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Editorial

Strategic Actions Agenda for Reducing Congenital Syphilis in Brazil: a multi-party, shared initiative

Syphilis is a curable and systemic sexually transmitted infection (STI) caused by *Treponema pallidum* bacteria. When left untreated, it progresses into different clinical manifestations that may last many years. Early syphilis includes the primary, secondary, and latent stages, while late syphilis comprises the late-latent and tertiary stages. Transmission via sexual intercourse is more common in the early stages and gradually decreases over time. It remains, however, transmittable from mother to fetus, most often within the uterus, potentially leading to abortion, preterm delivery, congenital manifestations, or even newborn death[1]. Having one STI increases the risk of acquiring HIV by two or three times.[2].

Syphilis still represents a challenge for public health, as the increasing number of infections both nationally and internationally is clear. According to the World Health Organization (WHO), more than a million cases of STIs are estimated per year. In 2012, it was found that 357 million new cases of curable STIs (gonorrhea, chlamydia, syphilis, and trichomoniasis) occured among adolescents and adults aged between 15 and 49 years, including 5.6 million cases of syphilis. As for syphilis in pregnancy, it is estimated that 143,000 fetal deaths, 62,000 neonatal deaths, 44,000 premature infants, and 102,000 children are infected worldwide due to lack of prenatal diagnosis and treatment[3].

In Brazil, from 2005 to June 2016, 169,546 cases of syphilis in pregnant women were reported. In 2015, the detection rate was of 11.2 cases of syphilis during pregnancy for every 1,000 live births (for a total of 33,365 cases)[4].

As to congenital syphilis (affecting infants under the age of one), from 1998 to June 2016, 142,961 cases were reported nationally according to the National Disease Notification Information System ("Sistema de Informação de Agravos de Notificação"). In 2015 alone a total of 19,228 cases were reported, at an incidence rate of 6.5 per 1,000 live births. A 20.91% increase in the total number of new diagnostics was reported between 2014 and 2015[4].

Among the policies adopted by the Ministry of Health (MOH), the "Rede Cegonha" (lit. "Stork Network") program was implemented within the National Unified Health System ("Sistema Único de Saúde" - SUS), through Decree no. 1,459 of June 24, 2011. This program aims to ensure the right to humanized care in reproductive planning, prenatal, delivery, and postnatal stages and infant care in health services, with a special focus on prevention, early diagnosis, and timely, adequate treatment of pregnant women (and their sexual partners) infected with syphilis during basic care[5].

As for syphilis diagnosis, the MOH's Department for Prevention, Surveillance and Control of Sexually Transmitted Infections, HIV/Aids and Viral Hepatitis ("Departamento de Prevenção, Vigilância e Controle das Infecções Sexualmente Transmissíveis, do HIV/Aids e das Hepatites Virais" - DIAHV), a branch of the Health Surveillance Secretariat ("Secretaria de Vigilância em Saúde" - SVS), managed to increase the distribution of rapid tests from 1,126.235 in 2012 to 6,169,145 tests in 2015[6]. Despite this increase, in 2015, 32.8% of pregnant women with syphilis received late diagnoses, that is, during their last three months of pregnancy[4]. Also important is the distance learning initiative "Telelab", a continued education program offering free diagnostics courses available at Http://telelab.aids.gov.br/. In 2016, the Technical Manual for Syphilis Diagnosis was published. It aims at increasing diagnostic possibilities as well as at guiding and supporting health professionals in managing and dealing with the disease[7].

In 2015, the National Commission for the Incorporation of Technologies in the Unified Health System ("Comissão Nacional de Incorporação de Tecnologias no SUS" - Conitec) drafted a report confirming, based on scientific evidence, that benzathine penicillin is the only option for safe and effective treatment for preventing congenital syphilis during pregnancy. As to penicillin's safety, no studies evaluating its use among pregnant women have shown occurrences of anaphylactic reactions. The risk of such reactions due to penicillin among the general population is very low[8].

The WHO has recognized the global shortage of benzathine penicillin[3]. In Brazil, the shortage began in June 2014 and, since then, the MOH has sought solutions with help from other sectors to normalize its supply. Although drug purchase is an attribution of states and municipalities, in 2016 the MoH conducted an emergency purchase of 2.7 million bottles of benzathine penicillin 1,200.000 IU and distributed among states, recommending that pregnant women and their sexual partners to be prioritized.

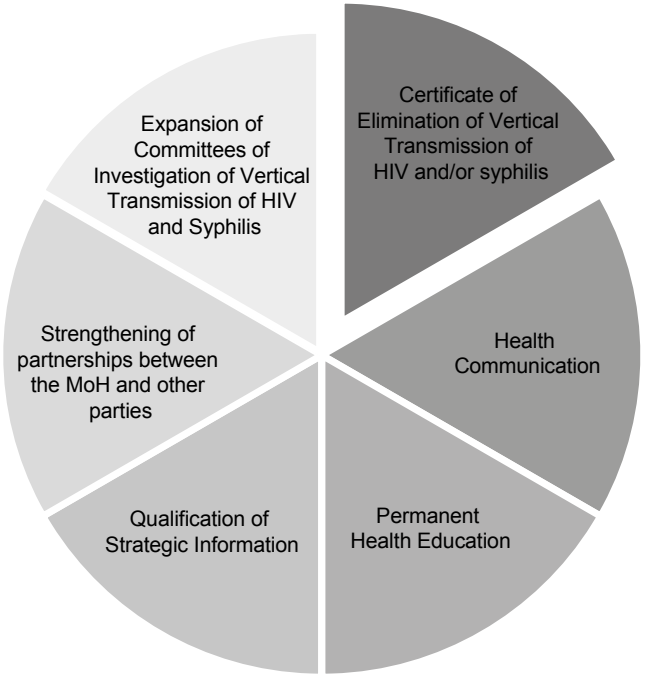
In this context, in October 2016 the Strategic Actions Agenda for Reducing Congenital Syphilis in Brazil was enacted as a result of a collective work, after internal articulation and strategical meetings with the National Council of State Health Secretaries ("Conselho Nacional de Secretários de Saúde"), the National Council of Municipal Health Secretaries ("Conselho Nacional de Secretários Municipais de Saúde"), non-governmental organizations, associations, and health professionals associations[9]. On this occasion, the Letter of Commitment was signed by all parties involved.

The implementation of these actions is coordinated dynamically by the DIAHV, and is thus subject to changes, additions, and updates during its implementation period – from October 15, 2016 until October 21, 2017. It is indispensable to monitor and evaluate its actions in order to enable effective, stable, and improving public policies focusing on the preventing congenital syphilis.

This Actions Agenda has as its main goal the reduction of congenital syphilis in Brazil, while also including specific goals such as early care of pregnant women and partners during the prenatal period[10]; increasing access to timely and adequate diagnoses and treatment; increasing awareness of health professionals on benzathine penicillin usage in primary care; disseminating health-related information among managers, professionals, and members of the community; the qualification of epidemiological surveillance; creating the committees for Vertical Transmission of HIV and Syphilis Investigation; and structuring the validation process for the national Certificate of Elimination of Vertical Transmission of HIV and/or syphilis in cities across the country.

This Agenda has six main sectors, under each of which strategic actions were devised for reducing congenital syphilis, as shown in **Figure 1**.

The MOH thus establishes public policies and supports local initiatives, highlighting that it is crucial to share responsibilities and join forces, effectively involving (federal, state, and municipal-level) managers, health professionals, members of communities, non-governmental organizations, and professional associations with the purpose of raising awareness of this disease throughout health practices as well as intersectoral actions; to implement preventive, diagnosis, care, treatment, and monitoring-related actions aimed at reducing cases of congenital syphilis in the country.



Source: DIAHV/SVS/MS, 2016
Department of Sexually Transmitted Infections, HIV/Aids and Viral Hepatitis of theHealth Surveillance Department of the Ministry of Health

Figure 1 - Actions to combat syphilis.

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Analysis of cytopathologic examination and hybrid capture in women receiving care in basic health units

Análise do exame citopatológico e captura híbrida em mulheres atendidas em unidades básicas de saúde

Deise Jaqueline Ströher[1], Thaís Delgado Brandolt Aramburu[2], Vinícius Tejada Nunes[3], Jacqueline da Costa Escobar Piccoli[4], Vanusa Manfredini[5]

ABSTRACT

Introduction: Cervical cancer is one of the most frequent cancers among Brazilian women and its relationship with the human papillomavirus (HPV) is well established. **Objective:** To analyze the presence of DNA/HPV using Hybrid Capture method for women in the city of Uruguaiana (RS). **Methods:** During the period of January to December 2015, 51 cervicovaginal samples were collected from patients who sought care at Basic Health Units in the city. After the collection, conventional and liquid-based cytological analysis was performed. **Results:** The results of the study indicate the prevalence of genital HPV infection in 5.9% of the samples; low-risk DNA/HPV was detected in 3.9% of patients of reproductive age (PIR); and 2.0% of PIR presented high-risk DNA/HPV. By stratifying the prevalence of HPV in age, we found positivity between 16 and 31 years. **Conclusion:** Conventional cytology is often inconclusive and, in such cases, using molecular biology methods that detect the DNA/HPV presence would be very useful.

Keywords: Neoplasm; Papillomaviridae; ; Molecular biology.

RESUMO

Introdução: O câncer de colo de útero é um dos mais frequentes entre as mulheres brasileiras e a sua relação com o Papilomavírus Humano (HPV) é bem estabelecida. **Objetivo:** Analisar a presença de DNA/HPV por meio do método de captura híbrida em mulheres no município de Uruguaiana (RS). **Métodos:** No período compreendido entre janeiro e dezembro de 2015, foram coletadas 51 amostras cervicovaginais de pacientes que buscaram atendimento nas Unidades Básicas de Saúde do município. Após a coleta, foi realizada a análise citológica convencional e em base líquida. **Resultados:** Os resultados encontrados no estudo indicam a prevalência de infecção genital por HPV em 5,9% das amostras analisadas, sendo DNA/HPV de baixo risco em 3,9% e DNA/HPV de alto risco em 2,0% das infecções. Ao estratificar a prevalência de HPV por faixa etária, observou-se positividade entre 16 e 31 anos. **Conclusão:** A citologia convencional pode ser, muitas vezes, inconclusiva e, nesses casos, utilizar uma metodologia de biologia molecular que detecte a presença do DNA/HPV seria muito útil.

Palavras-chave: Neoplasia; Papiloma Vírus Humano; Biologia molecular,

INTRODUCTION

The cancer of the uterine cervix, also known as cervical cancer (CC), is caused by persistent infection of certain types of human papillomavirus (HPV). CC is the third most frequent tumor in the female population and the fourth leading cause of death of women by cancer in Brazil. According to the National Cancer Institute, the estimate for 2014 was 15,590 new CC cases[1].

It is estimated that 90% of women who developed CC were exposed to HPV[2]. HPVs involved in infections of the anogenital region can be classified according to their capacity to generate malignant neoplasias in the following categories: HPV of low oncogenic risk; HPV of high oncogenic risk; and genotypes of probable high risk[3].

Currently, more than 100 different types of HPV are known and about 20 of them are tropism for the squamous epithelium of the anogenital region (colon, vulva, perineum, perianal, and anal region)[4]. The World Health Organization (WHO), in partnership with the International Agency for Research on Cancer (IARC), identified types 16 and 18 as the main etiologic agents of CC. It is estimated that 75–80% of the female population will be infected before age of 50 years[5].

The CC prevention examination was implanted in the public network in Brazil in 1999 and aims at the early detection of neoplasia and its precursor lesions by periodic cytological analysis of the smear obtained by using the Papanicolaou technique. Statistical data show that effective screening can reduce the incidence of invasive forms of CC by up to 91%. However, the incidence of the disease remains one of the highest among malignant neoplasms occurring among Brazilian women[6].

Although cytopathological examination is the most widely used method for the screening of CC precursor lesions, its vulnerability to collection errors, slide preparation, and subjectivity in interpreting results may compromise its sensitivity and specificity. New techniques have been developed with the aim of improving the accuracy of the cytopathological examination, namely cytology in liquid medium[7].

Conventional cytology does not detect the HPV virus itself; it only detects the cellular changes caused by the virus. It presents great specificity, but it has limited sensitivity due to the variation

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in the interpretation of the results. However, when well performed, this test is still of fundamental importance in the screening of CC and its precursor lesions[8].

Hybrid capture is a signal amplification method that uses labeled ribonucleic acid (RNA) probes for hybridization to HPV targeting deoxyribonucleic acid (DNA). The second generation of this technique, the hybrid capture version II, is being used in diagnostic laboratories in addition to cytology. This method detects viral DNA in cervicovaginal materials by means of RNA probes capable of recognizing low-risk and high-risk HPV sequences. The sensitivity of this technique is comparable to that of the polymerase chain reaction (PCR), in particular to detect high-grade lesions. This method is useful for determining viral load[8].

OBJECTIVE

To analyze the presence of DNA/HPV by means of the hybrid capture method in women receiving care at the Basic Health Units of the city of Uruguaiana (RS).

METHODS

Population Studied

The study included women of all ages and of various ethnic groups living in the city of Uruguaiana who were visiting Basic Health Units for routine gynecological examinations. The inclusion criterion was sexually active women of any age group living in the city of Uruguaiana (RS). The exclusion criteria included: women who were hysterectomized, undergoing gestational, or menstrual period, or with mental deficit that would hinder the understanding and completion of the questionnaire.

A total of 51 cervicovaginal samples were collected between January and December 2015. Cervical cytology was performed in two ways: the conventional method and cytology on a liquid basis.

The participation of women in the research was made through prior clarification and signing of the Informed Consent Form (ICF), according to resolution CN 196/96-MS and in accordance with the principles contained in the Declaration of Helsinki of the World Medical Association (1964, Reformulated in 1975, 1983, 1989, 1996 and 2000). All the objectives of this study and methodology are contained in a main study called "Elderly Women Project on the Uruguay River: Epidemiological Profile, Citomorphological of the Elderly of Uruguaiana, RS", which was evaluated and approved by the Ethics and Research Committee of the *Universidade Federal do Pampa* (CEP-Unipampa), of Rio Grande do Sul, under number 869.813.

CYTOPATHOLOGICAL EXAM

The conventional cytological smear was composed of two samples: scraped ectocervical and endocervical that were collected with Ayre spatula and endocervical brush. After collection, the material was immediately fixed with 95% alcohol. The cervicovaginal smears were sent to the Central Laboratory of Public Health (LACEN - RS).

Diagnoses were performed using the nomenclature based on the Bethesda 2001 system and the Brazilian Society of Cytopathology (SBC). The cytopathological changes were classified as atypicals of indeterminate significance of squamous and glandular cells (ASC-US / AGUS); low-grade intraepithelial lesions (LIEBG), which include CIN I; high-grade intraepithelial lesions (LIEAG), which include CIN II and CIN III; and cancer for squamous invasive carcinoma.

Tests for DNA/HPV

The hybrid capture method (DM Molecular), processed by Digene technology – a molecular hybridisation technique associated with monoclonal antibodies, allows the detection of 1 pg/mL of HPV DNA, equivalent to 0.1 copy of virus per cell. It is considered positive when RLU/PCA ratios for group A viruses (6, 11, 42, 43 and 44) and / or RLU / PCB for group B viruses (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) are equal to or greater than 1.

Statistical Analysis

For the analysis of the results, the women were classified according to the age range in patients of reproductive age (PRA) and patients of non-reproductive age (PNRA). The data were plotted in the program Microsoft Office Excel and later analyzed using the program GraphPad Prisma, expressed in percentage.

RESULTS

The mean age of the women assisted during the study period was 42.7 years, with ages ranging from 16 to 86 years. **Table 1** shows the demographic, behavioral, and reproductive variables of women receiving care at the Basic Health Units of the municipality of Uruguaiana and classifies reproductive and non-reproductive age. The table shows that 66.7% of the study participants are of PRA, in the age range of 16–45 years. Patients classified as PNRA, in the age range of 46–86 years, corresponded to 33.4% of the women participants.

Regarding the marital status of the study participants, the majority of PRAs were single (35.3%) or married (41.2%). On the other hand, in the PNRA group, 41.2% stated that they did not have fixed partners, were widowed or lived without partners at that time.

Regarding the educational level, there was a higher frequency of women (35.3%) who reported having completed elementary education in the PRA group. Among the PNRAs, it is noteworthy that 11.7% are illiterate, and 11.7% have completed elementary education.

It was also found that 70.6% of the PRAs started sexual activity at an age less than or equal to 16 years and 29.4% declared that they started sexual activity after 16 years of age. In contrast, 70.6% of the PNRAs reported having had their first sexual intercourse before reaching the age of 16.

When asked about the use of contraceptives, 55.9% of the PRAs and 82.3% of the PNRAs answered that they were not using oral contraceptives. Regarding the number of partners, 91.2% of the PRAs reported having had one to two sexual partners in the last

year. In the PNRA group, 64.7% said they had not had any sexual partners in the past year.

According to the distribution of the results of the cytopathological examination shown in **Figure 1**, 47 (92%) patients presented the cytology within the limits of normality, while 4 (8%) patients presented cell alteration.

In all cases, the cytological alterations were LIEBGs. Regarding the age group, the altered results were distributed in PRA, between 26 and 32 years.

Table 2 shows the results of the viral load by the hybrid capture test. The prevalence of genital HPV infection in the analyzed samples was 5.9%, all in PRA, and in 3.9% of infections, low-risk DNA/HPV and 2.0% high-risk DNA/HPV. When stratifying the prevalence of HPV in the age group, positivity was observed between 16 and 31 years. Positive results were not observed for the PNRA test.

DISCUSSION

Studies in Latin America have detected an association between CC risk and the following sexual habits: number of partners, early sexual activity, and use of oral contraceptives, among others[9]. In the present study on behavioral factors, 91.2% of the PRAs reported having had one to two sexual partners in the year and 70.6% had started sexual activities at an age less than or equal to 16 years. On the other hand, in our study most of the PRAs (55.9%) were not using oral contraceptives.

Table 1 – Demographic, behavioral and reproductive variables of women assisted at the Basic Health Units of the municipality of Uruguaiana, classified in reproductive and non-reproductive age.

VARIABLE	PRA (16-45 years)	PNRA (46-86 years)
Age	34 (66.7%)	17 (33.4%)
Marital status		
Single	12 (35.3%)	6 (35.3%)
Married	14 (41.2%)	4 (23.5%)
Others	8 (23.5%)	7 (41.2%)
Educational level		
Illiterate	0	2 (11.7%)
Incomplete elementary	8 (23.5%)	13 (76.5%)
Complete elementary	12 (35.3%)	2 (11.7%)
Incomplete high-school	7 (20.6%)	0
Complete high-school	7 (20.6%)	0
Higher education	0	0
Onset of sexual activities		
≤16 years	24 (70.6%)	12 (70.6%)
>16 years	10 (29.4%)	5 (29.4%)
Use of contraception		
Yes	15 (44.1%)	3 (17.7%)
No	19 (55.9%)	14 (82.3%)
Number of sexual partners during the year		
None	0	11 (64.7%)
1 to 2	31 (91.2%)	5 (29.4%)
More than 2	**3 (8.8%)**	**1 (5.9%)**

PRA = patients in reproductive age
PNRA = patients in non-reproductive age

Roteli-Martins et al.[10] indicate that 76.4% of patients reported onset of sexual activity between 14 and 20 years. This scenario poses even greater risks for HPV susceptibility when the onset of sexual practice is devoid of condoms. The practice of younger women's sexual activity is greater than that of older women. This fact is confirmed in our study, which indicated that 64.7% of the PRNAs say they had not had sexual partners in the past year. It can be said that the sexuality of the elderly woman is surrounded by prejudices and failures of the information about the aging process and the changes in sexuality in different age groups, especially for older age groups[11].

