



Cervicovaginal shedding of hepatitis C viral RNA is associated with the presence of menstrual or other blood in cervicovaginal fluids

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ABSTRACT

Background: The role of sexual activity in hepatitis C virus (HCV) transmission remains controversial. Studies to date have not explored the relationship between HCV shedding in cervicovaginal fluids and the presence of menstrual or other blood.

Objectives: Since cross-sectional studies may underestimate the prevalence of viral shedding, we performed a 56-day longitudinal study of cervical HCV shedding.

Study design: Women self-collected cervicovaginal swabs for 56 consecutive days, while keeping a diary of menses and genital symptoms. Swabs were tested for HCV RNA and cellular DNA by quantitative PCR, and hemoglobin by spectrophotometry.

Results: Sixteen women contributed a total of 701 cervicovaginal swabs (mean collection period 48 days, range 18–56). Detection of HCV RNA was associated with detection of hemoglobin. Premenopausal women were more likely than post-menopausal women to have HCV RNA detected in cervicovaginal fluids. For premenopausal women, detection of HCV RNA was more likely during menstruation (OR = 56.4) or when hemoglobin was detected in cervicovaginal fluids, even if menstruation was not occurring (OR = 35.4). No woman post-hysterectomy had HCV RNA detected in cervicovaginal fluids on any day, regardless of whether hemoglobin was detected.

Conclusions: Our findings are consistent with a low likelihood of sexual transmission of HCV. The results suggest that shedding of HCV RNA in the female genital tract is associated with the presence of blood, and requires the presence of a cervix. Clinicians should consider advising premenopausal women who are concerned about transmitting infection that infectivity may increase during menstruation.

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1. Background

While injection drug use is recognized as the primary mode of hepatitis C virus (HCV) transmission in the United States,^{1,2} the role of sexual activity in HCV transmission remains controversial. Sexual risk factors, including number of sex partners² and history of sexually transmitted diseases,^{3–5} are associated with an increased risk of HCV infection, yet studies of monogamous serodiscordant heterosexual couples have repeatedly demonstrated a lack of sexual HCV transmission.^{6–8}

There is some evidence that sexual transmission of HCV may be possible if associated with blood exposure. Investigation of a cluster of seven acute HCV infections in a sexual network of men who have sex with men found that after parenteral risk factors were excluded, unprotected active and passive fisting were associated with HCV transmission.⁹ Given the risk of trauma to the rectal mucosa during fisting, this suggests that blood exposure during sexual activity may increase the risk of HCV transmission.

Because of the lack of evidence of sexual transmission of HCV in monogamous partners, the CDC has not recommended barrier precautions for long-term monogamous couples.¹⁰ For heterosexual discordant sexual partnerships in which the woman is HCV-infected, no specific recommendations regarding transmission risks during menstruation have been made. However, advice regarding the safety of sex during menses is commonly sought by couples concerned about transmission of HCV, and this potential mode of transmission begs further study.

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Several cross-sectional studies have reported detection rates of HCV RNA in cervicovaginal fluids ranging from 0¹¹ to 36%.^{12,13} Two studies specifically excluded cervical samples that were contaminated by hemoglobin assessed by spectrophotometry¹⁴ or by the presence of erythrocytes assessed microscopically.¹² The remainder did not assess the presence of blood or hemoglobin in connection with detection of HCV RNA.^{13–16} Thus, studies to date have not explored the relationship between HCV shedding in cervicovaginal fluids and the presence of menstrual or other blood in the female genital tract.

2. Objectives

Longitudinal studies of HIV, HSV, HHV-8, and HCV have demonstrated that virus shedding in body fluids is often intermittent; as such, cross-sectional studies may substantially underestimate the true prevalence of viral shedding.^{17–20} We therefore chose to implement a longitudinal study on cervical shedding of HCV, since daily collection of cervicovaginal fluids allows evaluation of the pattern of HCV shedding in relationship to menstrual flow for each study participant. Here we present data on detection of HCV RNA in cervicovaginal fluids in pre-menopausal, post-menopausal, and post-hysterectomy women who collected daily cervicovaginal swabs over 56 days.

