

A population-based study of cervical cytology findings and human papillomavirus infection in a suburban area of Thailand



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ABSTRACT

Despite the high incidence of cervical cancer in Thailand, large population-based studies on cervical cytology and HPV prevalence and genotype distribution are rare. This study aimed to determine cervical cytology results and the prevalence and distribution of HPV among Thai females in Bangkhayaeng subdistrict, Pathumthani province, Thailand. Of 4681 female inhabitants, aged 20–70 years, 1523 women finally participated in the study. Cervical samples using liquid-based cytology were collected during February–August 2013 and analyzed for HPV genotype by the LINEAR ARRAY® HPV Genotyping Test (Roche, USA). All participants with abnormal cytology or HPV positivity underwent colposcopy and biopsy. Of 1523 eligible women, 4.1% had abnormal cytology including ASC-US (2.4%), LSIL (1.0%), and HSIL (0.5%). The HPV infection rate was 13.7%. The prevalences of high-risk, probable high-risk, and low-risk HPV types were 5.6%, 3.5%, and 6.8%, respectively. The most common high-risk HPV types detected were HPV-16 (1.31%), HPV-51 (1.25%), and HPV-52 (1.25%). The most common probable high-risk and low-risk HPV types detected were HPV-72 (1.51%), HPV-62 (1.38%), and HPV-70 (1.18%). The rates of CIN2–3 and cancer in this cohort were 1.4% and 0.3%, respectively. In conclusion, HPV prevalence in this study was lower than reported in studies conducted in Western countries or other Asia countries, despite the high prevalence of CIN2+ and cancer. HPV type screening results of the general population in Bangkhayaeng subdistrict were similar to those reported in other countries, with HPV-16 the most common type. However, higher frequencies of HPV-51 and HPV-52 were observed. Despite the availability of a free screening program in this area, the participation rate remains low.

1. Introduction

Per GLOBOCAN 2012, cervical cancer is the fourth most common cancer in women worldwide with 528,000 new cases reported annually (Ferlay et al., 2013). It is also the fourth most common cause of cancer death in women with 266,000 deaths occurring worldwide in 2012. Almost 70% of the global cervical cancer burden is found in less developed countries. Despite the decreasing worldwide incidence, cervical cancer remains a critical health problem in Thailand with > 8000 new cases diagnosed annually (rate, 16.7 per 100,000 persons). Additionally, it is the second most common cancer and a leading cause of cancer death in Thai women after breast cancer (Ferlay et al., 2013).

High-risk human papillomavirus (HPV) types are now recognized as a causal factor of cervical cancer (Walboomers et al., 1999). The distribution of HPV genotypes varies according to ethnic, geographic, and behavioral factors (Bruni et al., 2010). Increased understanding about

geographical distribution of HPV genotypes in Thailand may aid in development of cervical cancer control policies.

A limited number of population-based studies about cervical cytology and HPV infection in Thailand have been performed. Therefore, we conducted a study in Bangkhayaeng subdistrict, Pathumthani province to evaluate cervical cytology and HPV prevalence in all women residing in this area. A cervical cancer screening service using liquid-based cytology and HPV DNA testing was performed in all participants. Colposcopy was then performed for women with abnormal cytology results. Our study aimed to determine cytologic findings and HPV prevalence and genotype using cervical samples collected from women living in a suburban area of Thailand.

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2. Materials and methods

2.1. Study population

This study was conducted in Bangkhayaeng subdistrict, Pathumthani province, which is close to Bangkok, during February–August 2013. The study was approved by Chulabhorn Hospital's ethical committee for human research (No. 01/2556). Gynecologic oncologists from Chulabhorn Hospital cooperated with subdistrict health officers at a Bangkhayaeng health promotion hospital to educate women about cervical cancer and the free screening program.

House-to-house recruitment of all adult women aged 20–70 years residing in this subdistrict was conducted by subdistrict health officers of the Ministry of Public Health. Cervical screening by liquid-based cytology and HPV genotyping were performed. Exclusion criteria included absence of a cervix, history of abnormal cytology or cervical intraepithelial neoplasia, cervical carcinoma, active diseases for any types of cancer during the last 5 years, or inability to receive follow-up. Women who agreed to participate provided written informed consent and received a pelvic examination by gynecologic oncologists.

