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Prevalence of human papillomavirus infection in squamous cell carcinoma of the anal canal in a Northeast City in Brazil: viral genotyping and clinical aspects

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Abstract

Background: Anal cancer malignancies comprise about 1.5 to 3% of cancers from the gastrointestinal in which high-risk types of human papillomavirus (HR-HPV) is responsible for >80% of cases. The aim of this work was to detect and perform human papillomavirus (HPV) genotyping in squamous cell carcinoma specimens from the anal canal and to investigate the association between viral infection and histopathological and clinical aspects.

Methods: The presence of genotype-specific HPV DNA in formalin-fixed paraffin embedded tissue from 27 anal SCC samples from a reference cancer hospital of São Luís, State of Maranhão, Brazil was performed by Linear Array HPV Genotyping Test and the INNO-LiPA HPV Genotyping Assay. Fisher's Exact test and Chi-square test were performed in order to evaluate the association between HPV type and clinical and morphological variables. *P* values less than 0.05 were considered statistically significant.

Results: Average age of patients at the time of diagnosis was 54.96 years ± 15.81; 74.07% of patients were female. Vegetative ulcers represented the most common type of lesion (22.22%). The lesions ranged in size from 2.1 cm to 5.0 cm and mostly were well-differentiated (70.38%). Lymph node involvement was observed in 26% of the patients. Molecular evaluation revealed that HPV infection was detected in 81.48% of the lesions, and the most common type found was the oncogenic HPV 16. Statistical analysis indicated that the clinical and histopathological variables were not associated with HPV infection.

Conclusions: Our results indicate that anal SCC rarely occurs in the absence of HPV and emphasize the predominant role of HPV16. The evaluation about genotype-specific prevalence of HPV in anal SCC is important to assess the potential benefit of HPV vaccination.

Keywords: Anal cancer, Papillomavirus infections, Association, Molecular typing

Background

Squamous cell carcinoma (SCC) compromises more than 70% of all anal cancer malignancies, and represents 1.5 to 3% of cancers of the gastrointestinal tract [1, 2]. Persistent infection with high-risk types of human papillomavirus (HR-HPV) is responsible for >80% of cases. SCC mainly affects women over the age of 50 years; among men, SCC predominantly affects individuals in the age range of 20 to 49 years [3]. There are few epidemiological data for anal human papillomavirus (HPV) infection in Brazil. According to Instituto Nacional de Câncer (INCA), in Brazil, anal cancer accounts for 1 to 2% of all colon tumors and 2 to 4% of all cancers that affect the large intestine [4]. This rate rises to alarming proportions in specific risk groups, such as in homosexual men and in HIV-positive patients. Palesfky et al. [5] reported that the incidence of anal HPV was 61 and 93%

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in HIV-seronegative and HIV-seropositive homosexual male patients, respectively. In women, the corresponding values were 42 and 76%, respectively.

Although HPV infection is essential for the development of anal SCC, the exact frequency of this disease is not known. In Brazil, the data regarding this type of cancer are included in the statistics for colorectal cancer. Therefore, epidemiological data representative of a specific region is essential for implementing particular guidelines for treatment and prevention, e.g. an effective vaccination schedule, especially in high-risk groups. HPV infection, a sexually transmitted disease (STD) with potentially malignant development, that affects both men and women, is of global concern. There is a lack of studies about anal cancer in the Northeast region of Brazil, where social-economic conditions are precarious. Therefore, a thorough study of the prevalence and genotyping of HPV in patients diagnosed with anal cancer in São Luís, a city of the Northeast region, is potentially important for the prevention and early diagnosis of this disease. In addition, the understanding of HPV prevalence and knowledge of the viral subtype distribution constitute important epidemiological information that can assist the development of local or regional public policies to prevent HPV and of new vaccines.

The aims of this study were to detect and assess genotype-specific HPV prevalence in patients with anal SCC and to examine the association between viral presence and clinicopathological aspects of disease.

