

Transvaginal ultrasound probe contamination by the human papillomavirus in the emergency department

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ABSTRACT

Objective To determine if human papillomavirus (HPV) DNA can be detected on the transvaginal sonography (TVS) probe in the emergency department (ED) and whether the current barrier method plus disinfection can prevent HPV contamination of the TVS probe.

Methods This was a two-part cross-sectional study. In the first part, surveillance samples were taken from the TVS probe for HPV DNA detection daily for 2 months. In the second part, patients presenting with early pregnancy complications were identified in the ED and high vaginal swabs were taken for HPV DNA testing. Several probe swabs were taken to identify if contamination was possible in cases where the procedure was done on an HPV carrier.

Results A total of 120 surveillance samples were obtained, nine of which (7.5%) tested positive for HPV DNA. In the second part, 76 women were recruited, of whom 14 (18.4%) were HPV carriers. After the procedure and disinfection of the probe, three out of the 14 probe samples (21%) were HPV DNA positive.

Conclusions HPV is commonly encountered in the ED and contamination of the TVS probe with HPV is possible. Although it is difficult to prove the viability and infectivity of the virus, vigilant infection control measures should be maintained.

INTRODUCTION

Human papillomavirus (HPV) is the most common sexually transmitted disease worldwide with 10%–20% of both men and women having molecular evidence of HPV infection.¹ The cumulative risk of acquiring HPV infection is reported to be 45% at 3 years after the first sexual relationship, and the overall prevalence is 25% in sexually active young women.² Most infections are subclinical and transient. Risk factors for infection include earlier age of coitarche, more sexual partners, smoking and reduced immunity.^{3,4} Nevertheless, infection is common even in those without identifiable risk factors.

HPV is a circular double-stranded DNA virus consisting of more than 150 genotypes. There is a well-established relationship between cervical neoplasia and certain HPV subtypes, namely types 16, 18, 31, 33 and 45. Persistent infection with these high-risk HPVs may lead to abnormal cervical cell changes, increasing the risk of cervical cancer.⁵ Among those high-risk HPV types, types 16 and 18 together cause about 70% of all cases of cervical cancer.⁶ Other types of sexually transmitted HPV (type 6 and 11) are responsible for genital condylomata.

Bedside ultrasound examination is gaining importance in the everyday practice of the emer-

gency department (ED). The use of ultrasonography is mainly focused on biliary disease, intrauterine pregnancy and abdominal aortic aneurysms,⁷ as well as looking for peritoneal fluid and pericardial tamponade in trauma patients.⁸ Many studies have demonstrated that emergency physician performed ultrasonography can be very useful in the management of early pregnancy bleeding,^{9–12} with potential reductions in the length of inpatient stay under the care of the gynaecology team. Patients identified as having an intrauterine pregnancy can be safely discharged from ED with proper advice and an early follow-up appointment at an early pregnancy assessment clinic. This decreases treatment time in the ED by 55%, and saves total costs of 63% per patient without major adverse outcomes.^{13,14} In our department, there was a dramatic reduction in the number of gynaecological admissions from 75% to 26% when we introduced this in 2009.¹⁵

The use of transvaginal sonography (TVS) has consequently become more popular in EDs in recent years. The TVS probe is routinely protected by a condom, acting as a physical barrier to contamination. Studies have shown that the perforation rate of these condoms ranged from 0.9% to 5%.^{16–18} One large scale study showed that the condom perforation rate was 2%, with 65% of the leakage points being <10 cm from the tip.¹⁸ With these expected perforation rates, staff are advised to follow the proper steps for disinfection of the TVS probe after each scan to prevent cross-infection.

There are few studies on this issue of TVS probe contamination with HPV in the ED. It is unclear whether the current disinfection method is sufficient to clear up the virus in cases of contamination.

The aims of the study were (1) to determine if any HPV DNA could be detected on the TVS probe and its contamination rate and (2) to evaluate if HPV DNA was detectable on a TVS probe which was used on patients with confirmed vaginal or cervical HPV infection despite following the recommended barrier method and disinfection procedure.

MATERIALS AND METHODS

Study design

Two independent cross-sectional studies were conducted.

Setting

The studies were conducted in the ED of a teaching hospital in Hong Kong which has an annual ED attendance of around 150 000 persons. The study

took place between December 2011 and February 2012. It was approved by the Local Institutional Clinical Research Ethics Committee.

