Epidemiological surveillance of Human Papillomavirus (HPV) genotypes in oral and genital tracts and the impact of HPV vaccination on male university students from Rio de Janeiro state, Brazil

Vigilância Epidemiológica dos genótipos de papilomavírus humano (HPV) nos tratos oral e genital e o impacto da vacinação em estudantes universitários do sexo masculino do estado do Rio de Janeiro, Brasil

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ABSTRACT

Introduction: Human Papillomavirus (HPV) infections are of significant concern in men, given its potential impact on their health and the risk of transmission to partners. Understanding and addressing this infection in men is crucial to evaluate the effectiveness of vaccination in reducing HPV-related diseases. **Objective:** To assess the impact of HPV vaccination, potential genotype shifts, and adverse effects, through a prospective study conducted with male university students. **Methods:** The study involved 286 volunteers who were examined at Sexually Transmitted Disease Clinics at the Universidade Federal Fluminense in Niterói, Rio de Janeiro, Brazil. The HPV prevalence was evaluated using generic PCR, genotyped by DNA microarray and monitored adverse effects. **Results:** The findings of this study revealed the absence of moderate or severe adverse effects. Genetic shifts were observed, including the disappearance of oncogenic HPV types 16 and 18. Surprisingly, even after completing the full vaccine regimen, students still harbored HPV11 in the oral tract. Furthermore, persistent HPV 6 and 11 infections were identified in three students, who had pre-existing infections prior to vaccination, at the follow-up visit. Multivariate analysis uncovered independent associations, notably an increased risk of HPV infection in the oral tract among men who have sex with men. HPV prevalence rates remained low both before and after the vaccination scheme (T0: 14.7%, T1: 8.7%). Even after the full vaccination scheme, the prevalence remained similar at T2 (14.6%), with no statistically significant differences recorded. HPV11 emerged as the most prevalent type throughout the study, followed by HPV6. Vaccine genotypes were detected in a significant proportion of samples at T0 (85.4%), T1 (89.5%), and T2 (100%). **Conclusion:** Overall, this study suggests that vaccination may represent a promising approach to reducing HPV-related health risks. These findings shed light on the potential benefits and challenges of HPV vaccination, emphasizing the need for continued monitoring and vaccination efforts.

Keywords: HPV. Vaccination. Genital tract. Genotype.

RESUMO

Introdução: As infecções por papilomavírus humano (HPV) são de grande preocupação em homens, dada sua possível influência na saúde deles e no risco de transmissão para parceiros. Compreender e abordar essa infecção em homens é fundamental para avaliar a eficácia da vacinação na redução de doenças relacionadas ao HPV. **Objetivo:** Avaliar o impacto da vacinação contra o HPV, possíveis alterações genotípicas e efeitos adversos, por meio de um estudo prospectivo realizado em estudantes universitários do sexo masculino. **Métodos:** O estudo envolveu 286 voluntários examinados em Clínicas de Doenças Sexualmente Transmissíveis na Universidade Federal Fluminense, em Niterói, Rio de Janeiro, Brasil. A prevalência do HPV foi avaliada por *polymerase chain reaction* (PCR) genérico e genotipada por microarranjo de DNA, e foram monitorados os efeitos adversos. **Resultados:** Os resultados deste estudo revelaram a ausência de efeitos adversos moderados ou graves. Observaram-se mudanças genéticas, incluindo o desaparecimento dos tipos oncogênicos do HPV 16 e 18. Surpreendentemente, mesmo após a conclusão do esquema completo de vacinação, os estudantes ainda abrigavam o HPV 11 na cavidade oral. Além disso, foram identificadas infecções persistentes pelo HPV 6 e 11 em três estudantes que já tinham infecções preexistentes antes da vacinação e na visita de acompanhamento. A análise multivariada revelou associações independentes, especialmente um aumento no risco de infecção pelo HPV na cavidade oral em homens que têm relações sexuais com homens. As taxas de prevalência do HPV permaneceram baixas tanto antes quanto depois do esquema de vacinação (T0: 14,7%, T1: 8,7%). Mesmo após a conclusão do esquema de vacinação, a prevalência permaneceu semelhante em T2 (14,6%), sem diferenças estatisticamente significativas registradas. O HPV 11 emergiu como o tipo mais prevalente ao longo do estudo, seguido pelo HPV 6. Genótipos da vacina foram detectados em uma proporção significativa de amostras em T0 (85,4%), T1 (89,5%) e T2 (100%). **Conclusão:** No geral, este estudo sugere que a vacinação pode representar uma abordagem promissora para a redução dos riscos à saúde relacionados ao HPV. Esses achados lançam luz sobre os benefícios e desafios potenciais da vacinação contra o HPV, enfatizando a necessidade de monitoramento contínuo e esforços de vacinação.