2.2. Sample collection and preparation

Samples were obtained using a cytobrush by gynecologic oncologists of Chulabhorn Hospital. The brush was then placed in preservative fluid in the BD SurePath Pap test kit (BD Diagnostics-Tripath, Burlington, NC, USA) for liquid-based cytology and HPV DNA testing. Investigators performing HPV typing were blinded to cytology results. All cervical cytology slides were interpreted by standard protocol by qualified pathologists at Chulabhorn Hospital using the Bethesda 2001 system.

2.3. HPV genotyping

HPV genotyping was performed using the LINEAR ARRAY® HPV Genotyping Test (Roche Molecular System, Inc., Branchburg, NJ, USA) according to the manufacturer's protocol. This kit can identify 37 HPV types: 12 high-risk (HR) types (16/18/31/33/35/39/45/51/52/56/58/59), 8 probable high-risk (PR) types (26/53/66/67/68/70/73/82), and 17 low-risk (LR) types (6/11/40/42/54/55/61/62/64/69/71/72/81/83/84/IS39/CP6108), which are classified by oncogenic potentiality.

2.4. Diagnostic procedures

Cytologic specimens were classified by the Bethesda 2001 system into atypical squamous cells of undetermined significance (ASC-US), low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL), and cancer. All women with abnormal cytology results (ASC-US or more severe) and those with any HPV types underwent colposcopy, endocervical curettage, and cervical biopsy. Biopsy of a visible lesion was then performed. For patients with no visible lesion, a random biopsy was performed. Endocervical curettage and biopsy specimens were then analyzed by qualified pathologists at Chulabhorn Hospital.

2.5. Statistical analysis

We used descriptive statistics to determine distribution and frequencies of HPV, age, and cytology results. Frequency tables were used for qualitative variables. Odds ratios (ORs) with 95% confidence intervals (CIs) were used for relative risk evaluation.

3. Results

3.1. Participation rates

Of 4681 women aged 20–70 years from Bangkhayaeng subdistrict, 1780 women (38.0%) participated. Of these, 257 women were excluded, thus 1523 women were finally enrolled in this study. The main reasons for exclusion were residence outside of Bangkhayaeng subdistrict, refusal to participate, or did not show up for appointments.

3.2. Demographic characteristics

The mean age of participants was 44.5 years (range, 20–70 years). The proportions of participants aged 20–30 years and 31–70 years were 10.9% and 89.1%, respectively. Most women (96.4%) had had sexual intercourse and reported contraception use (71.0%). Eighty-four percent was multiparous and 68% was premenopausal. Nearly half of the participants' educational level was elementary school (44.7%), followed by high school (26.9%), bachelor's degree (12.3%), and vocational school (11.9%). Only 1.9% of the cohort had an education level below the standard education level.

3.3. Prevalence of abnormal cervical cytology and HPV infection

All specimens were sufficient for sequencing. There were 1292 (84.8%) women with normal results (normal cytology/HPV-negative) and they received follow-up appointments at 5-year intervals. Twenty-three (1.5%) women had abnormal cytology/HPV-negative, 168 (11.0%) women had normal cytology/HPV-positive, and 40 (2.6%) women had abnormal cytology/HPV-positive. Women in these three groups underwent colposcopy with endocervical curettage and cervical biopsy to acquire histologic diagnoses. Fig. 1 demonstrates pathology results of these groups.

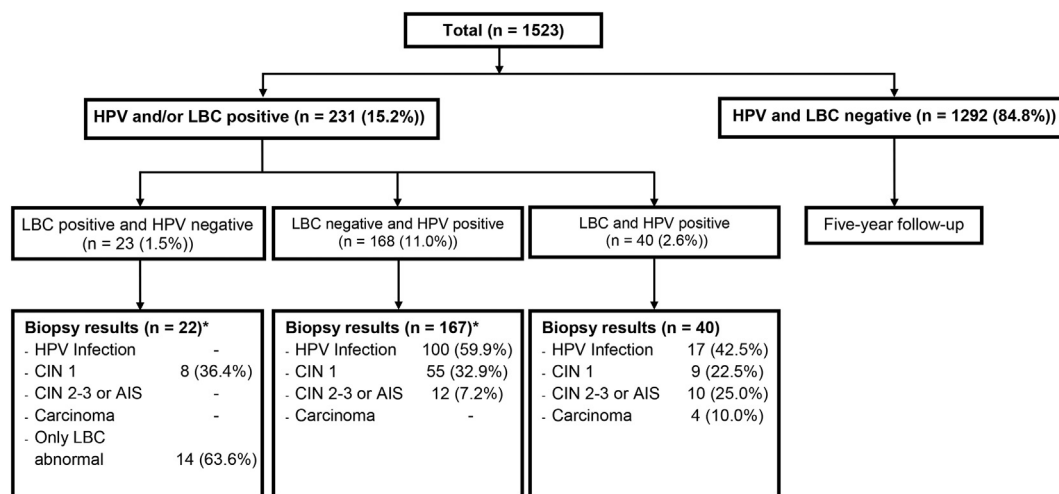
The rates of CIN2+ lesions [cervical intraepithelial neoplasia (CIN) 2–3, adenocarcinoma in situ (AIS), and carcinoma] in the abnormal cytology/HPV-negative group, normal cytology/HPV-positive group, and abnormal cytology/HPV-positive group were 0%, 7.2%, and 35.0%, respectively. Of 1523 women, CIN2–3/AIS were found in 22 women (1.4%). Invasive cervical cancer was found in four women (0.3%).

