

Increased Frequency of Bacterial Vaginosis and *Chlamydia trachomatis* in Pregnant Women with Human Papillomavirus Infection

Cléber Sérgio da Silva^a Sheila Jorge Adad^b
Maria Azniv Hazarabedian de Souza^b Ana Cristina Macêdo Barcelos^a
Ana Paula Sarreta Terra^b Eddie Fernando Candido Murta^a

^aDiscipline of Gynecology and Obstetrics and ^bDepartment of Biological Science, Faculdade de Medicina do Triângulo Mineiro, Uberaba, Minas Gerais, Brazil

Key Words

Papillomavirus, human · Bacterial vaginosis · *Chlamydia trachomatis* · Pregnant woman

Abstract

The aim of this study was to verify the presence of bacterial vaginosis (BV), *Candida* sp, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, to determine the prevalence of tobacco use and measure vaginal pH (VpH) in pregnant women with (n = 26) and without (n = 26) human papillomavirus (HPV) infection, and make comparisons between these 2 groups. HPV, *C. trachomatis* and *N. gonorrhoeae* were diagnosed using hybrid capture, BV using clinical criteria, and *Candida* sp via cultures. A digital pH meter was used to measure VpH. The frequencies of *Candida* sp were 19.2 and 23.1% (p = 1), and VpH was 4.4 ± 0.4 and 4.3 ± 0.4 (p = 0.23), in the HPV-positive and HPV-negative groups, respectively. Compared to the group of pregnant women without HPV infection, those with HPV infection had a significantly higher prevalence of tobacco use (50 vs. 11.5%; p = 0.006), BV (53.8 vs. 15.4%; p = 0.007), and *C. trachomatis* (34.6 vs. 7.7%; p =

0.039). No case of *N. gonorrhoeae* was diagnosed. All cases of *C. trachomatis* and BV had high-grade HPV infection.

Copyright © 2004 S. Karger AG, Basel

Introduction

It has been demonstrated that the frequency of human papillomavirus (HPV) infection in the lower genital tract ranges from 13 to 46%, depending on the population studied and the diagnostic method utilized [1]. The intermediate and high-risk (carcinogenic) types of HPV are responsible for more than 95% of the world's cervical cancers [2]. For this reason, there is interest in studying this virus, as well as the factors predisposing towards the acquisition of this infection.

The vaginal environment is a complex and delicate ecosystem that shelters a wide variety of different microorganisms [3]. The normal vaginal flora is in a state of constant change and is at physiological equilibrium [4]. Various infectious processes that occur in the vagina result from disequilibrium within this ecosystem, as is the

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2004 S. Karger AG, Basel
0378-7346/04/0584-0189\$21.00/0

Accessible online at:
www.karger.com/goi

Eddie Fernando Candido Murta
Discipline of Gynecology and Obstetrics, Faculdade de Medicina do Triângulo Mineiro
Av. Getúlio Guaritá s/n, Bairro Abadia
38025-440 Uberaba, Minas Gerais (Brazil)
Tel. +55 34 3318 5326, Fax +55 34 3318 5342, E-Mail eddiemurta@mednet.com.br

case of bacterial vaginosis (BV) [5]. Alterations in vaginal pH may occur due to BV [6].

Some epidemiological factors like tobacco use, youth and pregnancy have been correlated with an increased incidence of HPV [1]. It has been suggested that the presence of other sexually transmitted diseases may increase the risk of acquiring HPV in the genital tract. *Chlamydia trachomatis* and *Candida* sp have been diagnosed in approximately 6 and 25% of patients with HPV infection, respectively [7]. *C. trachomatis* frequency appears to be higher among pregnant [8] and non-pregnant women [9] who present HPV-positive cervical smears. The association between BV and HPV has been a focus for studies and a point of controversy [10, 11].

