THE JOURNAL OF
Obstetrics and Gynaecology Research

doi:10.1111/jog.13968 J. Obstet. Gynaecol. Res. Vol. 45, No. 7: 1260–1267, July 2019

Development and evaluation of a cervical cancer screening system in Cambodia: A collaborative project of the Cambodian Society of Gynecology and Obstetrics and Japan Society of Obstetrics and Gynecology

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Abstract

Aim: In Cambodia, the Japan Society of Obstetrics and Gynecology and the Cambodian Society of Gynecology and Obstetrics have an on-going project, started in 2015, for cervical cancer prevention and treatment. The project, currently aimed at factory workers, includes a women's health education program that leads into cervical cancer prevention by establishment of a system for early detection and treatment. It begins by health education, screening for human papillomavirus (HPV), followed by colposcopy and quicker treatment of earlier precursor lesions.

Methods: Rates for participant screening, HPV test positivity, cervical intraepithelial neoplasia (CIN) detection and distribution of HPV types were compared between two screening programs, factory-based and hospital-based. Some HPV test samples were divided into two, one of which was sent to Japan for a quality-control check of the Cambodian testing.

Results: The factory-based participant screening rate was 19% (128/681). HPV was detected more frequently in the factory-based program participants (12%) than in the hospital-based program participants (5%). Unfortunately, however, the rate of receiving proper secondary colposcopy screening among the HPV-positive females was significantly higher in the hospital-based program (94%) than the factory-based program (40%) ($P < 0.001$). The Cambodian laboratory HPV testing accuracy was 92.6%. HPV types demonstrated no significant difference between the two prevention programs.

Conclusion: We could successfully introduce HPV-based screening, starting from health education. However, low rate of screening, especially secondary screening for HPV positive factory workers was identified. Also, HPV testing could be further improved for accuracy through close monitoring.

Key words: Cambodia, cervical cancer screening, human papillomavirus test, low-resource country, polymerase chain reaction, probe-hybridization.

Received: February 22 2019.
Accepted: March 16 2019.
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Introduction

Thanks to human papillomavirus (HPV) vaccines and improved screening, cervical cancer is now largely an easily preventable disease, yet it tragically remains one of the leading causes of cancer death in women worldwide. Most of those deaths occur in low- to middle-income countries.[1] As a countermeasure, affordable cervical cancer prevention and control programs are being developed to decrease cervical cancer incidence, morbidity and mortality in these countries, in accordance with the World Health Organization (WHO)'s framework. These programs include community mobilization, education and counseling, HPV vaccination for prevention, screening for precancerous cervical lesions for earlier treatment, and diagnosis, treatment and palliative care for cases of invasive and metastatic cervical cancers.

According to WHO disease prevention guidelines, countries should consider introducing HPV vaccination as soon as sustainable financing can be secured and it is programmatically feasible to do so. Early detection by screening all women in a targeted age group, followed by proper treatment of any detected precancerous lesions, is a proven effective strategy for prevention of cervical cancer. Population-based cytology screening in low- and middle-income countries is often unsuccessful because the financial investments to establish and maintain the necessary level of healthcare infrastructure, including laboratory and skilled human resources, are not available or sufficient in most resource-limited settings.

In high-resource settings, testing for HPV as a primary screening tool is being incorporated into cervical cancer prevention programs. In low-resource settings, such as Cambodia, there is a newer lower-cost HPV test currently being tested, but it is less sensitive and less accurate that the polymerase chain reaction (PCR)-based test used in more affluent settings. In countries with only enough resources to provide the low-cost HPV testing, other procedures, including visual inspection with acetic acid (VIA) and colposcopy, can be combined with the HPV test to achieve more accurate screening.[1]

Cambodia has recently elevated itself to the level of a lower-to-middle-income country. However, its healthcare system has not kept pace; there is almost no concept of disease prevention, consequently a system for cervical cancer prevention has yet to be established. As a result, cervical cancer is the most common cancer in Cambodia women; the rates of age-standard incidence (23.8 per 100 000 women) and mortality (13.4 per 100 000 women) are much higher than estimates for the larger Asian region, or for the rest of the globe.[2] After successful reduction of maternal mortality, number of death caused by cervical cancer has been more than maternal death. Cervical cancer was added as a priority for the Ministry of Health in Cambodia (National Health Strategic Plan 2016–2020, MOH Cambodia).

