

DIAGNOSIS OF HUMAN PAPILLOMAVIRUS INFECTION BY ABNORMAL CERVICAL CYTOLOGY IS HIGHLY REPRODUCIBLE AFTER VAGINAL DOUCHING

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SUMMARY

Objective: To evaluate whether human papillomavirus (HPV) activity in symptomatic patients with abnormal Papanicolaou smears (minimal abnormal cytology) was affected by a complete vaginal douche, applied to the cervix prior to colposcopic examination.

Materials and Methods: A total of 132 women with abnormal Papanicolaou smears were prospectively registered in this study from 1999 to 2004. Two specimens were collected from each patient; one before and one after the cervix was swabbed with a complete vaginal douche during a colposcopic examination. We compared the HPV detection results before and after douching in the same patients. The samples were analyzed using the Hybrid Capture II assay.

Results: Before douching, 57.6% (76/132) of the samples were HPV-positive, compared with 58.3% (77/132) of the samples after douching. There was a strong correlation between the cervical HPV detection rates between the before and after douche samples ($p < 0.001$). The kappa statistic was 0.891 for the correlation (positive-positive, negative-negative) and the discrepancy (positive-negative) between the inter-douche rates of HPV activity. A comparison of the HPV assay before and after vaginal douching demonstrated a statistically significant relationship ($p < 0.001$). The sensitivity of the HPV test after douching was higher (97.8%) than that before douching (95.6%). The false-negative rate before douching in women with inflammatory smears was 3% (4/132) and the false-positive rate before douching in women with atypical squamous cells of undetermined significance was 2.1% (3/132).

Conclusion: Our results indicate that self-administration of a vaginal douche is not a reliable method of eradicating HPV infection. [*Taiwan J Obstet Gynecol* 2008;47(4):412-416]

Key Words: human papillomavirus, Papanicolaou smear, vaginal douche

Introduction

Cervical carcinoma is the second most common cause of cancer-related death in women worldwide. It has been well established that organized cytologic screening programs can substantially reduce the incidence of

morbidity and mortality from cervical cancer in developed countries [1].

Opinions differ regarding the benefits of using a douche, which is a universally accepted treatment for vaginitis, to aid health care of the lower genital tract. The efficacy of douching as a means of eradicating lower genital tract inflammation has been questioned [2-4]. Limited data exist in the literature concerning the effect of vaginal douches on human papillomavirus (HPV) activity. Our study investigated the effect of douching on the results of HPV tests.

Cervical cancer typically occurs in the fifth and sixth decades of life, and 27% of patients are aged over



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65 [5,6]. Delivering preventive care in primary care practices is often more difficult in rural areas than in urban areas. Medical practice also differs in rural areas, where there are fewer physicians and less contact with physicians [7]. Important limitations exist regarding the screening of samples obtained during pelvic examination, such as the fact that such examinations require a trained health professional working in a clinical setting. In addition, cultural inhibitions often render routine pelvic examinations unacceptable to symptomatic women from certain ethnic backgrounds. These factors limit the utilization of the screening services [8].

Epidemiologic and basic molecular biology studies support the concept that certain genital HPV strains are major risk factors for the development of cervical cancer and are responsible for 85–95% of human cervical carcinomas. HPV typing is important in order to identify women at risk of cervical cancer [9,10]. The clinical usefulness of HPV testing using the Hybrid Capture II (HC II) system has been evaluated in a number of studies, and it has been shown that this test can be used for the early detection of cervical precancer or cancer [11–15].

The aim of this study was to identify whether or not vaginal douching helped to eradicate HPV infection in women with mildly abnormal Papanicolaou (Pap) cytology. Possible effects on the sensitivity of HPV testing were also evaluated.

Materials and Methods

Participants who had mildly abnormal cytologic results for Pap smears using the Bethesda system were referred to the colposcopic clinic at Chang Gung Memorial Hospital, and were then recruited to this study. A total of 132 patients were enrolled from September 1999 to September 2004.

