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HPV and P16 expression in female genital tract and it's value in diagnosis

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ABSTRACT

Background: Cervical cancer is one of the most common cancers affecting women worldwide. Since the implementation of Pap smear screening, cervical cancer morbidity and mortality have declined drastically. The goal of this study was to evaluate the results of the expression of p16INK4a in normal uterine cervical epithelium, low-grade cervical intraepithelial neoplasia (CIN), high-grade CIN, squamous cell carcinoma (SCC), and adenocarcinoma of the cervix, in order to help draw a distinction between low risk and high risk patients with cervical lesions.

Materials and methods: This is prospective and descriptive study conducted in the Department of Pathology, Tertiary Care Teaching Hospital over a period of 1 year. Archival, formalin fixed tumour specimens from patients were retrieved from the department of pathology for immunohistochemical staining done at IMS, BHU, Varanasi by means of an anti-p16 monoclonal antibody. In total, there were 90 patients. We evaluated p16 expression for its clinicopathological significance. Immunohistochemical (IHC) staining was carried out by means of an avidin–biotin complex (ABC) immunoperoxidase method. We used similar protocols to those used previously for the study of p16 expression.

Result: HPV types and status in correlation with clinical parameters and expression of p16. Sixty out of 70 patients with PCV could be evaluated for HPV status. 16 were positive for high-risk HPV and 44 were HPV negative. The majority (11 out of 16, 68%) of HPV-positive patients were positive for HPV16. The others were positive for HPV45 (4 patients, 16.6%), HPV18 (2 patient, 8.3%), HPV35 (1 patient), HPV56 (1 patient), and HPV68 (1 patient). Human papillomavirus positivity was significantly correlated with strong p16 expression (p= 0.045). In all, 6 out of the 44 HPV-negative patients were negative for p16 immunostaining, while the remaining 86% showed varying expression: 31 out of 44 (70.5%) showed moderate or strong p16 expression.

Conclusion: This study suggests that women with HPV- and p16- positive vaginal cancer have an improved prognosis compared with those with HPV- or p16- negative vaginal cancer. Results for p53 were varied, and no conclusion could be reached. Only 12 studies could be included in the review, of which most were based on small populations. Hence, further and larger studies on the prognostic impact of HPV, p16, and p53 in vaginal cancer are warranted.

Keywords: Human papillomavirus, p16, p53, prognosis, survival, vaginal cancer

INTRODUCTION

Cervical cancer is one of the most common cancers affecting women worldwide. Since the implementation of Pap smear screening, cervical cancer morbidity and mortality have declined drastically. Nevertheless, the number of newly diagnosed cases worldwide is still significant, reaching about 400000 cases each year. [1] Epidemiologic and laboratory data supports the conclusion that human papillomavirus (HPV) is the etiologic agent for the vast majority of premalignant and malignant epithelial lesions of the cervical mucosa, as HPV DNA can be detected in 95% to 100% of all cases. [2]

Papillomavirus is a double-stranded DNA virus encased in a 72-sided icosahedral protein capsid. More than 120 types of HPV have been identified, which can be divided into high-risk, intermediate-risk, and low-risk types. The persistent high-risk type HPV infection of the cervical epithelium appears to trigger neoplastic progression. [3]

The protein p16INK4a (henceforth referred to as p16) is a cellular protein involved in cell cycle regulation, and its expression is tightly controlled in normal cells. In normal nondysplastic cells, p16 protein is expressed at a very low level and is almost undetectable by immunohistochemistry (p16 can be expressed physiologically in a few cells, especially those undergoing squamous metaplasia during this trans differentiation process). On the contrary, due to the transforming activity of the E7 oncogene of all high-risk human papillomavirus (HR-HPV) types, p16 is strongly overexpressed in dysplastic cervical cells and may be easily detected by immunohistochemistry (IHC). [4] Therefore, p16 may be considered a surrogate marker for the activated oncogene expression of HR-HPV in dysplastic cervical cells. [5]