Table 1 shows the overall HPV prevalence according to cytology result and prevalences of HR-HPV, PR-HPV, and LR-HPV. Of 1523 women, 4.1% (n = 63) had abnormal cytology results including ASC-US (2.4%, n = 37), LSIL (1.0%, n = 15), and HSIL (0.5%, n = 8). Other abnormal cytology results included ASC-H, atypical glandular cell, and squamous cell carcinoma (SCC), which were each found in 0.06% (n = 1) of women.

Overall, HPV was detected in 13.7% (n = 208) of specimens. The overall prevalence of HR-HPV was 5.6% (n = 86). When classified by cytologic findings, overall HPV and HR-HPV positivity rates in the normal cytology group were 11.5% and 4.1%, respectively. For the abnormal cytology group, HPV and HR-HPV positivity rates were 63.5% and 41.3%, respectively. In the LSIL group, HPV and HR-HPV positivity rates were 93.3% and 46.7%, respectively. In HSIL and SCC groups, HR-HPV was detected in all specimens.

HPV-16 prevalence was 1.31% (n = 20), which was the most common HR-HPV type followed by HPV-51 (1.25%, n = 19) and HPV-52 (1.25%, n = 19) (Fig. 2A). HPV-18 prevalence was only 0.53% (n = 8).

Overall, HPV infection was highest in young women aged 20–30 years, which decreased with increasing age but increased again in women aged 61–70 years (Fig. 2B). HPV prevalences stratified by age were as follows: 19.3% (20–30 years), 15.5% (31–40 years), 12.0% (41–50 years), 10.0% (51–60 years), and 16.8% (61–70 years).



* 1 case not done, n: number, HPV: human papillomavirus, LBC: liquid-based cytology, CIN: cervical intraepithelial neoplasia, AIS: adenocarcinoma in situ,

Fig. 1. Pathological diagnosis according to cervical cancer screening results.

3.4. HPV prevalence and cytology in each diagnostic category

Table 2 demonstrates HPV status classified by pathological results from tissue biopsy and risk evaluation. HR-HPV infection, especially HPV-16, had high oncologic potential for prediction of CIN2+ with ORs of 5.8 (95%CI 2.2–15.3) and 15.3 (95%CI 4.9–47.3), respectively.

HR-HPV was found in all women with invasive cervical cancer (4/4, 100%) and 16 of 22 women with CIN2–3 (72.7%; OR 4.6, 95%CI 1.6–15.2). HR-HPV type 16 was detected in 8 (36.4%) women with CIN2–3 (OR 11.9, 95%CI 3.0–47.0) and 3 (75%) women with SCC (OR 62.5, 95%CI 3.9–3302.5). Another cervical cancer case had HR-HPV type 33 and LR-HPV type 61 co-infection.

All four women with pathological result of invasive cervical cancer had abnormal cytology results (SCC, n = 1; HSIL, n = 1; ASC-H, n = 1; ASC-US, n = 1). Twelve (54.5%) women with CIN2–3 had normal cytology and 10 (45.5%) women had abnormal cytology.

3.5. Sensitivity and specificity of screening tests

Table 3 shows the calculated sensitivity, specificity, positive predictive value, and negative predictive value for CIN2+ detection. For HR-HPV testing, the sensitivity and specificity were 76.9% (95%CI 56.4–91.0%) and 95.6% (95%CI 94.4–96.6%), respectively. The sensitivity and specificity of HPV DNA testing were 100.0% (95%CI 86.8–100.0) and 87.8% (95%CI 86.1–89.5), respectively. For liquid-based cytology, the sensitivity and specificity were 53.8% (95%CI 33.4–73.4) and 96.7% (95%CI 95.7–97.6), respectively. HPV DNA

Table 1
Cytologic categories and HPV type detected.