Nevertheless, there is a scarcity of studies showing alterations in the vaginal flora and pH of pregnant women in association with sexually transmitted diseases (STD) with the presence of cervical HPV infection. We therefore proposed to make a study of pregnant women with HPV infection in order to verify the presence of BV, *Candida* sp, *C. trachomatis*, *Neisseria gonorrhoeae* and tobacco use, and to measure vaginal pH in comparison with a group of pregnant women without HPV infection.

Patients and Methods

We evaluated 26 pregnant women with a diagnosis of HPV infection between April 2000 and May 2001. Another 26 pregnant women without HPV infection were then selected as a control group, according to the attendance sequence. All patients attended at the gynecology and obstetrics outpatient service of Faculdade de Medicina do Triângulo Mineiro (FMTM). The individuals served there normally come from low socioeconomic and cultural backgrounds and have free public attendance. The criteria for inclusion in the groups were: pregnant women aged between 15 and 35 years; absence of bleeding at the time of examination; no utilization of oral antibiotics, fungicides and/or vaginal creams during the preceding 30 days; no sexual activity for at least 2 days preceding the time of sample collection, and no previous history of treatment for HPV. No patients with HPV infection had cervical intraepithelial neoplasia grades II or III.

For the investigation of BV and *Candida* sp, vaginal secretions were obtained by means of collection at the posterior vaginal fornix, utilizing 2 swabs and 2 Ayre spatulas. After collection, the 1st and 2nd swabs were placed into sterilized sample tubes identified as Nos. 1 and 2, which contained 0.5 ml of 0.9% physiological serum and 1 ml of deionized distilled water, respectively. The vaginal secretion collected with the 1st Ayre spatula was utilized to make a smear on a lamina slide for Gram staining, and the content of the other spatula was placed on another lamina slide for amine testing.

Microbiological Methods and Measurement of Vaginal pH

The swab inserted into tube No. 1 was utilized to achieve propagation in a Petri dish containing Saboraud's agar. The dish was placed in a glass cabinet at 35 °C in the presence of 5% carbon diox-

ide and observed at approximately 72-hour intervals over a period of up to 15 days, to see whether there was growth of *Candida* sp. This procedure was undertaken within a maximum time of 1 h after sample collection. The positive cultures were removed and propagated in chrome agar to identify the species of *Candida* sp.

For the investigation of BV, *C. trachomatis* and *N. gonorrhoeae*, the hybrid capture technique was utilized (Captura Híbrida® II System, DML 2000, Digene, Gaithersburg, Md., USA). Bacterial vaginosis was diagnosed when at least 3 of the following criteria were fulfilled: vaginal pH of >4.5; vaginal secretion of homogenous appearance with low viscosity and milky consistency, without flocculent or granular appearance, not curdled and not sticky or agglomerating; positive amine test, and presence of clue cells in the Gram smear.

The measurement of vaginal pH was done using a digital pH meter (Sentron, Zug, Switzerland) on a scale from 0 to 14. The device was calibrated once before starting the evaluations. The contents of tube No. 2 were utilized for this determination. After homogenization by spinning for approximately 10 s, the swab was removed and the pH meter probe was inserted into the tube, with its electrode at the extremity. The pH measurement was done digitally and instantaneously.

Statistical Analysis

For the statistical analysis, the two-sample t test, Mann-Whitney, Fisher and χ^2 tests were utilized. The results were considered to be significant if $p < 0.05$.

Ethical Committee Approval

This research was approved by the Research Ethics Committee of FMTM. Informed consent was obtained from all patients.

Results

Tobacco use was significantly higher in pregnant women with HPV infection (50.0%; 95% CI 29.9, 70.1) compared to pregnant women without HPV infection (11.5%; 95% CI 2.45, 30.14; $p = 0.006$; odds ratio 7.67; 95% CI 1.61, 47.68). The vaginal pH was 4.4 ± 0.4 in the HPV-positive group and 4.3 ± 0.4 in the HPV-negative group ($p = 0.23$, Mann-Whitney test). Among the 26 pregnant women with HPV infection, 7.7% (2 cases) presented low-risk HPV, 65.3% (17 cases) presented high-risk HPV and 26.9% (7 cases) presented an association of low- and high-risk HPV.

