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Prevalence of HPV-induced Anogenital Squamous Cell Carcinoma in Men in Kinshasa, Democratic Republic of the Congo

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Abstract

Anogenital squamous cell carcinomas are mostly HPV-induced but in the Democratic Republic of Congo (DRC) there is no HPV vaccination program or HPV screening program. The aim of this study was to determine the frequency of HPV-induced anogenital squamous cell carcinoma and the mutation of *p53*. In this descriptive cross-sectional study, performed immunostaining at p16, p53 and Ki-67 on 30 tissues of 10% formalin-fixed anogenital squamous cell carcinoma. Strong and diffuse expression of p16 was noted in 93% of cases; 66.6% of cases that had shown strong and diffuse expression of p16 and the 2 cases that had not shown p16 expression were not marked by the anti-p53 antibody; 8 cases that had shown strong and diffuse expression of p16 showed an expression of p53 less than 10%; 90% of cases that showed strong and diffuse expression of p16 showed a high expression of Ki-67, i.e. 40% to 90%; the 2 cases that showed no expression of p16 showed an expression of Ki-67 less than 10%; finally, a case that showed strong and diffuse expression of p16 showed no expression of Ki-67. In this study, the vast majority of male anogenital squamous cell carcinoma are HPV-induced, did not note any cases of mutation of *Tp53*. The introduction of the HPV vaccine and the national program for the early screening of HPV-associated lesions is an emergency in the DRC.

Keywords: Squamous Cell Carcinoma; Anogenital; Male; HPV prevalence; *p53* mutation; Kinshasa

Introduction

HPV infection has been widely known to the general public in the genesis of cervical cancer. In men, in the anogenital area, it is associated with cancers of the anus, penis, scrotum and perineum [1-3]. The increase in the prevalence of HPV infections due to HIV infection and the advent of homosexuality between men are at the root of the increase in the incidence of these once rare cancers in men [4,5]. A study of 262 men attending an STI clinic in Vancouver, Canada, showed that 70% of them were infected with HPV [6,7]. Anal cancer accounts for 3% to 6% of digestive cancers in France and its incidence is increasing [8]. This 90% HPV-related cancer is the second most HPV-induced cancer in the world after cervical cancer [5,9]. Surgical treatment of anal cancer is usually deleterious because of the difficulty of maintaining anal continence. The recurrence rate is about 50% for carcinoma *in-situ* [8]. The HPV status of this cancer is linked to a better prognosis (specific survival, progression-free survival and overall survival) [10].

Cancers of the penis, scrotum and perineum, on the other hand, are very rare and are associated with high morbidity and mortality and a serious psychological impact [11-14]. 30%-80%of these cancers are related to high-risk HPV [15]. Radical surgery is the basis of their management despite significant physical and psychosexual morbidity for those treated. Currently, conservative treatment has become a widely accepted approach due to equivalent oncological control established with satisfactory somatic and sexual health outcomes [16,17].

The interaction of HPV E6 and E7 oncogenes with cell cycle proteins leads to autonomic cell proliferation, nuclear and cytoplasmic accumulation of p16 [18,19]. Hence, the immunohistochemically demonstration of overexpression in squamous cell carcinoma tissue is thus considered as a Telltale marker of high-risk HPV infection [20-22]. In high-risk HPV-infected cells, the E6 protein of the virus interacts with the p53 protein, causing p53 to be broken down and thus preventing it from performing its role as a tumor booster. In these cells, the p53 protein is not overexpressed, but rather degraded [23,24].

HPV-negative squamous cell carcinomas lack p16 overexpression but generally show nuclear p53 overexpression in proliferative tumor cells [25,26]. The homogeneous or heterogeneous expression of p53 in this case is often related to mutations in *Tp53* [27].

According to several studies, radiation therapy, chemotherapy and immunotherapy resulted in better regression, longer disease-free survival and better overall survival in HPV-induced squamous cell carcinomas [10,28,29]. While several studies have shown that determining the mutational status of *TP53* in EC is clinically important in patients with this pathology because these mutations are associated with an increased risk of lymph node metastases and poor prognosis [30-33]. In the Democratic Republic of Congo (DRC), there is no primary prevention program with the human papillomavirus vaccine neither a national program for the screening and management of lesions associated with the human papilloma virus [29].

