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Colposcopically Observed Vascular Changes in the Cervix in Relation to the Hormonal Levels and Menstrual Cycle

Smita N. Joshi, MBBS¹, Soma Das, MBBS¹, Madhuri Thakar, MSc, PhD¹, Vikrant Sahasrabudhe, MBBS, MPH, DrPH², B. Kishore Kumar, MSc, PhD¹, Marianne Callahan, BA, MEd³, and Christine Mauck, MD³

¹National AIDS Research Institute, Pune, India

²Vanderbilt University School of Medicine, Institute for Global Health, Nashville, TN

³Contraceptive and Research Development, (CONRAD), Eastern Virginia Medical School, Arlington, VA

Abstract

Objective—We assessed colposcopically observed vascular changes occurring in the cervix in relation to cyclical hormonal variation in healthy women.

Materials and Methods—Thirty women with regular menstrual cycles and willing to remain sexually abstinent during a menstrual cycle were enrolled. Colposcopy was performed during the peak of the estrogen and progesterone levels.

Results—The mean ($\pm$ SD) diameter of the largest visible blood vessel differed significantly between the estrogenic phase (0.38 ± 0.14 mm) as compared with the progestogenic phase (0.47 ± 0.12 mm; $p < .01$). The blood vessels were more prominent and dense and had a well-defined outline during the progestogenic phase than the estrogenic phase; however, these differences were not statistically significant. There was borderline increase in the interleukin 8 level during the estrogenic phase.

Conclusions—Physiological changes of increased vascularity of the cervix observed colposcopically during the progestogenic phase are normal. If such changes do not correspond to the menstrual cycle phase in women using vaginal microbicides in early-phase clinical trials, presence of inflammatory markers should be evaluated. Elevated interleukin 8 during the estrogenic phase needs further evaluation.

Keywords

colposcopy; vaginal microbicides; blood vessels; cytokines; phase

Microbicides are listed as one of the “top ten most promising biotechnologies for improving global health” [1]. Many candidate products are being evaluated in clinical trials to demonstrate their safety and look for adverse events (phases I and II) and effectiveness

(phases IIb and III). Efforts to detect local adverse effects of vaginal products visually have been undertaken for more than a decade.

The World Health Organization (WHO) published a manual for colposcopy for vaginally administered products in 1995 which was revised by Contraceptive and Research Development and WHO at meetings of experts convened in 2000 and 2003. The most recent update, *Manual for the Standardization of Colposcopy for the Evaluation of Vaginal Products, Update 2004*, is available on the web sites of CONRAD and WHO (www.conrad.org and www.who.int/reproductive-health). The meeting held in 2003 addressed the ways of detecting the adverse effects of the vaginal products intended to reduce the risk of acquisition of HIV and sexually transmitted infections (STIs), before products move into phase III effectiveness trials [2]. Several research questions were identified, and changes in cervical vascular patterns by day of menstrual cycle or other hormonal influences were identified as an area deserving investigation [2, 3].

It has been known for many years that the blood supply of the uterus varies from time to time in a rhythmic manner [4–6]. It is known that progesterone causes hypertrophy of the cervix, and there is venous congestion in the vagina during pregnancy [4]. However, there are no reports on changes in the vascular pattern on the cervix that occur cyclically because of hormonal changes. This study was conducted to assess colposcopically visualized vascular changes occurring in the cervix in relation to cyclical hormonal variation (serum estradiol and progesterone) in healthy sexually abstinent women having regular menstrual cycles.

MATERIALS AND METHODS

This study was conducted by the National AIDS Research Institute in the clinic located in the Hirabai Cowasji Jehangir Medical Research Institute in Jehangir Hospital and Medical Center, Pune, India. The study was sponsored by Contraceptive and Research Development (CONRAD), USA, and was approved by the Ethics Committees of CONRAD and National AIDS Research Institute. Women were recruited from the community using the institutional community contact program. Consenting women were enrolled in the study if they were between the ages of 18 and 45 years, had regular menstrual cycles with a minimum of 28 days between menses, had a normal Pap smear at screening, were HIV-uninfected by licensed enzyme immunoassay and in a mutually monogamous relationship with their partner for 1 year, were willing to abstain from sexual intercourse during the study period, and were, in general, good health based on routine hematology and biochemistry. Participants were excluded from the study if they were menopausal, postmenopausal, or breast-feeding; had a clinically detectable genital abnormality; used any spermicide or spermicidally lubricated condom within the week before enrollment; or had participated in any other investigational drug trial within 30 days before screening. In addition, participants who were using an intrauterine contraceptive device; were pregnant; or had gynecologic surgery, irregular/breakthrough menstrual bleeding, vaginal bleeding during or after sexual intercourse, or signs consistent with an STI or other genital tract infection or trauma, as seen on a pelvic examination during screening or in the 3 months before screening, were excluded from the study.