One of the risk factors for HPV infection is limited level of education and low socioeconomic status. However, younger women still report a higher level of education[12]. Thus, it can be observed that the PRAs have a better level of schooling, since 35.3% of the women affirm that they have finished elementary school, unlike the PNRAs that affirm that they have not finished elementary school.

A higher level of education contributes to the reduction of the number of cases of the disease, since school attendance allow

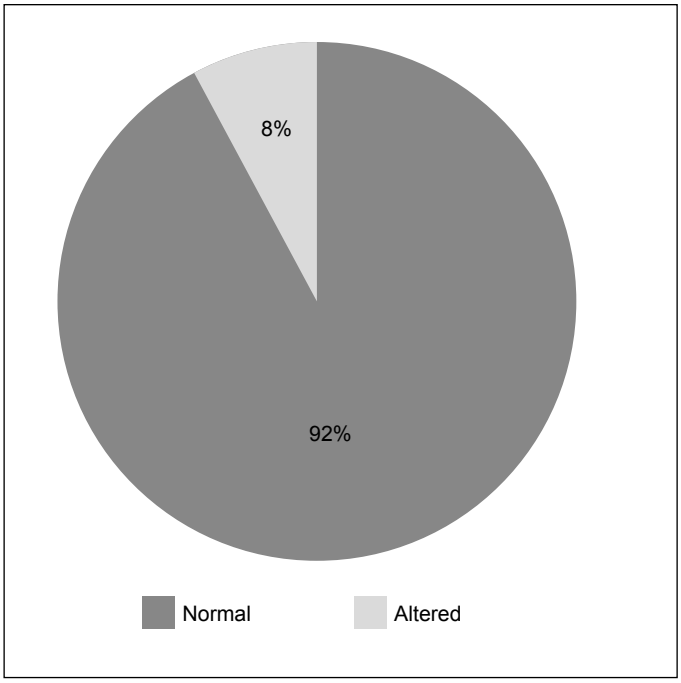

Figure 1 – Percentage of normal and altered cytopathologic reports of patients who underwent gynecological cytopathology in Basic Health Units of the city of Uruguaiana.

Table 2 – Results obtained in the cytology on liquid basis in relation to viral load by the hybrid capture test for oncogenic and non-oncogenic DNA/HPV.

DNA/HPV	n	Viral load	% of patients
RLU/PCA	1	1.02 pg/mL	3.9%
≥ 1pg/mL	1	1.00 pg/mL	
Non-oncogenic			
RLU/PCB			
≥ 1pg/mL	1	1.01 pg/mL	2.0%
Oncogenic			
Total	**3**		**5.9%**

students to reach a level of knowledge capable of influencing preventive measures when one has a better understanding about the disease[11]. However, in the present study, the highest prevalence of cases of HPV infection is still found in PRAs that have a higher level of schooling when compared to PNRAs.

Regarding the marital status of the women participating in the study, one can see a higher percentage of PRAs who declare themselves married or living with a partner. In this view, a study by Rama et al.[13] indicate that sexual activity of women with a stable union predisposes them to the virus. Indeed, it is understood that these women believe to live within a standard of reliability and safety in relation to the their partner and therefore do not recognize the need for STD prevention, which in turn makes them more vulnerable to HPV infection and other sexually transmitted diseases.

The literature indicates that a higher incidence of CC affects women between the ages 40 and 60 years, and that the cancer is not common in women under 20 years of age. However, 70% of cases of CC could be related to HPV, with the highest prevalence of HPV contamination in young women between the ages of 15 and 25, the period of sexual initiation. In this study, the prevalence of genital HPV infection was in the age range of 16–31 years. According to Roteli-Martins et al.[10], adolescents who are sexually active have the highest rates of infection, incidents, and prevalence.

The prevalence of genital HPV infection in the present study was 8.8% for both viral types (oncogenic and non-oncogenic). Data from the literature indicate a general prevalence of cervical infection by HPV ranging from 13.7 to 54.3%. For women with normal cytology, the prevalence of HPV infection in the cervix ranges from 10.4 to 24.5%[14]. The divergence between the literature data and the findings of this study may be related to the sample number and a high number of PNRAs.

Studies show that the highest prevalence of genital HPV infection is found in women under 25 years of age, with a progressive linear decrease after this age and reaching values below 5% after 55 years[13]. The fact that we did not have any positive results from HPV infection in PNRA in our study may be related to changes in sexual habits, which would make women less exposed. On the other hand, it is true that there is an increase in the number of STD/AIDS cases among the elderly.

According to Castle et al.[15], 15% of the women who underwent Pap smears may present a negative cytological result with positive hybrid capture for high-risk HPV. In this study, we observed this profile in 2.9% of the analyzed women.

When we compared the results of the positive hybrid capture DNA/HPV test with the conventional cytology of the study patients, we found that the result of conventional cytology is negative. Results such as these, discordant between cytological and molecular methods, may occur due to the observer's difficulty to interpret the slide, problems in the collection or even because of the high sensitivity of the molecular method.

CC is a highly preventable disease, with preventative measures including early detection and treatment of precursor lesions. Currently, in addition to conventional cytology and DNA/HPV detection several biomarkers are being studied. In this sense the evolution and improvement of new molecular techniques that allow for the evaluation of cellular alterations, and the simultaneous analysis of these markers may contribute to a significant decrease in the number of patients who develop CC.

CONCLUSION

Conventional colpo-cytology may be inconclusive and, in such cases, using a methodology that detects the presence of DNA/HPV would be very useful. Hybrid capture has been widely used in the routine of clinical analysis laboratories because it is faster to obtain the results as opposed to more complex methodologies, such as PCR, for example.

Conflict of Interest

The authors declare no conflict of interest.

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IMPACT OF *IN VITRO* CLINDAMYCIN ON THE COMBINATION OF CLINDAMYCIN AND KETOCONAZOLE ON EXOPOLYMER OF *CANDIDA* SPP BIOFILMS OF UROGENITAL ORIGIN

IMPACTO DE LA CLINDAMICINA IN VITRO EN LA COMBINACIÓN CLINDAMICINA-KETOCONAZOL SOBRE EL EXOPOLÍMERO DE BIOPELÍCULAS DE CANDIDA SPP DE ORIGEN UROGENITAL

Alicia Farinati[1,2], Daniyil Semeshchenko[1], Melina Marqués[1,2], Santalucia Martin[1,2]

ABSTRACT

Introduction: The vaginal mucosa has been widely used for administering antimicrobial agents to treat endogenous infections of the lower genital tract in pregnant and non-pregnant women. *Candida* spp. elaborates biofilms, and its formation is a complex process requiring that fungal cells establish multiple interactions with the medium. Biofilms are surrounded by an exopolymer matrix that can restrict the activity of antibodies, the diffusion of substances, and be associated with antimicrobials, therefore limiting its actions. General antimicrobials and particular anti-mycotic agents can face difficulties to access the cells within the exopolymer matrix. Many formulas used for empirical treatment have improper combinations with limited or null activity on the biofilms. The presence of molecules that cause its inhibition, thus eliminating the exopolymer matrix inducers, or by other mechanism, will allow the specific antimicrobial activity. **Objective:** To show that the activity of clindamycin used in dual formula with ketoconazole works on *Candida albicans* biofilm and on non-*albicans* species of *Candida*. **Methods:** We studied the activity of clindamycin and ketoconazole regarding the adherence and dispersion of biofilms from eight vaginal isolates of *C. albicans* and 7 of non-*albicans Candida*. The isolates were inoculated in three tubes with Sabouraud agar and a glass device to form the biofilm according to a known technique. *Adherence*: Each isolate was incubated for a six-hour period and a combination of clindamycin and ketoconazole from the material of ovules was added and conveniently diluted to one of the tubes of each isolate (62.5/260.4 ug/mL), considering 0 hour. *Dispersion:* The same dilution was added to another tube after 16 hours. The third tube was used as a control without antimicrobials. The reading was carried out with an optical microscope after 24 hours that the clindamycin and ketoconazole combination had been added and colored with crystal violet. They were then evaluated using photographic programs. The activity of clindamycin (62.5 ug/mL) and ketoconazole (260.4 ug/mL) was analyzed alone with a similar technique. We chose vaginal samples from seven patients with vulvovaginal candidiasis and studied them through the cell layer technique. The clindamycin and ketoconazole combination was used for studying the adherence and dispersion. **Results:** *Adherence:* Little influence of clindamycin and ketoconazole was seen in adherence regarding each control. *Dispersion:* The clindamycin and ketoconazole influence was seen in most of the isolates, especially in those of non-*albicans Candida* that showed higher presence of exopolymer matrix.The hyphae were only seen in 1 of 15 isolates of *Candida* spp after the clindamycin and ketoconazole were added at the 16th hour. In biofilms of clinical samples, neither hyphae nor mycotic elements were seen in 5 of 7 compared with the controls. **Conclusion:** According to these results, the use of a clindamycin and ketoconazole combination in biofilms of *Candida* spp results in proper penetration of the antimicrobial agent, which is seen by the biofilm dispersion during 24 hours. Clindamycin does not interfere with the action of ketoconazole, but it would promote its anti-*Candida* activity and would possibly modify surface and EP structures through inhibition of the molecules that facilitate its expression. The *in vivo* model promotes the immunomodulatory activity that *in vitro* models do not. Its combined use in dual formulas would facilitate the antimicrobial activity on *Candida* spp, therefore working as an inhibitor or modifier of the biofilms after dispersion of the exopolymer matrix.

Keywords: clindamycin; ketoconazole; *Candida* spp biofilm; exopolymer; vaginal and cervical infections.

RESUMEN

Introducción: La mucosa vaginal ha sido utilizada largamente para la administración de antimicrobianos destinados al tratamiento de infecciones endógenas del tracto genital inferior (IETGI) en mujeres embarazadas y no embarazadas. *Candida* spp elabora biopelículas (BP) y su formación es un proceso complejo que requiere que las células fúngicas establezcan múltiples interacciones con el medio. Las BP están rodeadas por un exopolímero (EPM) que puede restringir la actividad de anticuerpos, la difusión de sustancias y unirse a los antimicrobianos (AM), limitando su acción. Los antimicrobianos (AM) en general y los antimicóticos en particular (AMC) pueden tener dificultades para llegar a las células dentro del EPS. Muchas de las fórmulas que se emplean para el tratamiento empírico usan combinaciones inapropiadas con limitada o nula actividad sobre las biopelículas (BP). La presencia de moléculas que provoquen su inhibición anulando los inductores del EPM o por otro mecanismo, permitirá la actividad del AM específico. **Objetivo:** demostrar que la actividad de la clindamicina (CLI) en fórmula dual con ketoconazol (KET) actúa sobre BP *Candida albicans* (CA) y especies no *albicans* de *Candida*. (NAC). **Métodos:** estudiamos la actividad de clindamicina-ketoconazol (CK) sobre la adherencia y dispersión de BP de 8 aislamientos vaginales de CA y 7 de CNA. Se inocularon en 3 tubos con caldo Sabouraud y un dispositivo de vidrio para la formación de la BP según técnica ya descrita. *Adherencia:* Se incubaron durante 6 horas y se agregó una combinación de CK proveniente del material de óvulos, diluido convenientemente (62,5/260,4 ug/ml), a uno de los tubos de cada aislamiento tomándose como hora 0. *Dispersión:* esa misma dilución se agregó a otro tubo a las 16 horas. El tercer tubo quedó como testigo sin antimicrobianos. La lectura se efectuó con microscopio óptico a las 24 horas de agregada la combinación CK previa tinción con cristal violeta y se evaluaron con programas fotográficos. Por separado analizamos la actividad de CLI (62,5 ug/ml) y KET (260,4 ug/ml) con técnica similar. Seleccionamos las muestras de 7 pacientes que demostraron candidiasis vulvovaginal (CVV) y las estudiamos con la técnica de capas celulares. Se empleó la combinacion CK para el estudio de la adherencia y dispersión. **Resultados:** *Adherencia* se demostró poca influencia de CK en la adherencia con respecto a cada testigo. *Dispersión:* la influencia de CK se demostró en la mayoría de los aislamientos particularmente en los de CNA que mostraron una mayor presencia de EPM. Las hifas solo se observaron en 1/15 de los aislamientos de *Candida* spp cuando se agregó CK a las 16 horas. En las BP de las muestras clínicas no aparecieron hifas ni otro elemento micótico en 5/7 con respecto a los testigos. **Conclusión:** Según estos resultados el uso de una combinación de CK en BP de *Candida* spp, resulta en una adecuada penetración del AMC demostrada por la dispersión de la BP al cabo de 24 horas. Clindamicina no interfiere con la acción del ketoconazol sino que promovería su actividad anti-candida modificando posiblemente estructuras de superficie y la del EP por inhibición de las moléculas que facilitan la expresión del mismo. *In vivo* promueve la actividad inmunomoduladora que no se puede demostrar con este modelo *in vitro.* Su uso combinado en fórmulas duales facilitaría la actividad del AMC sobre *Candida* spp actuando como inhibidora o modificadora de las BP mediante la dispersión del EPM.

Palavras claves: clindamicina; cetoconazol; biopelícula; exopolímero; infecciones vaginales y cervicales.

INTRODUCTION

The vaginal mucosa has been widely used for administering antimicrobial agents to treat endogenous infections of the lower genital tract in pregnant and non-pregnant women. *Candida* spp. elaborates biofilms (BF), and its formation is a complex process requiring that fungal cells establish multiple interactions with the medium. They participate both in the colonization and in the progression of the disease[1-3]. Biofilms are surrounded by an exopolymer matrix (EP) that can restrict the activity of antibodies, the diffusion of substances and be associated with antimicrobials (AM), limiting its actions, and therefore they are significantly less susceptible to AM agents[4-6]. The access difficulty of general AM and particular anti-mycotic agents (AMC) to cells within the EP is phenotypically different of their corresponding planktonic or suspended cells[7,8]. Many formulas used for the empirical treatmen thave improper combinations with limited or null activity on the BF. The presence of molecules that cause its inhibition, thus eliminating the EP inducers, or by other mechanism, will allow the specific AM activity.

OBJECTIVE

To show that the activity of clindamycin used in combination with ketoconazole (CK) works on *Candida albicans* (CA) BF and on species of non-*albicans Candida* (CNA).

METHODS

We have analyzed the activity of CK regarding the adherence and dispersion of BF from eight vaginal isolates of CA and seven of CNA. The isolates were inoculated in three tubes with Sabouraud agar and a glass device to form the BF according to a known technique[9].

Adherence study: Each isolate was incubated in triplicate for a six-hour period and a combination of CK from the material of ovules was added and conveniently diluted to one of the tubes of each isolate (62.5/260.4 ug/mL), considering 0 hour.

Dispersion study: The same dilution was added to the second tube after 16 hours. The third tube was used as control without AM.

CLI and KET activity: we studied the activity of CLI (62.5 ug/mL) and KET (260.4 ug/mL) separately by using a technique similar to that previously described. The AM was added after the glass device had been incubated at the 6th (adherence) and 16th hours(dispersion).

Study of the CK and BF combination activity of clinical samples: we chose the samples from seven patients showing CVV that were studied by means of the conventional techniques (pH, amine test, microscopy and cultures). We made the BF by using the technique of cell layers[10]. The CK combination was used for the study of adherence and dispersion by applying the same concentrations used for the study regarding BF of CA and CAN. All glass devices with the BF of CA, CAN submitted to the activity of CK, CLI and KET were stained with crystal violet. The devices with cell layers were stained with Gram staining. The reading was carried out with a microscope (1000x) at 24 hours, and they were photographed with a digital camera and analyzed by means of a program for studying the EPM (Optical Imaging Software).

RESULTS

Biofilms of CA and CNA with CK

Adherence: Little influence of CK was seen in adherence regarding each control.

Dispersion: The CK influence was seen in most of the isolates, especially in those of CAN that showed more presence of EPM. The hyphae were only seen in 1 of 15 isolates of *Candida* spp after the CK was added at the 16th hour.

Biofilms of CA and CNA with CLI and KET

The analysis of BF, submitted to CLI activity, only enabled to observe that the EPM had been dispersed, and the BF of CA and CAN were the only ones that remained. In BF with KET addition, the persistence of the EPM was seen, even though there were some cases of certain alteration of the blastospores that indicate imperfect penetration (**Figure 1**).

Biofilms of CVV with CK

In BF of clinical samples, neither hyphae nor mycotic elements were seen in 5 of 7 compared to the controls (**Figures 2** and **3**).

DISCUSSION

The use of antibacterial and anti-mycotic combinations is very popular for local treatment of genital tract infections, whether by using gels, creams, or ovules and suppositories. They are an important option in the prophylaxis and treatment of superficial infections. In addition, they have low incidence of systemic toxicity and lower development of resistance than drugs of parenteral administration. It is known that 65% of human infections are associated with the formation of BF[7]. The genital tract is not different and we have showed, like other authors, that BF are formed in endogenous infections, such as bacterial vaginosis and CVV, and work as a real reservoir, therefore it is difficult to eliminate them and as such, they are usually associated with recurrent infections[7,10,11].

The combinations used do not always comprise the activity on the BF. We have chosen two of those that use different antimicrobials: clindamycin associated with ketoconazole and that including metronidazole, miconazole, gentamicin, neomycin, polymyxin, and centella.

Clindamycin belongs to the group of lincosamides, together with lincomycin. It is a synthetic derivate of lincomycin that was obtained in 1966. Owing to its larger activity, lower absorption through the gastrointestinal pathway and the larger spectrum, the previous one was replaced in the clinical practice.It was first introduced as an anti-staphylococcus. It was

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later seen as a powerful anti-anaerobe. Although the risk of colitis through *Clostridium difficile* has limited its use, this is a useful antibiotic for treating severe infections by anaerobic microorganisms. Lincosamides are composed of an amino acid (methyl proline) and a sugar (pyranose) that are united by an amide. In the clindamycin, the hydroxyl is replaced in the 7th position by a chloride atom.

Even though clindamycin is bacteriostatic, its bactericide action has been seen against some strains of *Staphylococcus*, *Streptococcus*, and *Bacteroides*.

