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Human Papillomavirus genotypes in cervical intraepithelial lesions among women at two referral centers in Brazil

Roberto Borges Bezerra [1], Ariani Impieri Souza [2,*], Rodrigo Alves Pinto [1], Miguel Angelo Martins Moreira [3] and Liz Maria de Almeida [4]

[1] *Department of Oncology, Instituto de Medicina Integral Prof. Fernando Figueira (IMIP), Recife, Brazil.*
[2] *Department of Research, Instituto de Medicina Integral Prof. Fernando Figueira (IMIP), Recife, Brazil.*
[3] *Associate Researcher, Instituto Nacional de Cancer (INCA), Rio de Janeiro, Brazil.*
[4] *Department of Epidemiology, Instituto Nacional de Cancer (INCA), Rio de Janeiro, Brazil.*

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Abstract

This study aimed to describe Human Papilloma Virus (HPV) genotypes and women with cervical intraepithelial neoplasia (CIN) sociodemographic characteristics at the oncology reference centers. A secondary data of 325 records on women with CIN were analyzed from a cohort study database conducted in two public institutions in the oncological service in the Northeast of Brazil, from July 2014 to February 2016. The HPV genotype analysis was carried out on 142 through viral DNA sequence after amplifying PCR technique and compared the sequences identified in the GenBank databases. The women were predominantly 25 to 39 years old. The 325 biopsies revealed 17.6% low-grade of cervical intraepithelial lesion (CIN1) and 82.4% high-grade of cervical intraepithelial lesion (CIN2 or CIN3). Among the 142 HPV genotypes the most prevalent was HPV-16 (51.7%), followed by HPV-35 (6.9%) and HPV-45 (6.2%). HPV-18 was in only 2.1%. There was an association between HPV-16 and high-grade lesions (CIN2 or CIN3) (p=0.008). Although HPV-16 was the predominant genotype in cervical intraepithelial lesions, especially high-grade lesions (CIN2 or CIN3), HPV-35 was the second most frequent in high-grade lesions in this population. This suggests that other HPVs may be as prevalent as those commonly known in some regions.

Keywords: HPV; CIN; HSIL; LSIL; Papillomaviridae; Cervical Intraepithelial Neoplasia; Uterine Cervical Neoplasms.

1. Introduction

Cervix cancer is the third most common cancer worldwide among women and its incidence is strongly influenced by economic and geographic situation [1]. In Brazil, the Ministry of Health / Instituto Nacional de Cancer José de Alencar Gomes da Silva (INCA), estimates for the year 2020 more than 16 thousand new cases of cervical cancer. As Brazil has continental dimensions and important economic and social contrasts, there is a wide variation of incidence in different regions of the country, ranging from 40.1 cases per 100 thousand inhabitants in the state of Amazon to 5.9 cases in 100 thousand inhabitants in the state of São Paulo. The estimate for the state of Pernambuco is 13 cases for every 100 thousand women, representing the second most common neoplasm among women in this state [2,3].

There are more than 100 HPV genotypes described, highlighting those considered of high risk (HPV16, 18, 31, 33, 34, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 70) and of low risk (HPV 6, 11, 40, 42, 43 and 44) for cervical cancer [4].

* Corresponding author: Ariani Impieri Souza; E-mail: ariani@imip.org.br
Department of Research, Instituto de Medicina Integral Prof. Fernando Figueira (IMIP), Recife, Brazil.

Studies on the prevalence of HPV types in the world show great variation between genotypes according to the researched region. In cases of invasive disease, it is observed that despite the slight variation in the distribution of HPVs worldwide, the most frequent genotypes are similar, regardless of the region studied [5-7].

After the exposure to HPV, most women will not have the virus, a small group will present latent infection, and some of these women will develop clinical alterations of the disease with pathological findings of cervical intraepithelial neoplasm (CIN) [8]. CINs, in turn, can progress to a more aggressive form or to spontaneous regression. The progression time of a cervical intraepithelial lesion until the development of a cervical cancer is, in general, longer and depends on the CIN grade [8,9]. WHO (2014) classifies CIN1 as a low-grade lesion (LSIL) and CIN2 and CIN3 as a unique histopathological entity point of view, classifying as a high-grade cervical intraepithelial lesion (HSIL) [10]

In addition to the HPV genotype present in the lesion, they are determinants in the immunologic carcinogenic process, genetic aspects, and are related to lifestyle, such as smoking [11-14].

