IN PRACTICE

Factors affecting vaginal pH levels among female adolescents attending genitourinary medicine clinics

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Objectives: Vaginal pH is related to hormonal status, and adolescents experience disturbed hormonal patterns following menarche. We assessed hormonal factors and risk of abnormal vaginal pH and bacterial vaginosis (BV) among adolescents attending genitourinary medicine (GUM) clinics.

Methods: In a cross sectional study adolescents within 5 years of menarche, ≤ 17 years, or with oligo-amenorrhoea were recruited. Vaginal pH and BV were assessed and among those not using hormonal contraceptives, estrone-3-glucuronide (E3G) and pregnanediol-3 α -glucuronide (P3G) concentrations were measured.

Results: Among 102 adolescents, 59.8% (61) had a high vaginal pH (>4.5), which was higher than the prevalence of BV, detected in 33% (34). No association was found between presence of sexually transmitted infections (STI) and vaginal pH. In logistic regression, after controlling for BV and condom use, vaginal pH was positively associated with cervical ectopy (OR = 2.5; 95% CI 1.0 to 6.6, $p=0.05$) and STI treatment history (OR = 2.5; 95% CI 0.9 to 6.5, $p=0.07$), and negatively associated with use of Depo-Provera (OR = 0.1; 95% CI 0.03 to 0.6, $p=0.003$) and recent onset (<12 months) of sexual activity (OR = 0.2; 95% CI 0.1 to 0.7, $p=0.004$). Among 23 adolescents not using hormonal contraceptives, a high pH occurred more often in abnormal compared to normal menstrual cycles (OR = 10.8; 95% CI 1.4 to 85.4; $p=0.026$). E3G concentrations were inversely correlated with vaginal pH in the follicular phase (Spearman: $r=0.51$; $p=0.024$).

Conclusions: Ectopy and abnormal menstrual cycles are common features of adolescence. Their presence is associated with increased risk of abnormal pH, and may also predispose to BV.

There is a striking correspondence between stage of the lifecycle and vaginal pH levels.¹ Vaginal secretions are acidic at birth and vaginal micro-organisms present in the newborn female are similar to those in her mother. Shortly after birth, when maternally derived oestrogen levels decline, vaginal pH rises to about 7. A variety of microbial species colonise the vagina but at lower concentrations than in adults, and lactobacilli are absent.² At puberty, in response to oestrogen acting on maturing epithelial cells, vaginal pH decreases to its normal adult level (pH 4 (SD 0.5)). Lactobacilli appear in the vagina, although vaginal pH frequently does not correspond with the presence or absence of lactobacilli.¹ After menopause the vaginal environment gradually reverts to the pre-menarcheal state. This lifecycle suggests that hormonal factors have a critical role in regulating vaginal pH levels. This is further supported by evidence of variation in vaginal physiology and pH levels during the menstrual cycle.^{3,4}

Although pH levels fall at puberty, adolescence represents a period of hormonal instability, which could affect vaginal pH. Adolescents have lower oestrogen levels than adults⁵ and irregular menstrual cycles persist for varying periods after menarche. In one study, adolescent vaginal pH mean values were inversely related to gynaecological age (years since menarche).⁶ Adolescents with high pH levels may be at increased risk of bacterial vaginosis (BV), a common polymicrobial syndrome characterised by high concentrations of aerobes and non-aerobes, absence of lactobacilli, and a high vaginal pH. Prevalence in British adolescents has not been described, but was 9% among women screened through general practices.⁷ Presence of BV is thought to increase susceptibility to chlamydia,⁸ herpes simplex virus,⁹ and HIV

infections.¹⁰ An abnormal adolescent pH could indirectly increase susceptibility to genital tract infections.¹¹

As part of a broader study of biological and hormonal risk factors for genital tract infections in adolescents, vaginal pH was measured as part of the clinical assessment of BV.¹² In this paper we investigated risk factors for abnormal vaginal pH among adolescents at high risk of sexually transmitted infections (STI).

METHODS

Study population

Adolescents attending three genitourinary medicine (GUM) clinics in Manchester were invited to participate.