	Total n (%)	Cytology, n (%)						
		Normal	ASC-US	ASC-H	LSIL	HSIL	AGC-FN	SCCA
n	1523	1460	37	1	15	8	1	1
HPV –	1315 (86.3)	1292 (88.5)	21 (56.8)	0 (0.0)	1 (6.7)	0 (0.0)	1 (100.0)	0 (0.0)
HPV +	208 (13.7)	168 (11.5)	16 (43.2)	1 (100.0)	14 (93.3)	8 (100.0)	0 (0.0)	1 (100.0)
HPV HR	86 (5.6)	60 (4.1)	9 (24.3)	1 (100.0)	7 (46.7)	8 (100.0)	–	1 (100.0)
HPV PR	54 (3.5)	45 (3.1)	4 (10.8)	–	5 (33.3)	–	–	–
HPV LR	104 (6.8)	89 (6.1)	5 (13.5)	–	8 (53.3)	2 (25.0)	–	–

*n: number, HPV –: HPV negative, HPV +: HPV positive, HR: high risk, PR: probable high risk, LR: low risk, ASCUS: atypical cells of undetermined significance, ASC-H: atypical squamous cells, cannot exclude HSIL, LSIL: low-grade squamous intraepithelial lesion, HSIL: high-grade squamous intraepithelial lesion, AGC-FN: atypical glandular cells, favor neoplasia, SCCA: squamous cell carcinoma.

testing had the highest sensitivity but lowest specificity. Conversely, cytology had the highest specificity but lowest sensitivity. All screening tests had very high negative predictive values ranging 99.2–100.0%.