Methods

Enrollment

Retrospective study performed in paraffin-embedded anal tumor tissue samples collected from two public reference hospitals in Maranhão, a city in the Northeast region of Brazil. The 27 samples included in the study were from patients diagnosed with anal cancer between the years of 2001 and 2011. Patient information and data on histopathological characteristics of tumors were obtained from medical records. As the samples were obtained from paraffin-embedded tumors, written informed consent was not required from the patients. The identity of the patients was not disclosed in this study. This work was approved by the Ethics in Research Committee of the University Hospital of the Federal University of Maranhão (n° 348.397).

Inclusion criteria:

Paraffin blocks and histological slides of anal tumors as a result of biopsy or surgical treatment at any follow–up in the archives of the Pathology Services. Exclusion criteria: Patients in which reports did not provide complete information.

HPV detection

The histopathological characteristics of the samples were reviewed by the pathologist, and blocks with tumor representativeness (over 50% of the total area of the fragment) were selected. After microtomy, the sections were deparaffinized and stored at 4°C for DNA extraction. The extraction of genomic DNA from the samples was performed using the QIAamp DNA FFPE Tissue Purification Kit (QIAGEN®) according to the extraction protocol suggested by the manufacturer.

The amplification and HPV genotyping was performed using the INNO-LiPA HPV Genotyping Extra kit (INNOGENETICS), which is capable of detecting 28 different HR types of HPV (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82), a number of low-risk HPV genotypes (6, 11, 40, 43, 44, 54,and 70), and the additional types 69, 71, and 74. Hybridized band signals corresponding to HPV types were visually interpreted according to the manufacturer's instructions.

Statistical analysis

The data was tabulated using the Microsoft Office® Excel 2010 application for calculation of frequencies, mean, median, standard deviation, minimum, and maximum of the variables used in the EpiInfo 7 program. The descriptive statistics for categorical variables were represented as a distribution of frequencies. Means, medians, standard deviation, minimum, and maximum values, and confidence intervals were used to represent numerical variables. Tables and graphs were constructed following the descriptive analysis. In order to evaluate the relationship between epidemiological and clinical data and the presence of HPV, the chi-square

Table 1 Age and clinical presentation of 27 patients diagnosed with anal cancer

	N = 27	Percent
Sex		
Female	20	74.07
Male	07	25.93
Age at diagnosis		
Mean age	54.96 ± 15.81	
≤ 42	04	14.81
42–52	11	40.75
53–63	06	22.22
64–74	02	7.41
75–86	04	14.81
Lesion area		
Anal edge	03	11.11
Anal canal	19	70.37
Anal edge and canal	05	18.52

Table 2 Pathologic characteristics of anal tumors from 27 patients diagnosed with anal cancer

Predominant morphology		
Ulceration	04	14.81
Vegetating	02	7.41
Ulceration and vegetating	06	22.22
Stenosing	01	3.7
Non evaluated	14	51.85
Size of the lesion (cm)		
≤ 0,5	01	3.7
0.6–2.0	08	29.63
2.1–5.0	15	55.56
≥ 5.1	03	11.11
UJCC/TNM Staging		
I	02	7.41
II	02	7.41
III	09	33.33
IV	0	0
Non evaluated	14	51.85
Tumor Grade		
Well Differentiated	06	22.22
Moderately Differentiated	19	70.38
Poorly Differentiated	01	3.7
Undifferentiated	01	3.7
Invasion		
Present	09	33.33
Absent	12	44.45
Not evaluated	06	22.22
Infiltration		
Anal canal wall	01	3.7
Perianal region	01	3.7
Perirectal fat	01	3.7
Vagina and uterine cervix	01	3.7
Stroma	01	3.7
Muscle layer	01	3.7
Perineural	01	3.7
Vulvar	01	3.7
Not evaluated	01	3.7
No infiltration	18	66.7
Lymph node involvement		
Yes	07	25.93
No	15	55.55
Not evaluated	05	18.52
Metastasis		
Yes	03	11.11
No	19	70.37
Not evaluated	05	18.52
Inflammatory process		
Yes	05	18.52
No	12	44.44
Not evaluated	10	37.04
Necrosis Presence		
Yes	02	7.41
No	12	44.44
Not evaluated	13	48.15

test and Fisher's exact test were used, and the results were considered statistically significant at 5% probability and for $p < 0.05$.