Knowledge on viral genotypes, most often associated with disease has enabled the development of vaccines against specific viral genotypes [15-17]. Currently in Brazil, the Ministry of Health, offers the public health services, the quadrivalent vaccine against HPV 16, 18, 6 and 11 types, although the nonvalent vaccine, which gives additional protection on HPV 31, 33, 45, 52 and 58 types have already been approved in the country [18-19]. The importance of the vaccine is the fact that, besides the expected action on the viral types included in its composition, it was described as a crossed protection on other types which are not present in vaccine.[19]. There is evidence of possible immunological crossed action for HPV-31, HPV-33, and HPV-45 types, but the impact of this protection and its potential benefit in preventing the development of cervical cancer is still poorly understood [20].

The strategy of immunization against the virus is essential. In determination on the types of HPVs circulating and monitoring the vaccine after its introduction. Brazil has great heterogeneity in its socioeconomic, cultural and knowledge about the prevalence of the types of HPV in various regions in the country will enable the adoption to adjust the measures in health policies by improving the resources and expanding benefits to the population [7].

This study aimed to describe the HPV genotypes and the characteristics of the population with precursor cervical lesions at two oncology reference centers in the Northeast of Brazil.

2. Material and methods

A hospital-based epidemiological study was carried out with analysis of secondary data from a multicenter study previously conducted and coordinated by INCA (Instituto Nacional de Câncer José de Alencar Gomes da Silva) in Brazil. This is a partial analysis of the project conducted in Recife, State of Pernambuco, between July 2014 and February 2016.

This sub-sample analyzed data of 325 women with low (CIN1) and high-grade (CIN2 and CIN3) cervical intraepithelial neoplasia. Of these, 142 samples were genotyped. The exclusion criteria in this study were invasive neoplasia (cancer), results on inflammatory and normal histopathological examination. The biological and sociodemographic variables, variables related to healthcare and lifestyle and variables related to women's gynecology and obstetrics history were described.

The data collection in the original study was done by face-to-face interview for the epidemiological data collection and the material collected of the cervix biopsies were sent for HPV genotyping. The biopsy was performed with a pull-out clamp and stored in cryotubes in 1 mL of RNA Later. The total DNA was extracted from the cells of the biopsy fragment using the QiaAMP DNA Mini Kit (Qiagen) and quantified in a Nanodrop spectrophotometer. To evaluate the HPV types, a segment of the L1 gene of the DNA viral was amplified by polymerase chain reaction (PCR) using the PGMY09/PGMY11 primer set and when necessary, by PCR nest using the PGMY9/PGMY11 indicators and GP5+/GP6+. The amplified products were purified with the "Ilustra GFX PCR and DNA Gel Band Purification Kit" and sequenced in the INCA Sequencing Platform in automatic sequence of DNA3730XL and 3130XL Genetic Analyzer (both from Applied Biosystems).

Sociodemographic variables and HPV types of the population studied were presented in frequency distribution. The association between HPV 16 and the cervical intraepithelial neoplasia grade were made through Pearson's chi-square test considering a 5% significance level. The original study from which the information for this analysis was taken from was approved by the Human Research Ethics Committee at the two institutions that participated in the study. CAAE: 24687713.8.0000.5201.

3. Results

325 records of women with histopathology confirming low- or high-grade cervical intraepithelial neoplasia (CIN1, CIN2 or CIN3) were included. In the characteristic's analysis of these women, there was a predominance in the 25 to 39 age (59.4%). Most were married (66.5%), had low schooling (only 6.5% went to university) and were unemployed (52.9%). 69.5% of the women, reported initiating sexual activity before the age of 18 and in 14.4%, before 14 years old. 37.0% of the women had, up to two sexual partners, and 25.5% reported greater than or equal to five sexual partners. The majority (96.6%) had already used some method of contraception, and the oral hormonal contraceptive was the most reported (78.7%), followed by a male condom (76.0%). (Table 1).

Table 1 Women's characteristics.

Characteristics	**N=325**	**%**
Age (in years)		
18-24 (adolescents and young people)	25	7.7
25-39	193	59.4
40-49	73	22.5
≥50	34	10.4
Marital status		
Single/ separated / widow	109	33.5
Married / Consensual Union	216	66.5
Schooling (years)		
0-3	61	18.7
4-7	126	38.8
8 to 11	117	36.0
> 11	21	6.5
Paid job		
Yes	153	47.1
No	172	52.9
Number of childbirths		
None	25	7.9
1-2	153	47.0
3-4	99	30.4
≥5	48	14.7
Age of onset of sexual activity		
<14 years old	47	14.4
14-17 years old	179	55.1
≥ 18 years old	99	30.5
Number of sexual partners		
Up to 2	120	37.0
3- 5	122	37.5
> 5	83	25.5
Types of contraceptives ever or currently used*		
Pills	256	78.7
Injectables	115	35.3
Intrauterine Device (IUD)	5	1.5
Male condom	271	83.4
Tubal ligation	130	40.0

* the sum (n) is greater than the total number of patients due to the possibility of using more than one method, so the sum is greater than 100%

Although 95.6% of the women had performed at least one Pap smear at some point in their lives, only 60% of these women knew that this exam could identify cervical cancer. More than half of the women (54.7%) informed that they performed the exam annually. They reported that they went to the health service for a routine asymptomatic examination (76%), and then the cytological alteration in the Pap smear was identified. The current use of tobacco was reported by 17.9%, and the other 18.4% were former smokers (Table 2).