The studies carried out in this country on lesions associated with the human papillomavirus have been of more interest to women, but for some time now, the phenomenon of homosexuality between men has been observed in the country's major cities, including Kinshasa, with the risk of infection with the human papillomavirus [34]. The aim of this study is to determine the frequency of induced anogenital squamous cell carcinomas HPV, the mutation of *Tp53* by immunostaining at p53 and the mitotic index by immunostaining at Ki-67 in order to contribute to the improvement of the management of this cancer in our communities.

Materials and Methods

We conducted a descriptive cross-sectional study in four Anatomy and Pathological Cytology Laboratories of the City of Kinshasa, namely the laboratories of the Department of Anatomy and Pathological Cytology of the University Clinics of Kinshasa, that of the National Institute of Biomedical Research (INRB); the Leboma Pathological Anatomy and Cytology Practice (ACP) and the NGANDA Hospital Laboratory. The present study covered a period of 10 years and 6 months, from January, 2012 to June, 2023. All available histopathological records and protocols were searched. The choice of the study period was guided by the availability of records and histopathological analysis reports from the above-mentioned laboratories.

Sampling was suitable for all cases of squamous cell carcinoma; 67 cases were identified in the registries and selected for this study after comparison with histopathological analysis reports. The variables of interest were age in year and sampling site. Only 30 paraffin blocks found and containing sufficient 10% formalin-fixed tissue were retained and intersected with the Reichert-Jung brand microtome for Immunohistochemical staining (IHC) at p16, p53 and Ki67. This staining was done with the Roche BenchMark GX IHC/ISH controller and the ultraView Universal DAB Detection Kit. These 30 cases were selected after re-reading of the Eosinophilic (EO) slides by two pathologists who all confirmed the histopathological diagnosis of squamous cell carcinoma.

P16 expression was detected by IHC staining using the Anti-p16 CINtec p16 Histology Antibody (E6H4 clone). ®The interpretation was positive if the cells were stained in the nucleus or combined in the nucleus and cytoplasm brown. Only squamous cell carcinoma tissues showing strong and diffuse cytoplasmic and nuclear expression of p16 were considered positive i.e. HR-HPV-induced. Expression of p53 was detected by IHC staining using the anti-p53 (DO-7) antibody. Homogeneous brown nuclear staining of all proliferating invasive cancer cells or heterogeneous staining with the presence of focal zones without p53 expression was considered positive and the absence of staining or ≤ 10% staining was considered negative. Only homogeneous and heterogeneous overexpression were considered to be cases with *TP53* mutation.

Ki-67 expression was detected by IHC staining using the Anti-Ki-67 antibody (clone30-9). The interpretation was positive if the tumor cells were stained only in the nucleus above 10% and negative below 10%. The reading of the IHC slides was performed by four pathologists who harmonized their point of view for the results presented. As the study was on archival material and not on individuals, informed consent was not required. Nevertheless, the data collection was carried out with the agreement of the managers of the laboratories that served as the framework for this study and the rules of anonymity and confidentiality were scrupulously observed. Descriptive analysis of frequencies and percentages allowed us to analyze the data.

Results

Out of 67 cases of anogenital squamous cell carcinoma diagnosed histologically in humans in Kinshasa during the period of this study, we performed immunostaining at p16, p53 and Ki-67 on 30 anogenital squamous cell carcinoma tissues fixed with 10% formalin and included in paraffin blocks **(Table 1)**.

Table 1: Distribution of cases by age and Site of Collection (SC).

Age/SC	Anus		Penis		Perineum		Scrotum		Total	
	n	%	n	%	n	%	n	%	n	%
≤ 40	1	7.7	0	0	0	0	0	0	1	3.4
41-60	4	30.8	4	40	4	80	1	50	13	43.3
≥ 61	8	61.5	6	60	1	20	1	50	16	53.3
Total	13	100	10	100	5	100	2	100	30	100

The squamous cell carcinoma is more observed over 60 years of age (53.3%) and then in the 41-60 age group (53.3%) with an extreme age of 27 and 85 years. The mean age was 59.3 years. The most observed site of development was the 13/30 anus (43.3%) followed by the 10/30 penis (33.3%) **(Table 2)**.