Participants

We recruited adolescents with young gynaecological age (within 5 years of menarche), young age (17 years or younger), or a specific complaint of oligomenorrhoea or amenorrhoea. Adolescents were excluded if premenarcheal, pregnant or breast feeding within the last 3 months, if treated for a genital tract infection within the last 4 weeks, or if they had started, or stopped, using hormonal contraception within the last month. All gave informed written consent, and if younger than 16 years, were assessed for Gillick competency (that is, sufficient intelligence and understanding to be treated without parental consent). The three NHS

Abbreviations: BV, bacterial vaginosis; E3G, estrone-3-glucuronide; GUM, genitourinary medicine; HPV, human papillomavirus; IQR, interquartile range; LH, luteinising hormone; P3G, pregnanediol-3 α -glucuronide; PCR, polymerase chain reaction; STI, sexually transmitted infections

local research ethics committees covering the participating centres approved the study. A questionnaire (pre-piloted) established contraceptive, menstrual, sexual, and pregnancy histories. Pictorial charts were used for self grading breast and pubic hair stages.¹³ Body mass index (weight (kg)/(height (m))²) was calculated. To calculate gynaecological age adolescents were asked if menarche had occurred within the last year, or between 1 and 2 years ago, 2 and 3 years ago, etc. The mid-point of the onset year of menarche was subtracted from the recruitment calendar age. As part of a standardised history and examination procedure, area of ectopy was drawn on a schematic diagram.

Collection of samples

Endocervical swabs were taken for culture of *Neisseria gonorrhoeae* and a combined endocervical swab and urine sample for *Chlamydia trachomatis*.¹⁴ For human papillomavirus (HPV) DNA detection a spatula was rotated through 360° within the transformation zone of the cervix and placed in saline. A blood sample was collected to measure antibodies to *Treponema pallidum* and HSV-2. Vaginal pH was measured by placing pH indicator paper (Whatman narrow range, UK) on the vaginal wall. A wet mount to detect *Trichomonas vaginalis*, *Candida albicans* and clue cells was prepared, as well as a Gram stain for BV. Vaginal pH was not assessed if adolescents were menstruating, and in a few cases examinations were abbreviated because of painful herpetic ulcers. Adolescents not using hormonal contraception were offered, and shown how to use, a fertility monitor (ClearPlan Easy Unipath) over one menstrual cycle, in order to characterise their cycles and the predicted peri-ovulatory luteinising hormone (LH) surge.¹⁵ Self collected urine samples were obtained on alternate days between days 12 and 26 of the menstrual cycle. These samples were stored frozen (−80°) at the University of Southampton Endocrine Unit for determination of estrone-3-glucuronide (E3G) and pregnanediol-3 α -glucuronide (P3G).

Analysis of specimens

All samples were returned to the virology laboratory, Manchester Royal Infirmary. Chlamydia samples were tested using a standardised commercial polymerase chain reaction (PCR) test (Cobas Amplicor, Roche Diagnostics), and HPV DNA samples using a generic GP5+/GP6+ primer mediated PCR system.¹⁶ A Gap-DH PCR was also performed to check the integrity of the DNA and for the presence of PCR inhibitors.¹⁷ HSV1 and HSV2 antibodies were detected using an IgG ELISA (Gull Laboratories Inc, USA). Gram stains for BV were assessed by one laboratory technician, following Nugent's criteria. A score of >6 defined presence of BV. All positive, and a selection of negative, slides (40/102) were re-read by a second technician. For five ambiguous slides, Amsel criteria were used to classify presence of BV. A time resolved fluorescence immunoassay for the measurement of E3G and P3G in urine was used which had low interassay and intraassay variation (<6%).¹⁸ All urinary hormone measurements were creatinine standardised.¹⁹

Data analysis

The menstrual cycle was defined as "disturbed" if the follicular phase was 21 days or more and the luteal phase less than 10 days, if the monitor registered no ovulatory event, and/or P3G concentrations remained below 2 μ g/ml following an LH peak or if no period occurred within 42 days of bleeding. Hormonal concentrations were transformed to a log scale. Median concentrations were determined before and after ovulation, and over the entire measured portion of the cycle. Correlations with vaginal pH were investigated, using Spearman's test for continuous variables and Mann-Whitney

Table 1 Characteristics of 102 female adolescents assessed for vaginal pH and bacterial vaginosis at GUM clinics