The prevalence of BV (53.84%; 95% CI 33.35, 73.4) and *C. trachomatis* (34.61%; 95% CI 17.23, 55.69) were significantly higher in pregnant women with HPV infection compared to pregnant women without HPV infection (15.38%; 95% CI 4.36, 34.87, and 7.69%; 95% CI 0.94, 25.12, respectively). The prevalence of *Candida* sp was not statistically significant between pregnant women with HPV infection (19.23%; 95% CI 6.56, 39.34) compared to pregnant women without HPV infection

(23.07%; 95% CI 8.98, 43.67). Table 1 shows the clinical data, the presence of BV, *Candida* sp, *C. trachomatis* and *N. gonorrhoeae*, and the number of cases of vaginal pH >4.5, among the pregnant women with HPV infection, in comparison with those without HPV infection. It was observed that BV and *C. trachomatis* were more frequent among pregnant women with HPV infection. All the *Candida* sp isolated belonged to the species *C. albicans*. There were 18 cases of BV (34.6%), 11 cases of *Candida* sp (21.1%) and 11 cases of *C. trachomatis* (21.1%). The simultaneous presence of BV and *Candida* sp was not encountered. BV and *C. trachomatis* were present together in 9 patients in the HPV-positive group, but not in any patients in the HPV-negative group. All the pregnant women with BV and *C. trachomatis* had high-risk HPV infection. The simultaneous presence of *Candida* sp and *C. trachomatis* occurred in 1 case in the HPV-positive group but did not occur in the group without HPV.

The vaginal pH among the HPV-positive women with bacterial vaginosis was 4.5 ± 0.3 , whereas it was 4.3 ± 0.4 among those without BV. The respective figures for the viral load (median and range) were 242.63 (1.17–2,537.68) vs. 3.33 (1.04–2,179.20; $p = 0.26$, Mann-Whitney test). Although the average pH and median viral load were observed to be higher in those with BV compared to those without BV, the differences were not statistically significant.

Discussion

Some risk factors associated with genital HPV infection, such as tobacco use, and its peak incidence at ages of <30 years and the greater incidence during the gestation, have previously been described by other authors [1]. In our study, we observed an increased prevalence of tobacco use, BV, and *C. trachomatis* in those with HPV compared to those without HPV. This association has previously been described among non-pregnant women [12]. Nonetheless, to our knowledge, it has not been described among pregnant women until now.

It has been suggested that the presence of other STD may increase the risk of acquiring HPV in the genital tract. This argument has been put forward in relation to the sexually transmitted pathogens that give rise to inflammatory lesions in squamous epithelial cells, as occurs with *Candida* sp [7]. The same has been said of the microorganisms that infect the columnar epithelium, giving the possibility of a process of squamous metaplasia, as occurs with *C. trachomatis* and *N. gonorrhoeae* [13].

Table 1. Distribution of pregnant women with HPV infection (HPV+) and without HPV infection (HPV-), according to clinical data, presence of bacterial vaginosis, *Candida* sp, *C. trachomatis* and *N. gonorrhoeae*, and the number of women with vaginal pH >4.5

	HPV+ (n = 26)		HPV- (n = 26)	
Age, years	22.3 ± 4.5		23.1 ± 3.9	
Gestational age, weeks	25.3 ± 7.8		24.1 ± 8.3	
Number of pregnancies	2.6 ± 1.4		2.5 ± 1.4	
	n	%	n	%
Bacterial vaginosis*	14	53.8	4	15.4
<i>Candida</i> sp	5	19.2	6	23.1
<i>C. trachomatis</i> **	9	34.6	2	7.7
<i>N. gonorrhoeae</i>	–	–	–	–
Vaginal pH >4.5***	14	53.8	6	23.1

* $p = 0.007$ (odds ratio 6.42; 95% CI 1.50, 31.75); ** $p = 0.0385$ (odds ratio 6.35; 95% CI 1.08, 55.43); *** $p = 0.0448$ (odds ratio 3.89; 95% CI 1.03, 15.67), Fisher's exact test.