Strong and diffuse cytoplasmic and nuclear expression of p16 was noted in 28 cases, or 93% of cases (**Figure 1A**). 20 cases (66.6%) who had shown strong and diffuse cytoplasmic and nuclear expression of p16 and the 2 cases who had not shown p16 expression were not labeled with the anti-p53 antibody (**Figure 1B**) ETV8 cases that had shown strong and diffuse expression of p16 showed an expression of p53 less than 10%. 27 cases, i.e., 90% of cases, which showed strong and diffuse cytoplasmic and nuclear expression of p16, showed a high expression of Ki67, i.e., 40% to 90% (**Figures 1C and 1D**). The 2 cases that showed no p16 expression showed Ki-67 expression less than 10% while one case that showed strong and diffuse cytoplasmic and nuclear expression of p16 showed no Ki-67 expression, these are all shown in **Figure 1**.

Discussion

According to several studies, anogenital squamous cell carcinoma in men is more HPV-induced and observed beyond the age of 50. In this study, anogenital squamous cell carcinoma was most common from the age of 60. The average age of our patients was 59 years [35-37]. In the USA, the average age of diagnosis is about 68 years old and about 80% of those affected are at least 55 years old at the time of diagnosis. In France and among blacks and Hispanics, the average age is 60 years old [11,33,38]. Globally, about 20% of people diagnosed with penile cancer are under the age of 40 [11]. In Canada, however, it is most often found beyond the age of 60. In the United States of America and China, squamous cancer of the anus is rare in people under the age of 35 and the average age of diagnosis is from age 60 [35-38]. In Canada, however, it is observed in people over 55 years of age.

In this study, the most observed site of development was the 13/30 anus (43.3%) followed by the 10/30 penis (33.3%). We did

Table 2: Evaluation of p16, p53 and Ki-67 expression in relation to the sampling site.

Sampling site IHC evaluation		Anus n (%)	Penis n (%)	Perineum n (%)	Scrotum n (%)	Total N (%)
p16	Positive	12 (92.3)	9 (90)	5 (100)	2 (100)	28 (93)
	Negative	1 (7.7)	1 (10)	0 (0)	0 (0)	2 (7)
p53	Positive	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Negative	13 (100)	10 (100)	5 (100)	2 (100)	30 (100)
Ki-67	Positive	12 (92.3)	9 (90)	4 (80)	2 (100)	27 (90)
	Negative	1 (7.7)	1 (10)	1 (20)	0 (0)	3 (10)

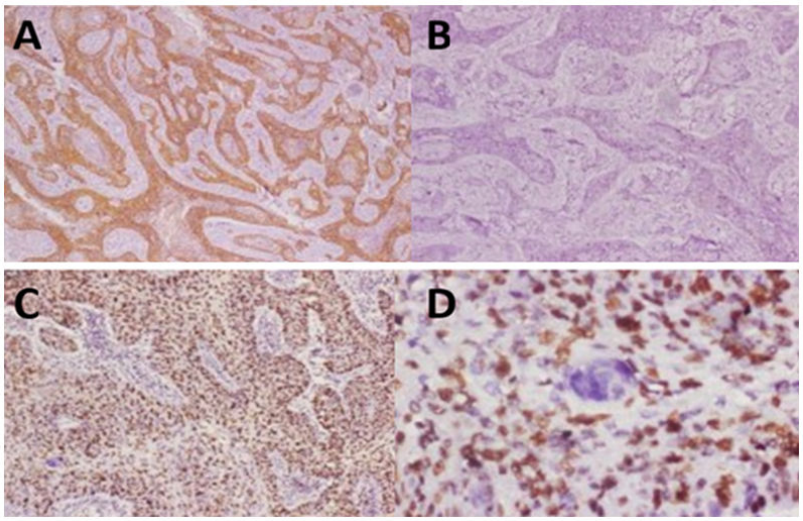

Figure 1: Cytoplasmic and nuclear expression of p16, p53 and ki67. **Note:** (A) Strong and diffuse cytoplasmic and nuclear expression of p16 in infiltrating well-differentiated squamous cell carcinoma of the penis (40x magnification); (B) Absence of nuclear expression of p53 in well-differentiated infiltrating squamous cell carcinoma of the penis (100x magnification); (C) Nuclear expression of ki67 in more than 90% of tumor cells in well-differentiated infiltrating squamous cell carcinoma of the penis (100x magnification) and (D) Nuclear expression of ki67 in more than 90% of tumor cells in well-differentiated infiltrating squamous cell carcinoma of the penis (400x magnification).

