Original Article

Clinicopathological aspects and prevalence of human papillomavirus in anal cancer

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Abstract

Anal cancer is relatively rare; however, its incidence has increased in recent years. Several risk factors are associated with the development of anal cancer, including age older than 50 years, low-fiber diet, chronic anal fistulas, smoking, multiple partners, anal intercourse practice, Human Immunodeficiency Virus infection and immunosuppression. However, the presence of human papillomavirus represents the main risk factor for the development of anal cancer. The aim of this study was to evaluate the clinicopathological aspects of a series of patients with anal carcinomas diagnosed in Hospital Araújo Jorge, Goiânia-Goiás, as well as the prevalence of human papillomavirus genome in these tumors. Clinical, pathological and socio-demographic data were collected from the respective medical files and paraffin blocks containing anal carcinomas specimens were used for DNA extraction and detection of human papillomavirus, by means of polymerase chain reaction, using short PCR fragment primers. Forty-three cases were selected and had the data analyzed, while 38 cases were tested for human papillomavirus genome detection. Among the evaluated patients, 62.8% were women; 53.4% of tumors were squamous cell carcinoma and 46.5% of the patients were aged between 60 and 75 years. Risk factors, such as smoking (39.5%) and alcoholism (20.9%) were recorded in the studied group. Lymph node metastases were detected in 30.2% of cases and 7.0% had distant metastasis. The detection of human papillomavirus DNA was positive in 76% of cases assessed and this was significantly associated with squamous cell carcinomas. Aggressive behavior and advanced stage of anal cancer described in this study highlight the need for preventive measures that contemplate these tumors, including vaccination against human papillomavirus.

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Introduction

Anal cancer is relatively rare, accounting for about 30,000 new cases a year worldwide, with a peak incidence between the ages of 58 and 64 years. It represents approximately 1.5% of all tumors of the digestive tract and 2–3% of colorectal tumors. The epidermoid carcinoma is the most frequent histological type, followed by adenocarcinomas (ADC), transitional cell or cloacogenic tumors, basaloid and mucus-epithelioid tumors. The epidermoid carcinoma is the most common cancer of the anus and is responsible for 85% of malignant lesions of this region.

The clinical presentation and severity of anal cancers depend on the size and location of the tumor in the anus. Small, mobile lesions, smaller than 2 cm have a probability of cure in 80% of cases, compared with <50% chance for tumors larger than 5 cm. It is known that 20% of patients are asymptomatic; however, anal bleeding and the sensation of a mass occupying the anal canal are the most frequent signs and symptoms, often mistakenly associated with hemorrhoidal disease.

The treatment of anal carcinoma can be surgical and clinical. Surgical treatment is based on local excision of the lesion or abdominoperineal resection of the rectum and anus, whereas the medical treatment is based on the anorectal segment preservation through chemoradiotherapy or radiotherapy alone.

Tumors of the anal canal are more common in females, while anal margin tumors are more frequent in males. Some studies have shown that although the prevalence of anal cancer is higher in women older than 60 years of age, the disease has become very common in men between 30 and 40 years, due to high association with infection by the Human Immunodeficiency Virus (HIV).

Several risk factors are known to be associated with the development of anal cancer, including age older than 50 years, low-fiber diet, chronic anal fistulas, smoking, multiple partners, history of anal intercourse (receptive anal sex), HIV infection, immunosuppression following organ transplantation and immunosuppressive drugs. However, the presence of HPV...
human papillomavirus (HPV) shows the main association with the development of anal cancer.\textsuperscript{2,8}

HPVs are small double-stranded DNA viruses, which belong to the Papillomaviridae family. Initially, these viruses were identified, cloned and sequenced from samples of cervical tumors, and subsequently established as important etiological agents of carcinogenesis in several human tumors.\textsuperscript{9,10} More than 200 types of HPV have been identified and each of them has particular tropism for specific anatomical sites.\textsuperscript{11}