Participants were screened for eligibility after signing a written informed consent form and collection of demographic data. Blood samples were collected for HIV and rapid plasma reagin (RPR) testing (confirmed with *Treponema pallidum* hemagglutinin assay), complete blood count, and biochemistry and coagulation studies. Urine samples were collected for pregnancy testing and *Neisseria gonorrhoeae* and *Chlamydia trachomatis* testing by polymerase chain reaction assay. A pelvic examination was conducted, which included vaginal pH measurement, collection of swabs for wet preparation for *Candida* species and *Trichomonas vaginalis*, vaginal smear for Nugent scoring, Pap smear, and bimanual examination.

Based on the presumptive eligibility by the pelvic examination and laboratory tests, the enrollment visit was scheduled between days 10 and 12 (ideally on day 12) counting the first day of menses as day 1. The volunteer was instructed to remain abstinent from the first day of her next menstrual cycle until the last study visit, which was scheduled to fall between days 22 and 24 (ideally on day 22). She was also asked not to use any vaginal products during this time. The 2 visits were typically scheduled during the peak of the estrogenic phase (follicular phase) and progestogenic phase (secretory phase) of the menstrual cycle, and blood samples were also collected for serum estradiol (E2) and progesterone levels. Pelvic examination at both these follow-up visits included vaginal pH measurement, swabs for wet preparation for candida and trichomoniasis, and vaginal smear for Nugent scoring. Colposcopy was done using a colposcope of Carl Zeiss (150 FC), Oberkochen, Germany, following the principles outlined in the *Manual for the Standardization of Colposcopy for the Evaluation of Vaginal Products, Update 2004* [7]. The study clinicians were blinded to the phase of the participants' menstrual cycle. Two clinicians were randomly assigned to perform the colposcopy.

During colposcopy, the uterine cervix was examined, and the color of the epithelium, prominence of blood vessels, density of blood vessels (coarse/dense), clarity of blood vessel definition, diameter of the blood vessels at maximum width, degree of cervical ectopy and fragility, hypertrophy of the columnar epithelium, and presence of cervical mucus were noted. To minimize interobserver variations, colored disks and sample diagrams were used to record cervical color, density of blood vessels, diameter of blood vessels, degree of ectopy, amount of cervical mucus, and others. Blood vessels were visualized with and without a green filter. Digital Image Management Software (DIMS; Denvu, Montreal, Canada) was used to measure the actual diameter of the vessel by measuring the distance between 2 widest possible points of the vessel diameter. As per the operating instructions, DIMS system was calibrated to compliment the colposcope magnification power setting used for the images to which the measurements were applied. All measurement values were expressed in millimeters. Ectopy was assessed using the Photo Atlas for Microbicide Evaluation [8], and the spinnbarkeit test was used for the viscosity of the cervical mucus [9].

Cervicovaginal lavage (CVL) specimens were collected during the estrogenic and progestogenic phase to evaluate the soluble markers of inflammation in the last 17 participants after a protocol amendment was approved. Cervicovaginal lavage specimens were collected using 10 mL of normal saline, placed immediately on ice in a 15-mL Becton Dickinson Falcon conical polypropylene tube and centrifuged within 30 minutes of sample

collection for 10 minutes at 1,000g at 4°C. The supernatant was then aliquoted into 3 vials and stored frozen at -70°C until tested for the following: interleukin (IL) 2, IL-4, IL-8, IL-10, granulocyte-macrophage colony-stimulating factor 34, interferon gamma, tumor necrosis factor α , macrophage inflammatory protein (MIP) 1 β , monocyte chemoattractant protein 1 and monocyte chemoattractant protein 2, eotaxin-2, MIP-1 α , and Regulated on activation, normal T expressed and secreted (RANTES). Cytokine and chemokine estimation was done using the Luminex Map Technology. Commercially available kits (Human Cytokine-8-Plex Assay from Biorad and Human Chemokine 5-Plex from Biosource, CA, USA) were used, and the levels of cytokines and chemokines were quantified using the Bio-plex Manager software 4.0 (Biorad, CA).