Methods

Our departmental protocol calls for the use of a condom (53 mm plain, Pleasure Latex Products, Selangor Darul Ensan, Malaysia) as barrier protection for the TVS probe for every examination. Dry tissue paper was used to wipe away the excess gel on the probe after condom removal, followed by the use of T-spray® (Pharmaceutical Innovations, Inc., Newark, New Jersey, USA). T-spray® is a bactericidal, fungicidal and virucidal disinfecting detergent, specifically designed for ultrasound probes and mammography compressor plates. The TVS probe was then left to dry in air for at least 5 min.

Specimen collection took place in the ultrasound room of the ED which is equipped with one transvaginal transducer. The instrument was available for all ED medical staff to use when clinically required. Cotton wool swabs were used to take samples from the TVS transducer head and to take the high vaginal swabs.

Part 1: surveillance study on contamination of the TVS probe

Two sets of samples were taken daily at 08:00 and 20:00 h for a 2-month period at times when the instrument was not in use. Although there was no formal blinding, the specimen collectors were unaware of how or by whom the instrument had been used or whether the probe had been cleaned. The transducer head up to 10 cm from the tip was swept in a 360° fashion. The cotton wool part was then stored in normal saline solution.

The samples were stored frozen until DNA extraction using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). The presence of HPV DNA in the samples was detected by PCR using consensus primers, PGMY09–11, which target a 450 bp region of the L1 gene. This consensus PCR covers more than 40 types of mucosal HPV. Briefly, the PCR was conducted in a 50 µl reaction mix containing 5 µl of extracted DNA, 200 µM deoxynucleotide triphosphates, 0.06 µM of PGMY09 and PGMY11 primers, and 1.25 µl of HotStarTaq Plus polymerase (Qiagen). The cycling conditions were as follows: activation of polymerase at 95°C for 5 min, 40 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 1 min, followed by a final extension at 72°C for 8 min. Amplification was visualised by agarose gel electrophoresis.

Part 2: serial samples from patients and the TVS probe

The investigator identified patients who presented with symptoms of possible early pregnancy complications and required a TVS as a component of routine clinical care. A total of four swabs were taken as follows. First, a high vaginal swab from the patient (swab 1) for HPV DNA detection was taken during the speculum examination. A probe swab (swab 2) was taken before patient contact to rule out pre-existing probe contamination. After evaluation with TVS, the condom was removed and another probe swab (swab 3) was taken. The probe disinfection procedure was then completed and the last probe swab (swab 4) was collected. Swabs 1–4 were sent for HPV DNA detection using PCR as described above.

Patients

Patients who were ≥18 years of age and confirmed to be pregnant with a positive urine pregnancy test, with a gestational age of ≤12 weeks, whose chief complaint was vaginal bleeding or abdominal pain were eligible for inclusion in the study. Exclu-

sion criteria included women with diseases causing immunosuppression or currently on immunosuppressive agents and patients with a confirmed spontaneous abortion (defined as products of gestation found in the genital tract on speculum examination).

Statistical analysis

χ^2 Test and Fisher's exact test were used to compare HPV positivity and risk factors for categorical data. Continuous variables were compared using the t test. All statistical tests were two-tailed and a p value of 0.05 or less indicated statistical significance. Data were analysed with SPSS software V.20 for Windows.

RESULTS

A total of 120 surveillance samples were collected over 60 days, of which nine (7.5%) were HPV DNA positive. Of the nine that were positive for HPV DNA, eight were collected in the first 20 days of the study period and two were consecutive samples. The results are summarised in figure 1. Despite an average contamination rate of 7.5%, the positive results were unevenly distributed. When we divided the study period into three parts (40 samples each), the contamination rate in the first period was up to 20%.

In the second part of the study, 78 patients were initially recruited but two of them were subsequently excluded as products of gestation were identified on speculum examination. Of the remaining 76 patients, most of them were from Hong Kong, three from Mainland China and two patients were from other countries. The mean age of the patients was 31.3 years (range 19–46 years) and their pregnancies had a median gestational age of 7.8 weeks (range 4–12 weeks). Most of the women were non-smokers (79%, n=60) and 4% (n=3) were ex-smokers. In all, 55% (n=42) of the women had had a normal cervical smear while 43% (n=33) had never had a cervical smear done. One woman (2%) had a history of a low-grade squamous cell intraepithelial lesion. Only one woman reported a history of sexually transmitted disease. The TVS revealed a viable fetus in 41% (n=31) of patients and 12% (n=9) of fetuses were non-viable. The TVS showed an empty uterus and uncertain viability in 18% (n=14) and 29% (n=22) of patients, respectively.