Characteristic	% (no)*
Frequency of sex (times per week)	
<1	23.5 (24)
1–4	46.1 (47)
≥5	30.4 (31)
Douching after sex (last 6 months) (n=91)	29.7 (27)
Intercourse during menses (last 6 months)	30.4 (31)
Became sexually active within the last 12 months	30.4 (31)
Did not use a condom at last intercourse	62.7 (64)
Lifetime number of partners (n=101)	
1	7.9 (8)
2–3	27.7 (28)
4–5	30.7 (31)
6–10	22.8 (23)
>10	10.9 (11)
Tanner breast stage	
3	7.9 (8)
4	44.6 (45)
5	47.5 (48)
Presence of cervical ectopy	45.1 (47)
Menstrual cycle length (n=98)†	
<21 days	11.2 (11)
21–42 days	64.3 (63)
>42 days	6.1 (6)
"Irregular"	18.4 (18)
Currently uses oral contraceptives (n=101)	57.4 (58)
Ever used Depo-Provera	19.6 (20)
Current or past smoker	82.4 (84)
Previous STI treatment	40.2 (41)
Reports intermenstrual bleeding	14.7 (15)

*n=102 except where indicated.

†For users of hormonal contraceptives, this refers to cycle length before contraception.

U tests for dichotomous variables. High vaginal pH was defined as pH >4.5.

To visualise the relation between pH and the continuous variables (age, gynaecological age, and years sexually active), smoothed curves were fitted using local regression (loess) to the proportion with high pH, fitting separate lines for BV positive and negative subjects.

Risk factors were assessed by logistic regression, including variables likely to mediate vaginal pH through maturation, hormonal or sexual activity effects. Condom use at last sex was selected as a surrogate marker for general use of condoms. Condom and BV status were included in the model as covariates. The effects were expressed as odds ratios with 95% confidence intervals (CI). The level of significance was assessed using likelihood ratio tests. Since BV is defined by a high vaginal pH, to identify differences in risk factors associated with vaginal pH between BV positive and negative participants, interactions between the risk factors and BV status were tested. None of these tests approached statistical significance, so the simpler non-interaction model is presented. Analyses were performed using the R statistical package, version 1.8.1.²⁰

RESULTS

Between September 2000 and December 2001, 310 sexually active adolescents were approached; 124 were ineligible and 59 refused, providing a sample of 127. The characteristics of 102 adolescents for whom a vaginal pH measurement was available are shown in table 1. Median calendar age was 17.9 years (interquartile range (IQR) 17.2–18.7), gynaecological age was 4.5 (IQR: 4.0–5.5) and body mass index 22.1 (IQR: 20.6–24.3). Ethnic distribution was 71% white (72), 17% black (17), 2% Asian (2), and 10% mixed race (10).

A high vaginal pH (>4.5) was detected in 59.8% (61) of adolescents and 33% (34) were BV positive. Four adolescents

Figure 1 Proportion of participants with BV at each pH increment, along with the individual Nugent scores (circles). Quadrants: (1) BV+, low pH. (2) BV+, high pH. (3) BV-, low pH. (4) BV-, high pH. The horizontal line transects the figure at a BV score of 6 and those above this line are BV positive. The vertical line transects the figure at a pH of <5, to distinguish low and high vaginal pH. The plotting symbols have been slightly offset to distinguish overlapping points. Individuals classified by Amsel criteria are omitted.

had intermediate flora (Nugent score 4–6). No differences were detected between ethnic groups in mean vaginal pH, or in the proportions with high pH or BV, but numbers were small. Figure 1 plots BV (Nugent) scores ($n = 97$) and shows the prevalence of BV for each pH increment. BV prevalence rose sharply between pH levels of 4.5 and 5.5, but 43% (28) of adolescents with abnormal pH had Nugent scores ≤ 6 (quadrant 4). Two individuals with a pH of 4.5 had Nugent scores above 6 (quadrant 1).

The two most common infections were HPV (66.2%, $n = 51$) and chlamydia (26.5%, $n = 27$). There was no significant association of HPV, chlamydia, HSV-2 antibodies, gonorrhoea or presence of mixed infections with high vaginal pH, or of candida ($n = 7$) with low pH. Recent antibiotic use did not affect pH levels. Time of most recent unprotected sexual intercourse was not known, but presence of sperm on

Gram stains was noted for two adolescents, one of whom had a high pH (7).