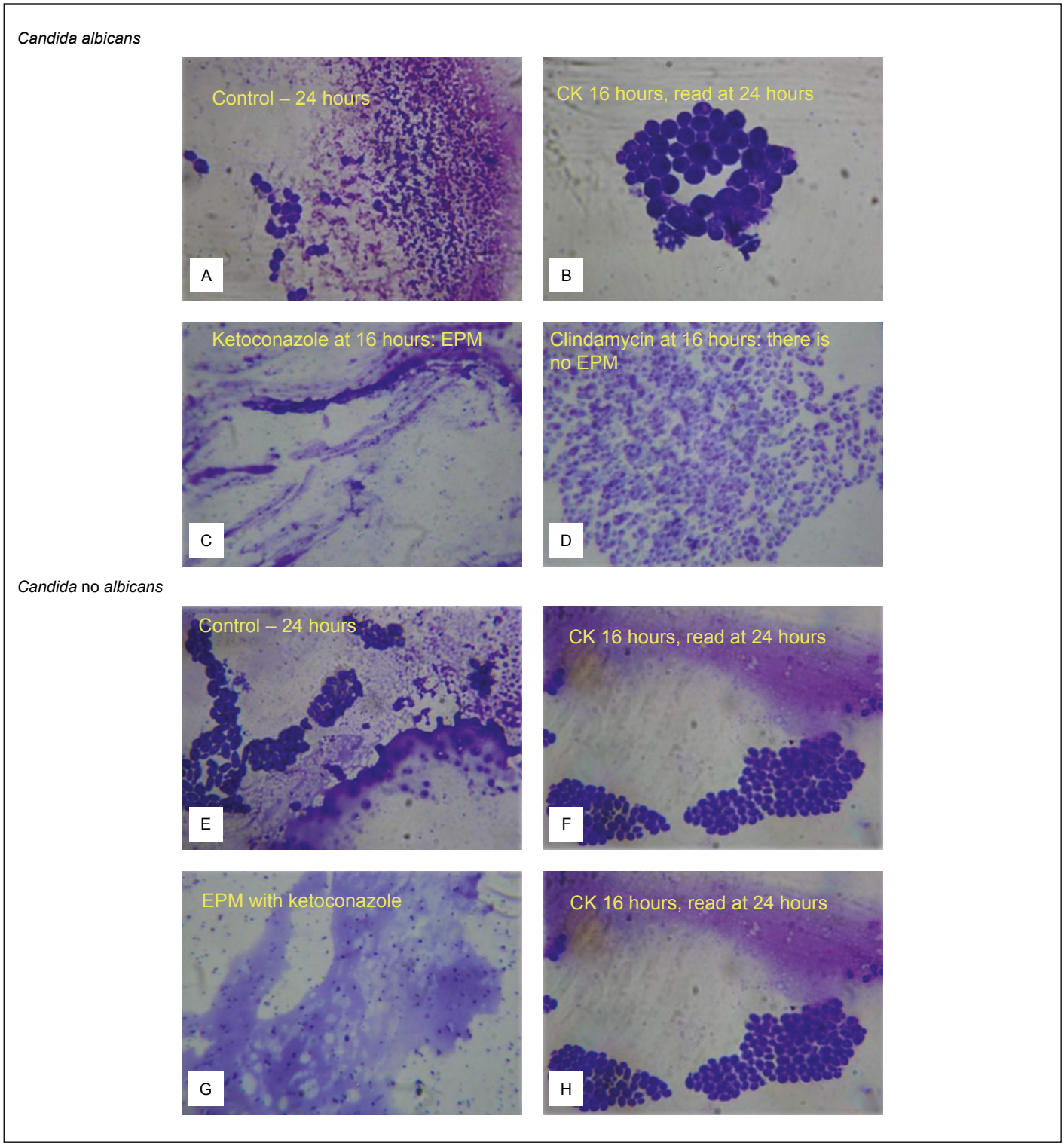

Figure 1 – Biofilms of *Candida albicans* and non *albicans Candida* with the control without antimicrobial exposure, exposure to Clindamycin plus ketoconazole (CK), clindamycin alone and ketoconazole alone.

It also inhibits the bacterial protein synthesis by connecting to the 50S subunit of the bacterial ribosome, thus preventing the beginning of the peptide chain.

The location of connection in the ribosome is the same for macrolide and chloramphenicol, therefore they inhibit their actions by competence and should not be used together because they are antagonistic. When used *in vitro*, they inhibit the production of staphylococci toxins associated with the toxic shock syndrome and prevent the production of BF. By changing the surface molecules, clindamycin facilitates the opsonization, phagocytosis, and intracellular death of bacteria, even in sub-inhibitory concentrations. The consequent alteration of the bacterial wall decreases the adherence capacity of bacteria like of *Staphylococcus aureus* to host cells and facilitates their destruction.

It also has a long-lasting post-antibiotic effect against some susceptible bacteria, possibility owing to the persistence of the drug in the ribosome union place. Its immunomodulatory activity *in vivo* is notorious and it is capable of suppressing the synthesis of toxins in *Streptococcus pyogenes*. Therefore, it is usually associated with the classical treatments using penicillin in severe infections[12].

Metronidazole is a molecule with anti-parasitic and anti-anaerobic activity that lacks immunomodulatory properties. The other antibacterial agents have different effects, but none of them works on BF or penetrate in its inner side.

The azoles that use both preparations have similar pharmacodynamics; however, similarly to all of them, they do not have the ability to penetrate through the EPM that form BF of *Candida* spp. They can work, but they do it slowly until they damage the mycotic elements.

The endogenous infections of the lower genital tract include the CVV, which affect a significant number of women at reproductive age. Topic and systemic medications are used for their treatment. The use of probiotics[13] is also suggested, since they would difficult the formation of hyphae, which is the most virulent form of *Candida* spp.

The pathogenesis of CVV is a multifactorial process and *Candida* spp may interact among themselves and with other microorganisms[14].

This is the reason for antimicrobial combinations. In order to eliminate the participating microorganisms, one has to obtain the penetration of the antimicrobial agents in the infection location and achieve proper concentrations[15]. The BF are usually resistant to many antifungal drugs[16].

Our results show that both the species used in the formation of BF and also those from the patients that we studied with CVV, show an EPM that would make the arrival of anti-mycotic agents difficult. The combined use of CLI and KET improves the activity of KET. The CLI facilitates the dispersion of the EP from the BF, even though it does not adhere. Thus, KET may act more freely on *Candida* cells. This is confirmed with the isolated action of CLI on BF of CA and CAN.

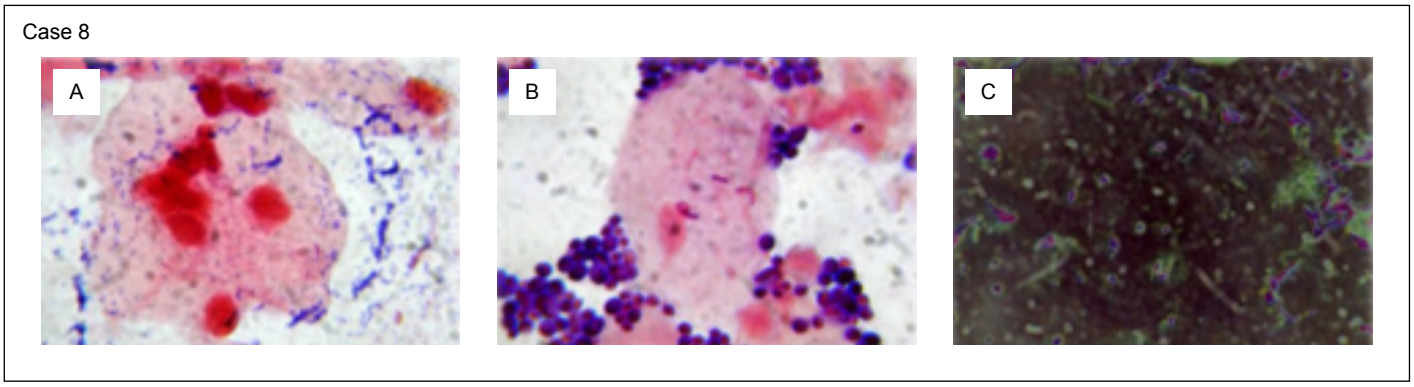

Figure 2 – Case 8 of CVV on cell layers. (A) Original material, presence of scarce mycotic elements and inflammatory response; (B) control cell layer: there is a BF of *Candida albicans*; (C) CK activity on the cell layer of *Candida albicans*: only altered scarce blastospores are seen and there is absence of EPM.

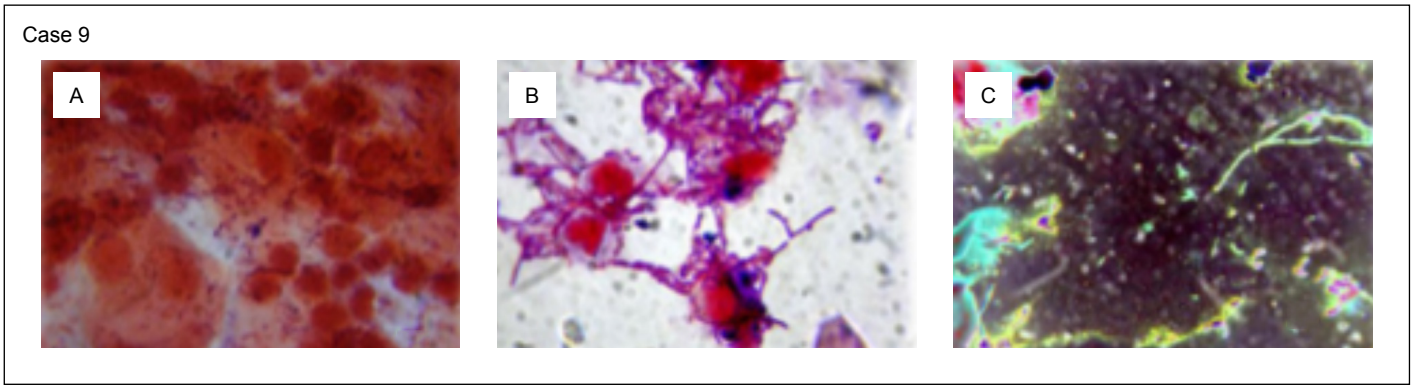

Figure 3 – Case 9 of CVV on cell layers. (A) Original material, presence of scarce mycotic elements and inflammatory response; (B) control cell layer: there is a BF of *Candida albicans*; (C) CK activity on cell layer of *Candida albicans*: only altered scarce blastospores are seen and there is absence of EPM.

It is clear that CLI does not have any action on yeasts, but it facilitates the action of KET and possibly of any other azole. When other antibacterial agents that do not disperse the EPM such as the CLI are used, the efficacy of anti-mycotic agents could be almost null. The activity would be done on the signal molecules, thus interfering with the EP structure that includes blastospores and hyphae. Some activity on the other EP components could be postulated. This would be better manifested *in vivo* since other microorganisms present in the genital tract could contribute to the formation of BF and to the structure of EP[17].

CONCLUSION

According to these results, the use of CK in endogenous vaginal tract infections because of *Candida* spp is more useful than the ketoconazole alone, due to the activity of clindamycin on the EPM architecture that presents the *Candida* spp biofilm. It is usually present in these infections, especially in recurrent or complicated ones.

Conflict of interests

The authors declare no conflicts of interest.

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Syphilis screening during prenatal development: missed opportunities in a public maternity hospital in Recife, Brazil

Rastreamento da sífilis no pré-natal: oportunidades perdidas em uma maternidade pública na cidade do Recife, Brasil

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ABSTRACT

Introduction: According to the norms issued by the Ministry of Health for the Prenatal and Birth Humanization Program, pregnant women should undergo two tests for syphilis detection. **Objective:** To evaluate missed opportunities for screening gestational syphilis and to identify factors associated with the missing application of the Venereal Disease Research Laboratory (VDRL) test during prenatal development. **Methods:** This cross-sectional study was undertaken in the maternity ward of a Unified Public Health System (SUS) hospital in the city of Recife in northeastern Brazil. We studied 460 women admitted for termination of pregnancy and/or abortion, between September and October 2013, who had at least one prenatal consultation. We conducted interviews and checked patients' prenatal care records and medical records. Women who did not take at least one VDRL test during prenatal development (reference category) were compared with those who did. Logistic regression was performed on the data collected from the 408 pregnancy records analyzed in order to identify factors associated with a failure to undergo syphilis screening. **Results:** 17.90% of the women in the sample did not take the VDRL test. In multivariate analysis, women who fit the following factors presented a greater chance of not having taken the VDRL test: facing difficulties in taking the test; attendance of the last prenatal consultation before the last trimester of pregnancy; attending less than six consultations; receiving prenatal care in hospital units which did not schedule subsequent exams; being 19 years of age or younger; having had three or more pregnancies. **Conclusion:** Results show that despite the high availability of prenatal care, actions aimed at preventing congenital syphilis still present low effectiveness.
Keywords: congenital syphilis; prenatal care; risk factors.

RESUMO

Introdução: De acordo com as normas do Programa de Humanização no Pré-Natal e Nascimento, do Ministério da Saúde, a gestante deve realizar dois exames laboratoriais para detecção da sífilis. **Objetivo:** Avaliar oportunidades perdidas no rastreamento de sífilis gestacional e identificar fatores associados à não realização do teste Venereal Disease Research Laboratory (VDRL) no pré-natal. **Métodos:** Estudo de corte transversal realizado em maternidade do Sistema Único de Saúde da cidade do Recife, no Nordeste do Brasil. Foram estudadas 460 mulheres admitidas por término da gravidez e/ou abortamento, entre setembro e outubro de 2013, que realizaram ao menos uma consulta de pré-natal. Foram realizadas entrevistas e consulta ao cartão de pré-natal e prontuários. As mulheres que não realizaram pelo menos um VDLR no pré-natal (categoria de referência) foram comparadas com aquelas que realizaram. Nas informações colhidas nos 408 cartões da gestante, foi utilizada a regressão logística para identificar fatores associados com a não realização do rastreio. **Resultados:** Uma parte correspondente a 17,90% das mulheres não realizou o VDRL. Na análise multivariada, apresentaram maior chance de não realizar o VDRL as mulheres que: enfrentaram dificuldades para realizar o exame; realizaram a última consulta antes do último trimestre da gravidez; passaram por menos de seis consultas; realizaram pré-natal em unidade que não realizava agendamento das consultas subsequentes; tinham 19 anos ou menos de idade; tinham três ou mais gravidezes. **Conclusão:** Os resultados mostram que, apesar da elevada cobertura da atenção pré-natal, persiste uma baixa efetividade das ações de prevenção da sífilis congênita.
Palavras-chave: sífilis congênita; cuidado pré-natal; fatores de risco.

INTRODUCTION

Syphilis is an infectious systemic disease of chronic evolution that may be sexually or vertically transmitted through pregnancy. The presence of this infection in pregnant women, and consequently in newborn infants, is a sign of failure in prenatal care, as early diagnosis and treatment of pregnant women, relatively simple measures, are quite effective in preventing its transmission to newborns[1].

In 2008, the World Health Organization (WHO) estimated the number of pregnant women infected worldwide at 2 million, with 80% having received prenatal care[2]. Due to its magnitude, syphilis remains a public health concern to this day[3].

In Recife, the incidence rate of congenital syphilis in 2011 for infants under 12 months of age was 15.4 cases per 1,000 successful deliveries, and the detection rate of syphilis in pregnant women was 8.7%[4].

In 2011, the city of Recife showed a high coverage of prenatal care, including 99.01% of pregnant women. Around 92.05% of consultations were initiated in the first trimester of pregnancy[5].

The WHO advises syphilis screening during prenatal care as a political and universal guideline covered under primary health care.

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The goal established for the elimination of congenital syphilis until 2015 recommends that at least 90% of pregnant women be tested[2].

According to the rules of the Ministry of Health's Prenatal and Birth Humanization Program, pregnant women should perform two syphilis detection tests. The conduction of two VDRL tests (one in the first trimester of pregnancy and the other in the last trimester) is recommended[6].

The "Projeto Sentinela Parturiente" study (roughly, "National Pregnancy Watch") revealed that 18.7% of pregnant women under prenatal care in northeastern Brazil did not take any VDRL tests in 2006. The study also indicated that, even among pregnant women who attended six or more prenatal consultations, 8.5% did not undergo any type of syphilis screening[7].

OBJECTIVE

To evaluate missed opportunities for gestational syphilis screening and to identify factors associated with the missing application of the VDRL test during prenatal development in a public maternity hospital in Recife, Pernambuco State.

METHODS

This is a cross-sectional study conducted in Recife, the capital city of the state of Pernambuco, Brazil, which has a population of 1,537,704 inhabitants according to the 2010 census[8].

The study was conducted in the public maternity hospital "Prof. Barros Lima," considered a reference in low risk pregnancies, which has 69 beds, 46 of which are reserved for obstetric procedures[9].

When calculating sample size in order to estimate the associations between explanatory variables and the main outcome, we used the Statcalc module of the Epi Info 3.5.2 software (Centers for Disease Control and Prevention, Georgia, United States), assuming a 95% confidence interval (95% CI), a statistical power of 80%, a proportion of outcome equivalent to 50% among the exposed individuals, and an Odds Ratio (OR) equal to 2. Thus, the estimated sample resulted in 400 women. Considering 10% of losses, a total of 440 women was obtained.

Between September and October 2013, all women admitted for delivery or abortion at the studied hospital who also lived in Recife and had undergone at least one prenatal consultation were included in the sample. 475 women were considered eligible, 96.8% (n=460) of whom were interviewed. There were 14 losses and 1 individual declined to participate.

Data were collected by in person interviews when women were still hospitalized after delivery procedures or, in cases of abortion, after curettage, using a structured, pre-coded questionnaire for recording information and transcribing medical and prenatal records, if present.

In addition to the respondents' socioeconomic and demographic characteristics, the questionnaire covered the proportion of women who began prenatal care in their first trimester of pregnancy; the number of pregnancies; the number of prenatal consultations; and the proportion of women who were not followed-up or who performed at least one VDRL test during prenatal care.

The 408 women (88.7% of the respondents) who presented their prenatal records at the time of the interview were included in bivariate analysis. The women studied were divided into two groups: those associated with the outcome (dependent variable), that is, no indication of VDRL tests in their prenatal records; and those not associated with the outcome (comparison group), that is, with one or more VDRL tests recorded during prenatal care.

Independent quantitative variables were categorized using clinical criteria and/or other bibliographical references or guided by the frequency distribution found for the independent variables. The association between the outcome and the independent variables was measured using the OR, with a 95% CI and a statistical significance level of 5% or less, as indicated by the χ^2 or the Fisher's exact test, when the expected value was equal to or less than five for one or more data cells.

The exposure category chosen for the reference line (used to classify exposed independent variables when calculating the OR) was the one which presented the greater proportion of the outcome. At this stage, variables with a p-value under 0.20 for association with the outcome of the study were selected for multivariate analysis.

During multivariate analysis, the logistic regression model was chosen, since the outcome studied is dichotomous. The following variables were included in the modeling process: years of education; number of total pregnancies; age in years; prenatal consultations in hospitals capable of scheduling subsequent consultations; awareness of the VDRL test; having faced difficulties to perform the VDRL test; attendance of the last prenatal consultation during the last trimester of pregnancy; the number of prenatal consultations provided; and prenatal care start date.

In this step, variables (which had previously presented a cut-off point) were transformed into dichotomous variables.

The goal of this step was to adjust the confounding effect and to investigate the presence of interaction among variables, in order to identify factors presenting a statistically significant association (predictor variables) with the main outcome – i.e. not undergoing VDRL tests – by calculating their respective adjusted OR, with a 95% CI.

In both of the above steps, the Epi Info 3.5.2 and the Statistical Package for Social Sciences (SPSS), version 20 (Chicago, United States) software were used.

This research project was submitted to the Ethics Committee of the Center for Health Sciences of the *Universidade Federal de Pernambuco* (UFPE) and was approved under opinion number 390,216.