not find any seminar study. Most studies on anogenital squamous cell carcinoma in men have addressed it in isolation according to the different locations; penile, anal, scrotal. During the cell cycle, in the G1 phase, the Cyclin D/CDK4 and Cyclin D/CDK6 (Cyclin Dependent Kinases) complex phosphorylate and inactivate the Retinoblastoma protein (pRb), resulting in the release of E2F transcription factors that control the expression of genes important for the transition of the cell from the G1 phase to the S phase and for its progression to the S phase by synthesizing cyclins E and A. The p16 protein is an inhibitor of CDK4 and CDK6 (Cyclin Dependent Kinases) that inactivate pRb and thus participates in the regulation of the cell cycle when it is attacked [18,19,37].

When squamous cells are infected with oncogenic high-risk HPV, the HPV E7 oncoprotein competes with the CD/CDK4 and CD/CDK6 complex to bind to pRb, resulting in the release of E2F1 from pRb and cell cycle activation leading to autonomic cell proliferation [18,19,38,39]. Disruption of the pRb-E2F1 pathway by E7 thus induces overexpression and accumulation of p16 in cells through a negative feedback loop [35]. Immunohistochemistry demonstration of strong and diffuse cytoplasmic and nuclear expression of p16 in squamous cell carcinomas has been considered a surrogate marker of persistent oncogenic high-risk HPV infection [20-22,40,41].

In this study, strong and diffuse cytoplasmic and nuclear expression of p16 was noted in 28 cases, i.e. 93% of cases, with 92% for the anus, 90% for the penis and 100% for the perineum and scrotum in the USA in 2017 [8] and in France in 2018 [9] had respectively noted a strong and diffuse expression of p16 in squamous cell carcinoma of the anus with a proportion of 70% and 80% of cases respectively in Germany 2015 [12], in France 2013 [13] and in Paraguay in 2011 [20] had on the other hand noted a strong and diffuse expression of p16 in squamous cell carcinoma of the penis with a proportion of 80%, 70% and 31%of cases respectively. These results corroborate with the literature review, 90% of anal cancer is linked to HPV [5,9] and 30%-80% of penile cancers are linked to high-risk HPV [11,20]. The lack of primary prevention by vaccination against HPV infection in our country is believed to be the basis for the high frequency of anogenital squamous cell carcinoma HPV induced in this study. The higher or lower frequency of anogenital squamous cell carcinoma in men observed in the USA and Europe despite HPV vaccination may be due to the fact that this country receives a lot of expatriates from countries where there is no HPV vaccine.

In HPV-infected cells, the HPV E6 protein binds to the p53 protein and p53 is a tumor suppressor protein because it is often responsible for repairing DNA damage, arresting the cell cycle, and apoptosis under stress at the cellular level. When the E6 protein binds to p53, it prevents its functions and causes it to be degraded by the ubiquitin and proteasome system, resulting in loss of control of genomic stability and cell resistance to apoptosis [19,23,24,25,27,42]. The E6 protein also interacts with cellular proteins, which possess a PDZ domain; proteins involved in the regulation of cell differentiation, adhesion and polarity. By binding to these proteins, E6 disrupts the architecture and cohesion of the infected epithelium and promotes tumor progression.

The E6 protein in HPV also stimulates the expression of telomerase, an enzyme that plays an important role in maintaining telomere length. This enzyme is normally inactive in somatic cells, which limits the number of possible cell divisions. By activating telomerase, E6 allows HPV-infected cells to divide indefinitely and acquire an immortal trait [19,23,24,27,42].

In immunohistochemistry, the expression of p53 in HPV-induced squamous cell carcinomas can vary depending on the type of tissue, the stage of the cancer, the type of HPV and the method of analysis used. Some studies have shown no or decreased p53 expression while others have shown overexpression or abnormal expression in these cancers [23,24,43].