P16 is a cyclin-dependent kinase (CDK)-4inhibitor. It is the product of the INK4a gene on chromosome 9 and specifically binds to cyclin D–CDK4/6 complexes to control the cell cycle at the G1-S interphase. P16 is integral to p-retinoblastoma (p-Rb) mediated control of the G1-S phase transition of the cell cycle; it puts a brake on the cell cycle by inactivating the CDKs that phosphorylate Rb protein. In preneoplastic and neoplastic cervical lesions associated with high risk HPV infection, there is functional inactivation of Rb by HPV E7 protein. This results in an accumulation of p16

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protein, because normally Rb inhibits transcription of p16. [6] P16 expression can also be regarded as a marker of E7 gene activity.

However, it is also clear that focal, or even diffuse, p16 expression in the cervix and other tissues may occur as a result of non-HPV related mechanisms. Despite the p16 overexpression in association with high risk HPV, there is no slowing effect on the cell cycle because Rb has already been blocked by the E7 oncoprotein. [7] The role of p16 immunohistochemistry as a diagnostic aid in gynecological pathology has recently been reviewed. [8]

Our objective was to investigate, through IHC, the expression of p16INK4a in biopsies of normal uterine cervical tissue as well as pre-cancerous and cancerous lesions. The goal of this study was to evaluate the results of the expression of p16INK4a in normal uterine cervical epithelium, low-grade cervical intraepithelial neoplasia (CIN), high-grade CIN, squamous cell carcinoma (SCC), and adenocarcinoma of the cervix, in order to help draw a distinction between low risk and high risk patients with cervical lesions.

MATERIALS AND METHODS

This is prospective and descriptive study conducted in the Department of Pathology, Tertiary Care Teaching Hospital over a period of 1 year.

Archival, formalin fixed tumour specimens from patients were retrieved from the department of pathology for immunohistochemical staining done at IMS, BHU, Varanasi by means of an anti-p16 monoclonal antibody. In total, there were 90 patients. We evaluated p16 expression for its clinicopathological significance.

Immunohistochemical (IHC) staining was carried out by means of an avidin–biotin complex (ABC) immunoperoxidase method. We used similar protocols to those used previously for the study of p16 expression. The specimens dewaxed in xylene for 15 minutes, and rehydrated with ethanol. The slides were treated with 3% hydrogen peroxide for 30 minutes at room temperature. After three washes in phosphate buffered saline, antigen retrieval was performed by microwaving in citrate buffer (pH 6) for five minutes. We used a 1/50 dilution of the monoclonal anti-p16 antibody and incubation was carried out overnight at 4°C. The sections were then incubated with secondary antibody for 30 minutes. Staining was performed using ABC reagents and 3,3`-diaminobenzidine/hydrogen peroxide as substrate. The sections were counterstained with Mayer's haematoxylin for 30 seconds and blued in Scot's tap water for three minutes. The normal epithelium adjacent to the tumour nests served as an internal positive control. Squamous cell carcinomas known to be positive for p16 were used in each run of the experiment as external positive controls.

HPV status: Briefly, analyses were performed in the Department of Microbiology, BHU, Varanasi. In extracted DNA obtained from a 10-mm thick section of paraffin blocks, the preceding section of which had been used for morphological diagnosis. These sections of archived tumour biopsies were dewaxed with xylene-ethanol. DNA was extracted by a MagNA Pure LC Robot according to the manufacturer's instructions.

HPV detection and typing: The quality of DNA samples was analysed using a Beta-globin real-time PCR using 1 ml of the sample. All samples that we included for future analysis were b-globin positive. Human papillomavirus testing was performed by PCR amplification of a fragment in the L1 gene. Samples were tested for the presence of HPV DNA by amplifying 1 ml of DNA with the MGP primer system as previously described. The Bioplex 200 Luminex system was used for HPV detection and genotyping using multiplex bead-based hybridisation with Luminex technology as described by Schmitt *et al* (2006). Briefly, 10 ml of the biotinylated MGP-PCR product was mixed with beads coupled with different HPV probes. After 10 min of denaturation at 95 1C, the samples were hybridised at 41 1C for 30 min. After washing, streptavidin-Rphycoerythrin was incubated with the samples for 30 min at room temperature. One hundred beads of each HPV type from each sample were analysed using the Luminex system. Probes for 14 oncogenic, high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68a, and 68b, including probes for variant sequences of HPV18, 35, 51, and 58) and 22 non-oncogenic types including low-risk and possible high-risk types (6, 11, 26, 30, 40, 42, 43, 53, 54, 61, 67, 70, 73, 74, 81, 82, 83, 86, 87, 89, 90, and 91) were used. Eleven negative controls (H2O) and eight positive controls (HPV plasmid pools) were included in each test.