In 2012, the Japanese Society of Obstetrics and Gynecology (JSOG) implemented a joint cooperative program to improve Cambodian women's health status by supporting the Cambodian Society of Gynecology and Obstetrics (SCGO). After several years of communications and technical exchanges, in 2015 the Japan International Cooperation Agency (JICA) Grassroots Technical Cooperation Project was signed into effect by the three participants: the Cambodian Ministry of Health, the SCGO and the JSOG. Funded by the Japanese Ministry of Health, Labour and Welfare and JICA, the project's strategic focus was a model for women's health education and cervical cancer prevention for female factory workers. This was to be accomplished by establishment of a system of early detection of cervical cancer using HPV testing followed by secondary colposcopy for any HPV-positive cases, and immediate treatment of any early lesions by loop electrosurgical excision procedure (LEEP) or by conization (using the Taniguchi-Shimodaira procedure).[3]

This system for cervical cancer education, prevention and treatment has now been running in Cambodia for 3 years, from 2015 to the present. We have evaluated the successes and weaknesses of this joint program, and we present our results herein.

Methods

The program of women's health education was conducted by teams of SCGO gynecologists, midwives and SCGO Secretariat staff at five factories located within Cambodia's Phnom Penh Special Economic Zone (PPSEZ). The factory-based program consisted of five learning sessions, each covering one of the following five topics: hygiene, women's health, family planning, pregnancy care and cervical cancer. After finishing all five sessions, the procedure for further cervical cancer screening was explained and recommended to the participants. The Cambodian cervical cancer screening project and study was approved

by both the Cambodian Ministry of Health and the Osaka University Medical Hospital.

Testing for HPV was used for the initial screening method. All cervical samples collected for HPV testing were sent to one of Cambodia's national hospitals, where trained laboratory technicians conducted the 'care HPV assay' (Qiagen) and scored the samples as either positive or negative. Using probe-hybridization technology, the care HPV test can detect the 14 highest-risk types of HPV, including 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68, at a cost per test of US$5.40.

In random cases, the samples were divided into two, one of which was sent to Japan for a quality control check of the Cambodia HPV test results. In Japan, the HPV types were analyzed by a more sensitive PCR-based method. DNA samples sent from Cambodia were amplified using PGMY09/11 primers for HPV L1 region and HLA primer as internal control and the PCR products were used for reverse blotting hybridization (PGMY-CHU assay).[4,5] In this assay, 31 HPV types (HPV6, 11, 16, 18, 26, 31, 33, 34, 35, 39, 40, 42, 44, 45, 51, 52, 53, 54, 55, 56, 57, 58, 59, 66, 68, 69, 70, 73, 82, 83 and 84) were detected. Our genotyping quality was equivalent to that of genotyping system used in the National Institute of Infectious Diseases in Japan, which had been evaluated as proficient in the HPV DNA proficiency panel study conducted by WHO HPV Laboratory Network.[6,7]

With no reliable information on the age-specific prevalence of HPV genotypes in Cambodia, or in this specific subpopulation of women, the criteria for screening eligibility was decided to be: the woman must be above age 25 years and ever married, who had at least one sexual partner, who had fully attended the health education program, and who was not known to be pregnant at the time of testing. Women who tested positive for HPV were referred to one of three national hospitals (Calmette, Khmer-Soviet and the National MCH Center) to undergo secondary screening by colposcopy. The diagnostic and therapeutic strategies we used are shown in Figure 1.

Apart from the factory-based education and screening program, any woman who was a regular admittance at one of the three program-participating national hospitals, who had previously experienced intercourse and who wished to have HPV and cervical cancer screening, were accepted into what we termed the hospital-based program for screening. Acceptance into this hospital-based program occurred without the type of prior health education received by the women in the factory-based program.

After two and half years of running the program, we have evaluated the overall Cambodian cervical cancer screening and prevention project. In particular,

