Incidence of human papillomavirus and other sexually transmitted agents in Brazilian women with cervical atypia from a public health care service of Pernambuco, Brazil

Micheline Oliveira Lobo Pereira da Costa [1,2], Gabriela Oliveira Lobo Pereira da Costa [4], Pedro Henrique Rodrigues Pontes [5], Maria Tereza dos Santos Correia [1], Paulo Roberto Eleutério de Souza [3]

[1] Departamento de Biologia, Universidade Federal de Pernambuco (UFPE), Recife, PE, Brazil; [2] Laboratório Central de Saúde Pública do Estado de Pernambuco (LACEN), Recife, PE, Brazil; [3] Departamento de Biologia- Universidade Federal Rural de Pernambuco(UFRPE), Recife, PE, Brazil; [4] Departamento de Medicina-Universidade Maurício de Nassau (UNINASSAU), Recife, PE, Brazil; [5] Departamento de Medicina- Faculdade de Medicina de Olinda(FMO), Olinda, PE, Brazil

ABSTRACT

Aim: This study was conducted to assess the correlation between infection with high-risk genotypes of human papillomavirus (HPV) and with various types of microflora, in particular Chlamydia trachomatis (CT), in patients with different stages of cervical damage recruited from the public health care service in the State of Pernambuco, Brazil.

Methods: A population-based cross-sectional study was initially conducted in 525 women aged 18 to 64 years. Among these women, 87 were diagnosed with cytopathological changes of different degrees based on analysis of Pap smear samples at the Central Public Health Laboratory of Pernambuco (LACEN), during the period from April to November 2011. All of these women underwent routine colposcopy. The presence of HPV and CT was verified by means of polymerase chain reaction. To assess whether the presence of these and other genital infections were associated with cervical intraepithelial changes, Fisher's exact test was used.

Results: Of the 87 women diagnosed with cytopathological changes in this study, 73 (83.9%) were positive for CT infection and 72 (82.8%) for HPV infection, with co-infections detected in 65 cases (90.3%). Of the patients who had HPV infection, 93.1% had colposcopic changes and 68.96% had histopathological agreement. There was a statistically significant association between HPV-CT co-infection and the presence of cervical tissue damage (p=0.037). The most prevalent HPV genotypes were types 16 and 31 in low-risk cervical injuries; while for the high-risk injuries, the most common genotypes were 16, 31, 33 and 18. Other micro-floras found were *Gardnerella vaginalis* (GV) in 31 (35.6%) cases, *Coccus sp* in 17 (19.5%), *Candida sp* in 8 (9.2%), *Trichomonas vaginalis* in 5 (5.7%), *Lactobacillus sp* in 4 (4.6%), and herpes simplex virus in 1 (1.2%). However, no correlations were found between the presence of these microorganisms and the type of cervical lesion.

Conclusions: Genital infections, in particular CT infections, need to be investigated and dealt with appropriately, given that co-infections with HPV are associated with an increased likelihood of low-grade lesions, which can develop into more serious lesions, such as cervical carcinomas.

KEYWORDS

HPV infection, cervical lesion, genital co-infections.

Introduction

Cervical cancer is a serious public health problem worldwide. The World Health Organization (WHO) reports that cervical cancer is the fourth most common type of cancer in women in general, and the second most common type of cancer in women living in less developed regions of the world. In 2018, about 570,000 new cases were diagnosed and 311,000 women died from the disease [1]. In Brazil, according to the National Cancer Institute (INCA), 16,590 new cases of cervical cancer are expected to be recorded during 2020, with an estimated risk of 15.43 cases per 100,000 women, making this the third most frequent form of cancer [2,3]. In Pernambuco (PE), a state in the northeast of Brazil, the estimated rate for 2020 is approximately 43.74 per 100,000 women [3-5]. Unlike other types of human cancer, cervical cancer is a preventable disease, due to its slow

Article history
Received 28 Jun 2020 - Accepted 03 Aug 2020

Contact
Micheline Oliveira Lobo Pereira da Costa; michelineo@uol.com.br
Doctor specialized in Obstetrics and Gynecology, Department of Biological Sciences at the Federal University of Pernambuco (UFPE)
Rua Leonardo Bezerra Cavalcanti, 300, 52060-035 Recife, Brazil.
Tel: +55 (81) 4101-8740 / +55 (81) 9 9972-7008

evolution, with a long period from the development of precursor lesions to the onset of cancer, which when detected early, has a virtually 100% chance of being cured [2,4].