RESULTS

Table 1 refers to the socioeconomic and demographic characteristics of the respondents. 60.65% (n=279) of respondents were aged between 20 and 34 years. Among the participants, 53.69% (n=247) attended formal education for 9 to 11 years. Concerning race/skin color, 44.10% (n=203) of respondents declared themselves as brown-skinned (in Brazilian Portuguese, "pardo" is used to refer to Brazilians of mixed ethnic ancestries, commonly a mixture of white Brazilian, Afro-Brazilian and Native Brazilian). With regard to marital status, 75.22% (n=346) of women reported being in a domestic partnership.

Table 2 refers to characteristics related to the respondents' current pregnancy. The category "two or more pregnancies" included 58.90% (n=271) of the participants. 69.20% (n=308)

reported starting prenatal care consultations during the first trimester of pregnancy. 67.22% (n=283) reported undergoing over 6 prenatal consultations.

Among the respondents, 89.20% (n=403) of women reported concluding prenatal care in the last trimester of pregnancy. The majority of respondents, 79.10% (n=364), received prenatal care in clinics of the Family Health Program (in Portuguese "*Programa Saúde da Família*"). 75.90% (n=349) of interviewees received prenatal care in a clinic capable of scheduling subsequent consultations. Around 48.30% (n=222) of women reported that community health workers (CHW) do not perform their monthly home visits.

Table 3 includes variables referring to factors related to VDRL testing based on respondents' interviews and their medical and prenatal records. 66.10% (n=304) of interviewees reported receiving no information regarding sexually transmitted diseases (STD), such as syphilis, during prenatal care; whereas 58.90% (n=271) had no knowledge of VDRL tests. Among the respondents, 60.65% (n=205) of them reported some difficulty in performing the tests requested during prenatal care. Of these, 33.72% (n=114) complained that collection centers were too far from their homes; 27.22% (n=92) reported delays in scheduling exams or in receiving their results; whereas 14.80% (n=50) reported that the queues to schedule exams were too long.

Among the 408 women who provided their prenatal records, 17.90% (n=73) had not performed the VDRL test during the prenatal period. Furthermore, among those who performed VDRL test, 44.78% (n=150) did so during the first trimester of pregnancy. None of the prenatal records analyzed described some rapid testing for syphilis.

Table 4 shows the bivariate analysis of the association between the independent variables studied and the outcome of the study (that is, the absence of VDRL testing during prenatal care). Such variables are those which showed a statistically significant p-value.

Table 1 – Socioeconomic and demographic characteristics of women admitted to a public maternity hospital after birth procedures or curettage in 2013. Recife, Pernambuco.

Variables	% (n)
Age	
13 to 19 years	31.09 (143)
20 to 34 years	60.65 (279)
35 years or +	8.26 (38)
Years of schooling	
Unable to read or write	1.30 (06)
1 to 4 years	4.35 (20)
5 to 8 years	36.52 (168)
9 to 11 years	53.69 (247)
12 years or +	4.13 (19)
Race/skin color	
Brown (parda)	44.10 (203)
White	30.00 (138)
Black	25.90 (119)
Religion	
Without Religion	24.10 (111)
Evangelical	38.30 (176)
Catholic	35.00 (161)
Other	2.60 (12)
Marital status	
Domestic partnership	75.22 (346)
Single no partnership	24.78 (114)
Monthly gross income in minimum wages	
Below 1	33.26 (153)
Between 1 and 4	60.65 (279)
Above 4	1.52 (07)
Didn't know/ did not inform	4.56 (21)

Table 2 – Characteristics related to the current pregnancy and to prenatal care of women admitted to a public maternity in 2013. Recife, Pernambuco.

Variables	% (n)
Number of pregnancies (including the current one)	
1	84.80 (390)
2 or +	15.20 (70)
Start date of prenatal care[a]	
1st quarter	69.20 (308)
2nd quarter	28.30 (126)
3rd quarter	2.50 (11)
Number of consultations[b]	
1 to 5	32.78 (138)
6 or +	67.22 (283)
Time of the last prenatal consultation[c]	
1st quarter	6.20 (28)
2nd quarter	4.60 (21)
3rd quarter	89.20 (403)
Location of last prenatal consultation[*]	
Family Health Program (FHP) clinics	79.10 (364)
Public Hospitals	18.90 (87)
Health Centers	9.80 (45)
Private Clinics	3.26 (15)
Appointment scheduling	
With schedule	75.90 (349)
Scheduled by herself	14.10 (65)
Through Community Health Workers (CHW)	9.30 (43)
Could not answer	0.70 (03)
Visited monthly by community health workers (CHW)	
Yes	44.80 (206)
No	48.30 (222)
There are no Community Health Workers	7.00 (32)

[a]15 women did not answer; [b]39 women did not answer; [c]8 women did not answer; [*]One or more answers were acceptable; FHP: Family Health Program; CHW: community health workers.

Table 5 presents the multivariate analysis, which enabled us to conclude that women who reported experiencing some difficulty in taking the VDRL test during prenatal care had a ten times greater chance of not undergoing the examination if compared to those who reported no such difficulties.

Table 3 – Characteristics related to Venereal Disease Research Laboratory (VDRL) testing, according to respondents' interviews and data collected from their prenatal records for women admitted to a public maternity hospital, in 2013. Recife, Pernambuco.

Variables	% (n)
Information regarding syphilis during prenatal care[a]	
Received no information	66.10 (304)
Received information	32.80 (151)
Knowledge of VDRL testing[b]	
Knows or has heard about it	41.10 (186)
Never heard about it	58.90 (271)
Difficulties in taking the VDRL test[*]	
No difficulties	39.35 (133)
Collection center too far	33.72 (114)
Delay in receiving exam	27.20 (92)
Long waiting queues	14.80 (50)
Lack of materials in the laboratory	1.48 (05)
Poor care by the health service	1.18 (04)
Other reasons	2.60 (09)
VDRL testing (based on prenatal records)[c]	
Took the test	82.10 (335)
Did not take the test	17.90 (73)
Period of pregnancy of the 1st VDRL test[c]	
1st quarter	44.78 (150)
2nd quarter	43.58 (146)
3rd quarter	11.64 (39)

[a]5 women did not know how to answer; [b]3 women did not know how to answer; [c]408 women provided prenatal records; [*]One or more answers were acceptable.

Table 4 – Bivariate analysis between variables and the outcome (VDRL testing during the pre-natal care) for women admitted to a public maternity hospital in 2013. Recife/Pernambuco.

Variable	Outcome		OR	95%CI	p-value
	N/VDRL	Y/VDRL			
Years of education					
≤8 years	40	130	1.90	1.14 – 3.20	0.012
≥9 years	33	205	1.00	-	
Number of pregnancies					
≥3	31	91	1.98	1.17 – 3.30	0.010
≤2	42	244	1.00	-	
Age					
≤19 years	30	99	1.66	0.99 – 2.80	0.055
≥20 years	43	236	1.00	-	
Appointment scheduling of prenatal consultations					
No	32	53	4.15	2.40 – 7.20	0.000
Yes	41	282	1.00	-	
Has heard about the VDRL test					
No	53	183	2.20	1.26 – 3.84	0.005
Yes	20	152	1.00	-	
Difficulties in taking the VDRL test					
Yes	70	220	12.20	3.70 – 39.60	0.000*
No	3	115	1.00	-	
Last prenatal consultation during the 3rd quarter of pregnancy					
No	28	18	10.90	5.60 – 21.40	0.000
Yes	45	317	1.00	-	
Number of consultations (based on prenatal records)					
≤5	55	89	8.40	4.70 – 15.10	0.000
≥6	18	246	1.00	-	
First prenatal care consultation during the 1st quarter of pregnancy					
No	35	88	2.60	1.50 – 4.30	0.000
Yes	38	247	1.00	-	

*using Fisher's exact test; N/VDRL: without VDRL; Y/VDRL: with VDRL, OR: Odds Ratio; 95%CI: 95% confidence interval.

Table 5 – Final multivariate analysis model: factors associated with not undergoing VDRL testing during prenatal care among women admitted to a public maternity hospital, in 2013. Recife, Pernambuco.

Variables	OR_{Crude}	$OR_{Adjusted}$	95%CI	p-value
Difficulties in taking the VDRL test (yes/no)	12.20	10.11	2.96 – 34.60	0.000
Period of the last consultation (1st, 2nd, or 3rd quarter)	10.90	5.46	2.27 – 13.12	0.000
Number of prenatal consultations (≤ 5 / 6 or+)	8.40	3.73	1.89 – 7.36	0.000
Age in years (≤ 19 / 20 or +)	1.66	2.89	1.37 – 6.09	0.005
Number of pregnancies (3 or more /1 or 2)	1.98	2.38	1.12 – 5.07	0.025
Subsequent consultations scheduled after the first consultation (no/yes)	4.15	2.35	1.17 – 4.72	0.016
Heard about VDRL tests (no/yes)	2.20	1.77	0.90 – 3.47	0.099

OR: Odds Ratio; 95%CI: 95% confidence interval.

Having one's last prenatal consultation before the last trimester of pregnancy resulted in having a five times larger chance of not taking the VDRL test during pregnancy. Attending less than six consultations during prenatal care contributed to a four times greater chance of not taking the VDRL test.

Pregnant women under 20 years of age were three times more likely to not undergo VDRL testing during prenatal care than older women. However, women with three or more pregnancies and women who were treated with no prior scheduling at prenatal services were two times more likely to not undergo VDRL testing than those of the opposite group.

DISCUSSION

Syphilis is a disease with serious consequences for women and babies born to HIV+ mothers. The application of nontreponemal serological tests during prenatal care is indicated in several national and international publications[2,6,10-12] as essential for the control and prevention of vertical transmission of syphilis. The high rate of vertical transmission of syphilis and its low detection during pregnancy stem from flaws in prenatal care[12.13]. With early diagnosis and treatment of pregnant women during prenatal care it is possible to prevent congenital syphilis and reduce the risk of miscarriages, serious neonatal infections, and perinatal death[2].

In this study, the proportion of women interviewed who provided prenatal records at the time of interview and who had not taken at least one VDRL test during prenatal care was 17.90%. Although this value is lower than the one found in a 2004 study conducted in public maternity hospitals in Recife[14], is still above the national average[7] of 13.50%. However, it is similar to the estimate found in the northeastern state of Fortaleza (CE) of 20.20%[15].

After logistic regression analysis adjusted for confounding variables, the chance of not undergoing at least one VDRL test during pregnancy remained higher for women in the following categories: aged under 20 years; multiparous, with three or more pregnancies; having undergone the last prenatal consultation before the last trimester of pregnancy; attending less then six prenatal consultations; receiving care at a hospital incapable of scheduling subsequent prenatal consultations.

A study conducted in the United States[16] showed that women aged under 20 years are two times as likely to not undergo screening for syphilis diagnosis during pregnancy when compared with older women.

Other studies[17] that investigated factors associated with the absence of VDRL testing during the prenatal period found similar results, documenting a four times higher chance of taking the VDRL test among women who underwent their last prenatal consultation during the first quarter of pregnancy. A multicenter national study[18] found that women who underwent three or more prenatal consultations were three times more likely to have taken the VDRL test.

Attending six consultations and starting prenatal care early, as recommended by the Program for Humanization of Birth and Childbirth Care and the Rede Cegonha[19,20], are necessary to expand coverage and ensure compliance with the established goals related to the screening of gestational syphilis during prenatal care. A failure to comply with the minimum number of visits and the premature interruption of prenatal care reduce the possibility of VDRL testing and of receiving its results in a timely manner.

Guaranteed scheduling of subsequent consultation proved a factor that favors the diagnosis of syphilis during pregnancy, possibly through strengthening pregnant womens' access to health services[6].

However, the results suggest that one obstacle in universalizing screening for syphilis during prenatal care is in the access to VDRL testing and its results. More than two thirds of the women interviewed reported encountering difficulties in implementing the VDRL in laboratories, indicating the following as main challenges: the distance between the household and the pick-up unit; the delay to receive the results of the examination; and the existence of extensive queues.

The deployment of the rapid test for the detection of infection can correct this difficulty because it will be performed in the clinic itself and the result will be issued in a matter of minutes. In June 2011, by means of Decree No. 1.459/2011, the Ministry of Health[19] introduced this test in primary care. However, at the time of completion of the present study there was still no record of rapid testing for syphilis in the records of pregnant women studied.

This strategy can ensure the treatment of maternal syphilis even in early pregnancy, when it is more effective for the prevention of vertical transmission[21].

The study demonstrated that most of the women had prenatal care in clinics of the FHP, probably due to the decentralization of prenatal care and the strengthening of the basic network by the Ministry of Health[22]. While there should be an increased efficiency in the control of vertical transmission of syphilis in comparison to other models of care[23], it was not evident.

However, approximately one-third of the interviewees reported having less than six visits and starting the prenatal care after the first quarter; moreover, half mentioned the lack of follow-up home visits by CHW. There is evidence that the contact with the CHW may influence the early onset and greater adherence to the activities of the pre-natal care, including undergoing examinations[24].

Although the educational activities are considered an essential part of pre-natal care, particularly in basic units, a little more than 50% of the respondents reported they had heard of the VDRL and 33% have received some information about syphilis in prenatal care. These findings suggest limited adherence to the guidance on the prevention of congenital syphilis in prenatal care. A study conducted in the state of Pernambuco[25] reported inadequate performance in health education as one of the factors that determine the current maintenance of congenital syphilis.

In order to achieve the goal of completing the VDRL test by 90% or more of pregnant women during prenatal care, as recommended by the Ministry of Health[10] and the WHO[2], it is necessary to undertake efforts to improve the early engagement of pregnant women, increase awareness of examination requests for those professionals who provide prenatal care in the first consultation, and ensure access to laboratory examination in a timely manner.

One of the limitations of this study may be the possibility that the estimated proportion of women who had not taken the VDRL test is underestimated since it was based on the medical records of the pregnant women and some professionals may not have registered the examination even if it has been performed.

Another limitation concerns the generalization of results, because the type of motherhood studied does not include pregnant women or women with more severe complications of abortion. Furthermore, the results may not represent the reality of all women who had not attended the prenatal care in the public municipal health department.

CONCLUSION

The loss of opportunity for the diagnosis of syphilis among pregnant women cared for in the public municipal health department also indicates the need for strengthening prenatal care in the primary care network of SUS, in particular with respect to the promotion of action for prevention and control of syphilis and other STDs. This would include educational measures, in addition to providing the means for screening syphilis in pregnancy, preferably in basic health units.

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Conflicts of interest

The authors declare no conflicts of interest.

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Analysis of students' knowledge about human papillomavirus

Análise do conhecimento de escolares sobre papilomavírus humano

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ABSTRACT

Introduction: The human papillomavirus is the main risk factor for cervical cancer and is also a risk factor for oropharyngeal and penile cancer. Adolescence is the period of greatest risk of infection by the virus, because many people begin the sexual life during this period. Information about human papillomavirus is fundamental for prevention. Objective: To evaluate the adolescent's knowledge about human papillomavirus and to disseminate information about prevention, transmission, and infection. Methods: Cross-sectional study that was carried out in a school with 390 participants, who were aged 11–18 years. A questionnaire with objective questions, descriptive analysis, and the χ^2 test were used. The statistical significance level was established as $p<0.05$. Informative lectures were carried out after the data analysis. Results: A total of 188 participants were females. The average age was 14.4 years. Among the participants, 91.28% had already heard about human papillomavirus and 91.54% of them answered that it is a virus. A total of 43.08% participants knew what the acronym stood for. The sexual intercourse was indicated by 81.03% of the participants as the main route of transmission; however, only 48.46% indicated that both genders could be infected. With regard to prevention, 59.48% of participants mentioned condom use, 12.56% of them alluded to late start of sexual activity, 53.38% of them mentioned vaccination, and 28.02% of participants indicated sexual education. Mainly female participants referred to vaccination and condom use ($p<0.05$). The clinical symptoms of this infection were correctly indicated by 70% of the adolescents. Conclusion: Most of the participants were aware of the basic information about human papillomavirus. However, their awareness of the possibility of both genders becoming infected and about prevention is poorer. Female participants showed higher knowledge level.

Keywords: communicable disease control; Papillomaviridae; adolescent; sexually transmitted diseases.

RESUMO

Introdução: A infecção pelo papilomavírus humano é o principal fator de risco para o câncer de colo uterino e fator de risco também para câncer de orofaringe e pênis. A adolescência constitui o período de maior risco de infecção pelo vírus, pois é nela que muitos iniciam a vida sexual. Informações sobre o papilomavírus são fundamentais na sua prevenção. **Objetivo:** Avaliar o nível de conhecimento dos adolescentes acerca do papilomavírus humano e informar sobre prevenção, transmissão e infecção. **Métodos:** Estudo transversal, realizado em um colégio, tendo 390 participantes, de idade entre 11 e 18 anos. Utilizou-se questionário com perguntas objetivas, análise descritiva e teste do χ^2. O nível de significância foi estabelecido em $p<0,05$. Realizaram-se palestras informativas após análise de dados. **Resultados:** Um total de 188 participantes eram do sexo feminino. A idade média foi de 14,4 anos; 91,28% já ouviram sobre papilomavírus humano e 91,54% responderam que é um vírus. Dos participantes, 43,08% souberam o significado da sigla e 81,03% apontaram as relações sexuais como principal maneira de transmissão, contudo, somente 48,46% apontaram que ambos os sexos podem ser infectados. Sobre prevenção, 59,48% se lembraram do uso de preservativo, 12,56% do início tardio da atividade sexual e número reduzido de parceiros, 55,38% da vacinação e 28,20% da educação sexual. Vacinação e preservativos foram lembrados principalmente pelo sexo feminino ($p<0,05$). Um total de 70,00% dos adolescentes souberam as manifestações clínicas da infecção. **Conclusão:** A maioria dos participantes conhece as informações básicas sobre o papilomavírus humano. Todavia, o conhecimento é menor sobre ambos os gêneros poderem ser infectados e formas de prevenção. As participantes do sexo feminino apresentaram maior nível de conhecimento.

Palavras-chave: controle de doenças transmissíveis; Papillomaviridae; adolescente; doenças sexualmente transmissíveis.

INTRODUCTION

Human papillomavirus (HPV) presents more than 100 serotypes, of which approximately 40 infect the genital tract and 18 of these cause most of the uterine cancers[1]. In 1983, Harald zur Hausen found that types 16 and 18 are present in 70% of the biopsies performed in cervical cancer patients[2]. HPV is usually sexually transmitted; however, it can also be transmitted through contact and via mother to fetus[3]. It is manifested through benign or malign skin lesions, epidermodysplasia verruciformis and benign or malign mucosae lesions[3]. In men, the asymptomatic form of manifestation is the most common, although there is a chance of symptom manifestation. Thus, the male sex is considered a virus spreader[2]. It is worth noting that the infection has high prevalence in both sexes, 75–80% of the population will be infected throughout their lives, and half of the new cases are found in the three first years of sexual activity[4-6].

HPV infection is considered the main risk factor for cervical cancer, which is the second most common cancer in Brazilian women[7]. In 1 year, HPV is responsible for approximately 500 thousand new cases of cervical neoplasm worldwide, causing approximately 231 thousand deaths of women[8]. Furthermore, this infection is also a risk factor for oropharyngeal and penile cancers, which is manifested in men[9,10].

The use of condoms, the reduced number of sexual partners, periodic gynecological examinations, and prophylactic vaccines against HPV are the main protection factors against the development of cervical cancer and of HPV-associated diseases[11].