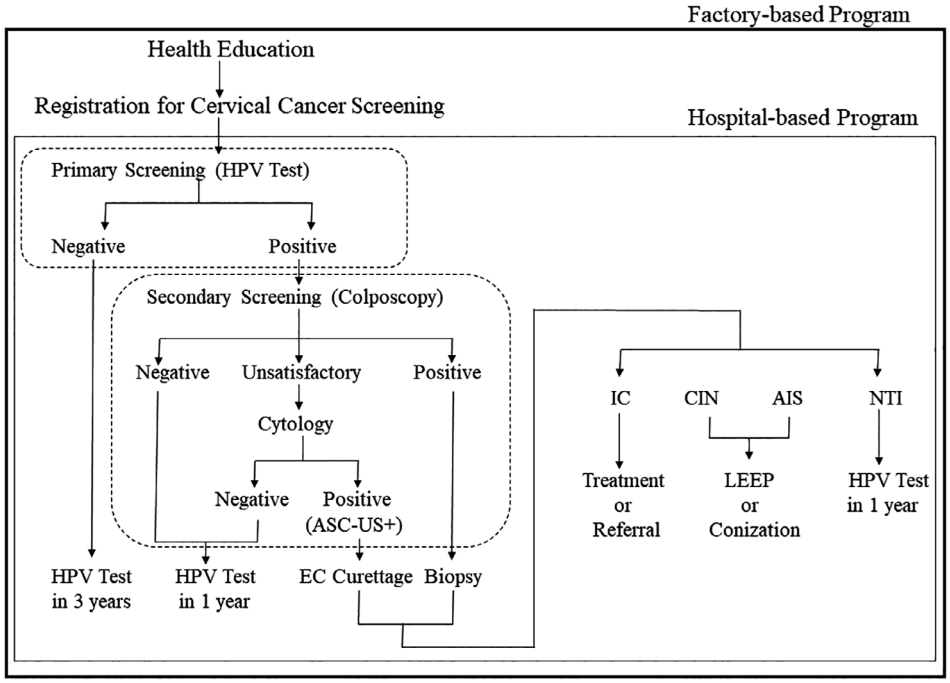

Figure 1 Change in the number of cervical cancer cases, by histological types, during the period of 1976–2010, by organizer of screening program (fine square: hospital-based program; bold square: factory-based program).

we examined the rates for participant cervical screening, HPV positive tests and detection of CIN. Using Fisher's exact test, rates were compared between the factory-based and hospital-based programs. Using the chi-square test, the age distribution of the screening participants and the distribution of HPV types were compared between the two types of programs. The level of statistical significance was set at $P = 0.05$. The accuracy of the HPV testing conducted in the Cambodian national labs was evaluated based on results of testing the same samples done in Japan. The prevalence of HPV types was determined based mainly upon the results of the HPV tests conducted in Japan.

Results

Health education

The first session for woman's health education was held at a PPSEZ factory in August of 2016. Since then, health education sessions were conducted at five factories, for a total of 14 additional times. The sessions were taught by SCGO gynecologists, midwives and SCGO Secretariat. Among the total of 2597 female PPSEZ factory workers who participated in the education sessions, by our criteria, 681 women were deemed eligible for cervical cancer screening tests. Among the eligible participants, 205 (30%) registered to receive the HPV test, which was the first round of cervical cancer screening. Among those 205, 128 participated in screening conducted between June 2017 and April 2018 (Table 1), for a screening rate of 19% (128/681), for that time period.

Table 1 Program participants[†]

	Factory-based	Hospital-based	*P* value
Women's health education	2597		
Age distribution			
15–19	492	—	
20–24	1025	—	
−29	588	—	
30–39	347	—	
40–49	18	—	
50–59	2	—	
Unknown	1734	—	
Eligible for cervical cancer screening (age distribution is unknown)	681		
Registered for cervical cancer screening	205	339	
Age distribution			<0.01
20–24	75	0	
25–29	48	39	
30–39	33	103	
40–49	5	107	
50–59	0	74	
60 and above	0	16	
Unknown	44	0	
Participated in cervical cancer screening	128	315	
Age distribution			<0.01
20–24	4	0	
25–29	58	30	
30–39	57	88	
40–49	7	108	
50–59	1	70	
60 and above	0	1	
Unknown	1	0	

[†]Chi-square test was used to compare age distributions between the two groups.

Comparison of the factory-based and hospital-based programs

Separate from the factory-based programs described above, on three occasions, from 2016 to 2018, a hospital-based program of cervical cancer screening was also conducted by SCGO gynecologists in the project's three participating national hospitals. Comparing the two screening programs, 93% of the women participants in the factory-based program were in their twenties or thirties, whereas in the hospital-based program they were much older, only 37% of participants were in that young age group (Table 1).

High-risk types of HPV were detected more frequently (12%) in the factory-based program participants than those in hospital-based program (5%) (Table 2). The rate for receiving secondary colposcopy screening among the HPV-positive females was significantly higher (94%) in the hospital-based program than for the factory-based program (40%) ($P < 0.001$).

