Human Papillomavirus 16 lineage D is associated with high risk of cervical cancer in the Brazilian Northeast region

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Abstract

Background

Like HPV types, different lineages also appear to have different carcinogenic capabilities. Studies have shown that oncogenic HPV specific to the genotype lineage is associated with different risks for the development of cervical intraepithelial neoplasia (CIN2 / CIN3) and cervical cancer. This study aims to analyze the genetic diversity of HPV 16 genotype in cases of cervical intraepithelial neoplasia (CIN2/CIN3) and cervical cancer in women from the Northeast region of Brazil.

Methods and Results

A cross-sectional hospital-based study conducted in the Northeast region of Brazil from 2014 through 2016. In the sample, there were 196 cases of HPV-16 variant (59 cervical intraepithelial neoplasia - CIN2/CIN3 and 137 cases of cervical cancer). Difference of proportion test was used to compare groups of CIN2/CIN3 and cervical cancer by viral lineage (p -value <0.05) in respect to HPV-16 lineage prevalence.

The percentage of lineage frequencies by histopathological diagnosis showed a borderline difference of lineage A in the CIN2/CIN3 group compared to the cervical cancer group ($p = 0.053$). In relation to lineage D, the proportion was higher in cancer cases (32.8%) compared to the CIN2/CIN3 group (16.9%), p -value of 0.023.

Conclusions

HPV16 lineage A was the most frequent in both CIN2/CIN3 and cervical cancer samples, while lineage D predominated in cervical cancer, suggesting a possible association of HPV-16 lineage D with cervical cancer.

Introduction

Cervical cancer is the fourth cancer in incidence and mortality among women worldwide [1]. In Brazil, it is the third type of cancer in incidence and fourth in mortality among women [2,3]. Several studies have shown that the presence of Human Papillomaviruses (HPV) is a required, although not sufficient, factor for cervical cancer development. The viral genotype HPV16 is the most prevalent in cervical cancer, being considered by the International Agency for Cancer Research as a Group 1 carcinogenic agent for humans [4]. The study of genetic diversity of HPV16 allowed to characterize that specific viral lineages are associated with a higher risk for cervical intraepithelial neoplasia (CIN2/CIN3) and cervical cancer development [5-8]. For HPV16, the lineages were initially named according to their geographical prevalence, being placed in five groups: European (EUR), Asian (As), Asian-American (AA), African 1 (Af1) and African 2 (Af2) (9-12). In 2013, Burk, Harari and Chen proposed a new alpha-numeric nomenclature for all HPV lineages based on differences of the complete viral genome sequence. The lineages for

HPV16 were named: A (corresponding to EUR and As), B (corresponding to Af1), C (corresponding to Af2), and D (corresponding to AA) [13].

There are evidences for higher pathogenicity of lineages B, C and D of HPV16 associated with a higher viral persistence in comparison with lineage A [4,14,15]. Additionally, the lineages B, C, and D were also associated with a higher risk for CIN2/CIN3 and cervical cancer [14, 16, 17]. The D lineage was also associated with adenocarcinoma [18]. A longitudinal study had shown that HPV16 sub-lineages D2 and D3, appear to be more associated with CIN2/CIN3 and cervical cancer, when compared to other lineage/sub-lineages [16,19]. Similar characteristics were reported for lineages of other HPV genotypes as HPV 18 and HPV 45 [20].

In this study we analyzed the genetic diversity of HPV 16 genotype in cases of cervical intraepithelial neoplasia (CIN2/CIN3) and cervical cancer in women from the Northeast region of Brazil. In this region, cervical cancer was the second in incidence, excluding non-melanoma skin cancer, (17.6/100,000) [2], being the third in mortality by cancer (6.7/100.000) among women for the year 2019 [3]. This work was part of a multicenter study on the HPV genotypes prior to HPV vaccine introduction in the National Immunization Program and it was carried out in two other cities in Brazil (Rio de Janeiro - RJ and Belém - PA) [21,22].

Materials And Methods

Study population

A cross-sectional hospital-based study was carried out in two health units of Recife City, capital of Pernambuco State, in Brazil, (Pernambuco Cancer Hospital and Prof. Fernando Figueira Integral Medicine Institute - IMIP) between July, 2014 to December, 2016. Women aged 18 years and older, with pap smear test indicative of high-grade intraepithelial lesion (CIN2/CIN3) or cervical cancer, who were referred for a colposcopy, were invited to participate in the study. Women who had a biopsy and received a histopathological result of CIN2/CIN3 or cervical cancer were included in this analysis. The exclusion criteria considered women with previous oncological treatment for cervical cancer (cancer surgery, radiotherapy, or chemotherapy) or cognitive or physical disabilities that could prevent them to answering a questionnaire.

