Correlation between squamous intraepithelial lesions (SILs) and bacterial vaginosis

G. Vetrano, A. Pacchiarotti, G. Lombardi, V. Cimellaro, M. Verrico, S. Carboni, R. Corosu

Department of Gynaecology, Perinatology and Childhealth, University of Rome 'La Sapienza', Rome (Italy)

Summary

Bacterial vaginosis (BV) is a condition that seldom occurs in prepuberal girls or postmenopausal women, suggesting a hormonal component in its aetiology. The precise mechanisms by which BV arises are not fully understood. One proposed mechanism suggests that carcinogenic nitrosamines act either independently or via human papilloma virus (HPV). Human papillomavirus is known to be associated with the development of squamous intraepithelial lesion (SIL). Still today the relationship between BV and SIL is debated. Many confounding factors regarding the relationship between BV and SIL include the presence of HPV and/or other sexually transmitted diseases. In a case-controlled study the correlation between BV, SIL and the presence of HPV was evaluated. BV was diagnosed according to standard criteria: vaginal pH > 4.5; positive amine test or 'whiff' test ; presence of clue cells and abnormal discharge. High risk-HPV testing by PCR was performed. X_2 Pearson analysis was applied for statistical evaluation of data. The results of the study have shown that BV is not associated with SIL.

Key words: Bacterial vaginosis; Squamous intraepithelial lesions; HPV.

Introduction

Bacterial vaginosis (BV) is a condition that seldom occurs in prepuberal girls or postmenopausal women, suggesting a hormonal component in its aetiology [1, 2].

BV is a treatable condition where the normal lactobacilli-dominated vaginal flora are replaced by a mixture of anaerobic organisms. The precise mechanisms by which BV arises are not fully understood [3].

The relationship between BV and squamous intraepithelial lesion (SIL) is still debated [4, 5]. Moreover, cytological changes (low SIL , high SIL) in women with BV have been found more often than in women without this condition [1].

Many confounding factors regarding the relationship between BV and SIL include the presence of HPV and/or other sexually transmitted diseases [6].

Recently a mechanism by which microorganisms might influence expression of oncogenic HPV has been revealed. McNichol evaluated the relationship between HPV16 oncogenes E6 and E7 and microbiologic environment. Lactobacillus sp. and expression of the E6 oncogene were associated with low-grade CIN or normal histology. Expression of HPV16 E6 and E7 genes in vitro was unaltered in the presence of bacteria. The results suggest that vaginal microorganisms are unlikely to alter the natural history of HPV-associated CIN by influencing HPV oncogene expression [7].

It has been suggested that BV could be important in the development of squamous intraepithelial lesions. One reason is that carcinogenic nitrosamines could be produced from abnormal flora and subsequently act on the cervix either independently or together with another agent, for example a virus [2, 3, 8-10].

Other studies have further investigated the production of nitrosamines by vaginal bacteria and the conclusions have been variable [11, 12].

The purpose of this study was to determine the correlation between BV, SIL and high-risk HPV.

Material and Methods

From 1991 to 2003, 504 women were eligible for the study. The patients who attended the centre of the Department of Gynaecology, Perinatology and Child Health University "La Sapienza" were divided into two groups: the first with 252 patients affected by SIL of different degrees and the second with 252 patients without SIL A structured questionnaire was administered regarding sociodemographic, gynaecologic and sexual behaviour characteristics. Exclusion criteria included pregnancy, use of antibiotics up to six months before, presence of HPV or other STDs and previous treatment to the cervix.

All participants gave their full informed consent.

Cytological diagnosis was formulated in agreement with the Bethesda System [13].

All patients were submitted to standard colposcopy using a 5% acetic acid solution followed by the Lugol test. Colposcopic findings were interpreted according to the international nomenclature [14].

The histological diagnosis was assessed by colposcopic direct biopsies in the areas revealing the greatest degree of abnormalities.

BV was diagnosed according to standard criteria: vaginal pH > 4.5; positive amine test or 'whiff' test; presence of clue cells on a gram-stained preparation of vaginal secretions and abnormal discharge.

High risk-HPV testing by PCR was performed.