Adolescence is the period that presents the greatest infection rates[11], because many people initiate their sexual lives during this period. Specific information about HPV is essential to prevent diseases associated with the virus.

This study was carried out at *Universidade do Vale do Itajaí* (UNIVALI) – Itajaí (SC), Brazil.

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OBJECTIVE

To assess the level of adolescents' knowledge about human papillomavirus and to promote the dissemination of information about its prevention, transmission, and infection.

METHODS

The Ethics and Research Committee of the *Universidade do Vale do Itajaí* (UNIVALI), under protocol no. 910.774, approved the research. Data remain under absolute secrecy, and they followed the standards established in the Resolution of the Brazilian National Health Council no. 466/12.

This is a cross-sectional and multicenter study conducted with 390 students aged between 11 and 18 years at *Colégio de Aplicação* from UNIVALI, in Itajaí, Santa Catarina, Brazil. Data collection was carried out after parents or guardians signed the free informed form and the adolescents signed a consent form. Participants who did not attend the collection day and who did not sign the Consent Form were excluded from the research, as well as those who did not receive parents' authorization.

For data collection, a questionnaire including objective questions was used to assess the level of knowledge about HPV, including questions such as: "What sex (male and/or female) can be infected with HPV?;" "What is the main route of HPV transmission?;" "What are the methods for prevention of HPV?."

In addition to the descriptive analysis, the χ^2 test was applied to establish homogeneity of proportions with a significance level of $p<0.05$.

Informative lectures were given after data analysis with the aim of clarifying the main doubts of students, as well as to increase their level of knowledge about HPV.

RESULTS

Among 390 participants, 188 were female (48.20%) and 202 were male participants (51.79%). The mean age among the interviewed adolescents was 14.41 years – 14.39 for female participants and 14.44 for male participants. The participants' minimum and maximum age was 11 and 18 years, respectively.

The study shows that 91.28% of the students had already heard about HPV, and the highest percentage was found for female participants (96.81%). Male participants' percentage was 86.14%.

When students were questioned where or by whom they had heard about HPV, the most frequent answer was "school," which was chosen by 33.59% of them. The second most common source of information was "television" (21.03%) and the third was "parents" (11.54%). The amount of male participants who answered "never heard," who "do not remember," or who used "the internet as a source" was higher compared with female participants, with a statistically significant difference ($p<0.05$). In addition, in the comparison of sexes, the number of female students who received information by physicians is statistically significant compared with male participants (**Table 1**).

Most of the students knew that HPV is a virus, therefore totaling 91.54%. In despite of it, only 43.08% were aware that HPV means human papillomavirus.

Of the total of interviewed adolescents, 81.03% indicated sexual intercourses as a route of transmission. Female participants had a higher rate of correct answers (92.02%), compared with male participants (70.79%), which is a statistically significant difference ($p<0.05$). Among the participants, 33.85% of the students answered that only women could be infected with HPV and 48.46% of the students answered correctly that both sexes could be infected (**Table 2**).

The use of condoms was indicated as a prevention action by 59.48% of the 390 interviewed adolescents. Late onset of sexual activity and decreased number of partners were indicated by only 12.56% of the interviewed adolescents. Among interviewed adolescents, 55.38% indicated vaccination as a HPV prevention. On the other hand, 28.20% of them indicated sexual education. The values of $p<0.05$ show a statistical significance between the level of knowledge of female students and that of male students (**Table 3**).

Of the 390 adolescents, 70.00% were aware that cancer, skin, and mucosa lesions could be possible manifestations of the disease. Once again, female students were more aware of that (78.72%), compared with male students (61.88%).

Table 1 – Source of information about HPV.

Variables	Female sex n (%)	Male sex n (%)	Total n (%)	p-value
Never heard about it	6 (3.19)	27 (13.37)	33 (8.46)	0.0003*
School	69 (36.70)	62 (30.69)	131 (33.59)	0.2090
Parents	25 (13.30)	20 (9.90)	45 (11.54)	0.2900
Television	40 (21.28)	42 (20.79)	82 (21.03)	0.9060
Internet	8 (4.26)	21 (10.40)	29 (7.44)	0.0210*
Newspapers	10 (5.32)	6 (2.97)	16 (4.10)	0.2420
Friends	6 (3.19)	3 (1.49)	9 (2.31)	0.2620
Physicians	17 (9.04)	2 (0.99)	19 (4.87)	0.0002*
Does not remember	7 (3.72)	19 (9.41)	26 (6.67)	0.0240*

HPV: human papillomavirus; *significant values.

DISCUSSION

Three hundred and ninety students participated in the study, 48.20% female and 51.79% male adolescents. Interviewed adolescents were aged between 11 and 18 years, with mean age of 14.41 years. Age range and sex distribution from this study are different of other outcomes. In a study conducted in Piauí with 218 interviewed subjects, the age range was between 17 and 34 years (78.00% female) [12]. On the other hand, in Ribeirão Preto, São Paulo, only women between 17 and 19 years were interviewed[13]; and at a private university from Pernambuco backcountry, a study included participants aged between 21 and 25 years, of which 65.70% were female[14]. The differences observed among this study and other studies occur owing to different methodologies adopted. This study is relevant because it collects data from a heterogeneous population regarding sex and younger age. This is due to the possibility of both sexes becoming infected by the virus and to the early onset of sexual activities.

Among the participants, 91.28% had already heard about HPV, which is similar to what was found in Piauí research, in which 87.00% knew about the virus[12]. However, in a study conducted with students from the third year of high school at an educational institution from São Gonzalo, Rio de Janeiro, Brazil, only 57.75% of the participants were aware of the virus[15]. The difference between this study and the one carried out in São Gonzalo possibly occurred because data from the latter were collected in 2006, a period when there were only few HPV prevention and vaccination campaigns. Similarity with the study carried out in Piauí may be explained because data were collected in 2014, close to the data collection of this study, in 2015, after the campaigns conducted by the Brazilian Ministry of Health (acronym in Portuguese – MS).

In this study, the main sources of HPV information were school, television, and parents, from the most to the least mentioned. Similar results were found in a cross-sectional study carried out in Portugal; however, in another order, as follows: family was the first source of information, followed by school, and television[16]. It is extremely important for health promotion purposes to know the path of information toward its target population, that is, to know what media should be used so that more people can acquire more knowledge of a subject. The more comprehensive the information media is, the greater the effectiveness of the health promotion policy and the higher the number of people who will benefit from it. It is noteworthy the fact that 6.67% of the interviewed individuals did not remember and 8.46% had never heard about HPV, being statistically significant the fact that most of these participants were men. Other two statistically significant results is that female participants receive more information from physicians than male participants, and male participants use more the Internet as a source of knowledge acquisition. The fact that participants receive more information from physicians may be explained by the lower search of male participants for health professionals.

At *Colégio de Aplicação* of UNIVALI (CAU) from Itajaí, Brazil, 91.54% of the participants knew that HPV is a virus, which is close to the result found among participants from a research performed at a university in Pernambuco backcountry, in which 93.40% of participants also knew the fact[14]. Therefore, it is assumed that the majority of population knows that it is a virus. However, only 43.08% of the CAU students know what the acronym stood for, in contrast with 60.30% in the research carried out in Ribeirão Preto, São Paulo[13].

Sexual intercourses were indicated as the route of transmission by 81.03% of the participants, which is higher than the value found in Portugal, in which 64.00% answered correctly[16]. Although it has not been approached in the questionnaire, HPV can be transmitted not only during sexual intercourse, but also through contact and through mother to fetus[4]. It is noteworthy that by knowing the possible routes of transmission, more search for prevention and care will be promoted.

In the study carried out in Piauí, 68.00% of the interviewed university students consider that both sexes can be infected with the HPV[12]. On the other hand, in this study, 48.46% of the interviewed people declared that men and women are more inclined to infection and 33.85% stated that only women are at such risk. Although almost half of them answered correctly, a significant number of participants believe that only women can be infected. It is assumed this misunderstanding occurs because the MS firstly determined that vaccination would be available in the Brazilian Unified Health System (in Portuguese – *Sistema Único de Saúde* – SUS) for free only for female adolescents aged 9 to 11 years[17]. The seriousness of this issue is that unclarified doubts may discourage male individuals from caring for disease prevention. However, it is noteworthy that although men usually present the asymptomatic form of the infection, they could be a great spreader of the virus, and therefore present chances of having the symptoms[3].

Table 2 – Who can be infected with HPV.

Variables	Female sex n (%)	Male sex n (%)	Total n (%)
Who can be infected with the virus?			
Only men	1 (0.53)	31 (15.34)	32 (8.21)
Only women	77 (40.95)	55 (27.22)	132 (33.85)
Men and women	100 (53.19)	89 (44.05)	189 (48.46)
Do not know how to answer	10 (5.31)	27 (13.36)	37 (9.49)

HPV: human papillomavirus.

Table 3 – HPV prevention.

Variables	Female sex n (%)	Male sex n (%)	Total n (%)	p-value
Condom	139 (79.93)	93 (46.03)	232 (59.48)	0.0000*
Late sex activity and low number of partners	28 (14.89)	21 (10.39)	49 (12.56)	0.1810
Vaccination	128 (68.08)	88 (43.56)	216 (55.38)	0.0000*
Sexual education	62 (32.97)	48 (23.76)	110 (28.20)	0.0430*

HPV: human papillomavirus; *significant values.

The study aimed at analyzing adolescents' knowledge about HPV, considering that adolescence is the period that presents the highest risk of this virus infection[11]. This is due to the risk factors for virus infection, among which the high number of sexual partners and the early onset of sexual activity, which increase virus exposure, stand out[18-20]. Although of great importance, only 12.56% of the participants mentioned that late onset of sexual activity or decreased number of partners is relevant method of prevention. In order to handle this lack of knowledge, more effective sexual education would be necessary; however, only 28.20% of the participants consider this initiative would facilitate prevention, being female participants those who noticeably mentioned this alternative more frequently than men. The use of condoms as a method for disease prevention was mentioned by 59.48% of the students. This is slightly below the result found in São Gonzalo, Rio de Janeiro, in which 65.50% of the participants had the same opinion[15]. Although most students deem necessary to use condoms for prevention, this number is still low, considering it is ideal that all people have a safe sexual activity. Among the participants, 55.38% consider vaccination as a method of prevention. Considering that only a few more than half of them mentioned vaccination, the lectures offered to students after the analysis of the questionnaires emphasized the importance of vaccine. The quadrivalent form used at SUS(sigla em português) vaccination campaign offers protection against serotypes 6, 11, 16, and 18, and confers 100% efficacy to prevent diseases associated with oncogenic viral forms[21]. It is important to emphasize that both quadrivalent and bivalent forms of the vaccine have led to a significant decrease in the incidence of persistent infections[21]. Results indicate that most participants who mentioned condoms and vaccination for prevention are female, with statistically significant results. Thus, it is assumed that the male sex is not being effectively involved in prevention campaigns against HPV.

HPV can be manifested as benign or malign skin lesions, epidermodysplasia verruciformis, and benign or malign mucosae lesions[4]. In the questionnaire administered, 70.00% of the participants answered that cancer, skin, or mucosae lesions are possible consequences of the infection. This percentage is high and differs from a study in Ribeirão Preto, São Paulo, in which 54.30% of the interviewed nursing students did not know the correct answer.

HPV has an estimated prevalence of 32.1%[22]. It has been estimated that 4 million adolescents aged 15 and 17 years become sexually active every year in Brazil[12]. Therefore, adding the onset of sexual life during adolescence to the greater risk of infection, it becomes very important that adolescents receive guidance about the transmission and prevention of sexually transmitted diseases (STDs). Since HPV is the most frequent STD worldwide[22], knowledge about the virus needs to be comprehensive. Furthermore, campaigns including the male audience directly are necessary, as their level of knowledge is poorer.

In this study, in addition to the assessment of the adolescents' level of knowledge, the conduction of lectures enabled to disseminate information and to provide more opportunities for the participants to learn. During the lectures, themes with the greatest knowledge gap were approached, and some incorrect concepts and common prejudice regarding the theme were demystified.

Most of the study participants know basic information about HPV. However, the level of knowledge was lower about the possibility of both sexes becoming infected and about prevention methods. Therefore, this study shows that health promotion and STD prevention campaigns should be stimulated. They would therefore increase society's knowledge, encouraging them to adopt prevention actions, as infection rates are high and lack of information facilitates the growth of the infection. In addition, it is worth noting that health policies should include both sexes and aim at informing and making population aware of the virus transmission, its infection risks, and possible consequences, as well as the importance of vaccination against HPV.

CONCLUSION

Most of the study participants know basic information about HPV. Nevertheless, knowledge about the possibility of both sexes becoming infected and its prevention methods is poor. Female participants showed higher level of knowledge.

Conflict of interests

The authors declare no conflict of interests.

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ABSTRACTS OF PRESENTED PAPERS

Regression of high grade squamous cervical lesion after HPV vaccine: case report

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Introduction: There is an unquestionable relation between a persistent infection by the human papilloma virus (HPV) and cervical cancer. The creation of vaccines against HPV represented an important milestone in the primary prevention of this condition. Nowadays, two vaccines are available against HPV 16 and 18, which are the subtypes responsible for 70% of the cases of cervical cancer and 50% of precursor lesions, respectively. Recent clinical trials suggest that these vaccines are also effective against the unfavorable evolution of cervical cancer precursor lesions. **Objective:** To report a case of regression of an HPV-induced squamous lesion after the vaccine, and collaborate with recent evidence showing that immunization can be useful as a therapeutic tool, replacing the surgical excision of the transformation zone. **Methods:** We present the case of a young woman with histopathology of cervical biopsy, cervical intraepithelial neoplasia (CIN) II, in association with cytopathic changes compatible with HPV — koilocytosis and binucleation. The treatment was conducted exclusively with vaccine against HPV and control, instead of high frequency surgery. At the time of biopsy, colposcopy was satisfactory and showed a transformation zone, with mosaic areas and sparse dots. Biopsy was carried out with Baliu instruments and was addressed to the mosaic area, partially removing the colposcopic change. The patient was informed about the risks and benefits of both options, that is, the treatment conducted exclusively with vaccine or high frequency surgery to completely remove the lesion. Both would have the proper posterior control, but she freely decided, after signing a consent form, to use the immunization conduct. Three doses of the quadrivalent vaccine were administered, without any other measure other than the control with colpocytology and colposcopy every six months. **Results:** In the six-month follow up, after the vaccine, the three colposcopies presented typical transformation zones, and the three colpocytology smears did not show the squamous intraepithelial lesion anymore. It is important to mention that the initial colposcopy and the histopathology of the respective biopsy suggested residual lesion, which rules out the possibility of completely removing the lesion through biopsy. **Conclusion:** This case report strengthens the inference that the vaccine for HPV can represent a useful therapeutic tool against cervical squamous lesions induced by this disease, leading to better prognosis; therefore, it corroborates the evidence reported in recent publications. However, it is necessary to conduct prospective studies with adequate sampling size, determination of the subtypes involved and long period of immunization in order to obtain solid evidence as to the use of vaccine for HPV in the treatment of squamous cervical lesions.

Keywords: HPV; vaccine; cancer.

Buschke-Löwenstein tumor in a patient with HIV

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Introduction: This is an invasive lesion first described in 1896. This tumor, known as giant condyloma acuminatum (GCA), is histologically benign, but the clinical behavior is malignant. GCA recurrence rate is equivalent to 66% after treatment and its fast growth is associated with immunity deficiencies. There is the possibility of malignant degeneration to squamous cell carcinoma. **Objective:** To describe the clinical case of a GCA in a patient with the acquired immunodeficiency syndrome. **Methods:** Case report presenting brief histories, clinical tests, and result of the histological analysis. **Results:** Low immunity characterized by CD4 count=190/mm^3, viral load of 4.25 log; use of ZDV+3TC+EFV for two months; the condition had begun six months earlier, with fast and progressive growth of the lesion; proctological examination, showing voluminous verrucous lesion, of about 12 cm in its largest diameter in the perianal region; rectosigmoidoscopy showing multiple minor lesions in the rectal mucosa; histology confirming the presence of coilocitosis. Surgical excision was conducted, with major resection of the lesion. **Conclusion:** The Buschke-Lowënstein tumor is a variant of the condyloma acuminatum and is related with the human papillomavirus (HPV), types 6, 11, 16, 18 and 54. Clinical evaluation must include proctosigmoidoscopy, abdominopelvic computed tomography, and endoanal ultrasound. To show the presence of HPV, it is necessary to conduct molecular techniques — in situ hybridization, hybrid capture, and polymerase chain reaction. The treatment of choice is surgery with radical excision. Complementary treatments are: cryosurgery, laser, electrocoagulation, hyperbaric oxygen therapy, chemotherapy, radiotherapy, use of interferon, imiquimod, and podophyllin. Differential diagnoses are: anal tuberculosis, spinocellular carcinoma, fungal disease, syphilis, keratoacanthoma, condyloma plana, pseudoepitheliomatous hyperplasia, lymphogranuloma venereum, and Bowen's disease. Abscesses, secondary infection, fistulae, necrosis, rectal stenosis, bleeding from the tumor or the operatory wound, urinary tract infection, ureteral obstruction, incontinence or anal stenosis, invasion into the pelvic cavity and recurrence are possible complications.

Keywords: AIDS; condyloma acuminatum; HPV; HIV.

Comparison of three HPV DNA detection methods: next_gen sequencing, multiplex-PCR and nested-PCR followed by sanger based sequencing

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Introduction: To date there is no in vitro culture method for HPV, neither a robust serotype response; therefore, the diagnosis of HPV infection is based solely on molecular methods for detection of HPV DNA and their sequences. Several laboratory tests are currently

available for HPV-DNA detection. The Multiplex-PCR for HPV-DNA has been used in several countries for screening and prevention of cervical cancer associated with cervicovaginal cytology. The PCR using the consensus primers MY09 and MY11 — forward and reverse — followed by Sanger based genetic sequencing is the most traditional technique used in scientific research, considered the gold standard for many years. Finally, a new technique has been recently described with promising results. The Next_Gen Sequencing (NGS) has been considered a transformer advent of modern genomics. NGS is not based on the Sanger method, and can sequence DNA at unprecedented speed, opening up new applications in biomedical research. This sequencing system can deliver data output ranging from 300 kilobases up to 1terabase in a single run. Its main difference is the use of primers tagged with initial sequences of specific nucleotides for each individual sample, which has been called primers with "barcodes" or labeled primers. However, NGS is not yet widely available, and its accuracy for the diagnosis of HPV has yet to be confirmed. **Objective:** To compare the diagnostic performance for HPV infection using three laboratorial techniques. **Methods:** Ninty-five cervicovaginal samples were randomly selected; each was tested for HPV DNA and genotypes using three methods in parallel: Multiplex-PCR; the Nested PCR, followed by Sanger sequencing; and the NGS with two assays — NGS-A1 and NGS-A2. The study was approved by the Brazilian National IRB — CONEP protocol 16,800. **Results:** The prevalence of HPV by the NGS assays was higher than that using the Multiplex-PCR — 64.2 versus 45.2%, respectively; p=0.001 — and the Nested-PCR — 64.2 versus 49.5%, respectively; p=0.003. NGS also showed better performance in detecting high-risk HPV (HR-HPV) and HPV16. There was a weak interobserver agreement between the results of Multiplex-PCR and Nested-PCR in relation to NGS for the diagnosis of HPV infection, and a moderate correlation for HR-HPV detection. Both NGS assays showed a strong correlation for detection of HPVs (k=0.86), HR-HPVs (k=0.91), HPV16 (k=0.92) and HPV18 (k=0.91). **Conclusion:** NGS is more sensitive than the traditional Sanger sequencing and the Multiplex PCR to genotype HPVs, with promising ability to detect multiple infections, and may have the potential to establish an alternative method for the diagnosis and genotyping of HPV.