Smoothed curves were fitted (fig 2) to explore trends in prevalence of vaginal pH by calendar age, gynaecological age and years of sexual activity, with pH plotted separately for BV positive and BV negative adolescents. With respect to calendar and gynaecological age, the pH curves were relatively flat for both BV positive and negative adolescents. Vaginal pH was lowest in the 12 months following onset of sexual activity. In logistic regression (table 2), controlled for presence of BV and condom use, the OR for a high vaginal pH was 0.2 (95% CI 0.1 to 0.7, $p = 0.004$) for adolescents who had only recently (within the last 12 months) become sexually active. There was also a significantly lower risk of abnormal vaginal pH among adolescents with a history of using Depo-Provera (OR = 0.1; 95% CI 0.03 to 0.7; $p = 0.003$). Risk of a high pH was increased among adolescents with cervical ectopy (OR = 2.5; 95% CI 1.0 to 6.6, $p = 0.05$), and a history of a previous sexually transmitted infection almost reached statistical significance (OR = 2.5; 95% CI 0.9 to 6.5, $p = 0.07$). There were no significant differences between BV positive and BV negative adolescents in the relation of these risk factors and pH in interaction tests.

Among 23 adolescents who monitored their menstrual cycle, a high pH was detected in 86.7% (13) of adolescents with a disturbed menstrual cycle compared to 37.5% (three) with a normal cycle (OR = 10.8; 95% CI 1.4 to 85.4; $p = 0.026$). There was no correlation between vaginal pH and median P3G concentrations. An inverse correlation of vaginal pH with E3G in the follicular phase of the cycle was observed (Spearman: $r = -0.51$; $p = 0.024$).

DISCUSSION

In this population 60% of adolescents had a high vaginal pH. Presence of cervical ectopy increased, and use of Depo-Provera decreased, the risk of abnormal pH. Adolescents were almost twice as likely to have an abnormal pH as to be BV positive. The prevalence of BV was, nevertheless, high (34%) compared to non-adolescents attending the same clinics (12.1%, unpublished data).

Our results are based on a single vaginal pH measurement and could be biased by measurement error or transient pH

Figure 2 Relation between vaginal pH and calendar age, gynaecological age, and number of years since participants became sexually active. Solid symbols denote BV negative and the open symbols BV positive. The lines are smooth lines fitted through the data using loess smoothing. Broken lines, vaginal pH curve for BV positives. Solid lines, vaginal pH curve for BV negatives. Data have been offset slightly to allow overlapping points to be distinguished.

Table 2 Odds ratios for associations between adolescent characteristics and a high vaginal pH (>4.5)

Characteristic	Odds ratio (95% CI)*	p (logistic)†
Age (per year)	1.4 (0.9 to 2.3)	0.15
Frequency of sex (per group)	1.1 (0.5 to 2.0)	0.88
Douches	0.6 (0.2 to 1.9)	0.36
Has intercourse during menses	0.5 (0.2 to 1.8)	0.29
Became sexually active within the past 12 months	0.2 (0.1 to 0.7)	0.004
No condom at last sex	0.8 (0.3 to 2.0)	0.57
Lifetime partners (per group)	1.0 (0.6 to 1.5)	0.95
Tanner breast stage (per stage)	1.5 (0.7 to 3.2)	0.27
Presence of cervical ectopy	2.5 (1.0 to 6.6)	0.05
Disturbed menstrual cycle‡	0.7 (0.3 to 1.9)	0.50
Body mass index (per kg/m ²)	1.0 (0.9 to 1.1)	0.70
Gynaecological age (per year)	1.0 (0.8 to 1.4)	0.81
Uses oral contraceptives	1.3 (0.5 to 3.5)	0.59
Ever used Depo-Provera	0.1 (0.0 to 0.7)	0.003
Current or past smoker	1.5 (0.5 to 4.7)	0.51
Previous STI treatment	2.5 (0.9 to 6.5)	0.07
Current intermenstrual bleeding	2.3 (0.5 to 10.3)	0.28

*CI, confidence interval.

†Adjusted for presence of BV and condom used at last sex.