Following signed informed consent, a careful inspection of the external genitalia and perianal area was performed to detect any evidence of HPV-related diseases. A vaginal speculum was then inserted and a sterile conical brush was used to collect cells for HPV DNA analysis. This applicator was a part of a commercial kit, i.e. the Digene Hybrid Capture II System (Digene Inc., Silver Spring, MD, USA) [14]. Cells were collected from the endocervix and the transformation zone and added to 1.0 mL of specimen transport medium. A colposcopic examination was then carried out with 5% acetic acid, according to the standard protocol. After douching with the acetic acid, a second HPV brush was inserted for cell collection. The entire transformation zone was observed for any lesions and

the colposcopic findings were recorded. If a lesion was seen, a punch biopsy specimen was taken from the most significant area.

Specimens were processed for HPV testing according to the manufacturer's instructions. The Digene Hybrid Capture II System is a sandwich capture molecular hybridization assay that employs chemiluminescence detection. The specimens were treated with alkaline denaturation solution at 65°C for 45 minutes and hybridized under high stringency with a mixture of specific RNA probes for HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. The resultant DNA–RNA hybrids were captured on the surface of the microtiter plate wells coated with an anti-DNA–RNA hybrid antibody. The immobilized hybrids reacted with the alkaline phosphatase-conjugated anti-hybrid monoclonal antibody. Light was emitted and measured as relative light units (RLUs) using a luminometer. The intensity of the light emitted was proportional to the amount of target DNA in the specimen. An RLU measurement greater than or equal to the positive control cutoff value (0.2 pg/mL HPV DNA) indicated the presence of HPV sequences in the patient's specimen, whereas an RLU measurement less than the cutoff value indicated the absence of HPV sequences.

The sensitivity, specificity, positive predictive value and negative predictive value were calculated using the final histologic diagnoses as the standard. Reliability was assessed by pairwise inter-douche vaginal HPV test comparisons using the kappa statistic. As a general guide, a kappa value > 0.75 represents excellent agreement beyond that expected by chance, values < 0.40 represent poorer agreement than that expected by chance, and values between 0.40 and 0.75 suggest fair to good agreement relative to that expected by chance. The association between HPV infection detection before and after vaginal douching was examined using the Chi-squared or Fisher's exact test. A *p* value of < 0.05 was considered statistically significant.

Results

The study group included 132 women who had minimal abnormal cytology on routine cytologic cervical examination. They, therefore, underwent colposcopic examination and/or directed biopsy with douche. All gave their informed consent to their participation in this study. The minimally abnormal smears included 27 cases of low-grade squamous intraepithelial lesions (LSIL); 52 cases of ASCUS (atypical squamous cells of undetermined significance) lesions; and 53 cases of inflammatory smears. HPV was found to be positive in

Table 1. Detection of human papillomavirus (HPV) before and after vaginal douching

HPV testing	n	Before douche		After douche	
		Positive Pap result	Negative Pap result	Positive Pap result	Negative Pap result
Inflammation	53	19	34	16	37
ASCUS	52	38	14	42	10
LSIL	27	19	8	19	8
Total	132	76	56	77	55

ASCUS = atypical squamous cell of undetermined significance; LSIL = low-grade squamous intraepithelial lesion.

Table 2. Comparison of rate of change in human papillomavirus (HPV) detection before and after vaginal douching

HPV testing	Pap results				χ^2	κ	p
	-/-	-/+	+/-	+/+			
Inflammation	34/37	0/0	3/3	19/16	41.013	0.872	<0.001
ASCUS	14/10	4/4	0/0	38/42	33.605	0.785	<0.001
LSIL	8/8	0/0	0/0	19/19	27.00	1.000	<0.001

ASCUS = atypical squamous cell of undetermined significance; LSIL = low-grade squamous intraepithelial lesion.

19 (70%) of the 27 LSIL smears, 38 (73%) of the 52 ASCUS, and 19 (35.8%) of the 53 apparently inflammatory smears.

Before vaginal douching, 76 of 132 (57.6%) subjects exhibited cancer-associated HPV activity. After douching, HPV infection was detected in 77 of the 132 (58.3%) subjects. In 94.7% of the samples (125/132), the HPV activity results were the same before and after vaginal douching (negative remained negative, and positive remained positive). Douching resulted in a minimal discrepancy rate of 5.3% (7/132), due to a shift in the vaginal douche population for high-risk HPV activity. This included tests where a negative result had turned positive (4/132) in the ASCUS group, or a positive result had turned negative (3/132) in the inflammatory group. Thus the detection of HPV activity was not influenced by a 5% acetic acid douche in 125 of the 132 (94.7%) swab samples studied ($p < 0.001$) (Tables 1 and 2). The HPV values in the seven swab samples that changed were very close to the upper limit of the negative control for the laboratory HC II test. The vaginal douche completely cleansed the area of cervical mucinous fluid, thus making the sampling of cervical cells easier and possibly increasing the HPV viral load of the samples (data not shown) (Tables 1 and 2). In this series of experiments, the use of self-administered vaginal cleansing with 5% acetic acid did not eradicate oncogenic HPV activity.