Epidemiologically, BV is associated with sexual activity, STD [14] and tobacco use [12]. Significant alterations take place in the vaginal ecosystem of women with BV, in particular a reduction in the number of lactobacilli that produce hydrogen peroxide and a large increase in the number of facultative anaerobic bacteria [15]. The association between BV and HPV is controversial in the literature. The case-control study by Castle et al. [10] found no significant association between BV and HPV. In contrast, a significant association was observed by Sikstrom et al. [11], which may be due to sexual behavior factors. We found a statistically significantly greater proportion of BV in pregnant women with HPV compared to pregnant women without HPV. Infection by HPV when BV is active may be facilitated by the presence of products derived from this abnormal microflora, such as sialidase enzymes, and the presence of amines [11]. Briselden et al. [16] have demonstrated positivity for such enzymes in 84% of women with BV. These enzymes have been implicated as factors in the virulence of various pathogenic organisms and they may promote virulence through their ability to adhere to, invade and destroy the mucosa [16]. Cauci et al. [17] have demonstrated that sialidases present maximum activity within the pH range from 4.5 to 5.5, and their activity diminishes drastically at the lower pH encountered among women with normal vaginal flora. It may be possible that this association occurs as a function

of reduced numbers of lactobacilli, which are responsible for controlling the vaginal micro-ecosystem, with consequent raising of the pH. The average pH for pregnant women with HPV infection in our study was close to 4.5. We also found in this study group of pregnant women, a higher percentage of pH >4.5 in those with HPV infection compared to those without HPV infection. Some studies have indicated that the viral load of HPV present in cells can be correlated with the severity or persistency of the cervical lesion [18]. In our study, the presence of BV had an association with HPV infection. Greater viral loads should therefore have been expected among such patients. However, even though the median for HPV positivity with BV was greater, we did not encounter a statistically significant difference in viral load between HPV-positive patients with and without BV. For this comparison, the sample size is small and consequently the power of the test is low, with only 17% power to detect at the 0.05 significance level, a difference of at least 450.2 in viral load.

Cavaliere et al. [19], studying the incidence of *C. trachomatis* in pregnant women, encountered a frequency of 41.5% among adolescents and 21.5% among adults, thereby indicating a tendency for this infection to occur at earlier ages. These authors [19] utilized cytological criteria for the diagnosis of HPV and found that 6 of the 7 HPV-positive adolescents examined had *C. trachomatis* infection, representing a frequency of 85.7%. However, Voog et al. [7], in a prospective study made at a clinic for STD, only found an infection rate of 6% for *C. trachomatis* in cervical smears from HPV-positive patients, in comparison with 12% for *Chlamydia* infection among HPV-negative women. In another study, HPV and *Trichomonas* infection were found more often among pregnant adolescents with *C. trachomatis* [8]. Lehmann et al. [9], using the polymerase chain reaction, found an infection rate of 10.3% for *C. trachomatis* in cervical smears from HPV-positive patients, vs. 1.7% in HPV-negative patients, and they concluded that there appears to be a correlation between cervical HPV and *C. trachomatis*. We also found greater frequency of *C. trachomatis* among patients with HPV infection. This is in accordance with the study results of other authors that suggest that *Chlamydia* infection increases the risk of invasive cervical cancer and is a possible cofactor with HPV in the etiology of squamous cervical cancer [20, 21].

In explaining our findings, some questions arose. It had to be considered whether the presence of genital co-infections, regardless of being sexually transmitted or not, could have had importance in the cell proliferation associated with HPV. This led us to consider whether women

who present BV and *C. trachomatis* in the vaginal flora could have greater predisposition to HPV infection, especially high-grade HPV. The risk factors for HPV infection are already well known and we do not have knowledge of any alterations to the vaginal flora that could play a role in predisposing towards this infection. It remains to be established whether the invasion of viruses or bacteria would be facilitated through microorganisms that favor each other. A better understanding of vaginal physiology and the possible direct or indirect relation between HPV infection, BV and *C. trachomatis* could lead to greater strides forward in the understanding of the physiopathology of HPV infection.