Accuracy of HPV testing in Cambodia

Of the 443 cases screened for HPV by the Cambodian national-level labs, a random 352 were re-analyzed for HPV genotypes in Japan. Among the 352 cases tested in both Cambodia and Japan, the Cambodian labs declared that 323 of the cases were HPV-negative and 29 cases HPV-positive. When these cases were

Table 2 Screening and treatment for cervical cancer

	Factory-based	Hospital-based	*P* value
Positive for HPV test	15/128 (12%)	17/315 (5%)	<0.01
Received secondary screening (Colposcopy±Cytology)	6/15 (40%)	16/17 (94%)	<0.01
Normal	4	3	
Unsatisfactory	0	6	
Cytology	—		
NILM	—	3	
ASC-US	—	1	
LSIL	—	2	
Abnormal	2	7	
Received biopsy/EC curettage	0	7	—
Insufficient material	—	2	
Cervicitis	—	2	
Condyloma	—	1	
CIN1	—	1	
CIN2	—	1	
CIN3+/AIS+	—	0	
Received LEEP/Conization	2/2 (100%)	4/7 (57%)	0.50
Histology			
CIN1	0	3	
CIN2	2	1	
CIN3+/AIS+	0	0	

AIS, Adenocarcinoma in situ; ASC-US, Atypical squamous cells of undetermined significance; CIN, cervical intraepithelial neoplasia; EC, Endocervical; HPV, human papillomavirus; LEEP, loop electrosurgical excision procedure; LSIL, Low grade squamous intraepithelial lesion; NILM, Negative for intraepithelial lesion or malignancy.

reanalyzed in Japan by a more sensitive PCR-based PGMY-CHU assay, of the 29 cases declared to be HPV-positive by the Cambodian labs, five (17%) were found to be HPV-negative by PCR. On the other hand, 32 (10%) of the 323 supposedly negative cases were judged to actually be HPV-positive, by PCR. Among these 32 misdiagnosed cases as negative for high-risk HPVs, three cases were falsely negative for HPV-16 and HPV-33 in one case, HPV-39 in one case, HPV-45 in two cases, HPV-51 in two cases, HPV-52 in three cases, HPV-56 in four cases, HPV-58 in one case, HPV-68 in two cases, HPV-16 and 58 in one case and HPV-51 and 66 in one case. The other 11 cases were positive for the other types which can be detected only by the PGMY-CHU assay that can detect 31 genotypes in Japan, but not by the Cambodian lab. These cases were not true false-negatives. There were 21 true false-negative cases. In total, the correctly diagnosed accuracy of the Cambodian labs was 92.6% (326/352), the false-negative rate was 1.4% (5/352) and the false-positive rate was 6.0% (21/352) (Table 3).

Prevalence of HPV types in Cambodia

The HPV types detected in the Cambodia samples by the PCR-based analysis conducted in Japan are shown in Table 4. The types of HPV present in the Cambodia samples were widely distributed and demonstrated no significant differences between the factory-based and hospital-based programs ($P = 0.16$). HPV-16 and HPV-18 were observed in four (3.1%) and two (1.6%) out of 128 factory-based program samples, respectively, and six (2.7%) and three (1.3%) out of 224 hospital-based program samples, respectively. The type most frequently detected in the factory-based program samples was HPV-51 (4.7%), whereas HPV-53 was the most frequently detected (3.6%) in the hospital-based program samples.

Discussion

Worldwide, 570 000 women are newly diagnosed yearly with cervical cancer and, for a number of socio-economic reasons, about 90% of these new cases occur in low-to-middle income countries.[8] Any practical prevention program must first aim to reduce the

Table 3 Cambodian laboratory human papillomavirus testing quality

Accuracy	False-positive	False-negative
92.6% (326/352)	1.4% (5/352)	6.0% (21/352)

Table 4 Human papillomavirus (HPV) type distribution in factory- and hospital-based programs