HPV infection seems to be associated with the majority of anal tumors, especially with squamous cell carcinomas. Among the genotypes most frequently associated with anal tumors, HPV 16 and HPV 18 are considered as high risk for the development of anal cancer. The overall percentage of anal cancers attributed to HPV is 90%, especially for genotypes 16 and 18, which correspond to 92% of cases, with some differences depending on the geographic region (75% in men × 91% in women). HPV 16 is the most prevalent type, found in over 70% of cases.\textsuperscript{1,9}

The constant practice of receptive anal sex apparently contributes to a higher frequency of anal lesions and also represents one of the main factors for the acquisition of HIV and HPV coinfection, important risk factors for anal intraepithelial neoplasia (AIN), considered the precursor lesion of anal squamous carcinoma. It is believed that the HIV virus is a cofactor that HPV needs to induce neoplasms that can progress to anal carcinoma.\textsuperscript{2}

**Objectives**

The aim of this study was to evaluate the clinical, pathological and sociodemographic characteristics of patients diagnosed with anal carcinoma in Hospital Araújo Jorge, Goiânia/GO, in the period 2004–2011, as well as the prevalence of HPV genome in these tumors.

**Methods**

**Study type and sample**

The study consisted of an epidemiological investigation that used clinicopathological data collected from medical records and analysis of paraffin blocks containing specimens of anal carcinomas. The study was approved by the Ethics Committee on Human Research of Hospital Araújo Jorge (HAJ), under number 272 288 in April 2013. As this was a retrospective study, there was no direct contact with selected patients and thus, it did not require the signing of the free and informed consent form. The prevalence of HPV was investigated in 43 specimens of anal carcinomas diagnosed in HAJ, in Goiânia/GO, in the period 2004–2011.

Patients included in the study were those with confirmed histopathological diagnosis of anal carcinoma and clinicopathological data available in their medical records. The detection of HPV included the cases in which the paraffin blocks were sufficient and were available for molecular analysis. Case selection was made from an active search of the records of the Department of Pathology of HAJ. The slides stained with hematoxylin–eosin related to each anatomopathological examination were reviewed and sections were prepared for DNA extraction.

**DNA extraction**

Genomic DNA was purified from tumor samples fixed in formalin and embedded in paraffin, according to standard protocols used in the Laboratory of Genetic Diversity of Pontifícia Universidade Católica Goiás (PUC Goiás). The deparaffinization and hydration steps were performed and DNA extraction and purification were performed using a commercial Wizard Kit (Promega). All purified DNA samples were tested for quality and integrity via amplification of a fragment of constitutive BRAF gene of 224 bp.

**Detection of HPV genome in tumor samples**

Detection of HPV DNA was performed by polymerase chain reaction (PCR) using SPF primers,\textsuperscript{12} which amplify a fragment of 75 base pairs of the viral genome and developed to promote sensitive amplification of the most clinically relevant HPV genotypes. The assay is capable of detecting the genotypes currently known as high risk HPVs (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73 e 82), as well as low-risk HPV genotypes (6, 11, 40, 43, 44, 54, 70) and some additional types. HPV detection was performed at the Laboratory of Bone Marrow Transplantation of Associação de Combate ao Câncer in Goiás and Laboratory of Genetic Diversity of PUC-Goiás. The PCR with SPF primers was performed in a final reaction volume of 25 μL, containing 2 μL of purified DNA, 10 mmol/L Tris–HCl, pH 9.0, 50 mmol/L KCl, 2.5 mmol/L MgCl\textsubscript{2} 200 mmol/L each of deoxy-nucleotide (dNTP), 10 pmol of each oligonucleotide primer, and 0.25 U of Taq Polymerase (Invitrogen, Brazil). The cycling conditions included: preheating for 1 min at 94°C followed by 40 cycles of 1 min at 94°C, 1 min at 45°C and 1 min at 72°C, with a final extension of 5 min at 72°C.