In this study, 20 cases (66.6%) who had shown strong and diffuse cytoplasmic and nuclear expression of p16 and the 2 cases that had not shown expression of p16 were not labeled with the anti-p53 antibody while 8 cases showed a low expression less than or equal to 10% in Austria 2017 [23], in Japan 2008 [27] and had noted a diffuse and homogeneous expression of p53 in squamous cell carcinoma with a proportion of 75%, 73% and 100% respectively. We believe that this difference in outcome may be justified by the lack of prevention of HPV infection in our country, which is the basis for a high frequency of anogenital HPV squamous cell carcinoma induced in this study. Primary prevention through HPV vaccination introduced in Japan, China and Austria thus justifies the high frequency of *p53* mutation observed in anogenital squamous cell carcinoma in men in these countries [44]. 27 cases, or 90% of cases, who showed strong and diffuse cytoplasmic and nuclear expression of p16, showed a high expression of Ki-67, i.e. 40% to 100%. The 2 cases that showed no expression of p16 showed less than 10% Ki-67 expression while one case that showed strong and diffuse cytoplasmic and nuclear expression of p16 showed no expression of Ki-67. In the case of cancer, high levels of Ki-67 often indicate rapid cell division, which indicates rapid progression of the cancer. Thus, the assessment of Ki-67 is generally used as a prognostic factor to guide the decision of adjuvant therapy but also defined as a predictive factor of response to neoadjuvant therapy [20,45].

The interest of this study is twofold because the determination of HPV and mutational status of p53 in squamous cell carcinoma has a therapeutic interest; induced HPV squamous cell carcinomas have a good prognosis while those showing a *p53* mutation have a poor prognosis. Secondly, in biomedical research, because to date, the HPV E6 and E7 oncoproteins and the p53 protein are potential targets for the development of new therapeutic strategies against HPV-related cancers and those with the *p53* mutation. Since the majority of these cancers are HPV-induced, a similar study with a large sample including *in-situ* hybridization or PCR for HPV subtyping is needed in order to advocate the insertion of the HPV vaccine in our country; but also, to make available the necessary data for clinical trials, given that currently research is more oriented towards conservative treatment, especially for penile carcinoma.

Conclusion

The elderly was more affected. The anus followed by the penis were the most noted locations. Nearly all of the man's anogenital

squamous cell carcinoma are induced by HPV and show a high mitotic index. No cases of *p53* mutation have been noted. There is a need to conduct a similar study including *in-situ* hybridization throughout the country with a large sample in order to determine the types of HPV implicated in the genesis of anogenital lesions in humans, which will not only make it possible to introduce the vaccine against this virus, but above all to organize the early screening and management of lesions associated with this virus on a national scale.

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Author Contributions

David AZAKO: Design, data collection, validation of histo-pathological and immunohistochemical diagnoses in the first first degree and writing of the article.

Gabriel KIKWEDI: Realization of the immunohistochemical technique.

Drs. Fabrice BOKAMBANDJA and Teddy MUKENDI: Confirmation and validation of histopathological and immuno-histological chemical diagnoses in the second degree.

Other authors: reading and amending the entire article before validating the latest version

Professor Dr. Bienvenu LEBWAZE : confirmation and validation of histopathological and immunohistochemical diagnoses in the third revision of its main scientific content and approval of the version to be published.