Epidemiological data and biological sample collect

After signed a consent form, the women were interviewed by trained nurses using an epidemiological questionnaire. The instrument was used to collect data about socioeconomic characteristics, knowledge about cervical cancer prevention, access to health services for diagnosis and treatment, hormonal and reproductive histories and tobacco use. Additional clinical information was obtained from medical records. A sample of the biopsy was stored in RNA-Later until nucleic acid isolation and was sent to the IMIP Research Laboratory.

DNA Isolation and HPV Identification

DNA was isolated from biopsies using the QIAamp DNA Mini Kit (Qiagen®). HPV detection was carried out by Polymerase Chain Reaction (PCR) amplification using primer sets PGMY07 and PGMY09 (23), reactions without PCR products were submitted to nested PCR with the primers GP5+/GP6+ (24). Samples negative for HPV DNA amplification after nested PCR were subjected to a PCR reaction for β -globin, and positive reactions for β -globin and negative for HPV nested PCR were considered negative for HPV. For HPV identification, PCR products were purified with Illustra GFX PCR DNA and Gel Band Purification Kit (GE Healthcare®) and subjected to DNA sequencing in both directions, using the Big Dye Terminator Cycle Sequencing Ready Reaction V3.1 Kit (Life technologies®), following the supplier's instructions, and sequenced in a ABI 3730xL capillary sequencer. The electropherograms of each sample were checked by eye and a consensus sequence of the bidirectional sequencing was subjected to HPV genotype identification using the Blast software (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>, [25]).

Among the 415 samples evaluated for HPV genotyping and DNA sequencing, the eight most common types were: HPV16 (58.6%), HPV45 (7.2%), HPV18 (7.0%), HPV35 (4.6 %), HPV58 (3.6%), HPV31 (3.1%), HPV33 (2.7%) and HPV52 (2.4%).

The HPV 16 was the most common in CIN2/CIN3 (53.9%) and cervical cancer (60.7%). A total of 243 samples presented the HPV16 genotype and lineage identification was carried out for 196 samples (80.7%).

HPV16 lineage identification

Samples with HPV16 were submitted to PCR amplification of the viral genomic regions *LCR* and *E6* in two overlapping fragments, following Vidal et al.,2016 [26]. The fragments were sequenced as describe above and the sequence obtained were aligned to HPV16 lineage reference sequences suggested by Burk et al., 2013[13]. HPV16 lineage identification was carried out by detecting a sequence signature, i.e. presence of specific nucleotides at specific sequence positions following Cornet et al.,2012[12]. HPV16 lineage nomenclature used in this work followed Burk et al.,2013[13].

Statistical analysis

Epidemiological data were stored using Epi-Info software and then linked to both clinical data and HPV DNA sequence data. The final database was analyzed using Stata v.15.0 [27]. Chi-squared test (or Fisher's exact test) was used to compare distribution of women by selected characteristics according to histopathological diagnosis. Difference of proportion test was used to compare the prevalence of viral HPV16 lineages in respect to CIN2/CIN3 or cervical cancer (p-value <0.05) .The lieneages B and C were grouped due to low frequency (Table 2).

The Institutional Ethic Committees approved all the procedures (Professor Fernando Figueira Integral Medicine Institute - CAAE 24687713.8.0000.5201; Pernambuco Cancer Hospital - CAAE 40349014.0.0000.5205).

Results

Selected characteristics for these 196 women, according to disease status (CIN2/CIN3 vs cervical cancer), are presented in Table 1. Women with CIN2/CIN3 were younger than women with cervical cancer. No statistical differences between these groups were found for oral contraceptive use or tobacco exposure. Number of childbirths were more frequent in women with cervical cancer than in women with CIN2/CIN3.

LCR and E6 regions were aligned with HPV16 reference sequences representing lineages A, B, C, and D, and the presence of specific SNPs were used to identify HPV16 lineage present in each sample. The distribution of HPV16 lineages was: 130 women had lineage A, 11 had lineages B/C (1B and 10, C) and 55 had lineage D.

The lineage frequencies by histopathological diagnosis showed a high frequency, with borderline significance for lineage A in CIN2/CIN3 when comparing to the cervical cancer group ($p=0.053$). Comparison of HPV16 lineage frequency by histopathological diagnosis showed a higher proportion of lineage D in cervical cancer than in the CIN2/CIN3 ($p=0.023$, Table 2).

Discussion

HPV 16 lineage A was the most frequent in the sample of study, in both CIN2/CIN3 and cervical cancer, while lineage D predominated in cervical cancer, suggesting an association of HPV16 lineage D with cervical cancer.