A study among women attending a STD clinic has demonstrated that *Candida* sp was present in 26% of the HPV-positive women, in comparison with 16% of the HPV-negative women [7]. A hypothesis has been raised that infection by *Candida* sp may activate latent HPV infection [7]. However, we did not encounter any significant difference between the groups with and without HPV infection. This may have been because we included only pregnant women in our study. In both groups, we only encountered *C. albicans*.

Genital infection by *N. gonorrhoeae* is one of the most frequent STD [22, 23]. Nonetheless, in our study we did not find any case of genital infection by *N. gonorrhoeae*, although capture assays permit simultaneous detection of HPV and other STD with high sensitivity [23].

Through this study, we have demonstrated a higher frequency of tobacco use and infection by *C. trachomatis* and BV among pregnant women who have high-grade cervical HPV infection. Further investigation, such as appropriate epidemiological studies with multivariate analysis will be necessary in order to truly demonstrate a correlation between these findings.

Our data allow us to conclude that these associations do not appear to be accidental. Other factors associated with *C. trachomatis* that might explain these co-infections, such as cervical immunological characteristics and variations in vaginal pH, may provide support for our results.

Acknowledgements

Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Sociedade de Medicina e Cirurgia de Uberaba (SMCU), Fundação Oswaldo Cruz (FIOCRUZ), and Coordenadoria de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) are thanked for financial support. Gilberto de Araújo Pereira and Uilho Antônio Gomes are gratefully acknowledged for their statistical support.