References

1. Burd EM (2003) Human papillomavirus and cervical cancer. Clin Microbiol Rev 16: 1-17.
2. Institut National du Cancer (2018) Papillomavirus and Cancer.
3. Mehanna H, Jones TM, Gregoire V, Ang KK (2010) Oropharyngeal carcinoma related to human papillomavirus. BMJ 340: c1439.
4. Clifford GM, Georges D, Shiels MS, Engels EA, Albuquerque A, et al. (2021) A meta-analysis of anal cancer incidence by risk group: Toward a unified anal cancer risk scale. Int J Cancer 148: 38-47.
5. Gargano J, Meites E, Watson M, Unger E, Markowitz L (2024) Human papillomavirus. Central for Diseases Control and Prevention.
6. Machalek DA, Poynten M, Jin F, Fairley CK, Farnsworth A, et al. (2012) Anal human papillomavirus infection and associated neoplastic lesions in men who have sex with men: A systematic review and meta-analysis. Lancet Oncol 13: 487-500.
7. van Rijn VM, Mooij SH, Mollers M, Snijders PJ, Speksnijder AG, et al. (2014) Anal, penile and oral high-risk HPV infections and HPV seropositivity in HIV-positive and HIV-negative men who have sex with men. PloS one 9: e92208.
8. Islami F, Ferlay J, Lortet-Tieulent (2017) International trends in anal cancer incidence rates. Int J Epidemiol 46: 924-938.
9. Abramowitz L, Jacquard AC, Jaroud F, Haesebaert J, Siproudhis L, et al. (2011) Human papillomavirus genotype distribution in anal cancer in France: The EDiTH V study. Int J Cancer 129: 433-439.
10. Rao S, Guren MG, Khan K, Brown G, Renehan AG, et al. (2021) Anal cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol 9: 1087-1100.
11. Bleeker MC, Heideman DA, Snijders PJ, Horenblas S, Dillner J, et al. (2009) Penile cancer: Epidemiology, pathogenesis and prevention. World J Urol 27: 141-150.
12. Hakenberg OW, Comperat EM, Minhas S, Necchi A, Protzel C, et al. (2015) EAU guidelines on penile cancer: 2014 update. Eur Urol 67: 142-150.
13. Brady KL, Mercurio MG, Brown MD (2013) Malignant tumors of the penis. Dermatol Surg 39: 527-547.
14. Micali G, Nasca MR, Innocenzi D, Schwartz RA (2006) Penile cancer. JAAD 543: 369-391.
15. Daling JR, Madeleine MM, Johnson LG, Schwartz SM, Shera KA, et al. (2005) Penile cancer: Importance of circumcision, human papillomavirus and smoking in *in-situ* and invasive disease. Int J Cancer 116: 606-616.
16. Djajadiningrat RS, van Werkhoven E, Meinhardt W, van Rhijn BW, Bex A, et al. (2014) Penile sparing surgery for penile cancer-does it affect survival? J Urol 192: 120-126.
17. Mahesan T, Hegarty PK, Watkin NA (2016) Advances in penile-preserving surgical approaches in the management of penile tumors. Urologic Clinics 43: 427-434.
18. Stankiewicz E, Kudahetti SC, Prowse DM, Ktori E, Cuzick J, et al. (2009) HPV infection and immunochemical detection of cell-cycle markers in verrucous carcinoma of the penis. Mod Pathol 22: 1160-1168.
19. Stankiewicz E, Prowse DM, Ktori E, Cuzick J, Ambroisine L, et al. (2011) The retinoblastoma protein/p16^{INK4A} pathway but not p53 is disrupted by human papillomavirus in penile squamous cell carcinoma. Histopathology 58: 433-439.
20. Cubilla AL, Lloveras B, Alejo M, Clavero O, Chaux A, et al. (2011) Value of p16^{INK4A} in the pathology of invasive penile squamous cell carcinomas: A report of 202 cases. Am J Surg Pathol 35: 53-61.
21. Reuschenbach M, Seiz M, Doeberitz CV, Vinokurova S, Duwe A, et al. (2012) Evaluation of cervical cone biopsies for coexpression of p16^{INK4A} and Ki-67 in epithelial cells. Int J Cancer 130: 388-394.

22. Snow AN, Laudadio J (2010) Human papillomavirus detection in head and neck squamous cell carcinomas. Advances in anatomic pathology 176: 394-403.

23. Kashofer K, Winter E, Halbwedl I, Thueringer A, Kreiner M, et al. (2017) HPV-negative penile squamous cell carcinoma: Disruptive mutations in the *TP53* gene are common. Mod Pathol 30: 1013-1020.

24. Feber A, Worth DC, Chakravarthy A, de Winter P, Shah K, et al. (2016) CSN1 somatic mutations in penile squamous cell carcinoma. Cancer Res 76: 4720-4727.