Results

Anal tumor biopsies were evaluated in 27 patients aged 32–85 years, with a mean age of 54.96 years and standard deviation of ± 15.81, presenting a higher prevalence between 42 and 52 years (Table 1). Among the patients involved in the study, 74.07% (20/27) were female. The demographic and distribution of clinical is shown on Table 1.

With regard to the location of the lesions, the anal canal was the most affected area. Vegetating and ulcerative lesions, observed in 22.22% of the cases, were the most commonly occurring type of lesion. Most lesions (55.56%) were between 2.1 and 5.0 cm in size.

Table 2 demonstrate the pathological characteristics of the anal tumors analyzed. According to UJCC/TNM staging, most tumors were grade III (33.33%) and moderately differentiated (70.38%). The findings indicate that invasion was present in 33.33% of the tumors examined, and that infiltration occurred in at least one of the structures (anal canal wall, perianal region, perirectal fat, vagina and uterine cervix, stroma, muscle layer, perineural and vulvar). Lymph node involvement was found in 25.93% of the tumors examined.

HPV DNA was detected in 81.48% (22/27) of the anal tumor samples. All tumors (100%) were found to be infected with high-risk viral types; HPV-16 was present in all samples (Fig. 1). Among the high-risk HPV types, types 18, 29, 39, 45, 52, 59, 66, and 68 were also present. HPV-11 was the only low-risk genotype detected. In addition, in 50.09% (12/22) of the samples, only one viral type (simple infection) was detected. HPV-16 infection, which is associated with high oncogenic risk, was found in all cases studied. The prevalence of multiple infections in the samples studied was 45.45% (10/22) (Table 3).

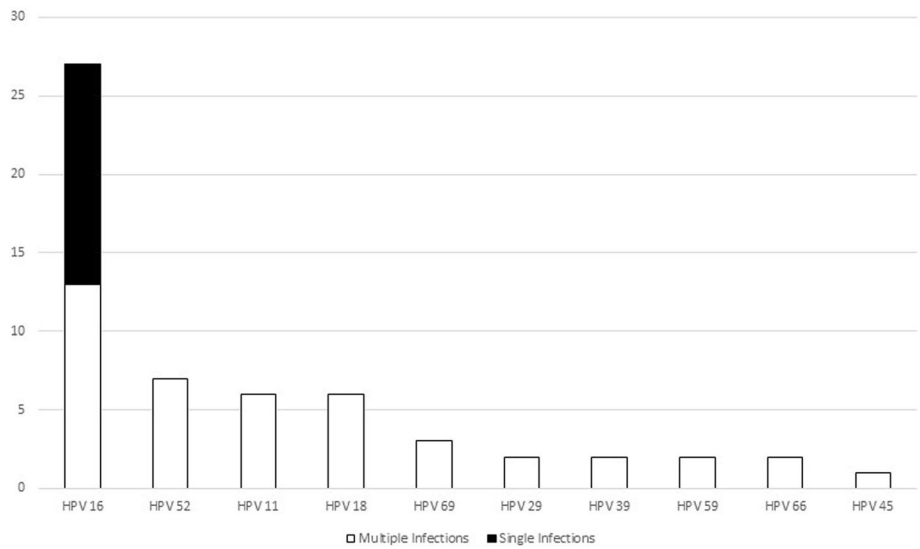

Fig. 1 The graphic shows the number of samples infected with the different HPV types. All positive samples were infected with HPV-16. HPV-52 was present in 7 samples; HPV-11 and HPV-18 in 6 samples; HPV-69 in 3 samples; HPV-29, HPV-39, HPV-59, and HPV-66 in 2 samples each; and HPV-45 in 1 sample

No association was found ($p < 0.05$) between HPV infection and histopathological and clinical variables (Table 4).