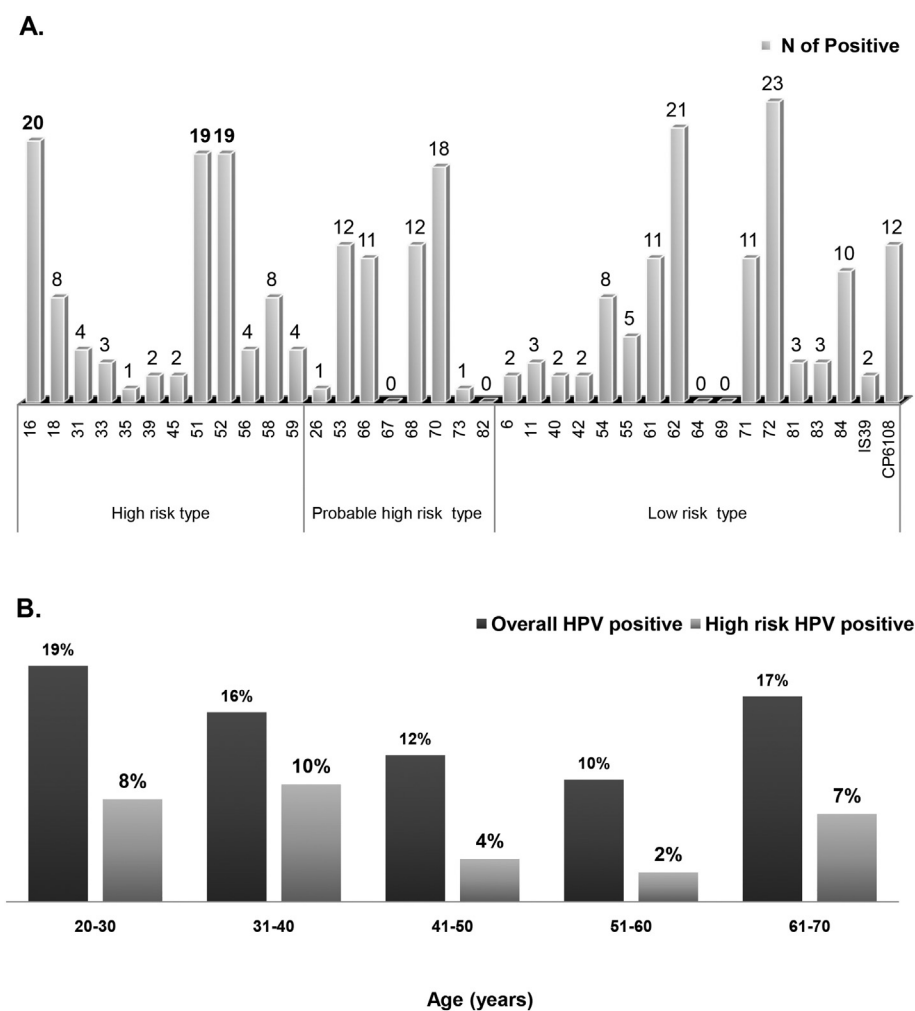
4. Discussion

We conducted a population-based study to evaluate cervical cytology results, HPV genotyping, and histologic diagnoses for all abnormal groups. This study was performed with less selection bias compared with hospital-based studies. The relation between screening results and histologic diagnoses demonstrated the efficiency of screening tests. To our knowledge, there has been no previous population-based study using liquid-based cytology and LINEAR ARRAY® HPV Genotyping Test with histologic diagnosis.

Geographic and ethnic variations in HPV prevalence and genotype distributions have been described (Bruni et al., 2010). This study revealed low HR-HPV prevalence (5.6% overall, 4.1% in women with normal cytology) in women from a suburban area of Thailand, which was lower than that reported in population-based studies from Western countries (24.0–28.3%) (Bonde et al., 2014; Tachezy et al., 2013). Compared with other population-based studies in Asia, this HR-HPV rate was lower than reported in Japan (17%), China (21.07%), India (10.3%), Indonesia (7.9%), and Nepal (6.1%), but marginally higher than reported in Bangladesh (4.2%) (Nahar et al., 2014; Sankaranarayanan et al., 2009; Sasagawa et al., 2016; Sherpa et al., 2010; Vet et al., 2008; Wang et al., 2015).

In comparison with our results, a population-based study from

Fig. 2. A. HPV genotype distribution (37 types) and B. HPV prevalence stratified by age group.



Northern Thailand with 2752 participants demonstrated an overall HR-HPV prevalence of 7.1% (4.1% in the normal cytology group) using Hybrid Capture 2 tests (Siriaunkgul et al., 2014). Another population-based study in Lampang and Songkla found an overall HR-HPV rate of 6.3% (Sukvirach et al., 2003).

The most common HR-HPV type observed in this cohort was HPV-16, followed by HPV-51 and HPV-52 (Fig. 2A). HPV-52 is particularly prevalent in Asian populations (Sasagawa et al., 2016; Vet et al., 2008; Wang et al., 2015). However, the number of women with each HPV type in this study was too low to definitively confirm the most common HPV type.

HPV prevalence appeared to be associated with age. HPV prevalence was highest in younger women (20–30 years), declined in older women (30–60 years), and increased again in women > 60 years. HPV

prevalence by age demonstrated a U-shaped curve, which is consistent with results from a previous study (Herrero et al., 2000). One potential explanation for the second peak in HPV infection may be a cohort effect that older women were more exposed to HPV. Alternatively, the second peak may indicate reactivation of latent HPV infections (Palefsky et al., 1999).

HR-HPV prevalence in each cytologic category correlated with disease severity: normal cytology (4.1%), ASC-US (24.3%), LSIL (46.7%), HSIL (100%), and SCC (100%). Overall HPV rates in normal cytology, ASC-US, LSIL, HSIL, and SCC groups were 11.5%, 43.2%, 93.3%, 100%, and 100%, respectively. Increased HPV positivity was observed with more severe abnormal cytology.

The importance of HPV infection, especially HR-HPV, for cervical cancer development was confirmed in our study. We observed

Table 2 Pathological results from tissue biopsy, HPV type detected, and risk evaluation.

Pathology	n	Detection of any HPV type n (%)	ORs for any HPV (95% CI)	HR-HPV n (%)	ORs for HR-HPV (95% CI)	HPV-16 n (%)	ORs for HPV-16 (95% CI)	HPV-18 n (%)	ORs for HPV-18 (95% CI)	HR-HPV non-16/18 n (%)	ORs for HR-HPV non-16/18 (95% CI)
Total	229	207 (90.4)		86 (37.6)		21 (9.2)		8 (3.5)		58 (25.3)	
Normal	131	117 (89.3)	1.0	48 (36.6)	1.0	6 (4.6)	1.0	5 (3.8)	1.0	37 (28.2)	1.0
CIN1	72	64 (88.9)	1.0 (0.4–2.4)	18 (25.0)	0.6 (0.3–1.1)	4 (5.6)	1.2 (0.3–4.5)	1 (1.4)	0.4 (0.0–3.1)	13 (18.1)	0.6 (0.3–1.1)
CIN2–3 or AIS or carcinoma	26	26 (100.0)	N/A	20 (76.9)	5.8* (2.2–15.3)	11 (42.3)	15.3* (4.9–47.3)	2 (7.7)	2.1 (0.4–11.5)	8 (30.8)	1.1 (0.4–2.8)

n: number, HR-HPV: high risk HPV, CI: confidence interval, ORs: odd ratios, CIN: cervical intraepithelial neoplasia, AIS: adenocarcinoma in situ, N/A: not available. * Significant (P value < 0.05).

