transformation. This is a fairly innocuous adjustment. On the other hand, premalignant cervical

intraepithelial neoplasia (CIN) and invasive carcinoma develop gradually after chronic infection brought on by hr-HPV types. In 1995, the World Health Organization (WHO) declared that HPV subtypes 16 and 18 in particular are carcinogenic [6-9]. In 2006, it became feasible to produce vaccines that limit HPV infection and hence prevent cervical cancer [4]. Numerous neoplasias, including genital warts, head and neck cancers (HNC), and anogenital malignancies (vulvar, vaginal, anal, and penile), have been shown to be associated with HPV infection [5, 6].

Two oncoproteins, E6 and E7, which are typically present in hr-HPV types, are expressed when an HPV infection occurs. These oncoproteins alter the differentiation of the cervical epithelium's basal cells genetically and epigenetically, preventing the epithelium from self-renewing. The HPV-encoded E7 oncoprotein can attach itself to regulatory proteins in host cells, especially the tumor suppressor gene retinoblastoma gene (Rb). This binding leads to the release of the transcriptional factor E2F from the E2F-Rb complex, allowing the release of transcription factors that drive uncontrolled progression of the cell cycle (Fig 1). The oncogenic effects of p16, a cell cycle checkpoint inhibitor protein, are usually triggered by the presence of hr-HPV and E7 oncoprotein [7, 10-14].

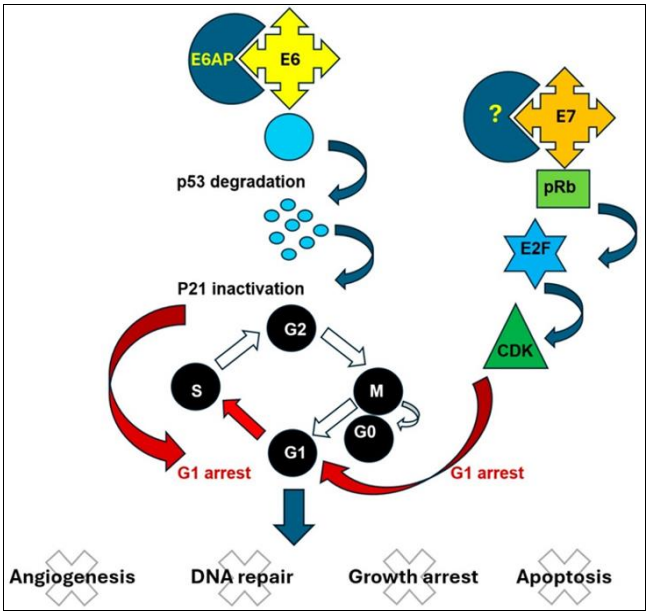

Fig 1: Mechanism of carcinogenic effect of HPV on cell cycle

Today, the detection of cervical HPV infections and associated epithelial changes can easily be done through cervicovaginal smear (CVS) screening tests. Similarly, in many institutions, polymerase chain reaction (PCR) from smear material can also be used to assess the presence of high-risk and other HPV strains. However, performing HPV typing on tissue samples is not typically a routine procedure. Moreover, the cost of HPV typing from CVS samples is quite high. In contrast, demonstrating p16 expression through immunohistochemical staining is very easy and cost-effective. According to a number of studies, p16 is a valuable biomarker, especially for anticipating the development of high-risk HPV-induced squamous intraepithelial dysplasia, intraepithelial neoplasia, and carcinoma. They have also found a significant relationship between the degree of cervical lesions and the distribution and intensity of p16 staining. Therefore, in order to diagnose numerous squamous cell carcinomas (SCC), particularly cervical malignancies, p16 expression is utilized as an alternative marker for HPV infection [8]. However, in squamous cell malignancies other than cervical carcinomas, the association between p16 overexpression and high-risk HPV has not been thoroughly investigated [14-21]. By thoroughly evaluating research in the literature that looked at p16 expression in various SCCs thought to be brought on by high-risk HPV infection, the significance of p16 as a diagnostic and prognostic biomarker was addressed in this study.