3. Study design

3.1. Subjects

This study was approved by the University of Washington Institutional Review Board. Female patients with chronic HCV were recruited from the Hepatitis and Liver Clinic at Harborview Medical Center, Seattle, WA. All participants had detectable serum HCV RNA levels at baseline. Women were instructed to insert two Dacron swabs into the vagina every morning until they met resistance at the back of the vagina, to rotate the swabs in a circle, and to wait at least 1 min before removing. Participants then placed the swabs into dry cryovials, snapped off the plastic handles, capped and placed the cryovials immediately into their home freezers. Women collected swabs for 56 consecutive days. After day 56 the swabs were transported on dry ice to the laboratory and stored at –80 °C until testing. Subjects completed a daily diary indicating the occurrence of menses, genital tract symptoms, and lesions. All subjects were tested for HIV and HSV-2 serostatus.

3.2. HCV RNA detection

To prepare cervical swabs for quantitative PCR, 200 µl of phosphate buffered saline (PBS) was added. The swab was agitated in the PBS for 10 s, removed, and discarded. The PBS buffer was mixed and 10 µl frozen for detection of hemoglobin. The remaining sample was added to 300 µl of Roche MagNAPure lysis buffer containing 10 mg/ml proteinase K and extracted using the Roche MagNAPure instrument (Total Nucleic Acid Small Volume kit/External Lysis Protocol). Three in-house developed quantitative RT-PCR amplification reactions were performed on the purified nucleic acid: HCV RNA, internal control plasmid (pAW109) added to the lysis buffer during the extraction procedure, and betaglobin to quantitate the amount of cellular DNA present in each sample.²¹

3.3. Validation of HCV RNA detection on swabs

To confirm the validity of frozen specimens, serial 1:10 dilutions of a high-level HCV-positive plasma sample were absorbed onto swabs. The swabs were removed and extracted either immediately

or after being frozen overnight. Linear viral yields were obtained and were indistinguishable between fresh and frozen specimens. To confirm the ability of the assay to detect cell-associated virus, HCV+ RNA replicon cells (Clone A, Apath, LLC, St. Louis, MO) were used to create another serial dilution series. 200 µl of the cell suspension was absorbed onto swabs, and the extraction procedure performed. Again, linear viral yields were observed indistinguishable from that of cell-free virus. The 95% detection limit for HCV after extraction from swabs was <40 IU/ml. Swabs with absorbed HCV+ RNA replicon cells were frozen and used as high and low controls for PCR runs over 3 months as part of this study. The swabs showed no loss of HCV RNA quantity over these 3 months, confirming the stability of frozen swab specimens. Post-study analysis of subject data showed no statistically significant change in the likelihood of detecting HCV RNA on swabs during the 56 day study period (OR 1.02, 95% confidence interval (0.99, 1.04)), demonstrating the validity of home storage of frozen specimens. To confirm that hemoglobin or blood did not interfere with the PCR, 10 µl of HCV RNA-whole blood was added to the cell suspension dilutions before absorption onto swabs. We observed no interference compared to suspensions without the addition of whole blood.

3.4. Hemoglobin detection on cervical swabs

Ten microliters of the PBS used to resuspend the swab material was analyzed with a BioTek PowerWaveX microplate spectrophotometer for the detection of Hemoglobin. The sample was diluted with 190 µl of PBS and then absorbance was read at 415 nm (oxy-hemoglobin) and 380 nm (turbidity correction) using a method modeled after plasma hemoglobin detection but excluding the bilirubin measurement (450 nm).²² For each sample, a corrected OD was calculated by the equation, corrected OD = [O.D. Sample 415 nm – O.D. Blank 415 nm] – [O.D. 380 nm – O.D. Blank 380 nm]. A 3-point hemoglobin standard curve with points at 60, 6, and 0.6 mg/dL, constructed from a patient sample with hemoglobin of 14.5 g/dl, was read simultaneously with the patient samples on a Sysmex XT-2000i analyzer. Samples with hemoglobin >6.0 mg/dl were considered positive.

3.5. Other laboratory testing

Serum HCV RNA was measured using the Bayer Quantiplex HCV RNA 2.0 Assay. Testing for hepatitis B surface antigen was performed using the Abbott Auzyme™ assay. Serologic assays for HIV and HCV were performed using the Genetic Systems HIV-1/HIV-2 Peptide EIA assay (Bio-Rad Laboratories) and the Abbott HCV EIA 2.0 diagnostic kit. Genotype testing was performed using the Abbott HCV Genotype ASR.²³ HSV serology by Western blot was performed by the UW Virology Laboratory at Seattle Children's Hospital.