Palavras-chave: HPV. Vacinação. Trato genital. Genotípica.

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INTRODUCTION

Globally, Human Papillomavirus (HPV) ranks as the most prevalent sexually transmitted infection (STI), ranging from 10% to 20% in both sexes[1]. Notably, while women have been extensively studied in the context of HPV-related diseases, men have received less attention despite serving as a reservoir for persistent HPV infection. Recent research unveiled that HPV can lead to severe conditions in men, with approximately 60% of penile cancers being attributed to

this viral infection[2]. Consequently, the significance of HPV vaccination in males remains a topic of ongoing investigation[3].

Furthermore, oral HPV infection is recognized as an STI, yet there is a noticeable dearth of studies examining the efficacy of HPV vaccines in preventing oral diseases[4]. Since the initial approval of HPV vaccines in 2006, they have demonstrated safety, high immunogenicity, and the ability to confer substantial protection against HPV and related ailments. Numerous national vaccination programs, even those achieving only 50% coverage, have demonstrated substantial benefits for females, such as reduced HPV infections, genital warts, and cervical neoplasia. Presently, over 90 countries have implemented HPV vaccination programs for young females, with some, like the United States, Australia, and Brazil, extending vaccination coverage to young males as well[5].

In 2014, the Brazilian National Immunization Program adopted the quadrivalent Gardasil® vaccine (4vHPV), targeting HPV types 6, 11, 16, and 18 for young girls, which was later expanded to young boys aged 9 to 14 years, in 2017. Data supports the efficacy of 4vHPV in preventing HPV types 16 and 18 infections, which account for 70% of cervical cancer cases and 50% of high-risk precancerous lesions. Additionally, it can prevent over 70% of genital warts and low-risk cervical lesions associated with HPV types 6 and 11. The overarching objective is to achieve a 90% vaccination coverage rate for both sexes or, at a minimum, 90% vaccination coverage for girls up to 15 years of age[5,6].

Despite the potential benefits of HPV vaccination for young males, limited research has explored its impact, with only a few focusing on university students. Notably, none of these studies investigated the vaccine's effectiveness in preventing oral HPV infections[3].

OBJECTIVE

The present study aims to assess the prevalence of HPV infection and its genomic diversity in asymptomatic young male university students at the Universidade Federal Fluminense, examining both oral and genital samples. Additionally, this study seeks to identify predictors of HPV infection and evaluate the impact of HPV vaccination, including genotype shifts and potential adverse effects. Data were collected prospectively, encompassing assessments prior to vaccination, after the second dose, and two years post-vaccination.

METHODS

Study type

This prospective cohort study with intervention and prevention components was conducted as a non-randomized, controlled, and single-blinded investigation.

Inclusion and exclusion criteria

Inclusion criteria encompassed male individuals aged 18 to 20 years from Universidade Federal Fluminense. Exclusion criteria comprised individuals who had previously received HPV vaccines, presented clinical HPV oral and anogenital lesions, were concurrently participating in other studies involving medical treatments or vaccines, exhibited a history of severe allergies or anaphylactic responses, were allergic to vaccine components, had autoimmune diseases, or were using medications that could potentially interfere with vaccine response.

Data collection instruments and procedures

a. At the study's commencement, subjects provided informed consent, which was thoroughly reviewed, discussed, and signed.
b. Volunteers completed a sociodemographic questionnaire.
c. Following a clinical examination, sample collection was performed at Time Point 1 (T1) prior to administering the first dose of the quadrivalent HPV vaccine (4vHPV). Each volunteer received a vaccine passport containing instructions for reporting adverse effects and return dates.
d. A second preventive brush sample was collected for molecular biology at Time Point 2 (T2), which occurred six months after the initial assessment and was concurrent with the administration of the second dose of 4vHPV.
e. A final preventive brush sample was collected for molecular biology after two years in the prospective study, which was the Time Point 3 (T3).
f. Variables analyzed in the questionnaire included age, address, marital status, family income, sexual activity, age at first intercourse, contraceptive usage, parenthood status, and the number of children.
g. As part of the study, volunteers presenting clinical lesions were assessed and treated in the Sexually Transmitted Diseases Sector of Universidade Federal Fluminense.