Materials and Methods

The study population consists of articles identified through searches using the keywords “Human Papilloma Virus,” “carcinogenesis,” “E6,” “E7,” “immunohistochemistry,” and “p16” in the databases Pubmed, Scopus, EBSCO, Medline, Google Scholar, Dergipark, and in thesis sources from the Networked Digital Library of Theses and Dissertations. 1510 of the 1570 articles that the search yielded were discarded because they did not fit the inclusion requirements. Forty of the 60 remaining items were disqualified because the entire text could not be accessed. Consequently, 20 articles that met the inclusion criteria were evaluated in terms of limitations and results (Fig 2).

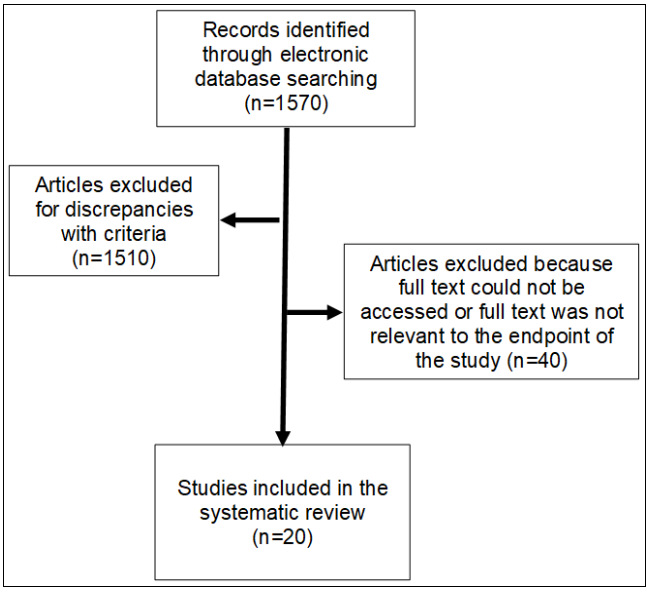

Fig 2: Preferred Reporting Items for Systematic Reviews (PRISMA) flowchart

The titles and abstracts of all articles found in the search were reviewed. The studies' whole texts were reviewed to see if they met the study's eligibility or exclusion criteria. The criteria and procedural steps used for the selection of articles can be summarized as follows:

Inclusion Criteria:

- Research articles on factors influencing HPV carcinogenesis, with a minimum of 20 patients.
- Full access to the article's complete text.
- The publication language is English or Turkish.
- The study was published between 2004 and 2024.

Exclusion Criteria:

- Case reports and review articles.

- Systematic review or meta-analysis articles.
- Research that dismisses the E6 and E7 factors, which have a significant role in the development of cancer.
- Studies that did not examine p16 in tissue immunohistochemically.
- Retrospective and prospective cohort studies.
- Research published before 2004.

The Preferred Reporting Items for Systematic Reviews and MetaAnalyses (PRISMA) criteria were adhered to in our systematic review [22].

Results

The systematic review, which assessed the existence of the primary oncogenes E6 and E7 in malignancies caused by the Human Papilloma Virus (HPV) and the tissue expression of p16 in tumors, looked at 20 publications in total that satisfied the inclusion criteria. The studies' findings regarding the tissue expression rates of p16 and the presence of the main oncogenes E6 and E7 in HPV-related cancer development, along with the year of publication and authors, are presented in table format (Table 1).

Table 1: The main characteristics for all studies included in the systematic review sorted by year of publication (n = 20)

First Author	Year	Tumor Location	N (Total)	HPV DNA	P16 (IHC)	E6/ E7
Bumrungthai S. [29]	2023	Cervix	152	96	49.3	96
Litsics A. [12]	2021	Larynx	41	53.7	11.1	53.7
Litsics A. [12]	2021	Hypopharynx	31	64.5	11.1	64.5
Zito Marino F. [9]	2021	Penis	60	35.0	46.6	35.0
Economopoulou P. [11]	2019	Oroparynx	22	63.6	63.6	45.4
Fujimoto M. [19]	2019	Cutaneous (genital skin)	136	5.8	10.0	5.8
Fujimoto M. [19]	2019	Cutaneous (extragenital)	107	66.7	73.6	66.7
Ruuskanen M. [14]	2019	Nasopharynx	150	14.0	14.0	14.0
Sudhakaran A. [15]	2019	Oral SCC	30	43.3	56.6	43.3
Lefevre M. [16]	2017	Oropharynx	296	26.1	33.0	26.1
Mills AM. [18]	2017	Anogenital/ HNSCC	102	84.3	87.2	82.3
Isoyeva T. [17]	2014	Oropharynx	102	63.0	56.8	63.0
Murao K. [20]	2014	Cutaneous (genital skin)	127	8.3	88.6	8.3
Murao K. [20]	2014	Cutaneous (extragenital)	9	66.7	88.6	66.7
Bussu F. [10]	2013	HNSCC	109	87.0	47.6	87.0
Portari EA. [24]	2013	Cervix	196	77.5	69.8	77.5
Ragendra S. [21]	2013	Esophageal AC	261	31.0	16.0	31.0
Gatta LB. [25]	2011	Cervix	72	52.0	66.1	52.0
Hoffmann M. [13]	2010	Tonsilla Palatina	39	53	25.6	28.2
Lambert AP. [28]	2006	Cervix	54	88.8	83.0	88.8
Queiroz C. [30]	2006	Cervix	60	68.3	58.0	68.3
Guimaraes MC. [27]	2005	Cervix	31	67.7	64.5	67.7
Kanao H. [23]	2004	Cervix	36	84.0	81.0	88.0