3.6. Statistical methods

Mixed effect methods²⁴ for logistic regression (with outcome detectable HCV RNA) or linear regression (with outcome level of HCV RNA) were used to account for repeated measures from the same individual.

4. Results

4.1. Study population

Sixteen women contributed a total of 701 cervical swabs over a mean collection period of 48 days (range 18–56 days), with 12 women collecting swabs on every day of the 56-day collection period (Table 1). The median age was 31 (range 17–82 years),

Table 1
Demographics, laboratory characteristics, and frequency of detection of HCV RNA in cervicovaginal fluids (N = 16).

Subject	Age	Status	Baseline serum, log ₁₀ IU/ml	Genotype	HSV2	HIV	# swabs collected	% swabs HCV+
1	27	Menstruating	6.42	3a	+	–	56	0%
2	37	Menstruating	7.36	1b	+	+	20	5%
3	38	Menstruating	5.17	1a	–	–	56	2%
4	43	Menstruating	5.78	1b	+	–	18	17%
5	49	Menstruating	7.26	1a	–	–	56	4%
6	52	Menstruating	6.51	1a	+	–	56	14%
7	56	Menstruating	6.11	1a	–	–	56	4%
8	49	Post-menopausal	6.45	1b	–	–	56	0%
9	53	Post-menopausal	6.69	1a	–	–	56	23%
10	54	Post-menopausal	5.98	2b	–	–	18	0%
11	57	Post-menopausal	6.55	1a	+	–	56	0%
12	57	Post-menopausal	5.98	1a	+	–	56	0%
13	59	Post-menopausal	6.72	1b	+	–	56	0%
14	40	Hysterectomy	6.65	1b	–	–	53	0%
15	47	Hysterectomy	6.33	1a	–	–	24	0%
16	50	Hysterectomy	6.24	1b	–	–	55	0%

and most participants were white (84%). One participant was HIV-positive and seven women were HSV-2 seropositive. The median serum HCV load was 6.4 log₁₀ IU/ml (range 5.17–7.36 log₁₀ IU/ml), and 14 women had genotype 1 HCV infection. Seven women were premenopausal, six were post-menopausal, and three had had total vaginal hysterectomies.

4.2. Detection of HCV RNA in cervicovaginal fluids

Thirty of 699 (4.3%) cervical swabs were positive for HCV RNA (Fig. 1), with a median viral load of 4,693 IU/ml (range 231–69,580 IU/ml). 53 of the 699 swabs (7.6%) were positive for Hb. All of the HCV-positive swabs were collected from 6 of the 16 women participating in the study; swabs from the remaining 10 women were uniformly negative. When cervicovaginal shedding of HCV RNA was examined univariately in a logistic mixed effects model, there was no association between a woman's age, genotype, serum viral load, or HSV serostatus and cervicovaginal shedding of HCV RNA. However, detection of Hb was associated with detection of HCV RNA on cervicovaginal swabs, with a three times greater likelihood of detection of HCV RNA on days when Hb was detected versus days Hb was not detected (3.3% versus 1.1%, odds ratio = 3.04 (95% confidence interval 1.20–7.71)).

When cervicovaginal HCV RNA was examined quantitatively in a linear mixed effects model, the mean viral load of HCV RNA detected when Hb was detected in cervicovaginal fluids was 0.45 log₁₀ IU/ml compared to 0.12 log₁₀ IU/ml when Hb was not detected ($p < .001$). Thus, there was a mean increase of 0.33 log₁₀ IU/ml or 2.16 IU/ml of HCV RNA detected in the samples with detectable Hb compared to those without (95% CI 0.15–0.51 IU/ml).

Premenopausal women were more likely than post-menopausal women to have HCV RNA detected in cervicovaginal fluids on at least 1 day (86% versus 16%, $p = 0.02$). For premenopausal women, detection of HCV RNA was more likely during menstruation (OR = 56.4, 95% CI (23.0, 138.3) or when hemoglobin was detected in cervicovaginal fluids, even when menstruation was not occurring (OR 35.4, 95% CI 12.2, 102.6). Interestingly, no woman who had had a hysterectomy had HCV RNA detected in cervicovaginal fluids on any day, regardless of whether hemoglobin was detected.

