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Editorial

Challenges in promoting HPV vaccine in the era of web 2.0: understanding the tricks and tropes of anti-vaccinologists

The National Geographic magazine cover from March 2015 issue states that there is a war on science: climate changes do not exist, evolution has never happened, moon landing was fake, genetically modified food is evil, and vaccinations can lead to autism.

In order to analyse anti-vaccine communication strategies, we need to review the content of web 2.0 media, such as anti-vaccine websites, blogs, social media, articles as well as rebuttal actions from pro vaccine advocates. We have to agree that the anti-vaccine activists have made very good use of the Internet. Opinions are presented as evidence-based information. Web 2.0 media like websites, blogs, social media and classical communication tools such as paper media articles in journals, television and publication in obscure scientific journals are easy to find on the Internet and frequently come up first following a search on browsers. Tropes used by the anti-vaccination movement (i.e. oft-repeated mottos, phrases, and rebuttals) include:

- "I'm not anti-vaccine, I'm pro-safe vaccines": denying one opposes vaccination, instead claiming they are for safer vaccines and further research.
- "Vaccines are toxic!": listing potentially toxic vaccine ingredients, while providing disingenuous explanations of their dangers (a.k.a. the "toxin gambit").
- "Vaccines should be 100% safe": because absolute safety cannot be promised, vaccination is therefore flawed and dangerous.
- "You can't prove vaccines are safe": demanding vaccine advocates demonstrate vaccines do not lead to harm, rather than anti-vaccine activists having to prove they do.
- "Vaccines didn't save us": attributing improvements in health over recent decades to factors other than vaccines (e.g. better sanitation).
- "Vaccines are unnatural": designating something "natural" to be the better option (e.g. naturally acquiring immunity from diseases rather than from vaccination).
- "Choosing between diseases and vaccine injuries": framing vaccination choices as restricted between undesirable outcomes (e.g. catching a disease versus serious vaccine side-effects).
- "Galileo was persecuted too": invoking the names of subjects persecuted by scientific orthodoxy, implying ideas facing close-mindedness will eventually gain acceptance (a.k.a. the "Galileo gambit").
- "Science was wrong before": citing prior instances of scientific errors to imply that scientific evidence supporting vaccination is also in error.
- "So many people can't all be wrong": implying anti-vaccine claims are true because many people support such ideas.
- "Skeptics believe…": ascribing false reasons to vaccine supporters, which are then easily attacked.
- "You're in the pocket of Big Pharma": claiming those supporting vaccines do so because they are hired by pharmaceutical companies (a.k.a. the "pharma shill gambit").
- "I don't believe in coincidences": rejecting that health problems can occur coincidentally after vaccination.
- "I'm an expert on my own child": redefining expertise, where parents are the experts on their own children while medical authorities are discounted.

All these anti-vaccine publications, interactions and user-generated content have become ubiquitous. They foster a new postmodern paradigm of healthcare that has shifted from doctors to patients. The legitimacy of science is questioned, and expertise has a new meaning. The techniques used by the anti-vaccination movement are cunning, for not only their protests are camouflaged in unobjectionable rhetoric such as "informed consent", "health freedom", and "vaccine safety", but they also take advantage of the current postmodern medical paradigm. Calls to "do your own research before vaccinating" dovetail with the postmodern characteristics of patient empowerment and shared decision-making, where individuals play a more involved role in their healthcare.

It is likely that anti-vaccine websites can influence whether people vaccinate themselves or their children. The types of rhetoric used by the anti-vaccination movement can be convincing, despite lacking scientific support for their claims. This includes actions such as skewing science, shifting hypotheses, censoring dissent, and attacking criticisms. Health professionals have little time to discuss these raised issues and will frequently skip the issue of vaccination over going in a long discussion explaining the safety and reasons of vaccine further legitimizing those issues. Strong statements from health care professionals remain an important reason why parents decide to vaccinate their kids.

What solutions exist to quell these fears? Some proposals include "immunizing" against misinformation through education using emotional or even harnessing social media in return — such as by creating web-based decision aids about vaccination using real-time Internet tracking to determine public attitudes or launching

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social media campaigns. While it is important to attempt such efforts and combat the misinformation that exists, it is doubtful that the anti--vaccination movement can ever be completely quashed. For instance, emotional narratives about vaccine side effects have been found to increase risk perception and uptake intention to a greater extent than statistical information, demonstrating the power of emotional appeals and anecdotes over educational efforts. With many people desperate for answers, invested in their belief systems, and distrustful of authorities, it is unlikely that "the facts" alone will ever sway the truly committed. Some individuals choose to disregard the evidence, and are essentially denialists.

However, the pendulum is swinging. Whereas prior coverage was supportive and largely unquestioning, popular media outlets have begun to lambaste the anti-vaccination movement. Various articles have condemned the movement and its representatives. A PBS Frontline documentary did not portray the movement in a positive light and Robert F. Kennedy Jr.'s error-laden article on vaccines and thimerosal was removed from Salon.com, after acknowledging that keeping posted a corrected version was a disservice to the population. Such coverage is distinct from past reporting in that it largely ignored the journalistic mantra of "balance", where both sides of the issue are afforded equal time and respect, thus equating the positions. A particularly large blow to the anti-vaccine movement credibility was dealt by investigative journalist Brian Deer, whose series of articles exposed Andrew Wakefield's research as fraudulent. Australia has forced the Australian vaccination network to change its name to the Australian Vaccination-Skeptics network and was stripped of its fundraise capacity. In Canada, the Canadian alliance to support immunization has ended after the Toronto Star removed an article that was strongly biased on Gardasil and won its cause since the journal withdrew the article from its archive.

Thus, it is possible that the minds of deeply invested anti--vaccine activists may never be changed. Therefore, it is for both the laypersons with genuine questions or worries about vaccines and the healthcare professionals who work to ease their fears, that keeping abreast of the methods of persuasion discussed here is essential. Recognizing anti-vaccine tactics and tropes is imperative for an awareness of the disingenuous arguments used to cajole, and convert audience gives individuals the tools to think criticize the information they encounter online. It is through such recognition that truly informed choices can then be made.

I will conclude citing Hillary Clinton's tweeter feed from February 3rd 2015: "The Earth is round, the sky is blue and vaccines work. Let's protect all our kids. #grandmothersknowbest".

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Influence of human papillomavirus infection on the vaginal microbiome of women with immunocompetency

Influência da infecção por papillomavirus humano no microbioma vaginal de mulheres imunocompetentes

Karinne Cisne Fernandes Rebouças[1], José Eleutério Junior[2], Renata Mírian Nunes Eleutério[3], Kathiane Lustosa Augusto[1], Karla Maria Rêgo Leopoldo[1]

ABSTRACT

Introduction: The influence of vaginal infections on the natural history of human papillomavirus (HPV) is still unclear. **Objective:** To determine if patients with low-grade squamous intraepithelial lesions (LSILs) and HPV have more vulvovaginitis than patients with normal liquid-based cervical cytology who were negative for HPV. **Methods:** This is a cross-sectional study including 322 patients who underwent cervical exams. One hundred and sixty-seven of these patients had LSILs on cervical cytology and were simultaneously hybrid capture 2 (HC2)-positive for HPV, and the remaining 155 patients were negative for malignancies and intraepithelial lesions by cytology and HC2-negative for HPV. The prevalence of vaginal infections in both groups was compared using the χ^2 test without Yates' correction. **Results:** Among the patients with HPV and LSILs, the most common vaginal infection was vaginosis (8.98%) compared to candidiasis (12.9%) in the patients without LSILs and HPV. No significant differences were found in the prevalence of vaginosis between the two groups (p=0.53). Candidiasis was statistically more prevalent in patients without LSILs and HPV (p<0.001). **Conclusion:** An association was found between the presence of *Candida* and the absence of HPV. Although vaginosis was more frequent among patients with LSILs and HPV, it was not statistically significant.

Keywords: papillomavirus infections; Papanicolaou test; vaginitis.

RESUMO

Introdução: A influência das infecções vaginais na história natural do papillomavirus humano (HPV) ainda é incerta. **Objetivo:** Determinar se pacientes com lesões intraepiteliais escamosas de baixo grau (LIEBG) e HPV têm mais vulvovaginites que aquelas com citologia cervical em meio líquido normal e testes negativos para HPV. **Métodos:** Este é um estudo transversal, que incluiu 322 mulheres que fizeram exames de colo. Cento e sessenta e sete destas tinham LIEBG na citologia oncótica e foram simultaneamente positivas para HPV na captura híbrida 2 (CH2). As outras 155 tiveram citologias negativas para neoplasia intraepitelial e malignidade e eram CH2 negativas para HPV. A prevalência de infecção vaginal nos dois grupos foi comparada usando o teste do χ^2 sem correção de Yates. **Resultados:** Entre as pacientes com HPV e LIEBG, a infecção vaginal mais comum foi a vaginose (8,98%), enquanto que, no grupo sem LIEBG e sem HPV, foi a candidíase (12,9%). Nenhuma diferença estatisticamente significante foi encontrada na prevalência de vaginose entre os dois grupos (p=0,53). Candidíase foi estatisticamente mais prevalente nas pacientes sem LIEBG e HPV (p<0,001). **Conclusão:** Foi encontrada uma associação entre a presença de *Candida* e a ausência de HPV. Embora a vaginose tenha sido mais frequente em pacientes com LIEBG e HPV, esse dado não foi estatisticamente significante.

Palavras-chave: infecções por papillomavirus; teste de Papanicolaou; vaginite.

INTRODUCTION

The cervicovaginal milieu is a very complex environment, where glandular secretions, exfoliated epithelial cells and many microorganisms, both pathogenic and nonpathogenic, interact. Many infections can occur in the lower female genital tract and disrupt this intricately balanced vaginal ecosystem[1]. Moreover, a disrupted cervicovaginal environment due to a change from a protective bacterial population to a non-protective population can affect an individual's susceptibility to other infections[2].

The most common infectious disorders in the cervicovaginal milieu are related to *Candida sp*., *Gardnerella vaginalis*, *Trichomonas vaginalis*, *Chlamydia trachomatis* and human papillomavirus (HPV)[1]. Among these, only those caused by *Trichomonas*, *Chlamydia* and HPV are considered sexually transmitted infections (STIs). HPV is more prevalent in sexually active young women, and its manifestations vary from latency to intraepithelial lesions and carcinomas in the lower female genital tract[3].

The influences of bacterial vaginosis, trichomoniasis and candidiasis on the natural history of HPV have been well studied, but the results remain unclear. Some studies have reported an association between *Candida* and papillomavirus[3], but most studies have found no relationship[1,4]. *Trichomonas* is also uncertainly associated with HPV; some studies have indicated a positive relationship[5,6], while other studies have not[1,4].

However, the biggest controversies lie in the study of the relationship between bacterial vaginosis (BV) and HPV. BV, which is caused by a decrease of *Lactobacilli* and a predominance of anaerobic bacteria in the vaginal flora, is among the most common causes of vaginal complaints in women of childbearing age[7]. Many studies have shown a positive association between BV and HPV, even more consistently than those observed for other vaginal infections. King et al.[1], in a longitudinal multi-site investigation including 756 women with human immunodeficiency virus (HIV) and 380 uninfected women at high-risk for HIV, found that bacterial vaginosis was associated with increased prevalence and incidence of HPV and delayed clearance of infection, even with paired risk factors.

Study carried out at the Hospital Geral de Fortaleza (HGF) – Fortaleza (CE), Brazil.

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A meta-analysis by Gillet et al.[7] also showed a positive association between BV and uterine cervical HPV infection, even when the number of sexual partners was matched.

Several hypotheses that support this association have been postulated. In BV-negative women, hydrogen peroxide-producing *Lactobacilli* dominate the vaginal microbiome and are part of the main defense mechanisms. Loss of these protective microorganisms and other changes in the vaginal milieu related to BV could facilitate the survival of other sexually transmitted agents and are risk factors for developing vaginal infections[7]. Other alterations in vaginal fluids induced by BV can explain this association, such as reduced levels of secretory leukocyte protease inhibitor (SLPI), increased levels of mucin-degrading enzymes and changes in the production of immunological factors, such as cytokines[8-10].

Most studies on this topic have found an association between BV and HPV but have not established a cause-effect relationship. Whether HPV-positive women are more likely to develop BV is unclear[7].

Although an association between BV and HPV has been suggested, there is no evidence that BV increases the risk of squamous intraepithelial lesions (SILs) or cervical cancer[11-13]. Bacterial vaginosis seems to affect only the viral incidence and prevalence but not the persistence of HPV infection. This explains the lack of effect of BV infection on the development of SILs[3,14]. In a cohort study by Mao et al.[15], a time lag analysis suggested that HPV infection usually precedes BV detection. Therefore, patients with papillomavirus have an increased risk for vaginosis. However, there are a few studies evaluating the influence of viral infections and subclinical lesions on the cervicovaginal milieu and the possibility of an increased risk of acquiring other infections.

OBJECTIVE

The purpose of the current study was to determine if patients with HPV-induced, low-grade squamous intraepithelial lesions (LSILs) have a higher prevalence of vaginitis.

METHODS

This is a retrospective cross-sectional study performed using a database of cervical pathologies from a private laboratory in Fortaleza, Brazil, between January 2009 and May 2012. The cervical cytology results were collected. Women with a history of prolonged corticotherapy or transplants were excluded. Furthermore, negative tests for HIV, hepatitis B and C and VDRL were established as inclusion criteria. A total of 322 women were included.

This study was approved by the Research Ethics Committee of the Hospital Geral de Fortaleza, Brazil.

All cervical cytologies were assessed with a liquid-based Pap test, using the SurePath (BD Diagnostics, Burlington, NC, USA) and ThinPrep (Hologic, Inc., Bedford, Massachusetts, USA) assays. The Bethesda System 2001 was used to conduct the cytological diagnosis[16].

The presence of at least 20% of clue cells in the smear was diagnosed as vaginosis[17]. The presence of blastoconidia or pseudohyphae was diagnosed as candidiasis.

HPV infection was determined using the hybrid capture 2 (HC2) test (Qiagen AG, Garstligweg 8, CH-8634, Hombrechtikon, Switzerland), which was performed using the same material obtained for the cervical cytology assays. The material was processed according to the manufacturer's guidelines for the identification of high-risk HPV-DNA (HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68). Quantitative results were also obtained and expressed in relative light units (RLU), indicating the viral load. Each test was processed with positive and negative controls in triplicate.

The patients were divided into two groups. The first group included women who exhibited negative cytology for malignancies and SILs and were HC2-negative for HPV. The second group included women with LSILs and evidence of HPV infection. There were 155 patients in the first group and 167 patients in the second group.

The prevalence of vaginal infections was compared between the groups. Only HPV-positive patients with LSILs were assessed, whereas patients with latent infections or high-grade lesions were excluded because of their greater activity and increased viral replication[18].

The statistical analyses were performed with GraphPad InStat, 3.10 version (GraphPad Software Inc., San Diego, California, USA). The prevalence of vaginal infections in both groups was compared using the χ^2 test without Yates' correction to determine the p-value and the 95% confidence interval (95%CI).

RESULTS

The mean age of the HPV-positive group was 29.2 years, with a standard deviation of 10.2 years. The mean age for the HPV-negative women was 35 years, with a standard deviation of 11.52 years. Patients with HPV were significantly younger ($p<0.0001$).

In the patients that were HPV positive, the most common vaginal infection was vaginosis (15 cases; 8.98%) (**Figure 1**), whereas candidiasis was the most common infection in the patients that were HPV negative (20 cases; 12.9%) (**Figure 2**). The infections identified in

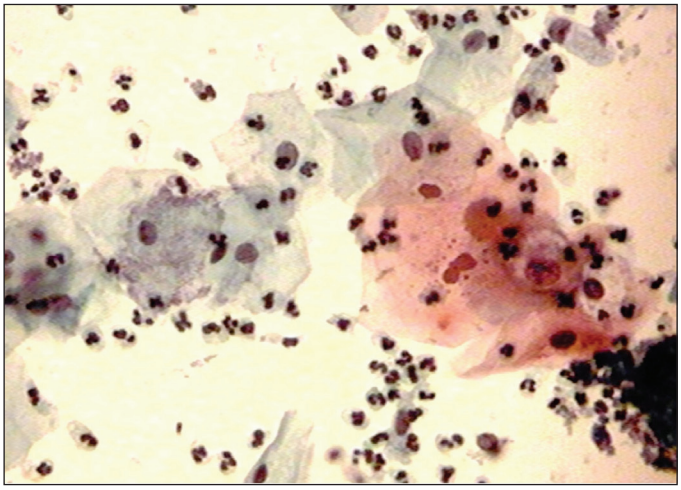

Figure 1 – Cytological analysis of a specimen exhibiting a low-grade squamous intraepithelial lesion (and positive hybrid capture 2 for high-risk human papillomavirus) with clue cells, suggesting bacterial vaginosis (SurePath 400x).

the HPV-positive patients with LSILs and the HPV-negative patients without lesions are detailed in **Figures 3 and 4**, respectively.

The difference in the prevalence of vaginosis between patients with and without HPV was not statistically significant (p=0.53). However, the frequencies of *Candida* infection were significantly different between groups (p<0.001), as shown in **Figure 5**.

DISCUSSION

Women with HPV were statistically younger (p<0.0001), which is consistent with the peak incidence of the disease. Dunne et al.[19] reported that the highest prevalence of HPV was among women aged from 20 to 24 years, followed by a gradual decline in prevalence to 59 years of age.

Gardnerella was the most frequently identified bacteria using cytology in the HPV-positive patients (8.98%), whereas in the HPV-negative patients, *Candida* was the most frequently identified pathogen (12.9%), which is consistent with the findings of Murta et al.[20], who also observed that *Candida* was the most frequent agent in a group without HPV (23.9%; 13.8% in HPV-positive patients; p<0.001).

These findings are consistent with the hypothesis that the local cervicovaginal milieu plays a role in an individual's susceptibility to HPV infection because women who carry *Candida* spp. are likely to possess a healthy *Lactobacillus*-dominated vaginal microbiome, in contrast to women with bacterial vaginosis[21]. Dols et al.[22] showed that, in women with HPV, the prevalence of *Lactobacillus crispatus* was significantly reduced and that there was a shift in the composition of the *Lactobacillus* microbiota following HPV infection. The leading hypothesis concerning these associations is that the absence of protective lactobacilli increases the biological susceptibility of acquiring STIs upon exposure[7]. Women with a loss of *Lactobacillus*-predominant vaginal microflora are more likely to acquire HPV. In contrast, women who have a vaginal flora dominated by *Lactobacillus* species tend to have fewer HPV infections but are more likely to develop candidiasis.

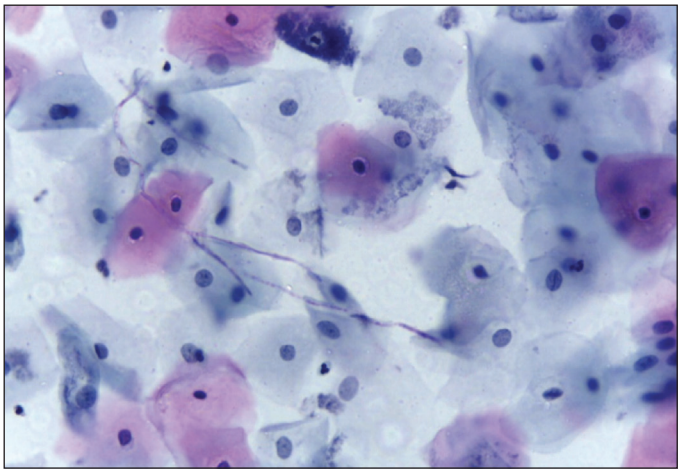

Figure 2 – Cytological analysis of a specimen negative for lesions (and negative hybrid capture 2 for high-risk human papillomavirus) exhibiting the presence of *Candida* morphotypes (SurePath 400x).

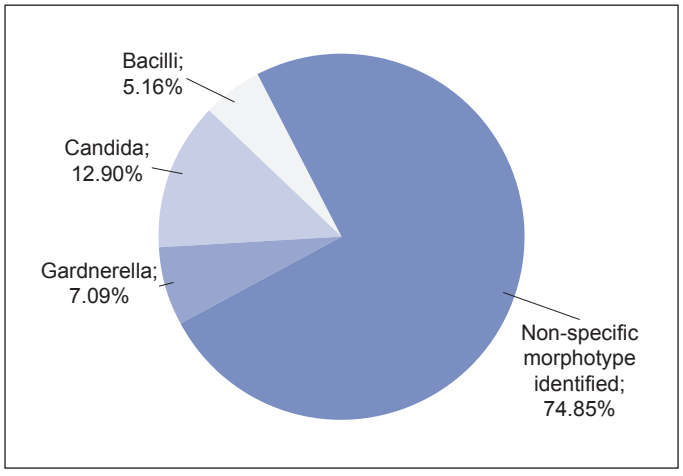

Figure 4 – Cytological findings obtained by liquid-based Pap tests in 155 women who were hybrid capture 2-negative for human papillomavirus and did not have malignancies or intraepithelial lesions.

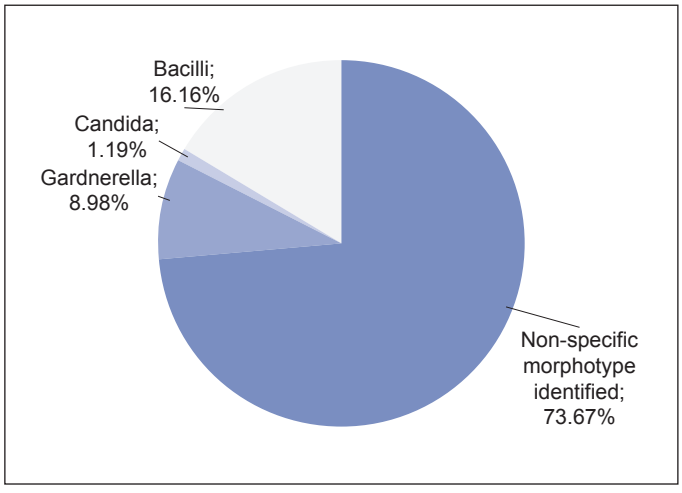

Figure 3 – Cytological findings obtained by liquid-based Pap tests in 167 women who were hybrid capture 2-positive for human papillomavirus and had low-grade squamous intraepithelial lesions.

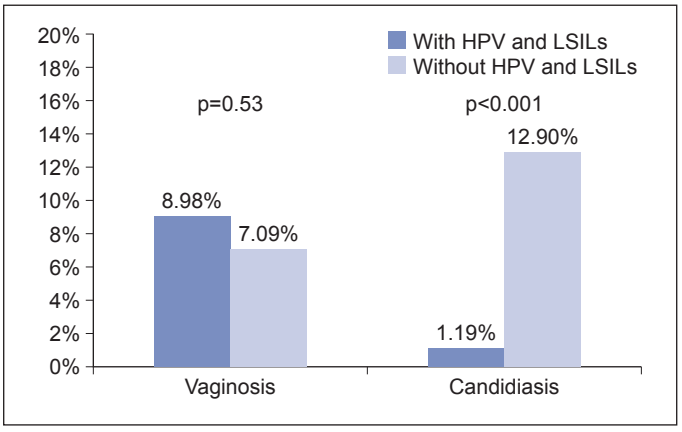

Figure 5 – Prevalence of vaginosis and candidiasis in 167 women who were hybrid capture 2 (HC2)-positive for human papillomavirus (HPV) and had low-grade squamous intraepithelial lesions (LSILs) and in 155 women who were HC2-negative for HPV and did not have malignancies or intraepithelial lesions.

The presence of *Candida* is not associated with an increased risk of acquiring HPV[23]. These findings are consistent with those of Watts et al.[24], who demonstrated that candidiasis was not related to high-risk papillomavirus infection in HIV-negative women.

There was no significant difference in the prevalence of BV between the two groups (with or without HPV).

Some studies have suggested that BV is associated with HPV acquisition. A meta-analysis of 12 studies including a total of 6,372 women indicated a positive association between BV and HPV infection, with an overall estimated *odds ratio* of 1.43 (95%CI 1.11–1.84)[7]. Nogueres et al.[3] found a positive association between *Gardnerella* and HPV. Vaginosis was observed in 9.1% of patients who were HC2-negative for HPV, while vaginosis was observed in 22.4% of HC2-positive patients (p=0.012). Guo et al.[25] reported that, compared to women without BV, women with BV had a lower clearance of HPV infection. All these findings can be explained by the hypothesis that susceptibility to HPV and the immune system's ability to clear HPV can be affected by vaginal bacterial infections, which disrupt the balance of the vaginal microbiota[7,26].

In our report, this association was not observed. Some of the limitations of the present study may explain the discordance among BV findings. There was a low prevalence of vaginal infections, which may be a result of our sampling from a private clinic, thus including patients with high social-economic status and fewer risk factors. Another limitation is that the microbiome evaluation was conducted only using Pap tests and disregarded other criteria such as the Nugent score and the Amsel clinical criteria.

There are other studies related to our findings regarding BV and HPV[4,27]. Zheng et al.[28] found a higher prevalence of BV in an HPV-positive group compared to a control group, which is similar to our results. However, this association was not statistically significant. In another study, bacterial vaginosis was found in 12% of subjects with LSILs and 4% of subjects with a normal Pap result; however, this result was not statistically significant either[29].

These data conflicts may be the result of the different methods applied in each study. A positive relationship between BV and HPV was observed in a population of sex workers, which was very different from our sample group[21]. Murta et al.[20] also found an association between vaginosis and papillomavirus; however, this study used a cytological diagnosis of HPV, which is significantly less sensitive than HC2.