STATISTICAL CONSIDERATIONS

Data from ultrasound and color Doppler studies have revealed that more than 90% of women have well-defined outlines of blood vessels in the progestogenic phase, whereas just more than half of women have well-defined blood vessels in the estrogenic phase [10]. The study was planned to detect a statistical difference of 30% in the proportion of women having well-defined blood vessels in the estrogenic and progestogenic phase of the cycle (assuming >90% of women have such changes in the progestogenic phase). A sample size of 30 women was determined to detect this difference at 90% power and with a type I error rate of 0.05, by collecting paired observations (estrogenic phase and progestogenic phase) on each participant and assuming a correlation coefficient (degree of independence between the 2 matched pair cohorts) of 0.2 [11]. Statistical analysis was done using SPSS 14.0 for Windows (SPSS, Inc., Chicago, IL). McNemar test was used to test for marginal homogeneity for each of the colposcopically observed vascular changes (predictors of interest) in the paired observations (estrogenic phase and progestogenic phase in each participant). In case the total of marginal discordant pairs was less than 25, a 2-tailed exact test, based on the cumulative binomial distribution, was used.

RESULTS

Sixty women were screened from January to July 2006, and 30 participants fulfilling the eligibility criteria were enrolled. The last follow-up visits were completed in August 2006. All 30 participants completed the scheduled follow-up visits within the protocol-specified window period mentioned above. HIV prevalence was 1.7% among the screened participants. The mean age of the enrolled study participants was 31.3 years (SD, 5.6 years). Most women (25/30; 83.3%) were living with their spouse. Twenty-four (80%) of the 30 enrolled women had some education. Twenty-seven (90%) were parous, and 3 (10%) were nulliparous. Female sterilization was the most common method of contraception, being used by 19 (63.3%) of 30 women. Two participants were on oral contraceptive pills during the study period and were excluded from the analysis.

Mean hormonal levels during the estrogenic and the progestogenic phase are reported in Table 1. There was a statistically significant difference in the mean progesterone level ($p < .001$) but not in the estrogen level ($p = .8$) during the 2 phases of the menstrual cycle. The mean ($\pm$ SD) vaginal pH was 4.3 ($\pm$ 0.3) during both phases of the menstrual cycle (data not

shown). None of the participants, except for 1, had bacterial vaginosis using Nugent scoring during either the estrogenic or the progestogenic phase of the menstrual cycle (data not shown).

Colposcopic photographs of the vessels on the cervix using the DIMS software during the proliferative and secretory phase showed a subtle change in the appearance during the 2 phases of the menstrual cycle (Figure 1). Colposcopic observations and their comparisons during the estrogenic and progestogenic phases of the menstrual cycle are reported in Table 2. The mean ($\pm$ SD) diameter of the largest visible blood vessel differed significantly between the estrogenic phase (0.38 ± 0.14 mm) and progestogenic phase (0.47 ± 0.12 mm; $p < .01$; data not shown). Three other parameters also differed significantly by phase, all having to do with cervical mucus: visible cervical mucus flowing from the external os was seen in 18 (81.8%) of 22 participants during the estrogenic phase and 8 (36.4%) of 22 participants in the progestogenic phase ($p < .01$); the amount of mucus was moderate/abundant in 17 (77.3%) of 22 participants during the estrogenic phase and 8 (36.4%) of 22 participants during the progestogenic phase ($p = .02$); and the Spinnbarkeit test was positive in 9 (40.9%) of 22 participants during the estrogenic phase and 2 (9.1%) of 22 participants in the progestogenic phase ($p = .03$). The cervix was more fragile during the estrogenic phase (7/28; 25.0%) compared with the progestogenic phase (2/28; 7.1%), although this difference did not quite reach statistical significance ($p = .06$). The blood vessels were also more dense in the progestogenic phase as compared with the estrogenic phase ($p = .06$).

There was no statistically significant difference in any of the other parameters. The proportion of women having a moderate/large amount of cervical ectopy or epithelial hypertrophy was identical in the estrogenic and progestogenic phases. The color of the cervix was slightly more pink/red during the progestogenic phase, and the blood vessels were more prominent, well defined, and denser in the progestogenic phase than in the estrogenic phase, but these differences were not statistically significant (Table 2).