The total number of patients in the reviewed studies was 2,223. When the total number of patients, tumor localization, and HPV and p16 positivity rates were compared using the Chi-Square Goodness of Fit test, tumor location was found to be statistically substantially correlated with both HPV presence ($p<0.001$) and p16 expression ($p<0.001$). HPV and p16 positivity were most frequently observed in anogenital squamous cell carcinomas (SCC), particularly in cervical cancer. In the nonparametric Spearman correlation test, HPV positivity and p16 expression were shown to be significantly positively correlated ($P=0.001$, $r=0.723$). In the partial correlation test, conducted to determine the effect of tumor localization, a significant relationship was again observed, although some influence of localization was noted ($P = 0.023$, $r = 0.548$). Linear regression analysis showed that the p16 positivity rate was statistically significant in predicting HPV positivity ($p=0.029$).

When comparing the positivity rates of HPV ($p=0.103$), E6/E7 ($p=0.077$), and p16 ($p=0.274$) across 10 tumor locations (cervix, oral cavity, oropharynx, hypopharynx, nasopharynx, larynx, tonsil, penis, anus, and other skin-mucosal areas) there were no statistically significant differences discovered using the nonparametric Kruskal-Wallis test. However, when tumors were categorized into three groups based on tumor location—anogenital region, head and neck region, and others—p16 positivity was found to be statistically significantly higher in anogenital cancer cases ($p = 0.036$) according to the nonparametric Kruskal test. Similarly, HPV positivity ($p = 0.011$) and E6/E7 positivity ($p = 0.008$) were also significantly associated with tumor localization. However, in two studies examining skin biopsies, when genital and extragenital skin were not differentiated, the statistical significance was lost.

Discussion

Comprising over 170 distinct varieties, HPV is a tiny, non-enveloped, double-stranded DNA virus that infects cutaneous and mucosal epithelia and promotes cellular growth. Roughly one-third of all virus-related cancers are caused by high-risk HPV strains. About 5% of all human cancers develop due to HPV, with high-risk type HPV16 accounting for nearly half of all cervical cancers. Numerous studies have demonstrated that certain cutaneous HPVs contribute to the development of non-melanoma skin malignancies in addition to high-risk mucosal HPV types. The E6 and E7 proteins are important in carcinogenesis, according to functional investigations on the virus's early gene products. By interfering with the host cell cycle and apoptosis regulation, these two proteins cause DNA damage, guarantee viral persistence, and avoid detection by the host immune system through a variety of methods. Therefore, most studies in the literature aim to demonstrate the E6 and E7 proteins using various methods [20, 23-26]. We made sure to include research that looked at the presence of E6 and E7 proteins in addition to our primary investigation of the

potential of p16 tissue expression as substitute markers for HPV infection.