25. Yang B, Hart WR (2000) Vulvar intraepithelial neoplasia of the simplex (differentiated) type: A clinicopathologic study including analysis of HPV and p53 expression. Am J Surg Pathol 24: 429-441.

26. Chaux A, Pfannl R, Rodríguez IM, Barreto JE, Velazquez EF, et al. (2011) Distinctive immunohistochemical profile of penile intraepithelial lesions: A study of 74 cases. Am J Surg Pathol 35: 553-562.

27. Yanagawa N, Osakabe M, Hayashi M, Tamura G, Motoyama T (2008) Detection of HPV-DNA, p53 alterations and methylation in penile squamous cell carcinoma in Japanese men. Pathol Int 58: 477-482.

28. Wiest T, Schwarz E, Enders C, Flechtenmacher C, Bosch FX (2002) Involvement of intact HPV16 *E6/E7* gene expression in head and neck cancers with unaltered p53 status and perturbed pRb cell cycle control. Oncogene 21: 1510-1517.

29. Brewer NT, Fazekas KI (2007) Predictors of HPV vaccine acceptability: A theory-informed, systematic review. Prev Med 45: 107-114.

30. Wang Y, Wang K, Chen Y, Zhou J, Liang Y, et al. (2019) Mutational landscape of penile squamous cell carcinoma in a Chinese population. Int J Cancer 145: 1280-1289.

31. Lopes A, Bezerra AL, Pinto CA, Serrano SV, de Mello CA, et al. (2002) p53 as a new prognostic factor for lymph node metastasis in penile carcinoma: Analysis of 82 patients treated with amputation and bilateral lymphadenectomy. J Urol 168: 81-86.

32. Ficarra V, Akduman B, Bouchot O, Palou J, Tobias-Machado M (2010) Prognostic factors in penile cancer. Urology 76: S66-S73.

33. Davody AP (2021) Penile cancer: Diagnoses, symptoms and treatments. Urologie.

34. ASCO (2023) Penile Cancer Causes, Risk Factors and Prevention.

35. ASCO (2023) Key Statistics for Anal Cancer. American Cancer Society.

36. Jullie S (2023) How Common is Anal Cancer? Medical News Today.

37. Pal A, Kundu R (2020) Human papillomavirus E6 and E7: The cervical cancer hallmarks and targets for therapy. Front microbiol 10: 3116.

38. Yu L, Fei L, Liu X, Pi X, Wang L, et al. (2019) Application of p16/Ki-67 dual-staining cytology in cervical cancers. J Cancer 10: 2654-2660.

39. Pauck A, Lener B, Hoell M, Kaiser A, Kaufmann AM, et al. (2014) Depletion of the cdk inhibitor $p16^{INK4A}$ differentially affects proliferation of established cervical carcinoma cells. J Virol 88: 5256-5262.

40. von Keyserling H, Kuhn W, Schneider A, Bergmann T, Kaufmann AM (2012) $p16^{INK4A}$ and $p14^{ARF}$ mRNA expression in Pap smears is age-related. Mod Pathol 25: 465-470.

41. Yu L, Wang L, Zhong J, Chen S (2010) Diagnostic value of $p16^{INK4A}$, Ki-67, and human papillomavirus L1 capsid protein immunochemical staining on cell blocks from residual liquid-based gynecologic cytology specimens. Cancer Cytopathol 118: 47-55.

42. Prétet JL, Charlot JF, Mougin C (2007) Virological and carcinogenic aspects of HPV. Bulletin de l'Académie nationale de médecine. 191: 611-623.

43. Kervarrec T, Touze A, Guyetant S, Samimi M, Jullie ML (2020) Expression de p40 expression dans les carcinomes à cellules de Merkel: Un potentiel piège diagnostique. Ann Dermatol Venereol 147: A226-A227.

44. Davey MG, Hynes SO, Kerin MJ, Miller N, Lowery AJ (2021) Ki-67 as a prognostic biomarker in invasive breast cancer. Cancers 13: 4455.

45. Bean SM, Eltoum I, Horton DK, Whitlow L, Chhieng DC (2007) Immunohistochemical expression of p16 and Ki-67 correlates with degree of anal intraepithelial neoplasia. Am J Surg Pathol 31: 555-561.