Keywords: PCR; molecular biology; laboratories; diagnosis.

Anogenital condyloma acuminata in a transplanted patient

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Introduction: Condyloma acuminata is caused by the human papillomavirus (HPV) and affects both genders. It is the most common cause of sexually transmitted disease (STD) of viral etiology in the USA. There are more than one hundred different subtypes of HPV. In Brazil, subtypes 16 and 18 are the ones mostly associated with squamous cell carcinoma. Subtypes 6 and 11 are mostly associated with benign condyloma acuminata — approximately 90% of the cases — and have low risk for intraepithelial neoplasia. **Objective:** To describe a case of anogenital condyloma acuminata in an immunosuppressed patient after liver transplantation. The case report refers to a male patient, 19 year-old, bisexual, with previous pathological history of liver transplant carried out four years earlier after the diagnosis of primary sclerosing cholangitis associated with ulcerative rectocolitis. At the moment, he is being regularly followed-up and treated with tracolimus (5 mg/day), everolimus (2 mg/day) and mesalamine (1,200 mg /day). His main complaint is: "penis pellets". History of current disease: onset of the condition 6 months ago, with lesions in the penis, perineum and perianal region. Dermatological examination showed several millimetric lesions and black verrucous papules in the dorsum penis and the perianal region. Diagnostic hypothesis: condyloma acuminata. Complementary tests: fast tests to verify the presence of the human immunodeficiency virus (HIV) and syphilis — negative results — and biopsies of fragments of the lesions in the dorsum penis and the perianal region for histopathological examination, which confirmed the hypothesis of condyloma acuminata. The therapy chosen was chemical cauterization using 70 and 90% trichloroacetic acid in two cycles — with a 14-day interval — in the lesions of the dorsum penis, followed by a treatment conducted at home with imiquimod in the anogenital lesions. He was informed that the treatment does not eradicate the virus, and was advised to return to the service in case of new lesions. Prolonged immunosuppression has increased the survival rates of transplanted patients. Health professionals must be aware that, as a result of this chronic immunosuppression — which influences the immune defense mechanisms of the patients and, consequently, leads to reduced cellular immunity — there is more susceptibility to different infectious dermatoses, such as the HPV infection.

Keywords: condyloma acuminata; HPV; immunosuppression.

BEST ABSTRACT POSTER CATEGORY

Early impact in reducing cervical abnormalities in Campos dos Goytacazes-RJ, Brazil, after introduction of the quadrivalent HPV vaccine for girls 11-15. It's time to think about HPV vaccine in boys?

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Introduction: Human papillomavirus (HPV) represents a significant concern to public and private health systems. The prevention of this condition is conducted by the combination of the use of Pap smear test, condoms, and HPV vaccines. Campos dos Goytacazes was the first Brazilian Municipality to implement, in September 2010, the quadrivalent HPV vaccine to girls aged 11–15 years in a hybrid strategy of vaccination — in schools and health centers. In 2014, vaccination for boys was introduced. **Objective:** To present the reduction in incidence of cervical abnormalities before and after the introduction of the quadrivalent HPV vaccine in the municipality. **Methods:** Historical cohort that evaluated the impact of HPV vaccination as a protective factor against low risk of HPV abnormalities. Results of the pap smear test obtained from the SISCOLO system of the Brazilian Minister of Health were categorized in low grade abnormalities (LGA) and high grade abnormalities (HGA). This preliminary study focused in LGA rates, which were estimated for 1-month period and stratified by 4 age groups (<20; 20–24; 25–30; >30 years) from 2007 to 2014. A quantitative comparison of LGA temporal trends before and after vaccination was done with Quasi Poisson regression analysis. The protective effect of the vaccine over time was evaluated by calculating the relative risk (RR) in each age group. **Results:** The study showed significant decrease of more than 60% in LGA in women aged <20 years, and almost 50% for the other groups. HPV vaccine was a protection factor, as RR result was equivalent to <0.0001 in all age groups. **Conclusion:** Although the studies show that the pre HPV neoplastic lesions may be reversible spontaneously, it is undeniable that the vaccine contributed greatly to the high reduction rates, associated with high vaccination coverage. These results are the first in Brazil and may address in the future the necessity to discuss the vaccination of boys in the context of the same results obtained in Australia.

Keywords: HPV; Human Papillomavirus Recombinant Vaccine Quadrivalent, Types 6, 11, 16, 18; uterine cervical neoplasms;

Infection at school: use of ludic methodologies about HPV and HIV

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Introduction: The promotion of health and the prevention of sexually transmitted diseases (STDs) are gradually being introduced in the school environment, even though there are many myths and taboos related to the approach of sexuality and ways to clarify these subjects, especially regarding the human immunodeficiency virus (HIV) and the human papillomavirus (HPV) among children and adolescents. **Objective:** To debate, with the scientific community, about the activity of the Academic League of Infectology and Parasitic Diseases at Universidade do Grande Rio (Unigranrio), Barra unit, in groups of health education addressed to children and adolescents of public and private schools of Rio de Janeiro. **Methods:** Qualitative study, experience report type. **Results:** Groups of educational intervention were created in a public and in a private school of Rio de Janeiro, including the participation of one hundred students, divided in two groups: one of children in a workshop, in the morning, and one of teenagers in a workshop, in the afternoon. Teachers and coordinators took part in the activities with members of the academic league. The activity began with the exposure of the story "Mariazinha Medrosa" (*Scared Mary*) and "José Vai com os Outros" (*José follows the crowd*), a couple that faced several conflicts and difficulties involving their families, friends, school, prejudice, anxiety and STD diagnosis. Then, there is a time to share opinions and views of the students about the story — told through images showing the scenes of the plot —, and, at the end of the reflection, the members of the league present a lecture and an educational debate. Afterwards, there is an activity with a human board game, including questions and answers about HIV and HPV, themes that were approached during the intervention. A box of questions was created, so the students could include questions that would be answered by the members and advisors of the league.

Conclusion: Despite the increasing number of public policies related to STDs, the school environment still needs efficient educational approaches using the language of the target audience, breaking barriers connected to the idea of children and teenagers as asexual beings, who should not be addressed to the search of knowledge regarding sexuality. It is necessary to develop more partnerships between the pedagogic staff and the students in the health field to carry out ludic and efficient approaches, in order to transmit knowledge about the subject. This also prevents mere lectures, which will have poor impact on the stimulation and comprehension of this target group.

Keywords: education in health; medicine; human papillomavirus; care; infectology.

Infectology day: STD and tuberculosis awareness actions of the academic league of infectious and parasitic diseases

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Introduction: Medical education and training of health professionals is generally permeated by challenges related to the student's development and encouragement for promotion, prevention, diagnosis, and treatment actions. The inclusion of the Academic League of Infectious Diseases in the Promotion and Prevention Front motivated the creation of a day of health campaigns related to sexually transmitted diseases (STDs) and tuberculosis, named Day I – Infectology Day. Recreational and informative activities assist in the creative development concerning approaches, intervention, and involvement of outpatients and medical students in campaigns on human immunodeficiency virus (HIV) and human papillomavirus (HPV), including members of the academic league in the group of health promoters. **Objective:** To communicate to the scientific community the activities on STDs, focused on HPV and HIV/AIDS, carried out by members of the Academic League of Infectious Diseases of *Universidade do Grande Rio* (Unigranrio) on the preventive campaign day. **Methods:** Qualitative study of the experience report type. **Results:** A group of nine students, executive members of the league, organized the event and prepared health education tents in front of the university's outpatient clinic. One tent was decorated with posters and photos related to healthy and preventive habits associated with HIV and HPV infection, as well as contraceptive methods and explanations concerning their use. Pamphlets were distributed to those leaving the tent. Inside the circuit of Day I, the visitors played the DST hopscotch game, in which they played dice and the number obtained corresponded to advancing one square and one question about HPV and HIV. In another tent, counseling on tuberculosis was offered by volunteers associated with the league. The groups distributed leaflets to patients leaving the outpatient clinic and the event was concluded with a lecture on STDs in the auditorium of Unigranrio, focusing on diagnosis and treatment of the main diseases in this category. At the end of the event, students and patients were invited to take pictures with signs supporting cervical cancer prevention and non-prejudice against HIV-positive patients. **Conclusion:** The Academic League of Infectious Diseases is a non-profit organization linked to the university and organized by students of the Medicine and Nursing courses under the guidance of a professor. The creation and expansion of health campaigns aimed at prevalent infectious diseases in the community activate the "teaching, research, and extension" tripod, which is essential for the inclusion of the student in the environment of preventive medicine and health promotion. This enables the development of health professionals who are committed to dialogue and reduce STD cases, prioritizing prevention by means of health education.

Keywords: infectious disease medicine; papillomaviridae; medicine.

Validation of the condom self-efficacy scale construct

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Introduction: Condoms are the barrier method of birth control that provides dual protection for both pregnancy and sexually transmitted infections (STIs) and is the most accessible and easy to use worldwide. Self-efficacy is a factor that influences the use of condoms and can be stimulated among adolescents to change behavior in sexual relationships. Thus, self-efficacy enhances adolescents' confidence in their own ability to use the condom, leading to a safer sexual activity. **Objective:** To validate the Condom Self-Efficacy Scale (CSE) construct with adolescents in Fortaleza, Ceará. **Methods:** The CSE was applied to 209 students aged 13 to 26 years who were sexually active and were regularly enrolled in a public education network school of the state of Ceará. Data were collected during the month of November 2014 using a questionnaire containing sociodemographic and sexual data, in addition to the CSE, a self-administered tool, in its Brazilian version. The validity of the construct was assessed by means of the factorial analysis, using analysis of the main components with varimax rotation. The test of choice for analyzing CSE homogeneity was the precision of internal consistency measured by Cronbach's alpha, which is more common with respect to reliability. The research was submitted to the Research Ethics Committee of *Universidade Federal do Ceará* (UFCE), through Platforma Brasil, and was approved under protocol no. 702.946/2014. **Results:** After the factorial analysis, the scale was composed of 14 items divided into 3 domains, similar to the original version of the scale, with only 2 items needing relocation. The reliability of the scale was verified by Cronbach's alpha, which varied from 0.638 to 0.788, obtaining the total value of 0.856, which denotes high internal consistency. A mean of 68.1 points from the total scale was obtained, and there was a statistically significant relationship between the total scale and the variables not having children (p=0.038), condom use (p=0.008), and condom use with a stable partner (p=0.036). **Conclusion:** It can be concluded that the Brazilian version of the CSE is a valid and reliable instrument to verify the self-efficacy of condom use among adolescents and young adults. This version can be used in clinical practice as well as in other studies to evaluate interventions that improve the self-efficacy of adolescents and young adults for condom use.

Keywords: condoms; scales; validation studies.

Adolecents' knowledge of HPV: an integrative review

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Introduction: Human papillomavirus (HPV) is a sexually transmitted infection (STI) that has as its main clinical manifestation the genital warts. Infection can be detected by three **Methods:** the DNA hybridization test, the visualization of condyloma acuminata in the genitals (or subclinical), and/or the Pap smear — oncocytic colpocitology. Knowledge of HPV prevention, clinical manifestations, and transmission is extremely important, especially in the adolescence phase, when initiation of sexual activity mostly occurs, often performed without the use of condoms, thus exposing teenagers to a greater risk of contamination by various pathogens. For this reason, adolescents need more information about the changes in their body, about physiology, anatomy, contraception, and disease prevention. **Objective:** To identify the scientific studies or the level of adolescents' knowledge of HPV infection. **Methods:** This is an integrative review, in which the bibliographic survey was conducted on publications at the health databases Scientific Electronic Library Online (SciELO), Latin American and Caribbean Health Sciences Literature (LILACS), and Science Direct in the last five years. This study began with the following guiding question: What knowledge do adolescents have about HPV? With this question in mind, data was collected from January to March 2016, using the following descriptors: HPV, adolescents, and sexuality. Full national and international articles related to the topic were included, with publications between 2010 and 2015. The criteria for exclusion of articles were those that did not meet the research objective. **Results:** With the combination of the descriptors, a total of 96 articles were found, but only 11 met the inclusion criteria, 8 from SciELO, 1 from LILACS, and 2 from Science Direct. Regarding the publication period, the year that presented the highest number of published articles was 2013, with 6 studies; followed by the year 2014, with 2 publications; and the years 2010, 2011, and 2015, with only 1 study each. The articles evaluated revealed the adolescents had little information about the subject. Most authors were unanimous in stating that adolescents had poor knowledge of the disease, and recommended health education studies on the subject. **Conclusion:** Based on the analysis of the scientific articles found, the adolescents were unaware of HPV infection, prevention methods, transmission, and treatment. Thus, nurses need to act at various levels of health care in order to promote community education, in addition to the training of teachers so that they also provide correct guidance to young people.

Keywords: sexuality; adolescent; papillomaviridae.

Academic league of gynecology and obstetrics: the extension experience for women's assistance and health care

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Introduction: Founded by a group of students interested in deepening theory and practice on women's health, the Academic League of Gynecology and Obstetrics of *Universidade do Grande Rio* (Unigranrio) develops actions with a focus on development,

disease prevention, and scientific knowledge activities, assisting in the improvement of scientific knowledge of medical students in the fields of health promotion, prevention, and follow-up with preceptors in that field. **Objective:** To discuss with the scientific community the activities carried out by the Academic League of Gynecology and Obstetrics of Unigranrio Barra da Tijuca in the fields of women's health and approach to and study of sexually transmitted diseases (STDs). **Methods:** This is a qualitative study of the experience report type. **Results:** The League of Gynecology at Unigranrio Barra is composed of 30 members, 6 of which are members of the board. The league initiated a new management this year by joining student associations involved in women's health from various educational institutions, expanding dialogue and partnerships with other universities. It holds monthly lectures and, this year, its theoretical and practical activities have turned to topics such as HPV and the preventive exam, climacteric, and breast cancer. It conducts clinical sessions with diagnostic challenges along the social pillar of the Academic Medical Center, where the members of the league themselves conduct the debate on clinical cases and their resolution. The league has an internship agreement with the Family Clinic associated with the university. Every year it performs preventive examination and offers counseling on HPV to women in the Unigranrio outpatient clinic. The activity is performed after class and a training workshop is organized by the league's advising teacher, with subsequent training in the experiences laboratory. The league is present in several Brazilian and international obstetrical gynecology congresses. Currently, the form of admission consists of approval in a recruitment process organized by the board, in which the student should write a summary about endometriosis and gestational diabetes. **Conclusion:** The growth of academic gynecology leagues that handles women's health is essential for the involvement of medical students and those from other health care courses with the assistance to patients, the expansion of experiences, and theoretical training, but above all, is essential for the organization of events that provide contact with the patients, expanding actions on prevention and attention to care.

Keywords: medicine; gynecology; health.

Evolution of usual VIN in an immunosupressed patient

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Introduction: Vulvar intraepithelial neoplasia (VIN) is characterized by the abnormal growth of the vulvar epithelium. It can be classified in: classic and differentiated VIN. Classic VIN is commonly associated with oncogenic human papillomavirus (HPV) and, in 19% of cases, with n invasive lesion. Differentiated VIN, in turn, is often related to vulvar dermatological conditions, such as Lichen Sclerosus, and is strongly associated with invasive lesions. The incidence of VIN has increased, especially in young women. Immunosuppression helps to accelerate the progression of the disease as immune defense is compromised. **Objective:** To report a case of an immunosuppressed patient, in which there was a progression from classic NIV to squamous cell carcinoma of the vulva, submitted to a successful surgical treatment in a University Hospital. Case report: LCP, 40 years old, HIV-positive, undergoing antiretroviral therapy – viral load of 2,235 copies in February 2016, smoker. She was assisted at the Vulvar Pathology Outpatient Clinic of *Hospital Universitário Antônio Pedro* (HUAP) with a diagnosis of VIN II, with the excision of the injury having been indicated in 2009 but only performed in 2010 owing to the abandonment of follow-up. In 2015, she returned with multiple hyperchromic, keratotic, and grayish lesions on the vulva, suggestive of classic VIN, as well as an ulcerated lesion between labia majora and minora on the left side associated with pruritus and local pain. A biopsy of the hyperchromic lesion was performed, whose findings were a high-level vulvar squamous intraepithelial lesion and ulcerated lesion with a moderately differentiated squamous cell carcinoma. She underwent vulvectomy and bilateral inguinal lymphadenectomy in 2016, with postoperative infection of the surgical wound, together with clinical treatment, vancomycin, and tazocin. Histopathology confirmed a moderately differentiated squamous cell carcinoma, with a maximum invasion depth of 5 mm; surgical margins and inguinal lymph nodes were free of neoplasia, requiring no complementary therapy. **Conclusion:** Women infected with the human immunodeficiency virus (HIV) have four to six times greater incidence of developing VIN. This incidence decreases with the use of antiretroviral therapy, which shows the importance of immunity in the evolution of the disease. The intensity and duration of smoking contribute to the increased incidence, since tobacco induces immunosuppressive effects, facilitating the persistence of HPV, favoring the process of carcinogenesis. Thus, HIV-positive smokers present an accelerated progression of VIN and increase in the risk of postoperative complications.

Keywords: vulva; disease; HIV; smoking.

Acrokeratosis verruciformis of hopf: a differential diagnosis with vulgar warts – a case report

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Acrokeratosis verruciformis of Hopf (AVH) is a rare autosomal dominant genodermatosis, characterized by the occurrence of polygonal verrucous lesions and symmetrical hyperkeratous papules on the dorsum of the hands and feet. Verruca vulgaris (VV) presents itself by the occurrence of single or multiple papular lesions with rough surface, of varied sizes, being able to converge and to form great masses, being caused by the human papilomavirus (HPV). This study aimed at evaluating the differences and similarities between AVH and VV, determining the best diagnosis for the patients. We have a case report of a male patient, 38 years old, white, retired farmer, with no schooling and history of mental retardation. His caregiver reports the occurrence of injuries in childhood and is not aware of cases in the family. Dermatological examination presents verrucous micropapular lesions with rough surface, located symmetrically on the back of the hands, sparing the palms, middle, and distal phalanges. There are also lesions related to sun exposure with important photo damage, intense elastosis in the areas of exposure, and presence of open comedones in the frontal and cervical region. AVH is a disease commonly developed in childhood and characterized by multiple flat polygonal verruciform papules and hyperkeratotic papillae sized 2-6 mm in diameter, firm, of reddish-brown skin color. There are reports of association with mental retardation. Of chronic evolution, the lesions do not regress spontaneously. In VV, infection occurs in the superficial layers of the skin or mucosa. HPV transmission occurs through direct contact with infected people and/or objects. Small wounds are necessary, which is why they are most common in areas of trauma. They manifest through papules with hyperceratoses that appear anywhere, being more common in the fingers (periungual) and in the dorsum of the hands. The peak incidence occurs between 12 and 16 years of age. This patient presents, besides mental retardation, lesions from infancy, with multiple symmetrical papules of small diameter sparing distal phalanges and without histories of trauma at the sites, being thus more suggestive of AVH. The classic findings in the histopathology of AVH are hyperkeratosis, acanthosis, papillomatosis, absence of acantholytic cleft, and cellular atypia, whereas VVs' are hyperkeratosis, hypergranulomatosis, acanthosis, and vacuolization of the cells of the upper epidermis. Therapy is frustrating in AVH and recurrences are frequent. VVs respond better to the different existing treatments and rarely leave scars. The patient's histopathology is underway, being a good tool for the differential diagnosis between the two diseases.