Table 2 Healthcare and lifestyle habits.

Feature	**N**	**%**
Knowledge about Pap Smear		
Yes	195	60.0
No	130	40.0
Periodicity of the preventive exam		
Never performed	14	4.3
Annually or more than once a year	178	54.7
Biannually or tri annually	25	7.7
With intervals of more than 3 years or irregularly	108	33.3
Age of first cytological examination		
Up to 24 years	238	73.2
25 or more	49	15.1
Do not know/ Never performed	38	11.7
Reason for consultation		
Some complaint has appeared	78	24.0
Gynaecological examination routine	247	76.0
Tobacco Exposure		
Currently smokes	58	17.9
Ex-smoker	60	18.4
Never smoked	207	63.7

The results of the 325 histopathological exams showed a prevalence of 17.6% of low-grade cervical intraepithelial neoplasia (CIN1) and 82.4% of high-grade (CIN2 or CIN3). Of the 142 HPVs genotyped, the highest prevalence was HPV-16 (52.1%), followed by HPV-35 (7.0%), HPV-45 (6.3%), HPV-58 (6.3%), and other types. (Table 3).

Table 3 Distribution of HPV and the grade of the cervical intraepithelial lesion in 142 women attended at two referral centers in the city of Recife, 2014-2016.

	CIN1		**CIN2 and CIN3**		
HPV type	**N**	**%**	**N**	**%**	Total
HPV-16	7	28.0	67	57.3	74
HPV-35	0	0	10	8.6	10
HPV-45	3	12.0	6	5.1	9
HPV-58	1	4.0	8	6.8	9
HPV-31	1	4.0	5	4.3	6
HPV-18	1	4.0	2	1.7	3
Other HPVs	12	48.0	19	16.2	31
Total	25	100.0	117	100.0	142

The association between the HPV type and the histological grade of the CIN was observed between HPV-16 and CIN2 or CIN3 (p= 0.008). (Table 4)

Table 4 Association between the HPV type and the histological grade of the cervical intraepithelial neoplasm.

	HPV-16		**Other HPVs**		**Pearson's chi-square test**
Histological grade	N	%	N	%	
CIN1 (n=25)	7	28.0	18	72.0	
CIN2 and CIN3 (n=117)	67	57.3	50	42.7	p = 0.008

4. Discussion

Our sample has sociodemographic and clinical characteristics like those found in other studies with women affected by cervical intraepithelial lesion or cervical cancer, which is typically a young, mixed skin color, with low socioeconomic level and low schooling [2,13,14].

A little more than half of the women knew that the Pap test had the ability to identify cervical cancer. This information was also found in a qualitative study conducted in Mozambique in 2015, and published in 2017, where most of the women researched were unaware of the importance of the preventive examination and only would seek the health service on some gynecological complaints [21]. Unlike our study, where 60% of the women knew the purpose of performing a Pap smear and 76% of them referred to perform the routine examination regardless of presenting symptoms, it is known that cervical intraepithelial neoplasia is characteristically asymptomatic and that the screening test should be performed independently of complaints [22]. The search for the health service, may it be gynecological complaints or clinical, should be encountered as an opportunity that health professionals have to offer patients the cervical cancer screening [23].

The high percentage of women, which draws our attention in this study, who had already had a preventive examination before the age of 25, which is the recommended age by Ministry of Health/INCA to start the cytological screening for cervical cancer, [2]. This high percentage of women who started the screening before the indicated age by the Ministry of Health/INCA can be justified by the early onset of their sexual activity, which in 70% of them occurred before the age of 18, and almost 15% before the age of 14.

Cervical cancer screening when is performed early in life, before the age of 25, there is no impact in reducing mortality as shown in a British study, which demonstrated that it would be necessary to treat between 300 and 900 women with CIN to prevent a single case of invasive cancer [24]. A systematic review of studies that followed women with CIN2 showed that half of the cases regress spontaneously within two years and that this regression rate is even higher in young women under the age of 30 [25]. In addition, another review of studies that evaluated obstetric outcomes on women undergoing CIN treatment found a higher risk of unfavorable obstetric outcomes such as prematurity [26]. This draws attention to the fact that, in general, the procedures are performed in young women and still without defined parity.