Out of 76 women, 14 (18.4%) had HPV DNA detected from the high vaginal swab (swab 1). Their demographic data and the swab results are summarised in table 1. About 78.5% showed no HPV contamination on the TVS probe after the procedure

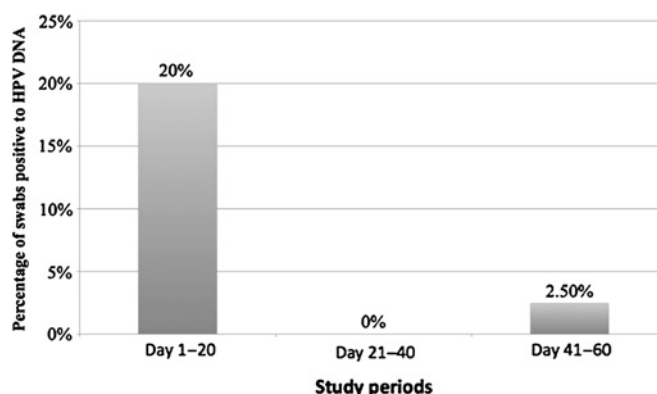


Figure 1 Result of surveillance transvaginal sonography probe samples (n=120). HPV, human papillomavirus.

Table 1 Demographic data and swab results for HPV DNA positive patients

Age (years)	Gestational age (weeks)	Smoking status	Previous pap smear	TVS result*	Swab 1†	Swab 2	Swab 3	Swab 4
30	10	Non-smoker	Normal	VF	++	–	–	–
41	10	Non-smoker	Never	NVF	++	–	–	–
31	10	Non-smoker	Never	VF	++	–	–	–
24	10	Non-smoker	Never	VF	+++	–	–	–
36	4	Smoker	Normal	UV	+++	++	–	–
24	6	Non-smoker	Normal	VF	+++	–	–	–
29	7	Non-smoker	Never	NVF	++	–	–	–
29	6	Non-smoker	Normal	EU	+++	–	–	–
46	11	Non-smoker	Normal	NVF	++	–	–	+
26	9	Non-smoker	Never	UV	+	–	–	–
34	12	Non-smoker	Normal	VF	+	–	–	+
20	6	Smoker	Normal	EU	+	–	–	–
23	10	Smoker	Never	UV	+	–	+	+
27	8	Non-smoker	Normal	VF	+	–	–	–

The HPV DNA PCR results were expressed semiquantitatively according to the intensity of the PCR amplicons.

*VF, viable fetus with fetal heart pulsation seen; NVF, non-viable fetus, fetal heart pulsation absent for fetal pole >1 cm; UV, uncertain viability, intrauterine sac seen without fetal pole or absent fetal heart pulsation in a fetal pole <1 cm; EU, empty uterus, no intrauterine sac identified.

†Swab 1, high vaginal swab of the patient; Swab 2, TVS probe swab before patient contact; Swab 3, TVS probe swab after procedure and before disinfection; Swab 4, TVS probe swab after standard disinfection.

HPV, human papillomavirus; TVS, transvaginal sonography.

following standard disinfection. Three out of 14 last samples (swab 4) taken after disinfection were HPV DNA positive. A comparison of the characteristics between HPV positive and HPV negative women is shown in table 2.

DISCUSSION

We found that 7.5% of surveillance samples were HPV DNA positive. It showed that either the ED staff did not fully comply with disinfection procedures after every ultrasound examination or the disinfection procedures did not remove all the HPV DNA. This finding confirms previous results that HPV DNA can still be found on medical instruments after sterilisation.⁶

HPV is a physically stable and resistant virus with long durability.¹⁹ A recent in vitro study demonstrated that it can survive on a wet surface for at least 7 days, and carries an infection ratio of 30%. Desiccation reduced its infectivity with a lower infection ratio of 10%.¹⁹

HPV is a very common infection. Our results showed an 18% carrier rate which is within the range of 10%–20% carrier rate reported worldwide.¹ It is spread predominantly through sexual intercourse, although other routes of transmission have been postulated.^{6, 20} A number of studies have demonstrated that HPV DNA can also be found in the environment. A study conducted in a genitourinary medicine clinic found mucosal HPV DNA present on the examination beds, the instrument handles and in the patients' washrooms.²¹ Even after proper sterilisation procedures, HPV DNA was still detected on the

medical instrument surfaces used in patients with confirmed HPV genital tract infection.⁶

The fact that most HPV DNA positivity concentrated in the first 20 days of the study period may be explained by the Hawthorne effect. Medical staff became more alert and possibly more thorough in cleaning the instrument (TVS probe) in response to learning that a study was going on. It seems likely that increasing alertness among staff may have led to meticulous disinfection which reduced the contamination rate in latter part of the study.