References

- 1 Koutsky L: Epidemiology of genital human papillomavirus infection. *Am J Med* 1997;102:3–8.
- 2 Bosch FX, Manos MM, Munoz N, Sherman M, Jansen AM, Peto J, Schiffman MH, Moreno V, Kuveman R, Shah KV: Prevalence of human papillomavirus in cervical cancer: A worldwide perspective. International biological study on cervical cancer (IBSCC) Study Group. *J Natl Cancer Inst* 1995;87:796–802.
- 3 Oleszczuk JJ, Keith LG: Vaginal infection: Prophylaxis and perinatal outcome: A review of the literature. *Int J Fertil* 2000;45:358–367.
- 4 Cibley LJ, Cibley LJ: Cytolytic vaginosis. *Am J Obstet Gynecol* 1991;165:1245–1249.
- 5 Faro S: Bacterial vaginitis. *Clin Obstet Gynecol* 1991;34:582–586.
- 6 Sagawa T, Negish H, Kishida T, Yamada H, Fujimoto S: Vaginal and cervical pH in bacterial vaginosis and cervicitis during pregnancy. *Hokkaido Igaku Zasshi* 1995;70:839–846.
- 7 Voog E, Bolmstedt A, Olofsson S, Ryd W, Lowhagen GB: Human papilloma virus infection among women attending an STD clinic correlated to reason for attending, presence of clinical signs, concomitant infections and abnormal cytology. *Acta Derm Venereol* 1995;75:75–78.
- 8 Chocephaiulkit K, Patamasucon P, List M, Moore B, Rodriguez H: Genital *Chlamydia trachomatis* infection in pregnant adolescents in east Tennessee: A 7-year case-control study. *J Pediatr Adolesc Gynecol* 1997;10:95–100.
- 9 Lehmann M, Groh A, Rodel J, Nindl I, Straube E: Detection of *Chlamydia trachomatis* DNA in cervical samples with regard to infection by human papillomavirus. *J Infect* 1999;38:12–17.
- 10 Castle PE, Hillier SL, Rabe LK, Hildesheim A, Harrero R, Bratti MC, Sherman ME, Burk RD, Rodriguez AC, Alfaro M, Hutchinson ML, Morales J, Schiffman M: An association of cervical inflammation with high-grade cervical neoplasia in women infected with oncogenic human papillomavirus (HPV). *Cancer Epidemiol Biomarkers Prev* 2001;10:1021–1027.
- 11 Sikstrom B, Hellberg D, Nilsson S, Kallings I, Mardh PA: Gynecological symptoms and vaginal wet smear findings in women with cervical human papillomavirus infection. *Gynecol Obstet Invest* 1997;43:49–52.
- 12 Peters N, Van Leeuwen AM, Pieters WJ, Hollema H, Quint WG, Burger MP: Bacterial vaginosis is not important in the etiology of cervical neoplasia: A survey on women with dyskaryotic smears. *Sex Transm Dis* 1995;22:296–302.
- 13 Koutsky LA, Galloway DA, Holmes KK: Epidemiology of genital human papillomavirus infection. *Epidemiology* 1988;10:122–163.
- 14 Schwebke JR: Diagnostic methods for bacterial vaginosis. *Int J Gynaecol Obstet* 1999;67(suppl 1):S21–S23.
- 15 Eschenbach DA, Williams BL, Klebanoff SJ, Young-Smith K, Critchlow CM, Holmes KK, Davick PR: Prevalence of hydrogen peroxide-producing *Lactobacillus* species in normal women and women with bacterial vaginosis. *J Clin Microbiol* 1989;27:251–256.
- 16 Briselden AM, Moncla BJ, Stevens CE, Hillier SL: Sialidases (neuraminidases) in bacterial vaginosis and bacterial vaginosis-associated microflora. *J Clin Microbiol* 1992;30:663–666.
- 17 Cauci S, Driussi S, Monte R, Lanzafame P, Pitzus E, Quadrifoglio F: Immunoglobulin A response against *Gardnerella vaginalis* hemolysin and sialidase activity in bacterial vaginosis. *Am J Obstet Gynecol* 1998;178:511–515.
- 18 Ho GY, Burk RD, Klein S, Kadish AS, Chang CJ, Palan P, Basu J, Tachezy R, Lewis R, Romney S: Persistent genital human papillomavirus infection as a risk factor for persistent cervical dysplasia. *J Natl Cancer Inst* 1995;87:1365–1371.
- 19 Cavaliere MJ, Maeda MY, Shirata NK, Shih LW, de Siqueira M, de Muelenare Correa MG, Oliveira HF: Cervico-vaginal *Chlamydia trachomatis* infection in pregnant adolescent and adult women: A morphologic and immunofluorescent study. *Arch Gynecol Obstet* 1993;253:175–182.
- 20 Lehtinen M, Hakama M, Luostarinen T, Hallmans G, Jellum E, Koskela P, Thoresen S, Youngman L, Hakulinen T: Joint effect of HPV16 with *Chlamydia trachomatis* and smoking on risk of cervical cancer: Antagonism or misclassification (Nordic countries). *Cancer Causes Control* 2000;11:783–790.
- 21 Smith JS, Munoz N, Herrero R, Eluf-Neto J, Ngelangel C, Franceschi S, Bosch FX, Walboomers JM, Peeling RW: Evidence for *Chlamydia trachomatis* as a human papillomavirus cofactor in the etiology of invasive cervical cancer in Brazil and the Philippines. *J Infect Dis* 2002;184:324–331.
- 22 Garland SM, Tabrisi SN, Chen S, Byambaa C, Davaaj K: Prevalence of sexually transmitted infections (*Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Trichomonas vaginalis* and human papillomavirus) in female attendees of a sexually transmitted diseases clinic in Ulaanbaatar, Mongolia. *Infect Dis Obstet Gynecol* 2001;9:143–146.
- 23 Ishi K, Suzuki F, Saito A, Kubota T: Prevalence of human papillomavirus, *Chlamydia trachomatis*, and *Neisseria gonorrhoeae* in commercial sex workers in Japan. *Infect Dis Obstet Gynecol* 2000;8:235–239.