The method of screening for cervical cancer in Brazil is the Pap smear, which should be offered to women aged 25 to 64 years who have already had sexual activity [2,4]. According to the WHO, the incidence of this cancer increases in women between 30 and 39 years of age, and reaches its peak in the fifth or sixth decade of life. Before the age of 25, human papillomavirus (HPV) infections and low-grade squamous intraepithelial lesions (LSIL) prevail; in most cases, these will spontaneously regress can therefore only be followed according to clinical recommendations [1,2,4].

Data obtained from experimental and epidemiological studies have shown that HPV is detected in 99.7% of cervical carcinomas. Of this total, 70% of infections are by types 16 and 18 [2,4]. Most genital HPV infections regress within two years, and only a minority of women develop a persistent infection that may eventually cause cervical intraepithelial neoplasia. Thus, although infection by viral types with high oncogenic risk is necessary to start the process of malignant transformation of cervical cells, it is not enough by itself [1,2]. Other factors may be involved in this process, such as immunological, environmental and genetic co-factors [2,3,6]. Among these factors, it is suspected that co-infection with agents responsible for other sexually transmitted infections (STIs), such as the human immunodeficiency virus (HIV), Chlamydia trachomatis (CT), herpes simplex virus (HSV), and other types of HPV, are related to the appearance of cervical lesions [6-11].

The persistent and simultaneous presence of several infectious agents could lead to chronic inflammation, which is considered to be important in the pathogenesis of various types of cancer, since about 90% of cases of cervical neoplasms arise from precancerous intraepithelial lesions, which were possibly originally induced by viral infections [6, 9-11].

The present study aimed to analyze the prevalence of several genital infectious agents, especially CT co-infection with HPV, in cervical alterations presented by patients from a public health service in the state of Pernambuco, Brazil.

Material and methods

A population-based cross-sectional study was initially conducted in 525 women aged 18 to 64 years between April and November 2011. Among these women, 87 were diagnosed with cytopathological changes of varying degrees, based on the conventional Pap smear test, analyzed by LACEN (Central Public Health Laboratory of Pernambuco). All of these women underwent colposcopy and/or cervical biopsy (if necessary). Women who were treated with radiation or chemotherapy for invasive cervical cancer were excluded from this study.

A standardized questionnaire was used to interview the subjects regarding their clinical history, sexual behavior, cultural habits and socioeconomic and living conditions. After consent, all women were examined by a gynecologist. The study had first been approved by the Research Ethics Committee of the Health Sciences Center of the Federal University of Pernambuco - CCS,UFPE (nº 105/09).

Colposcopy was performed according to the nomenclature classification of the International Federation of Cervical Pathology and Colposcopy (IFCPC-2011). Cytopathological changes were classified according to international convention (Bethesda System 2001, adapted by the Brazilian Society of Cytopathology). The samples were screened for the presence of HPV and CT infections as previously described [10].

Statistical analysis was performed using the Epi Info 3.5 for Windows STATCALC program. Cytology was reported according to the Bethesda System. The association of result and exposure was tested with Fisher's exact test and exposure to more than two categories with the non-parametric trend chi-square test. $P > 0.05$ was considered significant. The Kappa and McNemar tests were also chosen to better assess discrepancies. This was done using Epi Info statistical software version 5.0 or higher, with double entry. Initially, tables were drawn up with the distribution of frequencies for categorical variables, while for quantitative variables, measures of central tendency (mean, median) and dispersion (standard deviation, percentage) were used. To assess possible associations between HPV and CT co-infection and the different stages of cervical lesions, Fisher's exact test was used with a significance level of 5%. Odds ratios (OR) and their 95% confidence intervals (95% CI) were calculated for each test. Logistic regression analysis was performed to assess the significance of differences in the paired data in the same individual.