Vaccine characteristics

This study employed the Gardasil® 4vHPV, targeting HPV types 6, 11, 16, and 18. It is a sterile preparation administered intramuscularly. Each 0.5 mL dose contains approximately 20 mcg of HPV6 L1 protein, 40 mcg of HPV11 L1 protein, 40 mcg of HPV16 L1 protein, and 20 mcg of HPV18 L1 protein. The vaccine also contains excipients: 225 mcg of aluminum (as an amorphous adjuvant to aluminum hydroxyphosphate sulfate), 9.56 mg of sodium chloride, 0.78 mg of L-histidine, 50 mcg of polysorbate 80, 35 mcg of sodium borate, and water for injection. The vaccine is recommended for individuals aged 9 to 45 years and administered in a 0–2–6 months regimen[7,8].

Laboratory procedures

- DNA (deoxyribonucleic acid) extraction:

All samples used for the study were collected in TRIS-EDTA (ethylenediaminetetraacetic acid-tromethamine) buffer pH (potential of hydrogen) 7.2 and frozen at -20°C. After thawing, the samples were placed in a thermoblock at 56°C for two hours. The samples were submitted to the digestion process of their material through contact with 100 µL of proteinase K, containing a digestion buffer. The extraction protocol used was phenol-chloroform (Thermo Fisher®), as previously described[2]. The aqueous phase was transferred to a new tube, and the DNA was precipitated with 3M sodium acetate (pH 6.0; 1/10 volumes) and absolute ethanol (2.5 volumes). The DNA was suspended in 50 µL of mili-Q water and stored at -20°C.

- PCR (polymerase chain reaction) using generic primers:

Consensus primers (MY09/11) were used to detect generic HPV DNA. Amplification was carried out as previously described, using 50 ng of DNA sample in 50 µL of reaction mixture[9]. HeLa DNA was used as an HPV-positive control. The beta-actin primers were used as an internal control. Negative controls for checking contamination were included in all reactions. The PCR products were analyzed on 1.3% agarose gel stained with ethidium bromide for visualization of DNA under ultraviolet (UV) light, and a 100 bp DNA ladder was used as the molecular weight control pattern.

- Microarray technique for HPV typing by the LCD-Array Kit:

Samples positive for generic PCR but not genotyped by specific PCR were submitted to genotyping with the HPV 3.5® LCD-Array Kit (CHIPRON GmbH, Berlin, DE) according to the manufacturer's instruction. Briefly, PCR was performed using Primer Mix A (MY11/09) and B ('125') provided by the kit. The hybridization mix was composed of 5 µL of each amplified PCR product A and B. This mix was added to the LCD-Array slide. After staining and washing, the hybridization spots were scanned and analyzed by Slide Reader Software, as described by Kury et al.[10]

Statistical analysis

To determine the sample size, a test power of 95% was applied using the Epi-Info® network tool, based on estimated HPV infection incidence among the target population. With an assumed 20% HPV prevalence, a minimum sample size of 246 subjects was required.

Patient data were recorded as continuous and dichotomous variables in the EpiData® database, followed by univariate and multivariate analyses using Statistical Package for Social Sciences (IBM™, SPSS Statistics® software). Analyses explored the relationship between HPV positivity and specific variables among groups T0, T1, and T2. The strength of association was assessed using odds ratio (OR) with 95% confidence intervals (95%CI). Logistic regression was employed to estimate adjusted ORs based on variables selected via the Akaike Information Criterion (AIC). All hypothesis tests maintained a significance level of 0.05, and statistical analysis was conducted using SPSS Statistics.

Ethical considerations

The project and informed consent form were submitted to the Research Ethics Committee of the Faculty of Medicine of Universidade Federal Fluminense through the Plataforma Brasil system, receiving approval under protocol 1.788.830.