Anogenital malignancies are the most often linked tumors to HPV [27-31], followed by head and neck squamous cell carcinomas (HNSCC), especially oropharyngeal cancers. Globally, HNSCC is the sixth most often diagnosed kind of cancer. Oropharyngeal cancer has significantly increased, although the frequency of laryngeal and hypopharyngeal tumors is declining. It has been shown that 25-60% of HNSCC cases are associated with oncogenic HPV infections. The p16 gene is rendered inactive by deletion on the short arm of chromosome 9. On the other hand, the production of the viral proteins E6 and E7, which cause the cellular tumor suppressor proteins p53 and Rb to become inactive, is essential for HPV-induced carcinogenesis. There is debate on the natural pathway of transoral infection. HPV infections in the mouth can be frequent and frequently go away without any problems. Smoking has been found to increase the likelihood of developing HPV-related oropharyngeal HNSCC. The relationship between HNSCC and HPV can be demonstrated using established clinical diagnostic methods. Finding the HPV link could be crucial for choosing a treatment in the future, in addition to the traditional prognostic variables [10-15]. Recent studies indicate that particularly HPV-associated HNSCC cases have a better prognosis and may benefit from less aggressive treatments. But regrettably, it is more difficult to identify HPV infection in patients of HNSCC. Compared to cervical cancer, there is a substantially less link between HPV DNA levels and p16 tissue expression rates, which means that p16 tissue expression is not a useful surrogate measure [27-34].

Bussu *et al*. evaluated a study to determine the prevalence of HPV infection and the accuracy of various diagnostic methods in instances of HNSCC in different locations. According to this study, almost all of high-risk HPV infections were caused by HPV 16. The HPV DNA and E6/E7 mRNA testing showed a very high degree of correlation. Though it was shown to be very low overall, the association between immunohistochemistry p16 expression and HPV positive was strongest in oropharyngeal malignancies (%47.6) [10]. In contrast, Economopoulou *et al*. reported that HPV DNA positivity was detected by PCR in the liquid biopsy of 14 out of 22 oropharyngeal HNSCC cases (%63.6), with HPV E6/E7 positivity found in 10 of these cases, suggesting that liquid biopsy could be a useful non-invasive method. In the same cases, there was a correlation between the presence of HPV DNA and the p16 tissue expression rate [11]. Generally, high positivity for high-risk HPV is detected in only about 2% of HNSCC cases, while this rate is nearly 2/3 in oropharyngeal HNSCC. Since HPV-related HNSCC has a better prognosis, it is essential to show that HPV is present in these cancers. Currently, p16 expression is considered a good HPV marker in most studies. P16 expression and HPV presence in oral HNSCC were found to be highly correlated in Sudhakaran *et al*.'s research on oral submucosal fibrosis and HNSCC [15]. There are, however, very few papers on HPV's involvement in the development of laryngeal (LSCC) and hypopharyngeal HNSCC.

Lifscis and colleagues used molecular virology and immunohistochemistry (IHC) techniques to assess the status of hr-HPV DNA, p16, and E6/E7 oncoproteins in cases of laryngeal squamous cell carcinoma (LSCC) and hypopharyngeal squamous cell carcinoma (HSCC), as high-risk HPV viral proteins are generally believed to be associated with the presence of E6 and E7. According to their research, the prevalence of HPV16 infection was 64.5% for HSCC and 53.7% for LSCC. Most HPV16-positive tumor samples were progressed (Stage III or IV), and IHC further verified the presence of HPV16 E6/E7 viral proteins in tumor or dysplastic cells. On the other hand, only 11.1% of cases had p16 expression, and some of the positive cases had tumors that were HPV-negative. HPV16 is also the predominant hr-HPV type in laryngeal and hypopharyngeal SHCs. The absence of HPV E6/E7 oncoproteins in certain tumor samples, according to the researchers, may be the result of either different tumor formation pathways or a lack of viral integration. They said that it is not feasible to employ p16 expression as a marker of HPV infection in laryngeal and hypopharyngeal tumors, in contrast to cervical tumors [12].

In a study conducted by Hoffmann and colleagues examining 39 cases of tonsillar squamous cell carcinoma (TSCC), the HPV-DNA positivity rate was reported as 53%. However, E6/E7 oncoprotein mRNA was detected at a rate of 28.2%, and p16 expression was found to be 25.6%. The correlation between HPV DNA positivity and p16INK4a expression was statistically significant. As a result, it was noted that p16INK4a has the potential to influence the standard of care for HPV DNA-positive patients and should be tested [13].