‡<21 days, >42 days or irregular.

changes. Fluctuating pH values have been demonstrated in longitudinal studies,³ and may be attributed to menstrual cycle phase, recent sexual intercourse and presence of semen, or use of scented soap.⁴ Vaginal pH has not been characterised in adolescent menstrual cycles as younger subjects²¹ are usually excluded because of erratic cycle patterns. In our study, among the subgroup who monitored their cycles, pH levels were significantly lower in the follicular phase of the menstrual cycle, in association with elevated E3G concentrations. Almost all adolescents with an abnormal menstrual cycle had a high pH. Their cycles typically had long follicular phases, a late rise in oestrogen levels, and insufficient luteal phases. In disturbed cycles there may be more days when vaginal pH is raised, facilitating overgrowth of abnormal flora. One menstrual cycle study⁴ described a rise in pH before detection of BV but this was not confirmed by other research.³

It is uncertain whether the high pH levels associated with BV are a cause or product of the syndrome. Glycogen may be metabolised to lactic acid by vaginal bacteria and/or by the epithelium itself.²² One theory is that host factors cause vaginal pH to increase and lactobacilli to decrease, culminating in BV.²³ In our study, risk factors for an abnormal pH in BV positive and negative groups did not differ, although this would be expected if a high vaginal pH resulted from bacterial overgrowth. This suggests that, in adolescents, hormonal disturbances increase the vaginal pH and predispose to BV. It is noteworthy that risk factors associated with a high pH and BV in our study have been shown by others to predict BV. A reduced risk of BV was reported among women using hormonal contraception,²⁴ including Depo-Provera.²⁵ Hormonal contraceptives are often used to normalise the irregular adolescent menstrual cycle. Depo-Provera, by producing amenorrhoea, may protect against BV, as relapse of BV is associated with dysfunctional menstrual bleeding,²⁶ which is a common adolescent complaint.²⁷ Behavioural factors could also be implicated. Use of hormonal contraception and condoms were both associated with lower BV risk in a large Australian study.²⁸ It is reported that BV positive women use contraceptives or condoms less often at the start of a new sexual relationship.²⁹ This could explain the rise and leveling off of pH levels we observed after onset of sexual activity. As for ectopy, Mårdh *et al* reported a prevalence of 57.6% for women with BV compared to 43% with no infection ($p = 0.001$).³⁰ Ectopy is a consequence of

the oestrogen surge at puberty, and its frequency is lower in sexually experienced adolescents.

The higher frequency of chlamydial infection in subjects with BV reported in some studies could be mediated through cervical ectopy.^{8, 31, 32} This association was, however, mainly found in early studies using tests that were affected by the area of cervix available for swabbing. The higher chlamydia risk is usually attributed to loss of protective lactobacilli consequent to BV infection. However, a reverse situation cannot be ruled out. Peeters and Piot³³ suggested that, when mucopurulent cervicitis (often caused by chlamydia) is present, oxygen consumption by polymorphonuclear leucocytes decreases the redox potential and elevates vaginal pH, favouring development of BV. Schwebke *et al*³⁴ found that antimicrobial therapy for cervicitis, in addition to metronidazole, was necessary for cure of BV. In our study adolescents with abnormal vaginal pH or BV had no increased risk of current chlamydia infection (table 2), though history of treatment for a previous STI (mostly chlamydia) was associated with a higher risk of abnormal vaginal pH. This difference from other studies may reflect the high STI risk of the sample or different hormonal risk factors for BV and chlamydia.¹²

This study has a number of limitations. As a cross sectional study, causality cannot be attributed. The relatively small sample size means that some risk factors associated with vaginal pH may have been undetected. The sample size was limited because we selected adolescents of young gynaecological age, who constituted a subset of all adolescents attending GUM clinics. Vaginal pH patterns may be different in older, mature adolescents,³⁵ in black adolescents,⁶ and among those with less risky sexual behaviours. There are, however, no data on the natural history of BV and vaginal pH in low risk adolescents for comparison, and larger longitudinal studies are needed. Nevertheless, our study suggests that the adolescent hormonal milieu and onset of sexual activity disturb vaginal pH levels and increase susceptibility to BV.

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Key messages

- 60% of adolescents attending GUM clinics had a high vaginal pH
- Adolescents with cervical ectopy and irregular menstrual cycles are more likely to have a high pH
- Adolescents who use Depo-Provera have lower pH levels
- Unstable hormonal profiles may predispose adolescents to develop bacterial vaginosis

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CONTRIBUTORS

LB conceived the study and wrote the paper; EF contributed to study design, recruited participants, and helped draft the paper; DM, SPH, and SC advised on clinical issues, collected the data, and reviewed the paper; SR undertook the statistical analyses and contributed to writing the paper; PW and GB completed the hormone assays, advised on hormonal analyses and reviewed the paper; HCK reviewed the study design and analysis.

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