RESULTS

This study encompassed the final analysis conducted on 286 male students at Universidade Federal Fluminense (UFF), municipality of Niterói, Rio de Janeiro state, Brazil, spanning from 2016 to 2019. All 286 volunteers received medical attention at Sexually Transmitted Disease Clinics from UFF and provided both oral and penile samples. Subsequently, they were vaccinated with the first dose of 4vHPV, forming Group T0. Group T1 consisted of 218 students who returned for a second round of sample collection and received the second dose of 4vHPV six months later. Group T2 was comprised of 41 students who returned for sample collection two years after the initiation of the study. The study's flowchart is depicted in **Figure 1**.

In total, 504 doses of the 4vHPV vaccine were administered, achieving complete coverage (two doses) in 218 volunteers (76.2%). No severe adverse effects were reported. Following the first dose, 1.4% of students experienced mild symptoms such as fever or pain, and after the second dose, only 0.5% reported these minor effects.

The study encountered substantial attrition in the follow-up group, particularly during the final follow-up visit (85.6%), primarily due to the onset of the COVID-19 pandemic. Population demographics are detailed in **Table 1**.

By using MY09/11 generic PCR, it was detected HPV DNA in 42 out of 286 students (T0: 14.7%). In T1, 19 out of 218 (8.7%) were infected in oral and/or genital tract. After a two-year period of follow-up (T2), 6 out of 41 (14.6%) presented HPV DNA (**Table 2**).

HPV genotyping

Genotyping of HPV-positive samples was performed using a DNA Microarray assay (Greiner Bio-One, Germany). A profile of 30 different HPV genotypes was identified, including 13 benign types (06, 11, 42, 44, 54, 61, 62, 64, 72, 81, 84, 90, 91), 13 oncogenic

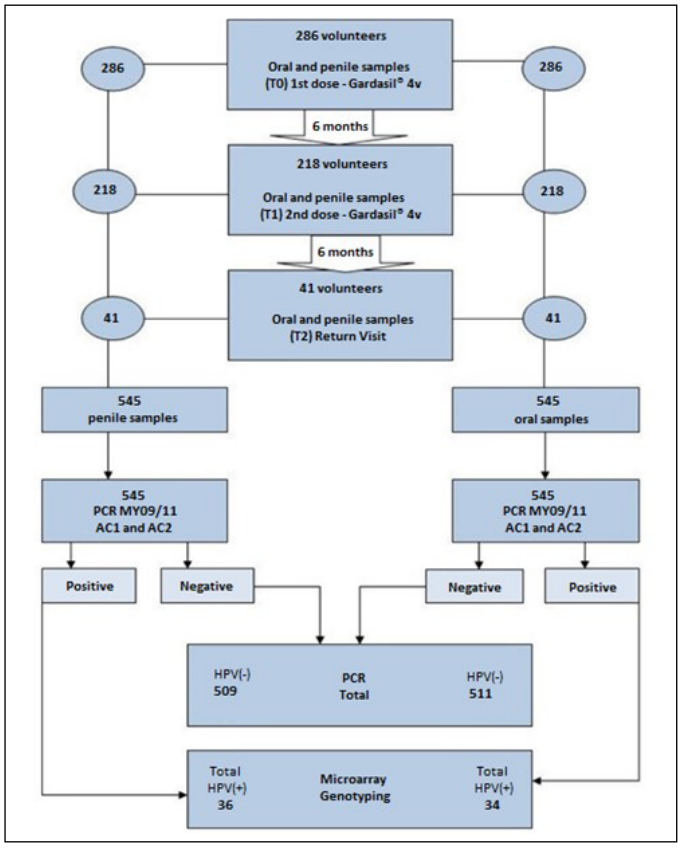

Figure 1. Flowchart of the study conducted on male students from Universidade Federal Fluminense, showing the enrollment and follow-up of the volunteers, including sample size, vaccine doses, and viral detection and typing.

types (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59), and 4 possible carcinogenic types (66, 73, 82). Notably, HPV11 emerged as the most prevalent type across all sampled time points. Among 67 HPV-positive samples, 44 (65.7%) featured HPV11, followed by HPV6 (13.4%), HPV66 (9%), and other types (6% each: HPV16, 42, 51, 52, 82; 4.5% each: HPV54, 59, 62, 73, 90; and 3% each:

Table 1. Socio-demographic and behavioral characteristics among university students, along the two-years follow-up study.