Cytological samples were collected in sterile 1.5 polypropylene tubes and resuspended in 100 µl of digestion buffer with

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proteinase K, incubated overnight at 37°C, and boiled for 5 min. Aliquots (10 µl) of each were used for PCR amplification. Each cytological sample was analysed by PCR for HPV open reading frame sequences using the following primers: HPV-16, 5'-ACC gAA ACC ggT Tag TATAAAAgC-3' and 3'-gAT CAT TTg TCT CTg gTT gCA AAT-5'; HPV-18, 5'-CAC ACC ACA ATA CTA Tgg CgCgCT-3' and 3'-CTg CTg gAT TCA ACg gTT TCT ggC-5'. Every amplification experiment included one negative and one positive control for each viral type. A portion of exon 15 of the human *APC* gene was routinely amplified as a positive control using the following primers: APC, 5'-gTCCTTCACA-gAAtgAAAgATg-3' and 3'-CTg CTT gAA gAA gAC ATA TgTTCg-5'. The sizes of the amplified fragments were 576, 360 and 520 bp, respectively. Amplification reactions were carried out in 100 µl of reaction buffer containing 50 mM KCl, 2 mM MgCl2, 10 mM Tris (pH 8.3), 200 µM each deoxynucleotide triphosphate, 2.5 units of Taq DNA polymerase (Perkin-Elmer-Cetus, Norwalk, CT), 100 pmol of each primer, and 10 µl of proteinase K-digested sample. Samples were denatured at 95°C for 5 min, followed by 40 cycles of amplification (denaturation at 94°C for 1.5 min, annealing at 55°C for 2 min, except APC, where annealing was at 40°C and 57°C, respectively, and extension was at 72°C for 2 min; the final extension was prolonged to 7 min). Amplified products (15 µl) were electrophoresed through 1.6% agarose gels. The gels were analysed by UV after staining with ethidium bromide [15].

Statistical analysis

X_2 Pearson analysis was applied for statistical evaluation of the data.

Results

The patients ranged in age from 18 to 61 years (median age 29).

Of the 252 patients with abnormal cervical cytology, 63 cases cytology revealed low-grade squamous intraepithelial lesions and 189 revealed high-grade squamous intraepithelial lesions. Histologic analysis of colposcopy-directed biopsies revealed 63 cases of CIN 1, 81 of CIN 2 and 108 of CIN 3.

HPV testing at diagnosis was positive for high-risk HPV (type 16) in two cases of CIN 1, in 81 of CIN 2 and in 108 of CIN 3, and HPV was negative for high-risk types in 61 cases of CIN 1.

Among the 252 patients in the first group affected by SIL of various degrees, 91 out of 252 presented BV, 124 out of 252 HPV and 37 out of 252 were negative. Among the second group 119 out of 252 revealed BV, 39 out of 252 HPV and 94 out of 252 resulted negative. The patients with negative findings were respectively, 37 out of 252 (15%) in the first group and 94 out of 252 (37%) in the second (Table 1).

The difference between the results of the two groups was statistically significant ($p < 0.00005$).

Table 1. — *Results of case and control groups.*

	Vaginosis + SIL (any grade)		Vaginosis without SIL	
	%	no.	%	no.
HPV	49	124	16	39
Bacterial vaginosis	36	91	47	119
Negative	15	37	37	94

$p < 0.00005$.

Discussion

In recent years intensive research has been done to define the risk factors that may be responsible for the development of SIL and cervical cancer. In the literature the association between HPV infection of the lower genital tract and SIL is well documented [16, 17]. The HPV infection rate is high among women with cervical cancer or SIL [18]. It is still unclear, though. why all women with this infection do not develop SIL [19]. Bacterial vaginosis could also be a sexually transmitted disease with a connection to SIL and cervical cancer: A correlation between BV and SIL has been suggested [19-21].

The present study suggests that there is not a significant correlation between SIL and BV; in contrast a significant correlation between BV without SIL was shown.

The frequency of BV was 36%; 91 out of 252 women in comparison to 47%, 119 out of 252 women affected by SIL. The correlation between HPV and SIL in both groups was respectively, 49% in the first group and 16% in the second.

Disturbed bacterial micro-flora is capable of producing potentially carcinogenic nitrosamines, especially sialidases, formally known as neuroaminidases, enzymes which cleave alpha-ketosidic linkages between the glycosyl residues of glycoproteins, glycolipids, or colomic acids and sialic acids [2, 8].

Examination of the relationship between nitrosamines and the presence of high-risk HPV demonstrated no significant correlation between the two and did not demonstrate a relationship between BV and CIN irrespective of the presence of high-risk HPV in cervical cells. Women with BV were not found to have CIN more frequently that women with normal flora and the quantities of nitrosaminies produced by women with BV did not differ significaty from women without BV [3].

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Address reprint requests to:
G. VETRANO, M.D.
Via A. Cadlolo, 9
00136 Roma (Italy)