A viral infection is the trigger of nasopharyngeal carcinoma. Although it is most frequently linked to Epstein-Barr virus (EBV), HPV and immunological variables may also contribute to carcinogenesis. In addition to being pattern recognition receptors that aid in the immune system's reaction to infections, toll-like receptors (TLRs) have also been connected to cancer. IHC revealed EBV positivity at 62% and p16 positivity at 14% in a study that examined the expression of TLR1, TLR2, TLR4, TLR5, TLR7, TLR9, and p16 in tumor tissues from nasopharyngeal cancer patients, as well as EBV-RNA and HPV E6/E7 mRNA. Using in situ hybridization (ISH), high-risk HPV E6/E7 mRNA and p16 positive were found to be 100% concordant in this investigation. However, due to the very low prevalence of HPV in nasopharyngeal carcinoma, investigating the presence of HPV or p16 as a marker for HPV is not a high priority for routine use in this carcinoma [14].

Nowadays, IHC-detected p16 expression serves as a stand-in biomarker for HPV-positive carcinomas. However, there may be discrepancies between p16 expression and HPV DNA test results because p16 expression is not a straightforward way to identify HPV-related proteins. For the purpose of evaluating HPV infection, a direct technique to identify HPV-related proteins in clinical samples is therefore preferred. Although the HPV oncoproteins E6 and E7 are known to cause carcinogenesis, it is unknown if these proteins are present in carcinomas. Using commercially available antibodies against the E7 protein, Kitamura and colleagues assessed the expression of these proteins in clinical oropharyngeal samples and HPV16-positive cancer cell lines. Anti-E6 antibodies, however, did not identify expression. The study demonstrated that in p16-positive regions of clinical samples with HPV16 DNA, the E7 protein was partially expressed. E7 protein was discovered in the p16-positive region of clinical samples that were both p16-positive and HPV16 DNA-positive, but it was absent

from p16-negative and HPV DNA-negative samples and p16-positive and HPV DNA-negative samples. Based on these findings, the authors contended that p16 positivity, HPV DNA positivity, and E7 positivity are related [8].

A poor prognosis for carcinomas is indicated by the detection of epithelial-to-mesenchymal transition (EMT) markers, which contribute to tumor invasion. In a study by Lefevre and colleagues involving 296 cases of oropharyngeal squamous cell carcinoma (OSCC), they looked at p16 tissue expression, HPV infection (HPV DNA, E6/E7 mRNA), EMT markers (E-cadherin, β-catenin, and vimentin), and survival results. They reported that 26% of their patients were HPV-positive, and 20.3% showed significant EMT expression. There was no discernible correlation between HPV status and EMT, and vimentin expression was linked to worse metastasis-free survival [16]. In a study conducted by Isayeva and colleagues involving 102 oropharyngeal carcinoma patients, it was suggested that the impact of HPV on carcinogenesis may vary according to racial differences [17]. In HPV-associated anogenital and HNSCCs, Mills *et al*. compared HPV DNA, p16 IHC, and HPV E6/E7 mRNA ISH tests. According to reports, the overall HPV DNA positive percentage was 84.3%. The test results showed ≥97% positivity rates for invasive squamous cell carcinoma and high-grade squamous intraepithelial lesions. The positive rate fell to 78% in intraepithelial low-grade squamous lesions. Reactive samples, on the other hand, nearly always produced negative results. The authors suggested that HPV positivity is important in distinguishing reactive lesions from neoplasms in differential diagnosis [18].

Essentially, 90% of cervical carcinomas are associated with HPV, and HPV vaccination is recommended to prevent infection. In contrast, this relationship is still debated in non-cervical anogenital/urogenital cancers. Even though HPV is less common in penile cancer (PC) than cervical cancer, PC linked to HPV constitutes a unique subgroup with a different biology and is typically linked to a better prognosis and better response to treatment than cancer unrelated to HPV. Studies conducted on PC cases have shown a general concordance between p16 expression detected by IHC and the presence of HPV. All neoplastic cells, with the exception of those in hyperkeratotic or parakeratotic regions, exhibit strong cytoplasmic or nuclear staining, indicating a strong positive connection between high-risk HPV and p16. The sensitivity of p16 expression in detecting HPV infection is modest (67%) despite the excellent correlation with molecular testing. High-risk HPV infection is one of the primary etiological paths in 30–50% of PC patients, according to prior research. In a study by Marino and colleagues involving 60 penile cancer cases, IHC was used to measure p16 expression, while ISH and PCR were used to determine the presence of HPV DNA and RNA. HPV positivity was found in 21 cases (35%), while p16 expression was shown in 28 cases (46.6%). The authors demonstrated that HPV positivity was more frequent in usual squamous cell carcinoma (SCC) compared to specific histotypes. In the same study, 5 of the 20 cases with p16 expression were HPV-negative. Additionally, p16 expression was positive in 13 HPV-negative cases. According to this study, p16 IHC has a positive predictive value of 54% and a negative predictive value of 100%, making it a highly sensitive (100%) and moderately specific (71%) test for PC patients. However, the p16 IHC specificity value rose to 89% and the positive predictive value to 75% when the intensity of p16 expression was taken into account [9].