Variables	T0 n=286 n (%)	T1 n=218 n (%)	T2 n=41 n (%)
Age (year-old)			
18	30		
19	53		
20	203		
Familiar income			
1 minimum wage*	19 (6.6)		
2–3 minimum wages	80 (28.0)		
4–5 minimum wages	80 (28.0)		
>6 minimum wages	107 (37.4)		
Students from Health Sciences graduation courses			
Yes	119 (41.6)		
No	167 (58.4)		
Sexual activity			
Yes	257 (89.9)	199 (91.3)	39 (95.1)
No	29 (10.1)	19 (8.7)†	2 (4.9)
Sexual debut			
≤18	228 (88.7)		
>18	29 (11.3)		
Sexual partners (T0)			
≤2	85 (33.1)		
>2	172 (66.9)		
Sexual partners (T0–T2)			
Stable		86 (42.2)	20 (51.3)
Not stable		113 (56.8)	19 (48.7)
Tobacco smoking			
Yes	20 (7.0)		
No	266 (93.0)		
Sexual practices†			
M/W	193 (75.1)		
M/M	47 (18.3)		
M/W/M	17 (6.6)		
Use of condom			
Always	92 (35.8)	93 (46.7)	20 (51.3)
Frequently	116 (45.1)	68 (34.2)	08 (20.5)
Sometimes	34 (13.3)	21 (10.6)	06 (15.4)
Never	15 (5.8)	17 (8.5)	05 (12.8)
Postectomization			
Yes	44 (15.4)		
No	242 (84.6)		
Phimosis			
Yes	01 (0.3)		
No	285 (99.7)		
HPV knowledge			
Yes	282 (98.6)		
No	04 (1.4)		
Recent history of STI			
Yes	15 (5.2)		
No	271 (94.8)		

*One minimum wage in Brazil corresponds to approximately U$ 200.00; †Sexual practices involving men that have sex with women (M/W), men that have sex with men (M/M) and men that have sex with both men and women (M/W/M).
T: time point (T0: samples + 1st dose of 4vHPV; T1: samples + 2nd dose of 4vHPV; T2: samples in follow-up visit); STI: sexually transmitted Infection.

HPV33, 39, 44, 45, 56, 61, 84, 91, 18, 31, 35, 53, 58, 64, 67, 70, 72, 81), occurring either as single or multiple infections (**Table 3**).

Vaccine genotypes were detected in 35 out of 42 HPV-positive samples (83.4%) at T0, coinciding with the first dose of vaccination. Six months later, at T1, 17 out of 19 samples (89.5%) harbored genotypes contained in the 4vHPV vaccine.

After a two-year follow-up (T2), six students exhibited HPV vaccine genotypes 6 and 11, with three displaying HPV11, one of whom had a recent infection, while the other two had persistent infections spanning the entire study duration. HPV6 was also detected in three out of six positive samples, with two students experiencing recent infections detected after completing the vaccine regimen.

Multivariate analysis

A multivariate analysis explored demographic, social, and behavioral factors in relation to HPV infection and high-risk (HR) HPV infection across the three study visits. Most variables showed no statistically significant correlation with HPV infection or HR-HPV infection, except for the specific instances:

In T0, penile HPV infection significantly correlated with students from Health Sciences graduation courses, as well as high-risk infections (HR HPV) ($p<0.001$).

In T1, the age of sexual debut ($p=0.016$) and the variable concerning sexual practices (men who had sex with men) ($p=0.046$) were significantly associated with both HPV and HR-HPV oral infections, but not with penile infections. Although the univariate model initially identified a greater number of sexual partners as a predictor of HPV and HR-HPV infections, it lost statistical significance in the multivariate analysis ($p>0.05$).

During the T2 visit, involving 41 students one year after completing vaccination and sample collection, no significant correlations were identified between variables and HPV infection.

Factors such as family income, condom use, tobacco smoking, and postectomy were not independently associated with HPV or HR-HPV. Importantly, nearly all students possessed prior knowledge of HPV infection, disease, and prevention (98.6%) (**Table 4**).