Research on the alleged associations between HPV infection and vulvovaginitis may have an impact on clinical practice because these associations can serve as a basis for a simultaneous screening policy. A greater understanding of vaginal physiology and the interactions that occur in the cervicovaginal milieu can also increase our understanding of HPV physiopathology and, thereby, optimize prevention and treatment strategies[7].

CONCLUSION

The cross-sectional design allowed us to observe an association between *Candida* morphotypes and the absence of HPV. There was also a higher frequency of vaginosis in the papillomavirus group, but this finding was not statistically significant.

Because this was a cross-sectional study, we were unable to determine whether a change in the vaginal microbiota preceded HPV infection or vice versa. Therefore, further research is needed to clarify this relationship.

Conflict of interests

The authors report no conflict of interests.

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Prevalence of cervical squamous intraepithelial lesion high grade according to age from Antônio Pedro University Hospital

Prevalência de lesão intraepitelial escamosa de alto grau do colo uterino de acordo com a faixa etária no Hospital Universitário Antônio Pedro

Rafaella Maia Paredes[1], Isabela Vieira do Lago[1], Susana Christina Aidé Viviani Fialho[2], Isabel Cristina Chulvis Do Val Guimarães[2]

ABSTRACT

Introduction: The targeted population for HPV infection has peak infection at young adults, but studies point to the emergence of a new peak of viral infection and injuries later, probably by changes in sexual behaviour, waning immunity over time or reactivation of latent infection. Whereas the cervical squamous intraepithelial lesion high grade, mainly cervical intraepithelial neoplasia grades III (CIN III) have significant potential for progression to invasive carcinoma, the procedures of choice for the diagnosis and treatment in the target population are essential for the prevention of cervix cancer. **Objective:** To determine the prevalence of CIN II/III among patients seen in Cervical Pathology Clinic of Gynecology Department at the University Hospital Antonio Pedro from May 1996 to May 2013, relating to age in which this diagnosis was made. **Method:** It was selected patients referred to the Cervical Pathology Clinic for altered cytology and diagnosed through biopsy guided by colposcopy with CIN II / III. They were segmented into the following age groups: 15 to 24, 25 to 34, 35 to 44, 45 to 54, 55 to 64, 65 to 74, 75 to 84 and 85 to 94 years old. **Results:** Between 25 and 64 years of age, there were 36.5% of patients in these age groups with CIN II/III. However, it was found that 19% of patients younger than 25 years and 14.2% at 64 years also had such a diagnosis. **Conclusion:** The target population according to Brazilian Ministry of Health would let 16.2% of women with high-grade lesions withouta diagnosis, which corresponds to 11.3% of all high-grade cervical lesions.
Keywords: Human papillomavirus 16; Human papillomavirus 18; cervical intraepithelial neoplasia; age groups; statistics & numerical data.

RESUMO

Introdução: A população alvo de infecções pelo HPV tem como pico adultos jovens, porém estudos apontam para um novo pico mais tardio, provavelmente por alterações do comportamento sexual, diminuição da imunidade ou reativação da infecção latente. Considerando que as lesões intraepiteliais escamosas de alto grau do colo uterino, sobretudo a neoplasia intraepitelial cervical grau III (NIC III) tem potencial significativo de progressão para carcinoma invasor, os procedimentos de escolha para diagnóstico e tratamento na populaçãoalvo são essenciais para a prevenção do câncer de colo uterino. **Objetivo:** Verificar a prevalência de NIC II/III dentre as pacientes atendidas no Ambulatório de Patologia Cervical do Serviço de Ginecologia do Hospital Universitário Antônio Pedro no período de maio de 1996 a maio de 2013, relacionando com a faixa etária em que este diagnóstico foi realizado. **Método:** Selecionou-se as pacientes encaminhadas ao Ambulatório de Patologia Cervical por citologia oncótica alterada e diagnosticadas, por meio de biópsia dirigida pela colposcopia, com NIC II/III. Foram segmentadas nas seguintes faixas etárias: 15 a 24, 25 a 34, 35 a 44, 45 a 54, 55 a 64, 65 a 74, 75 a 84 e 85 a 94 anos. **Resultados:** Entre 25 e 64 anos de idade, houve 36,5% de pacientes nestas faixas etárias com NIC II/III. Entretanto, verificou-se que 19% de pacientes com menos de 25 anos e 14,2% com mais de 64 anos também apresentavam tal diagnóstico. **Conclusão:** A população-alvo preconizada pelo Ministério da Saúde deixaria 16,2% das mulheres com lesões de alto grau à margem de um diagnóstico, o que corresponde a 11,3% de todas as lesões de alto grau do colo uterino.
Palavras-chave: Papillomavirus Humano 16; Papillomavirus Humano 18; neoplasia intraepitelial cervical; grupos etários; estatística & dados numéricos.

INTRODUCTION

Infection by the human papillomavirus (HPV) has become a pandemic, which has attracted the attention of health-care professionals. HPV is classified, according to oncogenic potential, as having low and high risk. Among the various types of oncogenics, HPV16 and HPV18 can be pointed out, as they are able to integrate themselves into the genome of the host and block cellular mechanisms that control proliferation and repair deoxyribonucleic acid[1-3].

HPV is transmitted through sexual intercourse. From this moment on, the infection can become latent, which is diagnosable only through molecular biology; subclinical, which is diagnosable through colposcopy and cytology; or clinical, viewed through the naked eye. However, the majority of infections behave in a transitory form. Persistent infection with HPV constitutes biggest risk factor for the occurrence of lesions[2,3].

The precursor lesions were screened for cervical cancer in three distinct forms, depending on the guidelines for each country: only by cytopathology, such as in Brazil; only with the HPV test; or through a combination of both cytopathology and HPV test. According to the guidelines from the American Cancer Society, the American Society for Colposcopy and Cervical Pathology, and the American Society for Clinical Pathology[4], the screening is performed in the following manner: women younger than 21 should not be screened, women aged between 21 and 29 should be screened only by cytology every

Study carried out at Universidade Federal Fluminense (UFF) – Rio de Janeiro (RJ), Brazil.
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3 years, and women aged between 30 and 65 should be screened preferably with cytology and the HPV test (co-test) every 5 years. Women more than 65 years of age with prior negative screening and no history of CIN 2 in the last 20 years should not be screened, whereas those more than 65 years of age with a history of CIN 2, CIN 3, or adenocarcinoma *in situ*, after either spontaneous remission or treatment, should continue the screening for at least 20 years after detection, even if they have surpassed the age of 65. For the confirmation, a colposcopy examination is performed[5,6].

When analyzing the natural history of CIN, CIN 2 is found to progress into CIN 3 in 22% cases and into invasion in 5% cases. Meanwhile, the progression rate of CIN 3 into invasion was more than 12%[7-9].

To treat the high-grade lesions of the cervix and, by consequence, prevent an invasive carcinoma, the recommended procedures are classic conization and large-loop excision of the transformation zone of the cervix[10].

OBJECTIVE

To analyze the cases of histopathological diagnosis of CIN 2 and 3 attending the Cervical Pathology Clinic at the Gynecology Department of the Antonio Pedro University Hospital, from May 1996 to May 2013, relating them to the age group of the patients.

MATERIALS AND METHODS

This was a retrospective study in which subjects were the patients referred to the Cervical Pathology Clinic at the Gynecology Department of the Antonio Pedro University Hospital for altered oncotic cytology, that is, atypical squamous cells of undetermined significance (ASC-US)[11] and diagnosed, through a biopsy by colposcopy, with high-grade cervical squamous intraepithelial lesion (CIN 2 and 3). The age group in which the diagnosis occurred was analyzed. The patients were divided into the following 10-year age groups: 15–24 years, 25–34 years, 35–44 years, 45–54 years, 55–64 years, 65–74 years, 75–84 years, and 85–94 years, in accordance to what was presented in a similar American study[12]. Women with a histopathological diagnosis of CIN 2 or 3 were included in the study.

RESULTS

From the 1,084 patients with altered cytology results in the 17 years of the retrospective study, 347 presented CIN 2 or 3 (**Table 1** and **Graph 1**).

When considering the altered cytology in the age group according to the Brazilian Ministry of Health (25–64 years), we had a total of 36.5% patients with high-grade lesions (CIN 2 or 3). However, 19% patients aged less than 25 years and 14.2% more than 64 years also presented the said histopathological diagnosis. Therefore, when the patients under 25-year and over 64-year age group were combined, 16.2% were found to be with CIN 2 or 3 (**Graph 2**).

When compared to the women in the age groups 21–24 years and 65–74 years, 23.5 and 16.3% patients were found to present high-grade lesions, respectively. Then, on adding the patients from these two age groups, 19.4% were found to be with CIN 2 or 3 (**Graph 3**).

From the 347 patients with histopathological diagnosis of CIN 2 or 3, we can determine the total number of women affected according to the age group (**Graph 4**).

It was found that 5.5% of women under 25 years and 5.8% over 64 years had high-grade cervical intraepithelial lesions. Thus, when combined, the patients from these two age groups, 11.3% were found to have CIN 2 or 3 (**Graph 5**).

Of the 347 patients with CIN 2 or 3, those aged between 21 and 24 and between 65 and 74 represented, respectively, 4.6% and 4.3% of total high-grade lesions, which corresponds to 8.9% cases with CIN 2 and 3.

DISCUSSION

This paper aimed at demonstrating the importance of a broader target population for the screening of cervical cancer, so that the lowest possible number of patients with CIN 2 or 3 can occur and, thus, provide epidemiologic impact on the reduction of new cases of cervical cancer. For this, it was necessary to analyze the age of the patients with precursor lesions and reflect on the need for changes in the age groups that were screened in Brazil.

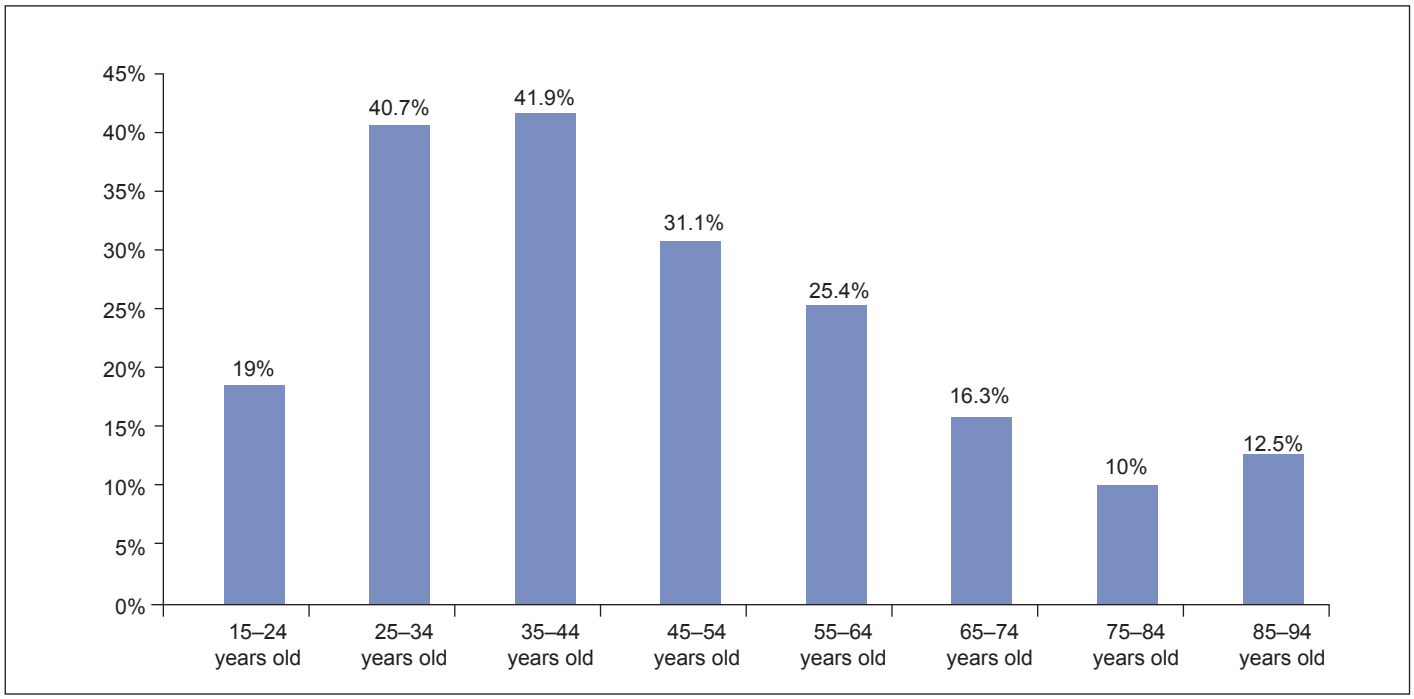
According to the guidelines of the Brazilian Health Ministry, the screening of lesions in the cervix should start at 25 years of age and end at 64[13,14]. In this study, when patients aged from 15 to 24 years were analyzed, 19% were found to have high-grade lesions. However, when patients aged 65 to 94 years were evaluated, 14.3% were found to have the said lesion, with this number being even more evident when only 65- to 74-year-olds were evaluated (16.3%). Consequently, according to this study, the target population determined by the Brazilian Ministry of Health would leave 16.2% women with high-grade lesions on the edge of a diagnosis, which corresponds to 11.3% of all high-grade cervical lesions, aged from 15 to 94 years. It is also known that a mere 30% Brazilian women undergo the screening of cervical cancer[15], which means it is likely that that there are an even higher number of women with high-grade lesions, in all age groups.

HPV infection has been more frequently reported among young, sexually active adults, between the ages of 18 and 25 years. When evaluating the natural history of the disease, we noticed that from the viral infection up to the appearance of an invasive carcinoma,

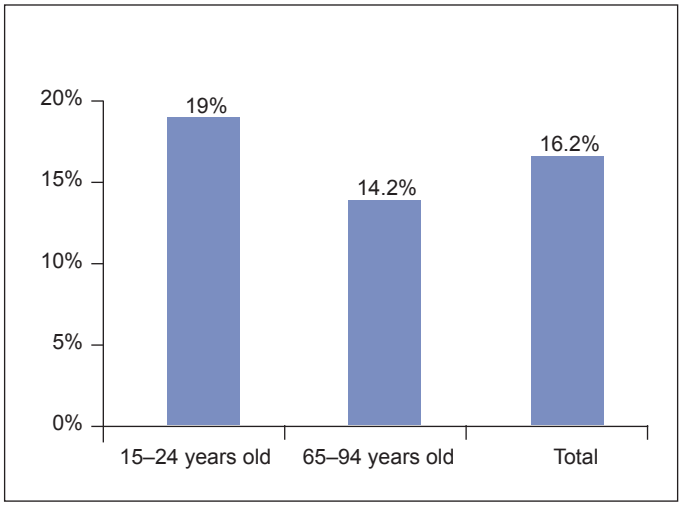
Table 1 – Number of patients per age group.

	15–24 years old	25–34 years old	35–44 years old	45–54 years old	55–64 years old	65–74 years old	75–84 years old	85–94 years old
Total	100	253	260	209	122	92	40	8
CIN 2 and 3	19	103	109	65	31	15	4	1
Percentage	19%	40.7%	41.9%	31.1%	25.4%	16.3%	10%	12.5%

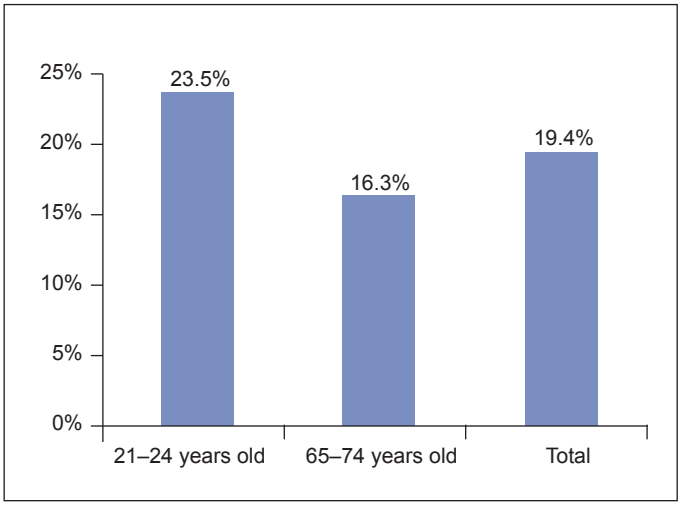
CIN: Cervical intraepithelial neoplasia.



Graph 1 – Prevalence of cervical intraepithelial neoplasia grade 2 and 3 for each age group.

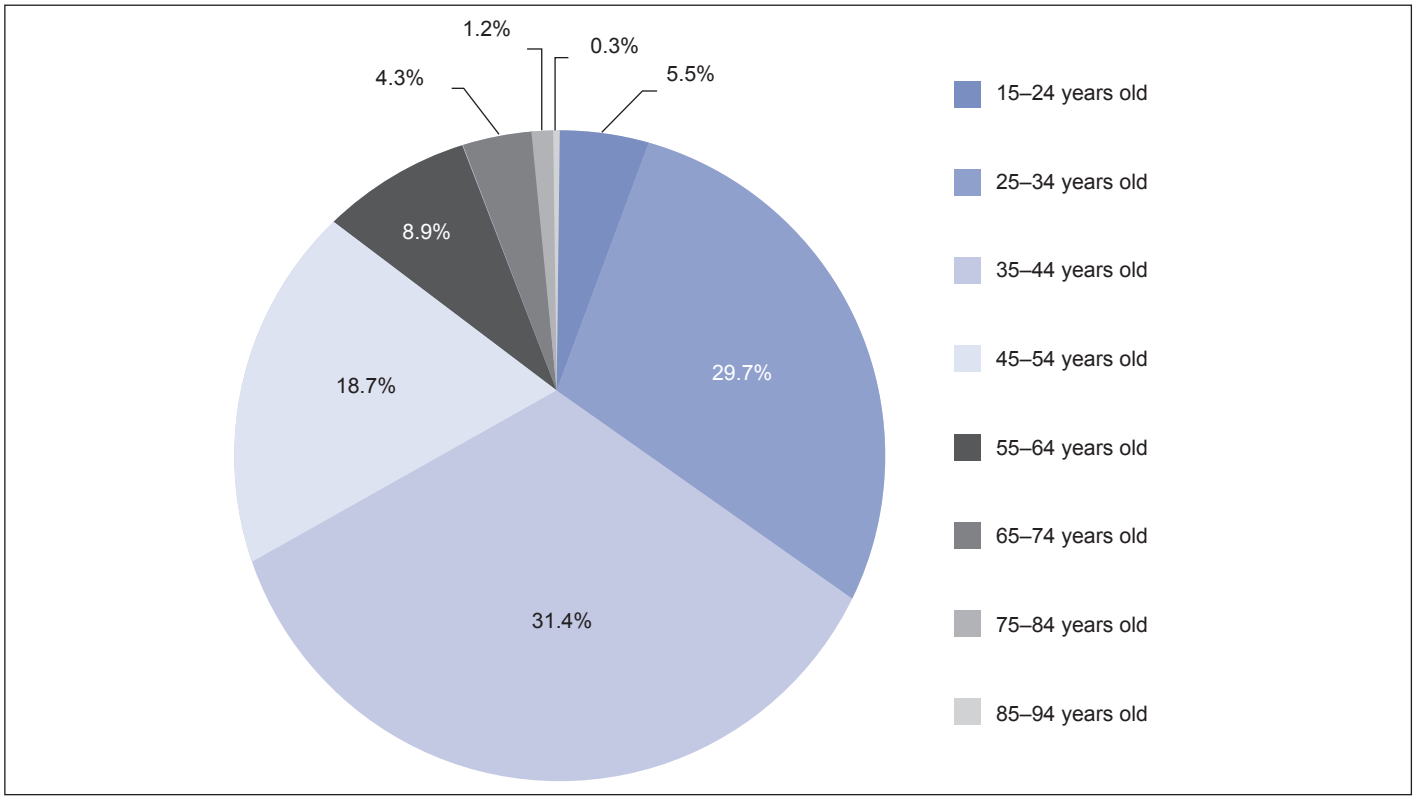


Graph 2 – Prevalence of cervical intraepithelial neoplasia grade 2 and 3 in those aged between 15–24 years and 65–94 years.

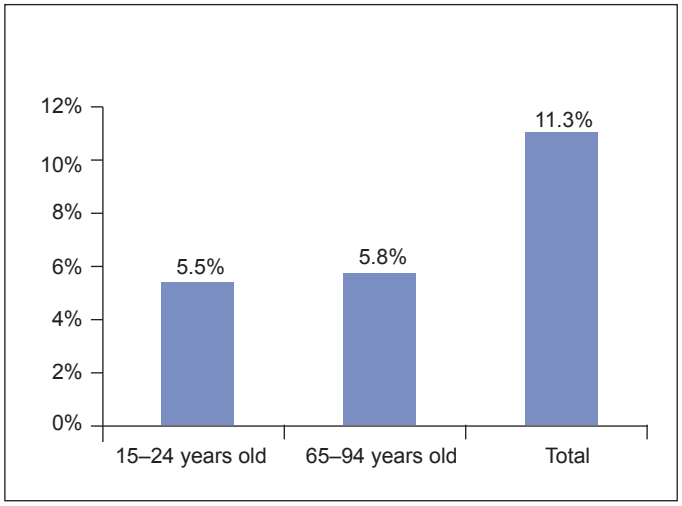


Graph 3 – Prevalence of cervical intraepithelial neoplasia grade 2 and 3 in those aged between 21–24 years and 65–74 years.

approximately 10 years passed. When the changes in the sexual behavior of the population are taken into consideration, mainly after the popularization of the medication against male impotence and female hormone therapy, and the decrease in the immunity or reactivation of the latent infection, mainly in elderly and immunosuppressed women[16], it is extremely important that we reflect on the existence of a new peak of incidence of later infection (therefore, a later appearance of these lesions) and discuss the necessity of amplifying the age group the screening covers, which has become more necessary in more advanced ages[12]. In this study, a significant number of patients over the age of 55 can be noted, revealing that a possible tendency in this direction will be exacerbated in the upcoming years. It is worth highlighting that the occurrence of CIN 2 and 3 in a 65-year-old patient, who would have been infected when around 55 years of age (allowing the possibility that she, currently, has an active sex life and new partners), is perfectly plausible and in line with the current reality. At this point, it is worth remembering that there is a tendency in amplifying the age group for women at a more advanced age due to a lack of studies, shown by the current recommendations from the WHO[13].



Graph 4 – Patients with cervical intraepithelial neoplasia grade 2 and 3 per age group.



Graph 5 – Percentage of patients with cervical intraepithelial neoplasia grade 2 and 3 from 15–24 years old and 65–94 years old, among the patients with this diagnosis.

In the case of the population under 25, with an initial sex life around 15 years of age, by 23 they could already have a premalignant or even a malignant lesion. A fact that is emphasized in the Brazilian population, which has estimates that indicate that more than 30% Brazilian women and around 47% Brazilian men initiate their sex life before the age of 14[17]. This premature start to a sex life leads some societies to opt for finding cytopathologic methods of the high-grade intraepithelial cervical lesions at 21 years of age, as was recommended in 2012 by the American Cancer Society, the American Society for Colposcopy and Cervical Pathology, and the American Society for Clinical Pathology[4].

Thus, returning to the results of our study, 23.5% patients between the ages of 21 to 24 have a high-grade cervical lesion. In the 15 to 25 age group, according to this study, 9.4% were found to have a high-grade lesion. These women would not be a part of the screening conducted by the Brazilian Ministry of Health. According to the folder, the reason for initiating the screening only at 25 years of age is based on studies that show even when cytology screening is performed in the young adult age group (21 to 24 years), there seems to be little to no impact in decreasing the number of cases of cervical cancer in screened or unscreened patients[18,19].

Regarding this age group of teenagers and young adults, some data are worth highlighting due to the importance of the infection in these groups. It is estimated that 13 to 38 % of young adults (20 to 24 years old), compared to 5 to 7% of those over 40 and sexually active, are infected by one or more types of HPV. This can be explained by the fact that this age group is initiating their sex life, a time at which there is characteristically a higher frequency of sexual activity, multiple partners, irregular use of barrier method contraceptives, and cervical fragility. Moreover, it is a known fact that having the first sexual encounter before the age of 18 is a risk factor for the development of cancer at an older age, with a risk three to four times higher of developing an invasive cancer compared to a first sexual encounter at 20 or older[20].

A detail from this younger age group is that younger women and teenagers still have not, in general, had offspring, and there is a discussion surrounding the increase in the risk of obstetric complications related to prior traditional conization, such as isthmus–cervical incompetence, leading to late abortion, premature labor, and/or low birth weight, when it cannot be avoided through cerclage[21-23].

It is noted, finally, that if in this population the patients from 21 to 24 and 65 to 74 years old were excluded, a total of 19.4% patients with high-degree lesions from these age groups and 8.9% among all high-grade lesions found would not have been a part of this diagnosis.

Regarding cervical cancer, it is relevant to know the prevalence of the disease and its behavior in each age group, and each population group, so that a deeper understanding of the profile of the disease can be had. This way, it becomes easier to equip the health-care system to decrease the frequency of the disease and produce progressively lower rates of invasive cervical cancer. The persistence of this neoplasia as an important cause of female mortality is unacceptable because it has a known natural history and, with the early diagnosis of the lesions, can be avoided in the majority of cases. Ample and early diagnoses of CIN 2 and 3 should, then, be prioritized, without forgetting that the public health groups should always be updated so as to mold their target population according to the changes in the population's needs and behavior[24].

Conflict of interests

The authors report no conflict of interests.