Scoring criteria for p16INK4a were no expression (negative); weak expression (<30% positive cells); moderate expression (31–50% positive cells); and strong expression (>50% positive cells).

RESULTS

Table-1: Patient and tumour characteristics

Parameters	Frequency	Percentage
Histology		
Squamous cell carcinoma	65	92.9

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Adenocarcinoma	2	2.8
Small cell carcinoma	3	4.3
Histopathological grade		
Well differentiated	7	10
Moderately differentiated	36	51.4
Poorly differentiated	27	38.6
FIGO stage		
I	36	51.4
II	14	20
III	12	17.1
IV	8	11.5
Tumour size		
<4 cm	31	44.3
4–8 cm	27	38.6
>48 cm	12	17.1
Tumour localisation		
Upper third	40	57.1
Lower third	14	20
All other locations	16	22.9

Table-2: Characteristics of tumour

Growth pattern	Frequency	Percentage
Exophytic	24	34.3
Ulcerating	40	57.1
Endophytic	6	8.6
Regional metastasis (inguinal node metastasis)		
Yes	8	11.5
No	62	88.5
Distant metastasis		
Yes	7	10
No	63	90

Table-3: p16 expression in relation to HPV status and different HPV types

		HPV positive N (%)	
p16 expression	**HPV negative N (%)**	**HPV16**	**Other HPV types (18, 35, 45, 56, 68**
None	6 (13.6)	1 (6.2)	
Weak (430%)	7 (15.9)		
Moderate (30–50%)	13 (29.6)	2 (12.5)	2 (12.5)
Strong (450%)	18 (40.9)	8 (50)	3 (18.8)
Total	44 (100)	16 (100)	

In table 3, HPV types and status in correlation with clinical parameters and expression of p16. Sixty out of 70 patients with PCV could be evaluated for HPV status. 16 were positive for high-risk HPV and 44 were HPV negative. The majority (11 out of 16, 68%) of HPV-positive patients were positive for HPV16. The others were positive for HPV45 (4 patients, 16.6%), HPV18 (2 patient, 8.3%), HPV35 (1 patient), HPV56 (1 patient), and HPV68 (1 patient). Human papillomavirus positivity was significantly correlated with strong p16 expression (p= 0.045). In all, 6 out of the 44 HPV-negative patients were negative for p16 immunostaining, while the remaining 86% showed varying expression: 31 out of 44 (70.5%) showed moderate or strong p16 expression.

DISCUSSION

This is, to the best of our knowledge, the first systematic review of the prognostic significance of HPV, p16, and p53 in vaginal cancer. Altogether 12 studies were included in the present review. Of seven studies reporting on HPV status as a prognostic factor, the majority found an improved survival for women with HPV- positive tumors. [9] For p16 expression status, three out of four studies found an improved survival among women with p16- positive tumors. [10] In contrast, findings for p53 expression status were mixed, and only one of six studies reported a statistically significant association with survival. [11] Most of the studies included small study populations, reflecting the rarity of the disease, and several studies did not adjust for important confounders such as age and tumor stage.