The mean ($\pm$ SD) level of IL-8 during the estrogenic phase was $411.2 (\pm 622.3)$ pg/ μ L, and during the progestogenic phase, it was $69.3 (\pm 84.4)$ pg/ μ L; this difference reached borderline statistical significance ($p = .05$). The mean ($\pm$ SD) level of IL-6 was $6.9 (\pm 8.3)$ pg/ μ L during the estrogenic phase and $3.1 (\pm 5.3)$ pg/ μ L during the progestogenic phase ($p = .16$; data not shown). Other detectable cytokines/chemokines were tumor necrosis factor α , MIP-1 β , monocyte chemoattractant protein 1 and monocyte chemoattractant protein 2, and RANTES, but there was no statistically significant difference in their levels during the 2 phases of the menstrual cycle.

DISCUSSION

The blood flow in the uterine arteries and their branches increases significantly in the luteal phase [10], and the objective of this study was to evaluate if this increased blood flow can be distinguished on the cervix during the luteal phase using colposcopy. In our study, the mean diameter of the largest visible blood vessel was significantly larger in the progestogenic phase. The blood vessels were likely to be more visibly prominent and more dense with a well-defined outline during the progestogenic phase of the menstrual cycle than the

estrogenic phase, although these differences were not statistically significant. This study suggests that the physiological changes of increased blood flow to the uterus and cervix during the progestogenic phase make the blood vessels on the cervix more prominent when observed by colposcopy. This also suggests that if vascular changes are observed during the estrogenic phase in women using a vaginal microbicide, the findings should be considered at least possibly attributable to the study product, and other markers indicating possible inflammatory effects of the product should be evaluated because inflammation of the female reproductive tract increases susceptibility to HIV-1 and other viral infections [12]. These might include inflammatory markers such as cytokines and chemokines (IL-1, IL-6, and IL-8), keeping in mind that in our finding, IL-8 was elevated in the normal estrogenic phase. Menstrual cycle day and prominence of blood vessels may be added to the colposcopy findings form when evaluating vaginal products so that further correlation with inflammatory markers may be done.

Interleukin 8 has been reported to be the most commonly detected chemokine in CVL samples [13]. It has also been reported that vaginal cytokines vary with the time of the menstrual cycle [14]. Plasma IL-8 has been reported to be elevated during the follicular phase of the menstrual cycle with no changes in the other cytokines/chemokines [15]. In the same study, in the vaginal compartment, as measured from CVL samples, the levels of IL-6 and IL-1 β were both 5-fold higher in the follicular than the luteal phase. It has been reported that IL-8 stimulates HIV-1 replication in macrophages and T lymphocytes [16]. Whether elevated IL-8 or IL-6 during the estrogenic phase of the menstrual cycle increases susceptibility of a woman to HIV acquisition needs further evaluation.

The ectocervix is lined by the multilayer stratified squamous epithelium, and the endocervix is lined by the single-layered columnar epithelium, which is more fragile and susceptible to trauma or disruption that may potentially increase the woman's risk to HIV acquisition during intercourse. The susceptibility to HIV and STI acquisition is not evenly distributed in the vagina and cervix. The cervix is fragile compared with the vagina and is the site of particularly high susceptibility to HIV and STIs [17]. The role of the cervix as the primary site for HIV infection is supported by data from rhesus macaques, suggesting that cervical mucosa seems to be the first site of infection after vaginal exposure to simian immunodeficiency virus; infected cells do not appear in the vaginal mucosa until the infection has become systemic [18]. In addition, the cervix is far more susceptible to infection by other sexually transmitted pathogens, including herpes simplex virus 2 [19], and is the principle site of infection for *C. trachomatis* and *N. gonorrhoeae* [19, 20]. Because the cervix is of prime importance in HIV transmission, knowledge of normal changes in the vascular pattern on the cervix in a woman with normal menstrual cycles is important in interpreting changes that might be visualized in women using investigational vaginal microbicide candidates.