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Vulvovaginitis and the treatment of asymptomatic partners: a systematic review and meta-analysis

Vulvovaginite e o tratamento de parceiros assintomáticos: uma revisão sistemática e metanálise

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ABSTRACT

Introduction: Treating sexual partners of women with vaginal candidiasis and bacterial vaginosis is a discussed topic. Despite the recommendations of international guidelines, doctors are still known to treat asymptomatic partners. **Objective:** To evaluate the influence of asymptomatic partner treatment in the cure and recurrence of vulvovaginitis in women. **Methods:** The following databases were searched using Mesh terms: PubMed, Embase, SciELO and CINAHAL. The selection criteria included randomized clinical trials published from 1982 to 2012. **Studies** involving pregnant women were excluded. Methodological quality was assessed using Jadad's scale. Review Manager 5.1 was used for statistical analysis. **Results:** Eight randomized clinical trials were included based on the chosen criteria: 1,088 women were enrolled. For bacterial vaginosis, the relative risk for cure was 1.00 (95%CI 0.95–1.05, p=0.13), and for recurrence 0.84 (95%CI 0.62–1.14, p=0.34). Vaginal candidiasis had a RR of 1.03 (95%CI 0.94–1.14, p=0.48) for cure, and 1.02 (95%CI 0.77–1.33, p=0.91) for recurrence. **Conclusion:** Treatment of asymptomatic sexual partners of women with vaginal candidiasis or bacterial vaginosis does not affect the cure or recurrence rates and may increase the risk of side effects and unnecessary financial costs.

Keywords: vulvovaginitis; vaginosis, bacterial; candidiasis; partner; treatment.

RESUMO

Introdução: O tratamento de parceiros sexuais de mulheres com candidíase vaginal e vaginose bacteriana é um assunto muito abordado. Apesar das recomendações estabelecidas nos manuais internacionais, este tópico ainda é muito questionado por um grande número de médicos que prosseguem desobedecendo estes manuais. **Objetivo**: Avaliar a influência do tratamento de parceiros assintomáticos na cura e recorrência de vulvovaginite em mulheres. **Métodos**: Foi realizada busca com descritores específicos nas seguintes bases de dados: PubMed, Embase, SciELO e CINAHAL. No critério de seleção foram incluídos ensaios clínicos randomizados publicados no período de 1982 a 2012. Estudos envolvendo mulheres grávidas foram excluídos. Na avaliação qualitativa, utilizou-se a Escala de Jadad. A análise dos dados foi realizada por meio do programa estatístico Review Manager 5.1. **Resultados**: Oito ensaios clínicos randomizados foram selecionados: 1.088 mulheres foram escolhidas. Na vaginose bacteriana, o risco relativo para cura foi de 1,00 (IC95% 0,95–1,05, p=0,13) e para recorrência foi de 0,84 (IC95% 0,62–1,14, p=0,34). A candidíase vaginal apresentou risco relativo de 1,03 (IC95% 0,94–1,14, p=0,48) para cura e de 1,02 (IC95% 0,77–1,33, p=0,91) para recorrência. **Conclusão**: O tratamento do parceiro sexual assintomático de mulheres com candidíase vaginal e vaginose bacteriana não afetaria as suas taxas de cura e recorrência, como também poderia causar efeitos colaterais e custos desnecessários.

Palavras-chave: vulvovaginite; vaginose bacteriana; candidíase; parceiro; tratamento.

INTRODUCTION

Vulvovaginitis (VV) is a common complaint and one of the most frequent reasons patients seek gynecologists[1]. Annually, about 10 million doctor appointments are attributed to symptoms and signs of vaginal discharge[2].

Although VV is a very relevant condition to women due to the high personal and financial costs ensued, women and medical community often minimize it. This causes constant incorrect diagnosis and treatment by both women and doctors[1], resulting in exaggerated use of antibiotics and antifungals.

The main causes of VV are well established: bacterial vaginosis (BV), vaginal candidiasis (VVC), and trichomoniasis (VT). However, several questions are debatable, such as best drug to be used, treatment regimen, and the most appropriate route of administration. Since VT has been confirmed as a sexually transmitted disease (STD), the treatment of an asymptomatic partner is uncontested[3-5].

Some studies suggest that the treatment of sexual partners of women with BV could reduce recurrence rates from 5 to 20%. Nevertheless, data evaluating the efficacy of this practice are controversial[6-8]. In a well-designed clinical trial, Mengel et al. found a reduction of recurrence rates in patients with BV whose partners were simultaneously treated[9]. Nonetheless, three other studies found no relationship between oral therapy of the partner and recurrence rates of women[10-12].

VVC cannot be established as a STD since the transmission of the agent does not necessarily cause VV. It is known that the incidence of VVC increases dramatically in the second decade of life, corresponding to the onset of sexual activity, when several factors (tissue trauma, deposition of semen in the vaginal cavity, exaggerated use of soaps and chemicals, hormonal changes) influence the vulvovaginal ecosystem[13]. The sexual transmission of Candida can occur during intercourse, but intercourse frequency and timing could

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influence the development of an acute crisis[14]. The practice of oral sex has also emerged as one of the risk factors[15]. Current studies have associated homosexual practices with an increase in the prevalence of Candida in female genitals[16]. On the other hand, some studies suggest that the role of sexual practice in the establishment of VVC has been amplified[17,18].

A recent study, which proposed to evaluate the transmission of genital candidiasis among heterosexual couples, could not prove sexual acquisition[19]. Such investigation evaluated the Candida species from couples and found that only 25% of men and women had the same species of Candida, differently from previous studies[15]. In other studies that have treated sexual partners of women with VVC, no increase in cure rates, decline, or recurrence was observed[17].

Currently, despite the existing technology for diagnosis and treatment of VV, the role of sexual transmission has yet to be defined. The clarification of this controversy could avoid unnecessary treatment of sexual partners, thus reducing costs, side effects, and conflicts within the couple.

This study proposes to systematically evaluate the influence of treatment of asymptomatic partner in the cure and recurrence of VV.

MATERIAL AND METHODS

This study adhered to the PRISMA guidelines[20].

Inclusion criteria

Randomized controlled trials published in the last 30 years to assess the effectiveness of partner's treatment in the cure and recurrence of VV.

Exclusion criteria

Women under 16 years of age, HIV positive, pregnant, asymptomatic, and sex workers were excluded of the study. These groups represent populations at increased or decreased risk for STDs, wherein the prevalence of disease differs from the general population. This could interfere with the sensitivity and/or specificity of the analysis in this review.

Search and selection of literature

Eligible studies were identified in the following databases: PubMed, Embase, SciELO, CINAHAL, and Google Scholar. The studies were determined in a literature search of databases following medical subject heading terms and/or text words (Mesh Terms): (Treatment*)* AND (Vulvovaginitis) OR (Candidiasis) OR (Moniliasis) OR (Vaginitis, Monilia) OR (Vaginosis) OR (Vaginitis) OR (Trichomonas)) AND (Partners) AND ((randomized controlled trial) OR (clinical trial) OR (follow-up) OR (prospective)) NOT (Pregnant Woman).

The bibliographies of the identified publications were reviewed for additional pertinent studies. No language restrictions were applied.

Two investigators (AKG and HMR) looked up for articles published until May 2012. After search in the databases, 513 potentially relevant papers were identified, 102 of which were excluded after review of titles. Then, the abstracts of the 411 remaining titles were read, removing 313 titles. Of the 98 remaining articles, 8 were duplicated among the databases, which left 90 articles for final reading and qualitative assessment by Jadad's scale[21]. This considers studies to be methodologically adequate when they obtain a score of 3 or more[21]. Thus, studies with three or more points (eight studies) were classified as of high methodological quality, and remained in the systematic review (**Figure 1**).

Data extraction

Several characteristics of the original articles were extracted and included in the systematic review. The data included last name of the first author, year of publication, country, number of subjects, type of VV studied, as well as type of intervention and results.

Analysis

Statistical analysis was done using Review Manager (RevMan) 5.1 to provide a group analysis of the results from the selected clinical trials. The pooled analysis was obtained by analyzing the combined results of the chosen studies using the random effect model, and then testing for heterogeneity using the χ^2 test. Homogeneity of the selected studies was carried out.

RESULTS

Bacterial vaginosis

Four randomized controlled trials were selected:

1. Verjtorp et al.[10] conducted a major double-blind randomized clinical trial with 117 women using 500 mg of metronidazole twice a day for seven days. Half of the partners were randomly treated with the same treatment regimen or placebo. Cure and recurrence rates were similar among women with treated (cure: 51/54 and recurrence: 13/54) or placebo partners (cure: 44/53 and recurrence: 14/53) (**Table 1**).
2. Moi et al.[4], in another double-blind randomized controlled trial with 241 women, treated with 2 g of metronidazole, and repeated two days later. The partners were randomly treated with the same dose of metronidazole. Cure and recurrence rates were similar among women with treated (cure: 115/119 and recurrence: 19/112) or placebo partners (cure: 111/113 and recurrence: 14/106) (**Table 1**).
3. Vutyavanich et al.[11] conducted a randomized clinical trial of 250 Thai women treated with 2 g of tinidazole and a partner randomly treated with placebo or tinidazole. Cure and recurrence rates were similar among women with treated (cure: 111/122 and recurrence: 43/117) or placebo partners (cure: 113/119 and recurrence: 33/126) (**Table 1**).
4. Colli et al.[5] carried out a double-blind randomized study with 131 Italian women who were treated with 2% clindamycin in the form of vaginal cream for seven days. The partners were randomly treated with oral clindamycin or placebo. Cure and recurrence rates were similar among women whose partners treated (cure: 66/69 and recurrence: 5/38) or not treated the cure: 65/69 and recurrence: 9/32) (**Table 1**).

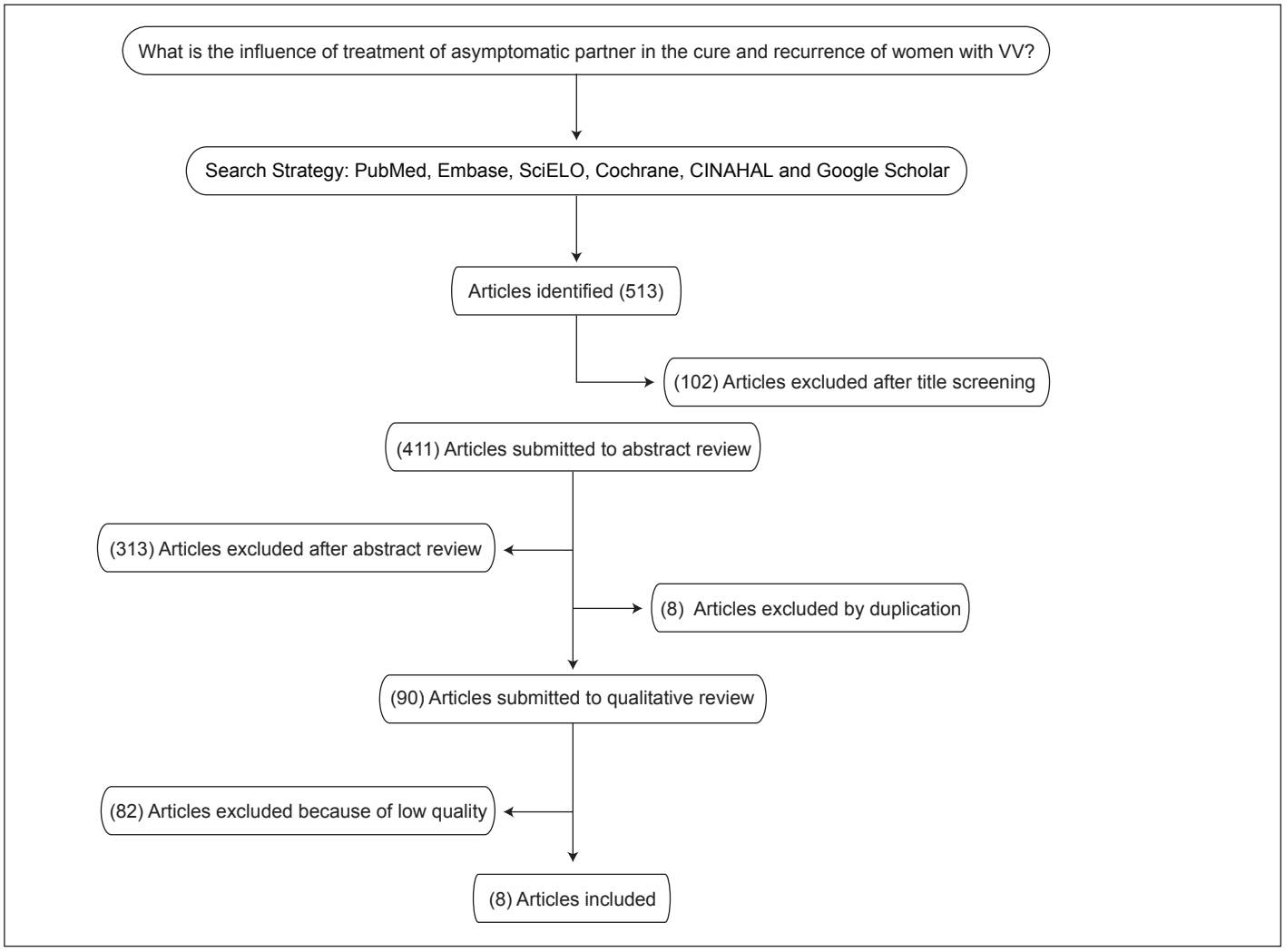

Figure 1 – Study selection.

Table 1 – Characteristics of selected randomized clinical trials for bacterial vaginosis and vulvovaginitis candidiasis.

Study	Country	Subjects	Randomized intervention	Results
Vejtorp et al.[10] (1988)	Denmark	107 non-pregnant women with BV	Women: 500 mg of Metronidazol twice a day/7 days Partners: 50% = same treatment, 50% = placebo	Treated: Cure: 51/54 Recurrence: 13/54 Placebo Cure: 44/53 Recurrence : 14/53
Moi et al.[4] (1989)	Denmark	241 non-pregnant women with BV	Women: 2 g of Metronidazol twice a day Partners: same randomized treatment	Treated: Cure: 115/119 Recurrence: 19/112 Placebo Cure: 111/113 Recurrence: 14/106
Vutyavanich et al.[11] (1993)	Thailand	250 non-pregnant women with BV	Women: 2 g of tinidazol Partners: randomized tinidazol or placebo	Treated: Cure: 111/122 Recurrence: 43/117 Placebo Cure: 113/119 Recurrence: 33/126
Colli et al.[5] (1997)	Italy	131 non-pregnant women with BV	Women: clindamycin 2% vaginal cream/ 7 days Partners: randomized oral clindamycin or placebo	Treated: Cure: 66/69 Recurrence: 5/38 Placebo : Cure:65/69 Recurrence: 9/32
Bishop et al.[2] (1986)	Belgium	117 non-pregnant women with VVC	Women : 200 mg 2 X day ketoconazole for 3 days Partners: randomized ketoconazole or placebo	Treated: Cure: 48/57 Recurrence: 13/48 Placebo Cure: 53/60 Recurrence: 19/53
Calderon-Marquez et al.[23] (1987)	Mexico	44 non-pregnant women with VVC	Women : 200 mg 2 X day ketoconazole for 3 days Partners: randomized ketoconazole or placebo	Treated: Cure:17/20 Recurrence:0/16 Placebo Cure:15/19: Recurrence:2/15
Fong et al.[24] (1992)	Canada	54 non-pregnant women with VVC	Women : 400 mg 2 X day ketoconazole for 7 days Partners: randomized 200 mg de ketoconazole for 5 days	Treated: Cure: 26/28 Recurrence : 8/26 Placebo Cure: 15/19 Recurrence : 9/28
Shihadeh et al.[25] (2000)	Jordan	144 non-pregnant women with VVC	Women : 400 mg 2 X day ketoconazole for 7 days Partners: half received randomized ketoconazole	Treated: Cure: 26/28 Recurrence : 8/26 Placebo Cure: 15/19 Recurrence : 9/28

The total RR for cure and recurrence was similar among women whose partners were treated or not for BV: cure RR=1.00, 95%CI 0.95–1.05, p=0.13; recurrence RR=0.84, 95%CI 0.62–1.14, p=0.34), as seen in **Figure 2**.

Vaginal candidiasis

Bisschop et al.[22] carried out a double-blind randomized clinical trial in Belgium with 117 women treated with 200 mg of ketoconazole twice a day for three days, whose partners were randomly treated with ketoconazole or placebo. Cure and recurrence rates were similar among women with treated (cure: 48/57 and recurrence: 13/48) or placebo partners (cure: 53/60 and recurrence: 19/53), as seen in **Table 1**.

Calderón-Marquez[23] performed a double-blind randomized study that included 44 women who used 50 mg itraconazole twice a day for five days, and their randomly treated partners. Cure and recurrence rates were similar among women with treated (cure: 17/20 and recurrence: 0/16) or placebo partners (cure: 15/19 and recurrence: 2/15), as in **Table 1**.

Fong[24] conducted a randomized clinical trial with 54 Canadian women who received 400 mg of ketoconazole for seven days. Their partners received 200 mg of ketoconazole for five days, or a placebo one. Cure and recurrence rates were similar among women with treated (cure: 26/28 and recurrence: 8/26) or placebo partners (cure: 15/19 and recurrence: 9/28), as in **Table 1**.

Shihadeh and Nawafleh[25] carried out a randomized clinical trial in Jordan with 144 women who received 400 mg of ketoconazole for seven days. Half of their partners received 400 mg ketoconazole for seven days. Cure and recurrence rates were similar among women with treated (cure: 57/72 and recurrence: 35/57) or placebo partners (cure: 53/72 and recurrence: 28/53), as further explained in **Table 1**.

The total RR for cure and recurrence was similar among women whose partners were treated or not for VVC: cure RR=1.03, 95%CI 0.94–1.14, p=0.48; recurrence RR=1.02, 95%CI 0.77–1.33, as in **Figure 3**.

Vaginal trichomoniasis

Interestingly, in the last 30 years no trials were performed to evaluate the treatment indication of partners of women with VT. The only randomized clinical trial was conducted over 30 years ago; however, it was not possible to include such trial in this study. In 1981, Lyng and Christensen[26] conducted a randomized clinical trial with 118 women, which found that the persistence of the infection was significantly higher in the group with no treatment of partners (14/59) compared to the group that did the treatment (3/59) (RR=0.21, 95%CI 0.06–0.71). This difference persisted in the subgroup of women who had sex with untreated partners. More recently, in a study testing the efficacy of intravaginal nonoxynol 9 for VT, Antonelli et al.[27] observed that women whose partners were treated with metronidazole had better cure rates compared to those whose partners were untreated. This study cannot be considered for this meta-analysis since the randomization tracking was not described.

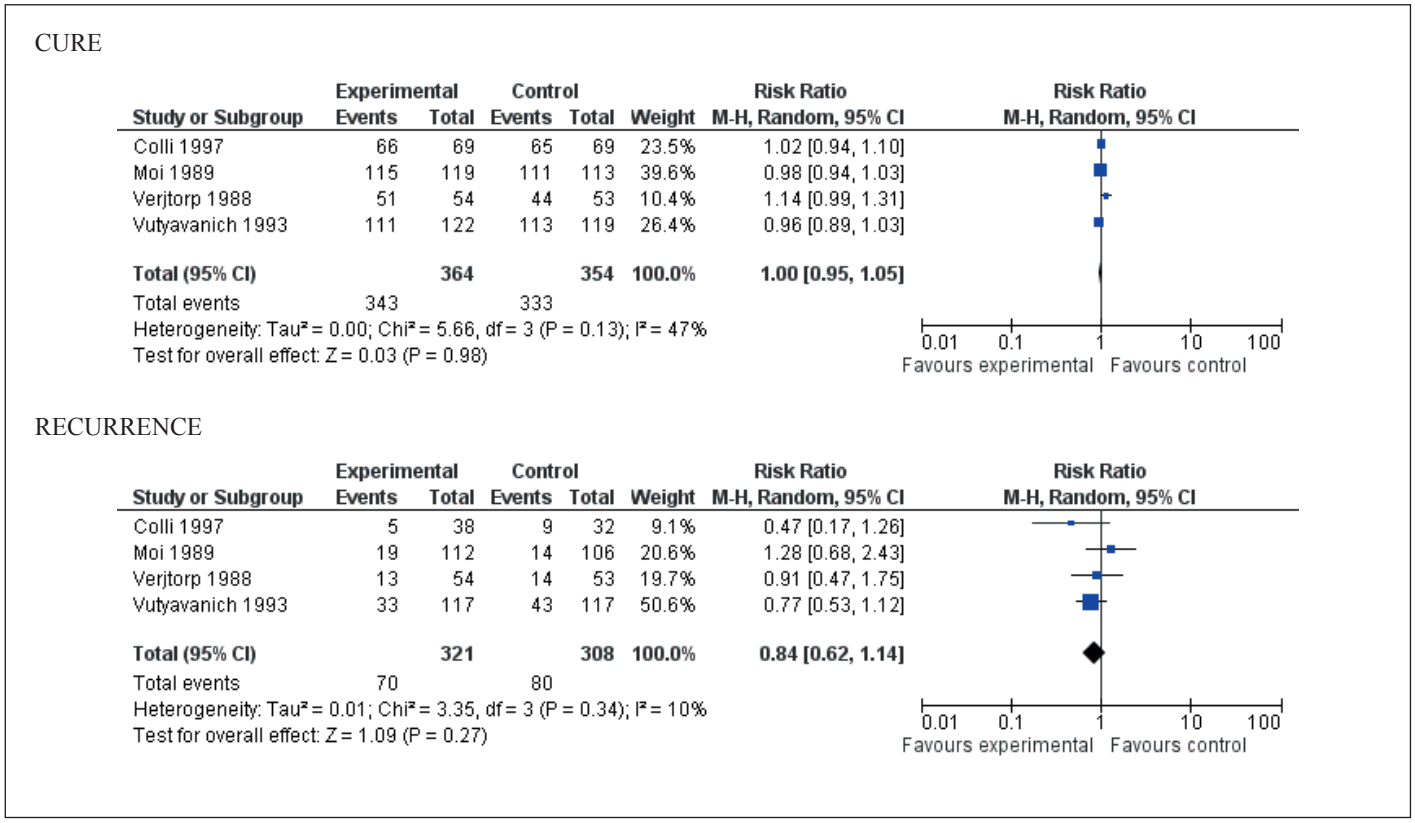

Figure 2 – Pooled analysis of selected bacterial vaginosis studies.

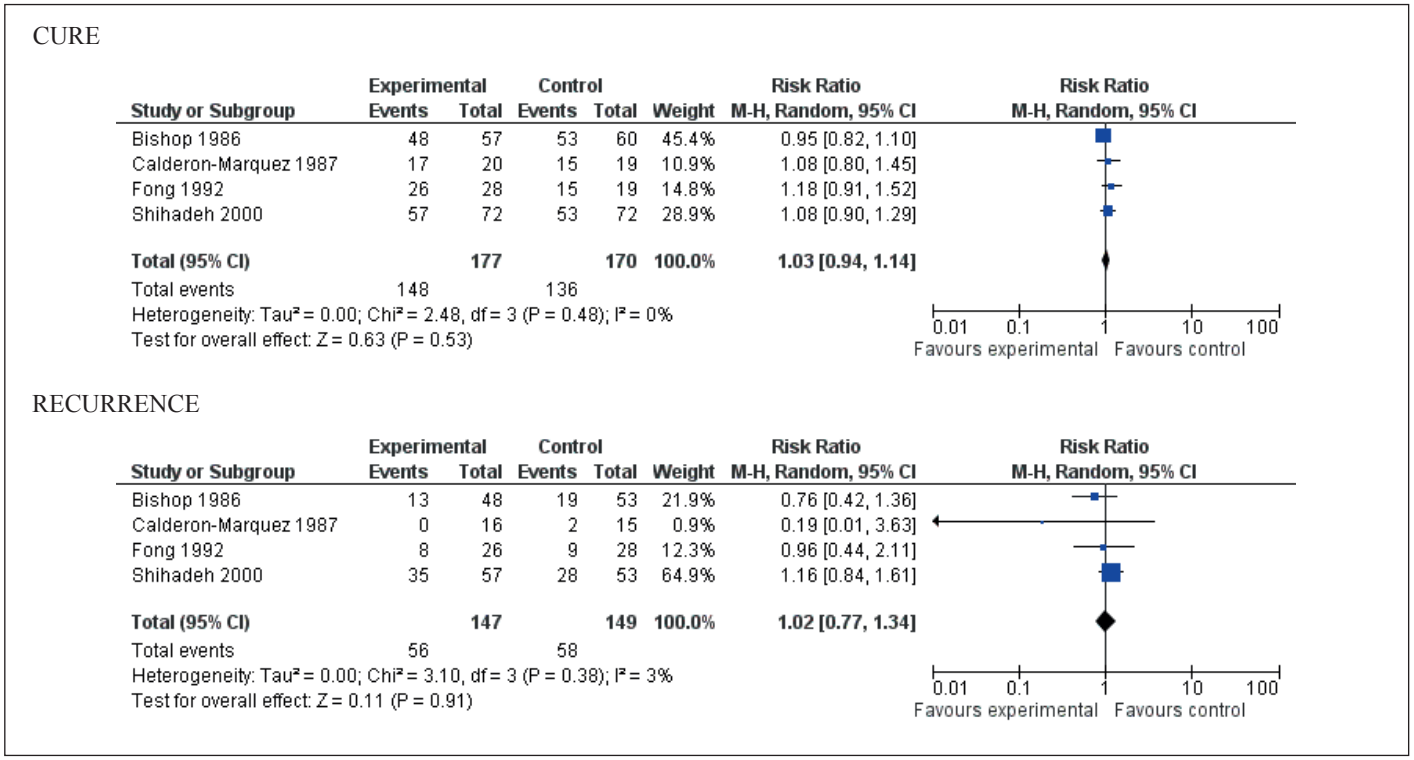

Figure 3 – Pooled analysis of selected vulvovaginitis candidiasis studies.