Table 3

Sensitivity, specificity, positive predictive value, and negative predictive value of screening tests for detecting CIN2 or more severe lesions.

Methods	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	ROC Area (95% CI)
HR-HPV detection	20/26 (76.9%) (56.4, 91.0%)	1431/1497(95.6%) (94.4, 96.6%)	20/86 (23.3%) (14.8, 33.6%)	1431/1437(99.6%) (99.1, 99.8%)	(0.86) (0.78, 0.95)
HPV detection	26/26 (100.0%) (86.8, 100.0%)	1315/1497(87.8%) (86.1, 89.5%)	26/208(12.5%) (8.3, 17.8%)	1315/1315(100.0%) (99.7, 100.0%)	(0.94) (0.93, 0.95)
LBC	14/26 (53.8%) (33.4, 73.4%)	1448/1497(96.7%) (95.7, 97.6%)	14/63 (22.2%) (12.7, 34.5%)	1448/1460 (99.2%) (98.6, 99.6%)	(0.75) (0.66, 0.85)
HR-HPV + LBC	21/26 (80.8%) (60.6, 93.4%)	1395/1497(93.2%) (91.8, 94.4%)	21/123 (17.1%) (10.9, 24.9%)	1395/1400 (99.6%) (99.2, 99.9%)	(0.87) (0.79, 0.95)
HPV + LBC	26/26 (100.0%) (86.8, 100.0%)	1292/1497(86.3%) (84.5, 88.0%)	26/231 (11.3%) (7.5, 16.1%)	1292/1292(100.0%) (99.7, 100.0%)	(0.93) (0.92, 0.94)

PPV: positive predictive value, NPV: negative predictive value, CIN: cervical intraepithelial neoplasia, CI: confidence interval, ROC: receiver operating characteristic, LBC: liquid-based cytology, HR: high risk.

significant ORs for HR-HPV and HPV-16 for CIN2 + development, but nonsignificant ORs for HPV-18 or HR-HPV non-16/18 (Table 2).

Interestingly, HPV DNA testing tended to identify more high-grade cervical lesions (HPV testing, sensitivity = 100%, HR-HPV testing, sensitivity = 76.9%) compared with liquid-based cytology (sensitivity = 53.8%). Therefore, HPV DNA testing may be a more sensitive method for cervical cancer screening.

This cervical cancer screening program had a low participation rate (38%), although the population was homogenous and had the same cultural background. The main reasons for low participation appeared to be relocation to an area outside of the study area and unawareness of the importance and availability of free cervical cancer screenings. Further studies exploring reasons for non-participation should be conducted to develop methods to increase participation rates.

Thailand is considering implementation of a national HPV vaccination program. HPV-16 was commonly observed in our cohort and the current vaccine against HPV-16/18 is suitable for vaccination strategies. HPV-18 prevalence was relatively low in this cohort, however, the number of participants was too low to make a definitive conclusion. As HPV-51 and HPV-52 were quite common in our cohort, a next generation HPV vaccine that covers five additional HR-HPV types (HPV-31/33/45/52/58 or a nonavalent vaccine) may be more appropriate for a national HPV vaccination program (Joura et al., 2015).

In conclusion, HPV prevalence and abnormal cytology in women in Bangkhayaeng subdistrict was relatively low in contrast to the high prevalence of CIN2 + and cancer compared with previous studies. The most common HPV type detected was HPV-16, however, higher frequencies of HPV-51 and HPV-52 were also observed in this study. HR-HPV testing may be superior to cytology for detection of high-grade cervical intraepithelial lesions. Nevertheless, there were several limitations of our analysis due to the small sample size of each group. Additional population-based HPV studies in Thai women may be necessary for development of an effective cervical cancer prevention program in Thailand.

Consent

Written informed consent was obtained from all participants.

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Potential conflict of interest

None.

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