Using computerized planimetry to measure cervical ectopia has been suggested [21]. In our study, there was complete agreement between the clinicians in the degree of ectopy during the menstrual cycle using standard colored disks. We also noted strong statistical correlation in the changes in the cervical mucus (its flow, amount, and spinnbarkeit test) during the proliferative and luteal phases of the menstrual cycle, suggesting that the colposcopic

evaluation was performed corresponding to the cyclical variation of estrogen and progesterone. The significant differences by phase in cervical mucus characteristics were as expected. There was no significant difference in the mean estrogen level in both the proliferative and secretory phase, probably because of the first rise in estrogen level before ovulation and second midluteal rise during the secretory phase of the menstrual cycle [9]. The difference in the progesterone level during the 2 phases of the menstrual cycle was highly significant as has been reported in the past [9]. We did not include sexually active women in the study to eliminate the confounding factors due to sex and deposition of the semen in the vagina and its effect.

CONCLUSIONS

This study demonstrates that the physiological changes in uterine blood flow during the progestogenic phase that have been confirmed using transvaginal color Doppler ultrasonography and other techniques are ultramicroscopic but can be visualized colposcopically as a significant increase in the vessel diameter. Therefore, if increased vascularity is observed in women using vaginal products during safety studies (phases I and II) during the estrogenic phase, these changes should be considered at least possibly attributable to the study product, and other markers indicating possible inflammatory effects of the product should be evaluated, keeping in mind that in our study, IL-8 levels were significantly elevated during the estrogenic phase of the menstrual cycle. Whether the risk of HIV acquisition in women is greater in the estrogenic phase than the progestogenic phase of the menstrual cycle needs further evaluation.

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Proliferative Phase

Secretory Phase

Figure 1.
Colposcopic photographs during the proliferative (estrogenic phase) and secretory phase (progestogenic phase).

Table 1

Mean Hormonal Levels by Menstrual Phase (n=28)

Mean hormonal levels	Proliferative phase ^a		Secretory phase ^b		p ^c
	Mean	SD	Mean	SD	
Estradiol (E2)	146.7	117.8	153.6	87.6	.8
Progesterone	0.8	1.02	9.4	6.1	<.001

^a Estradiol E2: reference range, 12.5 166.0 pg/mL; progesterone: reference range, 0.2 1.5 ng/mL.^b Estradiol E2: reference range, 43.8 211.0 pg/mL; progesterone: reference range, 1.7 27.0 ng/mL.^c Paired *t* test was used to calculate the *p* value.

Table 2

Paired Comparison of Vascular Changes and Related Findings in the Estrogenic Phase Versus Progestogenic Phase

Findings on colposcopy		Estrogenic/Follicular phase		Total	McNemar <i>p</i> value
Progestogenic/secretory phase	Color of cervix	Color normal	Color red		
	Color normal	18	2	20	
	Color red	5	3	8	
	Total	23	5	28	.45
	Presence of ectopy	Absent/small (<25%)	Moderate/large (>25%)		
	Absent/small (<25%)	22	0	22	
	Moderate/large (>25%)	0	6	6	
	Total	22	6	28	1.0
	Fragility of columnar epithelium	No	Yes		
	No	21	5	26	
	Yes	0	2	2	
	Total	21	7	28	.06
	Hypertrophy of columnar epithelium	No	Yes		
	No	19	2	21	
	Yes	2	5	7	
	Total	21	7	28	1.0
	Visible blood vessels on the cervix	Not prominent	Prominent		
	Not prominent	0	1	1	
	Prominent	4	23	27	
	Total	4	24	28	.37
	Well-defined outline of blood vessels	No	Yes		
	No	2	2	4	
	Yes	6	13	19	
	Total	8	15	23	.29
	Density of blood vessels ^a	Coarse	Dense		
	Coarse	14	0	14	
	Dense	5	4	9	
	Total	19	4	23	.06

Findings on colposcopy	Estrogenic/Follicular phase	Total	McNemar <i>p</i> value
Cervical mucus flowing from os			
No	No	23	.06
Yes	Yes	14	
Total		8	<.01
Amount of mucus		22	
Scarce	Scarce		
Moderate/abundant	Moderate/abundant	14	
Total		8	
Total Spinnbarkeit test		22	.02
Negative	Negative		
Positive	Positive	20	
Total		2	
		22	.03

Amount of mucus could be assessed, and spinnbarkeit test could be performed when cervical mucus was flowing from the external os.

^a Density was assessed only when the outline of the blood vessels was clearly defined.