Keywords: warts; acrokeratosis verruciformis of hopf.

"Preve em ação": awareness campaign of HPV vaccination and preventive actions

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Introduction: Human papillomavirus (HPV) infection is a public health issue owing to its association with cervical cancer, and therefore it is the most prevalent sexually transmitted disease (STD) in the world. Less than 15% of women in Brazil participate in cervical cancer prevention programs and in health educational measures. Awareness of the advantages of bivalent and quadrivalent vaccines is largely necessary at school, working along with children and adolescents' relatives. **Objective:** To discuss, with the scientific community, the relevance of ludic and active methodologies and the importance of increasing educational campaigns about prevention measures to relatives of children and adolescents at public schools. **Methods:** This is an experience study of qualitative approach. **Results:** First, scholars from *Universidade Estadual de Alagoas* (UNEAL) and *Universidade do Grande Rio* (Unigranrio) delivered lectures to relatives that lived in the community where the school is inserted, thus achieving an audience of 180 people, including men and women. The educational action was followed by the presentation of a theater play named "Is HPV a STD?," which was organized by the scholars and narrated, in a ludic and funny way, the story of patients with erroneous ideas and perceptions on STDs. It ended with a message on the importance of preventive measures and family support to break stigmas and myths associated with the theme. Brochures were distributed and a form was given to one member of each family, containing information about the perception of relatives on HPV and cervical cancer, as well as the identification of myths. The Free Informed Consent was signed.

Data collected from the activity were used only to prepare the school team for establishing the strategy of approaching relatives and students in subsequent campaigns. In the end of the activity, a choir presented a song parody about STDs and distributed the song lyrics to those participating in the event. **Conclusion:** HPV genital infection is the most prevalent STD in different age groups. In general, it occurs between 20 and 24 years of age. For controlling and combating HPV-related diseases, an educational and distinct work conducted by health and education teams at school and family clinics is essential, involving relatives of children and adolescents for breaking myths and clarifying doubts, and reinforcing the relevance of vaccination and general preventive measures.

Keywords: health education; human papillomavirus; medicine.

BEST ABSTRACT POSTER CATEGORY

STD antecedents and access to treatment among crack users from a Brazilian Northeastern state

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Introduction: Engagement in sex activities for drug acquisition, in addition to frequent unprotected sexual intercourses, has contributed to the increase of prevalence of sexually transmitted diseases (STDs) among crack users. Hence, access to health services has a fundamental role in the prevention and treatment of STDs. **Objective:** To estimate the prevalence of STDs and the access to health services for treatment among crack users from the state of Pernambuco, in Brazil. **Methods:** Prevalence study of a representative sample including crack users assisted at a social protection program — ATITUDE Program —, which was carried out from 2014 to 2015. Women aged 18 years or older who had used crack for at least 25 days in the last 6 months were eligible. Data were collected by means of a socio-behavioral questionnaire. Reporting wounds (ulcers) and small blisters or warts in the vagina or anus was considered an antecedent of STD. Pearson's χ^2 test was performed for data analysis, considering a 5% level of significance, with the support of the SPSS software, version 20. **Results:** 243 women were interviewed, most of them were aged between 25 and 34 years (45.3%); skin color was brown (66.3%); were single (70.4%), and had been living on the streets in the last 30 days (52.7%). With regard to crack, 79.4% of them referred using the drug compulsively before joining the ATITUDE Program. Regarding their sexual life, 54.7% had had more than 10 sexual partners during the 12 months before the interview; 66.7% had had sex in exchange of drug or money; and 55.6% did not use a condom in the last sexual intercourse. With regard to STD antecedents, 56.8% of interviewees reported vaginal discharge, 11.1% of them mentioned wounds (ulcers), 9.1% of them mentioned small blisters and 9.9% of them reported warts. Among women who reported at least one of these STD antecedents, around 15% did not seek the health service for treatment or orientations, whereas most (70%) of them sought this type of service. The most accessed health service was the family health unit (82.9%), followed by public hospitals (12.4%). As to the received orientations, 77.5% were advised to use condoms and 67.6% to inform their sexual partner about having a STD. Cure rate of STD antecedents among women who had access to health services for treatment (90.5%) was twice as high compared to those who reported not seeking a health service nor following orientations (40.9%) ($p \leq 0.05$). **Conclusion:** Access to health services was essential to treat and cure STD antecedents. Actions to facilitate the access of crack users to health services are important, such as the adjustment of working hours and execution of such activities in the streets.

Keywords: cocaine; crack; sexually transmitted diseases; health services.

Outubro Rosa [Pink October]: ludic methodologies in the development of projects regarding HPV and cervical cancer

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Introduction: Considered a public health issue, cervical cancer is the subject of fear and misconceptions in people's imagination. Such fact emphasizes the need for health educational interventions by using accessible and dynamic languages for the engagement of the population and disease's prevention. **Objective:** To discuss, with the scientific community, the relevance of ludic activities to approach human papillomavirus (HPV), cervical cancer, and breast cancer during *Outubro Rosa* campaigns. **Methods:** This is an experience report study of qualitative approach. **Results:** Activities of the *Outubro Rosa* campaign are included in an annual event organized by the Nursing department and count on the support of the Medicine program from *Universidade do Grande Rio* (Unigranrio). They occur simultaneously in several places at the University. The activities include providing services to patients in the outpatient clinic, performing lectures alternated between plays and songs about HPV, cervical, and breast cancers, as well as activities to raise awareness of patients in the waiting room. Groups of teachers, nurses, health students and members of the clown-doctors project were formed to work at the auditorium in theater plays, educational songs, and in the humanization of Unigranrio outpatient clinic. They provided a quiz for patients regarding HPV and cervical cancer concepts before patients were assisted by students wearing clown costumes and using white coats and stethoscope. Doubts on the themes were addressed by means of a game named *Jogo Rosa.* This is a game with questions and answers. All options were explained to patients including the reasons for right or wrong answers, followed by guidance, which applied humor and preserved the ludic aspect of the new educational and learning methodology. Risk factors, prevention methods, preventive examination performance, infection and HPV types, vaccine types for HPV, and importance of early diagnosis were discussed during the activities. The active and ludic approach obtained wide acceptance and interaction during the musical dialogue with choral formation among patients and scholars and final distribution of hearts in the waiting room as the campaign symbol and creation of bounds. **Conclusion:** Despite the growth of care to individuals with cervical and breast cancers, as well as higher investments on media communication interventions concerning HPV and cervical cancer, ludic activities supporting active comprehension and engagement are still poor. It is essential to integrate professionals and students from different areas in the formulation of dynamic strategies for awareness and prevention, using accessible language to target populations.

Keywords: care; health education; human papillomavirus; medicine.

A database analysis on vaccination against human papillomavirus in Sergipe state in 2014

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Introduction: The discovery of a vaccine to alter the natural course of cervical cancer and other cancers resulting from infection with human papillomavirus (HPV) has created high expectations in the medical community and in different segments of the population concerning the possibility of action at the primary level for the prevention of cervical uterine cancer and precancerous lesions. Prophylactic HPV vaccines have been developed since 1993, aiming at reducing infection and incidence of cervical cancer. After its approval, a great debate was raised involving the scientific community and the media on the risks and benefits of the new prevention method. The Ministry of Health of Brazil adopted, in 2014, the quadrivalent HPV vaccine as a preventive, but not therapeutic measure. It will be difficult to eradicate cervical cancer screening, since the vaccine does not provide protection against all oncogenic HPV subtypes or against other sexually transmitted diseases. Moreover, as vaccinating boys is not cost-effective, this group is not targeted to vaccination, although it is protected indirectly with vaccination of the female group, namely herd immunity. **Objective:** Analyze the data obtained from the vaccination program against HPV instituted in 2014 in the state of Sergipe, in girls aged 11 to 13 years, and quantify the target achieved by each micro-region of the state. **Methods:** Cross-sectional analysis, descriptive with retrospective component, using only secondary data from the State Department of Health, Sergipe, related to vaccination coverage against HPV in girls aged 11 to 13 years, from March 2014 to December 2014. Wilcoxon test was used for mean differences in the paired regions. **Results:** In the analysis of the study period, a total of 61,785 girls received the first dose of the vaccine (D1), reaching 103.25% coverage and of these, 30,561 received a second dose of the vaccine against HPV, generating 56.26% coverage. The quantity of second doses (D2) administered to girls decreased in all analyzed regions, as compared with the quantity of first doses (D1) administered in the same population. In this analysis, the 95% confidence interval were all small and all data were considered statistically significant at $p<0.001$. **Conclusion:** The study evaluated the HPV vaccine coverage in the state of Sergipe in 2014. We found that the regions have achieved the target vaccination of health state secretariat in the implementation of the first dose, but not achieved the same success in second doses.

Keywords: public health; papillomaviridae; vaccines.

Epidemiological profile of patients with cervical intraepithelial neoplasia at Santa Casa de São Paulo

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Introduction: Cervical cancer in Brazil is the second most common type of cancer among women. Human papillomavirus (HPV) infection is the main cause for the development of cervical cancer and cervical intraepithelial neoplasia (CIN). Screening is done by means of Pap smear tests, and one of the problems faced today is the low number of pap smears performed among women, which hinders early detection. **Objective:** To delineate the epidemiological profile of patients with CIN and to assess risk factors and possible standards involved in this population. **Methods:** A cross-sectional study was carried out including 82 patients that had been diagnosed with CIN by means of a general questionnaire. **Results:** Most of the patients were non-smoker, were aged around 38 years and belonged to the socioeconomic class C. With regard to the onset of sexual activity, the mean age was 18 years. Among the female patients, 57.31% did not use any contraceptive method in their first sexual intercourse; and among those who had used, 71.42% chose the male condom. On the other hand, among those in a current relationship, 79.03% had used contraceptive methods; however, only 32.65% had used the male condom. Knowledge of the subject was not sufficient; 26.61% of patients reported they did not know the meaning of a sexually transmitted disease (STD), 29.26% reported they were not aware of STD early diagnosis — even though all patients in the study are HPV-infected — and more than half of them did not know what HPV meant. The research showed that lack of knowledge mainly resides not only on the infection pathology — half of patients did not know which diseases can be HPV-caused —, but also on its transmission — it obtained 28.04% of correct answers — and on vaccination — 21.95% stated that there is no HPV vaccine. Among those patients diagnosed with CIN I (26.8%), 86.26% did not underwent the surgery and, among the CIN III patients (30.48%), 84% underwent the surgery that also had a correct, but incomplete, follow-up. All patients were aware of the oncotic cytology exam, 45.12% did not know its objective. The mean age of the first exam was 22.29 years old. **Conclusion:** We verified low adhesion to the use of male condom during their sexual intercourses, as well as a high number of smokers among the patients; therefore, we found important risk factors for the transmission and genesis of precursor lesions. Another relevant point is the perception of poor knowledge of the theme, which hinders prevention and adhesion to the treatment among this population, especially owing to the low educational level and social class of the interviewed subjects. This information is relevant for health promotion and prevention measures and it alerts to the key themes for future investments.

Keywords: cervical intraepithelial neoplasia; HPV; cervical cancer.

HPV and cervical cancer: reasons that influence elderly female subjects to not undergo Pap smear tests

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Introduction: The prevalence of elderly women that have an active and unprotected sex life is high. This may lead to high incidence of sexually transmitted infections (STI), such as the human papillomavirus (HPV). The age group recommended by the Brazilian Department of Health to undergo the preventive gynecological exam in women that had or have sexual activity — the Pap smear test — is between 24 and 60 years of age. However, it is important that older women also undergo Pap smear because women above 60 years of age are at risk of developing cervical cancer owing to the presence of HPV. **Objective:** To identify the reasons for low adhesion of elderly women to the prevention of cervical cancer. **Methods:** An integrative literature review was carried out in June 2016, based on the following guiding question: What are the reasons that influence elderly female subjects to not undergo the Pap smear test? Scientific productions were searched in the portal of the Health Virtual Library (BVS). The terms "adhesion", "aged," "prevention," and "cervical cancer" were used in a combined manner as keywords. The required inclusion criteria were articles between 2012 and 2016 that approached the theme and Brazilian and foreign complete works. The exclusion criteria included articles that analyzed elderly female subjects with cervical cancer. **Results:** Even though the number of women above 60 years old with HPV has been increasing, aged subjects continue facing difficulties associated with the cancer preventive exam. Among the reasons found for low adhesion are: unawareness, advanced age, insecurity, socioeconomic level, no partner, or low educational level — lack of information about sexuality in the third age is one of the main reasons why elderly female subjects do not undergo exams or perform gynecological prevention. Therefore, their behavior may facilitate STI contamination and increase their vulnerability for the development of cervical cancer. **Conclusion:** There are few studies on aspects concerning elderly women's sexuality that influence the routine performance of the Pap smear test; therefore, it is upon every health professional to provide guidance to elderly women on the importance of performing gynecological exams to prevent cervical neoplasm. The probability of early control and diagnosis of cervical cancer is believed to increase among this population, as they acquire knowledge and perform preventive practices.

Keywords: aged; prevention; HPV.

Nursing interventions for human papillomavirus prevention in primary health care

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Introduction: The Human papillomavirus (HPV) is a sexually transmitted infection (STI) capable of causing skin or mucosa lesions. The virus has more than 100 recognized types, of which types 16 and 18 can cause cervical cancer. As health professionals in Primary Care, nurses has several attributions, including the performance of preventive gynecological examinations such as the Pap Test, which is able to detect cell changes caused by HPV. It is considered the best method for cervical cancer and precursor lesions detection. **Objective:** Analyze the HPV prevention work performed by nurses in primary care. **Methods:** This is a literature review study. The Scientific Electronic Library Online (SciELO) and the Latin American and Caribbean Health Sciences (LILACS) databases were used in this study. The database search was conducted in June 2016, using the following descriptors: prevention, sexually transmitted diseases, nursing, HPV. The inclusion criteria consisted of articles published in the period from 2011 to 2016. **Results:** The primary role of nurses in primary health care is to prevent all types of diseases, among which is cervical cancer related to HPV. Through regular check-ups and health education activities, nurses administer quadrivalent vaccination to girls (within the age range recommended by the Ministry of Health); organize community group activities; provide guidance on the importance of wearing condoms on STI prevention; as well as perform Pap tests. Thus, in order to prevent HPV infection, in addition to early cervical cancer diagnosis, it is necessary to encourage preventive services aimed at reducing short and long term complication to minimize the effects of the disease. **Conclusion:** Nurses play a vital role in disease prevention and health optimization, and must have knowledge of proper patient evaluation. It is their duty to help patients truly understand the need to attend regular consultations and perform routine tests as a way of strategically preventing STIs and their complications.

Keywords: HPV; nursing; primary health care.

CD8 infiltration correlation and expression of its checkpoint proteins PD L1 and PD L2 with cervical carcinoma stages

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Introduction: The importance of CD8 infiltration in cancer prognosis has been underscored in recent times by the increased use of checkpoint inhibitors in patients with invasive tumors. **Objective:** To determine if CD8 infiltration and the expression of its two key checkpoint proteins, PD L1 and PD L2, vary between early stage (FIGO IA-IIA) and advanced stage (FIGO IIB-IVA) cervical cancer. **Methods:** A micro-array tissue with 61 cervical specimens was analyzed through immunohistochemistry for PD L1, PD L2, and CD8. Antigen presenting cells CD1a served as internal control. **Results:** CD8 and CD1a infiltration was evident in control tissues whereas little to no PD L1 and PD L2 was present in benign tissues. There was a three-fold increase in the number of CD8 cells in invasive cervical cancers with simultaneous dramatic rise in PT L1 and DP L2 expression (each $p<0,001$ *versus* benign control). There was a slight decrease in the numbers of CD1a cells in the malignant tissues, if compared to benign ones. No significant difference was found in either CD8 infiltration, or in PD L1 and PD L2 expression between early stage (FIGO IA-IIA) and advanced stage (FIGO IIB-IVA) cancers. **Conclusion:** Cytotoxic T cell infiltration and expression of two of its key checkpoint proteins (PD L1 and PD L2) remains constant as cervical cancers advance from early stage to late stage tumors. This suggests that the immune response may be equivalent in early and late stage cervical cancers.

Keywords: HPV; cervical intraepithelial neoplasia; cervical cancer

Prevalence of epithelial changes in the anal canal of females with human papillomavirus genital infections

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Introduction: Anal cancer is the second most common anogenital cancer caused by the human papillomavirus (HPV). In general, a history of anal intercourse is strongly associated with the occurrence of anal cancer. Persistence of HPV infections is considered the cause of this type of cancer. Similarly to the case of cervical cancer, we can be assumed that 85% of anal cancer cases that occur every year worldwide are caused by this virus as a result of a sexually transmitted infection (STI). **Objective:** To determine the prevalence of atypical anal lesions and changes in the anal cytology of women with genital HPV. **Methods:** This is a prospective cross-sectional study carried out at the Cervical Pathology Center of the *Universidade Federal de Sergipe* (UFS) from August 2015 to October 2015. Patients with genital condylomatosis and premalignant lesions were considered eligible for the study and underwent anoscopy preceded by anal cytology. Anoscopies and cytologies were performed in 34 patients. **Results:** Among the anoscopies performed, 7 (20.6%) of them resulted negative and the remaining anoscopies (79.4%) were positive and presented the following types of injuries: acetowhite epithelium, mosaic, and spiculated lesions. These patients were then referred to anorectal mucosal biopsy. Eleven (32.4%) of the cytologies performed were negative, whereas 18 (52.9%) were positive for the presence of koilocytes. Five (14.7%) of the tests proved unsatisfactory. No intraepithelial anal neoplasms (IANs) were detected in the cytologies. The mean age of patients was 25.5 years, with a standard deviation equal to 3.53535. 17.64% of genital lesions found were low-grade, whereas high grade ones were equivalent to 11.76%, and 70.58% were condylomas. **Conclusion:** There was a prevalence of positive results for both the anoscopies and cytologies performed. Cytologies showed no signs of IANs.

Keywords: Human papillomavirus; women's health; anal cancer.