The relationship between high-risk HPV and non-anogenital cutaneous squamous cell carcinoma (SCC) is controversial. In a study by Fujimoto and colleagues, HPV E6/E7 mRNA ISH results were positive in 66.7% of anogenital samples, but only 5.8% of samples from other regions. The authors concluded that since HPV E6/E7 mRNA is rarely detected in non-anogenital cutaneous SCC cases, p16 immunoreactivity has limited value for diagnosing non-anogenital cutaneous SCC [19]. Similarly, genital Bowen's disease (GBD) has been associated with high-risk HPV types. It is now known that HPV and extragenital Bowen's disease (EGBD) may be connected. Murao and colleagues found that HPV DNA was present in 66.7% of genital BD cases and 8.3% of extragenital BD cases in a study that examined the presence of HPV DNA in 142 Bowen's disease lesions. HPV types 6, 16, 33, 52, 56, 58, and 59 were found. IHC was used to analyze the expression of p16, pRb, and p53. There was no discernible difference between HPV-positive and HPV-negative BD in terms of p16 and pRb expression. Nonetheless, a robust association between p53 negative and HPV positivity was identified. The authors contended that the key to detecting HPV infection in Bowen's illness is negative p53 expression rather than p16 overexpression [20].

Studies on the connection between HPV and Barrett's esophagus (BE) and esophageal adenocarcinoma (EAC) are scarce. According to Rajendra and colleagues' study, 31% of 261 patients had positive HPV DNA. The study examined the prevalence of biologically active HPV in the esophagus epithelium of individuals with the Barrett metaplasia-dysplasia-adenocarcinoma sequence. Positive results for HPV DNA, p16INK4A, and E6/E7 mRNA were shown to be highly correlated with the severity of the disease, suggesting that HPV may contribute to the development of esophageal adenocarcinoma [21].

Cell cycle-regulating molecules have a key role in HPV-mediated cervical carcinogenesis. By attaching itself to the cyclin D1-CDK4/CDK6 complex and blocking the phosphorylation of the pRb protein, the tumor suppressor p16 protein regulates the expression of S-phase genes. The p16 protein is thought to be a helpful marker for detecting HPV-mediated premalignant and malignant cervix lesions because of its unique diffuse and robust immunostaining positivity [23, 24]. This is because the E7 oncoprotein's direct inactivation of pRb triggers a compensatory mechanism that stops the inhibition of p16INK4a gene transcription. As a result, the p16INK4a protein accumulates and is persistently overexpressed. A statistically significant rise in p16 and p21 expression was observed as lesions advanced from low-grade to high-grade and to cancer in the study by Portari and colleagues, which examined the expression of p53, p21, p16, cyclin D1, and Ki-67 in addition to the existence of HPV. In contrast, more severe lesions exhibited a substantial drop in cyclin D1 expression. Furthermore, a correlation between HPV-16 infection and p16 and cyclin D1 expression was found, indicating that the immunohistochemistry (IHC) assessment of cell cycle biomarkers is helpful in differentiating between various cervix precursor lesion groups and cervical carcinoma [24].

As possible molecular markers for diagnosis, the expression of the p16, Ki-67, and L1 proteins as well as HPV DNA are

being used. In the study by Gatta and colleagues, it was reported that L1 expression has an opposite association with malignant transformation, whereas the transformation potential of CIN1 is connected with the production of p16 and Ki-67 proteins [25]. The tumor suppressors p14 (ARF) and p16 (INK4A), which are encoded by the CDKN2A locus on human chromosome 9p21, respectively, improve the growth-suppressive properties of the Rb and p53 proteins. On the other hand, the p53 and Rb proteins are rendered inactive by the E6 and E7 oncoproteins of high-risk HPVs (hr-HPVs), which are directly associated with cervical carcinogenesis and aid in the development of tumors. It is unclear, therefore, how p14(ARF)/p16(INK4A) expression and cervical HPV infection are related.