HPV-type	6	11	16	18	31	33	34	35	39	40	42	44	45	51	52	53	54	55	56	58	59	66	68	69	70	83	84
Factory-based	0	0	4	2	2	2	2	0	1	1	1	1	0	6	2	4	4	0	0	2	0	3	0	0	3	0	2
(%)	0.0	0.0	3.1	1.6	1.6	1.6	1.6	0.0	0.8	0.8	0.8	0.8	0.0	4.7	1.6	3.1	3.1	0.0	0.0	1.6	0.0	2.3	0.0	0.0	2.3	0.0	1.6
Hospital-based	1	1	6	3	0	1	0	1	2	0	2	3	2	1	4	8	1	1	5	2	2	1	2	1	3	1	0
(%)	0.4	0.4	2.7	1.3	0.0	0.4	0	0.4	0.9	0.0	0.9	1.3	0.9	0.4	1.8	3.6	0.4	0.4	2.2	0.9	0.9	0.4	0.9	0.4	1.3	0.4	0.0
Total	1	1	10	5	2	3	2	1	3	1	3	4	2	7	6	12	5	1	5	4	2	4	2	1	6	1	2
(%)	0.3	0.3	2.8	1.4	0.6	0.9	0.6	0.3	0.9	0.3	0.9	1.1	0.6	2.0	1.7	3.4	1.4	0.3	1.4	1.1	0.6	1.1	0.6	0.3	1.7	0.3	0.6

burden of cervical cancer by reducing the incidence of high-risk HPV infections, and by earlier detection and better treatment of cervical precancerous lesions. Effective screening programs are essential to this endeavor, especially in low-resource countries where, again for any number of reasons, an HPV vaccine has not yet been introduced. However, what kinds of screening program should be established still depends on the available economical and medical resources of any given country.

Cambodia has only recently become a lower-to-middle income country; however, it still has many healthcare issues to overcome. For example, the most common cancer in women in Cambodia is cervical cancer, which, in similarly advanced countries elsewhere, is highly preventable. Beginning in 2012, with an urgent need for action to reduce the mortality in Cambodia from this disease, the SCGO and JSOG started to discuss how to establish an effective and sustainable joint strategy for cervical cancer protection. At last, in 2015, the JSOG and SCGO signed and began to implement a factory-based cervical cancer screening program that extends vertically – from women's health education through cancer screening to early treatment of premalignant lesions. The screening and treatment parts of this program were then applied within a parallel hospital-based prevention program.

In the pilot factory-based program, after receiving their women's health education series, the registration rate for initial cervical cancer/HPV screening, among the eligible factory females, was 31%, and the final rate for HPV screening testing) was 19% (128/681). This 19% screening rate is relatively low, compared to countries such as Finland, where the screening rate approaches 70%.[9] Cambodian PPSEZ factory workers are relatively young and educational level is not high. They do not understand the importance of keeping good health well. They were likely to have heard about woman-based cancer screening for the first time at their woman's health education seminars. It is thus clear that in the future effective health education sessions for women's health in general and cervical cancer screening should be further strengthened with information-recommendation leaflets, focusing on a woman's susceptibility to cervical cancer its repercussions, and the usefulness of screening programs. There are several reports that inexpensive educational leaflets about health promotion in the form of a comic strip are effective with youth.[10,11] On the other hand, direct communication or counseling with Factory

workers and managers are also to be considered under low awareness and literacy of women in Cambodia. A free-coupon program for cervical cancer screening, although more expensive, was also shown to be effective – and an acceptable alternative to the extreme costs associated with caring for advanced cases of cervical cancer.[12] Using these methods, screening rates should be improved and cases of actual cervical cancer reduced.

The HPV-positive rate (12%) was significantly higher in the factory-based program than the hospital-based program (5%). This was mainly because the participants in the factory-based program were significantly younger than those participating in the hospital-based program. This result is consistent with a previous report that showed the HPV infection rate in the United States was 44% in females aged 20–24, 27% in those aged 25–29 and 28% in those aged 30–39.[13]

The 94% rate for receiving secondary colposcopy screening was significantly higher in the hospital-based program than the 40% for the factory-based program. First barrier is the awareness: Recall system for HPV positive women was introduced from the second screening in April 2018. It seems effective with improved secondary screening rate (0 out of 4 [0%] in the first time in June 2017 to 6 out of 11 [54.5%] in the second time in April 2018). In the next step of the project, the importance of the secondary examination should be better explained to the factory-based program participants during health education and screening. In the early period of developing cancer screening system in Cambodia, face-to-face explanation by professionals, both with those who has positive or negative result, is important for future expansion, The second one is the geographic barrier: The females who tested positive for HPV in the factory-based program, in order to receive colposcopy examination, would have needed to travel to the national hospitals, and those hospitals are located by 1-h ride by bus from their PPSEZ work place. Medical clinics capable of providing affordable colposcopy should be built closer to the PPSEZ. Third one is the financial barrier: cost for the secondary examination at hospital. This barrier will be resolved, including early treatment, through being covered by the social insurance for factory workers since January 2018 under the movement toward Universal Health Coverage. The next issue to address is the development of early-lesion treatment procedures which the patients will accept and which can be easily accessed, including the LEEP and conization procedures. A one-day-stop-and-shop strategy that covers all aspects of cervical cancer prevention, ranging from an abbreviated intense education burst to screening and treatment.