5. Discussion

Overall, detection rates of HCV RNA on cervical swabs were low, with HCV RNA detected on only 30 of 699 (4.3%) cervical swabs collected. Many of these women had participated in a previous study, in which HCV RNA was detected in 72% of saliva samples.²⁰ Thus, detection rates of HCV RNA in cervicovaginal fluids were markedly lower than in saliva.

We report lower rates of HCV RNA detection than other published studies. However, since most published studies did not investigate the presence of blood in cervical samples or used insensitive methods to detect blood, it is difficult to compare our results with published data. Regardless, our findings are consistent with epidemiologic data supporting a low likelihood of sexual transmission of HCV.

For premenopausal women, detection of HCV RNA on cervical swabs was strongly associated with menstruation (OR = 56.4, 95% CI 23.0, 138.3) and with detection of hemoglobin, even when menstruation was not occurring (OR = 35.4, 95% CI 12.2, 102.6). In premenopausal women, HCV RNA was detected on 5 out of 318 days when hemoglobin was not detected, versus on 12 out of 31

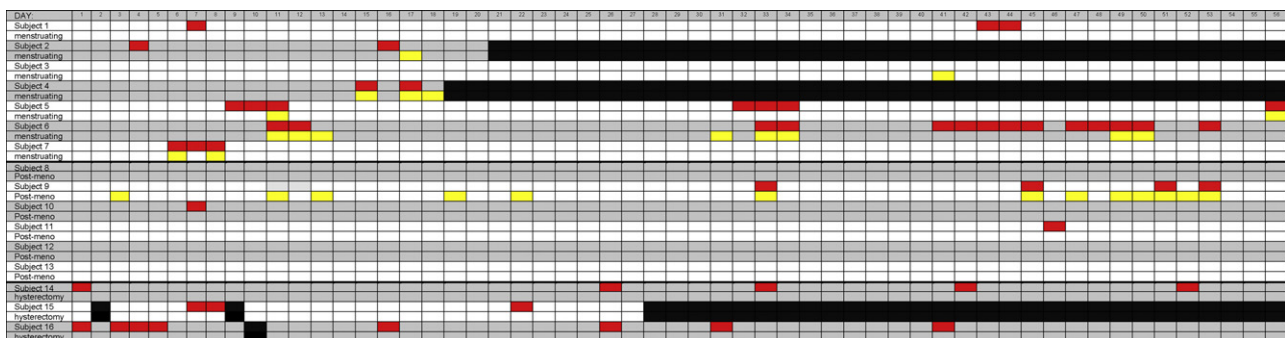


Fig. 1. Detection of hemoglobin and HCV RNA on cervical swabs collected over 56 days. Red indicates days that hemoglobin was detected; yellow indicates days that HCV RNA was detected; black indicates days that swabs were not collected.

days when hemoglobin was detected. In addition, the quantity of virus detected on days with hemoglobin present was significantly higher than on days when hemoglobin was absent. In one previous study, 15 cc of menstrual blood was collected on the first day of menses in 10 women with hepatitis C, and HCV RNA was detected in all samples.²⁵ To our knowledge, this is the only other published study examining the potential role of menstrual blood on the presence of HCV RNA in the female genital tract. Taken together, these two studies suggest that shedding of HCV RNA in the female genital tract is more likely to occur in the presence of blood.

HCV RNA was not detected on any day in any woman who had had a hysterectomy, even though hemoglobin was detected on 16 of 136 (12%) cervical swabs collected from such women. Belec et al. demonstrated that the presence of HCV RNA in cervicovaginal secretions was restricted to cellular fractions containing cervical cells as well as genital tract lymphocytes and monocytes.¹⁴ Manavi et al. localized the presence of HCV RNA to cervical lymphocytes, but not to cervical epithelial cells or cervical granulocytes.¹³ In light of these past studies, our data suggest that the presence of a cervix is necessary for shedding of HCV RNA in female genital tract fluids.

In conclusion, our data suggest that shedding of HCV RNA from the genital tract is more frequent in premenopausal women than in postmenopausal women or women who have had hysterectomies. In premenopausal women, HCV RNA is associated with the presence of blood in cervicovaginal fluids. Importantly, even in premenopausal women, detection of HCV RNA in cervicovaginal fluids is rare, and the viral load is low compared to serum. Clinicians counseling chronically HCV-infected women who are concerned about transmitting infection to their uninfected partners should consider advising women about the possibility that infectivity may increase during menstruation. Some women may choose to abstain from intercourse or use barrier precautions during their menses.

Declarations

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