Results

The average age of the 87 women selected in this study was 36.4 ± 11.9 years (mean ± standard deviation). The demographic characteristics, as well as the reproductive and sexual history of the 87 women are shown in Table 1. Of these women, 52.9% had received less than eight years of schooling. Most women (80.5%) were of urban origin, and 65.5% considered themselves black or brown. More than half of the selected women (62.1%) were married or in a stable civil union; 37.9% had a history of previous STIs and 41.38% did not use any contraceptive method. Only 14.9% were smokers, while 33.3% had consumed alcohol and 1.15% had tried or used drugs.

Table 2 highlights the distribution of cytolopathological changes detected by conventional Pap smear. It was found that 42.5% (37/87) of the patients had LSIL, 40.2% (35/87) of atypical squamous cells of undetermined significance, and 17.25% more serious changes.

Table 3 shows the distribution of genital infections and STIs in the 87 patients with cervical changes on conventional Pap smear test. For non-viral flora, with the exception of CT infection, the most prevalent STI was *Gardnerella vaginalis* (GV), found in 35.6% (31/87), followed by *Coccus sp*. in 19.5% (17/87), *Candida sp*. in 9.2% (8/87), *Trichomonas vaginalis* in 5.7% (5/87), *Lactobacillus sp*. In 4.6% (4/87) and HSV in 1.2% (1/87). For the infections described above, no association was detected with the degree of cervical intraepithelial injury ($p > 0.05$).

The incidence of CT infection was 83.9% (73/87), while

Table 1 Biological, socio-demographic characteristics and habits of 87 patients with cervical changes on conventional Pap smear test in the state of Pernambuco, 2011.

Feature	N	%
Origin		
- Rural	17	19.5
- Urban	70	80.5
Race		
- White	29	33.3
- Black / Brown	57	65.5
- Other	1	1.2
Education (years)		
≥ 8 years	41	47.1
< 8 years	46	52.9
Marital situation		
With partner (married / in a stable relationship)	54	62.1
Without partner (single / other)	33	37.9
Previous STD episode		
YES	33	37.93
NO	54	62.07
Smoker		
YES	13	14.9
NO	74	85.1
Drug use		
YES	1	1.1
NO	86	98.9

HPV infection was detected in 82.8% (72/87) of the selected women. Of the 43 women found to have HSIL, seven had only HPV infection and 36 were co-infected with CT; instead, all of the 29 women with LSIL were co-infected with CT (Table 3). Of the patients who had HPV infection, 93.1% had colposcopic changes and 68.96% had histopathological agreement. The most prevalent subtypes were HPV 16 in 48.6% of cases and

Table 2 Cervical cytopathological changes on conventional Pap smear test in 87 patients in the state of Pernambuco, 2011.

Cytopathological changes	N	%
ASC-US	35	40.25
LSIL	37	42.50
HSIL	07	08.05
ASC-H	06	06.90
AGUS	01	01.15
Carcinoma	01	01.15
TOTAL	**87**	**100%**

** ASC-US (atypical squamous cells of undetermined significance, possibly non-neoplastic), LSIL (low-grade squamous intraepithelial lesions), HSIL (high-grade squamous intraepithelial lesions); ASC-H (atypical squamous cells, lesion cannot be excluded); AGUS (atypical glandular cells of undetermined significance).*

Table 3 Distribution of genital infections and STIs in 87 patients with cervical lesions/changes on conventional Pap smear test in the state of Pernambuco, 2011.

