IOSR Journal of Nursing and Health Science (IOSR-JNHS)
e-ISSN: 2320–1959.p- ISSN: 2320–1940 Volume 13, Issue 2 Ser.2 (Mar. – Apr. 2024), PP 36-41
www.iosrjournals.org

Hpv Low Vaccine Coverage: Prevalence Of High-Risk Hpv Cases In A Vulnerable Region Of São Paulo, Brazil

Hannar Angélica De Melo Alverga[1], Ana Carolina Soares De Oliveira[2], Giovana De Oliveira Dichman[1], Gabriel Monteiro Pinheiro[3], Priscila Paruci[1], Neil Ferreira Novo[1], André Luis Lacerda Bachi[1], Expedito José De Albuquerque Luna[4] Marina Tiemi Shio[1], Ana Paula Ribeiro[1], Dennis Minoru Fujita*[4], Toni Ricardo Martins[5], Luiz Henrique Da Silva Nali[1]

Programa De Pós-Graduação Em Ciências Da Saúde, Universidade Santo Amaro, Rua Prof. Enéas De Siqueira Neto, 340, São Paulo, Brasil
Imtusp – Lab Virologia - Lim52-Hcfmusp, Av. Dr. Enéas De Carvalho Aguiar, 470, São Paulo, Brasil.
Hospital Escola Wladimir Arruda – Universidade Santo Amaro, Rua Prof. Enéas De Siqueira Neto, 340, São Paulo, Brasil
Fmusp, Av. Dr. Enéas De Carvalho Aguiar, 470, São Paulo, Brasil.
Ufam – Faculdade De Ciências Farmacêuticas

Abstract
Objectives*: The present study aimed to evaluate the HPV vaccine coverage and the current incidence in women who undergo routine cervical cancer screening at a university hospital in a socioeconomic vulnerable region of São Paulo, Brazil.*
Methods: *Data of HPV vaccine coverage was collected since 2017 in open data base of Brazilian Ministry of Health.*
Samples of cervical vaginal smears from women over 18 years old were collected at the Gynecology and Obstetrics outpatient clinic of "Hospital Escola Wladmir Arruda". All samples obtained were sent to the UNISA research laboratory to perform the cytopathology analysis and HPV genotyping by Papillocheck microarray. Moreover, sociodemographic data were collected through a questionnaire.
Results: *A total of 121 women (mean age of 45.66 years-old) participated in the study. Cytopathology analysis showed a 3.18% incidence of precursor lesions. Genotyping analysis revealed that 33/121 (27,3%) of the patients were infected with at least one of HPV genotypes. Importantly, most of the 23/33 were infected with high-risk HPV and 12/33 were coinfected with more than one HPV genotype. Among the genotypes, the most prevalent was HPV16 with 8,5% of total infected patients.*
Conclusion: *The low vaccine coverage in Brazil may increase the incidence of HPV in the next years, our findings shows that many hrHPV are frequent in the population of a poor region. Public health policies intervention should be resumed in order to avoid the increase of number of cases.*
Keywords: *Low Vaccine Coverage, HPV genotyping; Cervical cytology; cervical cancer, Brazil.*

Date of submission: 20-03-2024 Date of acceptance: 30-03-2024

I. Introduction

Human papillomaviruses (HPV) belong to a heterogeneous group of DNA viruses that present the ability to elicit the loss of stability of genomic DNA of the host cells. It is widely accepted that HPV is a worldwide issue for public health, particularly due to the fact that its ability, formerly cited, could induce tumorigenesis, which drives the host cells infected to the transformation and development of several types of cancer, especially cervical cancer[1]. Among the sexually transmitted infections (STI), HPV is the most prevalent virus and the role of high-risk HPV (hrHPV) in the development of cancer is well stablished[2].

It is noteworthy to mention that hrHPV types 16 and 18 are the most prevalent ones and are responsible for around 70% of cervical cancer[3–5]. Importantly, these types are present in the HPV vaccination distributed by the Brazilian Public Health System, therefore we might envisage a scenario in the next years where other hrHPV might be more prevalent.

It is important to emphasize that factors regarding the HPV genetic variation may interfere with the viral persistence, transmission, and progression to invasive cancer. In fact, this aspect, among the different high-risk genotypes, can contribute to disparities in the incidence of cervical cancer. Therefore, the study of specific types

of HPV has become fundamental for the real integration of the risks inherent to the development of cervical cancer [6,7].