In the second part of the study, three probe swabs were positive for HPV DNA after examining HPV carrier patients. Condom breakage may be a possible explanation and the rate of 3.9% as observed in this study is comparable with previous reports.¹⁸ On removal of the condom, the mucus or contaminated gel may contact the probe surface if the condom is turned inside out. Using a dry towel to wipe out the gel with an ammonia based disinfectant (T-spray®) may not be enough to remove the HPV DNA. After cleaning, the probe could also be contaminated by the environment or gloves which had previously been in contact with the external genitalia.

The detection of HPV DNA does not necessarily indicate the presence of viable virus, and thus its infectivity is unknown. To determine the infectivity of HPV is technically difficult as there is no feasible in vitro or in vivo culture system for HPV. Nevertheless, a good cleansing procedure should aim at removing any viral DNA.

Table 2 Demographic data of study subjects according to the human papillomavirus (HPV) results of high vaginal samples

	All (n = 76)	HPV positive (n = 14)	HPV negative (n = 62)	
Age (years) (mean ±SD)	31.3±5.48	30±7.22	31.6±5.04	p=0.34
Age range (years)	19–46	20–46	19–44	
Gestational age (weeks) (mean ±SD)	7.8±2.18	8.5±2.35	7.6±2.13	p=0.16
Normal previous pap smear	42 (55%)	8 (57%)	34 (55%)	OR=1.1 (95% CI 0.34 to 3.54), p=1.0
Smoker	13 (17%)	2 (14%)	11 (18%)	OR=0.77 (95% CI 0.15 to 3.9), p=1.0
Viable fetus	31 (41%)	6 (43%)	25 (40%)	OR=1.11 (95% CI 0.34 to 3.59), p=1.0
Non-viable fetus	9 (12%)	3 (21%)	6 (10%)	OR=2.5 (95% CI 0.55 to 11.7), p=0.35
Empty uterus	14 (18%)	2 (15%)	12 (19%)	OR=0.69 (95% CI 0.14 to 3.5), p=1.0
Uncertain viability	22 (29%)	3 (21%)	19 (31%)	OR=0.62 (95% CI 0.15 to 2.47), p=0.75

χ^2 Test and Fisher exact test were adopted for categorical data. t Test was adopted for continuous numerical data (age and maternity).

A TVS probe is considered to be a semicritical item which contacts mucous membranes or non-intact skin, according to the Centers for Disease Control and Prevention (CDC) guideline. This category of devices should be free from all microorganisms while small numbers of bacterial spores are permissible. Probe covers are encouraged to reduce microbial contamination but high-level disinfection procedures should be followed. High level disinfectant formulations can contain glutaraldehyde, glutaraldehyde with phenol/phenate, ortho-phthalaldehyde, hydrogen peroxide, and both hydrogen peroxide and peracetic acid.²² The exposure times vary with different compounds. The T-spray® which our ED is currently using is a quaternary ammonia based disinfectant which is recommended for non-critical equipment used on intact skin according to the CDC guideline. This may be an explanation of the failure to clear all HPV DNA on the probe surface. We have subsequently changed our TVS disinfectant method to the Tristel TRIO® wipes system (Tristel Solutions, Cambridge, UK) which is a chlorine dioxide based disinfectant.

We also observed that there was a significantly higher rate of non-viable fetuses found in the group of HPV carriers (OR 2.5). A previous study raised the possibility that HPV may be an aetiological agent of spontaneous abortions in early pregnancy.²³ Although the pathophysiology of this viral infection with respect to fetal pathology is not clear, our finding may add evidence to this observation. Future studies should aim at investigating the association between HPV carriage and spontaneous abortion.

Given these findings, proper disinfection with high level disinfectant should be done after each TVS procedure. It is not possible, on the basis of these results, to confirm or exclude the possibility of acquiring HPV infection through contaminated instruments. Further studies may well be targeted at cutaneous subtypes of HPV that could be transmitted through transabdominal ultrasound transducers; the prevalence of these may be even higher.

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Contributors MSTC, CSKP and CAG designed the study and reviewed and edited the final manuscript. MSTC did the data collection, literature review and wrote the first draft of the manuscript. CSKP supervised the laboratory work. YCMA performed the laboratory work. CAG supervised the study overall. All authors have seen and approved the final manuscript.

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Competing interests None.

Ethics approval Ethics approval was provided by Joint CUHK - New Territories East Cluster Clinical Research Ethics Committee.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement There are no additional unpublished data from the study.

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