HONORABLE MENTION POSTER CATEGORY

PCR detection of HPV DNA-specific sequences to L1 ORF using consensus primer and degenerated primer systems

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Introduction: Papillomaviruses present a circular double-stranded DNA containing approximately 8kbp, belong to the *Papillomaviridae* family, comprising 39 genera of high genetic variability. **Objective:** This study investigated the molecular detection of viral DNA amplifications in the L1 open reading frame (ORF), the most conserved region during polymerase chain reaction (PCR) and nested PCR (nPCR) using consensus primer and degenerated primer. **Methods:** DNA extractions — through swabs, blood and tissue collection — were performed using the QIAmp DNA mini kit according to manufactures's instructions. The internal quality control of the genomic DNA was performed by the β-globin gene using the primers GH20 (5`- GAAGAGCCAAGGACAGGTAC-3`)/PC04 (5`-CAACTTCATCCACGTTCACC-3`), that amplify 268bp. Partial amplification of the L1 gene was performed by PCR with MY09 (5`-CTCCMARRGGAWACTGATC-3`)/MY11 (5`-GCMCAGGGWCTATAAYAATGG-3`) oligonucleotides and degenerated primers as forward FAP59 (5`-TAACWGTIGGICAYCCWTATT-3`) and reverse FAP64 (5`-CCWATATCWVHCATITCICCATC-3`), which was designed originally by Swedish human samples amplifying a 478bp fragment. To increase the specificity and technical sensitivity of the target DNA, nPCR was performed using GP5+ (5`-TTTGTTACTGTGGTAGATACTAC-3`)/GP6+(5`-GAAAAATAAACTTGTAAATCATATTC-3`), that amplifies fragments of 150bp. In order to optimize the molecular reaction, samples of standard SiHa and HeLa cell lines ($3x10^6$ cells) were used as positives controls and a mixture without DNA as negative control. **Results:** PCR and nPCR products of human papillomavirus DNA-specific (HPV DNA-specific) bands were identified by gel electrophoresis at 1%. All samples tested were positive for the β-globin PCR gene showing some bands stronger than others. HPV DNA amplification was detected in all pairs of PCR primers. As expected, nPCR amplification efficiency increased using GP5+/GP6+ primers. **Conclusion:** This molecular system has been used for detection of HPV DNA-specific sequences to L1 ORF. The amplified products will be purified and sequenced. The choice of primers for clinical and epidemiological studies is critical in regard to the type of biological material to be investigated.

Keywords: deoxyribonucleases; papillomaviridae; PCR; oligonucleotides.

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HPV virus-like particles (VLP): morphological alterations of HPV-positive (SiHa and HeLa) human cervical carcinoma cell lines as possible prognostic markers of cervical cancer

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Introduction: Papillomaviruses constitute a family of epitheliotropic and mucosotropic viruses presenting closed circular double-stranded DNA genome. The expression of genes and the role of proteins involved in DNA damage repair pathways in cell lines as primary human keratinocytes (PHK) and HPV-positive — SiHa – HPV-16 and HeLa – HPV-18 — and HPV-negative (C33A) human cervical carcinoma cell lines, as well as immortalized keratinocyte cell lines — HaCaT, not tumor control — have been described as possible prognostic markers of cervical cancer. Some studies have investigated the ability of cytokine to inhibit the proliferation *in vitro* of normal and HPV infected keratinocytes, as well as the expression of E6 and E7 oncogenes. Cytokines include the growth factor (TGF-β), tumor necrosis factor (TNF) and interferons type I (IFN-α and IFN-β), which are produced by epithelial cells. The cytokine TGF-β has proved to be an inducer and inhibitor of growth of tumor cells not infected by HPV 16 and 18. This effect appears to be associated with inhibition of E6 and E7 expression. IFN-α inhibits transcription of E6 and E7 genes in HPV-18 infected HeLa cells and also inhibits the expression of the E7 protein of HPV-16. In contrast, few studies have investigated the cellular pathomechanisms and morphological changes in the host cell. **Objective:** This study reports ultrastructural cell morphology in samples of bovine papillomavirus (BPV) virus-like particles (VLP). Later we describe morphological alterations inside SiHa and HeLa cell lines ($3x10^6$ cells) through electron microscopy. Few studies have assessed the transmission of electron microscopy in different cell lines. **Methods:** For ultrastructural analysis, the specimens — warts and SiHa and HeLa cells — were embedded in epoxy resin, fixed in 1% glutaraldehyde and post-fixed in 1% osmium tetroxide. Later, the specimens were immersed in cacodylate buffer 0.2 M in sodium sucrose 0.7% and distilled water. The dehydration steps were performed. Warts and cell lines were included in epoxy resin and kept at 60°C to complete polymerization. Ultrathin sections and semi-thin sections were performed. **Results:** Morphologically, electron microscopy detected very electron-dense cells presenting well-developed mitochondria and rough endoplasmic reticula (rER), many vesicles and ribosomes in HeLa and SiHa cell lines. Cellular modifications similar to antigen-presenting cells, many activated mitochondriae and well-preserved vesicle transport were also observed. Furthermore, the presence of VLP and cellular junctions like desmosomes were also detected in BPV samples. **Conclusion:** These morphological alterations suggest high activity of HPV-positive — SiHa and HeLa — cell lines can be possible prognostic markers of cervical cancer.

Keywords: biomarkers; cells; papillomaviridae; ultrastructure; virus.

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Three-dimensional resources in the making of paradidactic books on sexually transmitted infections to the promotion of deaf patient accessibility

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Introduction: According to the Brazilian Institute of Geography and Statistics (IBGE), 23.9% of the population has some kind of disability, and 5.1% has some kind of hearing impairment, 1.12% suffering from severe hearing loss. By law, deaf people have a right for the Brazilian Sign Language (Língua Brasileira de Sinais - LIBRAS) and to bilingual education in school. However, in a scenario of linguistic minority, it is important to note the absence of physicians who can communicate in LIBRAS, as well as the lack of interpreters in various public services. An aggravating fact in this scenario is that high school is the highest educational level currently attained by most active interpreters, which may undermine the performance of their activities – translation and interpretation – in sensitive situations experienced by the deaf, such as medical appointments, due to either lack of knowledge about the subject and/or its scientific terminology. **Objective:** To develop materials in LIBRAS aimed at the health field which can be used in non-formal teaching environments such as doctor's offices and clinics. **Methods:** This study will be developed using a qualitative method. In our research, participants will be from the Institute of Dermatology Prof. Rubem Davi Azulay, Public Health in Dermatology Service (Leprosy/STD), 29th Clinic, *Santa Casa de Misericórdia de Rio do Janeiro* (SCMRJ). The development of a series of bilingual books (LIBRAS/Portuguese) that use three-dimensional (pop-up) resources is intended to facilitate a dialogue between physicians and their deaf patients, in order for them to understand the most prevalent sexually transmitted infections (STIs) in Brazil – chancroid, candidiasis, chlamydia, granuloma inguinale, gonorrhea, hepatitis B, genital herpes, human papillomavirus (HPV), syphilis, HIV/AIDS, and trichomoniasis. **Results:** Currently, there is a shortage of materials addressing ISTs available in sign language, and existing ones are usually very brief, exclusive and restricted to manuals describing contraceptive methods and some STIs and/or information about human reproduction. **Conclusion:** The edition of the

"Paradidactic Bilingual *pop-up Books*" series, which will promote the linguistic accessibility for the deaf through visual means, may generate an opportunity for integration between deaf and non-deaf persons. This strategy can help ensure that the deaf are able to gain access to knowledge about several concepts important to their health, enabling them to fully exercise their capabilities in society. The series is intended to be a more inclusive paradidactic approach for teaching about STI prevention not only to deaf individuals, but also to non-deaf ones, which may assist in reducing the STI incidence and enabling the exercise of sexual citizenship and respect between partners.

Keywords: education; sexually transmitted infections; health; deafness.

STDs in focus: educational actions with adolescents in the northeast region ofBrazil

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Introduction: The sexuality subject is surrounded by several taboos, questions, information deficiencies, and fears by the general population, especially among teenagers. However, the idea of addressing problems related to prejudices and the onset of sex life in schools hinders the introduction of interventionist actions, creating barriers that interfere with practices for health care education on the human papillomavirus (HPV), the human immunodeficiency virus (HIV) and adolescent pregnancy. **Objective:** Dissemination of ludic methodologies about HPV to adolescents in municipal schools and discussions on their use, which are included in an annual project on sexually transmitted diseases (STDs), conducted in a community in Alagoas. **Methods:** Qualitative study, experience report type. **Results:** The implementation of the project was conducted collectively – teachers, social workers, and health care professionals who work with the community in the area – after cases of sexual violence on the site were studied. After family members, students, and teachers were interviewed, we identified students' lack of knowledge on the subject. In addition, teachers do not address issues related to sex education and STDs. The project is conducted twice a year with male and female students enrolled in the school, aged 15 to 18 years. Questionnaires are administered before intervening on adolescents' general perception regarding HPV and STDs, for a future piecemeal approach related to questions and the youthful imaginary. The front for health care education on HPV, within the STD project is associated with the HIV/AIDS workshop, which is coordinated by medical and nursing students, school teachers and other volunteers, after a training lecture. Initially, adolescents are divided into small groups that have a monitor; this monitor conducts educational activities related to HPV and HIV, based on a primer developed by project members. Envelopes with fictional and real life stories of young people in situations such as those found in the community are distributed to pairs of adolescents. These stories involve issues associated with STDs, such as rape, adolescent pregnancy, pedophilia, and prostitution. Pairs report and discuss aspects of the stories, analyzing each case critically. Conclusively, monitors reinforce concepts regarding prevention, diagnosis, treatment, and ways of coping with the social conditions of vulnerability exposed. **Conclusion:** A strong resistance from the community and the team of education on the subject for children and adolescents was observed in literature and during the stages of project elaboration. Education and awareness about issues related to sexual health and STDs are still mistakenly confused for indirect stimuli for sexual practices. To change the perception as well as create an approach that is responsible and open is imperative, straitening bonds of trust and safety in the school and with health care professionals.

Keywords: HPV; adolescents; STD.

The used project: intervention on women's health in communities in the northeast region of bBrazil

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Introduction: Cervical cancer is the leading cause of cancer deaths among women and the second most common cause for this group in the world. The insubstantiality of women's perception of the disease, along with the fear of the diagnosis and the preventive exam, is a barrier that negatively influences the diagnosis and treatment process. **Objective:** To discuss the relevance of the work of educational groups for women's health focused on sexually transmitted diseases (STDs) and cervical cancer with the scientific community, encompassing preventive actions in the social and family history. **Methods:** Qualitative approach as an experience report. The program began in 2014 and is conducted twice a year with a group of 50 women from the Youth and Adult Education Program (EJA) in a municipal school in Alagoas, monitored by 10 volunteers, including medical, nursing, and social service students as well as teachers. **Results:** Activities start with groups being subdivided into rooms that hold health care workshops with different approach types, called the "Female Circuit". The workshops address issues such as women's rights, family planning, the role of women in the family and the community, violence against women, stories of great women in Brazil and the world, independence and health. **Conclusion:** The perceptions and knowledge about cervical cancer, the human immunodeficiency virus (HIV), and STDs in general are still contaminated by myths and misconceptions about prevention and diagnostic techniques. Confronting fears related to the diagnostic confirmation of a stigmatized illness and the situation of vulnerability involving women is essential as a front against cervical cancer. Such work must be carried out through a partnership between several fields that monitor women from the community in their school, family, and health care environments, acting in a clear and dynamic way in the target population.

Keywords: HPV; health promotion; STD.

Mapping of the disruption of the E1 and E2 genes during the integration of HPV16 as a tool in the assessment of progression of cervical diseases

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Introduction: Persistent infections with human papillomavirus (HPV) are associated with the development of cervical cancer, which is a significant cause of morbidity and mortality in women worldwide. To date, 12 types of HPV are considered oncogenic; HPV16 is considered the main type, comprising almost 50% of cases. Generally, integration occurs within the fragile regions of the E1 and E2 viral open reading frames (ORFs) and is considered a late event in cervical carcinogenesis. The elimination of the expression of E2 results in the transcriptional dysregulation of the E6 and E7 viral oncogenes. That leads to the increased expression of both oncoproteins, which target the p53 and pRb tumor-suppressor proteins, respectively; among other changes, the results are loss of cell cycle control, changes in DNA, and active telomerases. **Objective:** To evaluate the physical form – either episomal or integrated – of the HPV16 genome and establish the mapping of the disruption of the viral genome within the investigated region. **Methods:** Ten sets of pairs of primers that cover the entire E1-E2 region were used to assess the physical form of the HPV16 genome, as described in isolated polymerase chain reactions (PCRs). **Results:** Our results comprise 91 samples of patients infected with HPV16, with cervical lesions in different stages of progression. Of the analyzed samples, 16 (17.58%) were episomal, 38 (41.75%) were concomitant (mixed) and 37 (40.65%) were integrated. The E1A region was the one most frequently absent (66% – 60/91): 36 (39.56%) were fully disrupted and 24 (26.37%) were partially disrupted; E1A was followed by the E2C region (39.56% – 36/91): 13 (14.28%) were fully disrupted and 23 (25.27%) were partially disrupted. Literature demonstrates the predominance of disruption in the E2 region, but our results suggest the high prevalence of E1 disruption. **Conclusion:** The methods described are rather specific and enable the mapping of the fragile regions of the HPV16 genome. The absence of E1 and E2 is most common in high-grade squamous intraepithelial lesions (HSIL) and cancer; however, in a few cancer cases, episomal forms are found, suggesting that additional biomarkers are responsible for carcinogenesis.

Keywords: PCR; HPV, cervical cancer.

Support: *Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro* (FAPERJ) and *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)*.

Prevalence of anal HPV in a cohort of subjects infected with HIV-1

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Introduction: HPV is the primary etiologic agent of anogenital tract cancer. A higher prevalence and incidence of cancer and diseases associated with HPV have been observed in HIV-infected individuals. Neither the natural history of HPV infection nor the immune response that occurs in HIV/HPV coinfection, particularly in the anal mucosa, has been completely elucidated. **Objective:** To analyze the HPV prevalence as well as the clinical, epidemiological, and behavioral data in a cohort of HIV-seropositive subjects from the National Institute of Infectious Diseases, from the *Fundação Oswaldo Cruz* (Fiocruz), in Rio de Janeiro. **Methods:** The study included 114 subjects with a histopathological diagnosis

based on the anal biopsy. Polymerase chain reaction (PCR) and sequencing was performed for HPV DNA typing in anal discharge. Statistical analysis was performed using the SPSS 15.0 software. **Results:** HIV-infected patients with AIN II/III had CD4 + nadir < 50 cells/mm³ in comparison to normal patients (p = 0.01). The most prevalent HPV types in the anal secretion (by PapilloCheck®) were HPV16 (29.2%), followed by HPV52 (23.1%), both of which are high-risk oncogenic, followed by HPV44 and HPV55 (21.5%), which are low-risk. A total of 53.3% of the analyzed HIV-infected subjects have already been exposed to the four HPV types targeted by the current quadrivalent vaccine (HPV types 6, 11, 16, and 18). **Conclusion:** The data suggest that vaccination against HPV could be regarded as a prophylactic measure to reduce the risk of anal intraepithelial lesions in HIV-infected individuals.

Keywords: papillomaviridae; vaccines; homosexuality; women; anal cancer.

Quadrivalent HPV vaccine for males in Campos dos Goytacazes (RJ) – experience beyond the limits of the federal government

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Introduction: The human papillomavirus (HPV) is a sexually transmitted virus that causes genital warts and several types of cancer with high morbidity and mortality rates. In addition to being the primary cause of the cervical cancer agent, it is also related to the malignant transformation in the anus, penis, and oral cavity. The quadrivalent HPV vaccine Gardasil® was introduced in 2014 to the Brazilian National Immunization Program for girls aged 11–13 years. Remarkably, the municipality of Campos dos Goytacazes, in the state of Rio de Janeiro, using its own funds, introduced the vaccine into the municipal vaccination calendar on September 13th 2010, offering it for female residents aged 11–15 years. The introduction of this vaccine for boys aged 11–13 years started in March 2014 only in the municipality, simultaneously with the nationwide implementation for girls. Furthermore, there were other municipal recommendations for the quadrivalent vaccine that exceeded federal ones. Objectives: To describe the experience of the municipality of Campos dos Goytacazes with the introduction of an alternative HPV vaccination schedule, going beyond the limits of the ministry of Health. **Methods:** This is an experience report describing local decisions to overcome federal government limitations, recommending other indications for HPV vaccination, not only for teenagers. This report shows that the municipality created its own HPV vaccine limitations, based on international literature and other countries' successful experiences. The most important recommendations adopted were: HPV vaccination for boys aged 11–13 years; vaccination for HIV-positive males aged 9–26 years; and vaccination against recurrent genital warts and laryngeal papillomatosis. **Results:** HPV vaccination for boys in 2014 comprised 6,060 first doses and 3,750 second doses; the vaccination total covered 57.7% of boys. In 2015, the boys' interest in vaccination decreased, with a 45% of vaccination coverage. Since 2014, more than 120 men living with HIV/AIDS received at least one dose of the vaccine. The vaccination coverage for HIV/AIDS men was 80% for the target vaccination age. Moreover, 50 boys and men were vaccinated against recurrent genital warts and laryngeal papillomatosis. **Conclusion:** The Australian experience showed that the public decision to start vaccination of boys is not only political, but also cost-effective in reducing over 20% of cancers and genital warts related to HPV, at least. However, such result cannot be reached by the herd immunization of a girls-only vaccination program (e.g.: men who have sex with men). Furthermore, the Ministry of Health should immediately introduce a vaccination program for HIV-positive men into the health care system considering the benefits vaccines bring to the population by preventing anal and other HPV-associated cancers. Municipal studies are being conducted to verify the effectiveness of the HPV vaccine in reducing recurrent genital warts and laryngeal papillomatosis.

Keywords: HPV; Human Papillomavirus Recombinant Vaccine Quadrivalent, Types 6, 11, 16, 18; male.

BEST ABSTRACT ORAL PRESENTATION CATEGORY

Prevalence of papillomavirus in Brazil: the pop-Brazil study protocol

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Introduction: Cervical cancer is the third most common tumor in women and the fourth leading cause of death among this population in Brazil. Besides causing cervical cancer, the human papillomavirus (HPV) is also associated with penile cancer and genital warts. Moreover, the incidence of oropharyngeal cancer associated with HPV has increased considerably. Most studies conducted in Brazil include participants who attended specific health care facilities for screening or treatment; there are no studies covering the whole country. **Objective:** In this study, we will assess the prevalence of HPV in women and men aged 16–25 years in Brazil and its five geographic regions and examine demographic, socioeconomic, behavioral, and regional factors associated with the presence of HPV and its viral types. **Methods:** Participants will be recruited at Primary Health Care Centers (PHCs) from all 27 Brazilian state capitals and selected according to catchment area. Data will be collected by trained health care professionals – nurses or physicians – and at PHCs. Data will include sociodemographic factors such as age, race, ethnicity, income; behavioral data such as sexual behavior as well as use of tobacco, alcohol and other drugs; health information: history of parity/miscarriages, use of contraceptive methods, history of sexually transmitted diseases, genital warts, and oral lesions; knowledge of HPV; as well as vaccination and biological sampling for cervical, penile, and oral HPV. The recruitment of 7,935 subjects is anticipated. Roche's Linear Array® will be used to detect HPV genotypes. The prevalence will be estimated to each region's population size. Multilevel modeling will be used to examine risk factors associated with HPV positivity and certain genotypes. **Results:** The results of this study will provide a baseline to assess the impact of future vaccination. Our results will also provide information on the prevalence of papillomavirus across Brazilian regions, factors associated with HPV infection, and groups at higher risk for the disease. The concurrence of oral and genital infection among healthy subjects will also be evaluated. **Conclusion:** Surveillance planning and control measures are restricted by the lack of systematically collected national data. Therefore, this study will contribute to the epidemiological knowledge required to strengthen and redirect policies to control cervical cancer and HPV infection in Brazil.

Keywords: HPV; prevalence; Brazil.

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- Identifying strategies to prevent and treat HPV associated diseases
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- Decision making in HPV associated diseases
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