Interestingly, there is a lack of studies focusing on the diversity of HPV genotype infection in poor populations in areas with high density. This might be explained due to the infrastructure and costs for testing, which tend to be more difficult to assess in these areas [8]. However, understanding the profile of HPV infection in poor women might aid and emphasize the need for genotype screening in these women.

The Brazilian National Cancer Institute (INCA) registered 16710 new cases in Brazil in 2020[9]. According to the same institute for the years 2020/21, it was estimated 5.93 cases/100.000 women in São Paulo city, as well as 6.80 cases/100.000 women in São Paulo State[10]. Importantly, São Paulo is one of the wealthiest cities of Brazil, located at Southeast region where more than 11 million people lives in the capital and other 11million lives the cities around São Paulo[11]. South region of São Paulo is the largest area and comprises the largest population within the capital (2,5million people), mainly composed by young people[12].

Unfortunately, this region is one of the poorest ones, especially when considering the far south neighborhoods in the South region, where per capita income is around 0.4 of the Brazilian minimal wage[12].

For these reasons, the region may be a concerning place since cervical uterine cancer is considerably more prevalent in low-income areas[13]. Therefore, the aim of the study was to assess the frequency of HPV infection and to determine the genotypes present in cervical samples of women who lives in a poor area of São Paulo.

II. Methods

Design and population of the study

This is a cross-sectional, descriptive study with a qualitative approach conducted in the South region of São Paulo city. An area formed by neighborhoods with lowest income rates [11], presenting absence of public health care units focused on the HPV diagnostics or cytopathological findings, which turns the studied region in a vulnerable location.

Women who had an appointment at the gynecology outpatient clinic of Santo Amaro University were recruited, and the screening population was composed of women between 18-65 years old during July 2020 to June 2021, women were separated by age in order to analyze distinct risk of factor for transmission accordingly to age.

This study was approved by the ethical committee of Universidade Santo Amaro under protocol # 4.237.904. Patients who participated in the study signed the approved Consent Form

Cervical samples were collected from the squamocolumnar junction, and the whole brush head was transferred into a ThinpPrep cytological medium recipient (Hologic, USA). All samples were sent to University Santo Amaro's Research laboratory. All participants were not submitted to HPV vaccination.

The data of vaccine coverage were collected from de 2013 (first year of public campaign for HPV vaccination) to 2022 in online public health database of Brazilian Ministry of Health.

Sample preparation and DNA extractions

Around 5mL ThinPrep media of each sample were placed in Falcon tubes®, centrifugated at 1500g for 3 minutes. After that, the cell pellet was washed with sterile PBS, centrifugated again, the supernatant was discarded, and the cell pellet resuspended in the 1mL of PBS. An aliquot of 500µL was sent to perform the cytological test and another aliquot of 200µL was used to perform DNA extraction.

DNA was extracted by using Qiagen DNA Mini Kit(Germany), following the manufacturer's instructions. All samples were quantified in Nanopdrop (Thermofisher) and tested for GAPDH[14] housekeeping gene through Sybr Green RealTime PCR platform (Step One Plus – Apllied Biosystems, USA), in order to verify the quality and amount of DNA recovered.

Cytological preparation and testing

Around 1mL of the aliquoted cells was placed in a sterile glass slide and distributed throughout all the slide. Cells were fixed with Ethanol 95% and followed to pap coloring protocol. The smears in the slide were examined thoughtfully by a trained cytopathologist and then classified according to Bethesda protocol [15]. Cells were considered to present abnormal cytology if cytological findings presented atypical squamous cells of undetermined significance (ASC-US) or worse.

Molecular testing

DNA samples were screened for HPV-DNA by the PapilloCheck® (Greiner Bio-One), a polymerase chain reaction (PCR)-based DNA microarray system that can individually identify 25 HPV types, of which 12 high-risk HPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59) and other 13 HPV types (6, 11, 40, 41, 42, 43, 44/55,

53, 66, 68, 70, 73 e 82). The test cannot distinguish between HPV 55 and 44 due to cross-reactivity. The test was performed according to the manufacturer's instructions.

Statistical analysis

Descriptive data were shown in percentages. All data analyses were performed using the two-tailed Fisher's exact test, and the Chi-square test to verify the proportions period of study. The significance for all p values was set at 0.05.

III. Results

In 2022, for the first dose, 1,203,019 shots were delivered in girls from 09 to 14 years old and 1334926 shots for boys (same age group), and 991,425 booster shots were delivered in girls and 735,643 in boys.