Despite this, it is known that the onset of sexual activity at an early age is a well described risk factor and potential responsible factors as to longer exposure to HPV, higher biological vulnerability to STDs and more frequent presence of cervical ectopy that could favor the transmission of HPV virus, which may be present in this portion of women [27-28].

The number of sexual partners has also been reported as a risk factor for pre-malignant and malignant lesions as an independent risk factor [29]. In our study 1/4 of the women reported having six or more sexual partners, although the number of partners from whom this risk would be increased was not defined in the literature. The Asian meta-analysis conducted in 2015 found relative stability of risk when the number of partners reached 4 to 7 [29]. Although most studies refer of risk factors related to cervical cancer, such factors would be the same related to precursor lesions, since there is an evolutionary pathological and temporal relation in the natural history between the two entities [9,30].

HPV has been classified according to its oncogenic potential. Among the 142- genotyping performed, we found 90% of HPVs with high oncogenic potential. A large study conducted in Brazil in 2017 to assess the prevalence of HPV in a young population, sexually active and without gynecological disease, found positivity for HPV in 53.6% of the population in Brazil, being 38.6% high-risk HPV [31]. The high prevalence of HPV, both general and high-risk, evidenced in our sample, was much higher than found in the healthy population, this is justified by the fact that all the patients included in our study were referred to our service to continue the investigation because they presented some CIN-compatible lesion in the cytology examination, including the most with high-grade lesion.

The predominance of HPV-16 in both high- and low-grade lesions found in our study was expected. Similar findings for high-grade lesions were described in a meta-analysis conducted with studies in Latin America and the Caribbean, published in 2011, which showed a prevalence for Brazil of 52.7% of HPV type 16 and 9% of HPV type 18 [32]. A study conducted in the state of Pernambuco, published in 2010, included both high-grade lesions and cervical cancer by showing a prevalence of 62.5% of the HPV 16 genotype, followed by 23.6% for type 31 and no positivity for HPV 18 [33]. This evidence is in line with those found in this study, confirming the result of higher prevalence of HPV-16 in the Northeast region of Brazil.

Regarding to the other HPV types, the prevalence of HPV-35 in our study was higher than demonstrated in other studies conducted in Brazil [7,32,33]. The importance of this result is stressed since HPV-35 has not been described among those with higher prevalence in Brazil. HPV-35 together with HPV-16, HPV-18 and HPV-45 have already been described as the most related to invasive disease in sub-Saharan Africa [34]. Another African study showed HPV-35 as the most frequent type in women with and without cervical neoplasia submitted to oncotic cytology collection [35]. The analysis of these studies raises the discussion that the knowledge of the regional particularities of the population is a determining factor in the adoption of preventive measures, impacting, for example, on the benefit of vaccination.

The high prevalence found of HPV-35 is of extreme relevance since this genotype is not part of any of the currently available vaccines against HPV. If new studies confirm the high prevalence of HPV-35 in the precursor lesions or invasive lesions, it will become essential to follow up this type of HPV in the genesis of possible new cases of cervical cancer and the impact of immunization in our country.

It is known that about 30% of cervical cancer cases are not caused by HPV-16 and HPV-18, and therefore would not be directly protected by the quadrivalent vaccine made available by the Ministry of Health in Brazil [36] Current evidence suggests possible cross-protective effect, in which lesions by HPV types that are not included in the composition of the vaccine could be prevented, which generates an increased impact on the occurrence of the disease [37].

When analyzing the association between the HPV type and the histopathological result of the biopsy, we observed that the proportion of HPV-16 in CIN2 or CIN3 was significantly higher than in CIN1 suggesting that the most oncogenic and prevalent type in our environment is also related to the most aggressive lesions and prone to the development of invasive disease. Another relevant result is the high prevalence of HPV-35 and the fact that all women with this type of HPV have a high-grade lesion (CIN2 or CIN3), which leads us to question whether this genotype may also have high prevalence in our environment in more aggressive lesions, although the study does not have enough statistical power for such a conclusion.

This study has limitations. Among them, the use of a database designed to research cervical cancer, which may have involved the highest number of high-grade lesions found.

5. Conclusion

There was an association between HPV-16 and high-grade lesions (CIN2 or CIN3) (p=0.008). Although HPV-16 was the predominant genotype in cervical intraepithelial lesions, especially high-grade lesions (CIN2 or CIN3), HPV-35 was the second most frequent in high-grade lesions in this population. This suggests that other HPVs may be as prevalent as those commonly known in some regions.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Statement of informed consent

Informed consent was obtained from all participants included in this study.

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