The inter-douche vaginal reliability of the HC assay is shown in Table 3. The inter-douche agreement was generally higher for the positive group (84.2–100%) than for the negative group (71.4–100%). The positive group (positive–positive) agreements were 16/19

Table 3. Pairwise agreement before and after vaginal douche for human papillomavirus (HPV) detection*

HPV testing	Pap result	
	Agreement on positivity	Agreement on negativity
Inflammation	16/19 (84.2%)	34/37 (91.9%)
ASCUS	38/42 (90.5%)	10/14 (71.4%)
LSIL	19/19 (100%)	8/8 (100%)

* κ statistic = 0.89, $\chi^2 = 104.863$, $p < 0.001$. ASCUS = atypical squamous cell of undetermined significance; LSIL = low-grade squamous intraepithelial lesion.

(84.2%), 38/42 (90.5%) and 19/19 (100%), and the negative (negative–negative) agreements were 34/37 (91.9%), 10/14 (71.4%) and 8/8 (100%) in the Pap smears, indicating infection, ASCUS or LSIL, respectively. The kappa statistic was 0.891 for correlation (positive–positive, negative–negative) and discrepancy (positive–negative) of HPV activity, comparing vaginal douching as a whole. Comparison of the before and after vaginal douche HPV activities demonstrated statistical significance ($\chi^2 = 104.863$, $p < 0.001$).

Discussion

Cancer-associated HPV infection and the effects of vaginal douching are an issue for many women. However, there is a lack of adequate medical literature about self-administered vaginal douches and HPV infection. In our experience, Taiwanese women usually perform vaginal cleansing at home, despite symptomatic vaginal

bleeding and regardless of education. In the United States, 90% of women still perform self-bathing for lower genital tract infections [2]. Our use of a simple and safe test for detecting oncogenic HPV sheds light on the effect of vaginal douching on HPV infection/detection.

Our hospital in Taiwan has been using the HC II method to detect high-risk HPV, in addition to the minimal abnormal Pap smear method, for many years. The question of whether or not vaginal douching, self-bathing of the lower genital tract, or use of vaginal drugs can cause the HPV test to give false results has been posed previously, and we have attempted to answer this important question in this prospective study. Long-term infection with oncogenic HPV types seems to be one of the underlying factors responsible for the development of cervical cancer [4–6]. In our experience, the HC II assay has excellent sensitivity and has a good negative predictive value for the detection of cervical intraepithelial neoplasia or cancer. Overall, the sensitivity of the HC II assay in detecting high-grade lesions is 100%, versus 85.9% for standard cytology. However, its specificity (86.3%) and positive predictive value are not as high as the latter [16–19]. Thus, the HC II test is suitable for confirming negative results for cervical carcinoma [17–20].

A complete vaginal douche might be expected to reduce any cancer-associated HPV activity, because 5% acetic acid is able to cleanse the mucinous cervical transformation region and HPV-infected cervical lesions, so possibly causing the HC II test to yield false negative results. On the other hand, the douche may lead to improved sampling of infected cervical cells compared with reduced sampling of uninfected uterine cells. Age could also potentially affect the reliability of the test results. Older women (> 50 years old) have a drier lower genital tract due to menopause, as well as anatomic narrowing of the cervix opening, which may retain infected cells higher in the cervical canal and result in poorer viral sampling [19]. A vaginal douche with 5% acetic acid for 1 minute covering the cervical orifice induces relaxation of the cervical canal, which may allow sampling from lesions higher in the canal and thus lead to better detection.

In conclusion, douching does not eliminate the presence of oncogenic HPV in lower genital tract inflammation. Advances in the diagnosis of cervical cancer have resulted in an overall reduction in the number of deaths from cervical cancer. However, there is still a need for further education regarding cervical cancer and its risk factors. This study provides information that may be of use epidemiologically and for the future prevention and/or treatment of cervical cancer.

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