The current HPV vaccine coverage in Brazil (Table 1), considering the second shot, is estimated in 58.29% for girls (9-14 years old) and 38.39% for boys (9-14 years old) under the goal stablished in the National Program for Immunization that is in 80% for a safe vaccine coverage. The main target group of our study belongs to this period of free delivery of HPV vaccines that starts in 2013.

A total of 121 women were enrolled in the study. The detailed sociodemographic findings are described in Table 2. We have analyzed the data according to the age of patients who attended to the health care center. This was chosen to better analyze the patients accordingly to social conditions such as marriage and partners. Importantly, we did not any statistical difference among the frequency of HPV which is shown further or social conditions.

We were able to analyze 107 samples for cytopathological findings of the women enrolled in the study. Most of the samples revealed normal or inflammatory cytopathological analysis, the findings are described in Table 3.

Table 1. Cytopathological findings of the samples enrolled in the study. Legend LSIL: low-grade squamous intraepithelial lesion, HSIL: High-grade intraepithelial lesion, ASC-US: Atypical squamous cells of unknown significance.

Cytopathological findings	N (107)	%
Normal	49	45,79
Inflammatory	53	49,53
LSIL	1	0,93
HSIL	2	1,86
ASC-US	2	1,86

All samples were positive for GAPDH testing and presented DNA quantification higher than 50ng/µL. In table 4, we describe the frequency of HPV genotypes infection in our study. Genotyping analysis revealed a total of 33/121(27,3%) presented a positive result for some HPV type through the genotype testing. The analysis also revealed that most of the HPV+ patients (23/33 – 69,7%) were infected by one or more high risk HPV types (hrHPV), whereas 8/33 (24,3%) patients presented infection by low risk HPV (lrHPV). The detailed frequency of each HPV type in the samples are described in table 3.

Additionally, we have observed that 12/33 infected patients presented coinfection by more than one HPV type, The results revealed that there was no pattern of coinfection by hrHPV or lrHPV exclusively. From all infected patients that presented coinfection by more than one type of HPV, 5/12 (41,6%) were coinfected by two or more hrHPV, 6/12 (50%) patients were infected one or more hrHPV and lrHPV, and only 1 patient (8,4%)were infected by more than one lrHPV.

It is utmost to emphasize that as demonstrated in table 2, most of the women presented normal or inflammatory conditions on cytopathological analysis. However, 26 of these samples (24,3%) presented to be positive for at least one type of HPV.

Table 2. Frequency of HPV types detected in cervical samples.

HPV type	N(%)	HPV type	N(%)
hrHPV		*lrHPV*	
16	4(8,5)	**44/55**	6(12,7)
35	3(6,4)	**6**	4(8,5)
56	3(6,4)	**70**	3(6,4)
45	3(6,4)	**42**	3(6,4)
33	2(4,3)	**82**	1(2,1)
58	2(4,3)	**53**	1(2,1)
39	2(4,3)		
66	2(4,3)		

52	2(4,3)		
39	1(2,1)		
51	1(2,1)		
18	1(2,1)		
68	1(2,1)		
59	1(2,1)		
31	1(2,1)		
Frequency of HPV types detected in the samples	29		18

Lastly, in the present study, we also searched for any risk factor regarding the frequency of HPV infection. In this sense, no statistical difference was found regarding smoking, drinking, pregnancies, previous STI, number of sexual partners in the last 6 months, drugs, schooling, age of onset of sexual activity.

However, the overall number of sexual partners demonstrated to be statically different when comparing patients who were not infected by HPV (average 2,9 Standard deviation +-0,2), whereas patients who were infected by HPV presented (average 4 SD +- 0,1) $p<0,01$.

Although no difference was observed in the schooling of HPV-infected and non-infected patients, a total of 106/121 patients earns less than 2 minimal wages per month (around U$390) as a total income of the family, also all of these patients have low grading school.

IV. Discussion

The current HPV low vaccine coverage presents 58.29% for girls and 38.39% for boys in 9 to 14 years, was intensified by the COVID-19 Pandemic due to all efforts were focused on prevention, diagnose and control of this specific infectious diseases.