DISCUSSION

The medical literature and most researchers suggest that sexual partners of women with VV should not be treated[27]. However, some national health programs, including Brazilian health services, treat VV erroneously as a STD and leave treatment to the discretion of gynecologists. Unfortunately, this results in an excess of treatment that increases costs and causes unnecessary physical side effects. There are also serious social and emotional implications that cause conflicts to the couple due to the transmission of a STD. Very few studies consider the latter, or more importantly, the microbial resistance, a result from this practice.

Proponents of partner treatment argue that this practice could reduce recurrences in women, as well as new transmissions. However, our findings do not confirm these VT assertions. VT seems to be the only infectious VV wherein treating the partner increases the chances of cure and reduces recurrence. This being said, the only study that confirms this hypothesis, by Lyng and Christensen[26], was conducted in 1981. Besides this, it is accepted that VT is a protozoan, that cannot be found in the vaginal cavity under normal conditions and is not part of the vaginal flora. It is said that VT must be treated in both parties.

We believe that the ban on placebo use in clinical trials in recent years has impeded randomized trials[28]. Since VT is considered a STD, the consequence of prescribing placebo instead of the treatment is not ethically accepted. In vivo studies in animal models are a solution, even though they are difficult to perform. Even so, it is fundamental to encourage both studies in vitro and in animal models, which are already well-known for VVC, but not yet established for BV.

Contrary to VT, BV and VVC are caused by microorganisms that are part of the normal microflora composition, which sometimes assume the role of pathogens.

The pooled analysis suggested that a slightly lowered risk of recurrence was from the group of women with partners treated for BV-RR 0.84 (95%CI 0.62–1.14), however no statistically significant values were found for cure rates. There was no difference between the group of men who received a placebo and those who were treated with recurrence (1.00, 95%CI 0.95–1.05).

The pooled analysis of studies on VVC suggests that the evidence pointing to asymptomatic partner treatment is much weaker than for VB. The total RR for cure was 1.03 (95%CI 0.94–1.14), and for recurrence was 1.02 (95%CI 0.77–1.33).

Therefore, it is evident from these results that partner treatment does not significantly influence the outcome of cure and/or recurrence rates for BV and VVC.

This evidence can help the general practitioner to treat patients and their partners more adequately, thus avoiding the side effects of overtreatment.

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Human papillomavirus infection in healthy men from Rio de Janeiro, Brazil

Infecções por papilomavírus humanos em homens saudáveis do Rio de Janeiro, Brasil

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ABSTRACT

Introduction: Genital infections by human papillomavirus (HPV) are the most prevalent sexually transmitted viral diseases worldwide. Although the natural history of cervical cancer is better understood, there are still scarce information regarding the etiology of penile cancer, and the natural history of HPV infection in men is not yet fully elucidated. **Objective:** This study aimed to determine the prevalence of HPV infection in penile samples, from a clinically asymptomatic male population. **Methods:** A total of 550 samples were collected between January 2011 and July 2014 in different institutions in the State of Rio de Janeiro, including a dermatology clinic and a metallurgical company. The samples were collected from the anatomical regions of the glans and balanopreputial sulcus. HPV identification was made through the generic and type-specific Polymerase Chain Reaction (PCR), and Restriction Fragment Length Polymorphism (RFLP) techniques. **Results:** An overall prevalence of HPV infection was observed in 21.8% (120 subjects). The most prevalent HPV type was HPV 6 (35%), followed by HPV 16 (20.8%), HPV 11 (19.1%), HPV 31 (6.7%), HPV 33 (6.7%), HPV 45 (8.3%) and HPV 58 (3.3%). Hence, infection was associated with low-risk oncogenic types in 54.1% of the studied individuals, while high-risk oncogenic types were detected in 45.9% of them. The age of the studied subjects ranged from 18 to 65 years with a mean age of 28.4 years. **Conclusion:** According to our findings, we can infer that the prevalence of HPV infection among asymptomatic male population was considerably lower than the described in the literature, although in agreement with results reported in some recently published studies. We believe that the results may contribute to understand the features of circulation of HPV in male population, in order to evaluate risk-benefits and strategies of disease prevention.

Keywords: HPV; STD; men; asymptomatic; PCR.

RESUMO

Introdução: As infecções genitais pelo Papilomavírus humano (HPV) são hoje as mais prevalentes viroses de transmissão sexual em todo o mundo. Embora a história natural do câncer cervical seja melhor compreendida, pouco se sabe sobre a etiologia do câncer de pênis e a história natural do HPV no homem não está completamente elucidada. **Objetivo:** Este estudo teve como objetivo, determinar a prevalência do HPV em amostras penianas de uma população masculina assintomática. **Métodos:** Foram coletadas 550 amostras entre janeiro de 2011 e julho de 2014 em diferentes instituições do Estado do Rio de Janeiro, dentre elas: uma clínica de dermatologia e uma indústria metalúrgica. As amostras foram coletadas de sítios anatômicos como sulco balanoprepucional. A identificação do HPV foi feita pela Reação em Cadeia da Polimerase (PCR) genérica e tipo-específica, bem como pelo Polimorfismo do padrão de comprimento de fragmentos de restrição (RFLP). **Resultados:** A prevalência total da infecção pelo HPV foi de 21,8% (120 indivíduos). O tipo viral de maior prevalência foi o HPV 6 (35%), seguido pelo HPV 16 (20,8%), HPV 11 (19,1%), HPV 31 (6,7%), HPV 33 (6,7%), HPV 45 (8,3%) e HPV 58 (3,3%). A infecção foi majoritariamente associada a tipos de baixo risco oncogênico (54,1%), enquanto os genótipos oncogênicos foram detectados em 45,9% dos pacientes. A idade dos pacientes variou de 18 a 65 anos com média de 28,4. **Conclusão:** De acordo com nossos achados, podemos sugerir que a prevalência do HPV na população masculina assintomática foi consideravelmente menor do que a descrita em alguns estudos da literatura, mas em acordo com o reportado recentemente por vários autores. Acreditamos que estes resultados podem contribuir para a compreensão dos aspectos epidemiológicos associados à infecção no trato genital masculino, a fim de avaliar estratégias de prevenção de doenças associadas e avaliar o risco-benefício das diferentes abordagens aplicadas em Saúde-Pública.

Palavras-chave: HPV; DST; homens; assintomático; PCR.

INTRODUCTION

Although Human papillomavirus (HPV) infection causes the most prevalent sexually transmitted viral disease worldwide, the natural history of HPV infection have only been extensively studied in women, due to the prevalence of this disease and its well-established link to cervical cancer[1].

Most HPV infections in men are asymptomatic, and the male population is not routinely screened for HPV, so men may act as reservoirs of HPV infection, resulting in continuous transmission of both high-risk and low-risk HPV types to women[2]. Nevertheless, men have recently been recognized to manifest the pathological features of this disease, mainly through anogenital warts and neoplasias: anal intraepithelial neoplasia (AIN), penile intraepithelial neoplasia (PIN), and invasive carcinoma[3,4].

We believe that, since prophylactic HPV vaccines are recognized as effective in men, understanding the factors associated with HPV acquisition in men is critical to the development of public health strategies and preventive programs to control HPV infection[5]. Few studies have examined the epidemiology and risk factors associated with HPV infection in male population. In a systematic review, Dunne et al.[6] reported that half of the published studies concerning HPV prevalence among healthy subjects pointed out rates of infection of

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approximately 20%, although they may vary among different populations, sampling methods and diagnostic methodologies.

OBJECTIVE

This study aimed to describe the prevalence of HPV DNA among asymptomatic male subjects living in the State of Rio de Janeiro in order to evaluate the circulation of HPV infection among the studied population.

MATERIALS AND METHODS

Study design and participants

This cross-sectional study evaluated HPV infection in 550 asymptomatic men, treated in and recruited from several institutions of the city of Rio de Janeiro, namely: the STD clinic of Universidade Federal Fluminense, the dermatology clinic of Santa Casa da Misericórdia and a metallurgical factory from the Metropolitan region of Rio de Janeiro (MAENFE). The study was carried between January 2011 and July 2014, and aimed to evaluate the prevalence of HPV infection among asymptomatic men.

The participants did not present any clinical anogenital lesions related to the clinical characteristics of HPV infection. Exclusion criteria were: age under 18 years, and presence of anogenital lesions histopathologically compatible with HPV.

This study was approved by the Ethics Committee of Instituto Oswaldo Cruz from Fundação Oswaldo Cruz (CEP/IOC/FIOCRUZ, protocol no. 567/2010), and all subjects signed an informed consent. The social and epidemiological information (number of sexual partners, sexual behavior, circumcision, hygiene habits, tobacco use, history of STD, use of condom and anal intercourse) was collected from all participants using a structured questionnaire.

Samples

The volunteers were clinically evaluated for the occurrence of genital symptoms related to HPV. Individuals considered asymptomatic exclusively for this type of infection were invited to join this study. A total of 550 samples were collected from anogenital sites with a swab used for cytological exams, which was twisted clockwise three times in the anatomical site of sampling and, after that, kept in TE solution (10 mM Tris hydrochloride at pH 7.5, 1 mM ethylenediaminetetraacetic acid EDTA) (Invitrogen, USA) at -20°C until DNA extraction. Then, the participants signed the informed consent term and completed the social and epidemiological questionnaire.

DNA extraction, PCR amplification and genotyping

Samples were incubated for 4 hours at 56°C in 1mL digestion buffer (10 mM Tris hydrochloric acid at pH 8.3, 1 mM EDTA at pH 8.0, 0.5% Tween 20, 400 µg/mL proteinase K) (Invitrogen, USA), then extracted with phenol:chloroform:isoamyl alcohol (25:24:1) (Invitrogen, USA). DNA was precipitated with 300 µL 0.3 M sodium acetate plus 900 µL of ice-cold ethanol, washed with 70% ethanol, air dried, and suspended in 50 µL sterile water.

MY09/11 consensual primers for HPV detection, which amplify 450-bp DNA sequences at the *L1* region, were used to detect generic HPV DNA via polymerase chain reaction (PCR). Thirty-five amplification cycles were carried out in 50 µL reaction mixture (1 X PCR buffer, 200 mM deoxyribonucleoside triphosphates, 1.5 mM $MgCl_2$, 50 pmol each primer, 0.25 U Platinum®Taq DNA polymerase (Life Technologies®), 5 µL sample) using a DNA thermal cycler (Life Technologies, USA). Each cycle comprised denaturation at 94°C for 1 min, annealing at 55°C for 2 min, and chain elongation at 72°C for 2 min. The beta-actin primers Ac1 and Ac2 (0.1 pmol each), which amplify a 330-bp region of human DNA, were used as internal sample controls.

Genotyping was performed by PCR amplification with type-specific primers targeting the *E6* gene sequences of low-risk (LR) HPVs 6, 11 and 53, and high-risk (HR) HPVs 16, 18, 31, 33, 35, 45, 56, and 58, as previously described[7]. Thirty-five amplification cycles were carried out in 50 µL reaction mixture with denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and chain elongation at 72°C for 1 min.

For generic and specific genotyping, negative controls for background contamination were added to DNA templates. PCR products were analyzed on 1.3% agarose gel with ethidium bromide staining to visualize DNA under ultraviolet light, and their molecular weights were determined by comparison with a 100-bp DNA ladder[8].

Restriction fragment length polymorphism (RFLP) analysis for HPV genotyping

RFLP was performed following PCR amplification using the 450-bp amplicons from the MY09/11 PCR. Samples untyped by the type specific-PCR were submitted to digestion by a panel of six restriction endonucleases (BamHI, DdeI, HaeIII, HinfI, PstI, RsaI) (Invitrogen, Brazil). The pattern of length polymorphism of each sample was analyzed under ultraviolet (UV) light and compared with RFLP patterns for mucosal virus types, as described by Melgaço et al.[9].

Statistical analysis

A database was generated and analyzed using EpiInfo 8.0 (CDC). Biological data were compared using Fisher's exact test ($p<0.1$). Risk factors, HPV genotypes and sociodemographic features were evaluated. Associations of LR and HR HPV infections with social and epidemiological variables were examined.

RESULTS

The studied group was composed of 550 asymptomatic subjects, showing no clinically detectable HPV lesions. The average age of participants was 28.4 years, ranging from 18 to 65 years. The HPV-infected group presented an average age of 30.8, and HPV-negative subjects were 26.3 years old. No statistical differences were detected among them ($p>0.05$).

Regarding HPV infection, HPV DNA was detected in 21.8% of the patients (120/550). HPV 6 was the most prevalent type (35%, 42/120), followed by HPV 16 (20.8%, 25/120), HPV 11 (19.1%,

23/120), HPV31 (6.7%, 8/120), HPV33 (6.7%, 8/120), HPV45 (8.3%, 10/120) and HPV 58 (3.3%, 4/120). The HR types, 16, 31, 33, 45 and 58, were found in 45.9% of the cases. LR types 6 and 11 were the predominant types (54.1%). Multiple infections (e.g., HPV types 16 and 45, 11 and 58, 45 and 35 and 6 and 16) were found in 10.8% (13/120) of the samples. All the multiple infections were detected by the RFLP technique. Five samples (4.1%) presented HPV DNA according to MY09/11 PCR, but typing by both PCR specific primers and RFLP was inconclusive and are referred as HPV X (**Table 1**).

Age was the only socio-demographic factor that associated with risk of infection that could be analyzed, but no significant differences were found between infected and uninfected subjects (p>0.05).

DISCUSSION

Human papillomavirus (HPV) anogenital infections among healthy subjects presents prevalence rates ranging from 1.3% to 72% depending on the population studied and the diagnostic method used, but it is associated to 5% of all cancers worldwide[6]. Within these rates there is the cervical infection, which serves until now as a paradigm for understanding the carcinogenesis caused by high-risk HPV[1].

As penile carcinoma is a rare tumor and its etiology is still being discussed, little is known about HPV infection in men[10]. Recent studies have provided considerable evidence of the oncogenic potential of some HR-HPV types in the male anogenital tract[11,12]. Hence, HPV infection in men has increasingly become the object of research and discussion instead of being considered solely a source of transmission to women[13]. Although studies on HPV prevalence in male anogenital lesions have yielded highly variable results, we found a prevalence rate of 21.8%, which is similar to several studies from Brazil and other countries[7,14]. Nevertheless, an important study conducted in different countries (the HIM HPV in men study)[15] described HPV DNA rates greater than 70%. This high rate of HPV infection could be explained, in part, by the fact that the HIM study was conducted in subjects in the general population, not excluding individuals with clinical symptoms like warts.

Among the positive results for detection and typing of viral genetic material, we found a higher prevalence of HPV 6, followed by HPV 11, HPV 16, HPV 45, HPV31, HPV 33 and HPV 58 (Table 1). Dobao et al.[16], studying a similar population from Rio de Janeiro, also observed low variability in detected HPV genotypes (predominantly HPVs 6, 11 and 16). In agreement with the meta-analysis from Dunne et al.[6], these are the most prevalent HPV genomes, but the review pointed out that undetermined types were also commonly described. Different from other studies, multiple infections were not frequent in our sample (10.8%) and its role in disease establishment and progression remains inconclusive (Table 1), but recent studies point out that multiple-HPV infection can result in higher viral protein expression levels that can lead to genital disease. Prospective cohort studies linking sequential loss or gain of HPV types with cytological analysis are required to assess the impact of multiple HR-HPV infections on neoplastic progression[17].

Table 1 – Prevalence of HPV genotypes according to PCR and RFLP in male smears (n=120).

	n (%)
HPV 6	42 (35)
HPV 11	23 (19.1)
HPV 16	25 (20.8)
HPV 31	8 (6.7)
HPV 33	8 (6.7)
HPV 45	10 (8.3)
HPV 58	4 (3.3)
HPV X*	5 (4.1)
Co-infections**	**13 (10.8)**

*MY(+)PCR untyped by type-specific PCR and RFLP techniques.
**Individuals infected by more than one type of HPV.

We also observe statistical significance regarding HPV in older men, among which only high-risk types were detected (p>0.05).

It is interesting to note the absence of HPV 18 in these results, since this is considered one of the most prevalent types in the female population[18]. The absence of HPV 18 was consistent with other recent studies in male subjects[8,14]. On the other hand, the presence of HPV 45, whose prevalence has been shown to be increasing among the female population[1], was remarkable. HPV 45 is currently considered to be the second most prevalent type in cervical cancer cases in Brazil, being associated with insidious cases with difficult early detection[19]. These results draw attention to the increased circulation of this type of HPV in the world, including the male population, which highlights the relevance in considering its inclusion as well as other emerging viral types in future prophylactic vaccines, in order to extend the immunization coverage for the types with major clinical relevance.

It has been demonstrated that sampling and methodological strategies used in prevalence studies may have influence in diagnostic failure due to the characteristics of HPV infection sites in men and the lack of routine preventive screening to detect penile/anal lesions in men at risk for cancer development[20]. The samples used in our study were taken from the glans, corona, frenulum and coronal sulcus of the penis; locations which are known to have higher prevalence of HPV infection when compared to other anatomical sites of the male genitalia[14]. Even with this methodological approach, it is possible that individuals with HPV-related lesions in other sites of the penis might have been diagnosed as false negatives.

The HPV prophylactic vaccines are highly efficacious for the prevention of anogenital warts and precancerous cervical, vulvar, and vaginal lesions, prompting efforts to define its role in the prevention of male genital disease[12]. Although the protective efficacy of HPV vaccination in men has not been fully established, public policy discussions and cost-efficacy studies are necessary to support vaccination of boys, as of girls, at an early age, when they have engaged in limited or no sexual activity. In our study, nearly 75% of the studied subjects presented infection by genotypes covered by the quadrivalent vaccine, reinforcing the suggestion that this vaccine might be highly effective in reducing external genital lesions in young men[20].

CONCLUSION

We suggest that, for high-risk groups, an appropriate method of screening should be established as soon as possible, but we believe that the clinical knowledge of this pathology by physicians who gives assistance to this population should be encouraged in the short-term,

so that they can be alert and consider this pathology among the diagnostic hypotheses, even in the absence of anogenital warts. Education has relatively low cost, quick results and will be as or more effective than laboratorial methods for early diagnosis. At last, it is important to eliminate the idea, although deep-rooted, that HPV infection in men is not worthy of great concern, without recognition of its importance.

Conflict of interests

The authors declared no conflict of interest.

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Oral injuries as syphilis diagnosis: case report

Lesão oral como diagnóstico de sífilis: relato de caso

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ABSTRACT
Syphilis can present a variety of clinical manifestations as a consequence of the tropism that *Treponema pallidum* causes to various organs and body tissues. The oral cavity can be affected in all stages of the disease, therefore diagnosis requires knowledge of multidisciplinary health professionals. Even with the introduction of penicillin in the last century and initiatives of sexually transmitted diseases prevention, an increase in the incidence of syphilis has been observed, which indicates a public health issue. This paper presented the case of a patient with syphilitic chancre in the oral cavity, whose dental surgeon diagnosed an injury and referred her to the city sexually transmitted diseases ambulatory for treatment.
Keywords: syphilis; *Treponema pallidum*; sexually transmitted diseases.

RESUMO
A sífilis pode apresentar uma variedade de manifestações clínicas, justificada pelo tropismo que o *Treponema pallidum* tem para vários órgãos e tecidos do corpo. A cavidade oral pode ser acometida em todas as fases da doença; portanto, seu diagnóstico exige conhecimento de diferentes profissionais de saúde. Mesmo com a introdução da penicilina no século passado e com as iniciativas de prevenção das doenças sexualmente transmissíveis, observa-se aumento na incidência de sífilis, o que representa um problema para a saúde pública. Neste trabalho, apresentou-se o caso de uma paciente com cancro sifilítico em cavidade oral que fora diagnosticada e encaminhada por um cirurgião-dentista ao ambulatório de doenças sexualmente transmissíveis do município para tratamento da lesão.
Palavras-chave: sífilis; *Treponema pallidum*; doenças sexualmente transmissíveis.

INTRODUCTION

Syphilis is a sexually transmitted disease (STD), which could also manifest as congenital and, if untreated, can be potentially chronic or even fatal[1]. It is caused by a spirochete bacterium, *Treponema pallidum*, exclusive of human beings, which presents tropism for various organs and body tissues.

There is no cell membrane in *T. pallidum*; it is protected by an outer envelope with three layers of *N*-acetyl muramic acid and *N*-acetyl glucosamine. It has flagella that aid in its body rotation and can invade intact mucous membranes or broken skin facilitated by hyaluronidase. This bacterium also affects the regional lymphatic system and, through hematogenous dissemination, other body parts. It has the ability to divide transversely every 30 hours. It resists around 26 hours out of its environment, being destroyed by the heat and lack of humidity. It reddens lightly, hence the name pale, from the Latin word *pallidum*[2,3].

The penetration of the Treponema is carried out by small abrasions resulting from sexual intercourse. The response of the local defense results in erosion and ulceration, while the systemic dissemination causes the production of circulating immune complexes that can deposit themselves in any organ. The humoral immunity does not have a protection capacity, and the cell-mediated immunity comes later, which gives the *T. pallidum* multiplication and survival for long periods. In the histopathology, the most characteristic alterations are inflammatory infiltrate of plasma cells and perivascular lymphocytes, in which the endothelium presents proliferation and edema[2,4].

Owing to the wide variety of clinical manifestations, syphilis is divided into phases, with specific characteristics according to the clinical stage. However, patients infected with human immunodeficiency virus (HIV) may have atypical and exuberant symptomatology[5,6].

The early syphilis is defined by the manifestations of the disease within less than a year of evolution. After an average of 21 days of incubation, a papule, normally painless, appears at the inoculation site, evolving to the form of an ulcer with an elevated and a hardened edge. This alteration is called a hard chancre, characterized by the clinical period most typical of the disease, making the diagnosis and treatment easier. However, this fact is not routine in the care for patients with syphilis. The explanations by the patients to not seek a doctor in this stage are that the lesion is painless and goes unnoticed in most cases[7,8].

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The oral lesions are more frequent and varied in early syphilis and are described as syphilitic stains, syphilitic papules, and mucous patches. The oral chancres are found in 4 to 12% of the patients with early syphilis, and the most affected places are the tongue, the gums, the soft palate, and the lips[9,10].

Because of the diversity of manifestations that syphilis can present, its diagnosis acquires a multidisciplinary nature, involving various health-care professionals[8,11].

Despite the introduction of penicillin in the mid-20th century it has been impossible to eradicate this disease. Its incidence has increased in many parts of the world, and its resurgence is associated with, among other factors, the oral sex without protection, which seems to be potentially linked to the increase in the transmission and incidence of the disease[12-14].

The objectives of this study were to report a case of syphilitic chancre in the oral cavity and the importance of early diagnosis.

CASE REPORT

A 32-year-old female patient of mixed race was sent to the Public Health House, which operates the STD/AIDS program in the city of Valença, Rio de Janeiro, Brazil, by a dental surgeon. She reported menarche at the age of 12 years, first sexual intercourse at 16 years (she could not inform the number of sexual partners), being married, but having other partners. She had a pregnancy with vaginal delivery. The patient reported using oral contraceptives as a contraceptive method.

She mentioned the appearance of lesions that had been progressing for around a month, painless, in the palate region, and that she had undergone a biopsy by the dental surgeon. She reported the results of histopathological analysis of plasma cell inflammatory infiltrate and VDRL of 1/64, which was requested at the time of the pathological result by the same dental surgeon. The woman declared that she rarely made use of condoms, including in the practice of oral sex, and denied the presence of lesions in the vulva and/or vaginal discharge.

On examination of the oral cavity, the presence of a single lesion, of ulcer type, was observed, measuring about 1.5 cm, painless, with an elevated edge and a light background on the palate region **(Figures 1** and **2)**, and no cervical lymphadenopathy.

Therefore, a rectoscopy of the vulva and a speculum examination were performed, and there was an absence of lesions and/or leukorrheas.

On the basis of these conditions, the diagnosis of early syphilis was confirmed, and a treatment with benzathine penicillin (2,400,000 UI) in intramuscular dose was prescribed, and serology for HIV and hepatitis B and C was requested. After a month of treatment, a total remission of the lesion was observed **(Figure 3)**. The patient was monitored with VDRL, whereby the titers were decreasing: VDRL 3 months after the treatment was 1/32; 5 months, 1/16; 6 months, 1/2; and 1 year and 2 months, not reactant. The HBsAg, anti-HCV, and anti-HIV tests revealed nonreactive results.

DISCUSSION

Syphilis is a chronic infectious disease, which was first discovered in Europe in 1940. In the last three decades, there has been a general increase in the number of cases because of the pandemics of HIV virus. According to the World Health

Figure 2 – Syphilitic chancre in oral cavity.

Figure 1 – Syphilitic chancre in oral cavity.

Figure 3 – Absence of lesion after treatment with benzathine penicillin G.

Organization reports, more than 12 million new cases of syphilis occur every year. There are many reasons for the increasing prevalence of the disease. The main causes are the practice of unprotected anogenital sex and the ineffective search of the partner for treatment. Several factors also influence the growth of the number of cases including: patient negligence, absence of professionals in the Basic Family Health Units (UBSF), omission of the UBSF, medical misdiagnosis, delay in the results of VDRL, remote housing resulting in difficulties to access health-care centers, and lack of medicine for the treatment of the disease[15-18].