Infection by	Type of cervical lesion/change		Total n=87 (%)	OR (95%CI)	P
	High grade** n=50 (%)	Low grade * n=37 (%)			
Lactobacillus sp.					
- Present	3 (6.0)	1 (2.7)	4 (4.6)		
- Absent	47 (94.0)	36 (97.3)	83 (95.4)		
Coccus sp.					
- Present	12 (24.0)	5 (13.5)	17 (19.5)	2.00 (0.58-8.06)	0.28
- Absent	38 (76.0)	32 (86.5)	70 (80.5)		
Gardnerella vaginalis					
- Present	15 (30.0)	16 (43.2)	31 (35.6)	0.57 (0.21-1.50)	0.26
- Absent	35 (70.0)	21 (56.8)	56 (64.4)		
Trichomonas vaginallis					
- Present	4 (8.0)	1 (2.7)	5 (5.7)	3.10 (0.29-158.25)	0.39
- Absent	46 (92.0)	36 (97.3)	82 (94.3)		
Herpes simplex virus (HSV)					
- Present	0 (0.0)	1 (2.7)	1 (1.2)	-	0.43
- Absent	50 (100.0)	36 (97.3)	86 (98.8)		
Candida sp.					
- Present	6 (12.0)	2 (5.4)	8 (9.2)	2.36 (0.39-25.36)	0.46
- Absent	44 (88.0)	35 (94.6)	79 (90.8)		
Mixed flora					
- Present	20 (40.0)	16 (43.2)	36 (41.4)	0.88 (0.33-2.27)	0.83
- Absent	30 (60.0)	21 (56.8)	51 (58.6)		
Chlamydia trachomatis (CT)					
- Present	38 (76.0)	35 (94.6)	73 (83.9)	0.18 (0.02-0.92)	**0.036**
- Absent	12 (24.0)	2 (5.4)	14 (16.1)		
HPV and CT simultaneously	n=43	n=29	n=72		
- Present	36 (83.7)	29 (100.0)	65 (90.3)	–	**0.037**
- Absent	7 (16.3)	0 (0.0)	7 (9.7)		

** Low grade includes: ASC-US (atypical squamous cells of undetermined significance, possibly non-neoplastic) and LSIL (low-grade squamous intraepithelial lesions); ** High grade includes: HSIL (high-grade squamous intraepithelial lesions); ASC-H (atypical squamous cells, lesion cannot be excluded); AGUS (atypical glandular cells of undetermined significance) and carcinoma.*

HPV 31 in 22.2% (Table 4). There was a statistically significant association between the presence of CT infection and the presence of LSIL (p = 0.036) versus the presence of HSIL. HPV and CT co-infection was found in 90.3% (65/72) of the cases and a significant difference was observed when simultaneously compared HPV/CT co-infection with the degree of cervical intraepithelial lesions (p = 0.037).

Table 4 HPV subtypes in 72 patients in the state of Pernambuco, 2011.

HPV infection	Type of cervical lesion/change		Total infections by subtype n=72 (%)
	High grade (n=43)	Low grade (n=29)	
Subtype 16	20	15	35 (48.6)
Subtype 18	6	4	10 (13.9)
Subtype 31	9	7	16 (22.2)
Subtype 33	6	5	11 (15.3)

Discussion

Research into the etiology of cervical cancer has made substantial progress in the past two decades in both scientific and operational terms. For several years, the epidemiological profile of women with cervical cancer has been recognized as suggestive of involvement of STIs.

Various infectious agents, such HSV type 2, GV and CT have been proposed as co-factors in cervical carcinogenesis [6-10, 12-15]. With regard to non-viral flora, GV was the most frequent in our sample of women (35.6%), followed by other agents (Table 2). No association was found between the presence of these non-viral microorganisms and of HSV and the severity of intraepithelial lesions.

Inflammation of the cervical epithelium has also been recognized as one of the predisposing co-factors for cervical carcinogenesis, as disturbance of the vaginal flora is known to increase the risk of acquiring HPV infection, and have been showed that women cytologically diagnosed with bacterial imbalance had a significantly higher presence of cervical (pre) neoplasia [16]. This way, the cervical-vaginal environment plays a decisive role in susceptibility to HPV infection. Regarding GV infection, the women infected are likely to possess an unhealthy *Lactobacillus*-poor vaginal flora, as they should also be at risk for acquiring HPV infection.