Discussion

The results of the present study indicate that most patients with anal SCC was 54.96 years constituting a sample younger than that reported in the literature. According to several researches, the peak incidence of anal cancer is between the ages of 58 and 64 years [6–10]. The mean age of patients with anal cancer may had vary among studies, possibly because of the type of population studied and the presence of risk factors such as sexual orientation, immunosuppression (patients with HIV+ infection and transplant recipients), tobacco smoking, and alcoholism [6–10].

We did not evaluated the social economic status of our population but the study was developed on a very poor area of Brazil, with one of the lowest human development indexes. According to National Cancer Institute, cervical cancer and penile cancer are the second more incident type of cancer among women and men, respectively, in Maranhão [4]. Both cancer, as well as anal SCC, are associated with HPV infection and with poor social-economic status, health conditions and hygiene practices of the population. This condition may explain the younger status of our studied population.

Table 3 HPV prevalence and distribution according to oncogenic risk in 27 patients diagnosed with anal cancer

	N = 27	Percent
HPV -	5	18.52
HPV +	22	81.48
HR-HPV		
LR-HPV		
Single infection	12	50.09
Multiple infections	10	45.45

Our samples presented a high prevalence of HPV, which are consistent with those reported in the literature. Alemany and coauthors applied a retrospective approach (1986–2011) to evaluate 496 cases of invasive anal cancer for DNA-HPV presence in a multi-center study involving patients from Europe, North America, Latin America, Africa, and Asia. The prevalence of HPV infection for each continent was 87.6, 95.8, 90.4, 61.9, and 81.1%, respectively [10]. In Brazil, Aguiar and coauthors conducted a study of patients with anal carcinomas in Goiânia-Goiás, and found that 76.3% of the samples collected were positive for HPV [9]. The results of our study also corroborate the findings of Varnai and colleagues [11], Ramamoorthy and colleagues [12], de Vuyst and colleagues [13], and Rodel and colleagues [14], who reported the detection of HPV-DNA presence in 60.6, 80, 73, and 95.8% of anal cancer samples examined, respectively.

Torres Neto and colleagues [15] performed a retrospective study about mains demographics characteristics of anal cancer patients in Sergipe, another city of Northeast State of Brazil. However, they did not performed any HPV analysis.

Table 4 Association between clinical presentation and pathological characteristics in HPV infected patients with anal cancer

	HPV (+) $N = 22$	HPV (-) $N = 5$	p
Gender			0.1751[a]
Female	17	3	
Male	05	2	
Site of primary tumor			0.2386[a]
Anal Margin	3	-	
Anal Canal	15	5	
Anal Margin and Canal	3	3	
Lesion type			0.7809[a]
Ulceration	3	1	
Vegetating	2	-	
Ulceration and Vegetating	5	1	
Stenosing	1	-	
Non evaluated	10	06	
Size of the lesion (cm)			0.3657[a]
≤ 0.5	-	1	
0.6–2.0	6	2	
2.1–5.0	11	4	
≥ 5.1	03	-	
UJCC/TNM Staging			0.7542[a]
I	-	1	
II	2	-	
III	9	-	
IV	-	-	
Non evaluated	9	6	
Lymph node involvement			0.3133*
Yes	7	-	
No	11	5	
Non evaluated	2	2	
Inflammatory process			0.3017[a]
Yes	3	2	
No	12	2	
Non evaluated	6	4	

Significant p values < 0.05
[a] Fisher's test
* Chi-square

Infection with oncogenic, or high-risk, HPV-16 was detected in all cases. Other studies also report that HPV-16 was the main HPV type found in over 80% of carcinomas [10, 16–18].