Using the MY09/11 generic PCR, HPV DNA was detected in 42 out of 286 students at T0 (14.7%). At T1, 19 out of 218 students (8.7%) tested positive for HPV in oral and/or genital tracts. After a two-year follow-up (T2), six out of 41 students (14.6%) presented HPV DNA (**Table 2**).

DISCUSSION

HPV vaccination programs have demonstrated effectiveness in reducing the incidence of HPV infection, anogenital warts, and precancerous lesions in the female population across various countries, including Australia, Brazil, Denmark, the United States of America, New Zealand, the Czech Republic, and Sweden, as analyzed by ICO/IARC[11] and Ribeiro et al.[12]. Notably, countries that introduced the vaccine earlier, such as Australia, achieved significant reductions in genital warts, particularly in women under 21 years old. However, it is essential to acknowledge that follow-up studies on vaccinated male populations remain scarce. Recently, Lieblong et al.[3] emphasized

Table 3. Human papilloma virus genotypes profile in oral and penile samples, alone and/or in mixed infections along the two-year follow-up study by using microarray (Greiner-Bio, Germany).

HPV	Visits to the STD clinic		
genotypes	T0	T1	T2
06	04	02	03
11	29	12	03
16	01	03	
18	01		
31	01		
33		02	
35	01		
39	02		
42	02	02	
44	01	01	
45	02		
51	03	01	
52	02	02	
53	01		
54	01	02	
56	02		
58	01		
59	02	01	
61		02	
62	01	02	
64	01		
66	03	02	01
67	01		
70	01		
72	01		
73	02	01	
81	02		
82	03	01	
84	01	01	
90		03	
91		01	01

Classification of HPV genotypes[17]: Carcinogenics: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59; possibly carcinogenics: 53, 66, 67, 73, 82; and benign: 06, 11, 42, 44, 54, 61, 62, 64, 72, 81, 84, 90, 91.
STD: sexually transmitted disease; HPV: human papilloma virus; T: time point (T0: samples + 1st dose 4vHPV; T1: samples + 2nd dose 4vHPV; T2: samples in follow-up visit).

Table 2. Prevalence of human papilloma virus deoxyribonucleic acid in oral and penile samples along the two-year follow-up study by using generic polymerase chain reaction.

Visits	Students	Samples			Prevalence
Time points	n	Penile n (%)	Oral n (%)	Oral + Penile n (%)	Total n (%)
T0	286	19 (6.6)	21 (7.3)	2 (0.7)	42 (14.7)
T1	218	9 (4.1)	9 (4.1)	1 (0.5)	19 (8.7)
T2	41	2 (4.9)	3 (7.3)	1 (2.4)	6 (14.6)

T0: samples + 1st dose of 4vHPV; T1: samples + 2nd dose of 4vHPV; T2: samples in follow-up visit.

Table 4. Multivariate analysis showing statistical relevant correlations between HPV infection in oral, genital and in both sites and sociodemographic variables.

Variables	HPV (-) oral	HPV (+) oral	HPV (-) genital	HPV (+) genital	Pearson's test (p-value)	Monte Carlo Model (p-value)
Sexual behavior						
M/M*	34	05	-	-	0.042	0.046
M/W†	137	04	-	-		
Graduation course						
Health sciences	-	-	102	20	<0.001	
Others			135	5		
Sexual debut						
≤18	186	-	-	-	0.016	0.138*
>18	-	9	-	-		

*Men who have sex with men; †Men who have sex with women.

the crucial role that prophylactic vaccination in males can play in reducing HPV-related morbidity in both sexes.

This study observed an exceedingly homogeneous group of 286 male students, aged between 18 and 20, in good health, with no history of autoimmune diseases, cancer, or human immunodeficiency virus (HIV) treatment. A significant portion (over 37%) of the participants had a family income exceeding six minimum wages, indicating favorable economic conditions (**Table 1**). Importantly, nearly 80% of volunteers received both doses of the vaccine. This is particularly noteworthy since in developed countries with high vaccination coverage (ranging from 80 to 90%), the most substantial impacts could be observed[13].