Screening using the low-cost HPV hybridization test is thought to still be in the development stage. The accuracy of the HPV hybridization testing conducted by the Cambodian labs was 92.6%. False-negatives were called by the hybridization test in 38% of the actual HPV-positive cases (21/56, including four cases positive for very-high-risk HPV-16). In primary screening using the HPV hybridization test alone, the occurrence of false-negatives for the high-risk types of HPV was a serious mistake, especially for HPV-16 or 18. Although occurring possibly due to a technical error, the more likely scenario is that the copy number of HPV DNA genomes in those samples might have been insufficient to be detected by the hybridization technology used by the care HPV test system, whereas the more sensitive PCR-based test conducted in Japan could detect them. Quantitative PCR, which would have given relative copy numbers of HPV genomes in the various false-negative samples, was not conducted, so we cannot rule out operator error or presence of hybridization inhibitors in the samples as the source of false-negative signals from the Qiagen test. Likewise, probe hybridization could have detected a variant type of HPV that a primer-pair missed due to mismatches underlying the primer sites, meaning the PCR test would give the false-negative. Only metagenomic deep sequencing would resolve these issues, and that is as yet too expensive for a screening program in a limited-resource setting such as Cambodia.

This study is the first to demonstrate the HPV risk-subtype distribution in a screening setting in Cambodia. HPV types were widely distributed in both the factory and hospital programs. In the factory-based program, participants were mostly 40 or younger, and the two most dangerous HPV types, HPV-16 and HPV-18, were observed in 3.1% and 1.6% of the participants, respectively, whereas HPV-51 was the most frequently detected (4.7%). HPV types 51 and 53 are low-risk genotypes for development of invasive cancer although these cause squamous intraepithelial lesions. These data were reasonably consistent with those of Japanese females aged 20–25 years.[14] Notably, generation in both factory-based and hospital-based program are so young that various low-risk HPV genotypes can be detected widely in women. The sample size in this study is not so enough to analyze the prevalence of HPV genotype in Cambodia and future study would be needed in term of epidemiological interpretation of HPV prevalence. HPV subtype provides important information for HPV vaccination.

In the present analysis, a new system of cervical cancer prevention in Cambodia was implemented and evaluated. However HPV based screening was successfully introduced at factory settings, there were some problems encountered, including the lower accuracy of the low-cost HPV test that was used in-country, and the low secondary/follow-up colposcopy screening rate for the factory women who tested positive for HPV. These two issues must be forcefully addressed before this stage of our project can significantly progress. We need to improve HPV-based screening. A total of 92% might be acceptable for accuracy rate in Cambodia, but close monitoring and follow-up is still needed for future improvement.

The problems surrounding cytology, colposcopy and histological diagnosis during screening were not evaluated here. They will be addressed in a subsequent report. Our future screening strategies will be modified according to our evaluations of the program to date. We are expecting an introduction of the HPV vaccine into the Cambodian healthcare system, which might significantly change their situation. However, we still develop cancer screening program and make it function. For this purpose, HPV based screening is considered to be feasible in Cambodian context and can be continued. We (the JSOG and SCGO) will maintain our strong partnership to further develop an effective cervical cancer prevention system in Cambodia, which will serve to improve women's health in both our countries.

Acknowledgments

We would like to thank Dr G. S. Buzard, for his constructive critique and editing of our manuscript. We would like to express our sincere gratitude to Professors Ikuo Konishi and Tomoyuki Fujii for guidance on the promotion of this project and many Cambodian and Japanese doctors for supporting this project. This project was funded by Japan International Cooperation Agency Partnership Program and by the Program for International Promotion of Japan's Healthcare Technologies and Services by the Ministry of Health, Labour and Welfare.

Disclosure

Y. U. received lecture fees and a research grant (J550703673) from Hologic-Japan.

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