Given that syphilis can mimic many other clinical entities and that the initial manifestation of syphilis may be in the oral cavity, it is very important to include this disease in the list of differential diagnosis.

The anamnesis constitutes the primary basis, in association with clinical examination, of the search for a correct diagnosis. When one receives a patient with a history of sexual practices involving multiple partners, it is essential to investigate STDs . Furthermore, if the patient's main complaint is an oral lesion, the possibility of syphilis should be assessed, especially if this individual does not use condoms.

The patient in question already showed a risk behavior: background of multiple partners, rare use of condoms, and oral lesions from probable orogenital transmission. In Brazil, 62.45% of the women reported that oral sex is a part of the sexual act[19].

We highlight the role of the dental surgeon who noticed the lesion and made the diagnosis after conducting a biopsy of the lesion. Oral health professionals are the most likely to come across oral manifestations associated with the disease[15].

Finding the etiologic agent in the lesion through the dark field microscopy is the gold standard for the diagnosis of syphilis; however, in oral lesions, it may present a dubious result. The same goes for cytopathology, and this is because of the constant presence of other spiral bacteria morphologically similar to *T. pallidum*. Other diagnostic methods include detecting antitreponemic antibodies through nontreponemic VDRL techniques and through treponemic serology (FTA-ABS)[8,20,21].

The anatomopathologic study is not routinely performed for the diagnosis of syphilis. Because the basic pathologic finding in all the stages is edema, with the proliferation of endothelial cells and perivascular inflammatory infiltrate with lymphocytes and plasma cells being unspecific findings, other methods are necessary to confirm the diagnosis[2].

The sensitivity of the VDRL is 70% in early syphilis with a hard chancre, 99% in early syphilis with cutaneous manifestations, and about 75% in the late form[22]. It shows positive results from the fourth or fifth week after the infection, and it is the method of choice for monitoring patients with treated syphilis, because its result is expressed quantitatively[23-25]. The conversion to negative results occurs in approximately one year[16].

CONCLUSION

The clinical suspicion of syphilis is essential in the case of oral lesions in a patient who has multiple partners and does not use condoms, regardless of the sexual behavior. Syphilis is a disease that can simulate various other entities. The knowledge by health professionals of the oral manifestations in all its stages is of fundamental importance, so that they are able to perform a correct diagnosis and an early treatment.

Conflict of interests

The authors report no conflict of interests.

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Genital herpes on the penis and topic use of Uncaria Tomentosa: case report

Herpes Genital peniano e uso tópico gel de Uncaria Tomentosa: relato de caso

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ABSTRACT

Introduction: genital herpes is an infectious sexually transmitted disease that affects people from different socioeconomic strata, it is widespread throughout the world and a frequent cause of painful genital lesions in men and women. **Objective and Methods:** to report a case of a patient with penile ulcers initially treated with various topical and oral products, who finally used *Uncaria tomentosa* gel topically. The final diagnosis was genital herpes. **Results:** The symptoms of pain and burning had rapid remission. The clinical course was satisfactory and after a week, the patient, with no lesion, reported having had sexual intercourse without discomfort and returned to professional activities. **Conclusion:** the topical use of *Uncaria tomentosa* gel 50 mg/g in penile ulcers was well tolerated, and showed no side effects with relief of local symptoms.

Keywords: genital herpes; *Uncaria tomentosa*; treatment, STD.

RESUMO

Introdução: herpes genital é uma doença infectocontagiosa de transmissão sexual que acomete pessoas das mais diversas camadas socioeconômicas e está disseminada em todo o mundo, sendo uma causa frequente de lesões genitais dolorosas em homens e em mulheres. **Objetivo e Métodos:** descrever caso de paciente com úlceras penianas inicialmente medicado com vários produtos tópicos e orais que por último usou, topicamente, gel de *Uncaria tomentosa* e o diagnóstico final foi herpes genital. **Resultados:** os sintomas de dor e ardor tiveram rápida remissão. A evolução clínica foi satisfatória e após uma semana o paciente, já sem lesão, informou ter tido relação sexual sem incômodos e retomado as atividades profissionais de forma plena. **Conclusão:** o uso tópico de gel de *Uncaria tomentosa* 50 mg/g em úlceras penianas foi bem tolerado, não teve efeitos colaterais com alívio dos sintomas locais.

Palavras-chave: herpes genital; *Uncaria tomentosa*; tratamento, DST.

INTRODUCTION

Genital herpes (GH) is characterized as an infectious and contagious disease generally transmitted by direct sexual contact. However, in many cases the source of contamination is not known. Most times, GH is caused by the herpes simplex virus type 2 (HSV-2) and, in some cases, by the herpes simplex virus type 1 (HSV-1). HSV-1 and HSV-2 are DNA viruses, which are thermolabile and sensitive to ether, phenol and formaldehyde, being partially inactivated by ultraviolet radiation. However, they are resistant to cooling[1-3].

The disease is subject to repetition of crises and its period of incubation is from 1 to 26 days (mean of 7 days). The contact with exulcerated, ulcerated or vesicle lesions is the most common contamination pathway, but transmission can also take place through an asymptomatic person[1-3]. The disease is spread all over the world, affecting people from different socioeconomic strata in the populations[2,3]. Clinical manifestations can be divided into primo manifestation or recurrent. In general, the primo manifestation comes after symptoms such as erythema, burning sensation, mild pruritus and pain. Grouped vesicles appear on the erythematous base and stay there from four to five days; then comes the erosion. The whole process can last from two to three weeks. The appearance of a single lesion occurs in some cases. The genital condition often comes with fever, migraine, general indisposition and myalgia. Inguinal or femoral adenopathy occurs in approximately 70% of the cases[1-3].

In the recurrences, depending on many factors, lesions were less intense and lasted a shorter period of time in comparison to the primo manifestations. The treatment to eliminate the GH virus is still inefficient and aims at minimizing the effects and the duration of the crises[2]. Oral acyclovir, famciclovir and valacyclovir are the most effective medicines used to treat GH[2]. Topical antiviral therapy with acyclovir brings little clinical benefit[4,5].

OBJECTIVE

To describe the case of a patient with penile ulcers, diagnosed with GH, initially treated with different topical and oral products, finally using the topical gel of *Uncaria tomentosa.*

CASE REPORT AND DISCUSSION

A man in the sixth decade of life referred by a colleague general practitioner reported the presence of penile ulcers for more than 30 days.

He informed that the lesions were painful and did not disappear after using several medications, such as: polyvalent dermatological cream (with antibiotics, antifungal cream and corticoids), oral antibiotics (cephalexin, azithromycin, ciprofloxacin), bath with potassium permanganate solution, silver sulfadiazine and gentian violet.

The patient mentioned that, at night, before going to sleep, he felt pruritus on the glans and discomfort (not necessarily pain) in the penis and in the right scrotum. In the next morning, he observed two "blisters" on the glans. In the afternoon, the discomfort in the

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penis increased, with pain and burning sensation. At night, he presented general indisposition, as if he had the flu. Since he did not know what to do, he used a moisturizer and took a pain killer. On the next day, during shower, he rubbed the area and the blisters ruptured. Then, he noticed the area was inflamed and that the pain continued, even with the constant use of painkillers/anti-inflammatory. Since he did not feel any satisfactory improvement, he looked for a doctor on the sixth day after the onset of the problem, and was advised to use a polyvalent dermatological cream. He said he saw some improvement, but the lesions did not disappear. However, after some days, they returned.

He came back to the doctor, who prescribed oral acyclovir: 400 mg 3 times a day, for 5 days. Since he did not see any improvement, he decided to interrupt the treatment on the third day. He used several topical products, without satisfactory improvement. Then, finally, he decided on his own to apply gentian violet on the lesions.

He reported that this problem affected his quality of life. He missed work days, worked inefficiently, stopped traveling for business and did not have any sexual activity.

Then, he was advised to look for us. By telephone, at night, we asked him not to clean nor apply any product on the penis until our appointment, which took place in the next afternoon.

At clinical examination we observed the glans had several ulcerated lesions, with purulent inflammatory process and purple areas (**Figure 1**).

We collected scrape samples from the lesions for Gram bacterioscopy, stained cytology and polymerase chain reaction (PCR) for herpes simplex virus (HSV).

Figure 1 – Extensive ulcers, reported as painful, on the penis.

During the appointment, we applied Uncaria tomentosa on the affected area, 50 mg/g (free sample, lot 130688) and advised him to use the phytotherapy medicine 3 times a day, for 5 days. We also requested the serology for VDRL, anti-HIV, anti-HBs, anti-HCV and HBsAg.

Two days later, the patient returned and showed great evolution. He reported not feeling any discomfort in the region, and the process of lesion remission was clear (**Figure 2**).

In this occasion, we talked again and the good medical conduct indicated examinations also for the sexual partner. The patient said he would talk to his wife. However, he claimed she did not complain of genital alterations and that, not long ago, she had undergone a gynecological test, with normal results.

The patient used the phytotherapy cream for six days. However, on the last day he used it only once, because he did not think the product was still necessary, since he did not present any pain and the lesions were practically gone. Also, he had sexual intercourse again, using a condom, and went back to his professional activities.

Since all serological tests were non-reactive, we prescribed a vaccine against hepatitis B.

The stained cytology from the lesion scrape suggested cytopathic effect caused by the herpes virus (multinucleation) and the HSV-DNA test by PCR was positive. The Gram bacterioscopy was inconclusive.

The patient possibly presented a primo manifestation of GH, with consecutive crises, so that the length of the lesions was longer. On the other hand, the little time of use of oral acyclovir may not have been sufficient to reduce the time of clinical manifestation.

Many publications acknowledge[6-12] that the topical use of Uncaria tomentosa 50 mg/g has excellent results in the symptomatic treatment of genital lesions caused by the herpes virus.

Figure 3 shows a lesion-free genital area, two weeks after the phytotherapy treatment. However, we would like to point out that,

Figura 2 – Importante remissão das lesões penianas dois dias após início de uso tópico do fitoterápico *Uncaria tomentosa.*

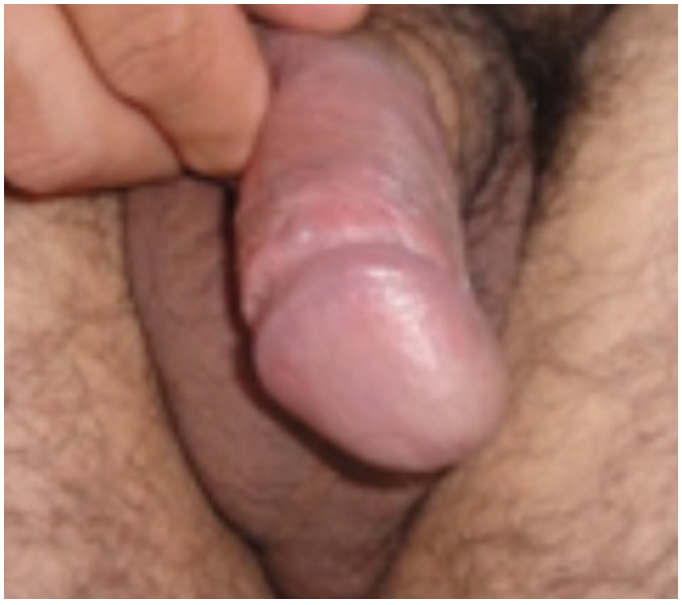

Figura 3 – Total remissão do processo ulceroso.

according to the information from the patient, the signs and symptoms completely disappeared in six days.

It is important to consider that good and efficient medical care involves the search for the etiological diagnosis of genital lesions, since the first appointment. On the other hand, the population should understand that self-medication often makes it more difficult to diagnose and cure the disease.

Despite our proposal, we did not talk to the person the patient had sexual intercourse with.

However, it is up to the medical assistant to pay attention and consider that lesions such as the ones presented by our patient may have a negative influence on quality of life, besides causing problems at work, such as absenteeism or presenteeism.

CONCLUSION

The topical use of *Uncaria tomentosa* 50 mg/g on penile ulcers was well tolerated, did not have side effects and was efficient to relieve the symptoms of GH.

Conflict of interests

The authors MRLP, JEJ and SMBC were collaborators in the monography of the product Imunomax® (*Uncaria tomentosa*), from the Herbarium Laboratório Botânico Ltda.

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ABSTRACTS OF PRESENTED PAPERS

ORAL 01 - Decrease in the expression of the Major Histocompatibility Complex II (MHC-II) and presence of Langerhans cells with immunosuppressive profile in human papillomavirus (HPV) - induced lesions

(Honorable Mention in the Oral Presentation Category)

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Introduction: Activation of T cells specific for Human Papillomavirus (HPV) in the cervical microenvironment has an important role in the eradication of virus infections and elimination of mutated cells. This activation depends on the expression of the major histocompatibility complex and cytokines produced by Langerhans cells in the lesion area. However, HPV genotypes of high oncogenic risk manipulate the immune system cells present in the infected cervical microenvironment, with the aim of not being eliminated, and an immunosuppressive role is induced in them through the production of IL-10 cytokines. **Objective:** To investigate the density of Langerhans cells that produce IL-10 and the class II MHC expression in cervical samples. **Methods:** Biopsies of the cervical epithelium without lesion (n=5), cervical intraepithelial lesions of low (n=10) and high degrees (n=10), and cervical carcinoma (n=10) were analyzed through immunohistochemistry, which were blocked in paraffin and previously submitted to histopathological evaluation and HPV-DNA detection. The co-expression of S100/IL-10 markers and the expression of MHC-II were also investigated. The manufactured slides were digitalized, and the software ImageJ analyzed them to determine the image area and density of immunomarked cells. The Research Ethics Committee of Universidade Federal de Mato Grosso do Sul (UFMS) approved this paper, under number 1628/10. **Results:** Among the samples without lesion, the co-expression S100/IL-10 was not seen, whereas 1.14 cells/mm^2 expressed the MHC-II. Among the lower lesions, 0.009 cells/mm^2 co-expressed S100/IL-10 and 0.73 cells/mm^2 expressed MHC-II. In the high-degree lesions, there were 0.23 cells/mm^2 co-expressing S100/IL-10 and 0.73 cells/mm^2 expressing MHC-II. Among the samples of cervical carcinoma, 0.32 cells/mm^2 presented a co-expression of S100/IL-10 and 0.37 cells/mm^2 presented the expression of MHC-II. **Conclusion:** The results indicate a negatively controlled microenvironment, which consists of a large number of immunosuppressive Langherans cells, and a decrease in MHC-II expression in the samples classified as carcinoma, followed by high-degree cervical intraepithelial lesions. These data suggest that the immunosuppression confirmed by cells and the deficient presentation of antigens through the decrease of MHC-II expression in HPV-induced lesions maintain a microenvironment that is favorable to viral persistence and neoplasm progression. This project was carried out in the Laboratory of Immunology and Molecular Biology of UFMS, with financial support of the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado (Fundect).

Keywords: papillomavirus infections; class II genes of the major histocompatibility complex; langerhans cells.

ORAL 02 - Human papillomavirus (HPV) and Chlamydia Trachomatis co-infection in positive and negative HPV samples obtained through self-collection and clinical collection

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Introduction: The Human Papillomavirus (HPV) is considered the most frequent etiological agent of Sexually Transmitted Infections (STI) in the world, and the main cause of cervical cancer. It is understood that this virus is capable of making associations with other microorganisms present in the vaginal tract. Among these pathogens, the *Chlamydia Trachomatis* is significant because it is a pathogenic bacterium, for the organism, capable of making the HPV infection stronger, thus increasing the risks of neoplasm progression. One of the problems that contribute to the evolution of the neoplasm development is the late diagnosis. Currently, the techniques for material collection are invasive, which makes population adhesion to the exam low, with the need of an alternative collection method in order to achieve a wider reach of the audience. **Objective:** To detect the Human Papillomavirus and *Chlamydia Trachomatis* co-infection in samples of positive and negative HPV uterine cervix through self-collection and clinical collection techniques. **Methods:** The paper was carried out with HPV positive samples obtained through self-collection and clinical collection (Research Ethics Committee of Universidade Federal do Mato Grosso do Sul, under number 383,072). One hundred and two samples from 51 HPV positive patients and 90 samples from 45 HPV negative patients were chosen. The analyses were done through conventional PCR, with modified Kl 1–F and Kl 2–R primers for DNA investigation of *C. trachomatis*. Then, the agarose gel electrophoresis was read in the transilluminator and photo documentation system. **Results:** Positive HPV samples were 1.96% positive for *Chlamydia trachomatis*. The samples from self-collection and clinical collection method had a concordance with regard to the DNA positivity for *C. trachomatis*. The DNA of bacteria was not detected in negative HPV samples. **Conclusion:** The self-collection technique had the same efficacy as the clinical collection; therefore, it is an advantageous option. More studies are needed to verify the association of *Chlamydia trachomatis* with the HPV virus, due to the low positivity index found. The project was carried out in the Immunology and Molecular Biology Laboratory, Universidade Federal do Mato Grosso do Sul (UFMS), with financial support of Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado (Fundect).

Keywords: papillomavirus infections; *Chlamydia trachomatis*; co-infection.

ORAL 03 - Human Papillomavirus (HPV) immunization coverage in the Basic Units of Health of Lerroville District in 2014, in Londrina city (Paraná), Brazil

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Introduction: Lerroville is one of the eight management districts of Londrina municipality, Paraná state, Brazil, with a population of 6,000 residents, of whom 71.9% live in the countryside and 28.1% in the urban area. Lerroville Basic Unit of Health (BUH) was opened in 1978, and was renovated and enlarged in 1995. It has several procedures, including immunization with vaccines. The staffs comprise many professionals, including two nurses, two physicians, eight nursing assistants, nine health community agents, one businessperson, one caretaker, two drivers, one physical therapist, one dentist, and one dental assistant. The *condylomata acuminate*, also known as genital warts, venereal warts, anal warts, or anogenital warts, is a Sexually Transmitted Disease (STD), caused by the Human Papillomavirus (HPV). There are more than 100 kinds of HPV in total, which are transmitted especially through sexual intercourse, or direct contact with infected skin or mucosae. The vaccine immunizes four of the most common HPV types: 6, 11, 16, and 18; the first two ones are associated with 90% of the genital warts and the last two with 70% of the cases of cervical cancer. The immunization target population is female adolescents, aged 11–13 years, who were not exposed to any HPV kind, and the goal of the Health Department is to vaccinate at least 80% of this population. In order to be vaccinated, the subject must show her vaccination card or an identification document. Each adolescent shall be given three doses to complete the protection. In these doses, the second dose must be given after six months; and the third, five years after the first dose. **Objective:** To present the HPV immunization coverage of the Basic Health Unit of Lerroville that was performed from March 10 to April 10 and from April 11 to July 25 in 2014. **Method:** A survey of the female population aged 11, 12, and 13 years was done through A/SIAB forms, and the data regarding the number of vaccines performed were entered in the Saúde Web system so that it was possible to find the total number of vaccines used and the calculation of immunization coverage. **Results:**

Table 1 – Number of used doses of the HPV vaccine, from March 10 to April 10, 2014, according to age range.

Age	Population	Number of used doses	Immunization coverage
11 years old	29	14	48.28%
12 years old	34	27	79.41%
13 years old	34	7	20.59%
Total	97	48	49.48%

Source: Sistema Informação da Atenção Básica (SIAB)

Table 2 – Number of used doses of the HPV vaccine, from April 11 to July 25, 2014, according to age range.

Age	Population	Number of used doses	Immunization coverage
11 years old	29	6	20.69%
12 years old	34	3	8.82%
13 years old	34	22	64.71%
Total	97	31	31.96%

Source: Sistema Informação da Atenção Básica (SIAB)

Table 3 – General total of used doses of HPV vaccine, from March 10 to July 25, 2014, according to age range.

Age	Population	Number of used doses	Immunization coverage
11 years old	29	20	68.97%
12 years old	34	30	88.24%
13 years old	34	29	85.29%
Total	97	79	81.44%

Source: Sistema Informação da Atenção Básica (SIAB)

Conclusion: The immunization coverage was beyond what the Department of Health determines, due to several factors, such as reorganization of the work process at USF and, especially, guidance to parents and girls who live in Lerroville district about immunization importance and efficacy.

Keywords: immunization coverage; papillomavirus infections; HPV.

ORAL 04 - Strategies of the Human Papillomavirus (HPV) immunization campaign for girls aged 11–13 years, in Aparecida de Goiânia (Goiás) in 2014

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Introduction: Human Papillomavirus (HPV) infection is common and produces several manifestations. In Brazil, HPV vaccine introduction in the immunization calendar aimed at preventing cervical cancer, thus reflecting on the decrease of incidence and mortality due to this disease. **Objective:** To report the strategies of the HPV immunization campaign for girls aged 11–13 years, in Aparecida de Goiânia (Goiás State), Brazil, in 2014. **Methods**: Case report study that describes the planning and operationalization of the HPV vaccination campaign with their respective immunization coverages. The estimated population receiving vaccination was of 13,137 girls aged 11–13 years. The Ministry of Health determined that at least 80% of the target population should be covered. For immunization coverage analysis, secondary data from the Sistema de Informação do Programa Nacional de Imunizações (Si-PNI) were used. **Results:** The main strategies used were: a) partnership between public and private schools; and b) availability of the vaccine in all health units. Twenty-two teams planned, coordinated, and operationalized the strategies, with each one in charge of seven schools. From the 155 existent schools in the city, only one did not accept taking part in the study. The campaign was done in 57 municipal schools, 62 state schools, and 35 private schools. Previous contact with all schools was performed, then the informed consent was sent to the parents, and the vaccination date was scheduled. In total, 10,912 (83.1%) girls aged 11–13 years received the first dose of the vaccine, 4,622 (35.2%) in health units and 6,290 (47.9%) at schools. The age group of 11–13 years old showed a very heterogeneous immunization coverage; the 11-year-old group showed 72.52% coverage; 12-year-old group showed 86.49% coverage; and 13-year-old showed 107.92% coverage. Only two side effects were reported: a lipothymia and a local reaction. The vaccines were administered in the dates determined by the National Program of Immunizations and are still available in health units for 17% of the girls who have yet not been vaccinated. **Conclusion:** The strategies were successful because they facilitated the selection of girls and ensured that the coverage determined by the Department of Health was accomplished. It has been seen that immunization of adolescents is part of a difficult tradition. It is necessary that health and education professionals and their managers in all levels, together, find means to get the best results in the immunization of the second dose in these populations. Although the vaccine administration for HPV prevention is essential, there is still the need of performing the essential examinations for preventing the cervical cancer.

Keywords: papillomavirus infections; vaccination; disease prevention.

ORAL 05 - Implantation of quadrivalent vaccine against Human Papillomavirus (HPV) in the city of Campos dos Goytacazes in boys aged 11–13 years and strategies used for immunization coverage increase

(Best paper in the Oral Presentation Category)

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Introduction: There are more than 100 different kinds of Human Papillomavirus (HPV). Among these, 30 affect the genital tract. Types 6 and 11 HPVs are considered of low risk and cause around 90% of the genital warts. The high-risk viruses are mainly types 16 and 18 HPVs, which have a higher probability of persisting and being associated with pre-cancer lesions and genital tumors. Rectal cancer had a 96% increase of its incidence in the last years in the male population. Pap test in women together with the use of condom for both genders is a strategy to identify and prevent this condition early. HPV vaccine is an addition to the protection portfolio. Campos dos Goytacazes (Rio de Janeiro, Brazil) has inserted the quadrivalent vaccine against HPV (types: 6, 11, 16, and 18), initially, for resident girls between 11 and 15 years old, since 2010. In 2014, from March 28, immunization for boys aged 11–13 years began in a hybrid strategy of vaccination in schools and in medical centers. **Objective:** To demonstrate all alternatives found to achieve immunization coverage for the three vaccine HPV doses, through the adoption of a vaccination hybrid strategy, combining the mobile vaccination in all public and private schools, the vaccine offer in fixed medical centers, and conducting lectures at schools for adolescents about HPV risks. **Methods:** Survey

of immunization coverage for the first HPV vaccine dose aimed at boys in the chosen age range. Data were taken from *Instituto Brasileiro de Geografia e Estatística* (IBGE) (10,000 boys), and the vaccination statistics were added to the vaccination information system. The Supporting Independent Immunization and Vaccine Advisory Committees (SIVAC) was also used, which the city acquired in order to register data of non-available vaccines in the immunization national program. Upon previous scheduling, the schools received the mobile vaccination. The vaccine was also offered in two fixed medical centers, especially for those who were absent at school and for children who do not study. **Results:** Until July 30, 2014, around 8,500 doses of HPV quadrivalent vaccine were given to boys, with an 85% coverage for the first dose. The strategies used to increase HPV coverage show that school vaccination accounted for around 80% of the coverage for the target population, while the fixed medical centers contributed with around 20% of the total coverage. **Conclusion:** The hybrid strategy of prevention through HPV vaccine associated with the use of health education strategies is efficient to adolescents in Campos dos Goytacazes, as correlated in international studies that used this method. Several other studies are being developed to assess the disease impact on the city and the acceptance level of the vaccine around the country.

Keywords: papillomavirus infections; vaccines; public health.