However, it is essential to recognize that, as reported by Dilley et al.[14], many countries implementing HPV prophylactic vaccines face low coverage rates, often attributed to misinformation and hesitancy. Brazil faces a similar challenge, with data from the Brazilian Ministry of Health indicating vaccine coverage as low as 55.4% in girls and 36.4% in boys[15]. Barriers to vaccination are multifaceted, including cost, healthcare service infrastructure, and social stigma. Young males tend to have less knowledge of HPV, underestimate the risks associated with sexual behavior, and have limited access to information about HPV and its associated diseases. These factors are significant impediments to successful vaccination efforts. Additionally, cultural, religious, and patriarchal gender values further exacerbate these challenges[16].

In several countries, the spread of fake news, particularly after the initial reports of severe adverse effects, has compromised the expected outcomes in HPV prevention[14]. However, after 15 years of vaccination, it is interesting to note that only minor adverse effects were detected, with just four students reporting fever, headache, and pain after the first dose.

In this study, HPV prevalence rates remained low both before and after the vaccination scheme (T0: 14.7%, T1: 8.7%). Even after vaccination, the prevalence remained similar at T2 (14.6%), with no statistically significant differences recorded. These results are consistent with global prevalence rates reported by the World Health Organization (WHO), as documented by Bruni et al.[1], which indicate prevalence rates of approximately 20% in healthy young men worldwide.

Genotyping using microarray technology revealed 13 low-risk and 13 high-risk HPV genotypes, along with three possible carcinogenic types (**Table 3**)[17]. HPV11 emerged as the most prevalent type throughout the study (T0: 70.7%, T1: 63.2%, T2: 50%). HPV6, classified as low-risk, was the second most frequent type detected (T0: 9.8%, T1: 10.5%). Notably, in T2, HPV6 reached the same prevalence rate as HPV11 (50%).

Vaccine genotypes were detected in a significant proportion of samples at T0 (85.4%), T1 (89.5%), and T2 (100%). It is worth highlighting the low prevalence rate found for HPV16 and HPV18, both of which are types included in the 4vHPV vaccine. Furthermore, no cases of HPV16 or HPV18 were reported after the two-year follow-up period. Given that Brazil has implemented HPV vaccination for young boys (ages 9 to 15), it is crucial to establish HPV genotype surveillance across different regions of the country, as previously suggested by Drolet et al.[13]. This is particularly relevant to monitor potential shifts in genotypes due to selective pressure or a potential decline in vaccine efficacy.

Interestingly, all students at T2 had at least one body site with a vaccine-type HPV infection. Among samples containing HPV11 DNA, only one represented a new infection acquired after the complete vaccine regimen. The other two cases were persistent HPV11 infections in the oral tract. Gillison et al.[18] proposed that HPV vaccination could contribute to the prevention of oral diseases, including cancer. However, the results of this study revealed vaccine types in previously HPV-negative students, even after completing the vaccination regimen. Therefore, further research is needed to evaluate this effect and its potential utility as a public health tool.

Among HPV6-infected patients, two recently acquired infections were identified, while one was characterized as a persistent infection in the genital tract. Although benign HPV types are rarely associated with malignancy, adverse outcomes have been documented[19,20]. Since vaccine types were persistently found in vaccinated students, it is plausible, as suggested by other studies[21], that there was no therapeutic effect associated with vaccination.

When samples were analyzed by the site of infection, it was noted that oral tract samples exhibited a lower prevalence of HPV compared to the study by Morán-Torres et al.[22]. These rates remained stable throughout the study (T0: 6.6%, T1: 4.1%, T2: 4.9%), with all samples exclusively infected by HPV11. Typically, such infections are transient, especially in a young population, as reported by D'Souza et al.[23]

Oral HPV infection, also considered an STI, is more common in men than in women and is often asymptomatic[24]. However, few

investigations have focused on its prevention. Despite its traditional association with factors like tobacco and alcohol use, poor oral hygiene, and specific genetic predispositions, the incidence of HPV-associated oropharyngeal cancer appears to be increasing[18,25]. Nevertheless, such findings are not consistently observed, with prevalence rates ranging from 1.3 to 9.2% worldwide, with HPV16 being the most prevalent high-risk type[26].