Cancer of the cervix is associated with persistent infection by oncogenic subtypes of the HPV virus, especially HPV-16 and HPV-18, which account for about 70% of cervical cancers [1,2,10]. HPV infection is very common; it is estimated that about 80% of sexually active women will acquire it over their lifetime. Approximately 290 million women worldwide have HPV, 32% of whom are infected with subtypes 16, 18 or both, but it is observed that cancer is a rare outcome, even in the presence of HPV infection. In other words, HPV infection is a necessary but not sufficient, factor for the development of uterine cervical cancer [1,2].

Most of the time, cervical HPV infection is transient and regresses spontaneously between six months and two years after exposure [2]. In the small number of cases in which the infection persists, it is caused by an oncogenic viral subtype, and can give rise to cervical precursor lesions (HSIL and adenocarcinoma in situ), whose identification and appropriate treatment prevent progression to invasive cervical cancer [2]. In our study, the most frequent subtypes were 16, 31, 33 and 18.

In addition to aspects related to HPV infection itself (subtype, viral load, single or multiple infection), other factors linked to immunity, genetics and sexual behavior also seem to influence the still uncertain mechanisms that determine the regression, persistence and progression of the infection to precursor lesions or cancer.

Several studies show that smoking, early sexual initiation, multiple sexual partners, multiparity and the use of oral contraceptives are considered risk factors for the development of cervical cancer [1,2,6-12,14,15]. Age also affects this process, with the majority of HPV infections in women under 30 years old regressing spontaneously, whereas above that age, persistence is more frequent [2].

The fact that we found a high rate of HPV infection (82.8%) in this study can be explained by the fact that we analyzed only women with cervical intraepithelial lesions. The most prevalent HPV subtypes found in this study were 16 and 31 (accounting for 48.6% and 22.2% of total infections, respectively). In the subgroup with LSIL, the most prevalent subtypes were 16 and 31 (accounting for 40.54% and 18.91% of infections, respectively), while in HSIL and carcinoma, the most prevalent subtypes were 16 and 18 (71.42% and 50%), which corroborates findings of several studies already published worldwide.

In the present analysis, there was a high frequency of CT infection in patients with cervical intraepithelial lesions (83.9%). HPV-CT co-infection occurred in 65 cases. Of the patients who had HPV infection, 93.1% had colposcopic changes and 68.96% had histopathological agreement. CT infection was more frequent in LSIL (p = 0.036) than in HSIL. This infection appears to increase susceptibility to HPV infection at the baseline level and facilitate access to basal epithelial cells by micro abrasions or alter cellular characteristics, increasing the viral load of the infection and facilitating persistence [7,13-17].

Concurrent CT infection can prevent the HPV infection from resolving by inducing a humoral-type immune response pattern. In addition, CT infection has been associated with hyperplasia of reserve cells and metaplasia, processes related to cervical carcinogenesis.

On the other hand, it is also believed that a modulation of the immune response and/or precipitation of an inflammatory response favors a subsequent infection by CT, increasing the rate of infectivity by CT in HPV-positive women [17]. Following same reasoning, the inflammatory response caused by CT, associated with other infectious agents, such as GV, Candida sp. and Trichomonas vaginalis, may cause greater local disruption, as suggested in other studies [6, 16].

In conclusion, genital infections, in particular CT, need to be investigated and treated properly, since HPV co-infections are associated with an increased likelihood of LSIL, which can progress to more serious injuries, such as cervical carcinomas.

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Acknowledgement: *The authors express their gratitude to the professionals of the Central Public Health Laboratory of the State of Pernambuco - LACEN-PE, the Center of Biological Sciences at the Federal University of Pernambuco (UFPE), and the Genetic Biochemistry and Sequencing of DNA Laboratory of Federal Rural University of Pernambuco.*

Conflict of Interest: *The author(s) declare(s) that there is no conflict of interest*