ORAL 06 - Human Papillomavirus (HPV) infection in genital and mouth of pregnant adolescents

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Introduction: Adolescence and pregnancy represent phases influenced by hormone changes that may predispose to Human Papillomavirus (HPV) infection in the genital area. Occurrence of an infection association between the genital and oral areas in adolescents and pregnant women has been suggested. However, it is still not known if the two conditions together increase this predisposal and association. It has been reported that HPV may infect the periodontium and strengthen the action of periodontopathogenic bacteria, causing the destruction of the teeth support tissues. **Objective:** The present study aimed at identifying a possible correlation of HPV infection in the mouth and cervix of 30 teenage pregnant girls (10–19 years old, according to the World Health Organization — WHO). **Methods:** An examination was performed in the genital area including the visual search for HPV-induced lesions (external genitalia, perineum, and anus). The injection of 2% acetic acid was done in the cervix. The acid-pigmented acetowhite areas were smeared. In the event of no pigmentation, smearing was done in the entrance of the endocervical channel. In the mouth, the virus research consisted of a clinical examination, i.e., a visual search for HPV-induced lesions in the oral mucosa. The complete periodontal examination, which includes determination of visible plaque indices, gingival bleeding, pocket depth, clinical level of insertion, and probe bleeding, was part of mouth examination. Samples of smears performed on tongue/palate and collection of supra-gingival (pool) and sub-gingival (four sites) dental bacterial biofilm were obtained. Smearing samples, collected in the genital and mouth, underwent cytopathological analysis and microarray testing (*Papillocheck®*). The molecular tests of Polymerase Chain Reaction (PCR) (MY09/11) and microarray were performed in biofilm samples. **Results:** Results were described. Fisher's exact test was applied to perform the associations between categorical variables. Kappa's test was used to assess the concordance between diagnosis methods. Group mean age was 15.2 years old (±1.3) and the average of gestational time was 28.8 weeks (±7.3). HPV-induced cell alterations that were identified using cytology were visualized in 3 (10%) smears in the cervix. The microarray testing found a virus in the cervix of 17 adolescents (56.7%), with higher prevalence of HPV16 (n=4; 23.5%) of high oncogenic potential. The citology did not find any HPV-induced cell alterations in any smear and the microarray did not detect the virus in any sample in the mouth. Twenty-two (73.3%) adolescents presented gingivitis at the time of periodontal examination, whereas 8 (26.6%) exhibited periodontitis. The PCR did not find the presence of HPV in any supra- and sub-gingival biofilm sample. The microarray testing identified the presence of HPV16 (low oncogenic potential) virus in the sub-gingival biofilm in a pregnant adolescent. There was no concordance between the used diagnosis methods (clinical *versus* cytological [k=0.103]; clinical *versus* molecular [k=0.198); cytological *versus* molecular [k=0.157]). There was a statistically significant association between adolescents with gingivitis and HPV presence in the cervix ($p<0.05$). **Conclusion:** Finally, there was no association between the presence of HPV in the mouth and cervix in the studied population.

Keywords: human papillomavirus 16; periodontal diseases; mouth; dental plaque.

ORAL 07 - Health practices and confrontation to Human Papillomavirus (HPV) of women living in rural cities: immunization scheme implications

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Introduction: It is observed that the Human Papillomavirus (HPV) vaccination in 70% of the girls younger than 13 years old combined with at least three Pap tests in women aged 35–45 years decreases the risk of cancer to 61%. The immunization scheme is composed of three doses: the first is offered at schools and basic units of health (BUH), the second is within a six-month interval, and the third, a reinforcement, in five years after the first dose. The last two doses are administered in the health unit. **Objective:** To analyze health practices and HPV confrontation of women living in rural cities. **Methods:** The sample comprised 421 women in reproductive age, from 16 rural cities in Paraíba State, of whom 15 women diagnosed with HPV were interviewed. A questionnaire was used about sexual and preventive practices, clinical aspects associated with HPV, and access to services, besides domiciliary interviews, which were analyzed through descriptive statistics and association and analysis of thematic categories. **Results:** The mean age was 35 years old (SD=8.13 years), distributed into the following age ranges: 18–29 (31%) and 30–49 years old (69%). The profile can be described as: married (75%), with elementary educational level (51%), income of up to two minimum wages (93%). The sexual beginning was on average after 18 years of age (SD=4.59), and for 23%, it happened between 10 and 15 years of age. With regard to the use of condom, 71% did not use it in their first sexual intercourse and only 12% affirmed using it constantly. The use of condom in the first sexual intercourse and thereafter is higher in the age range of 18–29 years (χ^2=26.961; p=0.000 and χ^2=12.565; p=0.002). In total, 24% declared never having gone to a gynecologist, a rate higher among older girls (χ^2=4.207; p=0.040); 11% have never taken a Pap test; 42% have never had ultrasonography; and 77% have never had mammography. In total, 17% mentioned the occurrence of Sexually Transmitted Diseases (STDs) (15 cases of HPV, 7 of candidiasis, and 1 of syphilis). The evaluation of health services obtained a mean score of 6.3 (SD=3.02), which was satisfactory. Among the common complaints was lack of physicians in a daily basis (76%), hospitals (74%) and equipment/lab for examinations (69%). The access was evaluated as easy (84%), with difficulties at scheduling appointments (40%), distance (23%), and difficulties at transportation (18%). The results from the interviews evidenced three categories: late diagnosis (embarrassment, lack of information, no apparent symptoms); diagnosis moment (fear, association with death, lack of clarifications); and treatment (access difficulty, transport need). **Conclusion:** Although there is appropriation in the analyzed age range in the immunization scheme of residents from rural cities, the other stages of the immunization scheme can be suffered due to the lack of contextualized information and structural conditions of health services that excessively make its access difficult.

Keywords: papillomavirus infections; papillomavirus vaccines; rural population health.

ORAL 08 - Concordance between self-collection and clinical collection in the detection of Gardnerella Vaginalis associated with Human Papillomavirus (HPV)

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Introduction: The human Papillomavirus (HPV) is directly associated with cervical cancer. Although it is pointed out as the main cause of this kind of cancer, there are many other related factors. Among these factors, the presence of co-infections with other microorganisms such as *Gardnerella vaginalis* (GV) bacterium, the target of this study, is significant. Despite a large number of studies in the area, some difficulties are still found in the screening routine of the Brazilian population, for example, the type of collection. An option suggests the use of self-collections followed by a viral identification through Polymerase Chain Reaction (PCR). This option would result in a wider population coverage and a significant gain of sensitivity, besides enabling the concomitant detection of other associated pathogens. **Objective:** To compare the DNA frequency of the *Gardnerella vaginalis* bacterium in positive and negative HPV samples that were stratified through self-collection and clinical collection. **Methods:** Samples of the cervix were collected through self-collection and clinical collection methods, from 51 HPV positive patients and 45 random HPV negative patients, which were stored at -20°C in sample banks for analysis. The patients considered as infected with HPV were positive for at least one of the collection methods, through PGMY-PCR in a previous study. In this investigation, the samples were assessed

regarding the presence of bacterial DNA through conventional PCR, using specific primers for the 16S region of *Gardnerella vaginalis*, according to the Laboratory standard protocol. The detection was done through 1.5% agarose gel electrophoresis, and the product was dyed using ethidium bromate. The Research Ethics Committee (REC) of Universidade Federal do Mato Grosso (UFMS) approved the study, under protocol number 383.072. The results were analyzed using the software Doc It-LS. **Results:** The positive HPV group had a positivity percentage for GV of 78.4, while the negative HPV group had a 66.6% positivity. If only the results were being considered based on the types of collection, in the positive HPV group, self-collection positivity was of 62.7% and 58.8% for clinical collection. **Conclusion:** *Gardnerella vaginalis* confirms what is suggested in literature. It is present most frequently in positive HPV samples. The self-collection method presents results close to those found in the clinical collection, thus indicating that it can be a viable option in the concomitant detection of other factors associated with HPV. This project was carried out at Universidade Federal do Mato Grosso do Sul (UFMS), with financial support of Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado (Fundect).

Keywords: papillomavirus infections; co-infection; *Gardnerella vaginalis*.

ORAL 09 - National program of Human Papillomavirus (HPV) immunization and vaccine in Brazil: adopted strategies and success in the results

(Honorable mention in the Oral Presentation Category)

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Introduction: The implementation of Human Papillomavirus (HPV) quadrivalent vaccine in the Immunization National Program (INP) of the Brazilian Ministry of Health began on March 10, 2014. It was based on vaccine efficacy studies to prevent cervical cancer and genital warts. The INP/Ministry of Health implemented the HPV vaccine in the modality of routine immunization in partnership with schools. It was also done in collaboration with some scientific and class societies and the Advisor Technical Committee in Immunizations (ATCI). The extended immunization scheme was adopted for the population aged 11–13 years in three doses administered at 0-, 6-, and 60-month intervals. The nominal registration was recommended at local level for ensuring the vaccination follow-up and active search for possible subjects lacking the subsequent doses, which is done through the system that provides an online graphic and numerical demonstration, with data added to the doses and immunization coverage (IC). **Objective:** To describe the INP/Ministry of Health strategy of implementing the HPV vaccine and the immunization results after the first dose. **Methods:** Ecological descriptive study using technical documents and secondary data of vaccination is available at <http:pni.datasus.gov.br> by INP/Ministry of Health. There was a description of the adopted vaccination strategy and preliminary results after the first vaccine dose in the country per age and Federate Unit (FU). **Results:** The determined IC goal was 80%, simultaneously to the performance of studies, to assess the impact of immunization. The first results regarding immunization coverage showed that more than 4,322,080 first doses of the vaccine were administered in a population estimated to be 5.2 million adolescents aged 11–13 years. The average IC with the first dose was 87.9%, with a variation from 83.41% in the 11-year-old group to 99.26% in the 13-year-old group. In 25 of the 27 FU, the IC surpassed 80%, with the best result in São Paulo (98.75%) and in Santa Catarina (92.64%). **Conclusion:** The immunization strategy with HPV confirms the success of INP in implementing new vaccines. The use of an online tool to follow the vaccination advance and create different assessment reports made possible the situation observance at real time. The partnership of INP/Ministry of Health with State and Municipal Departments of Health, scientific societies, and, mainly, schools was essential to obtain high IC. Ensuring their maintenance and searching for other strategies must be the target of INP to achieve good results in subsequent doses in order to accomplish the vaccination objectives.

Keywords: papillomavirus vaccines; HPV; prevention

POSTER 01 - Incidence of Human Papillomavirus (HPV) relapses in the clinic of sexually transmitted diseases in the years of 2012 and 2013 in Londrina (Paraná State, Brazil)

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Introduction: Londrina is a city in Paraná State, Southern Brazil, 381 km from Curitiba capital. The clinic of Sexually Transmitted Diseases, Human Immunodeficiency Virus and Acquired Immunodeficiency Syndrome (STD/HIV/AIDS) was implemented in 1992. The current urologist began his activities at such place in 1999, where he became a reference for users from the Brazilian Unified Health Systems (SUS) of Londrina and of the 17th Health Regional, which is comprised of 21 municipalities, in which Londrina is its headquarter. The users are assessed, diagnosed, and receive treatment. Later, they are sent to the services of the Center of Testing and Counseling (CTA) and Basic Health Units to take HIV, syphilis, and hepatitis B and C tests. **Objective:** To assess the relapse incidence of Human Papillomavirus (HPV) lesions after treatment in patients sent to Basic Health Units and to the clinic of HIV/AIDS, where the urologist treated them in the clinic of STD. **Methods:** This is a quantitative research, and data collection was done using clinical assessment and of patients' records in the years 2012 and 2013. The Human Papillomavirus, also known as HPV, is a virus installed on the skin or on mucosae that affects both men and women. It is transmitted sexually, vertically (mother–child), and rarely through fomites. In most cases, the HPV does not present symptoms and it is spontaneously eliminated through the organism. The incubation, i.e., period of time needed so that HPV infection manifestations are seen, is of around 2–8 months, but it can take many years. Treatment depends on factors such as patient's age, lesion type, extension, and location. **Results:** In 2012, 747 men received treatment in the STD clinic. Among these, 456 were diagnosed and received treatment for HPV, 75.9% were men treated in their first consultation, and 24.1% were relapses from 2004 to 2010; the predominant age range was from 20 to 39 years (69.3%), followed by 40–49 years (12.1%), 14–19 years (12.1%), 50–59 years (3.9%), and 60 years or older (2.6%). In 2013, the STD clinic received 980 men. Among them, 418 were diagnosed and received treatment for HPV, 74.2% were men treated in their first consultation, and 25.8% were relapses from 2005 to 2009 and 2011; the predominant age range was from 20 to 39 years (67.5%), followed by 14–19 years (14.6%), 40–49 years (9.8%), 50–59 years (6.7%), and 60 years or older (1.4%). According to some authors, the percentage of men presenting relapses, even after treatment with electrocauterization, was 24.8%. However, 33% relapses have been found in North-American men. **Conclusion:** HPV is highly contagious. One can be contaminated in only one exposure. Therefore, prevention and promotion measures are essential to control the diseases. Patients must be aware of condom use to reduce the risk of transmission to partner(s) who are not infected.

Keywords: incidence; papillomavirus infections; health of men; condylomata acuminada.

POSTER 02 - Adhesion of Human Papillomavirus (HPV) vaccine at health units, in Aparecida de Goiânia, in the year 2014

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Introduction: The Human Papillomavirus (HPV) is the most frequent sexually transmitted disease (STD). It has been estimated that around 50% of the sexually active population will be exposed to HPV at some time of their lives. Each year, about 4,000 women die of cervical cancer in Brazil. HPV vaccine prevents against infections through the viral types present in the vaccine, and, consequently, cervical cancer and disease load decrease. **Objective:** To report the adhesion of HPV vaccine in health units of Aparecida de Goiânia (Goiás State, Brazil), in 2014. **Methods:** An experience report case, describing the evaluation of adhesion to HPV vaccine in health units. In such city, there are 30 health units with vaccine rooms under operation (23 teams of Family Health — ESF; 3 Centers of Full Health Attention — Cais; and 4 Centers of Health). The estimated population to receive the vaccine in Aparecida de Goiânia was of 13,137 girls aged 11–13 years. The Ministry of Health adopted the extended vaccination scheme including three doses (0, 6, and 60 months). An 80% immunization coverage shall be achieved to show the impact of vaccination regarding collective health. Data were extracted from the Information System of Immunization National Program (Si-PNI) in order to assess the adhesion and immunization coverage. **Results:** The implementation of vaccine happened in 30 units of health after the entire team of vaccine rooms had been trained. The higher demand in the health units was in the campaign period. Until now, 2,741 (20.8%) people were immunized through the ESF, 1,276 (9.7%) in Cais, and 605 (4.6%) in Centers of Health. The health units have so far immunized 4,622 girls with a 35.2% immunization coverage; the total of immunized girls at schools is of 6,290 with a 47.9% coverage, resulting in an 83.1% of coverage. The vaccine search in health units was low compared to vaccination at schools. We believe that it happens due to some factors such as lack of parent's information regarding guidance to these adolescents, vaccine adverse reactions released in the media that happened in other states, religious reasons, and fear of inducing adolescents to early sex. **Conclusion:** The vaccine adhesion in health units was not representative, and it reached the goal expected due to the strategy at schools, which

facilitated the vaccine access to adolescents. Health professionals and authorities must be aware of their responsibility with regard to information about the use and efficacy of the vaccine so that parents and adolescents know the importance of this kind of prevention, in order that the adhesion to the second dose be effective. The strategies used in this city promoted access of target people; therefore, it was easier to obtain the immunization coverage that the Ministry of Health determines. These strategies shall be met in the second stage to accomplish the coverage.

Keywords: papillomavirus infections; vaccination; adolescent health.

POSTER 03 - Detection of APOBEC3-mediated hypermutation in Human Papillomavirus (HPV) genome and its role in the infection persistence in a HIV-positive pregnant women cohort

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Introduction: The cervical cancer is the second most frequent cancer among women around the world, and it is considered a public health issue, especially in developing countries. The development of cervical cancer needs the Human Papillomavirus (HPV) infection. The APOBECs enzyme family comprises a group of cytidine deaminases with the ability of adding mutations in sequences of DNA and/or RNA. These enzymes are able to restrict the pathogenesis of several viruses through a DNA edition mechanism known as hypermutation, causing G→A or C→T exchanges. A recent study observed evidence for the DNA edition of HPV16 through these enzymes in samples of pre-malignant cervical lesions; however, their role in the HPV infection and cervical cancer progression is not well established. **Objective:** This study aimed at investigating the presence of APOBEC3-mediated hypermutation in the genomes of high-risk HPV, and also verifying its relation with viral persistence and cytological alterations. **Methods:** Cervical smear samples of patients from a HIV-1-positive pregnant women cohort — who were followed-up by *Programa de Assistência Integral à Gestante HIV-positiva* of *Universidade Federal do Rio de Janeiro* (UFRJ) — were used. For hypermutation detection, the LCR region of HPV types 16 and 58 was widened, and then the technique of Polymerase Chain Reaction (PCR-3D) was used. The PCR-3D products were cloned and sequenced, posteriorly. For hypermutation statistical analyses, the software *Hypermut 2.0* was used. Until now, 15 HPV16 and seven HPV58 samples were successfully extended. There were no sequences of hypermutation in the seven HPV58 positive samples. **Results:** From the HPV16 samples, two presented evidence of hypermutation and sequences with monotonous substitutions – G→A e C→T. Twelve sequences of a sample were identified in the *Hypermut* program analysis as significantly hypermutated. **Conclusion:** It was seen that most of the editions happened in GpG and GpA dinucleotide contexts, which are representations of APOBEC3 enzyme actuation. In one of the patients who presented strongly hypermutated sequences, one evolution of normal cytology for a low-grade lesion (LSIL) during the time of studying was seen. Studies about the relation between HPV and APOBECs enzymes are another step for understanding the progression to cervical cancer. This study was carried out in the *Programa de Genética, Centro de Pesquisa do Instituto Nacional do Câncer* (INCA).

Keywords: papillomavirus infections; HPV; somatic hypermutation; immunoglobulin.

POSTER 04 - Human Papillomavirus (HPV) at Facebook: types of virtual communities and profile of their participants — search for new perspectives to care practice

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Introduction: The search for information encounters a strong ally on the network. Thus, inappropriate information may be described and used. Hence, social networks have a high number of users who are looking for information because they have communities directed to its transmission. Therefore, the present study was created to discuss social network communities that may aid the need of information about Human Papillomavirus (HPV) infection. **Objective:** To classify the kinds of HPV virtual communities available at Facebook and to identify the profile of participants in these communities. **Methods:** This is an exploratory and quantitative research using the communities available at Facebook® as data collection source. The keywords were indexed words: "HPV", "Cervical Cancer", and "HPV vaccine." The selection of communities and their participants followed inclusion and exclusion criteria. The communities presenting more interaction among their participants were chosen. They had 11,864 followers, and only 103 virtual identities (the participants were named, in the study, virtual identities because the same subject can have more than one identity in a virtual environment) were considered participants, i.e., expressing some kind of communication in the communities. The variables regarding virtual identities assessed age, gender, marital status, religion, city/state, profession, relationship interests, page search interest, and HPV carrier or not. **Results:** From the 9 communities, 4 were considered general information communities, 2 women information communities, 1 adolescent information community, 1 was directed to a school group campaign, and 1 focused on virtual campaign. Among these, users from the United States created 4, 3 were from Brazilians, 1 from Greeks, and 1 did not have any identification. The analysis of participants' profile obtained: 87% female and 54% reported their occupations, but the participants were not from the health industry. About 49.5% declared being HPV-infected. Around 34% were from the United States and 8% from Brazil. **Conclusion:** The higher rates for the female gender might be associated with women's search and "culture" of seeking medical care and follow-up through gynecological consultations. There was a higher frequency of North-American profiles than Brazilians. Most of the published information has a scientific basis and is a support to followers. The participants' self-statement as "virus carriers" shows that they are searching more for information than worrying about prejudice. The study shows how important is to disclosure correct data about HPV and the need of health professionals' participation in these communities to assess the "veracity" of the information, as no participants declared being a health professional.

Keywords: papillomavirus infections; uterine cervical neoplasms; social media.

POSTER 05 - Cytopathology as an efficient technique to track anal cancer precursor lesions

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Introduction: Despite the increase of incidence in the last decades, especially in women, recently, anal cancer is not considered a public health issue. Before this neoplasm has an invasive character, precursor lesions that are developed from the Human Papillomavirus (HPV) infection precede this neoplasm. Therefore, they are associated with the practice of receptive anal sex. It is also common in subjects with the human immunodeficiency virus (HIV).**Objective:** The research aimed at quantifying the patients who sought early diagnosis and detection of anal alterations through the anal cytology examination. **Methods:** This is a descriptive study using a quantitative data analysis in private domain bases. The information collection was carried out in a pathological anatomy laboratory in a city of Rio de Janeiro State. The collected and gathered data refer to January 2011 to December 2013. **Results:** At the time of data collection, there was a higher male demand. This gender presented an increasing quantitative each year. By the end, summing up three years, males represented 70%. With regard to the total volume of examinations, 4% represent the unsatisfactory diagnosis due to sample inadequacy for lack or absence of cellular components and 78% were negative for malignant neoplasm. In total, 18% indicate suspicious or positive tests for malignant neoplasm. From such parcel, 88% are men and 12% women. Diagnosis was divided according to nomenclatures of 2001 Bethesda System: Atypical Squamous Cells of Undetermined Significance (ASC-US and ASC-H for cases that high-grade lesions cannot be excluded), low-grade squamous intraepithelial lesion (LSIL), and high-grade squamous intraepithelial lesion (HSIL). For ASC-US diagnosis, the result was 88% among men and 12% among women. The LSIL was also more representative among men, with 96%. With regard to the Atypical Squamous Cells of Undetermined Significance, in which the possibility of a HSIL is not excluded, the representation was significantly higher among women, totaling 100%. However, in the lesion concluded as HSIL, there was a different reality, with men representing 78% of this high-grade precursor lesion. No cases of Invasive squamous-cell carcinoma were diagnosed. Considering the three-year-period of analysis, the total percentage for each diagnosis was: 8% ASC-US, 7% LSIL, 1% ASC-H, and 2% HSIL. **Conclusion:** Although there is a higher incidence of anal cancer among women, this paper found that the cytopathological examination search and precursor lesions were higher among men. The early diagnosis has a preventive profile, because it avoids the progression of these malignant lesions. The investigation was carried out at Laboratório Diagnóstico da América (DASA), from May to June 2014.

Keywords: cytology; anus; diagnosis; incidence.

POSTER 06 - Nursing and educational approach about Human Papillomavirus (HPV): experience report

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Introduction: The Human Papillomavirus (HPV) is an infectious agent expressed in lesions known as *condyloma acuminata* or genital wart. Normally, the virus is sexually transmitted, although other forms of transmission have been identified. **Objective:** To identify women's knowledge during nursing consultation about cervix AC regarding HPV. **Methods:** Experience report based on activities performed by nursing students and a professor in a medical center in Fortaleza (Ceará, Brazil) from February to June 2014. **Results:** Fifty-four gynecological consultations were performed. At data collection (anamnesis) and syndrome approach time, we found that few clients knew about the HPV. Some genital warts were identified in a pregnant adolescent when the prevention was being performed through analysis of the external genital region. In the opportunity, when the result came, it was possible to diagnose that the patient had HPV. **Conclusion:** The accomplishment of educational activities in the *Unidade de Atenção Primária à Saúde* (UAPS) *Dr. Alarico Leite* in Fortaleza, Brazil, was very important, especially in the syndrome approach, because it was possible to guide and inform about what is HPV and how one can "get" it.

Keywords: nursing; papillomavirus infections; health education.

POSTER 07 - Diversity of methylation patterns in long control region (LCR) of Human Papillomavirus 16 (HPV16) and Human Papillomavirus 18 (HPV18) in cervical cancer

(Best paper in the Poster Category)

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Introduction: Despite the promising results achieved by screening of asymptomatic women using Pap smears in the last decades and more recently with the advent of vaccines against Human Papillomavirus (HPV), cervical cancer is still a common disease with about 530,000 new cases and 275,000 deaths per year worldwide. For the cervical cancer (CC),viral integration has been suggested as an essential event during malignant transformation due to the loss of E2 repressive functions over E6 and E7 oncogenes. However, several studies have refuted this concept, since some HPV-related cancers lack integrated viral genome or potentially retain E2 gene functionally, suggesting that other events, such as DNA methylation, may contribute to the deregulation of E6 and E7 oncogenes in the HPV-induced carcinogenesis. **Objective:** To associate the methylation pattern of the CpG binding sites of HPV3' long control region (LCR) with HPV types and variants, clinical staging, and tumor kind. **Methods:** The analyzed samples were obtained from biopsies of patients at the Brazilian National Cancer Institute (*Instituto Nacional de Câncer* — INCA) ambulatory and diagnosed with invasive CC. HPV DNA detection was done through the Polymerase Chain Reaction (PCR), using the consensus primers PGMY09/11. By means of pyrosequencing, 5 CpG biding sites of 3' LCR of HPV16 and HPV18 were analyzed for methylation presence in 24 samples infected with HPV16 (21 invasive CC, 2 normal tissues, and 1 CASKI lineage), and 42 samples infected with HPV18 (39 invasive CC, 2 normal tissues, and 1 HeLa lineage). **Results:** Two groups were observed for HPV16, one including seven samples with high to intermediate methylation level (mean of 5 CpG biding sites of 74–26%) including CASKI (93%), and another group with six samples of low methylation level (20–2%) grouped with the samples without lesions/cancer (3.0% and 0.6%). A high methylation level was observed in adenocarcinoma compared to squamous cells carcinoma. For HPV18,a lower methylation pattern was observed, in which 33 of 38 samples with mean methylation level were lower than 20% and 6 samples with a higher methylation level (20–50%). In HeLa lineage and in normal tissue, a low methylation level was seen with means of 1.2% and 7.6%, respectively. **Conclusion:** A clear difference was observed in the methylation patterns between HPV16 and 18, in which HPV16 tends to have a higher methylation level. Additionally, differences in the methylation level between SCC and adenocarcinomas associated with HPV16 were seen, a finding that was not observed for tumors associated with HPV18, where both tumor types presented similar methylation levels. This work was supported by *Conselho Nacional para Desenvolvimento Científico* e Tecnológico (CNPq), *Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro* (FAPERJ), and *Instituto Nacional de Câncer*/*Ministério da Sáúde* (INCA/MS), *Institutos Nacionais de Ciência e Tecnologia para Controle do Câncer* (INTC).