Several studies have linked oral HPV infection, particularly HR-HPV, to changes in sexual behavior, such as an earlier age of first oral sex and a higher lifetime number of sexual partners, particularly among the younger population[3,23]. The results of this study revealed that sexual practices were the only variable significantly associated with HPV infection in the oral tract, with statistical significance p=0.001 and p=0.046, respectively, indicating a higher likelihood of infection in men who have sex with men. While sexual debut was initially associated with oral infection (p=0.016), the significance was lost after adjustment using the multivariate model (p=0.138) (**Table 4**).

Only penile samples exhibited HR-HPVs with a higher prevalence associated with an increasing number of sexual partners. However, its significance disappeared after the multivariate model, consistent with the studies by Vardas et al.[27] and Rocha et al.[28] Unexpectedly, students from Health Sciences had more HPV infections and more HR-HPVs compared to students in other academic programs, with statistically significant differences (p=0.001). This suggests that, despite having more information about STI, individuals in health sciences programs may not necessarily engage in safer sexual practices. Batista et al.[29] reported similar findings, suggesting that knowledge of HPV may not translate into the adoption of preventive measures.

While previous studies have linked HPV infections to factors such as family income and education level[30,31], these factors were relatively homogeneous in the population under study. Continuous condom use was infrequently reported, with less than half of the students using this resource. In South America, including Brazil, condom usage rates hover around 20%, making it an inefficient public health measure with low population adherence[5]. Circumcision, a well-established protective factor against HPV infection and related diseases[32], was evaluated, but the practice is uncommon in Brazil, and statistical analysis did not show a protective effect (p>0.05). Phimosis, which has also been consistently associated with malignancies of the male genital tract[2], was reported in only one case, limiting further analysis.

The study also investigated the students' history of STD once some authors suggested a potential link between other STDs and increased susceptibility to HPV invasion and reduced local immunity[33]. However, due to the small sample size, this variable could not be adequately evaluated.

The primary limitation of this study was the substantial loss of follow-up, particularly during the last follow-up visit, due to the onset of the COVID-19 pandemic. Consequently, variables could not be rigorously assessed through statistical methods. Additionally, there were technical and sample limitations. While well-established protocols exist for screening, diagnosing, and treating genital diseases in women, there is a notable lack of equivalent protocols for men and oral diseases in the general population, which may have influenced the results[34].

Nevertheless, the findings of this study offer promises regarding the population-level effects of HPV vaccination programs. Vaccination has been shown to reduce the circulation of oncogenic genotypes in Brazilian women living with HIV, as reported by Kury et al.[34] and reviewed by Drolet et al.[35]. The nonavalent Gardasil® vaccine, targeting more than seven HR-HPV types, has the potential to further enhance disease prevention[36].

The main challenge now is to increase population adherence, particularly considering the WHO call to action, with the goal of achieving high coverage rates and eliminating cervical cancer as a public health concern by 2030[5]. Furthermore, these results underscore the importance of continued genetic monitoring of HPV to identify any signals of potential waning vaccine efficacy or genotype replacement.

Strengths

This study adopted a prospective design, with a longitudinal approach, providing a dynamic view of HPV changes over time. Besides, literature data evaluating prospectively the impact of HPV vaccination on the male population are scarce.

Limitations

The study experienced a significant loss in follow-up, particularly during the last visit, attributed to the emergence of the COVID-19 pandemic, which may have limited the complete evaluation of variables.

CONCLUSION

This study demonstrated no significant vaccine adverse effects, and observed genetic shifts, including the reduction of oncogenic HPV16 and 18 strains. HPV11 persisted in the oral cavity post-vaccination, and we noted persistent HPV6 and 11 infections in three students. While promising, further research is needed to address limitations, emphasizing the ongoing relevance of HPV research in men.

Approval by the Human Research Ethics Committee

Universidade Federal Fluminense, Faculty of Medicine, under 1.788.830.

Participation of each author

KCS: Conceptualization, Investigation, Methodology, Writing – original draft. WMR: Data curation, Formal analysis, Methodology, Software, Writing – review & editing. MSP: Investigation, Methodology. HBA: Resources. TDG: Resources. CMHK: Resources. MRLP: Conceptualization, Project administration, Resources. SMBC: Conceptualization, Formal analysis, Funding acquisition, Methodology, Project administration, Supervision.

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Conflict of interest

The authors declare no conflicts of interest.

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