The main vaccine preventable diseases were neglected, with reduction in diagnoses that lead to a false perspective of these infectious diseases' decrease in the last 2 years, and a low engagement of the population towards vaccination motivated by the end of immunization awareness campaigns, the growth of anti-vaccination movements in the Pandemic period and misinformation [16]

These conditions promote a high population exposition that, in a general way, corroborate to our results with high frequency of different HPV genotypes, specially hrHPV in women who live in poor areas of São Paulo city. Although DNA testing for HPV diagnosis is recommended by WHO[17], HPV DNA molecular testing is still not affordable for many of public health services that works in the screening of HPV, probably due to the need for infrastructure and laboratory materials to perform the analysis.

Besides these observations, we also were able to demonstrate that many patients who presented normal and/or inflammatory cytopathological findings, presented infection by both lrHPV and hrHPV, which not only corroborates a previous report [18] but also highlights the need for early diagnosis of HPV presence since different HPV types were detected in these women. In fact, early detection may be a key to the prevention of cervical carcinoma, as well as to increasing the successful treatment rate[19–21].

The population of this study is predominately composed of poor people who live with less than 2 minimal wages as a total family income and by low education grade. Despite the frequency of HPV infections seems to affect all socioeconomic classes equally in the Brazilian population [22], the socioeconomic and educational status of these affected people can putatively impact their survival chances, especially due to difficulty in access to treatment and health insurance [23–25].

Moreover, socioeconomic status seems to be a major issue in order to the acceptance of HPV vaccination [26,27]. Therefore, our results, added to previous local findings [28–30], highlight the need for a broad vaccination campaign in all populations, particularly the poor people who are more vulnerable HPV related cancers[31], since this part of the population presents reduced chances of survival and also lower acceptance to vaccination, which includes HPV vaccination. In parallel, public health strategies should be implemented in order to increase molecular genotyping screening access in poor areas.

Not surprisingly, in our participant group, the main risk factor for HPV infection was the multiple sexual partners throughout the life of our volunteers, which corroborates the literature [32,33].

Besides, the frequency of HPV types found in this study was predominantly of hrHPV, although it was reported. that the frequency of HPV types was similar for hrHPV and lrHPV in some populations[34]. Therefore, our findings are concerning, since the detection of hrHPV DNA in cervical samples might predict malignant lesions [35].

Our study presents some limitations, such as the low number of samples collected, since, in fact, this might have impacted the real dynamics of the frequency of HPV genotypes in the studied population. In this regard, it is utmost of importance to cite that our study was enrolled during the worst moment of the SARS-CoV2 pandemic.

Therefore, many women who had appointments in the local clinics did not attend due to the necessary social distancing and isolation imposed by the pandemic. Taking into account this fact, further strategies for

sampling should be considered, such as self-sampling seems to be an important alternative strategy to increase the cervical cancer screening[36].

However, the results obtained in the diagnostic evaluations in our study (100% of persons unvaccinated for HPV) are more drastic than the percentage of vulnerability presented by the decreasing Brazilian vaccine coverage for HPV in the last years (under 50%), requiring attention to the resuming of sex education in schools and vaccination campaigns to raise awareness of the population, mainly in regions of extreme poverty to reduce the risk for HPV transmission.

V. Conclusion

The results of our study revealed concerning findings regarding to the high incidence of HPV infection, as well as the elevated hrHPV presence in young women with low socioeconomic status and low education grades.

In addition, we were able to show that many of the patients who present normal cytopathological findings also presented HPV DNA in their samples, including coinfections, which reinforces the need for molecular testing for the detection and screening of the HPV types. Future studies should focus on HPV genotyping screening in a larger number of participants and also in distinct cities in the same socio-economic conditions.

The low vaccine coverage allows an unusual incidence that could be prevented, however, the absence of health campaigns for awareness and education with special attention for young people, generates this scenario of extreme vulnerability.

CRediT authorship contribution statement

Alverga HAM: Conceptualization, Methodology, Formal analysis, Writing - original draft, Oliveira ACS Validation and Formal analysis, Dichman GO – Data Collection; Validation and Formal analysis, Pinheiro GM: Data Collection; Validation and Formal analysis, Paruci P: Validation and Formal analysis, Novo NF: Data Collection and Validation, Bachi ALL: Validation and investigation, Luna EJA - Revision, Shio MT - Data Collection; Validation and Formal analysis, Ribeiro AP – Methodology and Validation, Fujita DM – Investigation, Data Analysis and Writing – Original Draft, Martins TR – Formal Analysis and Validation , Nali LHS - Investigation, Conceptualization, Formal analysis, Writing - original draft, Writing - review & editing

Funding

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Competing Interests Statement

The authors state no conflict of interest to declare.

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