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The findings of this study, with an improved survival for women with HPV- positive vaginal cancer, are in line with results found in similar studies investigating survival after other HPV- related cancers. Within penile, vulvar, anal, and oropharyngeal cancer, HPV is relatively well- established as a prognostic marker, with HPV positivity signifying improved prognosis. Our findings are also in agreement with a previous review by Gadducci. [12] They included three studies (of which one41 was excluded from our review because no formal survival analysis was conducted) and concluded that HPV positivity is associated with improved survival. Block- type overexpression of the tumor suppressor protein p16 has been established as a prognostic factor in penile, vulvar, anal, and oropharyngeal cancer. [13] Our findings are in line with this, indicating that also in vaginal cancer, p16 positivity is a predictor of improved prognosis. Overexpression of p16 is established as a surrogate marker for a transforming HPV infection, which can explain why p16 can be used as a prognostic marker for HPV- related cancers. Studies of HPV- related cancers other than vaginal cancer, including oropharyngeal42 and anal12 cancers, have demonstrated that a combination of HPV and p16 testing is a better prognostic marker than using either HPV or p16 separately. Unfortunately, none of the studies included in our systematic review investigated prognosis for these markers combined.

Several studies have investigated p53 as a prognostic factor in vulvar, anal, and oropharyngeal cancer, suggesting an improved survival among p53- negative cases, although the evidence remains inconclusive. In our review, most studies did not demonstrate a prognostic value of p53 status, which is in agreement with the previous review by Gadducci who included two studies of p53. [14] The tumor suppressor protein p53 is involved in cell cycle control and apoptosis, and mutations of the TP53 gene or the interaction with HPV E6 can cause aberrant expression of p53 in the cell, hence it is conceivable that p53 could act as a prognostic marker.

Several hypotheses on the reasons for the difference in survival among HPV- related and non- HPV- related cancers have been investigated. In penile cancer, it has been indicated that the viral infection causes an increased immune surveillance, leading to a less aggressive development of the HPV- positive cancers. [15] In head- and- neck cancers, it has been shown that HPV positive cancers might possess a lower degree of gross genetic alterations or that the HPV infection in the tumor might influence the molecular profile of the cancer, leading to an increased sensitivity to radiotherapy. [16] The same has been proposed for vulvar cancer, but the results are conflicting. [17] With surgical intervention having a limited role in the treatment of vaginal cancer, most patients are treated with external radiotherapy and brachytherapy in combination with concurrent chemotherapy. [18] Therefore, a possible higher sensitivity to radiation therapy in HPV-related vaginal cancers could be of great clinical relevance when planning treatment and follow- up strategies as it could potentially be possible to reduce the radiation dose in HPV- positive cancers, thereby minimizing potential side effects.

CONCLUSION

This study suggests that women with HPV- or p16- positive vaginal cancer have a more favorable survival than women with HPV- or p16- negative cancer, whereas most studies did not find a prognostic value of p53. However, because of the limited sample size of existing studies, the current scientific evidence does not support any firm conclusions. Larger studies with the ability to adjust for other important prognostic factors are needed to improve our understanding of the correlation between HPV, p16, and p53 and prognosis after vaginal cancer. Furthermore, studies combining testing for HPV and p16 in vaginal cancer might also prove useful in the prognostication, as has been shown in tonsillar and base of tongue cancer.51 Such knowledge could potentially contribute to a more personalized and targeted treatment for vaginal cancer, as is being investigated in head and neck cancer,52 thereby maximizing treatment effectiveness while minimizing side effects and long- term treatment sequelae.

REFERENCES

1. Sung H, Ferlay J, Siegel RL, *et al*. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71:209- 249.
2. Chikandiwa A, Pisa PT, Sengayi M, Singh E, Delany- Moretlwe S. Patterns and trends of HPV- related cancers other than cervix in South Africa from 1994– 2013. Cancer Epidemiol. 2019;58:121- 129.
3. Hansen BT, Campbell S, Nygard M. Long- term incidence trends of HPV- related cancers, and cases preventable by HPV vaccination: a registry- based study in Norway. BMJ Open. 2018;8:e019005.
4. Shack L, Lau HY, Huang L, Doll C, Hao D. Trends in the incidence of human papillomavirus- related noncervical and cervical cancers in Alberta, Canada: a population- based study. CMAJ Open. 2014;2:E127- E132.
5. Bertoli HK, Baandrup L, Aalborg GL, Kjaer AK, Thomsen LT, Kjaer SK. Time trends in the incidence and survival of vaginal squamous cell carcinoma and high- grade vaginal intraepithelial neoplasia in Denmark - A nationwide population- based study. Gynecol Oncol. 2020;158:734- 739.
6. Alemany L, Saunier M, Tinoco L, *et al*. Large contribution of human papillomavirus in vaginal neoplastic lesions: a worldwide study in 597 samples. Eur J Cancer. 2014;50:2846- 2854.
7. De Vivar, A. D., Dawlett, M., Wang, J. P., Jack, A., Gong, Y., Staerkel, G., & Guo, M. (2015). Clinical performance of hybrid capture 2 human papillomavirus testing for recurrent high-grade cervical/vaginal intraepithelial neoplasm