Each PCR experiment was performed with positive and negative controls, previously tested in the Laboratory of Genetic Diversity of PUC-Goiás. A fragment of human BRAF gene with 224bp was amplified from DNA extracted from each sample to confirm the presence and the capacity of amplifying the extracted DNA from each sample.

**Statistical analyses**

The data obtained were coded and stored in a database in Excel software program and analyzed using GraphPad Prism. Clinicopathological and sociodemographic data were processed to descriptive statistical analysis and the data on HPV detection regarding the different variables were analyzed using Fisher’s exact test and Chi-square test, considering statistically significant differences the ones that resulted in p value ≤ 0.05.

**Results**

**Clinicopathological and sociodemographic characteristics**

A group of 55 cases of anal carcinomas was initially selected, but 12 of them were excluded from the analysis because three
cases received chemotherapy/radiotherapy before surgery and nine were colorectal ADCs extending to the anus. The remaining 43 cases were evaluated regarding clinicopathological and sociodemographic characteristics, which are described in Tables 1 and 2. All data were collected through medical chart review at HAJ.

Of the assessed patients, 62.8% were females. Approximately half of the sample (53.5%) was diagnosed with squamous cell carcinoma (SCC), while 37.2% were ADCs. Most patients (46.5%) were aged between 61 and 75 years and 39.5% were married. Risk factors such as smoking (39.5%) and alcohol consumption (20.9%) were recorded in the study sample. Data associated with sexual behavior and orientation, such as the number of partners, age at the first sexual intercourse and practice of receptive anal sex were not reported in the medical records.

In 63.2% of cases, the diagnosis was made by biopsy. The treatment chosen for the group was, in general, a combination of surgery, chemotherapy and radiotherapy, with 76.7% of patients being submitted to surgery. Lymph node metastases were detected in 13 patients (30.2%) and four (9.3%) had distant metastasis, with the lungs being the most prevalent site (50.0%). Although all patients were not followed for a long period of time, at the end of the data collection period, 11 patients had their deaths recorded in their medical files (25.6%).

**HPV detection in anal carcinoma samples**

Of the 43 samples initially evaluated, 38 had paraffin-embedded histopathological specimens available and sufficient for DNA extraction. All these samples were previously tested for endogenous control amplification, the human BRAF gene and provided DNA suitable for PCR amplification. Of the 38 samples, 29 were positive for detection of HPV DNA (76.3%). Differences between HPV(+) and HPV(−) tumors in relation to sociodemographic and clinicopathological features were evaluated and are described in Table 3. Significant differences \((p = 0.032)\) were observed in relation to SCCs and ADCs, confirming the association between HPV and anal SCCs. The other variables investigated were not statistically significant.

**Discussion**

Studies carried out in different countries are unanimous in stating that, despite the fact that it is considered a rare tumor, the incidence of anal cancer has increased steadily in recent years. The present study analyzes a retrospective series of 43 patients with anal cancer diagnosed in HAJ, in Goiânia-GO, in the period 2004–2011. The study highlighted the clinical, pathological and sociodemographic
Table 3 – Comparative analysis between the anal carcinomas HPV(+) and HPV(−).

<table>
<thead>
<tr>
<th>Variable</th>
<th>HPV(+) (n = 29)</th>
<th>HPV(−) (n = 9)</th>
<th>p</th>
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<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
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<td>Histological type</td>
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<td>Adenocarcinoma</td>
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<td>53.8</td>
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<tr>
<td>Diagnostic method</td>
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<td></td>
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<tr>
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<td>8</td>
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<td>Surgery</td>
<td>13</td>
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<td>Lymph node metastasis</td>
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* p ≤ 0.05, statistically significant difference; SCC, squamous cell carcinoma.

characteristics of patients, as well as the detection of HPV genome in the assessed tumor specimens. According to the results of the series, 46.5% of the patients were aged 61–75 years. Different studies have reported that the most prevalent age group for anal cancer is 50–70 years.13,14,16,17 The accumulation of exposure to different risk factors throughout life contributes to the higher prevalence of these tumors at older ages.