Keywords: papillomavirus infections; methylation; LCR; uterine cervical neoplasms.

POSTER 08 - Knowledge about Human Papillomavirus (HPV) vaccine

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Introduction: Human Papillomavirus (HPV) is a virus that is closely associated with cervical cancer and genital warts. The cervix malignant neoplasm is the second most frequent in the female population, and fourth cause of women's death for cancer, in Brazil. Therefore, there is a great investment on the primary prevention of this disease, with the development of vaccines against the main kinds of HPV. In the year of 2014, the HPV vaccine was added to the Brazilian National Immunization Calendar for female adolescents aged 11–13 years. Knowledge and acceptability of vaccine by professionals, health users, and their guardians are essential in the national panorama. **Objective:** The aim of this study was to develop an instrument to assess knowledge and acceptance of HPV vaccine in Brazilian adolescents. **Methods:** The study was divided into two phases: instrument formulation and cultural adaptation. A wide bibliographic research about the subject was done to develop the pilot questionnaire, which includes questions about knowledge and acceptability of HPV vaccine. Then, there was an agreement meeting with specialists and professors of Gynecology at *Universidade de São Paulo* (USP) with the aim of presenting the study proposal and discussing the pilot questionnaire in order to complement it. In the instrument cultural adaptation stage, the purpose was to obtain a questionnaire that had been culturally adapted to Brazilians, whose language is Portuguese. An 85% minimum comprehension rate was determined for each question, i.e., only 85% of the total of interviewed people should understand the question. From August 2013 to January 2014, 67 female adolescents aged 9–19 years, with or without sexual activity, presenting menarche; 51 adults together with their daughters in medical appointments; and 33 health professionals working in the adolescent's health area, spontaneously, were interviewed. No restrictions regarding sexual activity, ethnicity, school, and socioeconomic levels of the included patients were determined. **Results:** The questionnaire was prepared and adapted in four versions before the definitive model had been created: HPV Con. Questionnaire, which includes 30 questions distributed into six domains. The first one is about HPV knowledge; the second domain assesses HPV vaccine knowledge; the third covers the barriers concerning HPV immunization; the fourth is about HPV vaccine acceptance; the fifth is about previous history regarding HPV infection; and the sixth is directed to health professionals. Each question has three possible answers (no, yes, and not sure), besides the division in some questions between public or private network and an open field for "other" answers. The questionnaire was applied during three minutes on average. **Conclusion:** The first questionnaire was created adapted to Portuguese language to assess HPV vaccine knowledge and acceptance.

Keywords: knowledge; questionnaires; papillomavirus vaccines

POSTER 09 - Analysis of Human Papillomavirus (HPV) vaccine implementation in a unit of Médico Família program in Niterói: case study

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Introduction: Cervical cancer in Brazil is the second most common kind of cancer among women, estimated in 15,590 cases for 2014 and 5,160 deaths in the year 2011. In 2014, the HPV vaccine was incorporated into the Immunization National Program (INP) for girls aged 11–13 years, and, since 2015, for girls aged 9–13 years. **Objective:** The present study analyzed the implementation of Human Papillomavirus (HPV) vaccine in a unit of *Programa Médico de Família* (PMF) in Niterói (Rio de Janeiro, Brazil), where II Supervised Field Work subject is developed, from the Medicine course curriculum of *Universidade Federal Fluminense* (UFF). **Methods:** This is a qualitative study whose main instruments and research techniques included semistructured interviews with professionals of the PMF unit (physicians, nurses, health community agents, and nursing technicians), Word Free Association test with registered users, and non-participant systematic observation. **Results:** The health local management actions to implement the vaccine at local level were restricted to information about the target population and number of doses, and no occurrence of some kind of capacitation for the entire team was reported. In the Word Free Association test, most users associated "HPV" with the disease, and less frequently with STD. A great majority of interviewed women answered "I

don't know" to the item "HPV Prevention". "Cervical cancer" was associated with preventive/prevent or treatment. Most users reported that they never talked to professionals about cervical cancer prevention forms, although most of them stated having taken the Pap examination, in different periods. Only one-third of the interviewed women mentioned having talked to a PMF professional about the examination. A majority of them has already heard about the HPV immunization campaign, and TV was their main source. **Conclusion:** Stages involving population sensitivity and involvement of health workers in the incorporation of new technologies are part of the policy implementation process and essential factors to accomplish the objectives, which is a less privileged aspect in the vaccine implementation in the studied case. HPV immunization without a great involvement of all actors might create unreal expectations and, overall, mobilize the society with regard to the current prevention policies.

Keywords: papillomavirus infections; papillomavirus vaccines; family health.

POSTER 10 - Human Papillomavirus (HPV): prevalence and genotypes found in positive and negative women with human immunodeficiency virus 1 (HIV-1) in the University Hospital of Universidade Federal do Rio Grande (HU-FURG) and in the Basic Health Unit of Rio Grande (Rio Grande do Sul)

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Introduction: Human Papillomavirus (HPV) is the main risk factor for cervical cancer appearance, mainly in the presence of genotypes with high oncogenic risk. According to *Instituto Nacional do Câncer* (INCA), cervical cancer is the third most incident tumor in the female population in Brazil. **Objective:** To estimate HPV prevalence and its genotypes in two different clinical samples of women attended in the clinics of Gynecology and Obstetrics at the University Hospital (UH) and in a Basic Health Unit (UBS) in the city of Rio Grande (RS). **Methods:** 152 samples of cervical cells from women attended in the UH and 24 samples of cervical biopsies from women attended in the UBS were collected from February 2013 to May 2014. The samples were analyzed to verify the presence of HPV and genotypes through Polymerase Chain Reaction (PCR) fixed with MY09/11 and GP05/06 primers, type-specific PCR, and sequencing. The oncological cytology results from samples were obtained in the medical record. All patients answered a self-applicable questionnaire and signed the free informed consent. **Results:** From the 152 samples of the analyzed cervical cells, 27.6% (n=42) were positive Human Immunodeficiency Virus 1 (HIV-1) and 72.3% (n=110) were negative HIV-1. The mean age of patients was 28.7 years (SD±11.4), with the variation in age from 14 to 69 years. Among all patients, 42% reported not using condom during sexual intercourse and 57% did not know about HPV infection. In 25% of the samples (n=38), the HPV-DNA was found. There was a 29% (n=11) prevalence among positive HIV-1 women, and a 71% (n=27) among negative HIV-1 women. None of the oncological cytology examinations from 38 samples of positive HPV cervical cells showed the presence of a HPV-related lesion, differently from the 24 samples of biopsies collected at UBS that presented high-grade lesion cytopathological alterations (100%; n=24). The mean age of participants attended in the UBS was 35.5 (SD±9.4); with the variation in age from 16 to 56 years. In total, 66% of the patients reported not using a condom in the sexual intercourse and 21% did not know about the HPV infection. The HPV-DNA was seen in all samples of the analyzed biopsies (100%; n=24). With regard to HPV genotyping, the genotypes found in samples without cytopathological alterations were HPV16 (n=8); –66 (n=4); –45 (n=2); –44, 58, 31, 35, and 85 (n=1). The genotypes in samples of biopsies with alterations were HPV16 (n=15); –58 (n=2); –53 (n=1). **Conclusion:** The high HPV prevalence found in the PCR emphasizes the importance of this method of diagnosis. The HPV DNA detection tests associated with the cytopathological analysis may be useful tools in the prevention, identification, and follow-up of women with risk of developing cervical carcinoma.

Keywords: polymerase chain reaction; papillomavirus infections; HIV-1

POSTER 11 - Training multiplier youngsters to combat sexually transmitted diseases and Acquired Immunodeficiency Syndrome (AIDS)

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Introduction: HIV cases have recently increased among young people aged 13–24 years. We have seen the need of creating groups to discuss the theme among youngsters, because paired communication is more effective. **Objective:** To train adolescents that know specialists of the issue regarding Sexually Transmitted Diseases and Acquired Immunodeficiency Syndrome (STD/AIDS), pregnancy in adolescence, and prevention methods with the aim of making them multipliers. **Methods:** A participative and investigative methodology was used as it will be based on the participants' own experiences and knowledge, to discuss, make people aware, and give a new significance to the issue. The sample included 20 adolescents studying in the first grade of high school, of both genders, from a public school in the city of Promissão (São Paulo, Brazil). Data were obtained through a questionnaire and were analyzed using descriptive statistics. The control group sample had a total of 80% girls and 20% boys. **Results:** With regard to information collection about sexuality, around 96% mentioned parents, friends, and school. With regard to STDs contagion, 41.67% answered incorrectly in the first questionnaire, whereas in the second questionnaire, 26.32% answered incorrectly. About 80% did not answer that condom is the most efficient way to avoid an STD; however, around 50% do not know how to identify it. The sample in the test group, where meetings are developed, has 60% girls and 40% boys. Information about STD/AIDS, according to the first questionnaire use, is obtained from parents (35.7%), friends (25%), and school (25%). The second questionnaire showed a 15% increase in the report of STD information accomplishment at school (41.38%). With regard to STD/AIDS transmission ways, 51.6% of the interviewed subjects answered incorrectly to such question; in the second questionnaire, only 8.89% answered incorrectly. Therefore, there was a 41.72% decrease reported. When participants were asked about STD symptoms, 70% did not know how to answer it in the first questionnaire; on the other hand, in the second questionnaire, only 13.33% of them did not answer. Thus there was a 56.67% decrease reported. Young people know that condom and contraceptives prevent an undesired pregnancy, and the first analysis showed that 52.2% of them mentioned female condom and 47.8% contraceptives. The second questionnaire showed that 72.22% answered female condom, with a 20% increase compared to the first datum. In the first questionnaire, 63.4% of youngsters declared that condom is the most efficient way to avoid an STD. But, in the second questionnaire, there was an increase in this value to around 20%, i.e., 83.33% recognized that condom is the best method. **Conclusion:** There was a significant increase in the level of knowledge and involvement of youngsters in STD/AIDS-related issues.

Keywords: education; sexually transmitted diseases; acquired immunodeficiency syndrome.

POSTER 12 - Gardnerella Vaginalis AND Human Papillomavirus (HPV) co-infection in women infected with the Human Immunodeficiency Virus (HIV) or carriers of Acquired Immunodeficiency Syndrome (AIDS) in a care service specialized in HIV/AIDS in Amazon, Brazil

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Introduction: *Gardnerella vaginalis* infection in Human Immunodeficiency Virus (HIV)-infected women can increase genital infectiousness and susceptibility, favoring co-infections such as Human Papillomavirus (HPV). **Objective:** The study sought to determine the prevalence of *G. vaginalis* and HPV co-infection in women with HIV/AIDS and its associated factors. **Methods:** Cross-sectional study (2009–2011) of HIV-infected women or carriers of the Acquired Immunodeficiency Syndrome (AIDS) in an HIV/AIDS Specialized Service (SAE), in Amazon, Brazil. A questionnaire including sociodemographic, behavioral, and clinical variables was used. The vaginal content of Pouch of Douglas was collected for *G. vaginalis* diagnosis through Gram staining, cervical sample for HVP test/hybrid capture 2v2, and oncotic cytology. The analysis included frequency distribution; median and interquartile interval. The infection prevalence rate was measured by positive test presence (9% CI). The association tests were χ^2 or Fisher's test. The Research Ethics Committee approved the paper (number 1962-2009/FMT-HVD). **Results:** From the 374 women, 304 (81.3%) were included in the study. *G. vaginalis* was found in 121 (36.3%) cases and HPV in 187 (52.6%). From the total, 75 (24.7%) were co-infected with *G. vaginalis* and HPV, 12 (17.4%) had grade I Cervical Intraepithelial Neoplasm (CIN), 1 (1.4%) had CIN II/III; 23 (31.1%) presented TCD4+ lymphocytes ≤200 cells/mm^3, and 40 (56.3%) had HIV-1 viral load >1,000 copies/mL. The most frequent age range was 30–39 years (n=34; 45.3%), mean: 18–29 years (n=294; 38.7%); 35 (46.7%) studied for more than 9 years; 36 (48%) were married/marital familiarity; 46 (61.3%) had an income of until 1 minimum salary; 68 (90.7%) were not smokers; 38 (51.4%) had their first sexual intercourse at the age of >15 years; 51 (85%) used condoms with their partners; 48 (64%) used contraceptives; 14 (18.9%) were sex professionals; 46 (66%) had anal sexual

practice; 2 (2.7%) were homosexuals; 47 (62.7%) presented vaginal secretion; 43 (57.3%) had vaginal pruritus; 40 (53.3%) had pelvic pain; 13 (19%) had CIN grades I and II/III; 23 (31.1%) presented TCD4+ cell counting ≤200 cells/mm^3; and 61 (82.4%) had AIDS. **Conclusion:** The *G. vaginalis* and HPV co-infection associated with CIN is prevalent among women with HIV/AIDS in the studied SAE. Tracking of vaginosis, STD, and anti-HPV immunization shall be integrated for preventive measures to sexual and reproductive health of this female population.

Keywords: *Gardnerella vaginalis*; papillomavirus infections; HIV; acquired immunodeficiency syndrome; co-infection; women's health.

POSTER 13 - Human Papillomavirus Immunization: a timeless indication

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Introductio: Human Papillomavirus (HPV) is a sexually transmitted virus, considered one of the most frequent sexually transmitted diseases (STDs) worldwide. These are viruses capable of inducing skin or mucosa lesions. There are more than 200 kinds of HPV, of which about 45 infect the male and female anogenital region. On average, 20–50% of sexually active women are infected somehow with the virus. Since the HPV infections are not always correctly diagnosed, and there is a prevention impossibility in 100% of the cases with the use of condom, other possible ways of prevention need to be studied. As in other viral infections, the development of a vaccine against HPV infection was promising. A study in adolescents (boys and girls aged 9–15 years) demonstrated an excellent immunological reaction with high concentrations of antibodies for a long time, thus its possible efficacy in this group. **Objective:** This paper aimed at providing good results with regard to the decrease in lesion relapse, both in the skin (vulva or cervix) in patients with initial diagnosis of condylomata, or grades I and II Cervical Intraepithelial Neoplasms (CIN), who were later immunized against HPV. **Methods:** One patient who was initially diagnosed with vulvar condylomata underwent several chemical cauterizations with 90% trichloroacetic acid and electrocauterization, and four patients with initial diagnosis of I and II CIN, initially submitted to high-frequency surgery (HFS) if recurrent I and II CIN, presented, during follow-up, lesion relapse. Since these patients were young, aged 19 and 32 years, with no children, the HPV quadrivalent immunization in three doses was chosen, with biannual follow-up including preventive collection and coloscopy. **Results:** After two years following-up these patients, there was no relapse of vulvar or cervix lesions. The patient carrying vulvar condyloma was the only who still presented oral lesions, even after immunization. However, she reported that the warts in the oral cavity were already there before the beginning of vulvar lesions treatment with chemical and electric cauterization and "she was ashamed of reporting the presence of oral lesions". This fact made us reflect on including the oral cavity test when dealing with HPV patients. **Conclusion:** Based on the presented facts, HPV immunization in patients with lesions, regardless of the age, becomes an important tool in the therapeutic complementation, thus decreasing the risk of lesion relapse. This study is still being carried out in the clinic of Cervical Patology of Hospital Escola Álvaro Alvim, at Faculdade de Medicina de Campos.

Keywords: papillomavirus infections; condylomata acuminata; cervical intraepithelial neoplasm.

POSTER 14 - Human Papillomavirus (HPV) knowledge and cervical cancer prevention in adolescents immunized against HPV: an integrative review

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Introduction: Human Papillomavirus (HPV) types 16 and 18 are responsible for about 70% of the cervical cancer cases worldwide, and types 6 and 11 are found in 90% of the genital warts. Immunization is among the most important prevention measures of HPV transmission. Until now, two vaccines have been developed and registered in Brazil: quadrivalent (protects against HPV6, 11, 16, and 18) and bivalent (protects against HPV16 and 18). In 2014, the Brazilian Ministry of Health, through the Immunization National Program (INP), extended the Immunization National calendar with the introduction of quadrivalent HPV vaccine in the Brazilian Unified Health System, with the purpose of preventing cervical cancer and genital warts together with tracking actions. Its implementation will be gradual and is being initially offered with no charges for female adolescents aged 11–13 years. **Objective:** The present study aimed at analyzing the quality of information regarding such theme, the gaps in this production, and providing a knowledge synthesis following evidence levels and, from data domain, allowing the planning of new scientific productions. **Methods:** This is an integrative review study, carried out in June 2014 in such databases: Medline, Lilacs, PubMed, Embase, Scopus, and Web of Science. The theme was established and the following question was chosen: "What are the publications about HPV knowledge and cervical cancer prevention of adolescents aged 11 to 13 years, immunized against HPV?" The five databases were concomitantly researched. The keywords were used in Portuguese (DeCS): (*Vacinas contra Papilomavírus* or *Vacinas contra HPV* or *Vacinas contra Papilomavírus* Humano) and (*Adolescente* or *Adolescentes*) and (*Criança* or *Crianças*) and Knowledge, attitudes, and health practices filter. In English (Mesh): (Papillomavirus Vaccines or Human Papillomavirus Vaccines or Human Papilloma Virus Vaccines or HPV Vaccines) and (Child or Children) and (Adolescent or Teenagers or Teen or Teens) and (Health Knowledge, Attitudes, Practice or Knowledge, Attitudes, Practice). Studies with articles or abstracts published and indexed in the mentioned databases in the last 5 years followed the inclusion criteria. Articles that did not associate the theme with the studied population were excluded. For the publication selection, each title and abstract was thoroughly read to confirm if they considered the focus of this investigation and if they met the determined inclusion and exclusion criteria. **Results:** Based on the determined strategies, the research resulted in 453 publications and, after reading each title and abstract, all publications were excluded for not answering the paper questioning and not meeting inclusion criteria. **Conclusion:** The review enabled the identification of a knowledge gap on the investigated theme, and points out the need of further studies about such issue.

Keywords: HPV; papillomavirus vaccines; adolescent.

POSTER 15 - Recurrent Laryngeal Papillomatosis: immunohistochemical and molecular study

(Honorable Mention in the Poster Category)

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Introduction: Human Papillomavirus (HPV) are high-prevalence pathogens that determine persistent infections in human beings and are involved in the genesis of benign and malignant epithelial lesions of the anogenital tract, respiratory tract, and skin. HPVs 16 and 18 are associated with the development of cervical, head, and neck carcinoma; and HPVs 6 and 11 are the most frequently detectable types in the recurrent laryngeal papillomatosis (RLP) and anogenital warts. Among many factors, variations in the immune response are suggested as justifications for the different behaviors of these lesions with similar etiology. In HPV infections, an effective cellular immune response comprised of T CD4+ cells of Th1 profile and T CD8+ cells has a main role in its resolution and control. **Objective:** To perform an immunohistochemical and molecular study through HPV genotyping in patients with RLP and nasal and oral papilloma. **Methods:** The sample included 125 patients cared for at Hospital Santa Izabel (Salvador, Bahia, Brazil), from 2004 to 2012. The immunophenotyping study of inflammatory infiltrated cells was done using monoclonal antibodies and immunohistochemical reaction with the EnVision™ System. The Kit INNO-LiPA HPV Genotyping Extra (Innogenetics, Ghent, Belgium) was used for HPV typing, enabling the identification of 28 high- and low-risk HPV types. **Results:** In nasal cavity lesions, there were 11 cases, immunomarking for 7 CD3 (63.6%) and 5 CD8 (45.5%) was semi-quantified as moderate in most of the cases, CD68 evidenced the same scores in 3 (27.3%) cases with discrete, moderate and severe degrees. The auxiliary T lymphocytes (CD4) were negative in 10 (90.9%) cases. In the oral cavity (7), CD3 was positive in 5 (71.4%) and CD8 in 4 (57.1%). Immunomarking was negative for CD4 in most of the lesions, whereas it was moderate in 3 (42.8%) cases for CD68. In the larynx (18), CD4 15 (83.3%) and CD68 were negative in the majority of cases. CD3 was semi-quantified as moderate in 9 (50.0%), and CD8 as discrete in 8 (44.4%). In 125 of the analyzed cases, HPV was found in 106 patients (84.8%), 9 (7.2%) were invalid, and the HPV was not seen in 10 (8.8%). HPV16 was the most prevalent, being found in 50.0% of the cases, followed by 11 (43.4%), 52 (36.8%), 6 (34.9%), and 58 (30.2%). With regard to oncogenic HPV types, HPV16 was seen more prevalent in the larynx in 33.8% of the cases. **Conclusion:** A low expression of T CD4+ cells was seen, thus indicating that a possible deficiency of immune cellular response mediated by T-cells would facilitate the HPV infection persistence. The lower frequency of CD68 cells may have contributed for a less efficient immune cellular response in the

studied lesions. The HPV infection frequencies through the Polymerase Chain Reaction (PCR) in RLP, oral, and nasal cavity papilloma was 88.2, 85.3, and 73.9%, respectively. **Acknowledgements:** FIOCRUZ and *Instituto Nacional de Ciência e Tecnologia das Doenças Associadas ao Papilomavírus*.

Keywords: HPV; recurrent respiratory papillomatosis; papillomaviridae; larynx immunohistochemical.

POSTER 16 - Human Papillomavirus (HPV) oral infection in subjects infected with the Human Immunodeficiency Virus (HIV)

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Introduction: Human Papillomavirus (HPV) is one of most common sexually transmitted viral agents and is usually associated with anogenital diseases. They are classified as oncogenic (high risk) and non-oncogenic (low risk). Currently, there are only few studies about oral infection through this virus. A relevant question in the HPV oral infection is it can be developed through oral-genital contact or self-inoculation, or even be considered an independent event. Knowledge of a virus group tropism is a very important biological factor to understand how the viral variants of the same kind of HPV are developed in different ecological niches and how they induce pathogenic consequences in their hosts. Evidences are increasingly associating high-risk HPV presence of genital tract neoplasms, especially type 16, with oropharyngeal cancers. Some studies show that the prevalence of HPV oral infection is higher in subjects infected with the human immunodeficiency virus (HIV) than in negative HIV subjects. **Objective:** The aim of this study was to detect and typify HPV infection in oral smears of HIV seropositive patients, to analyze the viral genome of less frequent types in the oral mucosa and compare the obtained results to a negative HIV population. **Methods:** The study population comprised 75 HIV-infected subjects and the control group included 120 HIV negative subjects. Demographic and behavioral factors were obtained through a questionnaire. After DNA extraction from the samples using a commercial kit, HPV DNA detection was performed using MY09/MY11 generic oligonucleotides. The analysis of Restriction Fragment Length Polymorphism (RFLP) was performed to determine the HPV types. **Results:** According to the results obtained until now, we have seen that in 75 samples of oral mucosa smears of HIV positive patients, 53 (70.7%) were MY positive. Thus, 31 (77.4%) samples were typified through the used technique and, among them, 10 (18.9%) presented more than one HPV type. A total of 54 HPV types were found in these 31 positive samples: 23 samples presented undetermined HPV types (42.59%), 21 samples included HPV53 (38.88%), type 6 was found in 3 (5.55%), type 82 in 2 (3.70%), and type 52 in other 2 (3.70%). Types 45 (1.85%), 68 (1.85%), and 84 (1.85%) were each seen in one sample. From the 120 samples of oral mucosa smear of HIV negative subjects, 55 (45.8%) were positive MY. **Conclusion:** Regardless the HIV infection, both groups presented high frequency of HPV in the oral mucosa, and the prevalence of undetermined types reflects the presence of infection though non-genital types.

Keywords: papillomavirus infections; HIV; polymerase chain reaction.

POSTER 17 - Anal cytology: correlation between age range and diagnosis of cancer precursor lesions

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Introduction: Anal cytology aims at tracking cancer precursor lesions in the initial stage of Human Papillomavirus (HPV) infection. The receptive anal sex practice is associated with the development of this neoplasm. Subjects who began practicing it early, as well as those with multiple partners, and biological risk factors, ensure a higher possibility. The use of anal cytology has been more frequently in the last decades after registrations of cancer increase. **Objective:** To describe the profile of patients who took the anal cytology test, and to correlate their ages to diagnosis of positivity for malignant neoplasm. **Methods:** This is a descriptive study with a quantitative data approach in private domain bases. The survey was performed in a Pathological Anatomy Laboratory in Rio de Janeiro. Collected and compiled data refer to the period of January 2011 to December 2013. **Results:** There has been a 1.6% increase in average of the volume of examinations each year. There was a higher search of the male people who presented an increasing annual quantitative, representing a total of 70%. The female people had a different result, with a decreasing search and a total of 30%. Men had a mean age of 38 years and women, 41 years, with a general variation in age from 17 to 82 years. With regard to results, the total percentage for each diagnosis was unsatisfactory for evaluation due to cellular material inadequacy (4%) and negative for malignant neoplasm (78%). The remaining rate of 18% was divided into suspicious or positive results for pre-malignant or malignant alterations: Atypical Squamous Cells of Undetermined Significance (ASC-US) (8%) and ASC-H (High-Grade Squamous Intraepithelial Lesion cannot be excluded) (1%), Low-Grade Squamous Intraepithelial Lesion (LSIL), (7%) and High-Grade Squamous Intraepithelial Lesion (HSIL) (2%). The following data are presented according to the highest indices of lesion per age range. Higher incidence (50%) in patients aged 21–30 years was registered for ASC-US diagnosis. Patients aged 31 and 40 years had a 30% record. With regard to ASC-H diagnosis, there was an expressive registration of 100% for patients aged 81 and 90 years. The LSIL had a 62% incidence for the age of 31–40 years, followed by 20% for the age range of 21–30 years. No cases of cancer were recorded; however, High-Grade Squamous Intraepithelial Lesion (HSIL) was prevalent in very different age ranges, despite its relatively low percentage. Patients aged 51–60 years and 71–80 years had a 22.3% rate. This same number was also seen for younger patients aged 21–30 years. **Conclusion:** The periodic collection of anal material to analyze cells, mainly for risk subjects, might retard the evolution of initial lesions for more severe precursor lesions. The study was performed at Lab. Diagnóstico da América (DASA).