Female patients constituted the majority of the cases on our work. Alemany and co-authors [10] found that female patients constituted the majority of the cases of SCC (66.3%). Ouhoummanea and co-authors [8] found that the majority of incidence of anal cancer (60%) was in women. Aguiar and colleagues found that 62.8% of patients with anal cancer were women [9]. The results of a study of Czech patients with anal cancer by Tachezy et al. [6], in which the same number of patients was analyzed as in our study, revealed that 52.4% of the patients were female. Two other studies, conducted in Brazil, found that the occurrence of anal cancer was 62.8–78.8% among the female patients studied [19, 20].

The prevalence of anal SCC in women may be explained by the anatomical proximity between the vaginal opening and the anus. A study by Giraldo and co-authors [21] of 184 Brazilian women demonstrated a significant association between cervical intraepithelial lesions and anal intraepithelial lesions. Anal lesions were found to be present in 17.4% of patients with genital lesions, but only in 3.2% of patients without genital lesions. HPV infection in women may also be facilitated by non-sexual practices and self-inoculation (via vaginal secretions, transfer of fomites, and digital transfer), in view of the anatomical proximity between the vaginal opening and the anus [11, 22].

In what concerns the pathologic characteristics of the anal SCC samples, the most common morphology type observed on our work was the ulcerative form, presented a size of 2.1–5.0 cm, came from the anal canal region and moderately differentiated. However, the majority did not present invasion, infiltration, metastasis, and lymph node involvement. These clinical pathological characteristics are in accordance with others findings. Salati and Kadi [23] reported that the mean size of anal tumors investigated in their study was 3.9 cm. They report that spread of anal cancer is mainly local and regional. Usually, the tumor spreads into the ischiorectal fossae, the prostatic urethra and bladder in men, and the vagina in women. Anal cancer may spread via the lymphatic vessels (10–15%). Hematogenous spread develops in fewer than 10% of cases [23]. Studies have shown that the incidence of nodal metastasis in anal cancer is low (approximately 10%) [17, 24]. Ouhoummanea and co-authors [8] reported that, of the tumors studied, 43% were moderately differentiated.

No statistically significant association ($p < 0.05$) was found between HPV infection and clinical and histopathological variables. Other reports have also concluded that anal tumors are highly likely to be positive for infection with HR-HPV, regardless of the morphology of the tumor [6].

The understanding of HPV prevalence and knowledge of the viral subtype distribution constitute important epidemiological information that can assist the development of local or regional public policies to prevent HPV and of new vaccines. The present study, in agreement with other epidemiological and molecular studies, demonstrates that HPV infection is an important etiological agent of anogenital cancer [21, 25].

Conclusion

HPV DNA was found in 22 of the 27 analyzed samples (81.48%). The high-risk type HPV 16 was observed in 100% of the lesions while HPV-11 was the only low-risk genotype detected. The average age of the patients in the study was 54.96 years old. Prevalent lesions were larger than 2 cm, grade III (33.33%) and moderately differentiated (70.38%). The clinical and histopathological variables did not tend to have an association with infection by the HPV virus.

Abbreviations

HPV: Human papillomavirus; HR-HPV: High-risk human papillomavirus; SCC: Squamous cell carcinoma; STD: Sexually transmitted disease

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Availability of data and materials

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Authors' contributions

SJAG, FCBV and JMCS performed DNA extraction, viral analysis and patient data collection. Viral genotyping was performed under the supervision of LLV. FCBV, MDSBN and LMOB analyzed and interpreted the data. SJAG wrote the manuscript draft, which was read and edited by all the authors. All authors read and approved the final version of the manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable. According to Resolução 466 CNS, item "IV.8 - Nos casos em que seja inviável a obtenção do Termo de Consentimento Livre e Esclarecido ou que esta obtenção signifique riscos substanciais à privacidade e confidencialidade dos dados do participante ou aos vínculos de confiança entre pesquisador e pesquisado, a dispensa do TCLE deve ser justificadamente solicitada pelo pesquisador responsável ao Sistema CEP/CONEP, para apreciação, sem prejuízo do posterior processo de esclarecimento".

Ethics approval and consent to participate

This work was approved by the Ethics in Research Committee of the University Hospital of the Federal University of Maranhão (n° 348.397).

Publisher's Note

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