A higher proportion of cases of anal carcinomas analyzed in this series was detected in women (62.8%). This information was previously reported by other authors;1,13,14,16–18 however, plausible explanations for this observation are still inconclusive. Several hypotheses have been suggested, such as that women are more susceptible to HPV infection, by developing HPV-related lesions in several anatomical sites, such as the vulva, vagina and cervix and developing cervical cancer with a very high frequency.14 Men also have frequent anogenital HPV infection; however, the tumors associated with HPV are significantly less prevalent in males.

Our study showed that 39.5% of patients with anal cancer were married; however, another significant portion of patients were widowed (20.9%) and single (18.6%), a factor that could favor having a higher number of sexual partners. A higher proportion of married heterosexual men (72.2%) was also found in the DALING study,13 which assessed anal cancer in 306 patients from the North-American west coast.

Although smoking is considered a major risk factor for anal cancer, smokers did not represent the majority of cases in our study; however, a significant number reported smoking (20.9%), and moreover, this information was not available in many medical files (18.6%). Among the risk factors for anal cancer, smoking is considered very significant, both in women and men.13

As it is a rare type of cancer, the total number of cases of anal cancer analyzed in different series is, in general, small. The study by Abramowitz et al.1 has the largest series, with 362 cases diagnosed in 16 anatomical pathology centers spread throughout France. The second largest series13 analyzed 306 patients living in the state of Washington (USA) over a 12 year period, 1986–1998. The histological type of anal cancer most commonly associated to HPV infection is the SCC and, in this study, we found this tumor type in 53.5% of the assessed samples.

Our study evaluated the presence of the HPV genome in 38 cases of anal carcinoma and disclosed a prevalence of 76.3%. A review of the association between HPV and anal carcinoma
was made by reviewing articles published in major bibliographic databases (PubMed, SciELO, LILACS), using the terms anal cancer, HPV PCR and selecting those published from 2000 in English or Portuguese languages. The search resulted in nine selected studies and the results of HPV prevalence and genotyping in the anal carcinomas are summarized in Table 4.

Molecular investigations on the detection of the HPV genome in anal carcinomas indicate that anal cancer resembles cervical cancer in relation to HPV, that is, regarding squamous carcinomas, HPV is considered the most important risk factor for anal carcinogenesis.1,15

HPV infection is highly prevalent in patients with anal cancer, being considered a necessary cause for anal squamous cell carcinoma.19 In this study, an association between the presence of HPV and anal squamous cells was observed, i.e., HPV DNA was found in 90.9% of the SCCs. Some studies have detected the viral genome in 100% of samples of anal squamous cell carcinomas.17,20

However, in the largest series studied, the prevalence of HPV genome in anal carcinomas varied, ranging from 80.9%,14 88%13 and 96.7%.1 The different molecular methods used in viral DNA detection in these tumors certainly explain this discrepancy. Our study used generic primers (FPS) capable of amplifying a small fragment (75 bp) of the HPV genome, allowing the detection of viral DNA, even in samples of which DNA integrity was not of excellent quality.

Similar to cervical cancer, anal cancer seems to occur in the cells of the squamocolumnar junction, populated by a variety of precursor cells that originate different epithelia.17 Studies available in the literature show that anal carcinoma rarely occurs in the absence of HPV, reinforcing the role of the virus as the main risk factor for this tumor type.1

Regarding the HPV subtypes in anal carcinomas, our study did not include the analysis of present genotypes. According to the available literature, a significant predominance of HPV 16 is shown in relation to other genotypes in anal carcinomas (Table 4). Youk et al.20 and Tachezy et al.17 detected the presence of HPV 16 in 100% of the anal carcinoma samples analyzed. One explanation for this occurrence is that HPV 16 is closely involved in the development of anal SCC by promoting changes in the genome still at the phase of micro-invasive carcinoma, according to reports by Kagawa et al.16 in Japan.