Keywords: cytology; anus; intraepithelial neoplasm.

POSTER 18 - Prevalence and association of Human Papillomavirus in oral cavity and oropharynx carcinomas

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Introduction: The Human Papillomavirus (HPV) is a virus with great prevalence in carcinoma of the cervical region, as well as of other genital areas, but its prevalence and etiological relation in oral cavity and oropharynx carcinomas are being investigated. Several studies have reinforced this idea in the past years. When the virus is associated with other factors, such as smoking and alcoholism, the risks of carcinoma appearance in the oral cavity and oropharynx are increased. The presence of HPV16 genome increases such risk up to 50%. Unprotected oral sex or unprotected sex practice are very well described ways that lead to an increase in virus spreading and natural change of its location, since HPV is more common in the genital region. **Objective:** The study aimed at developing a theoretical basis for the development of a research project that is needed in the Central West region, with the purpose of estimating the HPV prevalence in head and neck carcinomas of patients cared at Hospital Araújo Jorge (HAJ), in Goiânia (Goiás, Brazil). **Methods:** This is a systematic review in literature from a bibliographic survey in LILACS and MEDLINE databases. The following keywords were used: *Oral cavity carcinoma*; *oropharynx carcinoma*; *Human Papillomavirus*. Papers that used the Polymerase Chain Reaction (PCR) as the HPV detection method and that associated the findings with carcinoma clinicopathological factors were included. **Results:** 13 studies that evaluated 1,216 cases of oral cavity and oropharynx carcinoma jointly were reviewed, and HPV was found in 36.45% of the cases, on average. In the 13 studies, HPV16 was the most prevalent genotype, present in 22–100% of HPV positive cases. There were more cases in male subjects, which also happened in cases where HPV genome was seen. The lower age mean in positive HPV cases was described in all studies. An inverse association between HPV presence and habits like smoking and alcoholism has been reported; therefore, HPV seems more prevalent in tumors of non-smokers and non-alcoholics. Thus, HPV was associated with smoking and alcoholism in some studies. A better prognosis and less recurrence has been reported for oral cavity and oropharynx carcinoma that present the HPV genome, as well as a higher prevalence of these tumors in younger subjects. **Conclusion:** The incidence of these tumors in young subjects has been increasing with time. They have been reported in studies carried out about oral cavity and oropharynx carcinomas association with HPV. Hence, and considering the high prevalence of HPV in oral cavity and oropharynx carcinoma in male subjects, it is extremely important that the HPV immunization campaign be extended to the male gender, as well. Recently, in Brazil, the HPV immunization campaign is exclusive to women, aged 11–13 years.

Keywords: papillomavirus infections; cancer; mouth; pharynx.

POSTER 19 - Anogenital Human Papillomavirus infection in men attending a dermatology clinic

(Honorable Mention in the Poster Category)

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Introduction: Human papillomavirus (HPV) infection causes one of the most prevalent sexually transmitted diseases (STDs) worldwide. The pathological and epidemiological features of HPV infection have been studied extensively in women due to the prevalence of this disease and its well-established association with cervical cancer. However, HPV infection is also an important concern in men due to the risk of transmission to women and the disease burden. **Objective:** We aimed at studying anogenital HPV infection in men. **Methods:** To achieve our goal, we evaluated a case series of 71 men attending a dermatology clinic in Brazil during an 18-month period with anogenital HPV infection. Clinical manifestations, laboratory findings, and sociodemographic factors were evaluated. Biopsy samples were subjected to histopathological analysis, generic and type-specific viral identification, and $p16^{INK4a}$ quantification. **Results:** The average age at diagnosis was 33 years. We observed little variation in identified viral types (HPVs 6, 11, 16, and 53), despite the inclusion of 16 HIV positive patients. The presence of high-risk HPV was associated with receptive anal sex ($p<0.05$), lesion malignancy ($p<0.01$), and $p16^{INK4a}$ expression ($p<0.05$). The HIV positive was correlated with HPV16 infection, presence of perianal lesions and high-grade lesions ($p<0.05$) diagnosed at a younger mean age than HIV negative patients ($p<0.05$). **Conclusion:** Our results demonstrate the unequivocal relationship between high-risk HPV infection and presence of high-grade lesions, HPV16 tropism in the anal epithelium, and the role of receptive anal sex as a risk factor for the development of high-grade anal lesions, which are early seen in HIV positive men who have sex with men. As high-grade lesions showed $p16^{INK4a}$ negativity but were associated with HPV16 presence, we believe that $p16^{INK4a}$ is a promising biomarker, but its use remains controversial requiring further research. The financial support was granted from Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (Faperj) (APQ1/2012) and Pró-Reitoria de Pesquisa, Pós-Graduação e Inovação da Universidade Federal Fluminense (Proppi/UFF).

Keywords: papillomavirus infections; cancer; anus; HIV; sexually transmitted diseases; polymerase chain reaction; genes, p16.

POSTER 20 - Can Human Papillomavirus (HPV) detection in oral mucosa suggest genital infection?

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Introduction: Human papillomavirus (HPV) is the etiological agent of cervical and anal cancers, and its pathogenic process has been elucidated, but little is known concerning the etiology of the oral infection and oral cancer. **Objective:** The aim of this study was to investigate whether oral infection could point out genital infection, determining the presence of HPV in both sites of infection. **Methods:** Oral scrapes from healthy mucosa and genital smears of condylomata lesions were evaluated by molecular methods. A hundred and ten samples from oral and genital sites were collected from patients attending the Sexually Transmitted Diseases Clinic from *Universidade Federal Fluminense*. **Objective:** To screen and type HPV DNA, generic MY09/11 Polymerase Chain Reaction (PCR), and type-specific PCR, followed by the restriction fragment length polymorphism (RFLP). **Results:** HPV was detected in 85.5% of genital lesions (n=55) and in 43.6% of oral mucosa samples. In 13 of the 55 (23.6%) studied cases, both sites were infected. The agreement between genital and oral types was high: 9 cases showed the same infected types in both mucosa. HPV11 were the most prevalent (n=7), followed by HPV6 (n=2) and HPV45 (n=1). Two cases showed mixed infections with HPV6/11 and one HPV11/45. Oral infection, separated by male and female, showed statistical significance (p=0.004), with markedly higher prevalence of oral infection on men. **Conclusion:** Our results indicate that HPV oral detection can suggest genital infection in half of the cases, but further studies are required to elucidate the natural history of HPV infection, mainly with regard to oral lesions.

Keywords: papillomavirus infections; mouth; genital; polymerase chain reaction.

Human papillomavirus infection in multiple sites

Infecção por papilomavírus humano em múltiplos sítios

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ABSTRACT

Introduction: Studies concerning human papillomavirus natural history have been focused on cervical infection and disease, but have scarcely described anal infections, especially in clinically health population. Hence, knowledge on HPV natural history is recently being investigated although viral maintenance in hosts is poorly understood. Detection on diverse, extragenital sites may add in the elucidation of infection-dissemination-reinfection cycle reported on the human genital tract. Besides that, it is evident the importance of an adequate screening of the viruses for appropriate diagnosis, mainly in the first steps of the neoplasia in order to provide a better prognosis. Our results pointed out HPV prevalence rates in genital lesions of 87% (80/92) and of 47.8% (44/92) for anal samples. We pointed out that this high prevalence is due to clinically detected lesions, that contributes to the risk of extragenital infections, mainly by host auto-inoculation. Despite the difference in samples size, we did not find statistical relevant differences related with genera (men 85.1% and women 92%; p>0.05), corroborating the idea of no tropism differences by sex. Nevertheless, for anal samples, statistically significant differences were found between men and women (68% of anal HPV infection in women against 40.3% in men (p=0.038). These results reveal that female anal infections are more frequent than in men, suggesting that infection by autoinoculation occurs and can render this site as an HPV reservoir, occasionally becoming clinical lesions. Hormonal profile, sexual behavior and differences among body sites can explain these differences here described. Our study is a preliminary evaluation of HPV infection in human multiple sites.

Keywords: papillomavirus infections; polymerase chain reaction; anus.

RESUMO

Introdução: Muitos estudos sobre a história natural do papilomavírus humano (HPV) têm focado nas infecções cervicais, mas pouco tem se falado sobre infecções anais em pacientes assintomáticos. Assim, a história natural do HPV ainda está em construção e os mecanismos de manutenção dele no organismo hospedeiro são pouco compreendidos. Entendemos que a detecção do vírus em sítios extragenitais poderá ajudar na compreensão da cadeia de infecção-disseminação-reinfecção pelo HPV no trato genital humano. Além disso, é evidente a importância de um rastreio adequado do vírus diante de diagnósticos clínicos, principalmente no período inicial da lesão, provendo, assim, um melhor prognóstico. Nossos resultados apontaram uma prevalência da infecção por HPV em lesões genitais de 87% (80/92) e nas amostras anais tal prevalência foi de 47,8% (44/92). Ressaltamos que nossa prevalência foi alta, mas estudamos indivíduos com lesões genitais clinicamente detectadas, aumentando, assim, o risco de infecção em outros sítios e confirmando a ocorrência da presença do HPV no hospedeiro, provavelmente decorrente de autoinoculação. Apesar da diferença entre o número de indivíduos dos sexos feminino e masculino em nosso estudo, não encontramos diferenças estatísticas relacionadas à presença do HPV em lesões genitais (H: 85,1% e M: 92%; p>0.05), corroborando que não há diferenças entre o tropismo por gênero referente às infecções genitais. Entretanto, no caso das infecções anais, a porcentagem foi estatisticamente diferente: 68% de HPV anal em mulheres e 40,3% em homens (p=0,038). Estes resultados apontam que a aquisição da infecção anal em mulheres, apesar de assintomática, é mais frequente do que em homens, sugerindo que esta mucosa possa ser infectada por autoinoculação e funcionar como um reservatório feminino, o que eventualmente poderá resultar em lesões. Tais discrepâncias da prevalência entre homens e mulheres poderiam ser explicadas pelo comportamento sexual, por efeitos hormonais e por diferenças entre sítios. Nosso estudo se trata de um levantamento preliminar da infecção por HPV em múltiplos sítios.

Palavras-chave: infecções por papilomavírus; PCR; ânus.

INTRODUCTION

The genital infection caused by the human papillomavirus (HPV) is a sexually transmitted disease (STD) that affects about 50% sexually active population[1]. HPV infections contribute to about 5.2% human cancers in the world population, including anal, genital, and oropharynx cancers[2,3]. HPV infection is the most common STD in the world. In the United States, approximately 14,000 people are infected with human immunodeficiency virus every year and 79,000 have persistent infection, whereas the majority of sexually active persons will detect infection by the virus at least once in a lifetime[4]. In Brazil, cervical cancer is the third most common type of cancer affecting women: about 15,590 new cases of cervical cancer were reported in 2014. Anal cancer ranks second among the most common types of cancer caused by HPV[5]. This neoplasm is relatively rare compared with cervical cancer in the population, with incidence of 1.5 per 100,000 people[6].

The incidence of anal cancer has been growing, and this type affects both men and women. However, in the general population, anal cancer is more common among women than among men[7]. Similar to cervical cancer, anal cancer is preceded by a series of pre-cancerous changes, that is, several degrees of anal intraepithelial neoplasia, raising the possibility that it can be avoided if detected early.

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The prevalence of HPV in different countries was nearly 100% in patients with cervical cancer; 40% in cases of penile, vulva, and vagina cancers; 90% in cases of anal cancer; 3% in cases of oral cancer; and 12% in cases of oropharyngeal cancer[8].

Cervical cancer is still the second leading cause of death by cancer among women[1]. The incidence of anal cancer continues to grow, and the presence of HPV in such cases is around 90%[7,9]. While it is already established that HPV is the causative agent of cervical cancer and anal carcinoma, little is known about the etiology of oral and even penile cancer[10]; however, it is accepted that HPV is a predisposing factor, along with alcoholism and smoking.

Many studies of natural history of HPV have focused on cervical infections, but little has been stated about anal infections in asymptomatic patients. Thus, the natural history of HPV is still under construction and its maintenance mechanisms in the host organism are little understood. We understand that the detection of the virus in extragenital sites could help in understanding the chain of infection-spread re-infection by HPV in the human genital tract. Furthermore, the importance of a proper screening of the viruses before clinical diagnosis is clear, especially in the initial period of the lesion, providing thus a better prognosis.

The objective of this study was to contribute to the knowledge about the natural history of HPV infection, investigating whether genital infection predisposes to anal infection by determining the presence of HPV infection in both sites. In addition, this study had the big purpose of confirming the diagnosis of genital HPV by polymerase chain reaction (PCR) in genital samples with lesion suggestive of HPV paired with anal mucosa samples.

METHODS

This was a cross-sectional study comprising 92 patients, totaling 184 samples, conducted in the Virological Diagnostic Laboratory of the Universidade Federal Fluminense (UFF). The samples were collected between 2009 and 2014 in the Sector of Sexually Transmitted Diseases of UFF and at Santa Casa de Misericordia of Rio de Janeiro. Two samples were taken from each patient: genital and anal. Genital samples were biopsies of benign lesions, whereas anal samples were only healthy mucosa smears.

The samples were collected in Tris-EDTA buffer (pH 7.2) and frozen at -20°C in the Sector of Sexually Transmitted Diseases of UFF and the Sector of Dermatology of Santa Casa. They were later transported to the Virological Diagnostic Laboratory of UFF and kept at -20°C.

DNA extraction

The method used was phenol-chloroform extraction. For inactivation of possible pathogens in the samples, the tubes were placed in a water bath at 56°C for 2 hours. Next, 100 µL digestion buffer was added (50 mM Tris-HCl (pH 8.5), 10 mM EDTA, 200 µg/mL proteinase K) in a water bath at 55°C for 3 hours or 37°C overnight. Continuing to extraction, phenol–chloroform method was used, starting with the addition of 1 mL phenol/chloroform/isoamyl alcohol (25:24:1; Lifetech) in each tube, containing around 500 µL sample.

The sample was homogenized for 5 minutes, and then centrifuged for 15 minutes at 8,000 rpm. Two layers were formed in the tube, from which the supernatant is transferred to a new Eppendorf tube. If the top aqueous layer was not clear, centrifugation was repeated. All procedures were performed by the hood and the residues were appropriately discarded in chemical waste.

Samples were mixed with a solution in the ratio 1:10 of 3 M sodium acetate (pH 6.0), then with absolute ethanol in the proportion of 2.5 times the volume of the aqueous layer. After mixing by inversion, the samples were frozen at -20°C overnight.

In the following phase, specimens underwent a 30-minute centrifugation at 14,000 rpm at 4°C. Then, the supernatant was discarded, 70% ethanol was used for washing it, and another 15-minute centrifugation at 14,000 rpm was performed. After discarding the 70% ethanol, the tubes went through drying process, for complete evaporation of the ethanol in a thermoblock at 60°C for 2 hours. Finally, the DNA adhered to the tube was resuspended in 50 mL distilled water and the samples were then stored at -20ºC.

Generic polymerase chain reaction with consensual primers MY09/MY11

Generic PCR allows detection of any HPV genotype using consensual primers MY09/MY11, which amplify a 450-pb fragment corresponding to L1 gene[11].

The reaction mixture was composed of 5 mL of 10´ PCR buffer (10 mM Tris-HCl (pH 8.0), 1 mM EDTA, 10 mM NaCl), 1 mL dNTP (200 mmol dATP, dCTP, dGTP, dTTP), 3 mL $MgCl_2$ (50 mM), 1 mL primer (50 pM) to 0.25 mL DNA Taq polymerase, totaling 50 µL reaction with the addition of the sample. We used primers for the human actin gene (Ac1 and Ac2) as DNA extract control, positive samples as positive control, and ultrapure water as negative control. Samples were subjected to 40 amplification cycles: 94°C for 1 minute (denaturation), 55°C for 1 minute (hybridization), and 72°C for 1 minute (extension). At the end of cycles, the stabilization phase was of 10 minutes at 72°C.

Polymerase chain reaction with internal primers GP5+/GP6+

For confirmation of positive or negative samples, the nested PCR was performed using primers GP5+/GP6+ (150 bp). The reaction mixture was composed of 5 µL of 10´ PCR buffer (10 mM Tris-HCl (pH 8.0), 1 mM EDTA, 10 mM NaCl), 1 mL dNTP (200 mmol dATP, dCTP, dGTP , dTTP), 3 mL $MgCl_2$ (50 mM), 1 mL primers (50 pM), and 0.25 mL DNA Taq polymerase, totaling 50 µL reaction mix with addition of 2 mL MY amplicon performed in the previous step.

The amplification reaction consisted of 35 cycles as follows: 94ºC for 4 minutes (pre-denaturation), 94°C for 30 seconds (denaturation), 45°C for 1 minute (hybridization), and 72°C for 1 minute (extension). Finally, samples were put at 72°C for 10 minutes (stabilization).

Polymerase chain reaction with specific primers

In this step, samples considered positive by a generic PCR amplification were submitted to specific PCR for HPV genotyping. The primers used were specific for the E6/E7 segment of HPV types 6, 11, 16, 18, 33, and 45 (synthesized by Invitrogen®), resulting in 89-230 bp. As a positive control for HPV types 16 and 18, DNA from HeLa and CaSki cell lines, respectively, were used. For other types, positive samples were used, and sterile distilled water was used as a negative control.

For the reaction to occur, a mixture was prepared containing 5 µL of 10´ buffer solution (10 mM Tris-HCl (pH 8.0), 1 mM EDTA, 10 mM NaCl), 1 µL of dNTP (200 µmol dATP, dCTP, dGTP, dTTP), 3 µL $MgCl_2$ (50 mM), 1 µL primer (50 pM), and 0.25 Platinum DNA Taq polymerase.

The amplification reaction consisted of 35 cycles as follows: 5 minutes at 94°C (pre-denaturation), 30 seconds at 94°C (denaturation), 30 seconds at 55°C (hybridization), and 1 minute at 72°C (extension). Finally, samples were left for 10 minutes at 72°C (stabilization).

Typing of human papillomavirus by restriction fragment length polymorphism

This technique was used for samples positive for generic HPV PCR by negative in specific PCR, which were . Restriction fragment length polymorphism (RFLP) uses PCR products of the L1 gene, that is, the generic PCR products with consensual primers MY09/MY11. Amplified DNA (3 µL) was added to 2 units of endonucleases *Bam*HI, *Dde*I, *Hae*III, *Hin*fI, *Pst*I, and *Rsa*I (10 U/µL–2 µL; Invitrogen®) and placed separately in 200 µL tubes for digestion. To maintain optimum enzymatic conditions, 1 µL buffer corresponding to each enzyme was added, as well as 4 µL TE buffer (0.5 M Tris (pH 7.4) and 0.5 M EDTA (pH 8.0)), totaling 10 µL in volume of reaction mix in each Eppendorf tube.

After the addition of all components, the enzymatic digestion proceeded at 37°C for 2 hours.

Results of pcr and restriction fragment length polymorphism

PCR results were revealed by electrophoresis in 1.5% agarose gel (Invitrogen®). The amplified products were stained with 2 µL xylene cyanol/bromophenol blue, using standard molecular weight phage λ of 100 bp (Invitrogen®). Then, the gel was stained with ethidium bromide, and the products were visualized on an ultraviolet transilluminator.

For disclosure of the RFLP technique, the total volume of sample (10 µL) was added to 2 µL xylene cyanol/bromophenol blue dye applied to 1.5% agarose gel (Invitrogen®), with the standard molecular weight (50 bp). The products of this digestion were subjected to electrophoresis for about 2 hours. After the run, the gel was stained with ethidium bromide solution. The fragments generated by digestion were visualized on an ultraviolet transilluminator. The samples were identified and typed according to the procedures by Melgaço et al.[11].

Statistical Analysis

Data of each patient and respective results were included in a database. Statistical analysis was performed using the features available in Epi Info software, version 6.2, and Fisher's exact test was applied to build tables with 2 ´ 2 comparative analysis.

RESULTS

This study consisted of 184 samples from 92 patients. We found 67.3% (124/184) positive and 48.3% (60/184) negative samples for HPV.

In 87% (80/92) genital samples, DNA of the virus was found, with a prevalence of 47.8% (44/92) in anal samples. Only 12 (13%) samples resulted in no genital lesion by the virus, whereas 48 anal samples (52.2%) were negative for HPV.

Regarding the prevalence of HPV according to gender, we found 25 (50 samples) female patients and 67 (134 samples) male patients positive for HPV. Among 50 female samples, HPV infection was found in 40 (80%). Among these 40 samples positive for HPV, 23 (57.5%) were genital lesions and 17 (42.5%) were anal mucosa material. Among 10 samples negative for HPV, 2 (20%) were genital samples and 8 (80%) were anal mucosa material. As for males, among the 134 samples studied, 84 (62.6%) had HPV and 50 (37.3%) did not. Of the total 84 samples positive for HPV, 57 (67.8%) were genital lesions and 27 (32.1%) were from the anal mucosa. As for the 50 negative samples, 10 (20%) were related to genital lesions and 40 (80%) to anal smears.

In the presence of viral types, low risk was found in 92.6% (76/82) infections (excluding mixed infections) and high risk in 7.3% (6/82). In viral typing, the most common genotype among the tested types was HPV 11 (43/82), followed by HPV6 (33/82), HPV18 (3/82), HPV16 (2/82), and HPV45 (1/82). Mixed infections were found in 37 samples of HPV 6:11; 1 sample of HPV 6:16; and 1 sample each of HPV types 6, 11, 16, 18, and 45.

Genital concurrence in two sites was found in 10 cases: 3 of HPV6, 5 of HPV11, and 2 of HPV6 and HPV11.

DISCUSSION

In Brazil, the incidence of the most common malignant diseases due to HPV, cervical cancer, is still high. A survey conducted by the Brazilian National Cancer Institute (INCA) showed about 17,000 new cases of cervical cancer per year. In 2013, about 4,160 women died from the disease. In 2015, about 16,000 new cases are expected to rise[12].

As for men, the natural history of infection is little understood. It is known that penile cancer may have HPV DNA present in 30 to 80% of cases[13-16].

Epidemiological estimates of the World Health Organization (WHO)[17] suggest that the prevalence of HPV infection worldwide is between 9 and 13%, equivalent to 630 million people. In genital lesions, this prevalence can reach 100%. This study showed positivity of 67.3% among 184 samples analyzed, as among genital lesions 87% were positive for HPV, which is close to the literature[18].

In the anal site without clinical lesions, 47.8% samples were positive (**Table 1**). In literature, these data vary widely and few studies have been published. The prevalence of HPV in anal site in heterosexual populations is very heterogeneous in these papers, ranging from 1.2, 8, and 24.8%[19-21]. We emphasize that the prevalence we found is high, especially for HPV types 6 and 11 (**Table 2**), but we studied subjects with genital lesions clinically detected, thus increasing the risk of infection in other sites and confirming the presence of HPV in the host probably due to self-inoculation.

Despite the difference between the number of female and male individuals in this study, we found statistical differences as to HPV in genital lesions (males, 85.1%; females, 92%; p>0.05), confirming that there is no difference between tropism by gender in genital infections. However, in the case of anal infections, percentages were statistically different: 68% anal HPV in women and 40.3% in men (p=0.038). These results suggest that the acquisition of anal infection in women, though asymptomatic, is more frequent than that in men, suggesting that the mucosa may be infected by autoinoculation and function as reservoir that may eventually result in injury in women[22]. Such discrepancies in the prevalence among men and women could be explained by sexual behavior, hormonal effects, and differences between these sites.

This study is a preliminary survey of HPV infection at multiple sites. The research is currently in progress, aiming to increase our sample and assess the natural history of HPV.

Table 1 – Prevalence HPV DNA by polymerase chain reaction in genital lesions and anal site.

Samples by site of infection	HPV infection		
	Males n (%)	Females n (%)	Prevalência total n (%)
Genital	57/67 (85.1)	23/25 (92)	80/92 (87)
Anal	27/67 (40.3)	17/25 (68)	44/92 (47.8)

HPV: human papillomavirus.

Table 2 – Human papillomavirus genotypes detected by polymerase chain reaction and specific restriction fragment length polymorphism in genital lesions and anal site.

Samples	HPV genotypes					
	HPV6	HPV11	HPV16	HPV18	HPV45	Mixed infections
Genital	20	30	2	1	1	26
Anal	13	13	–	2	–	13

HPV: human papillomavirus.
*In three anal samples, HPV typology could not be identified.

Conflict of interests

The authors report no conflict of interests.

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