According to a study, cervical cancers that are HPV-positive are strongly linked to overexpression of p14 and p16, while tumors that are HPV-negative are more frequently linked to decreased expression [23]. A study that looked at the predictive significance of p16INK4a and bcl-2 IHC expression in the development of cervical squamous intraepithelial lesion (SIL) showed no correlation between HPV16/18 presence and p16 and bcl-2 expression in the group with low-grade CIN. The presence of HPV16/18 and p16 overexpression were significantly correlated in the group with high-grade CIN, while there was no correlation with bcl-2 levels. According to the authors, p16 expression could be a useful indicator for forecasting how CIN will develop [27].

According to recent research, p16 expression may serve as a marker for cervical cells that are dysplastic or malignant. P16 expression was found in 83% of HPV-positive cervical samples in a study that used IHC to analyze p16 expression in cervical preneoplastic and neoplastic lesions and attempt to connect it with lesion severity. This finding supported the theory that the HPV-E7 protein's functional inhibition of pRb causes p16 expression to be upregulated in cervical lesions. Additionally, the study discovered a statistically significant correlation between p16 expression and the grade of cervical lesions [28].

A single molecular biomarker is now used to determine histological grade, which may help with differential diagnosis but is unable to forecast the likelihood of lesion progress. Since cancer develops through intricate processes, no one biomarker is able to reliably identify the disease and forecast its likelihood of progressing. Multiple biomarker modeling can be used to provide risk prediction scores. Predictions can be made by mathematical models using biomarker data. Risk scores for predicting the progression of cervical lesions ranged from 2.56 to 2.60 in cases of normal and low-grade squamous intraepithelial lesions (LSIL), and from 3.54 to 3.62 in cases where progression of precancerous lesions was predicted. This study aimed to develop score-based risk models to predict the progression of precancerous cervical lesions and to identify such lesions by evaluating the expression of multiple biomarkers. According to the study's findings, risk ratings derived from biomarker levels were helpful in detecting the emergence of precancerous lesions and forecasting the likelihood that cervical lesions would advance [29].

The disruption of the cycle process and modifications in the expression of certain proteins, including p16, cyclin D1, p53, and Ki67, result from the interaction of the E6 and E7 oncoproteins with cell cycle proteins. Ki67 and p16 expression levels indicated a stronger association with cancer progression than p53 and cyclin D1 in a study by Queiroz and colleagues that compared the changes in these proteins' expression at different stages of intraepithelial cervical carcinogenesis. Therefore, the authors specifically recommended the use of these two markers for follow-up. When applied to cervical biopsy samples, these monoclonal antibodies can detect high-grade lesions by increasing the cellular proliferation index (Ki67), differentiate CIN lesions, and identify lesions changed by oncogenic HPV. This could help distinguish between patients who require more intensive treatment and those who need a more cautious therapeutic approach [30-34].

Conclusion

HPV, a significant carcinogenic microorganism affecting the global population, is a DNA virus that primarily colonizes the urogenital system. In a number of systems, it may result in the formation of squamous cell carcinomas (SCC). In the reviewed studies, the highest correlation between E6/E7 positivity and tissue p16 expression was found in anogenital cancers, followed by oropharyngeal cancers. As a result, it is reasonable to draw the conclusion that cervical squamous cell lesions and the presence of high-risk HPV (hr-HPV) are closely related. Additionally, in urogenital cancers, p16 tissue expression is also associated with HPV positivity and prognosis. Therefore, the routine use of immunohistochemistry (IHC), a low-cost method, seems appropriate and rational for the histopathological diagnosis of anogenital and oropharyngeal cancers. Nevertheless, molecular tests to identify HPV infection in head and neck squamous cell carcinomas should also be carried out, given the very weak association between p16 expression and HPV infection in head and neck cancers, with the exception of the oropharynx.

Authorship contributions

G.U.Y; Conceptualization, Methodology, Investigation, Writing-Original Draft.

N.S.; Conceptualization, Methodology, Resources, Data Curation, Writing-Review&Editing.

G.D.; Conceptualization, Methodology, Formal Analysis, Visualization, Data Curation, Supervision.

Data availibity statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declaration of competing interest

No conflict of interest was declared by the authors.

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