It is already known that the genetic variation of HPV 16 can influence the risk of cervical cancer, varying its different lineages or sublineages in different regions of the world [8]. Previous studies attempted to demonstrate an association between non-European lineage (B/C/D) and a higher risk for developing CIN2/CIN3 [14,17]. and cervical cancer [17,28] when compared to European lineage (A). A major limitation of these studies was the grouping of lineage B, C and D into non-European lineages, which did not allow differentiation of the carcinogenic potential between these lineages. A recent study based on >3,200 women, comparing women without lesions and <CIN2 and women with CIN2 or CIN3 or invasive cancer found association between HPV16 sub-lineages D2 and D3 with CIN3 or cervical cancer [17]. Clifford et al. (2020) analyzing a sample of >7,100 women, comparing those with normal cells, or atypical squamous cells of undetermined significance [ASCUS], or Low-grade squamous intraepithelial lesion (LSIL)/CIN1, with those with invasive cervical cancer, found an association between lineage D and sublineage A4 with cancer diagnosis.

A recent study with women in Croatia confirms the predominance of HPV-16, mainly belonging to the European branch, as the most frequently associated with histologically confirmed HSIL and cervical cancer [29].

In this study, the A lineage of HPV 16 was the most frequent and although there was a trend of association with high-grade lesions (CIN2/CIN3), it was not possible to demonstrate a difference between these high-grade lesions and cervical cancer. A study carried out in another region of Brazil also found a high prevalence of the A lineage

of HPV16 in samples of high- and low-grade lesions, including cancer [30].

On the other hand, the D lineage of HPV showed an association with cervical cancer when compared to high-grade lesions and this reinforces the possibility that the HPV 16 lineage D is involved in the progression of HPV to cancer. Although this is a cross-sectional study, this study adds new data concerning the association of specific lineages of HPV16 with cervical cancer. Our data suggest that the lineage D could be more aggressive in respect to the progression of high-grade lesions to cancer, not restricting this association to comparisons between normal/low grade lesions and invasive cancer.

Declarations

Authors' contributions

LFLM : Conceptualization, Methodology, Formal analysis, Writing – Original Draft, Writing – Review and Editing. MAMM: Supervision, Resources, Conceptualization, Writing – Original Draft, Writing – Review and Editing. RAP: Conceptualization, Writing – Original Draft, Writing – Review. NB dos R: Methodology, Writing – Original Draft, Writing – Review and Editing.

SPF: Performed the Experiments, Writing – Review and Editing. JPBV: Performed the experiments, Writing – Review and Editing. LCT: Supervision, Resources, Performed the experiments, Writing – Review and Editing. AIS: Conceptualization, Writing – Original Draft, Writing – Review and Editing

LMdeA: Project administration, Funding acquisition, Conceptualization, Writing – Original Draft, Writing – Review and Editing.

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Data availability

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

Conflict of interest The authors declare there is no conflict of interests in the study.

Availability of data and materials

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

Ethics approval and consent to participate

The Institutional Ethic Committees approved all the procedures (Professor Fernando Figueira Integral Medicine Institute - CAAE 24687713.8.0000.5201; Pernambuco Cancer Hospital - CAAE 40349014.0.0000.5205).

All patients recruited in this study were accepted for information of the study by written informed consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1 - Distribution of women by selected characteristics according to histopathological diagnosis

Variable	CIN2/CIN3		Cervical cancer		P-value*
	n=59	%	n=137	%	
Age (in years)					
20 - 39	37	62.7	43	31.4	< 0.001
40 – 49	16	27.1	40	29.2	
50 – 59	6	10.2	23	16.8	
60 and over	0	0.0	31	22.6	
Number of childbirths					
None	3	5.1	3	2.2	0.07
1 – 2	28	47.5	43	31.4	
3 – 4	17	28.8	44	32.1	
5 – 6	5	8.5	14	10.2	
7 or more	6	10.1	33	24.1	
Oral contraceptive use					
Yes	42	71.2	88	64.2	0.34
No	17	28.8	49	35.8	
Tobacco exposure					
Yes	26	44.1	76	55.5	0.14
No	33	55.9	61	44.5	

(*) Chi-squared test or Fisher's exact test

Table 2 – Distribution of women according to HPV16 lineages frequency and histopathological diagnosis

Histopathological diagnosis	A		p-value*	B/C		p-value*	D		p-value*
	n	%		n	%		n	%	
CIN2/CIN3	45	76.3	0.053	4	6.8	0.635	10	16.9	0.023
Cervical cancer	85	62.0		7	5.1		45	32.8	
Total	130	66.3	-	11	5.6	-	55	28.1	-

(*) Test for proportion difference.