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in patients with an ASC-US Papanicolaou test result during long-term posttherapy follow-up monitoring. Archives of Pathology and Laboratory Medicine, 139(2), 219-224.

8. de Sanjose, S., Brotons, M., & Pavon, M. A. (2018). The natural history of human papillomavirus infection. Best practice & research Clinical obstetrics & gynaecology, 47, 2-13.
9. Joura, E. A., Giuliano, A. R., Iversen, O. E., Bouchard, C., Mao, C., Mehlsen, J., ... & Luxembourg, A. (2015). A 9-valent HPV vaccine against infection and intraepithelial neoplasia in women. New England Journal of Medicine, 372(8), 711-723.
10. Jentschke, M., Hoffmeister, V., Soergel, P., & Hillemanns, P. (2016). Clinical presentation, treatment and outcome of vaginal intraepithelial neoplasia. Archives of gynecology and obstetrics, 293(2), 415-419.
11. Faber, M. T., Sand, F. L., Albieri, V., Norrild, B., Kjær, S. K., & Verdoodt, F. (2017). Prevalence and type distribution of human papillomavirus in squamous cell carcinoma and intraepithelial neoplasia of the vulva. International journal of cancer, 141(6), 1161-1169.
12. Feldbaum, V. M., Flowers, L. C., & Oprea-Ilies, G. M. (2014). Improved survival in p16-positive vaginal cancers across all tumor stages but no correlation with MIB-1. American journal of clinical pathology, 142(5), 664-669.
13. Lorincz AT, Reid R, Jenson AB, Greenberg MD, Lancaster W, Kurman RJ. Human papillomavirus infection of the cervix: relative risk associations of 15 common anogenital types. Obstet Gynecol 1992; 79(3): 328-37.
14. Nam EJ, Kim JW, Hong JW, Jang HS, Lee SY, Jang SY, *et al*. Expression of the p16 and Ki-67 in relation to the grade of cervical intraepithelial neoplasia and high-risk human papillomavirus infection. J Gynecol Oncol 2008; 19(3): 162-8.
15. Klaes R, Friedrich T, Spitkovsky D, Ridder R, Rudy W, Petry U, *et al*. Overexpression of p16(INK4A) as a specific marker for dysplastic and neoplastic epithelial cells of the cervix uteri. Int J Cancer 2001; 92(2): 276-84.
16. Murphy N, Ring M, Killalea AG, Uhlmann V, O'Donovan M, Mulcahy F, *et al*. p16INK4A as a marker for cervical dyskaryosis: CIN and cGIN in cervical biopsies and ThinPrep smears. J Clin Pathol 2003; 56(1): 56-63.
17. Yoshida T, Fukuda T, Sano T, Kanuma T, Owada N, Nakajima T. Usefulness of liquid-based cytology specimens for the immunocytochemical study of p16 expression and human papillomavirus testing: a comparative study using simultaneously sampled histology materials. Cancer 2004; 102(2): 100-8.
18. Ekalaksananan T, Pientong C, Sriamporn S, Kongyingyoes B, Pengsa P, Kleebkaow P, *et al*. Usefulness of combining testing for p16 protein and human papillomavirus (HPV) in cervical carcinoma screening. Gynecol Oncol 2006; 103(1): 62-6.