HPV subtype 18 appears in global studies as the second most prevalent type of HPV in anal carcinomas, although the data are somewhat controversial. In nine studies found in the literature (Table 4), the oncogenic potential of subtype 18 is recognized in the natural history of anal carcinoma; however, only four studies performed HPV 18 genotyping in these tumors.

In the study by Ramamoorthy et al.,15 carried out in San Diego, USA, the prevalence of HPV subtype 18 was 65%, whereas in the study by Daling et al.13 performed in Seattle, HPV 18 was found in 6.9% of cases. In the study by Abramowitz et al.,1 carried out in France, the prevalence of HPV subtype 18 was not greater than 5.8%.

The study by Soares et al.21 carried out in Brazil, demonstrated the presence of HPV 18 genome in 15.2% of cases. This discrepancy may be related to HPV genotyping method, as the molecular technologies used in the studies were different.

Regarding the prognosis of patients with anal carcinoma, data available in the literature are still very scarce. In the present study, we observed a very aggressive behavior of these tumors, with 13 patients (30.2%) showing lymph node metastases and three patients (7.0%) with distant metastases: two with lung metastases, one with liver metastasis and one with brain metastasis. The aggressiveness of anal carcinoma can also be observed by the number of deaths recorded during the study period. Of the 43 patients included in the study, 11 died, representing 25.6% of the study sample.

The increased incidence of anal carcinoma and knowledge of the main factors involved in the etiology of these tumors stimulate the need to develop preventive measures for anal carcinoma. Such measures include full medical examination, with an investigation of the sexual history of the patient during anamnesis, careful clinical examination of the anogenital region, detection of precursor lesions (intraepithelial anal neoplasms), anoscopy and HPV vaccination, especially in the higher-risk groups.16

High-risk patients include promiscuous individuals with a high number of sexual partners, regular practice of anal intercourse, low immunity (HIV, immunosuppressed individuals) and past or current history of infection associated with anal lesions (condylomas, cervical intraepithelial neoplasia (CIN), vulvar intraepithelial neoplasia (VIN), vaginal intraepithelial neoplasia (VAIN) and vulvar, vaginal or cervical SCC). For this group, clinical examination with anal inspection, perianal and intra-anal cytology is recommended, as well as research of HPV genome in samples of anal neoplasia.14

The aggressive behavior and advanced stage of anal carcinomas observed in the present study highlight the need for prevention campaigns, which also include vaccination against
HPV. The studies by Abramowitz et al.\textsuperscript{1} in France and Komlos et al.\textsuperscript{18} in Slovenia emphasize the importance of further studies on HPV vaccination in the prevention of anal cancer, as most of these tumors are significantly associated with HPV infection, particularly with HPV subtype 16. Vaccination against high-risk HPV can have a big impact on the prevalence of anal carcinoma and its precursor lesions; however, more detailed studies on its potential power to prevent such tumors are still necessary.

This study has an important limitation regarding the collection of clinical, pathological and behavioral data of the patients. These data were scarce in most medical records, including the practice of receptive anal intercourse, history of HIV infection, presence of sexually transmitted diseases (STDs) or condylomas, among others.

The studies by Daling et al.,\textsuperscript{13} in the USA and by Abramowitz et al.,\textsuperscript{3} in France, also reported difficulties in obtaining these same data, both in interviews\textsuperscript{13} and during the review of medical files.\textsuperscript{1} Among the nine studies that evaluated HPV detection in anal carcinomas in the last 10 years, only these two\textsuperscript{1,13} analyzed the risk factors for anal carcinoma, in addition to HPV infection. The continuation of this study, with the analysis of a greater number of cases of anal carcinomas diagnosed in other oncology centers, as well as the genotyping of HPV in specimens of anal carcinomas is the objective of our team.

**Conflicts of interest**

The authors declare no conflicts of interest.

**References**